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1 **Understanding the digestibility and nutritional functions of rice starch**
2 **subjected to heat-moisture treatment**

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16

17 **Abstract:** In this study, rice starch with well-controlled digestion resistibility achieved by heat-
18 moisture treatment (HMT) was chosen as a supplementary diet for high-fat-diet-fed mice. Then, the
19 nutritional functions of HMT-modified rice starch were evaluated by the physiological and
20 biochemical indices, proliferation and distribution of intestinal microflora, and functional diversity
21 by putative metagenomes analysis. Compared with the native-rice-starch mice (DM) group, the
22 blood glucose, serum lipid, oxidative stress, and liver function metabolic levels/indices of the HMT-
23 rice-starch mice (HMT-DM) group were worse due to the declined level of slowly digestible starch
24 (SDS) in HMT-modified rice starch. Meanwhile, the species diversity index was observed to be
25 higher in the DM group and *Bifidobacteria* was identified as a type of bacteria related to the
26 relatively higher content of RS in HMT-modified rice starch. Overall, our results provide important
27 information for the rational design of rice starch-based health-promoting foods with nutritional
28 functions.

29
30 **Keywords:** Rice starch; heat-moisture treatment; digestion; nutritional functions; intestinal
31 microflora

32

33 **Nomenclature**

34	SDS	slowly digestible starch
35	HMT	heat-moisture treatment
36	RDS	rapidly-digestible starch
37	SDS	slowly-digestible starch
38	RS	resistant starch
39	TG	triglyceride
40	TCH	total cholesterol
41	HDL-c	high-density lipoprotein cholesterol
42	LDL-c	low-density lipoprotein cholesterol
43	MDA	Malondialdehyde
44	SOD	Superoxide dismutase
45	GSH-PX	Glutathione peroxidase
46	T-AOC	Total antioxidant capacity
47	ALT	alanine aminotransferase
48	ALP	alkaline phosphatase
49	AST	aspartate aminotransferase
50	OTUs	Operational Taxonomic Units
51	SEM	standard error of the mean

52

53 1. Introduction

54 With the improvement of living standard and the increased diversity of diet and disease spectra,
55 foods with nutritional functions such as physiological accommodation and disease prevention have
56 attracted increasing attention (Barratt, Lebrilla, Shapiro, & Gordon, 2017; Caballero, 2013; Link &
57 Reue, 2017). Therefore, designing personalized healthy foods with particular nutritional functions
58 has been one of the hotspots in food science. Rice, one of the most widely consumed staple food, is
59 mainly composed of starch. Modulating the digestibility and nutritional functions of rice starch have
60 a direct impact on human health.

61 For positive physiological effects and nutritional benefits, starch, depending on the rate and extent
62 of digestion, can be classified into three categories, namely rapidly-digestible starch (RDS), slowly
63 digestible starch (SDS), and resistant starch (RS) (Dhital, Warren, Butterworth, Ellis, & Gidley,
64 2017; Englyst, Kingman, & Cummings, 1992). Previous research has shown that RDS, after rapidly
65 digested in the digestive tract, releases glucose, which increases the level of serum glucose, promotes
66 insulin secretion, stimulates the liver and lipocytes to create fat, and increases the serum lipid level in
67 our body (Hung, Chau, & Phi, 2016; Lee et al., 2014). Meanwhile, SDS and RS can retard or restrain
68 the release of glucose, lower the glycemic index, improve the insulin sensitivity, promote the
69 secretion or expression of insulin and adipocyte-secreted factors, and prevent the fat from deposition
70 (Dhital, Bhattarai, Gorham, & Gidley, 2016; Wei, Sissons, Warren, Gidley, & Gilbert, 2016).
71 Interestingly, RS will produce a series of short-chain fatty acid metabolites at a lower pH. Many
72 health benefits and functional properties of RS have been reported including the prebiotic effect on
73 colon microbes (Shang et al., 2017), the adjustment of lipid metabolism (Nathalie et al., 2016), the

74 improvement in cholesterol metabolism (Newman et al., 2017), and the reduction in the risk of
75 ulcerative colitis (Bindels et al., 2017) and colon cancer (Si, Strappe, Blanchard, & Zhou, 2016; Si,
76 Zhou, Strappe, & Blanchard, 2016). However, gelatinized rice starch consists of over 95% RDS,
77 which can be easily digested and absorbed, leading to a high glycemic response. This negative
78 physiological effect of rich starch does not correspond to the modern concept of nutrition and health.
79 Therefore, recent research has focused on changing the multi-scale structure of rice starch under
80 different ways of processing or physical modification to regulate its digestion, absorption, and
81 metabolism in the human gastrointestinal tract (GIT) and to improve its nutritional functions.

82 Different ways of changing the starch multi-scale structure and regulating the starch digestibility
83 have been widely reported (Klein et al., 2013; Puncha-Arnon & Uttapap, 2013; Silva et al., 2017;
84 Tan et al., 2017). As a new physical modification method, heat-moisture treatment (HMT) only
85 involves the use of thermal energy and moisture, which has advantages such as environmental
86 protection, high efficiency, and safety (Wang, Zhang, Chen, & Li, 2016; Zavareze & Dias, 2011).
87 HMT has already played an important role in the green processing of starch (Arns et al., 2015; Hung,
88 Vien, & Phi, 2016; Pratiwi, Faridah, & Lioe, 2017; Silva et al., 2017). However, limited work has
89 been undertaken for evaluating the effect of HMT on the nutritional functions of starch in terms of
90 blood lipid metabolism, blood sugar metabolism, and intestinal microflora. Thus, it is necessary to
91 establish a systemic connection between digestibility and nutritional functions, which would
92 contribute to the development of healthy starch food by HMT.

93 Therefore, in this study, we investigated the physiological and biochemical indexes and the
94 proliferation and distribution of intestinal microbiota for 10 high-fat-diet-fed mice supplemented

95 with rice starch (the DM group) and another 10 with HMT-modified rice starch (the HMT-DM
96 group) to understand the alteration of nutritional functions by HMT. To the best of our knowledge, it
97 is the first study comprehensively comparing these detailed nutritional function parameters between
98 native rice starch and HMT-modified rice starch.

99

100 **2. Material and methods**

101 **2.1 sample preparation**

102 Native rice starch was purchased from the South China Agricultural University (Guangzhou,
103 China). The RDS, SDS and RS contents of this starch were 32.6%, 52.4%, 9.0%, respectively, which
104 was analyzed with a modified Englyst procedure according to a previous study in our lab (Wang et
105 al., 2018). The HMT of rice starch was conducted under a moisture content of 25%. The sample was
106 equilibrated at 4 °C for 24 h. Then, they were placed in a 500 mL screwed stainless steel reactor with
107 continuous rotation and heated with oil at 110 °C for 4 h, followed by cooling to room temperature.
108 During HMT, the starch granules were stirred by the paddle when the reactor was continuously
109 rotated. Subsequently, the treated samples were dried at 40 °C and then ground. The RDS, SDS and
110 RS contents of HMT-modified rice starch were measured to be 60.4%, 20.7%, 18.9%, respectively.

111 **2.2 Animals**

112 Twenty healthy male C57BL-6 mice (non-obese) of 20±5 g weight were purchased from the
113 animal house, the Academy of Military Medical Sciences (China). These mice were fed in a clean-
114 grade facility in the Laboratory Animal Center at Tianjin University of Science and Technology.

115 After one week's adaptive feeding with a basal diet, no difference in the body weight was observed.
116 These mice were divided into two groups randomly. One group of mice were fed a high-fat diet
117 supplemented with rice starch (the DM group) and the other group supplemented with HMT-
118 modified rice starch (the HMT-DM group). For both groups, the intervention was implemented for 8
119 weeks. Their body weights and food intakes were monitored weekly. Feces were collected on the day
120 of necropsy.

121 All animal procedures were approved by the Ethical Committee for the Experimental Use of
122 Animals in the Center for Drug Safety Evaluation, Tianjin University of Science & Technology
123 (Approval No: 13/051/MIS).

124 **2.3 Experimental diets**

125 The experimental high-fat diet was prepared by Research Diets (Choi, Gwon, Ahn, Jung, & Ha,
126 2013), which consisted of 54.5% carbohydrate source, 10% lard oil, 20% casein, 5% corn oil, 0.5%
127 cholesterol, 5% cellulose, 3.5% mineral mix (based on AIN76), 1% vitamins (based on AIN76),
128 0.3% methionine, and 0.2% choline bitartrate. The two groups of mice were fed with the
129 experimental high-fat diet where carbohydrate source was replaced by rice starch and HMT-modified
130 rice starch, respectively.

131 **2.4 Blood and tissue analysis**

132 At the end of the intervention period, the mice were fasted overnight, before the blood glucose was
133 recorded. Blood samples were taken from the arteria femoralis for the determination of physiological
134 and biochemical indexes. The liver tissue, epididymal white adipose tissue, and perirenal white

135 adipose tissue were quickly removed after sacrifice and then weighed. The liver tissue was
136 immediately stored at -80°C for further pathological slides.

137 **2.4.1 Analysis of blood lipids composition**

138 Blood lipid indexes evaluated in this study included triglyceride (TG), total cholesterol (TCH),
139 high-density lipoprotein cholesterol (HDL-c), and low-density lipoprotein cholesterol (LDL-c). The
140 specific measurement procedures and final calculations were performed according to the
141 specification of the TG, TCH, HDL-c, and LDL-c assay kits (Jian Cheng Biotechnology Co., Ltd.,
142 Nanjing, China).

143 **2.4.2 Analysis of oxidative stress index**

144 There are four main indexes for characterizing oxidative stress. Malondialdehyde (MDA) is a final
145 product of the lipid oxidation process and was measured according to an MDA assay kit (Jian Cheng
146 Biotechnology Co., Ltd., Nanjing, China). Superoxide dismutase (SOD) was determined using an
147 SOD assay kit (Jian Cheng Biotechnology Co., Ltd., Nanjing, China). Glutathione peroxidase (GSH-
148 PX) was measured using an assay kit (Jian Cheng Biotechnology Co., Ltd., Nanjing, China) and the
149 activity was calculated according to the manufacturer's instructions. The Total antioxidant capacity
150 (T-AOC) was determined using a T-AOC assay kit (Jian Cheng Biotechnology Co., Ltd., Nanjing,
151 China) according to the manufacturer's instructions.

152 **2.4.3 Determination of hepatic lipid metabolism enzymes**

153 The detection of the liver functional metabolism mainly includes three indices reflecting the
154 aspartate transaminase (Nathalie et al.), alanine aminotransferase (ALT), and alkaline phosphatase
155 (ALP) activities. The aspartate aminotransferase (AST) activity was measured by colorimetric
156 analysis using an AST assay kit (Jian Cheng Biotechnology Co., Ltd, Nanjing, China). The ALT
157 activity was measured by colorimetric analysis using an ALT assay kit (Jian Cheng Biotechnology
158 Co., Ltd, Nanjing, China). The ALP activity was measured by standard oxidation using potassium
159 ferricyanide.

160 **2.4.4 Liver histology and analysis**

161 The liver tissues were placed in 10% neutral formalin liquid for 12 h, which were then dehydrated
162 using 30%, 50%, 70%, 80%, 90%, 95% and 100% ethanol solutions, respectively, and washed in
163 xylol. Then, the liver tissues were embedded in paraffin (BMJ-III embedding machine, Jiangsu,
164 China). Finally, they were stained with hematoxylin and eosin (H & E) and observed by light
165 microscopy at 200× magnification.

166 **2.5 Microbial community analysis**

167 **2.5.1 Feces samples collection, DNA extraction, and 16S rRNA gene sequencing**

168 Feces samples were collected in a sterile container and immediately stored at -80 °C until further
169 processing. Total genome DNA from samples was extracted using the hexadecyltrimethylammonium
170 bromide/sodium dodecyl sulfate (CTAB/SDS) method (Caporaso et al., 2010). The DNA

171 concentration and purity were monitored on 1% agarose gels. The 16S rRNA genes of distinct
172 regions in V4 were amplified using the specific primer of 515F-806R. All polymerase chain reaction
173 (PCRs) were carried out with a Phusion® High-Fidelity PCR Master Mix (New England Biolabs).
174 The same volumes of 1× loading buffer (containing SYBR Green I) were mixed with PCR products
175 and electrophoresis was operated on 2% agarose gels. Sequencing libraries were generated using a
176 TruSeq® DNA PCR-Free Sample Preparation Kit (Illumina, USA) following manufacturer's
177 recommendations, and index codes were added. Finally, the library was sequenced on an
178 IlluminaHiSeq2500 platform and 250 bp paired-end reads were generated.

179 **2.5.2 Intestinal microbiota analysis**

180 After the removal of the low-quality sequences, pyrosequencing errors, and chimera, all the
181 sequencing reads were denoised. Sequences of >97% similarity levels were assigned to the same
182 Operational Taxonomic Units (OTUs) (Edgar, 2013). Representative sequences for each OTU were
183 screened for further annotation. The rare fraction curves and alpha diversity indices (Chao1, ACE,
184 Shannon, and Simpson) were performed using MOTHUR software, and the beta diversity (among
185 samples) was analyzed using principal component analysis (PCA). The function of intestinal
186 microbiota was performed by online phylogenetic investigation of communities by reconstruction of
187 an unobserved states program (PICRUSt, <http://picrust.github.io/picrust/>).

188 **2.6 Statistical analysis**

189 All analyses were conducted in triplicate and statistical analyses were performed using the
190 Statistical Package for Social Science (SPSS) software version 22. Body weight and food intake

191 were analyzed using General Linear Model repeated measures (Mixed Design ANOVA). P
192 interaction indicates the effect of time and group interactions. Once a significant difference ($P \leq$
193 0.05) was detected, post hoc multi-comparisons were performed by LSD adjustment. Other
194 physiological and biochemical indexes (blood glucose and insulin) were analyzed by one-way
195 analysis of variance followed by Tukey's multiple comparison analysis. Different lowercase letters
196 above the same column indicate a significant difference ($P \leq 0.05$). Results were expressed as mean
197 \pm standard error of the mean (SEM).

198

199 **3. Results and discussions**

200 **3.1 Body weight, and liver and adipose tissue weights**

201 **Fig. 1** shows the changes in body weight of the two groups of mice after the 8-week intervention.
202 The initial body weights between the two groups were not significantly different ($P > 0.05$), showing
203 the rationality of the grouping method. From the start to Week 4, there were apparent decreasing
204 trends of the body weight and food intake for both of the groups. Afterwards, both the body weight
205 and food intake gradually increased. At the end of Week 8, the body weights of mice in these two
206 groups were 30% higher than their initial values, and General Linear Model (GLM) analysis showed
207 no significant difference (week \times group interaction) between the two groups ($P > 0.05$).

208 After the 8-week intervention, the perirenal fat, epididymal fat, and total fat-to-body ratio of the
209 HMT-DM group mice were significantly higher than those of the DM group mice (**Table 1**). These

210 results indicate that native rice starch had better performance in controlling the fat tissue deposit in
211 contrast to HMT-modified rice starch.

212 3.2 Blood glucose, insulin and lipid levels

213 **Table 2** shows the blood glucose, insulin, and blood lipid levels of the two groups of mice.
214 Previous research has shown that the blood glucose level of C57BL-6 mice was normally 6–10
215 mmol/L (Rodríguez, Limón-Pacheco, Del Razo, & Giordano, 2016), which was slightly lower than
216 those of two experimental groups after the 8-week intervention. This observation indicated that both
217 native rice starch and HMT-modified rice starch could stabilize the blood glucose level and reduce
218 the speed of change into a hyperglycemia condition. In addition, the blood glucose level of the HMT-
219 DM group was significantly higher than that of the DM group ($P < 0.05$), which might be related to
220 the increased RDS content and greatly decreased SDS content after HMT. The result confirmed that
221 the SDS content has a strong impact on the regulation of the blood glucose level as also shown in
222 recent studies (Kittisuban, Lee, Supphantharika, & Hamaker, 2014; Wolever et al., 2016).

223 After the intervention, the serum insulin levels of these two groups were all within the normal
224 range (Seghers, Nakazaki, Aguilar, & Bryan, 2000). Nonetheless, compared with the DM group, the
225 HMT-DM group displayed a significantly higher serum insulin level ($P < 0.05$), indicating a higher
226 insulin resistance of the HMT-DM group than that of the DM group. Thus, the HMT-modified rice
227 starch intervention reduced the biological effect of insulin as well as the sensitivity of the insulin
228 receptor tissue to insulin.

229 According to a previous study (Roza, Possignolo, Palanch, & Gontijo, 2016), the degree of
230 dyslipidemia and the levels of TG, TC and LDL-c were all positively proportional to obesity, while

231 the HDL-c level was negatively related to obesity. It has been proved that the abnormal deposition of
232 TG in the liver and skeletal muscles can impair the activity of oxidase and affect the metabolism of
233 glucose and lipids (Zhang, 2016). In addition, it is widely believed that the abnormal changes in
234 individual blood lipid levels, such as increased TC, TG and LDL-c levels and a reduced HDL-c level,
235 could be the causes of coronary heart disease (Sugawara et al., 2000). In other words, the blood
236 lipids have a strong relationship with health. **Table 2** summarizes the blood lipid levels of the two
237 groups at the end of the intervention period. It can be seen that the TC and TG levels of blood lipids
238 in the HMT-DM group were significantly higher than those of the DM group ($P < 0.05$), while the
239 HDL-c level was significantly lower ($P < 0.05$). These results suggest that after the high-fat-diet
240 intervention for 8 weeks, the blood lipid indices of mice in the DM group were better than those of
241 HMT-DM group. In other words, the ability of rice starch in regulating the blood lipid levels was
242 actually reduced by HMT.

243 3.3 Oxidative stress indices

244 Oxidative stress causes cytotoxicity and the accumulation of reactive oxygen species in the
245 body (Reeves, Nielsen, & Fahey, 1993). In this study, the changes in MDA, SOD, GSH-PX and T-
246 AOC levels in the serum of mice were examined to understand the influence of native and HMT-
247 modified rice starches on these oxidative stress indices. After a high-fat diet intake, a large amount of
248 calorie led to exuberant energy metabolism and hyperglycemia status in mice, which then intensified
249 glucose oxidation to produce excessive oxidative products (Cristani et al., 2016). Meanwhile, the
250 stronger oxidative ability of excessive oxidative products than the antioxidant capacity could lead to
251 oxidative stress in the body. The reason for the reduced amounts of antioxidant enzymes could be the

252 rapid consumption and exhaustion of their storage in the body when fighting free radicals generated
253 during development of obesity (Sathiavelu, Senapathy, Devaraj, & Namasivayam, 2009). The results
254 (**Table 3**) show a statistically significant increase in serum levels of MDA and GSH-PX, as well as a
255 decrease in T-AOC level for the HMT-DM group compared with for the DM group ($P < 0.05$).
256 Overall, the serum oxidative stress status of mice in the DM group was more effectively improved
257 than that of mice in the HMT-DM group. Thus, it could be suggested that native rice starch,
258 compared with HMT-modified rice starch, could better suppress oxidant stress induced by the high-
259 fat diet.

260 3.4 hepatic lipid metabolism enzymes

261 The detection of hepatic metabolism enzymes mainly includes ALT, AST, and ALP. In the
262 present study, the activities of AST, ALT, and ALP are increased in the serum, which can be
263 considered an indication of liver damage. Previous studies have shown that cell membrane damage
264 leads to a more prominent increase in ALT and AST activities (Borlak, Chougule, & Singh, 2014).
265 The data in **Table 3** also show that the ALT and AST levels of mice in the HMT-DM group were
266 significantly higher than those in the DM group ($P < 0.05$). Thus, the increased levels of these
267 enzymes indicated that HMT-modified rice starch may have a significant impact on cellular
268 homeostasis. Overall, these results suggest that compared to the intervention with the HMT-modified
269 rice starch, native rice starch could alleviate liver dysfunction to a certain degree, and could prevent
270 or delay the abnormal liver function caused by fat accumulation.

271 **Fig. 2** shows that the liver tissue of mice in the DM group had a large amount of fatty
272 degeneration, with different sizes of lipid droplet vacuoles (marked by black circles) in the cytoplasm

273 and some degree of perivascular infiltration by inflammatory cells. While the liver tissue of the
274 HMT-DM group mice had visible steatosis as well, it contained fewer lipid vacuoles, higher vascular
275 permeability, and more perivascular infiltration than that of DM group did. In other words, the
276 resistance to abnormal liver function caused by the high-fat diet was reduced by the intervention with
277 HMT-modified rice starch.

278 3.5 Intestinal microflora

279 The gut is a complex, active, relatively balanced system, of which the type and flora amounts can
280 be changed when the body acquires different kinds of flora from the environment and food
281 (Clemente, Ursell, Parfrey, & Knight, 2012). Recent research has revealed the function of intestinal
282 microflora for the normal homeostasis of the human body (Hartstra, Nieuwdorp, & Herrema, 2016).

283 **Fig. 3A** shows the gut microbiota composition in mice after the 8-week intervention for the two
284 groups. The observed species indices in the DM group was significantly higher than those in the
285 HMT-DM group ($P < 0.05$), which indicates a higher intestinal flora abundance of the DM group.
286 This result confirmed that when RS reached the colon and degraded by microbes, the degradation
287 products would change the distribution structure of intestinal flora and reduce its species abundance.
288 In addition, the PCA analysis diagram based on OTU abundance showed two relatively separated
289 macroscopic distribution profiles, accounting for the apparent difference in the microbial structure
290 between the DM group and the HMT-DM group. To further analyze the difference in microbial
291 structure between the two groups, a weighted UniFrac distance matrix was constructed (shown in
292 **Fig. 3B**). Interestingly, the difference within the HMT-DM group was larger than that within the DM
293 group based on the level of a single sample of OTUs.

294 Then, we analyzed the statistical results of species percentage among different species
295 classification levels. At the phylum level (shown in **Fig. 3C**), bacteria between the two groups were
296 mainly composed of *Bacteroidete*, *Firmicutes*, and *Proteobacteria*. These three bacteria took up
297 about 45.8%, 31.6% and 17.7% in the DM group mice whereas those in the HMT-DM group mice
298 were 45.2%, 24.3.0%, and 24.4%, respectively. Thus, there were significant differences in the
299 percentage of *Firmicutes* and *Proteobacteria* between the two groups ($P < 0.05$).

300 At the family level, the microbial structure and relative abundance in the two groups of mice were
301 also shown in **Fig. 3C**. The proportions of *Ruminococcaceae*, *Lachnospiraceae*,
302 *Porphyromonadaceae*, and other bacteria in the DM group were slightly higher than that in the
303 HMT-DM group. The ratios were 7.1% vs. 4.2%, 7.6% vs. 4.1%, and 3.6% vs. 1.9% (DM vs. HMT-
304 DM), respectively ($P < 0.05$). At the same time, the proportions of some species of the intestinal
305 microflora in the HMT-DM group, namely *Helicobacteraceae*, *S24-7*, *Erysipelotrichaceae*,
306 *Desulfovibrionaceae*, and *Bifidobacteriaceae*, were significantly higher than that in the DM group,
307 and the ratios were 17.5% vs. 6.6%, 23.0 vs. 18.6%, 10.6% vs. 6.4%, 4.5% vs. 1.3% (HMT-DM vs.
308 DM), respectively ($P < 0.05$). Studies have shown that the digestive tract adenoma or cancer of the
309 organism are correlated positively with *Ruminococcaceae*, *Porphyromonadaceae*, and
310 *Lachnospiraceae* (Frank et al., 2007; Meehan & Beiko, 2014), and negatively with *Bacteroides*,
311 *Bifidobacteriaceae* and *Desulfovibrionaceae* (Panasevich et al.). Therefore, it could be speculated
312 that when RS entered the colon and degraded by microbes, the degradation products could
313 effectively inhibit the growth of some species of intestinal bacteria such as *Ruminococcaceae*,
314 *Porphyromonadaceae* and *Lachnospiraceae*, but meanwhile stimulate the growth of *Bacteroides*,

315 *Bifidobacteriaceae* and *Desulfovibrionaceae*, which could suppress the occurrence of digestive tract
316 adenoma and cancer.

317 Metagenomics analysis using the linear discriminant analysis effect size (LEfSe) method was
318 performed to compare the microbial community composition and its abundance diversity in feces
319 between the two groups of mice from the phylum, class, order, family and genus levels, and to
320 further select the dominant flora of microbial communities in the two groups. It can be seen in **Fig. 4**
321 that there were large differences in each level of microbial communities between the two groups.

322 According to the LDA score, the dominant bacteria in the HMT-DM group were *Bifidobacterium*,
323 while *S24-7*, *β -proteobacteria*, and *Proteobacteria* occupied important places in the DM group.
324 Some previous opinions considered that *bifidobacteria* were one of the probiotics associated with the
325 glucose and lipid metabolism (Martorell et al., 2016; Patterson et al., 2017). When the disorder of
326 glucose and lipid metabolism occurred, there was a decrease in the *bifidobacteria* content in the gut.
327 In contrast, a higher amount of *bifidobacteria* contributed to normalizing the blood glucose and
328 blood lipid levels. However, here, the content of *bifidobacteria* in the HMT-DM group mice was
329 higher than that in the DM group, though the indices on blood glucose and blood lipids in the HMT-
330 DM group mice were still worse than those in the DM group (see **Table 1** and **Table 2**). These
331 differences were mainly due to the high sugar environment produced by the high-fat diet, which led
332 to lower activity of functional enzymes in the gut. Therefore, SDS showed more powerful effects on
333 regulating blood glucose and lipids.

334 Besides, *Parabacteroides* and *S24-7* were pathogenic bacteria, which were closely related to the
335 incidence of cancer in the digestive tract. Our results showed that HMT-modified rice starch could
336 effectively inhibit the pathogenic bacteria, and reduce the occurrence of cancer in the digestive tract.

337 The results of animal experiments in our study indicate that physiological and biochemical indices
338 were mainly depended on the content of enzyme-resistant components (SDS + RS) in rice starch,
339 whilst the intestinal microflora was determined by the RS content.

340 We also assessed the functional diversity of the different putative metagenomes using PICRUSt
341 software (Langille et al., 2013), which allows the prediction of metabolic pathways from the 16S
342 rRNA reads. Most of the genes were detected to annotate in the pathways of environmental
343 information processing, genetic information processing, and metabolism. Moreover, the pathways
344 displayed a difference in the mean proportion between the DM and HMT-DM groups (**Fig. 5**). Some
345 pathways including carbohydrate metabolism, lipid metabolism, and membrane transport were over-
346 represented in the DM group, whereas the metabolisms of cofactors, vitamins and amino acids and
347 the cell motility were over-represented in the HMT-DM group. These results indicate that rice starch
348 associated with HMT may also influence the functional diversity, especially predicted by putative
349 metagenomes.

350 **4. Conclusions**

351 This research is focused on the nutritional function of rice starch after HMT, and the relationship
352 between digestibility and nutritional function was established. The results indicate that the
353 physiological and biochemical indices of the HMT-DM group were slightly worse than those of the
354 DM group due to the relatively lower content of SDS in HMT-modified rice starch. *Bifidobacteria*

355 were identified as a type of bacteria related to HMT-modified rice starch, which might be ascribed to
356 the relatively higher content of RS in HMT-modified rice starch, although the specific functions of
357 this type of bacteria require further studies. Moreover, some pathways influenced by these two
358 starches were annotated to reveal the functional metabolisms in the body. Thus, this work is of great
359 significance in modulating the nutritional functions of rice starch and is instrumental to the
360 development of starch-based healthy food.

361

362 **Potential conflict of interest statement**

363 The authors declare no competing financial interest.

364

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Figure captions

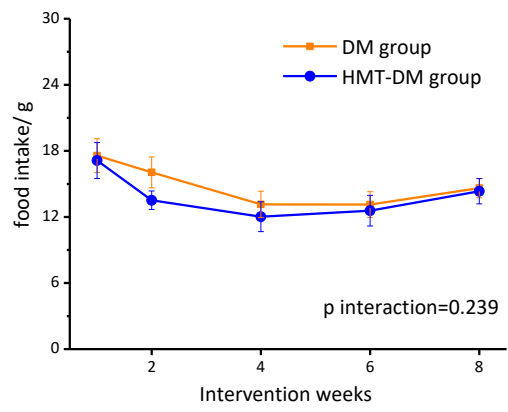
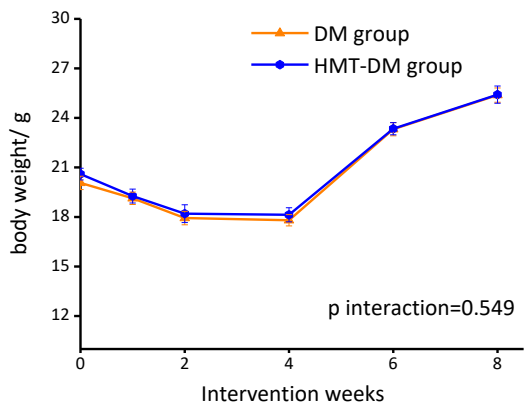
512 **Figure 1.** Changes in body weight and food intake of mice during the 8-week intervention for the
513 two groups. Statistical analysis was performed by General Linear Model repeated measures (Mixed
514 Design ANOVA). P interaction indicates the effect of time and group interactions.

515 **Figure 2.** Light microscope images of liver tissue of mice after the 8-week intervention for the two
516 groups (A: DM group; B: HMT-DM group)

517 **Figure 3.** Gut microbiota composition in mice after the 8-week intervention for the two groups. A:
518 Alpha diversity index; B: β diversity of intestinal microflora (left: PCA analysis; right: analysis of
519 weighted UniFrac distance matrix) (adjusted P value < 0.05); C: The relative abundance of bacterial
520 community at the taxa level (left: Phylum; right: Family) for the two groups (C1: DM group; C2
521 HMT-DM group)

522 **Figure 4.** Significant analysis of intestinal microflora in mice for the two groups (C1: DM group;
523 C2: HMT-DM group)

524 **Figure 5.** Function in KEGG module prediction using 16S data with PICRUSt. (A: KEGG genes
525 annotation; B: different notability functions between the two groups; C1: DM group; C2: HMT-DM
526 group).



527

Figure 1

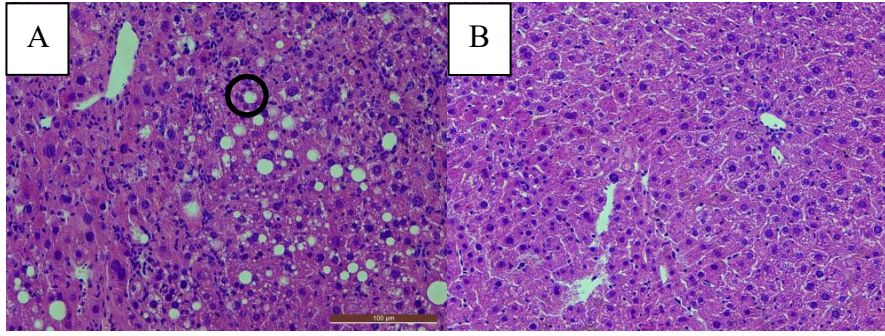
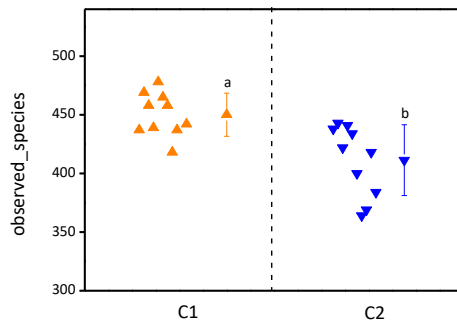
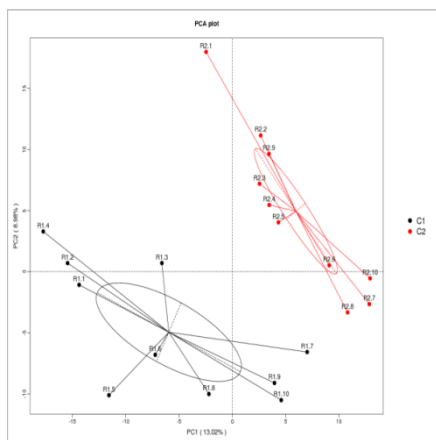


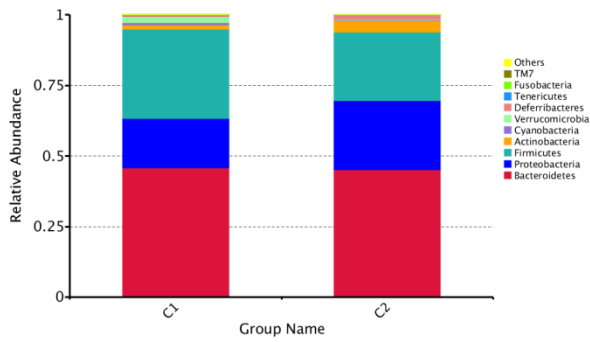
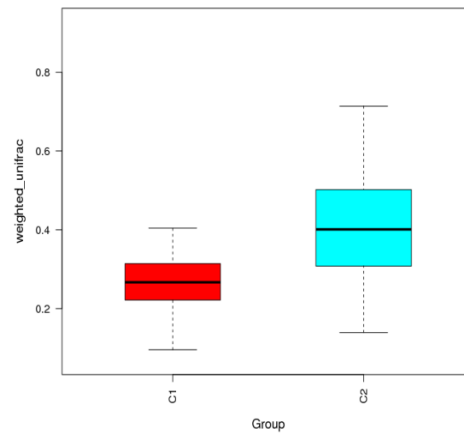
Figure 2



(A)



(B)



(C)

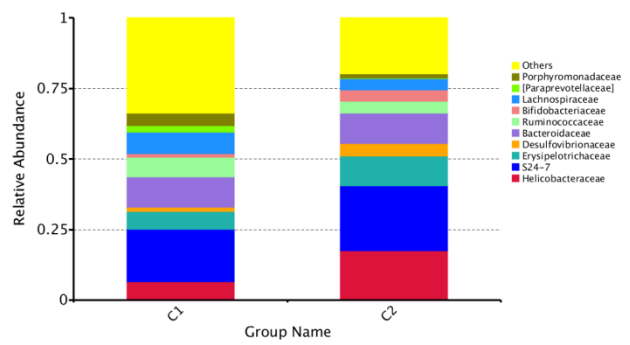


Figure 3

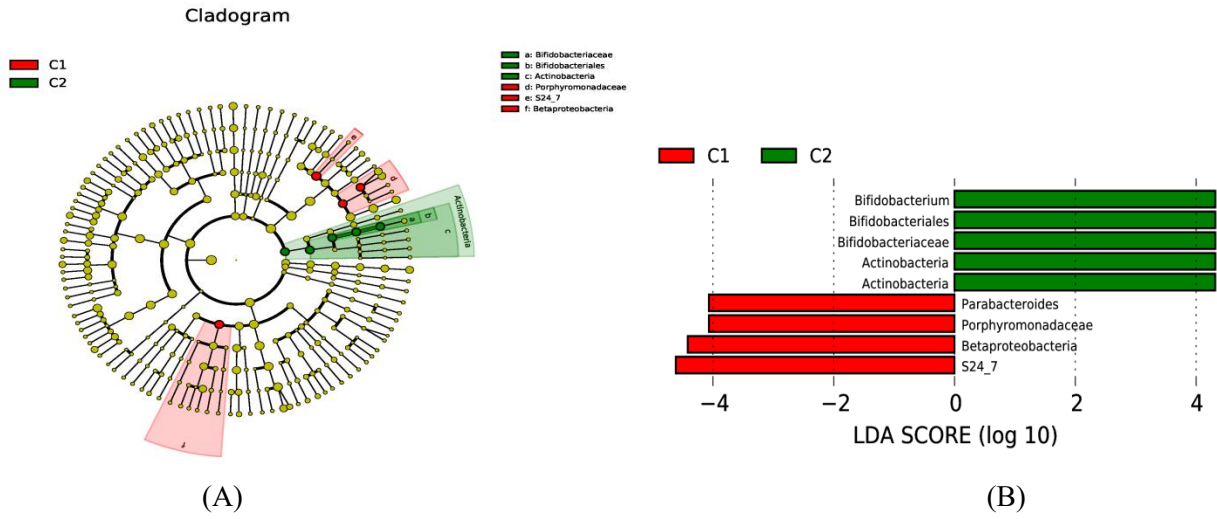


Figure 4

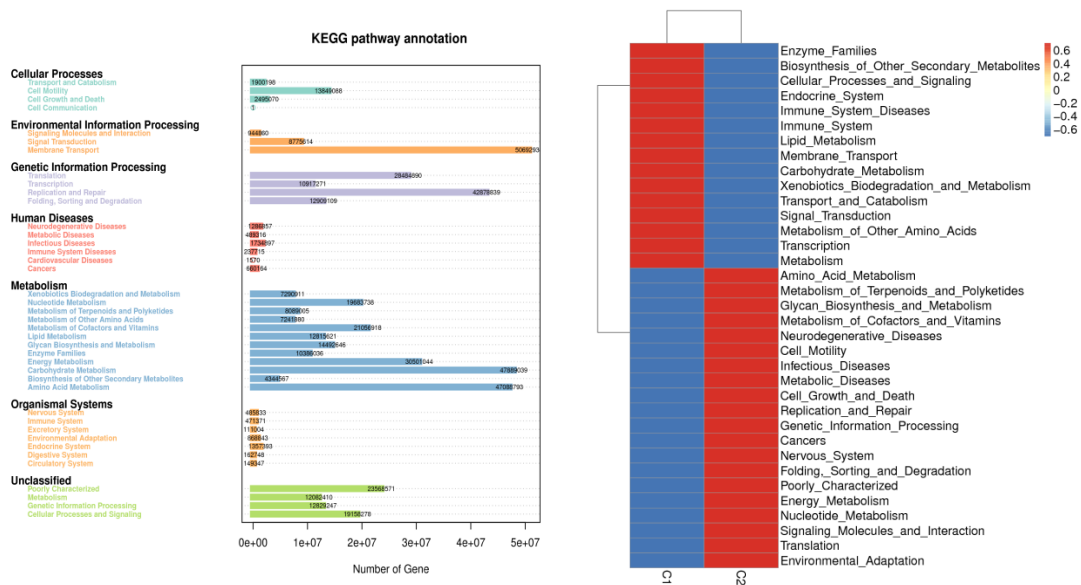


Figure 5

Table 1 Changes in perirenal fat, epididymal fat, fat-to-body ratio, and organ coefficient of mice after the 8-week intervention for the two groups (mean \pm SEM).

Group	Organ coefficient	Perirenal fat (g)	Epididymal fat (g)	Fat ratio (100%)
DM	0.054 \pm 0.0063 ^a	0.094 \pm 0.0074 ^a	0.418 \pm 0.0139 ^a	2.084 \pm 0.10 ^a
HMT-DM	0.058 \pm 0.0065 ^a	0.120 \pm 0.0102 ^b	0.464 \pm 0.0105 ^b	2.119 \pm 0.13 ^b

Different lowercase letters above the same column indicate a significant difference ($P \leq 0.05$).

Table 2 Changes in blood glucose, insulin levels, and serum lipid of mice after the 8-week intervention for two groups (mean \pm SEM).

Groups	Blood glucose (mmol/L)	Insulin (mU/L)	TC (nmol/L)	TG (nmol/L)	HDL-c (μ mol/L)	LDL-c (μ mol/L)
DM	11.12 \pm 0.28 ^b	0.115 \pm 0.002 ^b	1.922 \pm 0.059 ^b	12.629 \pm 0.789 ^b	104.676 \pm 5.310 ^a	88.551 \pm 2.384 ^a
HMT-DM	12.62 \pm 0.37 ^a	0.123 \pm 0.002 ^a	1.990 \pm 0.065 ^a	15.644 \pm 0.200 ^a	97.523 \pm 1.753 ^b	88.855 \pm 1.690 ^a

Different lowercase letters above the same column indicate a significant difference ($P \leq 0.05$).

Table 3 Changes in oxidative stress and liver function metabolic levels of mice after the 8-week intervention for two groups (mean \pm SEM).

Groups	MDA (nmol/mL)	SOD (U/mL)	GSH-PX (U/mL)	T-AOC (U/mL)	ALT (U/L)	AST (U/L)	ALP (IU/L)
DM	7.26 \pm 0.42 ^b	139.89 \pm 9.35 ^a	600.00 \pm 46.82 ^b	10.38 \pm 0.68 ^a	12.47 \pm 1.98 ^b	124.07 \pm 11.78 ^b	0.11 \pm 0.01 ^a
HMT-DM	8.41 \pm 0.99 ^a	141.50 \pm 5.68 ^a	715.86 \pm 35.47 ^a	9.52 \pm 0.23 ^b	15.15 \pm 0.88 ^a	137.13 \pm 11.15 ^a	0.12 \pm 0.01 ^a

Different lowercase letters above the same column indicate a significant difference ($P \leq 0.05$).