Original Paper

Effects of IRW and IQW on Oxidative Stress and Gut Microbiota in Dextran Sodium Sulfate-Induced Colitis

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Key Words

Inflammatory bowel disease (IBD) • Microbiota • IRW • IQW

Abstract

Background/Aims: There are known links between inflammatory bowel disease (IBD) and changes in the microbiota of the gut and inflammation and oxidative stress. In this study, a colitis model induced by dextran sodium sulfate (DSS) in mice is used to evaluate whether the presence of bioactive peptides IRW (Ile-Arg-Trp) and IQW (Ile-Gln-Trp) peptides is advantageous. **Methods:** The mice were arbitrarily assigned to the following four groups: (i) control (untreated), (ii) dextran sodium sulfate (DSS) treated, (iii) IRW-DSS treated, and (iv) IQW-DSS treated. For 7 days, the control group subjects had unrestricted access to untreated drinking water, whereas the drinking water supplied to the subjects in the DSS, IRW-DSS, and IQW-DSS groups during this period consisted of 5% DSS solution. The colonic lesions were scored after hematoxylin and eosin staining. Serum antioxidant capacity was analyzed by 2,2'-azino-bis(3-ethylbenz-thiazoline-6-sulfonic acid) (ABTS) radical cation decolorization test and the microbiota in the colonic contents were sequenced by HiSeq2500 PE250. **Results:** The presence of DSS reduced daily weight gain, enhanced histopathology scores, and inhibited antioxidant enzyme expression. Superoxide dismutase, catalase, and glutathione peroxidase activities in the DSS-induced colitis model were significantly enhanced ($P < 0.05$) in the presence of dietary IRW and IQW. Furthermore, the Simpson index was significantly increased ($P < 0.05$) in the presence of dietary IRW and IQW compared to the control group. IRW and IQW increased the abundance of *Coprococcus_1*, *Ruminococcaceae_UCG-014*, and *Desulfovibrio* compared to the control group and DSS group. Furthermore, IQW decreased the abundance of *Bacteroides* in relation to the control group, but increased *Parabacteroides*.

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In addition, IRW increased the level of *Anaerotruncus*, *Oscillibacter*, and *Ruminiclostridium_9* compared to the control group. **Conclusion:** This study concludes that the presence of IRW or IQW can mitigate DSS-induced oxidative stress by improving the activities of antioxidant enzymes, increasing intestinal microbial diversity and enhancing the abundance of gut microbiota, which may help maintain the homeostasis of host health and microenvironment in a DSS-induced mouse model, thus providing a potential further treatment for IBD patients.

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Introduction

Inflammatory bowel diseases (IBD) comprise a variety of persistent and recurrent ailments of the gastrointestinal tract leading to major inflammation and injury to the rectum and gut. Two significant forms of IBD are Crohn's disease (CD), recognized by chronic intermittent transmural inflammation usually involving the terminal ileum but potentially affecting any part of the gastrointestinal tract, and ulcerative colitis (UC), recognized by inflammation of the colonic mucosa that varies in full extent from the rectum to the proximal colon [1]. Given that the determinants of genetic susceptibility are moderately consistent within the community at large, the pathogenesis and rising incidence of CD and UC worldwide are probably strongly dependent upon environmental determinants. The links between IBD and a range of environmental determinants, such as diet and the use of antibiotics [2], social standing and early life exposure to microorganisms [3], and exposure to microorganisms throughout life, are evidenced by epidemiological, clinical, and experimental research. Several of these determinants may have a significant impact on the range of microbiota present in the gut [4].

Significant differences between the microbiota of IBD patients and healthy individuals, such as reduced diversity and bacterial load along with the development of various characteristics of the condition known as dysbiosis, have been demonstrated via the examination of the microbiome using 16S rRNA sequencing [4-6]. Further in-depth investigations on CD and UC sufferers have demonstrated measurable decreases in *Firmicutes* (particularly *Clostridium* groups) and numerous beneficial bacterial species from genera such as *Bacteriodes*, *Eubacterium*, and *Lactobacillus* and an increase in *Proteobacteria* [6]. Studies have also shown that the matrix metalloproteinases, which are the primary mediators of tissue damage and are responsible for provoking matrix degradation, epithelial cell detachment, and ulceration, are activated by the inflammation response [7]. Although evidence suggests that microbes are essential in driving the inflammatory process, some species from a range of genera, such as *Bifidobacterium*, *Faecalibacterium*, and *Lactobacillus*, may in fact safeguard their hosts' mucosa from unwarranted and potentially harmful inflammatory responses [8-10].

Although a number of medical treatments, such as steroids, immunomodulators, and antibodies, are available to IBD patients, their application is restricted by safety issues surrounding detrimental effects, such as malignancy and infection [11, 12]. Studies have demonstrated the successful inhibition of cytokine-induced inflammatory protein expression in vascular endothelium, at least in part via the modulation of the NF- κ B pathway, in the presence of two novel peptides, namely IRW and IQW, which are derived from the egg white protein ovotransferrin [13-15]. In addition to this anti-inflammatory effect, the observed advantages of these peptides have included antioxidant effects [16]. The capacity for dietary constituents to function as modulators for intestinal inflammatory disease has been demonstrated by previous work examining nutritional regulation by amino acids [17-19]. This study examines the impacts of the presence of dietary IRW or IQW on oxidative stress and colon microbiota in dextran sodium sulfate (DSS) induced colitis.

Materials and Methods

Experimental design

The Chinese guidelines for animal welfare were observed in the design of the experimental strategy. The approval of the Animal Care and Use Committee of Hunan Agricultural University was obtained. The experiment used 40 female ICR mice weighing approximately 22.5 g. The subjects were arbitrarily assigned to the following four groups: (i) the control group (n = 10), (ii) the DSS-treated group (n = 10), (iii) the IRW-DSS group (n = 10), and (iv) the IQW-DSS group (n = 10). All of the mice were kept in polycarbonate cages in a room with a 12-h cycle of light and dark under conditions of controlled temperature ($25 \pm 3^\circ\text{C}$) and humidity ($50 \pm 5\%$). The mice in groups (i) and (ii) were given a basic diet and ready access to clean drinking water for 7 days, whereas the mice in groups (iii) and (iv) received a basic diet with drinking water containing 30 mg/ml IRW or IWQ for the same period of time. Commencing on day 7, all of the mice, except for those in group (i), were given ready access to drinking water containing 5% DSS solution for a further 7 days [17]. This drinking water was prepared by dissolving DSS (molecular weight 5,000, supplied by Kayon Biological Technology Co. Ltd.) in tap water to obtain a 5% (wt/vol) DSS solution. On the morning of day 15 (the end of the experimental period), each mouse was weighed to assess the average daily weight gain and then each mouse was sacrificed and colon length and weight were measured as detailed in our previous publication [17]. Colon tissues and contents from each mouse were also collected, immediately frozen in liquid nitrogen and retained at -70°C for further analysis.

Histological analysis of colon tissues

The terminal colon was removed and fixed in 10% formalin, after which paraffin-embedded sections were prepared and stained with hematoxylin and eosin at Hunan Agricultural University for histological grading. Scores were assigned ranging from 0 (no change) to 4 (extensive infiltration and tissue damage) based on the examination of the following six criteria: cryptitis, erosion, edema, goblet cell hyperplasia, inflammation, and ulceration. Lesions were scored from 0 to 4, where 0 = no colitis or epithelial thickening; 1 = increased number of mucosal leukocytes and/or minor epithelial cell hyperplasia; 2 = multiple inflammation sites, leukocytic infiltration of mucosa and submucosa, and/or noticeable epithelial cell hyperplasia (increase in crypts by 2 to 3 times); 3 = major leukocytic infiltration of mucosa and submucosa, ulceration, depletion of mucin-secreting goblet cells, and/or significant epithelial cell hyperplasia (increase in crypts by 3 to 10 times); and 4 = extensive transmural leukocytic infiltration, crypt abscesses, and/or extreme epithelial cell hyperplasia (increase in crypts by more than 10 times).

Serum antioxidant capacity and antioxidant enzymes

The 2, 2'-azino-bis(3-ethylbenz-thiazoline-6-sulfonic acid) (ABTS) radical cation decolorization test was used to measure the total antioxidant capacity of mice treated as described in the previous sections. For this procedure, a mixture containing 10 mM ABTS solution and 2 mM hydrogen peroxide was prepared and stored overnight in the dark at 4°C . The resulting ABTS $^{\bullet+}$ solution was then diluted by a ratio of 1:10 to acquire an absorbance of approximately 0.31 at 660 nm. A buffer (1,000 μL , pH 3.6), plasma (10 μL), and ABTS $^{\bullet+}$ (25 μL) were sequentially added to a cuvette and the absorbance at 660 nm was measured after 5 min. A blank was included in each assay and the tests were carried out in triplicate. The results are presented in micromolar equivalents of ascorbic acid, an antioxidant normally found in the plasma [20]. Superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) kits supplied by the Jiancheng Bioengineering Institute, Nanjing, China, were used to evaluate the antioxidant enzyme activities, defined as the quantity of enzyme degrading 1 μmol of substrate per minute [21]. The results are presented in units per mg of protein.

Microbial community analysis

The V3-V4 region of the 16S rRNA gene of the microorganism in the colonic contents was amplified after extracting DNA for pyrosequencing. Briefly, the V4-V5 region of the 16S rRNA gene of the intestinal microflora of piglets was amplified using specific primers 515F (5'-GTGCCAGCMGCCGCGGTAA-3') and 926R (5'-CCGTCAATTCMTTGTAGTTT-3'), which amplify almost all bacteria and archaea with few prejudices against specific groups. The PCR product was sequenced by HiSeq2500 PE250 (Illumina, U.S.). For this analysis, the raw data were screened and assembled using Quantitative Insights into Microbial Ecology

(QIIME) (<http://qiime.org/>), an open-source software pipeline that uses PycGooTooKit6 to handle the problem of sequential data from original sequences to interpretation and database deposition [22], and Fast Length Adjustment of SHort reads, a software tool that splices paired-end reads and extends the reads to find their correct overlap [23]. For each sample, the reads identified as chimeras were removed to generate a collection of effective sequences. The sequences were analyzed and the operational taxonomic units (OTUs) were determined with an identity threshold of 97%, using the QIIME and UPARSE pipeline (<http://drive5.com/uparse>) applications [22]. The RDP classifier (Version 2.2, <http://sourceforge.net/projects/rdpclassifier/>) was used to assign taxonomic data to a selected representative sequence for each OTU [24]. The RDP classifier and the Greengenes database (<http://greengenes.lbl.gov/cgi-bin/nph-index.cgi>) were used to assign taxonomic classifications. The abundance-based coverage estimator (ACE), bias-corrected Chao1 richness estimator, Shannon and Simpson indices, and Good's coverage were used to obtain alpha diversity values for colon bacterial communities at a sequence depth of 31,460 for all samples.

Statistical analysis

The Statistical Package for Social Science (SPSS v19.0, U.S.A.) was used to conduct the statistical analysis. One-way analysis of variance was used to evaluate the differences between groups, with the results presented as the mean $\pm$ SEM. Differences were regarded as statistically significant when $P < 0.05$. Before this analysis, a Shapiro-Wilk test was used to evaluate data normality, whereas the Levene test was used to verify the homogeneity of variance. A principal coordinate analysis of overall microbial diversity based on an un-weighted Unifrac metric was conducted using Bray-Curtis distance to compare all of the samples.

Results

The most frequent clinical index in IBD is weight loss. As such, the central strategy was to evaluate the average daily weight gain following DSS treatment. As indicated in Fig. 1A, the DSS group exhibited a significant reduction in daily weight gain ($P < 0.05$), which was significantly remedied by treatment with dietary IRW or IQW ($P < 0.05$). Although Fig. 1B indicates that the administration of DSS resulted in considerably more extreme colitis in the colons of mice from the DSS, IRW-DSS, and IQW-DSS groups compared to the control group, the presence of IRW and IQW resulted in significantly reduced ($P < 0.05$) disease scores.

The results of the ABTS assay measuring the plasma antioxidant capacity are presented in Fig. 2. Although the total antioxidant capacity was significantly decreased ($P < 0.05$) in the DSS, IRW-DSS, and IQW-DSS groups relative to the control groups, the total antioxidant capacity in the IRW-DSS and IQW-DSS groups was significantly higher ($P < 0.05$) than that

Fig. 1. Influence of dietary IRW and IQW on (A) body weight (g) and (B) colon histopathology scores. The results are expressed as mean $\pm$ SEM. * indicates significant difference between groups ($P < 0.05$; $n = 10$).

Fig. 2. Antioxidant enzymes in the colons of mice following administration of dietary IRW or IQW. The results are expressed as mean±SEM. * indicates significant difference between groups ($P < 0.05$; $n = 10$).

in the DSS group (Fig. 2A). Fig. 2B-D presents the antioxidant enzyme activities. Significant reductions in SOD and CAT activities, along with GPx activity ($P < 0.05$), were indicated for the DSS group relative to the control group. The administration of dietary IRW or IQW led to significant enhancement of SOD, CAT, and GPx activities ($P < 0.05$) relative to the DSS group, although no measurable difference in GPx activities was noted in the control, IRW-DSS, and IQW-DSS groups. These outcomes indicate that the antioxidative effect of dietary MR results in significantly enhanced SOD, CAT, and GPx enzymatic activities.

The V3-V4 regions of the 16S rRNA were detected in all of the fecal samples collected at necropsy. In total, 334, 370 eligible sequences were acquired, with samples from the control, DSS, IRW, and IQW groups furnishing 83, 184, 83, 229, 82, 749, and 85, 208 raw tags, respectively. Trimming, assembly, and quality filtering furnished 72, 295, 71, 741, 72, 538, and 74, 567 effective tags for analysis, from which 681 ± 36 , 631 ± 50 , 590 ± 27 , and 666 ± 67 OTUs were respectively detected for the control, DSS, IRW-DSS, and IQW-DSS groups. The selected sequences can adequately determine the great majority of bacterial diversity parameters, based on the normalized subsamples of 51, 568 readings per sample. Table 1 presents the results of the diversity indexes (Shannon and Simpson), community richness (Chao1 and ACE), and coverage (Good's coverage estimator) calculations. Fig. 3A indicates that IRW-DSS treatment significantly enhanced the Shannon index ($P < 0.05$) relative to the control and DSS groups. Fig. 3B indicates that both the IRW-DSS and IQW-DSS significantly enhanced the Simpson index ($P < 0.05$) relative to the control group. The community richness (Chao1 and ACE) and the coverage (Good's coverage estimator) demonstrated no statistically significant differences.

Table 1. Alpha diversity indices of the fecal bacterial communities of mice. * indicates significant difference between CON vs other groups ($P < 0.05$; $n = 8$); # indicate significant difference between DSS vs other groups ($P < 0.05$; $n = 8$)

Item	CON	DSS	IRW	IQW
Raw tags	83,184±2,041	83,229±2,407	82,749±2,569	85,208±1,613
Effective tags	72,295±1,831	71,741±2,107	72,538±2,276	74,567±1,299
OTU	681±36	631±50	590±27	666±67
Shannon	5.46±0.19	5.54±0.18	5.87±0.03	6.23±0.09*
Simpson	0.91±0.02	0.94±0.01	0.96±0.00*	0.96±0.00*
Chao1	687.57±21.23	632.29±46.08	576.50±7.45	644.88±63.35
ACE	718.55±20.20	654.22±49.83	597.58±3.42	668.48±68.63
Coverage	0.998±0.000	0.998±0.000	0.998±0.000	0.998±0.000

Fig. 3. Shannon and Simpson indexes of colon microbiota in the four different groups. * indicates significant difference between groups ($P < 0.05$; $n = 8$).

The taxon-dependent analysis was applied to the fecal samples to examine the taxonomy of the intestinal microbiota, leading to the detection of seven phyla. Fig. 4 presents the relative abundance of the detected species, indicating the predominance of *Bacteroidetes*, *Firmicutes*, and *Proteobacteria*. For the control group, the relative abundances were 66.30% *Bacteroidetes*, 28.40% *Firmicutes*, and 4.21% *Proteobacteria*, whereas the DSS group showed 58.93% *Bacteroidetes*, 31.46% *Firmicutes*, and 7.97% *Proteobacteria*. The IRW group revealed relative abundances of 47.31% *Bacteroidetes*, 43.76% *Firmicutes*, and 7.55% *Proteobacteria*. The IQW-DSS group demonstrated relative abundances of 46.24% *Bacteroidetes*, 47.07% *Firmicutes*, and 5.00% *Proteobacteria*.

Table 2 lists the most abundant genera. For the *Bacteroidetes* phylum, the relative abundance of genus *Bacteroides* increased in the DSS group relative to the control and IQW-DSS groups ($P < 0.05$), whereas the relative abundance of *Parabacteroides* increased in the IQW group relative to the control and DSS groups ($P < 0.05$). For the *Firmicutes* phylum, the

relative abundance of the *Coprococcus_1* genus was enhanced by the IQW treatment ($P < 0.05$) relative to the other three groups, whereas the relative abundance of *Ruminococcaceae_UCG-014* was enhanced by both the IRW and IQW treatments ($P < 0.05$) relative to the control and DSS groups. For the *Proteobacteria* phylum, the abundance of genus *Desulfovibrio* was enhanced relative to that of the control group in both the IRW and IQW groups ($P < 0.05$).

Table 2. Composition of the fecal bacterial communities of mice at the genus level. * indicates significant difference between CON vs other groups ($P < 0.05$; $n = 8$); # indicate significant difference between DSS vs other groups ($P < 0.05$; $n = 8$)

Phyla	Genus	CON	DSS	IRW	IQW
Bacteroidetes	Alloprevotella	23.86±4.13#	11.79±5.30*	2.16±0.69*	1.18±0.24*
	Bacteroides	11.46±1.39#	18.49±2.96*	13.14±2.03	9.65±1.50#
	Parabacteroides	2.14±0.23	2.23±0.50	1.31±0.14	1.13±0.18#
Firmicutes	Anaerotruncus	0.36±0.08	0.51±0.17	0.83±0.14*	0.75±0.11
	Coprococcus_1	0.29±0.05	0.52±0.08	0.76±0.12	1.48±0.36*#
	Erysipelatoclostridium	1.02±0.43	1.77±0.57	0.57±0.12	0.71±0.18
	Lachnospiraceae_NK4A136_group	7.07±1.43	7.82±2.70	10.01±2.82	12.24±3.76
	Oscillibacter	0.35±0.05	0.77±0.27	1.07±0.16*	0.83±0.14
	Roseburia	0.70±0.14	0.78±0.19	2.43±1.31	0.74±0.13
	Ruminiclostridium_9	0.50±0.01#	1.55±0.46*	1.69±0.27*	1.40±0.27
	Ruminococcaceae_UCG-014	0.41±0.07	0.67±0.05	1.77±0.16*#	2.47±0.49*#
	Desulfovibrio	0.38±0.05	0.70±0.18	1.08±0.19*	1.16±0.28*
	Helicobacter	2.05±0.55	4.80±1.51	4.47±0.90	1.53±0.57#
Proteobacteria	Parasutterella	0.80±0.23	1.14±0.39	0.30±0.05#	1.24±0.17

Fig. 4. Microbiota of the colon in the four groups at the phylum level.

Discussion

This study examines the impacts of two different peptides (IRW and IQW) on DSS-induced colitis in mice to model the pathology of IBD, a persistent inflammatory condition of the gastrointestinal tract that includes CD and UC. The mice treated with DSS displayed clinical symptoms and pathological developments comparable to those associated with UC in humans, such as body weight reduction, diarrhea, and blood in the feces. The outcomes of this study indicate that IRW and IQW decrease DAI scores, weight loss, and oxidative stress in the presence of DSS, thus significantly inhibiting DSS-induced colitis. Moreover, IRW and IQW increased the diversity of microbial diversity in the colon and decreased the abundance of *Bacteroides*, but enhanced *Parabacteroides*, *Anaerotruncus*, *Oscillibacter*, and *Ruminiclostridium_9*.

IBD is linked to significant alterations in the constitution, diversity, and metabolic function of the extensive, diverse, and complex community of microbiota found in the human gut. High-throughput screening was used to assess the microbiological community present in the mice. The diversity of the sample microbiological community, in terms of species richness and species evenness, is indicated by the Shannon and Simpson indices. For communities with identical species richness, an increased diversity is reflected by increased species uniformity. Microbial microecology in the intestine of mice is associated with the development of DSS-induced colitis [21, 25, 26]. Research has found that the compositions and varieties have changed in DSS-induced mouse models, such as those of *Bacteroides*, *Lactobacilli*, *Parabacteroides*, *Enterobacteriaceae*, and *Anaerotruncus* [27, 28]. Yeom et al. reported significantly higher Shannon diversity indices for the control group compared to the DSS group; furthermore, DSS increased the ratio of *Bacteroidetes*, but reduced the proportion of *Firmicutes* [29]. Ritchie et al. detected no significant differences between the control group and rats treated with DSS at 47 days after treatment and colonic injury showed a negative relation to *Lactobacillus*, *Actinobacteria*, *Firmicutes*, and *Lactobacillales* [30]. However, at the same amount of time after treatment, DSS rats given Sumac bran displayed significantly enhanced richness ($P = 0.0199$) and measurably greater diversity indices than control rats given Sumac bran. Similarly, DSS rats given Hi Tannin black bran displayed significantly enhanced richness ($P < 0.001$) and diversity ($P = 0.013$) than control rats administered Hi Tannin black bran. These differences may have occurred because the experimental rats were also given an effective experimental diet with a specific function in DSS treatment [30].

Due to the central role of gut microbiota in IBD, sufferers are generally administered short-term antibiotic treatment to ameliorate intestinal inflammation [31, 32]. In the IBD animal model, the proportion of symbiotic organisms, such as *Helicobacter*, *Clostridium*, and *Enterococcus*, has changed [33]. Differences in microbiota among experimental groups were compared at the phylum level by Yeom et al., who reported that the levels of *Bacteroidetes* were significantly enhanced (by 44.9%) and the levels of *Firmicutes* were significantly reduced (by 34.4%) relative to the control group following DSS treatment. Accordingly, the *Bacteroidetes* to *Firmicutes* ratio in the gut microbiota was greater for the DSS group than for the control group [30, 34]. Although the *Bacteroidetes* and *Firmicutes* phyla are predominant in healthy gut microbiota, Zhou et al. reported significant reductions in these species, along with significant increases in *Proteobacteria* and *Actinobacteria* in patients with IBD [35]. Munyaka et al. detected 14 phyla, 5 of which (*Bacteroidetes*, *Deferribacteres*, *Firmicutes*, *Proteobacteria*, and *Verrucomicrobia*) were abundant, constituting more than 1% of the population. The remaining nine (*Acidobacteria*, *Actinobacteria*, *Cyanobacteria*, *Fibrobacteres*, *Lentisphaerae*, *Planctomycetes*, *Spirochaetes*, *TM7*, and *Tenericutes*) were rare, constituting less than 1% of the population. No significant differences in the abundant phyla ($P > 0.05$) were noted for the DSS-treated mice and the control group [36].

At the genus level, Yeom et al. found that DSS treatment led to significantly enhanced proportions of *Clostridium*, *Bacteroides*, and *Enterobacter* relative to the control group [29]. Kang et al. examined the microbiota in fecal samples, identifying genera that increased in the presence of IBD (*Akkermansia*, *Bacteroides*, *DQ532165_g*, *Bifidobacterium*, *EF406806_g*, and *Prevotella*) and others that decreased under the same condition (*DQ326408_f_uc*, *EF406830_g*, *EF602759_f_uc*, *EF602759_g*, *EF603943_g*, *EU381732_f_uc*, *EU381732_g*, *Erysipelotrichi_uc_g*, and *Lactobacillus*). When the microbiota communities in the small intestinal fluids were examined at the genus level, the major bacteria (*Turibacter*, *Pseudomonas*, and *EF406806_g*) showed small compositional changes, their respective abundances on the first day being 54.2%, 16.5%, and 6.1% and on the second day being 54.1%, 15.3%, and 8.1%, respectively. By contrast, the abundance of *EF602759_f_uc* decreased in the presence of IBD from 9.2% on day 1 to 0.7% on day 2 [37]. The differences in the results of Kang et al. and those of this study may be due to the former's use of the metagenomic analysis method and the fact that they examined samples from small intestinal fluids. Moreover, the up-regulation of antimicrobial peptides can mediate the composition of intestinal microorganisms and protect the host from foodborne and other pathogens in the IBD model [38, 39].

Conclusion

In brief, this study indicates that 7 days of treatment with DSS induces symptoms of colitis, such as reduced growth and antioxidant enzyme inhibition, whereas the provision of dietary IRW or IQW ameliorates these effects by up-regulating the antioxidant enzymes, thus relieving oxidative stress. Hence, this study concludes that dietary IRW or IQW may provide a subsidiary treatment for IBD patients. Changes in alpha-diversity in IBD following treatment with DSS, IRW, and IQW, along with species changes at the phylum and genus levels, are also examined. Although the impacts of dietary IRW and IQW on DSS-induced colitis and the microbiota of the gut are demonstrated, the relationship between the antioxidant effects of IRW and IQW and the microbiota of the gut remains unresolved.

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Disclosure Statement

All co-authors are aware of and agree with the contents of this manuscript. There is no financial interest to report.

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