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Original article

Treatment with mannose oligosaccharides reverses the intestinal injury in the acetylsalicylic acid-treated rat model

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Abstract

Mannose oligosaccharide (MOS) has been shown to promote animal growth, maintain intestinal health, and activate the intestinal immune system. However, the question of whether MOS can stimulate the immune system and alleviate acetylsalicylic acid (ASA)-induced gut damage remains unresolved. The purpose of this study was to investigate the impact of MOS pretreatment on the immunological and anti-inflammatory capabilities of rats with ASA-induced intestinal injury. Thirty-six male Sprague-Dawley rats were divided into 6 groups and were fed with 0 (negative control), 100, 300, 600, and 800 mg/kg·Body weight (BW) of MOS for 3 weeks. From day 8, rats were fed with 200 mg/kg BW of ASA for 14 days to induce intestinal injury. The growth performance, viscera index, serum and intestinal immunity, intestinal inflammation and morphology of ASA-induced intestinal injury rats with or without MOS administration were investigated. In MOS deficient rats, oral treatment of ASA causes severe intestine damage and immunological dysfunction. In a rat model, 600 mg/kg BW MOS can lower the expression of inflammatory markers and effectively increase liver index, serum interleukin-2 (IL-2), lysozyme contents, intestinal secretory immunoglobulin A (sIgA) and mucus volume, intestinal villus height, crypt depth and villus height/crypt depth in comparison to the ASA group. These results imply that providing rats with MOS at the appropriate dosage can significantly improve their immune system and successfully shield the intestines from ASA damage. MOS is therefore expected to be a promising gut immunopotentiator for enhancing intestinal health in animals.

Keywords: mannose oligosaccharide, acetylsalicylic acid, intestinal injury, serum immunity

Introduction

Changes in temperature, environment, and diet can cause stress in animals, and in some extreme cases, intestinal dysfunction, commonly manifested as diarrhea, results in decreased food intake and increased mortality (De Oliveira et al. 2019, Pluske et al. 2019). As a result, mitigating intestinal damage serves as an effective means to enhance nutrient absorption and safeguard the welfare of animal husbandry (Chen et al. 2021). The gut is considered the body's largest immune organ and plays a crucial role in digesting and nutrient absorption (Elderman et al. 2017). A healthy digestive system has an intact intestinal villi structure, which may improve the body's capacity to digest and absorb nutrients (Landín et al. 2012). In addition, as the initial line of defense against foreign substances, gut mucosal immunity can assist the animal's immune system (Zou et al. 2021). Intestinal injuries and disorders can be brought on by modifications to the breeding habitat, the diet that the animals are provided, and the conditions of transit. Inflammation and abnormalities in the digestive tract follow, resulting in diarrhea, a slower metabolic rate, stunted growth, and eventually death (Yi et al. 2017, Chen et al. 2021).

The establishment of a successful intestinal injury model is the base for investigating intestinal problems in the animal model (Cipriani et al. 2013, Lai et al. 2015). Acetylsalicylic acid (ASA) is a non-steroidal anti-inflammatory drug (NSAID) that can harm the mucosa of the stomach, causing erosion and ulcers (Sugimoto et al. 2000). In the meanwhile, ASA primarily damages the stomachs, duodenums, and posterior intestinal tract of animals, as well as the intestinal mucosa, intestinal tight junctions, and immune system. It is an effective drug for producing gastrointestinal inflammatory injury (Suyama et al. 2018, Mortensen et al. 2019). In our previous study (Chen et al. 2020), we demonstrated that giving 200 mg/kg Body weight (BW) ASA for 14 consecutive days can successfully induce intestinal injury model in the Sprague Dawley (SD) rat model. We found the body weight gain, liver index, serum interleukin-2 (IL-2) content and lysozyme (LZM) activity, intestinal mucosa secretory immunoglobulin A (sIgA) content, intestinal villus height (VH), crypt depth (CD) and their ratio were all decreased significantly. According to recent research, intestinal immune malfunction may contribute to the emergence of NSAIDS-induced intestinal mucosal injury (Kim et al. 2019, Watanabe et al. 2020, Cai et al. 2021). Consequently, one way to treat intestinal inflammation is to find natural additives that can strengthen the immune system of the body and maintain intestinal health without having harmful side effects.

Mannose oligosaccharide (MOS) synthesized from the cell wall of *Saccharomyces cerevisiae* has been shown to promote the proliferation of beneficial bacteria in the intestine, increase animal food intake, and improve growth performance (Halas and Nochta, 2012, Cheng et al. 2019, Mohammad et al. 2019). In addition, MOS also has immunological features such as phagocytosis, superoxide anion generation, and lysozyme activity (Mohammad et al. 2019). It has been extensively utilized in food, medicine, and animal nutrition because of its ability to activate B lymphocytes and natural killer cells (Sun et al. 2019). Other studies found that MOS supplementation significantly improved broiler intestinal morphology, and increased the VH and CD ratios in young broilers (Iji et al. 2001) and weaned piglets (Shen et al. 2009). Mannan compounds can directly enhance the immune system of pigs, stimulate the intestinal mucosa locally, raise IgA secretion in different parts of the mucosa, and consequently initiate the host's systemic immune response (Halas and Nochta 2012). Davies et al. (2004) discovered that supplementation of phosphomannan in a dose of 2 g/kg to the diet for 21 days altered the amount of jejunum intrinsic T cells. MOS, in its capacity as a functional oligosaccharide, being regarded as a potential substitute immunopotentiator for enhancing the intestinal health, growth, and development of animals (Hutsko et al. 2016, Yan et al. 2019, Wang et al. 2021).

MOS may enhance intestinal health, promote intestinal growth, and stimulate the intestinal immune system, according to these results. Nonetheless, the potential of immune system stimulation to mitigate the intestinal harm induced by ASA remains unknown. The present investigation examined the impact of MOS pretreatment on the anti-inflammatory and immunological capacities of rodents that had intestinal damage induced by ASA. Our findings may pave the way for a novel method of enchancing the intestinal health of animals.

Materials and Methods

Chemicals

MOS (PW120) was obtained from Angel Yeast Co. Ltd. (Yichang, China), while ASA was acquired from Henan Fengmu Biotechnology Co. Ltd (Zhengzhou, China).

The ELISA kit for LZM, IL-2, and sIgA was purchased from Jiancheng Biologic Project Company (Nanjing, China). The RNA Isolation kit was procured from Shenggong Bio Inc. (Shanghai, China). The Reverse Transcriptase kit and SYBR qPCR kit were obtained from Takara Bio Inc. (Shiga, Japan).

The qTOWER 3G real-time PCR system were obtained from Analytik Jena (Thuringia, Germany). All the other chemicals were analytical grade.

Experimental animals

36 six-week-old male Sprague-Dawley (SD) rats weighing 150-180 g were obtained from Henan Experimental Animal Center (Henan, China). The rats in the present study were taken care of in complete compliance with the National Institutes of Health's guidelines for the Care and Use of Laboratory Animals (NIH, 1996). The protocols were accepted by the animal care review committee of the Henan University of Technology with the approval number HAUTETHI-2017-0485.

Experimental design and feeding condition

36 healthy SPF grade SD male rats (body weight: 150-180 g) were randomly assigned to one of six groups (n=6 per group): Control group, ASA group, 100 mg/kg BW MOS group (MOS 100 group), 300 mg/kg BW MOS group (MOS 300 group), 600 mg/kg BW MOS group (MOS 600 group) and 800 mg/kg BW MOS group (MOS 800 group). The rats were allowed to eat and drink freely in a temperature range of 20-25°C and a humidity range of 40-60%. After 1 week of adaptation, the experiment lasted for 3 weeks. The food intake and body weight of the rats were recorded daily. The control group was given normal saline intragastrically for 21 days. The rats in the MOS and ASA groups received varied dosages of MOS and normal saline intragastrically for 7 days as their assigned group dosage. An intragastric injection of 200 mg/kg BW ASA was administered for 14 days. On the 22nd day, the rats were fasted but had unrestricted access to water. On the 23rd day, the rats were sedated with ether after being weighted, and blood was collected from their eyeballs. The rats were dissected after being euthanized via neck dislocation. Rat spleens, livers, and thymuses were dissected, cleansed in sterile normal saline, dried, and weighed. After separating the intestines, jejunum tissue was taken for immunological index examination. A portion of the intestinal tissue was kept in 10% formalin buffer for paraffin sectioning, which utilized to examine intestinal morphology and assess VH and CD.

Growth performance and viscera index

The body weight gain (BWG), average daily gain (ADG), average daily feed intake (ADFI), feed conversion rate (FCR), food consumption, and body weight were recorded at the beginning and end of the study to assess the rat's overall growth. The liver, spleen, and thymus were dissected at the end of the experiment, and the carcass was cleaned and dried with normal saline before being weighed and recorded using the equation below:

spleen index (%) = [spleen mass (g) / body mass (g)] × 100

liver index (%) = [liver mass (g) / body mass (g)] × 100

thymus index (%) = [thymus mass (g) / body mass (g)] × 100

Immunity ability

Serum immunoassay

Rat blood was coagulated at room temperature and centrifuged at 2000 rpm for 20 min to prepare the serum. After that, the serum was carefully extracted and the levels of LZM and IL-2 were determined using an ELISA kit (Jiancheng Biologic Project Company, Nanjing, China) following the manufactory's instructions.

Determination of sIgA content

Jejunum tissue (5 cm) near the duodenum was sliced open on the gauze, and the intestinal mucosa was scraped with glass slides and placed in a centrifuge tube, which was stored at -80 °C. The presence of sIgA in the intestinal mucosa was determined using the ELISA kit (Jiancheng Biologic Project Company, Nanjing, China) in accordance with the manufacturer's instructions.

Determination of intestinal mucus

The method to determine the intestinal mucus volume was following the report of Suyama et al. (2018). In short, a portion (approximately 5 cm in length) of the intestine (10 cm from the pylorus) was chosen for homogenization, and 1 mL phosphate buffer solution (PBS) was added to form a suspension. Centrifuge at 15000 rpm for 15 minutes at 4°C. After incubating with periodate (0.1%, 100 µL) at 37°C for 2 hours, 100 µL Schiff reagent was added and incubated at room temperature for 30 minutes. The absorbance (OD) value, representing the amount of mucus, was determined at 555 nm utilizing the Spark 10M Multimode Microplate Reader (TECAN, Mannedorf, Switzerland).

Intestinal morphology

The tissue from rats' jejunum tissue was removed after dissection, fixed for 24 h in 4% paraformaldehyde, dehydrated using a concentration gradient of ethanol, translucid and embedded in paraffin. It was then cut into 5 µm thin slices using a microtome, stained with hematoxylin and eosin (HE), dehydrated, sealed, and exa-

Table 1. Quantitative real-time polymerase chain reaction forward and reverse primer sequences.

Gene	Gene accession number	Primer sequence 5′-3′
TNF-α	Forward primer	GAGATGTGGAACTGGCAGAGGA
	Reverse primer	TCAGTAGACAGAAGAGCGTGGTG
IL-6	Forward primer	CCTACCCCAACTTCCAATGCT
	Reverse primer	GGTCTTGGTCCTTAGCCACT
IL-1β	Forward primer	CTCGTGGGATGATGACGACC
	Reverse primer	AGGCCACAGGGATTTTGTCG
β-actin	Forward primer	CCCATCTATGAGGGTTACGC
	Reverse primer	TTTAATGTCACGCACGATTTC

Table 2. Effects of MOS on the growth performance in rats.

Groups	BWG (g)	ADG (g/d)	ADFI (g/d)	FCR
Control	188.17 ± 6.24	6.28 ± 1.42	32.31 ± 1.56	0.20 ± 0.04
ASA group	150.26 ± 9.35	5.30 ± 0.76	31.39 ± 0.78	0.17 ± 0.02
MOS 100 group	156.62 ± 3.88	6.01 ± 0.39	32.76 ± 0.95	0.19 ± 0.02
MOS 300 group	163.66 ± 13.60	6.13 ± 1.05	32.00 ± 0.58	0.19 ± 0.03
MOS 600 group	160.96 ± 18.10	5.93 ± 1.08	30.91 ± 0.69	0.19 ± 0.03
MOS 800 group	171.48 ± 17.82	6.10 ± 1.15	31.15 ± 0.29	0.19 ± 0.04

MOS means mannan oligosaccharide; BWG means body weight gain, ADG means average daily gain, ADFI means average daily feed intake, and FCR means feed conversion rate. MOS 100-800 means 100-800 mg/kg BW MOS.

mined under a microscope to observe the histopathology. The morphology of intestinal villi in jejunum tissue from each group was observed and analyzed using an inverted light microscope (Echo Laboratories, CA, USA). In addition, the VH and CD were measured, and the ratio of VH/CD was calculated.

qRT-PCR analysis

An animal total RNA Isolation kit (Shenggong, Shanghai) was used to extract RNA from jejunum tissue following the manufacturer's instructions. The Reverse Transcriptase Kit (Takara, Japan) was utilized for cDNA synthesis, and the temperature settings were 15 min at 42℃, followed by 5 min at 95℃ for deactivation.

On a qTOWER 3G real-time PCR system (Analytik Jena, Germany), the SYBR qPCR Kit (Takara, Japan) was used to run qRT-PCR under the following conditions: 94℃ for 30 s, followed by 30 cycles of 72℃ for 60 s, followed by 60℃ for 30 s. Table 1 lists the PCR primer sets for gene identification. Using the $2^{-\Delta\Delta CT}$ approach, the relative mRNA expression levels of proinflammatory cytokine genes (TNF-α, IL-6, and IL-1β) were adjusted based on the β-actin expression.

Statistical analysis

The data were presented as mean ± SEM, and the statistical analysis was carried out using SAS 9.1 statistical software and one-way ANOVA. The significant difference was defined as $p<0.05$.

Results

Effects of MOS on the growth performance of rats

Table 2 shows the daily gain and feed conversion rate (FCR) of rats in all groups. The daily gain and FCR in the ASA group were 15.61% and 15% lower than in the control group, respectively. The groups administered with MOS at doses of 100, 300, 600, and 800 mg/kg BW showed better growth performance compared to the ASA group, with daily increases of 13.40%, 15.66%, 11.89%, and 15.09%, respectively. The feed conversion efficiency rose by 11.76% in all groups compared to the ASA group.

Effects of MOS on viscera index of rats

Table 3 presents that the thymus index and spleen index in the ASA group were lower than in the control group, decreasing by 11.76 % and 13.04 %, respectively ($p>0.05$). Organ indicators in the MOS groups showed improvement to different extents as compared to the ASA group. The liver index of the MOS 600 and MOS 800 groups showed a substantial increase compared to the ASA group ($p<0.05$), with increments of 17.03% and 27.30%, respectively. Furthermore, there was no

Table 3. Effects of MOS on viscera index in rats.

Groups	Spleen index /%	Thymus index /%	Liver index /%
Control	0.23 ± 0.03	0.17 ± 0.02	4.24 ± 0.2^{ab}
ASA group	0.20 ± 0.02	0.15 ± 0.02	3.70 ± 0.35^{c}
MOS 100 group	0.24 ± 0.04	0.17 ± 0.02	4.01 ± 0.22^{abc}
MOS 300 group	0.21 ± 0.02	0.18 ± 0.04	4.24 ± 0.37^{abc}
MOS 600 group	0.24 ± 0.03	0.17 ± 0.03	4.33 ± 0.27^{ab}
MOS 800 group	0.24 ± 0.02	0.18 ± 0.03	4.71 ± 0.23^{a}

MOS means mannan oligosaccharide, ASA means acetylsalicylic acid, MOS 100-800 means 100-800 mg/kg BW MOS. The data were presented as mean ± SEM. Superscript letters indicate significant differences ($p<0.05$).

Table 4. Effects of MOS on serum cytokine and lysozyme in rats.

Groups	IL-2 (pg /mL)	LZM (pg /mL)
Control	245.45 ± 8.48^{ab}	2243.35 ± 124.87^{a}
ASA group	178.76 ± 6.30^{b}	1602.75 ± 113.42^{b}
MOS 100 group	229.57 ± 26.44^{ab}	1891.17 ± 100.69^{ab}
MOS 300 group	269.20 ± 49.13^{a}	1977.25 ± 160.97^{ab}
MOS 600 group	255.79 ± 40.25^{a}	2009.85 ± 156.26^{a}
MOS 800 group	230.09 ± 23.13^{ab}	2183.40 ± 133.43^{a}

MOS means mannan oligosaccharide, ASA means acetylsalicylic acid, IL-2 means interleukin-2, LZM means lysozyme, MOS 100-800 means 100-800 mg/kg BW MOS. The data were presented as mean ± SEM. Superscript letters indicate significant differences ($p<0.05$).

Table 5. Effects of MOS on intestinal mucus volume and sIgA content in rats.

Groups	Mucus volume	sIgA (pg/mL)
Control	3.03 ± 0.22^{a}	359.35 ± 19.25^{c}
ASA group	2.58 ± 0.12^{c}	325.52 ± 31.40^{c}
MOS 100 group	2.62 ± 0.04^{c}	391.80 ± 50.47^{c}
MOS 300 group	3.01 ± 0.23^{ab}	400.83 ± 55.50^{c}
MOS 600 group	3.00 ± 0.20^{ab}	644.75 ± 73.27^{b}
MOS 800 group	2.89 ± 0.29^{b}	845.80 ± 85.87^{a}

MOS means mannan oligosaccharide, ASA means acetylsalicylic acid, sIgA means secretory immunoglobulin A, MOS 100-800 means 100-800 mg/kg BW MOS. The data were presented as mean ± SEM. Superscript letters indicate significant differences ($p<0.05$).

significant difference in the organ index between the MOS group and the control group.

Effects of MOS on inflammatory cytokine IL-2 and antimicrobial enzyme lysozyme

Table 4 shows that the serum IL-2 and LZM content of rats in the ASA group decreased by 21.76% and 28.6%, respectively, as compared to the control group. The serum IL-2 concentration in MOS groups from 300 to, 600 and 800 mg/kg BW was increased by 42.61%, 35.51%, and 21.90% ($p<0.05$), respectively, as compared to the ASA group. The serum LZM content in MOS groups from 600 to 800 mg/kg BW increased by 31.64% and 43.01%, respectively ($p<0.05$). Meanwhile, levels of serum IL-2 and LZM levels were consistent between the two highest dose groups of MOS 600 and MOS 800, suggesting that administering 600 and 800 mg/kg BW MOS orally may provide the maximum protective effect in our animal model.

Effects of MOS on intestinal sIgA levels and mucus volume in rats

Table 5 shows that the intestinal mucosal sIgA in the ASA group decreased by 12.19% compared with the control group ($p<0.05$). Compared to the ASA group, intestinal mucosal sIgA content in MOS groups from 100, 300, 600, and 800 mg/kg·BW was increased by 21.75%, 24.55%, 100.34% and 162.82%, respectively. In the meantime, sIgA levels were considerably greater in the MOS 600 and 800 groups compared to the ASA and control groups ($p<0.05$). Furthermore, MOS

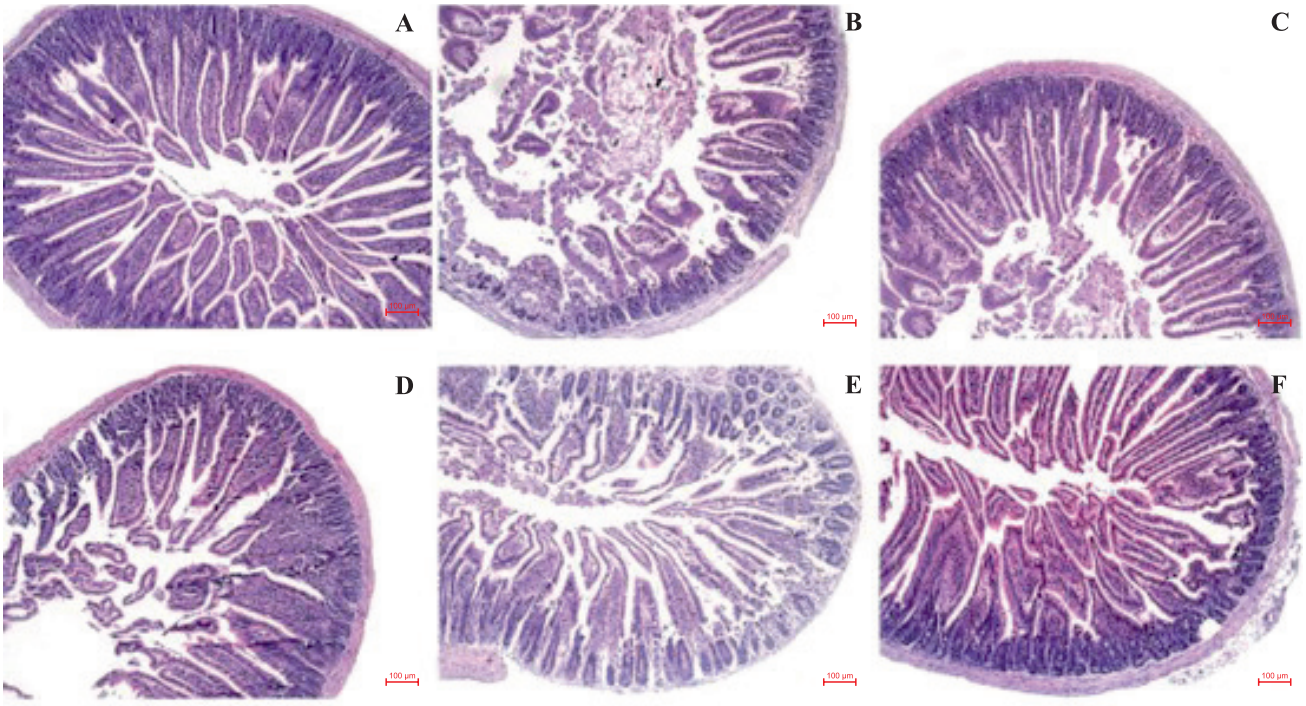

Fig. 1. Intestinal villous morphology of rats (×100). A. Control group, B. ASA group, C. MOS 100 group, D. MOS 300 group, E. MOS 600 group, F. MOS 800 group. MOS means mannan oligosaccharide, ASA means acetylsalicylic acid. MOS 100-800 means 100-800 mg/kg BW MOS.

Table 6. Effects of MOS on intestinal morphology in rats.

Groups	VH (μm)	CD (μm)	VH/CD
Control	532.52 ± 52.34^{a}	120.32 ± 12.12^{a}	3.92 ± 0.31^{a}
ASA group	362.19 ± 32.27^{d}	112.14 ± 11.32^{bc}	3.24 ± 0.31^{b}
MOS 100 group	407.42 ± 37.73^{c}	104.80 ± 14.16^{c}	3.92 ± 0.28^{a}
MOS 300 group	475.00 ± 31.33^{b}	125.49 ± 19.44^{a}	3.86 ± 0.56^{a}
MOS 600 group	523.09 ± 29.69^{ab}	135.06 ± 17.19^{a}	3.91 ± 0.41^{a}
MOS 800 group	562.91 ± 34.74^{a}	143.22 ± 7.03^{a}	3.89 ± 0.27^{a}

MOS means mannan oligosaccharide, ASA means acetylsalicylic acid, VH means villi height, CD means crypt depth, and VH/CD means villi height/crypt depth ratio, MOS 100-800 means 100-800 mg/kg BW MOS. The data were presented as mean ± SEM. Superscript letters indicate significant differences ($p<0.05$).

300, 600, and 800 considerably raised intestinal mucosal volume by 21.25%, 21.13%, and 16.53% ($p<0.05$) in comparison to the ASA group.

Effects of MOS on intestinal morphology of rats

Figure 1 depicts a microscopic image of the morphological structure of intestinal villi in rats. From the figure, it is evident that the intestinal villi in the blank control group exhibit an intact and well-defined structure. In contrast, the ASA group displays disrupted villi morphology with obvious signs of inflammatory damage. However, with MOS treatment, significant improvements in both villus morphology and inflammatory status are observed. As shown in Table 6, the intestinal VH of MOS groups from 100, 300, 600, and 800 increased by 14.07%, 32.99%, 46.45%, and 57.60% compared to the ASA group, respectively. Intestinal mucosal CD increased by 16.04%, 24.89%, and 32.44% in the MOS 300, 600, and 800 groups, respectively, compared to the ASA group. Similar to the intestinal VH/CD ratio, which was 17.63%, 15.84%, 17.59%, and 16.86% greater than the ASA group, the VH/CD ratio in the MOS group was also higher.

Effects of MOS on inflammatory cytokine gene expression in the intestine

Our data show that TNF-α, IL-6, and IL-1β mRNA expression levels were considerably higher following ASA treatment compared to the control group ($p<0.05$) (Fig. 2), suggesting that ASA may activate the upstream of an inflammatory pathway. Additionally, a decrease in the levels of TNF-α, IL-6, and IL-1β mRNA expression

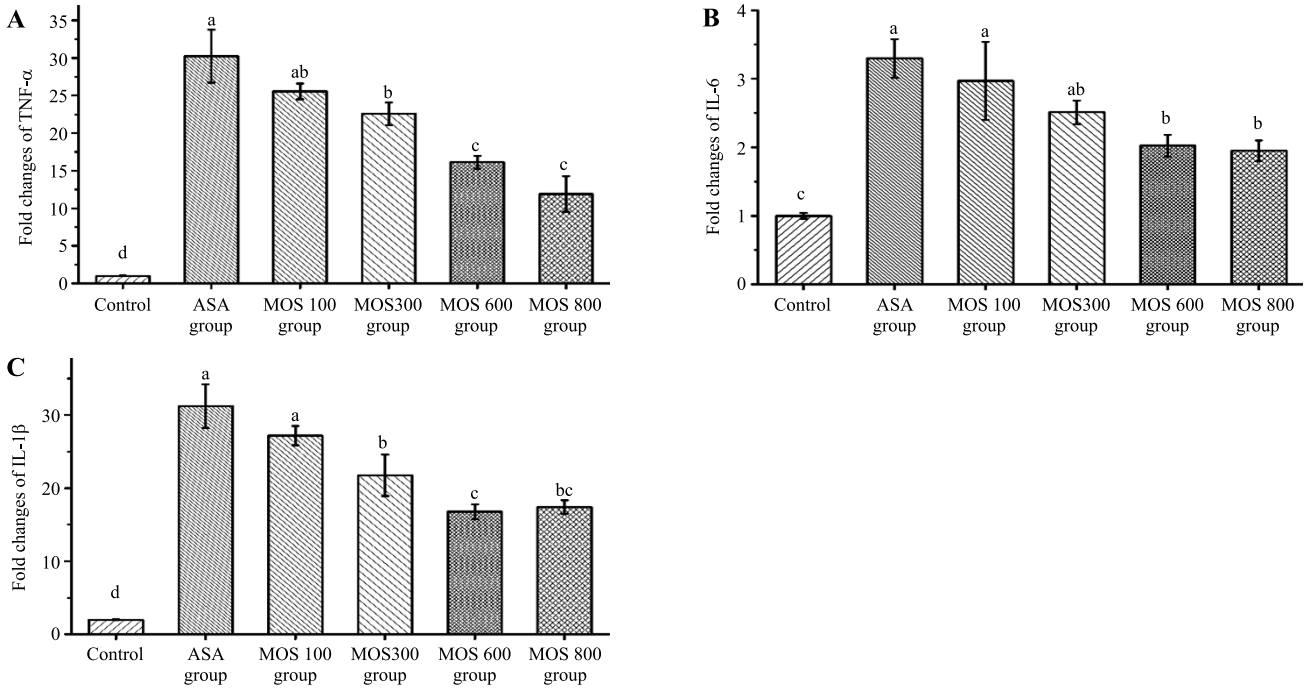

Fig. 2. Relative mRNA expression of proinflammatory cytokines in intestinal of rats. A. TNF-α, B. IL-6, C. IL-1β. MOS means mannan oligosaccharide, ASA means acetylsalicylic acid. Superscript letters above the columns in each panel indicate significant differences ($p<0.05$).

was observed with an increase in MOS levels, suggesting that MOS can mitigate intestinal inflammation by obstructing the inflammatory pathway. When comparing the MOS group's mRNA expression levels of TNF-α, IL-6, and IL-1β to the ASA group's vehicle control at 600 mg/kg, there was a significant down regulation of 44.7%, 38.6%, and 46.2% ($p<0.05$), respectively. Furthermore, when MOS supplementation levels of 600 mg/kg were compared to MOS supplementation levels of 800 mg/kg, there was no further reduction in mRNA expression levels of those inflammatory markers.

Discussion

No studies have been reported on the effects of MOS on ASA-induced enteritis, despite research on its effects on constipation, obesity, and growth performance in rats. Our results suggest that mannan-oligosaccharide can protect the intestinal barrier and potentially prevent enteritis.

MOS supplementation may partially promote growth in rats with ASA-induced enteritis, as mentioned before. Our findings align with those reported by Jahanian and Ashnagar (2015), who observed no significant impact of mannan supplementation on the performance of laying hens. Additionally, studies indicate that supplementing the diet of Caspian trout (*Salmo trutta caspius*) with oligosaccharides alone or in combination with other prebiotics has minimal impact on growth performance. However, a combination of oligosaccharides and probiotics can notably enhance feed conversion rate and daily gain (Mohammad et al. 2019). Therefore, MOS supplementation may not have effectively enhanced rodent growth since ASA medication damaged the intestinal barrier, impacted the intestinal flora, and decreased beneficial bacteria. Furthermore, short-term MOS administration did not restore normal probiotic levels, which impacted MOS metabolism in the colon. In future research, it is crucial to extend the duration of MOS treatment and explore the interaction between MOS and the intestinal flora. This approach will facilitate a deeper comprehension of MOS's mechanism in mitigating intestinal injury among rats.

The thymus, spleen and liver are significant immune organs, and their indexes can be utilized to evaluate the overall immune health of the body (Attia et al. 2017). The thymus is a primary immunological organ that generates T lymphocytes and secretes thymic hormones (Thapa and Farber. 2019). The spleen is a peripheral immunological organ that serves as the hub for both humoral and cellular immunity (Li et al. 2019). The liver is a vital metabolic and immune organ in the human body, performing a variety of functions including synthesis, metabolism, excretion, detoxification, and immunity (Tarasenko and McGuire. 2017). The immunological organ index correlates positively with the body's immune state. According to a study performed by Veronika et al. (2012), the MOS can improve disease resistance and stress resistance by increasing

blood glucose transportation into immune organs and assisting immune function maturation, promoting plasma cell production, lengthening immunological memory time, and significantly improving disease resistance and stress resistance. The study found that supplementing with MOS at a dosage of 600-800 mg/kg BW improved the spleen and thymus index and significantly enhanced the viscera index in rats with ASA-induced enteritis. The results were comparable to those of Wu (2018) who fed growing rabbits with konjac mannan oligosaccharides. An increase in the immune organ index suggests an enhancement in the body's immunological activity. Various factors such as different forms of mannan oligosaccharides, dietary composition, animal species, developmental stage, and feeding conditions might affect the growth and development of animal immune organs (Chen et al. 2021).

MOS has an immune-enhancing function and can boost the body's immunity (Attia et al. 2017). IL-2 plays a crucial role as an inflammatory cytokine in the immune system's response to combat microbial infections. It can initiate a series of immune responses by stimulating T cells and inducing the release of T cells associated cytokines such as TNF and IFN-γ (Ma et al. 2021). LZM has antibacterial activity by efficiently hydrolyzing the mucopolysaccharide of pathogenic bacteria and breaking down bacterial cell walls to create peptioglycan (Saito et al. 2019). Our results were similar to a previous report by Vlies et al. (2012), who demonstrated that indigestible oligosaccharides can efficiently drive T cells to perform immunological functions, significantly increasing the level of IL-2 in the blood, and boosting immune response. Meanwhile, Geraylou et al. (2012) discovered that an appropriate amount of oligosaccharide can raise the concentration of LZM in serum, and increased LZM activity can minimize the degree of damage caused to the body by inflammatory agents.

Intestinal mucus serves as the body's first line of defense against pathogens, successfully blocking the pathogen's invasion and enhancing the intestine's immune system by secreting the immunological protein sIgA. Following intestinal mucosal injury caused by 5-fluorouracil, the animals had weight-loss, exfoliation of their intestinal villus, aggregation of inflammatory cells, and a significant drop in blood sIgA levels (Wang et al. 2019). In addition, increased intestine sIgA secretion could improve the antioxidant and immunological capacities of piglets (Zhu et al. 2017). Our results showed that in rats with ASA-induced enteritis, intestinal mucus volume, and sIgA level were significantly increased by MOS administration. MOS can reverse the decrease of sIgA content and mucus volume induced by intestinal mucosal injury, as well as reduce intestinal damage induced by bacteria, antigens, and other sources (Chen et al. 2021). In the meanwhile, MOS can increase disease resistance and decrease infection rates by limiting the rate at which potentially harmful microbes attach to intestinal epithelial cells (Torrecillas et al. 2011, Zhu et al. 2014). MOS could also contribute to an increase in the number of acidic mucus-secreting cells per unit area of the intestinal mucosa and the number of eosinophils and LZM activity (Torrecillas et al. 2011), which increased harmful pathogenic bacteria breakdown and elimination from the body. As a result, increasing mucus secretion on the mucosal surface of the host is an effective way to prevent pathogenic bacteria colonization and destroy pathogenic bacteria (Zhu et al. 2014).

Animal growth performance is strongly associated with the structure of the intestinal morphology. Intact intestinal structure and functional integrity are crucial for facilitating the body's digestion and absorption of nutrients (Tiwari et al. 2020). Our results showed that the intestinal VH of MOS groups from 100, 300, 600, and 800 increased significantly compared to the ASA group. Increased villus height suggests an increase in the contact area between the digestive tract and nutrients, as well as an increase in the ratio of intestinal villus height to crypt depth, implying improved intestinal digestion and absorption capability (Shalaei et al. 2014, Mohammadsadeghi et al. 2019). Furthermore, the study demonstrated that including mannan oligosaccharides enhanced the intestinal morphology of rats with intestinal injury and significantly increased the VH/CD ratio. Supplementing rats with 600 and 800 mg/kg BW MOS can restore the intestinal villus morphology to normal levels, suggesting an enhanced digestion and absorption ability in the intestinal system. This was consistent with the findings of Mourao et al. (2006), who showed that both intestinal VH and VH/CD ratios were significantly changed, even though there was no effect on weight gain and daily food consumption in the fat rabbits. The positive impacts of MOS on intestinal structure are mainly linked to controlling the resident microorganisms, decreasing pathogen presence and its harmful effects on intestinal cells, and promoting the growth of beneficial bacteria (Agazzi et al. 2020). However, some investigations found that intestinal morphology was unaffected by MOS therapy; the reasons for this could be associated with the MOS supply, the type of animal used, or the research design (Giannenas et al. 2016, Anjos et al. 2019).

There is a direct correlation between inflammatory biomarkers and the occurrence of enteritis. Activated immune cells like monocytes and macrophages secrete inflammatory cytokines, initiating a series of inflamma-

tory reactions (Yu et al. 2017). Local tissues and blood vessels release a high number of pro-inflammatory substances, including TNF-α, IL-1, and IL-6, during the initiation and progression of enteritis (Yu et al. 2017). An imbalance of anti-inflammatory mediators can contribute to disease progression and irreversible consequences by supporting chronic inflammatory responses. Inflammatory cytokines such as TNF-α, IL-1, and IL-6 can lead to an increase in vascular dilatation and permeability. The acute-phase protein, complement, which is released by neutrophils and mononuclear cells, can aggravate the inflammatory response and cause lasting damage to the vascular bed and its surrounding tissues (Yin et al. 2014). The study showed that rats administered ASA developed severe enteritis, leading to an inflammatory reaction. MOS, particularly at elevated concentrations, significantly decreased the production of proinflammatory cytokines and alleviated inflammation in the intestines.

Conclusions

Our findings show that 600 mg/kg BW MOS treatment can successfully reduce ASA-induced intestinal damage and improve the immunity of intestinal injury rats by modulating serum cytokines, immune cells, proinflammatory cytokines, and intestinal morphology. An effective dose of 600 mg/kg BW of MOS is best for repairing ASA-induced intestinal damage in rats. Additional studies are required to determine the effects of MOS on intestinal flora, its metabolites, and its influence on other intestinal diseases.

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References

Agazzi A, Perricone V, Zorini FO, Sandrini S, Mariani E, Jiang XR, Ferrari A, Crestani M, Nguyen TX, Bontempo V, Domeneghini C, Savoini G (**2020**) Dietary mannan oligosaccharides modulate gut inflammatory response and improve duodenal villi height in post-weaning piglets improving feed efficiency. Animals-Basel 10: 1283.

Attia Y, Al-Khalaifah H, Ibrahim M, Al-Hamid A, Al-Harthi M, El-Naggar A (**2017**) Blood hematological and biochemical constituents, antioxidant enzymes, immunity and lymphoid organs of broiler chicks supplemented with propolis, bee pollen and mannan oligosaccharides continuously or intermittently. Poult Sci 96: 4182-4192.

Cai GF, Wu Y, Wusiman A, Gu PF, Mao NN, Xu SW, Zhu TY, Feng ZA, Liu ZG, Wang DY (**2021**) Alhagi honey polysaccharides attenuate intestinal injury and immune suppression in cyclophosphamide-induced mice. Food Funct 12: 6863-6877.

Chen YJ, Wang JR, Qiao HZ, Su LL, Tang GF, Huang J, Zhao YL, Gao WT (**2021**) Effects of mannose oligosaccharide on repair of intesinal injury induced by aspirin in rats. Chinese J Anim Nutr 33: 3523-3531. https://kns.cnki.net/kcms2/article/abstract?v=FC2wxXHna7psXyPQp1jyoFY_EP9XoXFTYTRVGknZ_4RSI6LqmF3aRoS3ykCQbSKLCsPoiG9yEuF_Ksn9VXO-n8BLbyMlplsW0bxGGubv_rQ0jp7wFhGcuzMTov3j7XNXSUBM-DT7LhuFlTV-3Da7dlA==&uniplatform=NZKPT&language=CHS.

Chen YJ, Wang JR, Su LL, Gao WT, Zhao YL, Duan EZ, Wang JJ, Lou P (**2020**) Establishment of intestinal injury model induced by aspirin in rat. Chinese J Anim Nutr 32: 898-904. https://kns.cnki.net/kcms2/article/abstract?v=FC2wxXHna7o_p7k4sM_Jux5Js9aCML2rX-FS_b4oA1WZ04DMc8TDAG-ix9iHpZKz38TvlwflnVRE72vt3cTCukcsKWqkFnvOblQjVg3VG0sVTGmq48C4j2IMUmrUT5K6e3vu0ZnXqJQ78K9Z2gu0pw==&uniplatform=NZKPT&language=CHS.

Cheng YF, Chen YP, Chen R, Su Y, Zhang RQ, He QF, Wang K, Wen C, Zhou YM (**2019**) Dietary mannan oligosaccharide ameliorates cyclic heat stress-induced damages on intestinal oxidative status and barrier integrity of broilers. Poult Sci 98: 4767-4776.

Cipriani S, Mencarelli A, Bruno A, Renga B, Distrutti E, Santucci L, Baldelli F, Fiorucci S (**2013**) Activation of the bile acid receptor GPBAR1 protects against gastrointestinal injury caused by non-steroidal anti-inflammatory drugs and aspirin in mice. Brit J Pharmacol 168: 225-237.

Davis ME, Maxwell CV, Erf GF, Brown DC, Wistuba TJ (**2004**) Dietary supplementation with phosphorylated mannans improves growth response and modulates immune function of weanling pigs. J Anim Sci 82: 1882-1891.

de Oliveira AC, Vanelli K, Sotomaior CS, Weber SH, Costa LB (**2019**) Impacts on performance of growing-finishing pigs under heat stress conditions: A meta-analysis. Vet Res Commun 43: 37-43.

de Souza TC, Landín GM, García KE, Barreyro AA, Barrón AM (**2012**) Nutritional changes in piglets and morphophysiologic development of their digestive tract. Vet Mexico 43: 155-173.

Dos Anjos CM, Gois FD, dos Anjos CM, Rocha VD, de Sa e Castro DE, Allaman IB, Silva FL, Carvalho PL, Meneghetti C, Costa LB (**2019**) Effects of dietary beta-glucans, glucomannans and mannan oligosaccharides or chlorohydroxyquinoline on the performance, diarrhea, hematological parameters, organ weight and intestinal health of weanling pigs. Livest Sci 223: 39-46.

Elderman M, Sovran B, Hugenholtz F, Graversen K, Huijskes M, Houtsma E, Belzer C, Boekschoten M, de Vos P, Dekker J, Wells J, Faas M (**2017**) The effect of age on the intestinal mucus thickness, microbiota composition and immunity in relation to sex in mice. PloS One 12: e0184274.

Geraylou Z, Souffreau C, Rurangwa E, De Meester L,

Courtin CM, Delcour JA, Buyse J, Ollevier F (**2012**) Effects of arabinoxylan-oligosaccharides (AXOS) on juvenile Siberian sturgeon (Acipenser baerii) performance, immune responses and gastrointestinal microbial community. Fish Shellfish Immun 33: 718-724.

Giannenas I, Doukas D, Karamoutsios A, Tzora A, Bonos E, Skoufos I, Tsinas A, Christaki E, Tontis D, Florou-Paneri P (**2016**) Effects of Enterococcus faecium, mannan oligosaccharide, benzoic acid and their mixture on growth performance, intestinal microbiota, intestinal morphology and blood lymphocyte subpopulations of fattening pigs. Anim Feed Sci Technol 220: 159-167.

Halas V, Nochta I (**2012**) Mannan oligosaccharides in nursery pig nutrition and their potential mode of action. Animals-Basel 2: 261-274.

Hogenkamp A, van Vlies N, Thijssen S, Dingjan G, Knipping K, Garssen J, Knippels L (**2012**) Effects of short-chain galacto- and long-chain fructo-oligosaccharides on systemic and local immune status during pregnancy. J Reprod Immunol 94: 161-168.

Hutsko SL, Meizlisch K, Wick M, Lilburn MS (**2016**) Early intestinal development and mucin transcription in the young poult with probiotic and mannan oligosaccharide prebiotic supplementation. Poult Sci 95: 1173-1178.

Jahanian R, Ashnagar M (**2015**) Effect of dietary supplementation of mannan-oligosaccharides on performance, blood metabolites, ileal nutrient digestibility, and gut microflora in Escherichia coli-challenged laying hens. Poult Sci 94: 2165-2172.

Jami MJ, Kenari AA, Paknejad H, Mohseni M (**2019**) Effects of dietary b-glucan, mannan oligosaccharide, Lactobacillus plantarum and their combinations on growth performance, immunity and immune related gene expression of Caspian trout, Salmo trutta caspius (Kessler, 1877). Fish Shellfish Immunol 91: 202-208.

Kim MW, Kang JH, Shin E, Shim K, Kim MJ, Lee C, Yoon Y, Oh S (**2019**) Processed Aloe vera gel attenuates non-steroidal anti-inflammatory drug (NSAID)-induced small intestinal injury by enhancing mucin expression. Food Funct 10: 6088-6097.

Lai Y, Zhong W, Yu T, Xia ZS, Li JY, Ouyang H, Shan TD, Yang HS, Chen QK (**2015**) Rebamipide promotes the regeneration of aspirin-induced small-intestine mucosal injury through accumulation of β-catenin. PloS One 10: e0297646.

Li Y, Yue HG, Yang SY, Yuan DD, Li LX, Zhao JH, Zhao LT (**2019**) Splenomegaly induced by anemia impairs T cell movement in the spleen partially via EPO. Mol Immunol 112: 399-405.

Ma TT, Li C, Zhao FQ, Cao J, Zhang XY, Shen XR (**2021**) Effects of co-fermented collagen peptide-jackfruit juice on the immune response and gut microbiota in immunosuppressed mice, Food Chem 365: 130487.

Mohammadsadeghi F, Afsharmanesh M, Ebrahimnejad H (**2019**) The substitution of humic material complex with mineral premix in diet and interaction of that with probiotic on performance, intestinal morphology and microflora of chickens. Livest Sci 228: 1-4.

Mortensen B, Murphy C, O'Grady J, Lucey M, Elsafi G, Barry L, Westphal V, Wellejus A, Lukjancenko O, Eklund AC, Nielsen HB, Baker A, Damholt A, Vlieg JE, Shanahan F, Buckley M (**2019**) Bifidobacterium breve Bif195 protects against small-intestinal damage caused by acetylsalicylic acid in healthy volunteers. Gastroenterology 157: 637-646.

Mourão JL, Pinheiro V, Alves A, Guedes CM, Pinto L, Saavedra MJ, Spring P, Kocher A (**2006**) Effect of mannan oligosaccharides on the performance, intestinal morphology and cecal fermentation of fattening rabbits. Anim Feed Sci Technol 126: 107-120.

Paul AL, Saki AA, Tivey DR (**2001**) Intestinal structure and function of broiler chickens on diets supplemented with a mannan oligosaccharide. J Sci Food Agric 81: 1186-1192.

Pluske JR, Miller DW, Sterndale SO, Turpin DL (**2019**) Associations between gastrointestinal-tract function and the stress response after weaning in pigs. Anim Prod Sci 59: 1-8.

Saito H, Sakakibara Y, Sakata A, Kurashige R, Murakami D, Kageshima H, Saito A, Miyazaki Y (**2019**) Antibacterial activity of lysozyme-chitosan oligosaccharide conjugates (LYZOX) against Pseudomonas aeruginosa, Acinetobacter baumannii and Methicillin-resistant Staphylococcus aureus. PloS One 14: e0217504.

Shalaei M, Hosseini SM, Zergani E (**2014**) Effect of different supplements on eggshell quality, some characteristics of gastrointestinal tract and performance of laying hens. Vet Res Forum 5: 277-286.

Shen YB, Piao XS, Kim SW, Wang L, Liu P, Yoon I, Zhen YG (**2009**) Effects of yeast culture supplementation on growth performance, intestinal health, and immune response of nursery pigs. J Anim Sci 87: 2614-2624.

Sugimoto N, Yoshida N, Yoshikawa T, Nakamuara Y, Ichikawa H, Naito Y, Kondo M (**2000**) Effect of vitamin E on aspirin-induced gastric mucosal injury in rats. Dig Dis Sci 45: 599-605.

Sun Z, Wang T, Demelash N, Zheng S, Zhao W, Chen X, Zhen YG, Qin GX (**2019**) Effect of yeast culture (Saccharomyces cerevisiae) on broilers: A preliminary study on the effective components of yeast culture. Animals-Basel 10: 68.

Suyama Y, Handa O, Naito Y, Takayama S, Mukai R, Ushiroda C, Majima A, Yasuda-Onozawa Y, Higashimura Y, Fukui A, Dohi O, Okayama T, Yoshida N, Katada K, Kamada K, Uchiyama K, Ishikawa T, Takagi T, Konishi H, Itoh Y (**2018**) Mucus reduction promotes acetyl salicylic acid-induced small intestinal mucosal injury in rats. Biochem Biophys Res Commun 498: 228-233.

Tarasenko TN, McGuire PJ (**2017**) The liver is a metabolic and immunologic organ: a reconsideration of metabolic decompensation due to infection in inborn errors of metabolism (IEM). Mol Genet Metab 121: 283-288.

Thapa P, Farber DL (**2019**) The role of the thymus in the immune response. Thorac Surg Clin 29: 123-131.

Tiwari UP, Fleming SA, Abdul R, Muhammed S, Jha R, Dilger RN (**2020**) The role of oligosaccharides and polysaccharides of xylan and mannan in gut health of monogastric animals. J Nutr Sci 9: e21.

Torrecillas S, Makol A, Benítez-Santana T, Caballero MJ, Montero D, Sweetman J, Izquierdo M (**2011**) Reduced gut bacterial translocation in European sea bass (Dicentrarchus labrax) fed mannan oligosaccharides (MOS). Fish Shellfish Immunol 30: 674-681.

Torrecillas S, Makol A, Caballero MJ, Montero D, Ginés R, Sweetman J, Izquierdo M (**2011**) Improved feed utilization, intestinal mucus production and immune parameters in sea

bass (Dicentrarchus labrax) fed mannan oligosaccharides (MOS). Aquac Nutr 17: 223-233.

Wang T, Cheng K, Yu CY, Li QM, Tong YC, Wang C, Yang ZB, Wang T (**2021**) Effects of a yeast-derived product on growth performance, antioxidant capacity, and immune function of broilers. Poult Sci 100: 101343.

Watanabe T, Fujiwara Y, Chan FK (**2020**) Current knowledge on non-steroidal anti-inflammatory druginduced small--bowel damage: a comprehensive review. J Gastroenterol 55: 481-495.

Wu FY, Cui J, Xia XR, Liu TT, Gu ZL, Chen BJ (**2018**) Effects of konjac mannan oligosaccharide on nutrient digestibility, immune organ index and serum indices of growing rex rabbits. Feed Industry 39: 8-13. https://kns.cnki.net/kcms2/article/abstract?v=FC2wxXHna7rUJpFCGPRJI2kfAF2TW8yqRzZOI-X3g-xicTfy4lXz9_JSPk9EHmjoHdQK_u-Cut75hd3j8ylbCvyIB45vnfprAMlnLSTlXjyGkaqjwFWzMnKUzKsgQfJmOXRczTqmO9G8OyU-mqiihg==&uniplatform=NZKPT&language=CHS.

Yan SK, Shi RJ, Li L, Ma SB, Zhang HB, Ye J, Wang JM, Pan JR, Wang QX, Jin X, Liu XB, Liu ZG (**2019**) Mannan oligosaccharide suppresses lipid accumulation and appetite in western-diet-induced obese mice via reshaping gut microbiome and enhancing short-chain fatty acids production. Mol Nutr Food Res 63: 1900521.

Yi D, Hou YQ, Xiao H, Wang L, Zhang Y, Chen HB, Wu T, Ding BY, Hu CA, Wu GY (**2017**) N-Acetylcysteine improves intestinal function in lipopolysaccharides--challenged piglets through multiple signaling pathways. Amino Acids 49: 1915-1929.

Yin H, Pan XC, Wang SK, Yang LG, Sun GJ (**2014**) Protective effect of wheat peptides against small intestinal damage induced by non-steroidal anti-inflammatory drugs in rats. J Integr Agric 13: 2019-2027.

Yu CC, Xiong YJ, Chen DP, Li YL, Xu B, Lin Y, Tang ZY, Jiang CL, Wang L (**2017**) Ameliorative effects of atractylodin on intestinal inflammation and co-occurring dysmotility in both constipation and diarrhea prominent rats. Korean J Physiol Pharmacol 21: 1-9.

Yu Z, Yu RL, Shao JW, Yang Z, Wang H (**2019**) Study on repair effect of artificial artificial Isaria cicadae on intestinal mucosal injury induced by 5-fluorouracil in rats. China Pharmacy 30: 2973-2979. https://kns.cnki.net/kcms2/article/abstract?v=FC2wxXHna7qqMRqnMN6Ua4GgXOelY-z4D9_xiRM3h8Ro0tmA_YfLOQGF9aOXNGh0JVU5B3UXfy9jO-nQgMaes-V1odScSTQOHiNsmmHdR4B47WqTEWNO8TYeOX8mEA_f_gopAm9uo_Xw10oTR4opVQ==&uniplatform=NZKPT&language=CHS.

Zhu C, Wang L, Wei SY, Chen Z, Ma XY, Zheng CT, Jiang ZY (**2017**) Effect of yeast Saccharomyces cerevisiae supplementation on serum antioxidant capacity, mucosal sIgA secretions and gut microbial populations in weaned piglets. J Integr Agric 16: 2029-2037.

Zhu ZM, Zhu WM, Lan HB, Cui XD (**2014**) Research development of Saccharomyces cerevisiae: Nutritional manipulation and application in aqua feed. Chinese J Anim Nutr 26: 3550-3560. https://kns.cnki.net/kcms2/article/abstract?v=FC2wxXHna7rm6nZo2NdW52AMf6cbsAQYhdx1m9XSgv8J8cFzjOTycdcp_uTikVzlGjYHWMgnkMROgxB8M1CnOAtA9k57XRl2S3ONt-ay6lTOgtd7whFm6XHqw0CUY8yBJMLy_2Egq7TMUL7hbbHnnA==&uniplatform=NZKPT&language=CHS.

Zou JF, Liu C, Jiang S, Qian DW, Duan JN (**2021**) Cross talk between gut microbiota and intestinal mucosal immunity in the development of ulcerative colitis. Infect Immun 89: e00014-21.