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Influence of High-soluble Fiber Diets on the Gut Microbiota and Inflammatory Markers: A Comparative Study in Aged Mice

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ABSTRACT

Background: Chronic low-grade inflammation and gut microbiota dysbiosis are linked to aging, while the consumption of soluble fiber in the diet may help control both conditions. The purpose of this study was to assess the effects on gut microbiota composition, systemic inflammatory biomarkers and intestinal barrier integrity markers-specifically, plasma lipopolysaccharide-binding protein and serum amyloid A of a 10-week dietary intervention supplemented with 15% functional fibers (oats, carrots and sourdough) in an aged mouse model.

Methods: The groups included control and high-soluble fiber diets (HSFD), as well as oats, carrots and sourdough bread. Blood, fecal samples and muscle function tests were obtained after the intervention to evaluate the composition of the gut microbiota and inflammatory markers.

Result: Dietary supplementation with functional fibers dramatically improved physical performance and body composition in aged mice compared to the control group. This was demonstrated by increased muscle mass, grip strength and motor coordination. At the same time, it improved metabolic health by lowering fasting blood sugar and improving lipid profiles. The sourdough group had the lowest levels of pro-inflammatory cytokines, such as C-reactive protein (CRP), interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) (1.58, 8.62 and 11.85 pg/mL), respectively. All HSFD groups also showed decreased levels of endotoxemia markers, such as serum amyloid A (SAA) and lipopolysaccharide-binding protein (LBP). Beneficial bacteria, including *Bifidobacterium* (13.17%), *Lactobacillus* (10.16%) and *Akkermansia* (3.08%) were identified to be more abundant in the sourdough group than in the control group, according to a quantitative PCR investigation of the gut microbiota. In aged mice, HSFD, especially those composed of sourdough, oats and carrots effectively enhanced metabolic health, decreased inflammation and altered the composition of the gut microbiota.

Key words: Aging, Dietary soluble fiber diet, Gut microbiota, Inflammation.

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INTRODUCTION

The gastrointestinal tract is the primary symbiotic site for commensal microbes and studies have shown that the intestinal microbiome has a major impact on human health and illness by Douglas *et al.* (2020) and Shao *et al.* (2025). The majority of the bacteria that colonize the gastrointestinal tract are anaerobic and the overall number of these germs is vast and varied, accounting for nearly thirty percent of the dry weight of feces by Metwaly *et al.* (2020). Human immunity, nutrition and metabolism are all impacted by the gut microbiota. In addition to producing different vitamins and metabolizing nondigestible carbohydrates, the gut bacteria could also prevent pathogen invasion by Parker *et al.* (2022) and Mossad *et al.* (2022). Diet, age and the environment can affect the gut microbiome's composition, while antibiotics are considered the most significant environmental influences at the moment by Shi *et al.* (2021) and Kiewiet *et al.* (2021). The last ten years of life are frequently linked to morbidity, chronic illnesses and a lower quality of life, which fuels the growing need for primary care by Li *et al.* (2022). Strategies to enhance the quality of later life are essential for public health concerns by Vailati-Riboni *et al.* (2022). Health depends on the balance of gut microbes and dysbiosis leads to a number of functional abnormalities and illnesses, including autoimmune, neurological, degenerative diseases, impaired gastrointestinal barrier function, food absorption, metabolic and endocrine disorders with tumor development by Zagato *et al.* (2020). Cognitive decline and physical frailty are influenced by age-associated dysbiosis. Fortunately, a number of behaviorally adjustable factors, including nutrition, which in turn can affect gastrointestinal physiology. The gut microbiota aims to slow down aging and reduce related functional losses by Ojo *et al.* (2021). Numerous metabolic, cognitive, immunologic and gastrointestinal advantages of dietary fiber have long been recognized as beneficial to mammalian health by Muthyala *et al.* (2022). In addition to raising the risk of obesity and colon cancer, a diet low in fiber has been demonstrated to cause cognitive deterioration.

Since the gut microbiota plays a significant role in both health and disease. The purpose of this study was to assess the effects on gut microbiota composition, systemic inflammatory biomarkers and intestinal barrier integrity markers-specifically, plasma lipopolysaccharide-binding protein and serum amyloid A of a 10-week dietary intervention supplemented with 15% functional fibers (oats, carrots and sourdough) in an aged mouse model.

MATERIALS AND METHODS

Animal study and experimental details

The Ethics Committee approved all the experimental techniques under the approval number (8816/6709) issued by the Gulf Countries Association of Sciences. Mice were acquired from the Animal Holding Unit of the Biological Science in Jeddah, Saudi Arabia. They were kept at a temperature of 27 to 30°C and 60% humidity, with a 12-hour light and 12-hour dark cycle. The Biology Laboratory at Taif University in Saudi Arabia housed aged C57BL/6 J male mice (22 months of age and 22-30 g weight at the time of the initial study evaluation). Animals were checked every day for indications of pain, discomfort, distress during the course of the study. Body weight and other aspects of health were routinely recorded. The study excluded any animal exhibiting severe signs of discomfort, such as hard respiration, extreme lethargy, weight loss over 20%. CO_2 inhalation was used for euthanasia in order to reduce suffering. They also measured muscle mass, grip strength and the rotating rod test in addition to collecting blood and stool. A total of 24 healthy male mice were selected at random and divided into 4 groups, each dietary group (n=8).

Diets

Control mice were fed either conventional chow (LabDiet 5001), which consists of ground corn, soybean, beet, dried fish, oats, alfalfa, enhanced as (23% protein, 4.5% fat, 6% fiber, 8% ash, 12% moisture) with some minerals and vitamins or the customized High-Soluble Fiber Diet (HSFD) by Okamoto *et al.* (2019). Mice were housed according to their diet and were kept in different cages. Various HSFD diets for oats (*Avena sativa)*, carrots (*Daucus carota)* and flour (*Triticum aestivum)* were obtained from a local market in Taif City. Oats and carrots contain β-glucan and pectin as soluble fibers, respectively. While sourdough is rich in prebiotics and was previously prepared from the pre-fermented dough without additives, the bread was obtained and dried at a temperature of 50°C. HSFD diets were freeze-dried, ground and added as 15% to the ordinary chow.

Experimental design

Following a week of acclimation feeding period, male mice were given free access to water during a 20-hour fast. Each week, mice were given 150 g of chow and 250 mL of water in each cage. Food consumption was recorded between 18:00 and 22:00 after implementing the tailored diet ad libitum over 10 weeks, when the circadian rhythm was at its most active. At the end of each week, the amount and feed left over were weighed to record the food intake. Each mouse's used bedding, such as feces was gathered, combined with fresh bedding and then redistributed to each cage every week when the cages were changed by Hutchinson *et al.* (2023). Body weight was measured regularly at 18:00 and recorded in grams with a sensitivity of 0.001 g (FA2004B, SHSI Co., Ltd., Shanghai, China) until the end of the experimental period. Prior to each mouse being sacrificed, the identical fasting and 4-hour ingestion test technique was applied. Four hours after the start of the dark cycle, mice were put in a clean, empty cage to collect fecal samples, which were then frozen at -80°C. Mice were transcardially perfused with sterile ice-cold phosphate-buffered saline (PBS) and euthanized by CO_2 asphyxiation four hours after the dark phase began. Cardiac punctures were used to get blood for the biomedical tests. Fig 1 presents the summary of the current study.

Fasting blood glucose

Fasting blood Sugar (FBG) was assessed during the 9th and 10th weeks. Prior to measurement, mice were fasted for 11 hours from 6 pm. (with unlimited access to water) to create baseline metabolic conditions. Under minimum restraint, blood was extracted from the tips of the tails or by sampling the tail veins. FBG was expressed in milligrams per deciliter and measured using a glucometer (GA 3, Hunan, China). The measurement was done at a constant time of day to reduce the impact of the circadian rhythm. Following the collection of the last blood sample, the mice were fed again. Mean values from triplicate readings were utilized to reduce sample and device variability by Xie *et al.* (2023).

Physical examinations

Assessments of physical examinations were conducted after ten weeks of the experimental diets' consumption. Seven to eight weeks following the start of the food intervention, the grip strength and rotating rod tests were evaluated. Until sacrifice (10 weeks), since muscle mass was removed, feces and blood were gathered.

Grip strength

Forelimb grip strength was assessed using a computerized grip strength meter (Ugo, Basile, Italy) in compliance with the manufacturer's instructions in order to evaluate general muscular strength. The device is made up of a horizontal metal bar that the mouse automatically grasps and a precision force sensor attached to it. Mice were allowed to hold the bar with their forepaws or all four limbs after being gently raised by the base of their tail. The mouse was pushed steadily rearward in a horizontal plane until it released the bar once a solid hold was established. The maximum forces previously applied to release were automatically recorded by the instrument and expressed (in grams). The results are standardized to the corresponding body weight (g) and show the muscle force (gf) acquired in n = 3 trials per mouse, with a 5-minute gap between sessions, with brief resting periods between trials and the average of the highest three values was utilized for analysis to decrease variability and ensure consistency. For measuring neuromuscular function in rodents, this approach has received extensive validation by Takeshita *et al.* (2017).

Motor coordination by rotarod (Latency to fall)

The rotarod device was utilized to evaluate mice's motor coordination, balance and neuromuscular function; the latency to fall (measured in seconds) served as the main endpoint. There were six channels in the rotating rod instrument and each one had a rotating rod. When a mouse slipped off the rod, the device halted and noted the distance traveled and the time of fall. Mice were trained over a period of three to four days following acclimating to the testing environment and the rotarod apparatus (ZH-YLS-4C, China) in order to reduce unpredictability and promote task learning. Several trials, typically three per day for three days, were conducted with rest intervals in between to reduce fatigue. The fourth day was designated as the official testing day. For statistical analysis, the best trial or mean delay to fall over trials was utilized. On test days, mice were put on the spinning rod, which began slowly (about 4 rpm) and increased steadily (up to 40 rpm) over a certain amount of time (300 seconds). The rotation started each time and the trials stopped when the mouse fell off the rod (breaking a sensor or stepping to the platform) or, if the mouse stayed on the rod the entire time, until a cut-off delay was achieved. A test was repeated if a mouse fell during the first 30 seconds of the test, which was deemed an unintentional slip by Shan *et al.* (2023).

Muscle mass

Following the experiment's humane termination, the mice's back limbs were meticulously dissected to reveal the quadriceps femoris muscle. After that, the muscle was carefully separated and removed. Immediately, a precision microbalance with a sensitivity of 0.001 g or higher was used to assess the quadriceps' moist mass. Accurate evaluation of muscular atrophy or hypertrophy after death

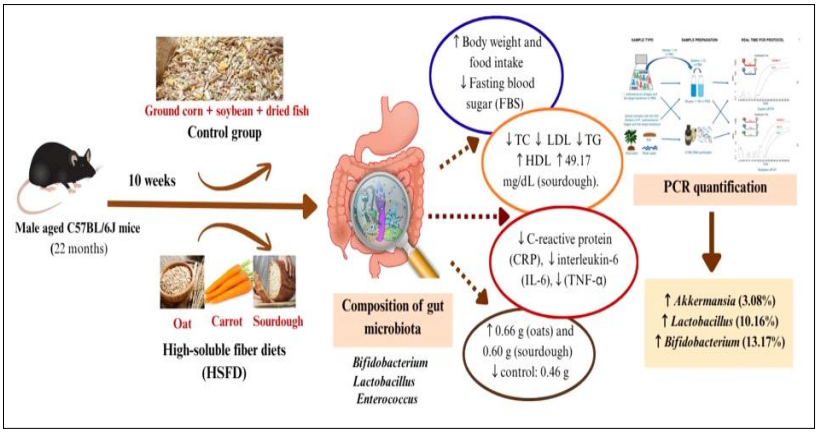


Fig 1: A schematic overview of the animal study.

was made possible by this measurement by Abbassi-Daloii *et al.* (2023).

Serum lipid profile

Serum levels of total cholesterol (TC), high-density lipoprotein (HDL), low-density lipoprotein (LDL) and triglycerides (TG) were evaluated in order to measure lipid profile in aged mice by Zhao *et al.* (2023). Serum was separated by centrifuging the samples at 1,800 rpm for 10 minutes at 4°C. Following the manufacturer's instructions, quantitative analysis was carried out using commercial enzymatic colorimetric kits (MAK043 - MAK045 - MAK266). Every measurement was carried out in triplicate and the results were expressed in (mg/dL).

Serum inflammatory cytokines

The enzyme-linked immunosorbent assay (ELISA) kits designed for mouse samples (Sigma-Aldrich, USA) were used to detect the serum levels of inflammatory markers as tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6) and C-reactive protein (CRP). The process was completed in line with the manufacturer's guidelines. Blood samples were drawn, allowed to coagulate at room temperature and then centrifuged at 2,000 rpm for 10 minutes by Singh *et al.* (2024). Prior to analysis, the collected serum samples were kept at -80°C. A microplate reader (RT-6100, Shenzhen, China) was used to read ELISA plates at a wavelength of 450 nm. The systemic inflammatory response in the experimental groups was evaluated using the detected levels of TNF-α, IL-6 and CRP and the concentrations were expressed as (pg/ml).

Serum lipopolysaccharide-binding protein and amyloid A

Lipopolysaccharide-binding protein (LBP) serves as a stand-in for the function of the gut barrier since it depicts the internal exposure of bacterial lipopolysaccharide (LPS). The enzyme-linked immunosorbent assay (ELISA) kits from Sigma-Aldrich, USA, were used to quantify LBP and amyloid A (SAA) in mouse blood (LBP: RAB1136; SAA: RAB1126). Blood was diluted 500 and 200 times using the dilution buffer that was supplied, drawn, allowed to clot at room temperature and then centrifuged for 10 minutes at 2,000 rpm for LBP and SAA, respectively by Hutchinson *et al.* (2023). Prior to testing, the serum samples were kept at -80°C. The ELISA process was carried out in line with the kit's instructions. A microplate reader (RT-6100, Shenzhen, China) was used to measure absorbance at 450 nm. The levels of SAA (µg/mL) and LBP (ng/mL) were determined in triplicate.

PCR analysis of gut microbiota

Mice's feces were then kept at -80°C until analysis. Following the manufacturer's instructions, microbial DNA was isolated using a stool DNA extraction kit (GenEluteTM, Sigma-Aldrich, USA). The bacterial 16S rRNA gene was then sequenced from fecal DNA using a SYBR Green-based master mix; each reaction was carried out in triplicate on a real-time PCR machine (SLAN-96P, Changsha, China). Approximately 250 mg of the frozen stool was suspended in 800 µL of buffer in the Power Bead Pro tubes after dilution in Diethyl Pyrocarbonate-treated water and incubation at 65°C for 10 minutes according to the assay of by Thorpe *et al.* (2018). Gut microbial compositions were examined and the relative abundance of *Akkermansia*, *Lactobacillus* and *Bifidobacterium* was assessed by using real-time PCR genus-specific primers. The ΔΔCt method was used to calculate each genus' abundance, which was then normalized to the levels of the entire bacterial 16S rRNA gene and expressed as a percentage of the total gut bacteria.

Statistical analysis

The mean ± SEM was used to represent all data. SPSS version 27 (SPSS, Chicago, IL) was used to perform statistical analysis (IBM, USA). Two-way analysis of variance (ANOVA) was used to analyze the parametric data and a Tukey HSD adjustment was used to evaluate pairwise comparisons. $P<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Food intake

All mice in the trial showed no discernible changes in their hair, activity level, or fecal characteristics across the various diet groups, indicating little stress and an overall well-being of mice. The mice's body weight and food intake were raised in almost groups compared to the control. Mice were fasted for 20 hours before being fed the high-fiber diets on day 0 in order to examine their palatability. During the first week, the food consumption patterns of all groups were similar, with the exception of the carrots group (4.67 g/day). During the first week, the oat and sourdough groups may tasteless appetizing than the other diets (2.07 to 2.11 g/day), while by the sixth week of feeding, nearly all groups showed very similar outcomes, with food intake gradually declining. Oats-treated mice group had slightly greater food intake compared to the beginning of the experiment period, especially the 7th and the 10th weeks (2.95-1071 g/day), respectively, Table 1. The present findings were consistent with those of Hu *et al.* (2025), who discovered that mice fed HSFD showed superior hepatic transcriptome and metabolomic profiles as well as healthy aging signatures that were comparable to the control group.

Body weight

As expected, mice in all groups exhibited a steady increase in body weight over the course of the ten weeks, in contradiction to food intake; however, by the tenth week, the aged mice fed oats were lighter 37.26 g compared to the control 40.04 g, (Fig 2). Further, the rise in body weight was decreased with the fermented sourdough 35.98 g. The fiber alteration might offer an innovative approach for optimizing nutrients by Li *et al.* (2024). HSFD has been shown to improve a number of other health benefits, such as weight control, decreased appetite and a lower risk of

developing a number of chronic diseases by Hossain *et al.* (2025).

Fasting blood sugar

Significant differences in the fasting blood sugar (FBS) levels between the four experimental groups (control, oats, carrots and sourdough) indicated that dietary intervention may have an impact on glycemic management. At 132.47 mg/dL, the control group's fasting blood sugar level was the highest, significantly exceeding the generally recognized normal fasting range (usually <100 mg/dL, with 100-125 mg/dL being considered pre-diabetic and ≥126 mg/dL being indicative of diabetes). Mild blood glucose elevations are frequently observed as a typical aspect of aging. The absence of dietary change, this increased score most represents a baseline level of poor glucose regulation. The groups that consumed sourdough, oats and carrots had the lowest average FBS levels, at 103.64 mg/dL, 109.35 mg/dL and 115.71 mg/dL, respectively, (Fig 3). Although the mean levels of all three intervention groups remain over the optimal threshold (less than 100 mg/dL), their relative decreases in comparison to the control group point to possible glycemic advantages of these foods. The fermentation process, which modifies starch structure and enhances gut flora to lower the glycemic index and increase insulin sensitivity, may be the reason for sourdough's excellent performance. In addition to having fiber and antioxidants, carrots also have natural sugars, which might be responsible for their comparatively higher FBS value as compared to sourdough and oats by Gill *et al.* (2021); Barone *et al.* (2024) and Hossain *et al.* (2025). The present findings were consistent with those of Hu *et al.* (2025), who examined how HSFD replicates aging-related dietary restriction indicators in mammals.

Grip strength

The comparison of the experimental groups' grip strengths suggested that dietary changes could help with better muscular function. As predicted, aging considerably

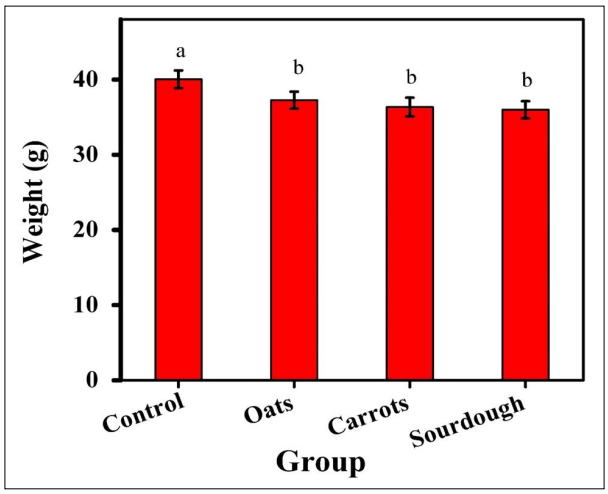


Fig 2: Effect of various HSFD on body weight of aged mice.

Table 1: Food intake for aged mice during the study period (10 weeks).

Groups	1	2	3	4	5	6	7	8	9	10
Control	3.55±0.21^{b}	3.10±0.22^{b}	3.87±0.18^{b}	3.12±0.23^{b}	3.57±0.23^{c}	3.11±0.22^{c}	4.02±0.23^{b}	3.07±0.19^{c}	3.14±0.21^{b}	3.05±0.21^{c}
Oats	2.07±0.25^{c}	4.67±0.24^{a}	4.79±0.21^{a}	4.85±0.24^{a}	4.23±0.25^{b}	4.02±0.21^{b}	4.99±0.21^{a}	3.55±0.18^{b}	3.45±0.19^{b}	3.78±0.22^{b}
Carrots	4.67±0.24^{a}	4.98±0.23^{a}	4.85±0.23^{a}	4.57±0.22^{a}	4.66±0.21^{a}	4.97±0.18^{a}	4.38±0.19^{b}	4.53±0.23^{a}	4.86±0.18^{a}	5.07±0.19^{a}
Sourdough	2.11±0.23^{c}	2.37±0.15^{c}	2.66±0.22^{c}	3.01±0.21^{b}	3.12±0.19^{d}	3.12±0.23^{c}	3.16±0.22^{c}	3.24±0.19^{bc}	3.12±0.22^{b}	3.17±0.18^{c}

Data are presented as mean±standard deviation (SD) with significant differences (p<0.05) among mice groups.

decreased the grip strength in mice, (Fig 4). Nonetheless, the sourdough group's grip strength was significantly increased by HSFD. While all dietary groups displayed higher values, the sourdough group achieved the highest grip strength (193.06 g), followed by the oats group (187.34 g) and the carrots group (169.94 g). The control group displayed the lowest grip strength (152.10 g). These results indicated that muscle strength and the functional meals were positively correlated. The fermentation process of sourdough may be responsible for its superior results, as it can improve muscle protein synthesis by reducing anti-nutritional agents and increasing the bioavailability of amino acids. According to Kim *et al.* (2023), middle-aged adults' grip strength increased when they supplemented with fermented protein as opposed to non-fermented protein sources. Oats may also promote muscle metabolism and neuromuscular function since they are high in β-glucans, dietary fiber and minerals as magnesium. Similarly, β-carotene, an antioxidant found in carrots, can lower oxidative stress and aid in muscle repair, albeit with a less pronounced effect. According to Zhang *et al.* (2022), reported the improvement in muscle function and strength in older persons who consume fermented foods and a healthy diet.

Roting rod

The rotarod test, which measures balance and motor coordination [19], revealed that all dietary groups performed better than the control group, (Fig 5). The control group showed the least amount of motor coordination (45.23 s), while the groups who consumed oats, carrots and sourdough diets had the largest latency (57.16, 54.48 and 51.6 s), respectively. These enhancements imply that consuming oats, carrots and sourdough might have improved balance and neuromuscular coordination. Oats' β-glucans and antioxidants, which have been demonstrated to lower systemic inflammation and promote neurological function, may be the reason for the oats group's better performance. Through increased metabolic stability and gut-brain axis regulation, previous studies have connected diets rich in whole grains and fiber to improved cognitive and motor function by Pato *et al.* (2025). β-carotene and other carotenoids with neuroprotective qualities might have improved the carrot group Choi *et al.* (2025). Even while it still outperformed the control, sourdough might have had less of an impact since it included fewer neuroactive elements. Nevertheless, because it was fermented, it might still have some moderate cognitive and neuromotor advantages. Overall, the results were consistent with the theory that dietary factors may influence motor function, possibly via antioxidant and anti-inflammatory processes by Ullah *et al.* (2023). This implies that age-related motor deficits were successfully reduced by HSFD.

Muscle mass

In comparison to the control group (0.46 g), the oats group (0.66 g) and the sourdough group (0.60 g) had significantly higher muscle mass, while the carrots group (0.51 g) showed more moderate gain, (Fig 6). The positive impact of oat-derived fiber was previously shown: supplementing

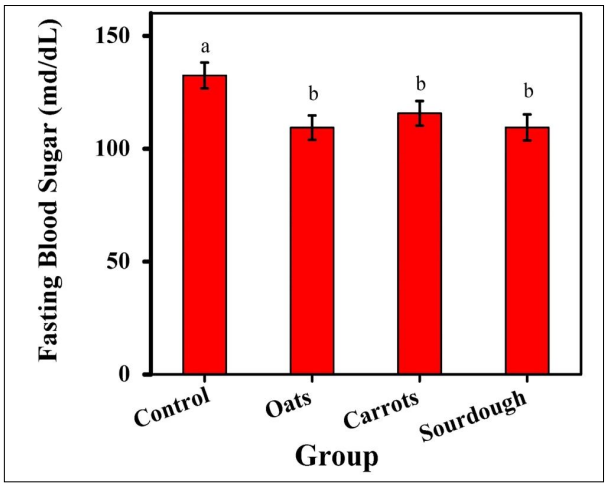


Fig 3: Effect of various HSFD on fasting blood sugar of aged mice.

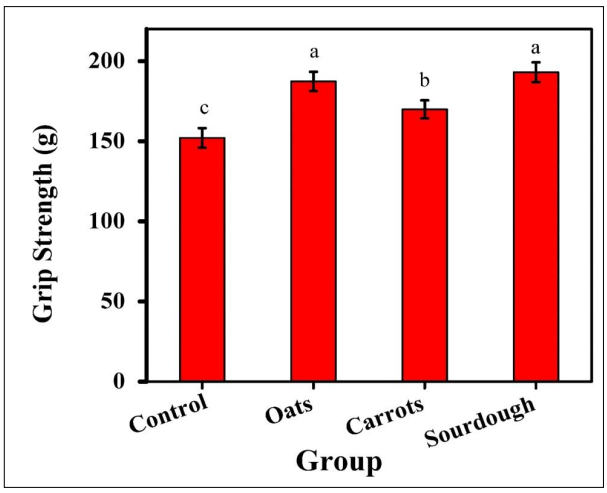


Fig 4: Effect of various HSFD on grip strength of aged mice.

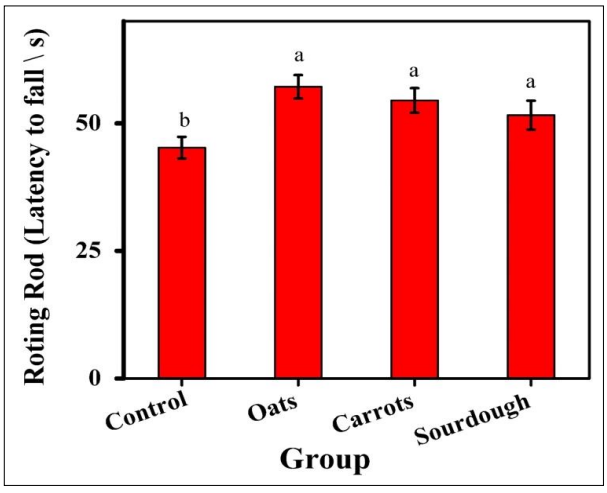


Fig 5: Effect of various HSFD on roting rod test of aged mice.

with oat bran improved oxidative stress, decreased systemic inflammation and improved skeletal muscle impairments. Additionally, a recent study found that (98 weeks) fed mice a whole-food, HSFD (3× standard chow) for 10 weeks showed that females performed better on the treadmill and that the quadricep body weight ratio increased sex-dependently, while the gains were not consistent between the sexes. However, sex, baseline nutritional condition and fiber type probably affect the extent of these effects by Fielding and Lustgarten (2024). These findings provide credence to the theory that by regulating the gut muscle axis, fermentable fiber may help maintain or slightly enhance muscle mass with age.

Lipid profile

The lipid profiles of aged mice fed HSFD (carrots, oats and sourdough) were significantly improved than those of the control group. In particular, the control group had the lowest HDL (40.23 mg/dL), the highest (TC 165.87 mg/dL), LDL (93.82 mg/dL) and TG (119.76 mg/dL). The oats group slightly raised HDL to reach 43.65 mg/dL while decreasing TC to reach 118.87 mg/dL, LDL at 69.25 mg/dL and TG to reach 93.08 mg/dL. The sourdough group further exhibited improvement (TC 112.05, LDL 63.45, TG 89.16 and HDL 49.17 mg/dL), while the carrots group displayed comparable patterns (TC 123.45, LDL 71.97, TG 97.21 and HDL 43.73 mg/dL), (Fig 7). According to these results, oats and sourdough, which are high in fiber, had a greater impact in reducing lipids in aged mice than carrots. Our results were in agreement with the recent research in C57BL/6 mice, supplementing with cereal fiber restored raised TC, TG and LDL caused by fatty diets by Han *et al.* (2019). Furthermore, by suppressing lipogenesis and improving intestinal cholesterol outflow, diets high in fiber improved lipid metabolism by Bulsiewicz (2023). Particularly for serum TG, fermented carrots had a stronger action than raw carrots. Thus, HSFD attenuates age-related and diet-induced hyperlipidemia in mice, as evidenced by the observed decreases in TC, LDL and TG and the rise in HDL.

Inflammatory cytokines

Diets rich in fermentable fibers (oats, carrots and sourdough) significantly reduced inflammatory processes in aged mice. TNF-α (21.03/ pg/mL), IL-6 (14.76/ pg/mL) and CRP (3.44/pg/mL) were all the greatest control group. TNF-α (12.54 pg/mL), IL-6 (8.89 pg/mL) and CRP (1.76 pg/mL) were significantly lower in the oats group, (Fig 8). Carrots group showed reduced inflammation (1.98, 14.02, 10.11), whereas the sourdough group showed the lowest levels (1.58, 11.85, 8.62) for CRP, TNF-α and IL-6, respectively. According to these findings, dietary fiber reduced low-grade inflammation linked to aging, with oat and sourdough sources that had the strongest impacts. In diabetic or metabolic mouse models, high-fiber diets have been demonstrated to reduce TNF-α and IL-6 in comparison to controls. CRP indicator of acute phase inflammation, was decreased in diabetic cases after the consumption of dietary fibers, according to Ojo *et al.* (2021).

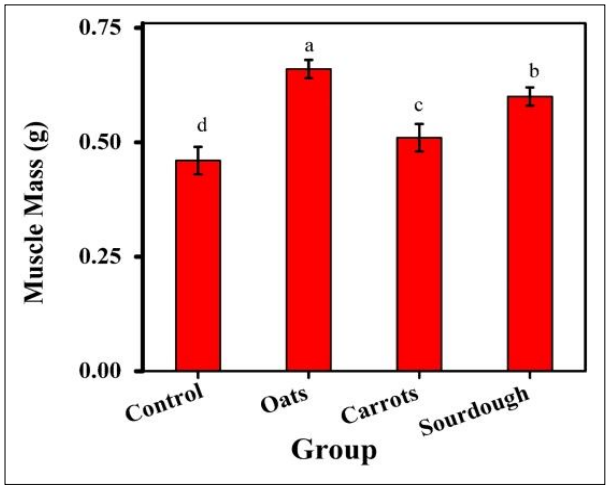


Fig 6: Effect of various HSFD on muscle mass of aged mice.

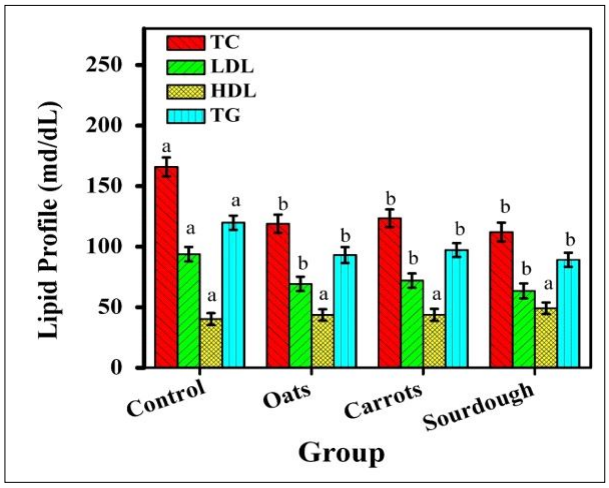


Fig 7: Effect of various HSFD on lipid profile of aged mice.

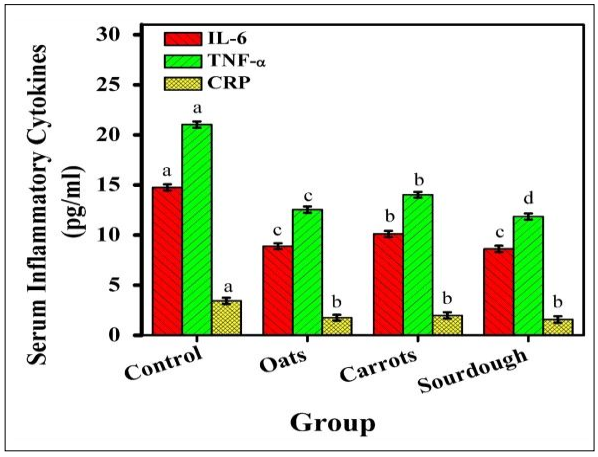


Fig 8: Effect of various HSFD on inflammatory cytokines of aged mice.

Lipopolysaccharide-binding protein and amyloid A

LBP is associated with obesity, resistance to insulin, persistent inflammatory conditions and dyslipidemia and is frequently used to signal endotoxemia, which may be caused by disruption of the intestinal barrier [45]. Systemic indicators of endotoxemia and acute-phase inflammation decreased in aged mice fed HSFD (oats, carrots and sourdough) as compared to the control diet, Fig 9. LBP was 2.54 ng/mL and serum amyloid A (SAA) was 10.03 µg/mL in the control group, while SAA was significantly lower in the oats group (7.61 µg/mL, LBP 1.77 ng/mL), moderately lower in the carrots group (7.95 µg/mL, LBP 1.86 ng/mL) and intermediately lower in the sourdough group (8.62 µg/mL, LBP 2.13 ng/mL), (Fig 9) (a and b). These modifications indicated that fermentable fiber reduced systemic LBP, which binds LPS and the ensuing acute-phase SAA response by inhibiting the translocation of microbial LPS over the gut barrier by Hutchinson *et al.* (2023). The effects of dietary fiber on serum LBP were not statistically significant, although it did affect neuroinflammation. Related studies have demonstrated that dietary fiber supplementation decreased various acute-phase proteins and indicators of endotoxemia in animal models of metabolic disease, despite the paucity of studies directly detecting SAA or LBP in elderly mice after fiber interventions Lassenius *et al.* (2011) and Liu *et al.* (2025). Liu *et al.* (2025) showed that supplementing diabetic mice with oat and wheat fiber dramatically lowered their serum levels, indicating systemic anti-inflammatory effects linked to decreased endotoxemia. The significant role of dietary fiber in reducing age-associated endotoxemia and the ensuing inflammatory activation through gut-microbiota-barrier axis modulation was the primary objective of the observed decreases in SAA and LBP in HSFD groups.

Gut microbiota

All HSFD groups demonstrated increases of beneficial organisms, including *Bifidobacterium* (5.11%), *Lactobacillus* (4.79%) and *Akkermansia* (1.15%), in contrast to the control group, which showed relatively low abundance. The largest enrichment was seen in the sourdough group, where *Bifidobacterium* increased to 13.17%, *Lactobacillus* to 10.16% and *Akkermansia* to 3.08%, (Fig 10). The increases in the oat and carrot groups were comparable and, albeit marginally smaller. These changes in microbial composition accord with fermentable fibers' well-established prebiotic benefits, which include preferentially fostering the growth of commensal organisms that can produce SCFAs, boost gut barrier integrity and improve mucosal immunity by Thorpe *et al.* (2018). In aging and metabolic issues, *Akkermansia* muciniphila is known to help preserve gut homeostasis and reduce inflammation. In aged hosts, it has also been demonstrated that *Lactobacillus* and *Bifidobacterium* alter intestinal permeability by Hutchinson *et al.* (2023). *Bifidobacterium* and *Lactobacillus* are the principal producers of these SCFAs, which are crucial for regulating the immune system and reducing inflammation. Louis and Flint (2017) have discovered that a number of gut bacteria such as

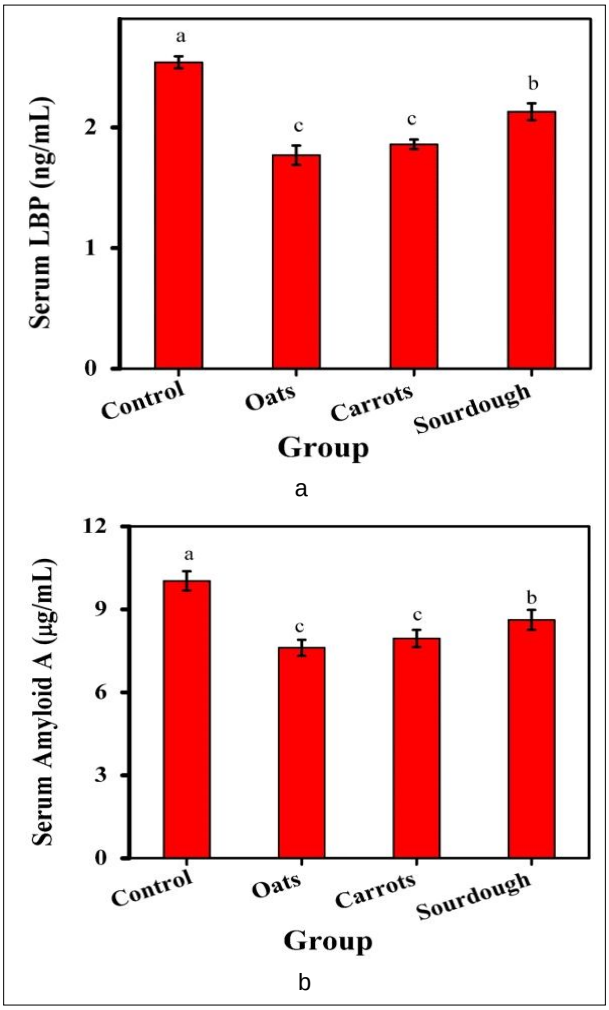


Fig 9: Effect of various HSFD on (a) LBP and (b) SAA of aged mice.

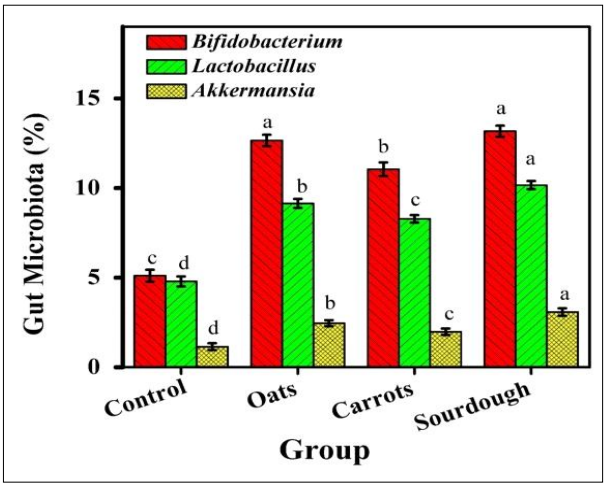


Fig 10: Effect of various HSFD on gut microbiota of aged mice.

Faecalibacterium, Roseburia and *Bifidobacterium*, were not unique to aging mice and that dietary fibers strengthen the beneficial microbiota and its anti-inflammatory compounds, hence promoting gut and systemic health by Sarkar *et al.* (2021) and Sreelekshmi *et al.* (2024). Thus, the anti-inflammatory and metabolic advantages linked to fiber consumption in aged mice were probably influenced by the microbial changes observed in the current investigation.

CONCLUSION

The current study showed that feeding aged mice diets rich in soluble fiber from oats, carrots and sourdough considerably enhanced a number of age-related health indicators. In addition to the positive changes in lipid profiles, such as higher HDL and decreased LDL, TC and TG, these dietary interventions resulted in decreased FBS levels, enhanced muscle growth, grip strength and motor coordination. Additionally, TNF-α, IL-6 and CRP, which are indicators of systemic inflammation, were considerably lower, especially in the group that was fed sourdough. The anti-inflammatory potential of these diets was further supported by decreased levels of endotoxemia-related proteins, such as serum SAA and LBP. PCR analysis showed significant increases in beneficial gut microbes, such as *Bifidobacterium*, *Lactobacillus* and *Akkermansia*, suggesting that soluble fiber intake modulated gut microbiota composition in a way that may contribute to improved metabolic and immune function. These findings suggest that including soluble fiber-rich foods in the diet may offer a promising, non-pharmacological strategy to mitigate age-related metabolic dysfunction, chronic inflammation and gut dysbiosis. Future research ought to investigate the long-term effects of high-soluble fiber diets on aging-related metabolic and inflammatory disorders in humans to confirm these findings.

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Availability of data and materials

Data is provided within the manuscript files.

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Conflict of interest

The authors declare no conflict of interest.

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