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Impact of galacto-oligosaccharides on prebiotic potential in the intestinal microbiota fermentation and health status in an animal model

Amna Ashraf BAJWA[1], Sanaullah IQBAL[1], Muhammad SOHAIB[1]*, Michael CONLON[2], Muhammad NASIR[1], Aftab Ahmad ANJUM[3]

Abstract

The present study was conducted to assess selected galacto-oligosaccharides (GOS) effects on short-chain acids (SCFA), microbiota variability in *in vitro*, and health benefits using an animal model. In the *in-vitro* anaerobic batch, fermentation was applied to different groups divided by a varied amount of GOS sources, mixtures, and prebiotics. Results reported that SCFA for inulin contributed significantly higher for acetic, propionic, and butyric acids, and resistant starch (RS) showed a non-significant effect for acetic and propionic acids whereas the combined effect of GOS and RS showed higher values for parameters. For bacterial enumeration of *bifidobacteria* compared to individual GOS, synergistic effects were documented. The Sprague-Dawley rats given GOS under western diet influence relative to a high-fat diet alone observed after 1 and 4 weeks documented significant levels for acetic and butyric acid production, whereas body and organ weights for cecum tend to increase after 4 weeks of dietary intervention ($p<0.05$). Microbiome data using gene sequencing revealed a higher proportion of firmicutes and lower Bacteroides in control rats, which means Lachnospiraceae family abundances were higher in HF+GOS group. Overall, GOS fermentation showed an increment in the *bifidobacterial* population and tend to raise levels of SCFA in rats fed on a high-fat diet alone, whereas non-significant variation was reported in microbiome diversity after intervention.

Keywords: galacto-oligosaccharides; *in vitro* fermentation; short-chain fatty acid; high-fat diet; body weight.

1 INTRODUCTION

The occurrence of long-lasting diseases like cardiovascular complications, colorectal cancer, and type II diabetes is increasing nowadays, resulting in an immense burden on society in terms of health, well-being, and productivity. Among all other factors, sedentary lifestyles and western diets are major contributors to non-communicable diseases in humans. Western diets are considered to be rich in animal protein, saturated fats, refined carbohydrates, and sodium, resulting in an increase in risk factors and disease burden. They are low in fiber as there is less consumption of fruits and vegetables. The variation in gut microbial community composition can be linked to changes in dietary patterns, whether long- or short-term. The process underlying this may involve an increase in fecal mass, which in turn can happen to a lesser extent by fermentation that involves bacterial propagation (Conlon and Bird, 2014).

Diet is one of the important factors as it can be involved in the provision of substrates that might help in improving the microbiota residing in the large intestine, especially the large bowl. The addition of a diet high in fiber compared to the common western diet stimulated noticeable variations in the fecal microbiota, even though there was not as major a shift from the *Bacteroides* to the *Prevotella* enterotype (Wu et al., 2011). Dietary fiber has gained attention in Western countries nowadays as they have lower dietary fiber consumption in general and also the variety of substances present in it, so there is a bigger concern to explore dietary fiber type and related mechanisms that can produce a positive impact on human health. Dietary fiber is considered a crucial part of the human diet, and studies have also shown its role in controlling and treating different disease conditions like bowel movement disorders, obesity, and heart-related disease complications (Sleeth et al., 2013). Likewise, the end products and metabolites that are produced by colonic microbiota fermentation can result in a variety of health benefits. Similarly, the fermentation of carbohydrates by bacteria results in the production of organic acids, including short-chain fatty acids after fermentation that are involved in different beneficial functions like energy for tissues, the bowel epithelium, and other bacterial communities. Additionally, pathogen and harmful bacterial activity is lowered by decreasing pH, which ultimately results from these weak short-chain fatty acids. The key SCFA produced by this fermentation process includes butyrate, propionate, and acetate, which has specific roles in body functions as they make up to 90% of the total fatty acids produced by the fermentation process. Butyrate is one of the important SCFAs due to its role in the defense mechanism against colon cancer as well as being involved in the regulation of colonocyte differentiation and apoptosis, which can stimulate the exclusion of dysfunctional cells (Fung et al., 2012). Propionate and acetate

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[1]*University of Veterinary and Animal Sciences, Department of Food Science and Human Nutrition, Lahore, Pakistan.*
[2]*CSIRO Food and Nutrition Flagship, Kintore Ave, Adelaide, Australia.*
[3]*University of Veterinary and Animal Sciences, Institute of Microbiology, Lahore, Pakistan*
*Corresponding author: muhammad.sohaib@uvas.edu.pk

are oxidized by and are involved in lipogenesis and gluconeogenesis processes that have a crucial role in fat metabolism and energy production in living organisms. The SCFA functions and mechanism of action depend on the quantity and type of fibers utilized as substrates, like butyric acid. Concentration can reach its maximum when oligosaccharides, resistant starch types, and β-glucan are used as substrates, whereas acetic and propionic acids are high when pectin and guar gum are used as a substrate, respectively (Nilsson & Nyman, 2005).

High-fat diets also affect the composition of the gut microbiota, resulting in increased levels of bacterial-derived lipopolysaccharides (LPS) in living organisms, possibly due to improved absorption in the intestine (Moreira et al., 2012). LPS are involved in metabolic diseases and considered inflammatory agents. Many studies have shown that western-style diets result in a microbial population associated with the Bacteroide enterotype compared to diets with plant polysaccharides, which are accompanied by the *Prevotella* enterotype (Wu et al., 2011). The classic western-style diet provides nearly 50 g of carbohydrates that can be fermented by microbiota residing in the colon, with the major substrate being dietary fiber, while other substances can include non-starch polysaccharides (NSP) that can contribute 20%–45% of dry matter that can ultimately be delivered to the colon. Nearly 10% and 8% of contribution in dry matter is provided by sugars, oligosaccharides, and starch, respectively. A minor portion of substrates for the microbiota of the colon is provided by sugar alcohols, as they are not absorbed by the small intestine (Salem, 2018). Similarly, one of the researcher's groups reported that other dietary substrates, including proteins, lipids, polyphenols, catechins, lignins, tannins, and nurturing colonic microbiota, including 90% polyphenols, have a noteworthy effect on microbiota in living organisms (Deschasaux et al., 2013).

The proximal part of the colon is involved in most bacterial fermentation due to the major access to the substrate in this part of the living organism compared to the distal colon, where carbohydrates are less available. In the distal part of the colon, the SCFAs are absorbed more readily, which makes the fermentation of proteins more proficient, along with the production of alkaline metabolites that can increase the pH in the distal part (from 5.8 to 7.0–7.5). Furthermore, the proximal part has the highest SCFA (~100 mM) compared to the distal part, where a gradual drop-off is reported. Also, the SCFA has a potential role in energy production as it encourages specific types of sodium-coupled transport in the colonocyte apical membrane that can facilitate electrolyte recovery and energy production (Binder et al., 2010).

It is commonly believed that variation in dietary substrates can produce a minimal effect on the whole microbial population in the gut, which also includes structural conformation and composition. Due to emerging prebiotics, this concept has been challenged, and there is growing acceptance that minor variations in dietary strategies can alter microbiota composition and physiological functions related to them (Macfarlane & Dillon, 2007). The prebiotics and dietary fibers terminologies are mostly used in substitution, but prebiotics are more engaged in specifically promoting microbiota that are involved in health benefits, which also differentiates them from dietary fiber. Additionally, the prebiotics are involved in encouraging fermentation processes that promote the increment of especially *bifidobacteria* and *lactobacilli* and SCFA production by fermentation processes (Venter, 2007). The *bifidobacteria* assist in retaining the mucosal barrier (Barry et al., 2010), and prebiotic substrates stimulate their growth, apparently at the cost of Bacteroides, clostridia, and coliforms. Literature has reported that prebiotics are also involved in a variety of health-promoting benefits, like colorectal diseases, immunomodulation, and diarrhea suppression (Ito et al., 2002). Although nearly all types of carbohydrates are involved in functions that classify them as prebiotics, galacto-oligosaccharides are considered the most vital among reported substances due to their functional role (Tuohy et al., 2005).

A variety of resident saccharolytic bacteria in the colon can ferment the popular prebiotic inulin. Several studies have been done on exploring the diverse beneficial gut flora's preference for fermenting inulin, particularly *bifidobacteria* and *lactobacilli*. Results have reported that consuming enough inulin as a prebiotic may help maintain gut health, reduce the risk of infection, and enhance stool quality, along with improving microbiota, pH, short-chain fatty acids, and frequency. This inulin-type prebiotic's advantageous physiological effects also included control of obesity and diabetes as well as elevation of blood lipid levels and absorption of minerals in humans (Chen et al., 2019).

Functional foods, which offer health advantages beyond basic nutrition and can lower the risk of chronic diseases, are receiving a significant amount of attention today. One of the most frequently utilized components in functional foods is resistant starch (RS), which is classified as a form of dietary fiber. The dysbiosis of the gut microbiota has been linked to a number of disorders, including allergies, colorectal cancer, and chronic inflammatory diseases. Prebiotics like RS, on the other hand, may be administered to lessen the symptoms of inflammatory bowel disease and the risk of colorectal cancer. It has been suggested that these effects may be mediated by an increase in the number of particular bacterial groups and their fermented products after RS treatment, even if the processes underlying these findings are not completely understood (Herrmann et al., 2017).

Considering the scenario, the present study was conducted to compare a variety of groups of different types of commercial GOS preparations on microbial populations and immunological factors using *in vitro* and animal model trials provided a western-style diet that can contribute to poor health outcomes. The short-term impacts (1 week) of dietary polysaccharides on microbiota populations, in general, are poorly understood, and knowledge of the time required to affect significant beneficial shifts in microbial populations or other biochemical and physiological changes may assist in optimizing dietary strategies that can impact human health.

2 MATERIALS AND METHODS

2.1 Chemicals and substrates

All chemicals utilized in the present study were of analytical/chromatographic grade, purchased from Merck and Sigma.

The two B-GOS preparations used in this study were donated for research purposes; one was OLIGOMATE® 55N from Yakult Pharmaceutical Industry Co., Ltd. Liquid syrup that has 4′-galactosyl lactose as the main constituent and 55% or more GOS; the other was Vivinal® GOS powder from Friesland Campina Domo, which has high levels of GOS (69%) and low levels of monosaccharides (5%). Other substrates for comparison include resistant starch Hi-maize® 958 (Starch Australasia, Lane Cove, NSW, Australia); positive and negative controls used were inulin and blank, respectively.

2.2 Fecal sample preparations

For the collection of fecal samples, three human participants meeting the requirements of fitness, being free from any disease, having no diet-related limitations, and consenting to the provision of fecal specimens for study were selected. Additionally, the participants who were not on antibiotics for at least 90 days before contributing fecal samples were included in the study. Afterward, in the anaerobic compartment, fecal samples were shifted, containing the same fecal quantity from every participant, which was diluted by 10% (w/v) using sterile phosphate-buffered saline (PBS) at 0.01 M maintained at a pH of 7.2. Afterward, the suspension was homogenized and frequently agitated during sample inoculation in every test related to the fermentation process for further processing.

2.3 Batch fermentation process

Batch-type fermentations using anaerobic conditions, which are already adapted processes, were utilized for the evaluation of prebiotics (GOS) from two different sources in combination to access variation in individual and combined prebiotics on microbial arrangements and fermentation properties by following the guidelines of Zhou et al. (2013). The fermentation time for prebiotics and fresh specimens of feces used under an anaerobic environment for 12 h during the experimental period in the aerobic compartment (Bactron IV Anaerobic Chamber, Sheldon Manufacturing Inc., Cornelius, United States) with GOS samples at 1.5% (w/v) as substrate sources in the media for fermentation for treatment and blank samples. Inulin was used in the same quantity to serve as a positive control, whereas media composition for fermentation was done using trypticase at 2.5 g/L along with 125 L micromineral, 250 mL macromineral, 250 mL buffer, 1.25 mL resazurin solution, and 0.1% (w/v) of distilled water. Similarly, the composition of the micromineral suspension was 100 g of $MnCl_2.4H_2O$ along with 132 g of $CaCl_2.2H_2O$, 80 g of $FeCl_3.6H_2O$, and 10 g of $CoCl_2.6H_2O$ per liter of distilled water. Likewise, the formulation of macromineral suspension was KH_2PO_4 (6.2 g), Na_2HPO_4 (5.7 g), and $SO_4.7H_2O$ (0.6 g), whereas fermentation media of 1 L volume with reducing solution was made using 33.5 mL of $Na_2S.9H_2O$, 6.25 g of cysteine hydrochloride, and 40 mL of 1 M NaOH dissolved per liter of distilled water, followed by sterilization at 121°C for 15 min. Additionally, modifications related to pH for fermentation media were done at a pH of 7.2, whereas the concentration of fecal inoculum was 10% (w/v) in each fermentation trial, and incubation trials using an orbital mixer incubator were completed at 37°C along with agitation at 80 rpm for 12 h with the remaining products that were further utilized for analysis of short-chain fatty acids (SCFA) and bacterial population.

2.4 Short-chain fatty acid analysis

The effect of GOS prebiotics on short-chain fatty acids (SCFAs) was evaluated using the protocol of McOrist et al. (2008) with a slight modification. For the analysis, all test substances (1 mL) were subjected to centrifugation at a speed of 2,000 *g* for 10 min at 4°C prior to the addition of internal standard heptanoic acid (3 mL), followed by 10 µL inclusion of 1 M phosphoric acid to the supernatant (300 µL). All samples during analysis were placed in ice to inhibit SCFA volatilization during processing. Afterward, proceed to the insertion of a sample (0.2 µL) filtered prior to the gas chromatograph (model 7890A; Agilent Technologies, Santa Clara, CA, United States) using a particular flame ionization detector and a capillary column (Zebron ZB-FFAP, 30 m × 0.53 mm × 1.0 m, Phenomenex, Lane Cove, NSW, Australia). The carrier gas employed was helium, with an oven temperature of 90°C kept for 1 min that was later enhanced from 20°C/min to 190°C held for 2.5 min; temperature readings for the injector and detector set to 210°C; and septum purge at 3.0 mL/min along with gas flow at 7.7 mL / min. The final SCFA identified was estimated by matching peak areas relative to standards, and quantification was done in mol/mL. The SCFAs identified included acetic, propionic, butyric, isobutyric, valeric, isovaleric, and caproic acid.

2.5 Microbial population enumeration by quantitative real-time polymerase chain reaction

The 12 h of fermentation resulted in samples from which 2 mL were obtained and subjected to centrifugation for 5 min at a speed of 2,655 *g* at 4°C. For purposely extracting DNA, the kit was used after bead beating, namely, PowerMag® (27500-4-EP; MO BIO Laboratories, United States), which is conveniently utilized for epMotion® and optimized with few amendments to the procedure. In the feces sample, the addition of glass beads and PowerMag® Microbiome Lysis Solution for about 650 µL that was heated before was done, followed by centrifugation for 5 min. The addition of proteinase K (600 mAU/mL, Qiagen, Hilden, Germany) in the supernatant was followed by heating for 10 min at 70°C. Afterward, PowerMag® inhibitor removal solution was added, followed by incubation for 5 min at −20°C after heating. The centrifugation process was completed at a speed of 20,817 g for 5 min, and the remaining supernatant was used in the MO BIO Deep Well Plate, where 5 µL of RNAse was added further. The extraction process was performed with the assistance of the manufacturer's procedure (epMotion-protocol-27500-V2.dws), with a slight modification to achieve optimization for epMotion® 5075 (Eppendorf, Germany). For the elution of DNA, assistance with RNase-free water using ClearMag® was achieved, and for finding DNA concentrations and transparency, the spectrophotometric method was employed using a NanoDrop spectrophotometer (Thermo Fisher Scientific, United States), followed by storage at −80°C. The purpose for amplifying PCR along with detection was achieved using Q-PCR (Bio-Rad Labo, Hercules, United States) with the use of primers that are particulate to the genus. In short,

each reaction mixture had a nucleic acid dye SsoFast™ EvaGreen® (Bio-Rad Laboratories, Hercules, CA, United States) of 5 µL with bovine serum albumin (BSA) from Promega (Madison, United States) along with primers at concentrations of 300–600 nM in a 0.2 µL volume and 0.06–0.12 µL water (sterile) so that the final total volume is 7 µL, whereas the purpose of quantification is to use extracted DNA at 3 µL of 3 ng/L from each sample. Finally, every cycle involved the detection of fluorescent products. For microbial population estimation, Cq values were used along with melting curve analysis after amplification and differentiation of target products of PCR. For obtaining standard curves, eight tenfold serial dilutions for bacterial groups, including *bifidobacterium* along with *Bacteroidetes*, *lactobacillus*, and total bacteria, were chosen for exploring particular bacterial genera involved in affecting the gut health of the target living organism.

2.6 Animal housing, treatments, and sample collection

For the evaluation of GOS's potential to improve the health status of living organisms, Sprague-Dawley male rats were purchased from the Animal Resource Centre, University of Adelaide, and kept in a small animal unit (CSIRO, Food and Nutrition Adelaide, SA 5000, Kintore Ave, Australia). Prior to the study ethical approval was taken by CSIRO Animal Ethics Committee (SA), Australia. The animals were kept in cages made of wire in the animal room, where the temperature was maintained at about 23°C and a light-dark cycle of 12 h was maintained, and they were permitted free connection to food and water. To avoid ingestion of bedding materials that can compromise dietary treatments, wire cages were utilized. The ceramic huts were placed in each cage to provide environmental enrichment, whereas rats acclimatized for 7 days to the environment after receiving them and were allowed to take *ad libitum* water and a standard diet without any restriction. Afterward, weight measurements and labeling of rats with ear tags were completed, and then they were allocated to their respective treatment groups as per plan. The rats in each treatment were provided with a western-style (WD) diet containing a high amount of protein and fat along with a low level of fiber, with or without GOS supplementation, and free access to water. After 1 week of research trial, 8 rats consuming WD+GOS and 8 rats consuming WD alone were sacrificed, whereas the remaining rats in each group were killed after completing a total of 4 weeks of dietary intervention study time; the details of treatment groups for rats are provided in Table 1.

The composition of the western diet involved an alteration in the AIN 93G diet with elevated 20% protein and 25% fat components, whereas levels of corn starch in the diet were lowered to accommodate increases and were fed in pellet form. Additionally, the rats were given free access to a diet with or without GOS supplements. The commercially available yakult, Oligomate 55 N, a syrup was added at a rate of 2% (mixed in diet). This dose equates to the level generally regarded as safe (GRAS) for this product in humans and has previously been given to animals like rodents and pigs, resulting in significant increases in beneficial bacteria and beneficial short-chain fatty acids production in these animals.

During experimental execution, the rats were weighed twice a week (days 3 and 7 of each week), whereas, at trial completion, the rats were anesthetized using 4% isoflurane/oxygen and killed to enable tissue collection for further analysis. Once animals have lost pedal reflexes during anesthesia, the digesta collected for analyses, followed by the diaphragm and aorta, was then cut, and the heart was removed to ensure animals were dead, organs were collected, and their weights were recorded. Furthermore, cecal digesta (feces) were collected, followed by frozen storage for subsequent analyses such as short-chain fatty acids and bacterial enumeration by a quantitative real-time polymerase chain reaction (qRT-PCR) assay.

2.7 Effect of galacto-oligosaccharides on the organ weight of rats

For evaluating the effect of GOS on body composition and organs weights in rats given a western diet (high fat) alone or in combination with GOS for 1 and 4 weeks, organs like the liver, spleen, small intestine, thymus, and cecum were cleaned and subjected to weight measurements using an electronic balance for the impact assessment of GOS using the protocol as described by Dyer et al. (2008).

2.8 Short-chain fatty acid analysis

For evaluation, the effect of GOS dietary intervention on the production of short-chain fatty acids (SCFAs) was analyzed using the procedure as described by McOrist et al. (2008), with minor adjustments in the method for the animal study.

2.9 Statistical analysis

For *in vitro* fermentation, the resulting SCFA and bacterial calculations are presented in triplicate as mean±SD. In order to compare the means of groups belonging to different treatments, a one-way analysis of variance (ANOVA) was employed. For computing differences among means, Duncan's test was used for further analysis, with significance at $p<0.05$. All observations were analyzed using IBM SPSS Statistics 22 (IBM Corporation Software Group, Somers, NY, United States). For the animal study, the resulting SCFA, organs weights, and body

Table 1. Description of study groups for the GOS dietary intervention study.

Groups	Description
G_1	Rats were provided with a western diet and galacto-oligosaccharides for 1 week
G_2	Rats were given a basal diet, not provided with galacto-oligosaccharides supplementation, and killed after 1 week
G_3	Rats were provided with a western diet and galacto-oligosaccharides for 4 weeks
G_4	Rats were given a basal diet, not provided galacto-oligosaccharides supplementation, and killed after 4 weeks

weights were compared using a two-way ANOVA under the CRD approach (Cohort-CoStat-2003 software version 6.33).

3 RESULTS AND DISCUSSION

The gastrointestinal region is considered to be a tremendously complicated ecosystem that comprises nearly 10^{11} CFU (colony-forming units) of bacteria per gram of intestinal lumen. The well-being of living organism/hosts is significantly influenced by this huge community of microbiota. The microbiota of the colon is involved in the fermentation of indigestible organic material in the upper portion of the gut, which mainly consists of resistant starch, whereas the carbohydrates remain unbroken, like fructans, along with proteins and amino acids. The SCFA is considered to be the chief end major product of fructans metabolism in the colon, including prominently acetates along with propionate, butyrate, *etc*. (Roberfroid et al., 2006).

3.1 Short-chain fatty acids during in vitro fermentation

The results (Table 2) regarding short-chain fatty acid (SCFA) concentrations produced by batch-type fermentation (12 h) process using different concentrations of GOS in single and combination along with control groups (positive and negative) showed that different compounds produced during the process include acetic, propionic, butyric, isobutyric, isovaleric, valeric, and caproic acids. Among acetic acid, the highest concentration was reported in the positive control, having 150 mg inulin as 54.67±8.30 followed by T_1 as 41.78±9.29, T_2 as 38.96±6.8, and the lowest in the negative control as 11.4±3.01. Similarly, for propionic and butyric acid production, the highest concentrations were documented in T_{10} as 13.31±2.47 and T_{10} as 9.5±4.4, whereas the lowest concentrations were documented in T_4 as 2.85±1.93 and T_4 as 0.63±0.24, respectively. Contrary to this, it was observed that the lowest concentrations of isobutyric acid, isovaleric acid, valeric acid, and caproic acids were in the treatment of T_4 as 0.01±0.02, T_6 as 0.07±0.01, T_4 as 0.05±0.02, and T_4 as 0.05±0, respectively. Additionally, the highest concentrations of isobutyric, isovaleric, valeric, and caproic within a treatment were observed in groups as follows: T_{11} as 4.14±0, T_{11} as 4.73±0, T11 as 5.11±0, and T_{11} as 5.67±0. Meanwhile, the lowest concentrations of the same acids were documented for groups T_4 as 0.01±0.02, T_6 as 0.07±0.01, T_4 as 0.05±0.02, and T_4 as 0.05±0.01, respectively. Additionally, treatment groups of T_1 and T_2 having 75 mg GOS 1 and 2 were observed with higher concentrations of acetic acid as 41.78±9.29 and 38.96±6.8, propionic acid as 6.47±1.04 and 7.45±1.39, and butyric acid as 3.56±2.93 and 5.33±2.34 compared to the treatment groups of T_8 and T_9 with 75 mg and 150 mg RS, as in acetic acid (13.59±0.54 and 18.5±1.39), propionic acid (4.74±0.72 and 6.18±0.43), and butyric acid (1.99±0.44 and 4.41±0.89). However, the treatment groups of T_1 and T_2 (75 mg GOS 1 and 2) were observed with lower concentrations of isobutyric acid (0.05±0.01 and 0.04±0.01), isovaleric acid (0.09±0.02 and 0.08±0.02), valeric acid (0.09±0.03 and 0.09±0.02), and caproic acid (0.06±0 and 0.05±0.01) as compared to the treatment groups of T_8 and T_9 with75 mg and 150 mg RS, as in isobutyric acid (0.08±0.01 and 0.11±0.03), isovaleric acid (0.19±0.04 and 0.28±0.09), valeric acid (0.15±0.03 and 0.28±0.01), and caproic acid (0.06±0.01 and 0.1±0.02).

Table 2. Dietary arrangement of the western-style diet.

Composition of Control (Western-Type) Diet	
Ingredients	***g/kg* diet**
Corn starch	350
Wheat bran	50
Sucrose	100
Tert-butylhydroquinone	0.014
Casein	250
L-Cystine	3
Choline bitartrate	2.5
Vitamins (AIN 93)	10
Fat blend (canola and palm oils)	200
Minerals	35

The findings of our study are in accordance with McOrist et al. (2008) who reported that inulin resulted in enhanced production of SCFA production owing to fermentation. Our results indicated that all prebiotic groups showed significantly higher values for acetic acid compared to the blank group, except for resistant starch. They also reported a relative assessment of varied prebiotics *in vitro*, and all substrates resulted in an elevated quantity of acetic acid production, which is also in line with our study findings. There was no dose-dependent relationship for prebiotics, which also indicated that higher quantities did not affect the production of SCFA, which also indicates various aspects involved in SCFA production, including the origin of the substrate, specifically its chemical nature, quantity, and appearance, like particle size, along with solubilization binding with complex food systems. This might be the reason that our results showed that 75 mg of GOS_1 (the vivinal source) was significantly different compared to RS (75 mg and 150 mg, respectively) and GOS_2 (150 mg) in terms of acetic acid production. The microbial SCFA is ominously involved in refining the health concerns of the host in the interior of the gut and the boundary, and there is an extensive spread of pathways involved in acetate production among bacterial populations residing in the gut of living organisms. Along with acetate conversion to butyrate due to some cross-feeding procedures in which there is bacterial conversion into varied metabolites. Likewise, another study by Frost et al. (2014) also reported similar patterns in terms of propionic acid and inulin mechanisms for the production of short-chain fatty acids.

According to the study by Eswaran et al. (2013), dietary fibers of various types can be differentiated by their fermenting properties in the colonic lumen. The ones with greater fermenting properties include inulin, oligofructose, and psyllium, which are considered soluble dietary fibers relative to those with lesser fermenting abilities, including cellulose and hemicellulose, which are insoluble forms of dietary fiber. Our results showed that the combined effect of GOS_2 + RS (75 mg + 75 mg) showed higher values compared to the blank and other groups. The resistant starch showed an increment after raising its quantity, indicating a slight effect of dose, whereas there was a significant decrease in the concentration of propionic acid with increasing amounts of both sources of commercially available galacto-oligosaccharides (GOS_1 and GOS_2).

The variations in SCFA form production as per different reported literature might be due to the fact that variations in bacterial groups in fecal samples or procedures involved in fermentation. Propionate has possible health-enhancing properties that might be due to its cholesterol-lowering, anti-inflammatory, anti-lipogenic, and anti-carcinogenic actions, whereas the processes involved in propionate and butyrate metabolism have gained specific concerns and curiosity nowadays owing to their involvement in diseases inflammatory mechanisms. The butyrate-resulting bacteria are less common in ulcerative colitis, and the population of parallel propionate-producing bacteria diminished in children more prone to asthma. Additionally, long-term diet and varied food combinations can affect the microbiota of the intestine and SCFA arrangements in living organisms, whereas varied fermentation processes due to substrates' varied chemical nature, fermentation process time, and microbial variety can also influence the process and end products. For butyric acid, GOS_1 and GOS_2 showed significant differences in contrast to inulin, which even showed elevated levels compared to all other prebiotic groups as indicated by *in vitro* fermentation, and a higher level of butyrate was seen in the dose of GOS_2, which did not elevate butyrate production but rather lowered their concentration. This is in contrast with the study by Jung et al. (2015), who reported that butyrate production was elevated by using a large amount of GOS and FOS at 10 g/L using *in vitro* fermentation contacting fecal microbiota galacto-oligosaccharides.

As per our study findings, 75 mg of GOS_2 showed comparably high values compared to other groups, although they remain insignificant when used in a mixture with resistant starch (GOS_2+RS 75 mg), RS 75 mg, GOS_1 75 mg, and blank group. This is a particular concern regarding the bonding between butyrate production and the underlying fermentation of starch as a substrate. The elevated butyrate levels can be manifested by several studies incorporating starch as a substrate in animal feeding models, and for colon epithelium, butyrate is considered a desired substrate as it is engaged in the provision of energy as well as avoiding risks related to colon cancer. Although butyrate is involved in obstructing the development of neoplastic cell lines in some cases, the lowest value was shown by 150 mg GOS_1, which was significantly different from inulin, blank,75 mg GOS_2, and 150 mg RS and inulin but similar to other groups.

3.2 Bacterial enumeration of different prebiotics

According to results of GOS evaluation of bacterial populations including *Bifidobacteria*, *Lactobacillus*, *Escherichia coli*, *Bac prev*, *enterococcus*, and total bacteria *in vitro* in batch type fermentations after 12 h, T_2 (75 mg GOS_2) has highest *bifidobacterial* population as 8.95±0.17, followed by T_3 (75 mg GOS_1 + 75 mg GOS_2) as 8.88±0.18, T_5 (150 mg GOS_2) as 8.82±0.05, T_1 (75 mg GOS_1) as 8.70±0.25, and lowest in control as 7.16±0.224. Similarly *lactobacillus* population highest count was reported in T_1 as 5.04±0.26, followed by T_9 as 4.78±0.24, T_3 as 4.73±0.12, T_6 (75 mg GOS_1 + 75 mg RS) as 4.71±0.03, T_5 as 4.68±0.16, T_2 as 4.61±0.19, T_{10} (positive control with 150 mg inulin) as 4.17±0.20, and lowest in T_{11} as 3.48±0.049. Comparing T_1 to others like T_2, T_3, T_4 (150 mg GOS_1), T_5, T_6 (75 mg GOS_1 + 75 mg RS), T_7 (75 mg GOS_2 + 75 mg RS), T_8 (75 mg RS), T_9 (150 mg RS), T_{10}, and T_{11}, *Escherichia coli* count for T_1 was highest as 8.54±0.16, while group T_{10} was lowest as 7.67b±0.37as compared to the negative control with media only and other groups. Additionally, the 12-h fermentation process also revealed that *Bac prev* population in T_{10} (positive control: 150 mg inulin) had the highest count as 6.32±2.50, followed by T_9 as 4.29±1.52 and T_{11} (control) as 1.28±1.478. Likewise, *Enterococcus* population count T_1 as 5.85±0.03 depicted an increased bacterial count compared to other groups T_2 (75 mg GOS_2) as 5.18± 0.23, T_3 (75 mg GOS_1+75 mg GOS_2) as 5.15±0.44 and the lowest in T_{11} as 4.59±1.266. According to total bacterial count (T_3), the 75 mg GOS_1 + 75 mg GOS_2 group showed the highest count of 9.92±0.10 and the lowest in T_{11} (negative control with media only) as 8.94±0.094, as mentioned in Table 3.

Table 3. Short-chain fatty acids concentration (µmol/mL±SD) in batch-type fermentations after 12 h in different prebiotics galacto-oligosaccharide samples and control*.

Groups	Acetic	Propionic	Butyric	Isobutyric	1sovaleric	Valeric	Caproic
T_1 (**75 mg GOS_1**)	41.78±9.29 ^{b}	6.47±1.04 ^{bcd}	3.56±2.93 ^{bcd}	0.05±0.01 ^{cdef}	0.09±0.02 ^{cd}	0.09±0.03 ^{cd}	0.06±0 ^{c}
T_2 (**75 mg GOS_2**)	38.96±6.8 ^{b}	7.45±1.39 ^{bc}	5.33±2.34 ^{b}	0.04±0.01 ^{cdef}	0.08±0.02 ^{d}	0.09±0.02 ^{cd}	0.05±0.01 ^{c}
T_3(**75 mg GOS_1+75 mg GOS_2**)	33.31±8.28 ^{bc}	4.19±1.99 ^{cde}	1.15±0.72 ^{cd}	0.04±0.01 ^{cdef}	0.08±0.02 ^{d}	0.08±0.04 ^{cd}	0.06±0.01 ^{c}
T_4 (**150 mg GOS_1**)	22.72±1.67 ^{cd}	2.85±1.93 ^{e}	0.63±0.24 ^{d}	0.01±0.02 ^{f}	0.14±0.12 ^{cd}	0.05±0.02 ^{d}	0.05±0 ^{c}
T_5 (**150 mg GOS_2**)	38.91±16.52 ^{b}	4.58±2.03 ^{cde}	1.57±0.8 ^{cd}	0.03±0.03 ^{fe}	0.09±0.01 ^{cd}	0.08±0.02 ^{cd}	0.06±0.02 ^{c}
T6 (**75 mg GOS_1+75 mg RS**)	34.46±0.49 ^{bc}	6.35±1.92 ^{bcd}	1.8±0.77 ^{cd}	0.04±0 ^{fed}	0.07±0.01 ^{d}	0.08±0.02 ^{d}	0.08±0.04 ^{c}
T_7 (**75 mg GOS_2+75 mg RS**)	33.24±3.66 ^{bc}	8.74±2.67 ^{b}	3.17±0.67 ^{bcd}	0.06±0.01 ^{edc}	0.14±0.04 ^{cd}	0.15±0 ^{c}	0.07±0.01 ^{c}
T_8 (**75 mg resistant starch**)	13.59±0.54 ^{d}	4.74±0.72 ^{cde}	1.99±0.44 ^{bcd}	0.08±0.01 ^{c}	0.19±0.04 ^{b}	0.15±0.03 ^{c}	0.06±0.01 ^{c}
T_9 (**150 mg resistant Starch**)	18.5±1.39 ^{d}	6.18±0.43 ^{bcd}	4.41±0.89 ^{bc}	0.11±0.03 ^{bc}	0.28±0.09 ^{b}	0.28±0.01 ^{b}	0.1±0.02 ^{cb}
T_{10} (**positive control 150 mg inulin**)	54.67±8.3 ^{a}	13.31±2.47 ^{a}	9.5±4.4 ^{a}	0.07±0.03 ^{b}	0.14±0.07 ^{d}	0.28±0.09 ^{d}	0.12±0.08 ^{b}
T_{11} (**negative control with media only**)	11.4±3.01 ^{d}	3.98±0 ^{de}	4.5±0 ^{bc}	4.14±0 ^{a}	4.73±0 ^{a}	5.11±0 ^{a}	5.67±0 ^{a}

*Values are Mean±SD; means with different letters are significantly different with respect to columns ($p<0.05$).

The prebiotics influence considered for particular modulation is related to the development and evolution of *bifidobacteria* in a network of microbial groups in the intestine. The bacteria involved in encouraging the well-being of the host include, prominently, *bifidobacteria* and *lactobacilli*. Scientific studies focused on utilizing pure cultures and revealed the selective metabolism of GOS in contrast to other prebiotics, FOS and lactulose, by strains of *bifidobacterium*. Several *bifidobacterial* strains in verification studies for their consumption of a wide range of prebiotics indicated varied results related to substrate availability and variation within bacterial species and strains. This was proved by another study, which reported that not only *bifidobacteria* but also strains of *lactobacilli* and *pediococcus* use GOS substrates when examining their capability regarding GOS consumption through a wide range of lactic acid bacterial strains. Additionally, the flawless association was shown in the middle of β-galactosidase and capacity of strains consuming GOS along with tri- and tetra-saccharide using the capability of lactic and di- and monosaccharide by *L. rhamnosus*. Also, the consumption of GOS regarding *B. adolescentis* capability is more proficiently endorsed due to enzymatic *ß-galactosidase* occurrence with GOS but not lactose (Baghel et al., 2005).

The results of our study revealed that all treatment groups of prebiotics raised levels of *bifidobacteria*, and GOS specifically lowered pathogen populations like *clostridias*. There was prebiotics utilization as a source of the substrate where restricted batch culture was in anaerobic conditions with controlled temperature and pH and fecal homogenates were used as inoculum, which is in line with a study that revealed a bi-effect on the *bifidobacteria* population when GOS or FOS were utilized and fecal homogenates in batch culture. Our results showed GOS had no dose-dependent response within the same and different commercial sources, even when used at different concentrations (Bouhnik et al., 2004).

Resistant starch is involved in the provision of energy consequent from diet to bacteria residing in the colon. The combined mixture of GOS_1 and GOS_2 with RS from both sources showed a non-significant effect on *bifidobacteria* production of a prebiotic, which may be due to resistant starch masking its effect. This is in line with where combinations of polydextrose and GOS PDX/GOS were incorporated in infant formulas and given to infants for 30–60 days. The results showed no significant variation in terms of *bifidobacteria* from the reference line to 30 and 60 days, in contrast to remarkable variation for *B. infantis* at 30 days and *B. longum* at 60 days from the starting position compared to the control with treatment groups comprising PDX/GOS. Contradictory to that increment in the *bifidobacterial* population observed in the cecum and colon using quantitative PCR technique in rats provided diets rich in RS potato RS with casein or beef in cooked form Paturi et al. (2012).

Similarly, Lasrado and Rai (2022) reported variation in the *bifidobacterium* population by RS provision, where the initial results of consuming RS 1 g/day in human trials lasted 14 days. The combination of RS with oligosaccharides like fructo-oligosaccharides might work together to elevate the prebiotic effect, raising beneficial bacteria significant to health (*bifidobacteria* and *lactobacilli*). The alterations in the chemical structure of two different prebiotics (xylo-oligosaccharides and GOS) can influence fermentation, which is considered specific to bacterial incorporation, to improve host health, as grouping two or more varieties may work together to enhance the prebiotic effect, leading toward better response (Lasrado & Rai 2022).

In vitro studies combining inulin and oligofructose effects related to prebiotic properties revealed a *bifidobacterial* community with a favorable fermentation pattern. In comparison to other bacteria like *E. coli* and *Clostridium perfringens*, which remained in diminishing quantity when their ability to ferment was related to other standard carbohydrates in batch culture. The increase in *bifidobacteria*'s ability to ferment substrate sources when utilizing pure cultures was revealed when matched to glucose-assessed inulin fermentability, which was maximum and fast due to the highest butyrate-releasing ingredient when evaluated relative to rye, oat, bran, and wheat. According to 12 out of 16 *lactobacillus* and 7 out of 8 *bifidobacterium* varieties, 7 out of a total of 28 showed the capability of fermentation related to inulin substrates along with oligofructose in a specified strain. Similarly, modulation of *bifidobacteria* by inulin in an *in vitro* batch study using human fecal microbiota reported that gut health can be improved by utilizing substrates like inulin that are involved in fermentation and consequent *lactobacilli* and *bifidobacteria*, in contrast to *enterobacteria* and butyrate, proved their role in host gut health (Jung et al., 2015).

For *bifidobacteria*, GOS_2 (75 mg) and a combination of both galacto-oligosaccharide sources, GOS_1+GOS_2 (75 mg), showed maximum production compared to other prebiotic varieties, which also showed significant differences compared to inulin culture. For resistant starch, no dose-dependent relationship was observed at lower and higher concentrations. The combined mixture of GOS_1 and GOS_2 with RS from both sources showed a non-significant effect on *bifidobacteria* production, whereas our results showed GOS had no dose-dependent response within the same and different commercial sources when employed at different concentrations. For *Escherichia coli*, *Bac prev*, and *Enterococcus*, there was a non-significant response within groups for different prebiotics. Although 75 mg GOS_1 showed maximum values compared to positive control inulin but remained non-significant with others for *Escherichia coli*.

3.3 Evaluation of short-term GOS incorporation in a western-style diet in Sprague-Dawley rats

3.3.1 Effect of GOS incorporation on body and organ weights in rats

The results regarding the effects of GOS on body weight and organs of rats during the dietary intervention (4 weeks) showed the results of body weight, cecum full, and cecum empty among treatment groups, i.e., high-fat control (western) and HF + GOS, as presented in Table 4. The body weight was increased more in the high-fat control (359.75±29.93) than in the HF + GOS (354.37±27.97) after 4 weeks of intervention, whereas the cecum full weight was increased in HF+GOS (2.50±0.28) than in the HF control (2.36±0.38) after dietary intervention. Consequently, it was observed that cecum

Table 4. Evaluation of galacto-oligosaccharide on bacterial population by *in vitro* in batch-type fermentations*.

Groups	*Bifidobacteria*	*Lactobacillus*	*Escherichia coli*	*Bac prev*	*Enterococcus*	Total bacteria
T_1 (**75 mg GOS_1**)	8.70^{cba} ±0.25	5.04^{a} ± 0.26	8.54^{a}±0.16	1.48^{b} ±1.49	5.85^{a}±0.03	9.91^{a}±0.20
T_2 (**75 mg GOS_2**)	8.95^{a} ±0.17	4.61^{ba}± 0.19	8.05^{ba}± 0.37	2.92^{ba}± 0.14	5.18^{a}± 0.23	9.75^{ba}±0.03
T_3 (**75 mg GOS_1 + 75 mg GOS_2**)	8.88^{a}± 0.18	4.73^{ba}± 0.12	8.20^{ba}±0.17	3.58^{ba}± 0.41	5.15^{a}± 0.44	9.92^{a}±0.10
T_4 (**150 mg GOS_1**)	8.42^{cba} ±0.43	4.54^{ba}± 0.27	8.22^{ba}± 0.10	2.83^{ba}± 0.40	4.82^{a}± 1.09	9.68^{ba}±0.03
T_5 (**150 mg GOS_2**)	8.82^{ba}±0.05	4.68^{ba}± 0.16	8.04^{ba}± 0.10	3.62^{ba}± 0.66	4.97^{a}± 1.07	9.74^{ba}± 0.02
T_6 (**75 mg GOS_1 + 75 mg RS**)	8.24^{cba}±0.25	4.71^{ba}±0.03	7.98^{ba}±0.07	3.15^{ba}±0.12	4.74^{a}±0.91	9.44^{b}±0.08
T_7 (**75 mg GOS_2 + 75 mg RS**)	7.77^{cd}± 0.30	4.34^{b}±0.22	8.32^{ba}±0.15	1.58^{b}±1.59	5.03^{a}±0.26	9.35^{b}±0.12
T_8 (**75 mg Resistant Starch**)	7.82^{bcd}± 0.63	4.54^{ba}±0.23	8.03^{ba}±0.26	3.27^{ba}±0.16	4.74^{a}±0.30	9.37^{b}±0.46
T_9 (**150 mg RS**)	7.75^{ba}± 0.19	4.78^{ba}±0.24	7.84^{ba}±0.09	4.29^{ba}±1.52	5.59^{a}± 0.08	9.49^{ba}±0.15
T_{10}(**Positive control 150 mg inulin**)	8.59^{cba}± 0.03	4.17^{a}±0.20	7.67^{b}±0.37	6.32^{a}±2.50	5.29^{a}± 0.25	9.61^{ba}±0.01
T_{11}(**Control with media only**)	7.16^{d}±0.224	3.48^{c}±0.049	7.76^{b}±0.104	1.28^{b}±1.478	4.59^{a}±1.266	8.94^{c}±0.094

*Values are Mean±SD; means with different letters are significantly different with respect to columns ($p<0.05$).

empty weight showed a decreasing trend in HF + GOS by 0.54 ± 0.05 more than in HF control by 0.61 ± 0.07 at trial expiry.

Dietary fiber is considered vital to lowering weight gain, particularly the water-soluble component of dietary fiber, which is sticky or more viscous. As the dietary fiber is engaged in low consumption of food that leads to a raised satiety level and lowers nutrient assimilation in the small intestine, which might be responsible for lower weight gain, our results also indicated that body weights were significantly increased ($p<0.05$) after 4 weeks for both control and GOS-treated groups, which is in accordance with the fact that the incorporation of a high-fat diet contributed to weight enhancement compared to a diet with low fat. However, the addition of dietary fiber decreased the effect, which is in contrast to our results where there was no significant change in body weights between treatment groups and controls. Another reason for the lack of change in body weight might be due to the production of SCFA, where results show a non-significant impact, and SCFA as a result of the microbiota of the colon might contribute to a lower weight gain (Jakobsdottir et al., 2013).

Dietary fiber incorporation might involve elevating cecal content and tissue involved in cecal weight, whereas cecum full and empty wall weight were significantly increased ($p<0.05$) after 4 weeks for the control and GOS-treated groups. Contrary to this, the oligosaccharide FOS showed increased cecal weight and cecal wall weight when matched with a low-fiber diet. As the chemical nature of GOS and FOS is different, that might be why the response was variable, as a high-fat diet might not be responsible for affecting cecal contents as indicated in a high-fat diet. It is also one of the reasons that study times were less likely to have any effect on it, as raised spleen weight is in accordance with the fact that a high-fat diet and a low-fat diet for 2 and 4 weeks showed no impartion or variation in spleen weight, which might be considered an indication of systemic inflammation (Jakobsdottir et al., 2013).

3.3.2 Effect of GOS incorporation on short-chain fatty acids

The results of the impact of dietary galacto-oligosaccharides on short-chain fatty acids (SCFA) including acetic acid, propionic acid, butyric acid, isobutyric acid, valeric acid, isovaleric acid, and caproic acid in rats for 1 and 4 weeks of study duration on cecal, consisting of two groups, namely, high fat (HF) and high fat along with GOS (HF+GOS) are presented in Table 5, which indicated that the HF+GOS group showed increased production compared to the HF group under acetic acid treatments. Similarly, under treatment with propionic acid, the HF group showed a higher increase in the HF group than the HF+GOS group. Additionally, butyric acid production was higher in the HF+GOS group than in the HF group, whereas after 4 weeks of treatment with isobutyric acid, the HF group exhibited a significant increase and the HF+GOS group showed a non-significant decrease in their counts. In contrast to HF+GOS, the HF group reported a higher count for valeric acid treatment. Likewise, after receiving isovaleric acid treatment, the HF group showed an increase, whereas the HF+GOS group reported a decrease in the count. Our study findings also showed that the HF+GOS group demonstrated a greater rise than the HF group with caproic acid treatment. The short-chain fatty acids (acetic acid, propionic, butyric, and isobutyric acids) after consumption of a high-fat diet with GOS in combination showed comparatively lower concentrations ($p<0.05$) compared to control after 1 and 4 weeks (Table 4). The results might be the reason that high fat has masked the effect of galacto-oligosaccharide, resulting in a lower concentration of SCFA, and a profusion of fat in diet led to a lowering of SCFA, which is considered a consequence of diminished bacterial performance in the colon. Additionally, it has been reported that high-fat diets are considered destructive due to their role in weight gain owing to the provision of more energy and lower SCFA production (Wang et al., 2017). Likewise, Henningsson et al. (2002) also reported elevated SCFA formation with an extended experimental time period and decreased fecal quantities of butyric acid that can be observed in ulcerative colitis patients compared to healthy subjects. Additionally, butyric acid production was observed to increase in fecal samples when subjects who consumed β-glucan-enriched oat fiber suffered from ulcerative colitis and related signs and symptoms toward betterment (Lührs et al., 2002).

Table 5. Effect of galacto-oligosaccharides dietary intervention on different body organs including colon, cecal spleen, and thymus weight*.

Parameters	Treatment groups		Statistical significance of the effect		
Weight (g)	**HF control**	**HF+GOS**	**Diet**	**Time**	**Diet×time**
Body weight					
1st week	259.37±22.67	251.62±14.29	**ns**	*	**ns**
4th week	359.75±29.93	354.37±27.97			
Colon weight full					
1st week	2.27±0.54	2.09±0.35	**ns**	**ns**	**ns**
4th week	2.29±0.63	2.37±0.25			
Colon empty					
1st week	1.54±0.26	1.11±0.90	**ns**	**ns**	**ns**
4th week	1.21±0.2**5**	1.36±0.30			
Cecum full					
1st week	2.06±0.22	2.16±0.43	**ns**	*	**ns**
4th week	2.36±0.38	2.50±0.28			
Cecum empty					
1st week	z0.65±0.03	0.68±0.10	**ns**	*	**ns**
4th week	0.61±0.07	0.54±0.05			
Spleen					
1st week	0.85±0.24	0.72±0.06	**ns**	**ns**	**ns**
4th week	0.80±0.06	0.82±0.06			
Small intestine					
1st week	6.36±0.73	6.28±0.27	**ns**	**ns**	**ns**
4th week	6.81±0.65	6.55±0.52			
Thymus					
1st week	0.85±0.24	0.72±0.06	**ns**	**ns**	**ns**
4th week	0.80±0.06	0.82±0.06			

*Values are Mean±SD of eight replicates; means with different letters within a row differ significantly ($p<0.05$).

The raised production of SCFA involved in lowered gastric emptying rate by the process of ileocolonic brake might be significant for glycemic-related response and fullness. One of the researcher groups (Fava et al., 2013) reported an increment in SCFA in fecal samples after the provision of a diet enriched in fat for 24 weeks. Although the fecal concentration of SCFA did not show the actual content of the colon due to the fact that more than half of it was assimilated from the colon (Macfarlane & Macfarlane, 2012). In contrast to our results, groups given a mixture and guar gum resulted in the highest cecal amount of propionic and butyric acids, whereas the addition of dietary fiber like guar gum raised the level of cecal butyric and propionic acids. Though total SCFA production was relatively lower when compared with other studies using rat models, the underlying cause might be due to the extended time period between blood withdrawal and completing the animal study, whereas gut microbiota conformation of a diet high in pectin, guar gum, and fiber-free consequent in varied results that showed a variety of fibers can affect the microbial community. The diet high in guar gum resulted in raised Bacteroides concentrations, which in turn are related to acetic and propionic concentrations in the colon. Guar gum also tends to increase the levels of other bacterial groups linked to SCFA, and a fiber-free diet results in more negative factors like liver cholesterol, triglyceride, and inflammation. Although results were more obvious in a high-fat diet than a low-fat diet, consumption of a high-fat diet primarily resulted in decreased SCFA in cecum and circulation but slowly recovered with the advancement of experimental time. The enhanced concentration of succinic acid in rats following HFD had an extended study period, whereas the butyric acid amount was lowered in rat groups (Jakobsdottir et al., 2013).

Consistent with our findings when observed, rats fecal content in diets raised in specific types of oligosaccharides like chito-oligosaccharides revealed SCFA parallel amounts compared to the HF group. From microbiota data, due to raised levels of *lactobacillus* and *bifidobacterium*, specifically allobaculum and blautia, it is confirmed that SCFA might be raised. Another study also revealed that *bifidobacteria* and *lactobacilli* even grow but are not enhanced to a significant level. Similarly, the SCFAs, including acetate butyrate propionate, are the end products of substrates like carbohydrates in microbial fermentation that are involved in metabolic roles and significance in intestinal health (Kao et al., 2018).

The addition of fructo-oligosaccharide and resistant maltodextrin at high dosages in high-fat diets influences microbiota

Table 6. Impact of dietary galacto-oligosaccharides on cecal short-chain fatty acids concentrations in rats*.

Short-Chain Fatty Acids	HF+GOS	HF	Statistical significance of the effect		
			Diet	Time	Diet×Time
Acetic acid					
1st week	58.75±3.84	51.11±7.71	*	**ns**	**ns**
4th week	61.68±8.93	52.31±10.30			
Propionic acid					
1st week	12.63±3.90	11.77±0.93	ns	**ns**	**ns**
4th week	12.71±1.51	11.89±2.18			
Butyric acid					
1st week	15.41±2.64	11.32±1.51	*	**ns**	**ns**
4th week	18.55±4.15	11.93±5.09			
Isobutyric					
1st week	0.85±0.21	1.00±0.23	*	ns	ns
4th week	0.82 ±0.14	1.15±0.17			
Valeric acid					
1st week	1.46±0.22	1.46±0.17	**ns**	*	**ns**
4th week	1.55±0.22	1.63±0.27			
Isovaleric acid					
1st week	0.65±0.16	0.74±0.21	*	**ns**	**ns**
4th week	0.63±0.10	0.88±0.14			
Caproic Acid					
1st week	0.68±0.38	0.22±0.14	*	*	**ns**
4th week	1.01± 0.20	0.36±0.31			

***Values are Means±SD of eight replicates (p<0.**05).

and fecal SCFAs such as acetic acid, propionic acid, butyric acid, and other SCFAs, which also indicates the beneficial effect of fibers involved in gut microbiota and influencing intestinal health due to SCFA. Butyric and propionic are considered to be beneficial with respect to roles like the stimulation of pro- and anti-inflammatory indicators existing in the gut of living organisms. Although our study findings indicated that a high-fat diet was incorporated with fibers like oat and guar gum, the SCFA levels were raised, but with respect to barley products, a non-significant impact on SCFA compared to a high-fat diet was observed. The concentrations of isovaleric, valeric, and isobutyric, which are generally present in lesser amounts, also increased, which is also in line with the findings of our study (Sivaprakasam et al., 2016).

3.4 Microbial population by gene sequencing

From a statistical point of view, the Kruskal-Wallis test for all groups showed a non-significant response (p=0.398) when compared to the high-fat group at 7 days with the HF group at 28 days (p=0.90), which means the control was non-significant with time. Also, comparisons with HF GOS treatment groups at 7 and 28 days showed no significance ($p \leq 0.05$). Comparison of HF_{28} with $HFGOS_{28}$ showed (p=0.32) a non-significant effect of various treatment groups. Alpha rarefaction curves showed that HF GOS_{07} showed a higher response compared to $HFGOS_{28}$, which also indicated more diversity within HFGOS on the 7th day of study than on the 28th day of trial, which means, in a short time, GOS produced more effect than prolonged time,

The microbial analysis by gene sequencing showed that the phylum level for different treatments groups (HF+GOS) at the 1st and 4th weeks showed a greater abundance of firmicutes and lower Bacteroides, with no significant difference observed compared to the control (HF) at both study intervals. The phylum Actinobacteria also showed less abundance, especially in treatment groups, whereas at the order level, Clostridiales were more abundant in the high-fat diet (control) after the 1st and 4th weeks. The *lactobacillales* from class bacilli showed relatively high abundance in treatment groups (HF+GOS) compared to control at the 4th week. The *Turicibacter* genus, family (*turicibacteraceae*), *Blautia* genus, and *Ruminococcus* genus, family (*Lachnospiraceae*), showed more abundance of prebiotics in diet (HF+GOS) after the 1st and 4th weeks relative to control (Figures 1 and 2).

4 CONCLUSION

The present study reported a non-significant response for the *in vitro* batch fermentation considering the dose-response relationship for both varieties of galacto-oligosaccharides that actually belong to different commercial sources, whereas acetic, propionic, and butyric acid content in the group having inulin (the positive control) was significantly higher compared to other groups. For propionic acid, group GOS_2 with RS (75 mg+75 mg) showed higher values compared to the negative control, as well as revealed that combination with dietary fiber resulted in higher values of propionate. For isobutyric, valeric, isovaleric, and caproic acids, all groups significantly differ from the negative control, but there is no difference within the groups observed. From bacterial enumeration, *bifidobacteria* growth on substrate GOS_2 (75 mg) and a combination of both GOS sources, GOS_1+GOS_2 (75 mg), showed maximum production compared to other prebiotic varieties. From animal study findings, the SCFA after consumption of GOS showed non-significant results for the treatment group given high-fat +GOS with a high-fat

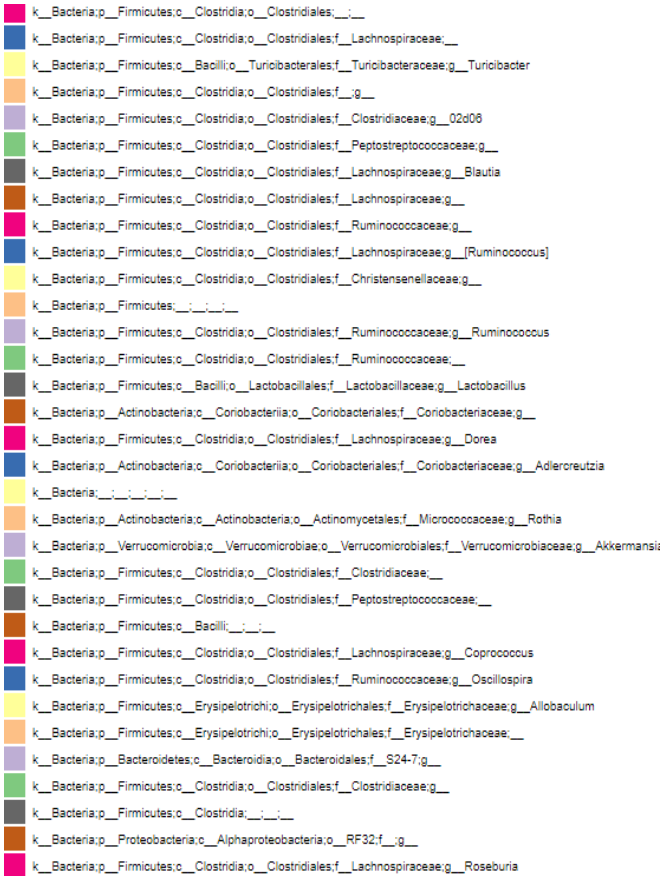

Figure 1. Relative frequency of microbiome in treatments groups regarding the effect of galacto-oligosaccharides in the high-fat diet at the genus level.

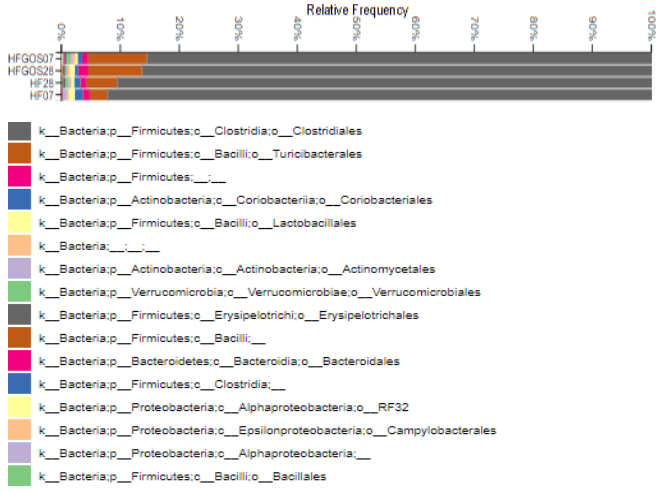

Figure 2. Relative frequency of microbiome in different treatments groups at the order level.

diet alone (control), but an increase in concentrations was observed after 4 weeks. For caproic acid, significant differences with respect to treatment and time were seen. Conclusively, *in vitro* fermentation of GOS from different sources showed non-significant effects, but in terms of *bifidobacteria*, a positive control impact was observed, and GOS addition particularly has not influenced body and organ weight in rats.

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