The Impact of Oat and Fruit Consumption on Short-Chain Fatty Acid Production

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The Impact of Oat and Fruit Consumption on Short-Chain Fatty Acid Production

by

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Popular Science Summary

Have you ever taken into account how the food we eat affects our gut health? Our gut microbiome, consisting of trillions of microorganisms, is responsible for producing essential compounds like short-chain fatty acids (SCFAs) that maintain our overall health. Recent scientific research has revealed the fascinating relationship between our diet, gut microbiota, and overall well-being.

Fatty acids are tiny molecules that our bodies use as a source of energy. SCFAs are a specific type of fatty acids made by the friendly bacteria living in our guts. These bacteria help us digest the food we eat, especially dietary fibre. When we consume certain types of fibre, our gut bacteria break it down through a process called fermentation. As a result of this fermentation, SCFAs are produced. The three most common SCFAs are called acetate, propionate, and butyrate.

SCFAs have some important functions in our bodies. They provide energy to the cells lining our intestines and help keep our digestive system healthy. They have also been linked to various aspects of our health, including immune function and even mental well-being. Recently, researchers have started looking into the presence or absence of these molecules in connection with brain disorders like Alzheimer's and Parkinson's disease.

Therefore, we want to find out what kind of fibre and sort of diet can increase the production of SCFAs in our gut, as these molecules are beneficial for our health. We analysed the blood samples of mice fed different diets to better understand the impact of specific foods on SCFA levels. This approach brings us closer to understanding which types of food are beneficial and can be used in the prevention of certain diseases.

So remember, when you sit down for a meal, you're not just feeding yourself but also nourishing the trillions of microbes residing in your gut. Choose wisely and keep your gut buddies happy!

Abstract

This study explored the importance of short-chain fatty acids (SCFAs) in maintaining gut health and how different diets affect their levels. Blood plasma and serum samples from mice subjected to two intervention studies on oats and fruits were analysed to determine SCFA concentrations.

Using a one-way ANOVA, the study compared SCFA levels in five different diet groups focused on fruit supplementation, followed by additional statistical analyses to compare specific diet pairs. The study also investigated the impact of oat bran and whole oats supplementation and examined age-related differences in SCFA levels.

The analysis revealed that valeric acid significantly differed between the standard feed (STD) and a high-fat diet supplemented with fruits (HF+F). Furthermore, all diets had higher SCFA concentrations than the standard diet. A low-fat and high-fat diet (supplemented with fruits) was found to increase iso-butyrate, butyrate, valerate, and total SCFA levels. Literature research found that carbohydrates, fibre and fat intake are primary factors affecting faeces SCFA levels. Additionally, literature research suggested polyphenols as prebiotic compounds that promote SCFA production. Oat supplementation led to a significant increase in SCFA concentrations, although the type of oat fraction used did not significantly impact SCFA serum levels. Age-related differences were observed, with older mice exhibiting higher SCFA concentrations, particularly acetic acid.

The findings provide valuable insights into the relationship between diet composition and SCFA levels. The higher concentrations of SCFAs in all diets compared to a mainly plant-based chow diet suggest the influence of animal-based products on SCFA production. Future research should analyse the correlation between SCFA levels in different body fluids and establish reliable biomarkers. Combining fibre and polyphenol-rich foods could create functional foods with exciting health benefits. Last but not least, studying food components like fat can help understand how these nutrients influence gut microbiota and SCFA levels.

Keywords: short-chain fatty acids (SCFAs), diet, gas chromatography, serum, mice.

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Abbreviations

AD	Alzheimer's disease
BMI	Body mass index
BOB	Bio-processed oat bran
BWO	Bio-processed whole oats
DF	Dietary fibre
EI	Energy intake
GIT	Gastrointestinal tract
GC	Gas chromatography
HF	High-fat
HF+F	High-fat + fruits
HDACs	Histone deacetylases
IS	Internal standard
LF	Low-fat
LF+F	Low-fat + fruits
PD	Parkinson's disease
RS	Resistant starch
SCFAs	Short chain fatty acids
Tg	Transgenic
WHtR	Waist-to-height ratio

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1. Introduction

1.1 The gut microbiome

The human gut contains more than 10^{12} microorganisms essential to the host's physiology, such as immune system maturation, protection against pathogens, regulation of glucose and lipid metabolism and growth. The gut flora is a complex community that interacts with the host and other microbes to regulate biological processes vital for optimal health. Our understanding of the biological activities of the gut microbiota, as mentioned earlier, has significantly improved over the past ten years.

The mutualistic symbiosis between individuals and their microbiota, which refers to a healthy balance of bacteria in the gut, can be disrupted, influencing the development of a range of chronic illnesses such as type 2 diabetes, autoimmune disorders, metabolic disorders, different types of cancer and neurodegenerative diseases like Alzheimer's (AD) and Parkinson's (PD). These disorders can be due to lifestyle changes due to an underlying inflammatory status of the microbiome, which may influence gut microbiota symbiosis owing to the loss of advantageous, protective bacteria, which is relevant given that most microbiome-related disorders have considerably grown over the past century [1].

1.2 Diet and effect on short-chain fatty acid (SCFA) production

Short-chain fatty acids (SCFAs) are produced by the gut microbiota in the large intestine through the fermentation of dietary fibre. These include acetic, propionic, iso-butyric, butyric, iso-valeric, valeric, heptanoic, and caproic acids, each with varying physiological effects that benefit the host. A diet that promotes SCFA production is essential for health, but Western-style diets, rich in processed carbs, animal fats, and protein, are often low in dietary fibre [2]. While research on the implications of dietary fibre for cognition is still in its infancy, animal studies using fibre-rich diets have shown promising results in areas such as exploratory behaviour, recognition memory, and attentional set-shifting ability. Therefore, maintaining a healthy diet high in fibre is essential for good health.

Dietary fibre (DF) is a component of plant-based foods that often passes through the digestive system without being broken down as the gastrointestinal tract (GIT) falls short of those enzymes. However, bacteria possess these enzymes, and therefore they can degrade this component. DF comes in two forms: soluble and insoluble [4]. Insoluble fibres, like cellulose and hemicellulose, have a bulking effect on faeces as gut bacteria do not easily break them down. Soluble fibres, on the other hand, are digested by gut bacteria and produce SCFAs [1]. There are three subclasses of DF based on their monomeric units: non-starch polysaccharides, resistant

starch (RS), and non-digestible oligosaccharides. SCFAs can also be produced from undigested proteins or peptides. The amount of SCFAs produced is determined by the DF's physical and chemical properties and the microbiome's makeup [4]. SCFAs regulate the pH in the large intestine, which affects the gut microbiota's composition since a higher SCFA concentration will cause the pH to decrease [5].

The transformation of DF to SCFAs can take place using various methods, such as the Wood-Ljungdahl route or succinate pathway, and involves different bacteria, including *Lactobacillus* spp. for acetate production, *Bacteroides* spp. for propionate production, and several Firmicutes species for butyrate production [4, 5, 6].

1.3 Link between SCFAs and health

As previously mentioned, the quantity of SCFAs produced relies on the type of DF in the diet and the initial colonic microbiota composition. An animal study was conducted to illustrate this. Germ-free mice were given bacterial communities from "healthy" overweight individuals (BMI=30). They were then fed different types of DF, including cellulose, inulin, pectin, or a fibre mix (resistant starch (RS) type 2 and 4, pectin, inulin, and short-chain fructose-oligosaccharides). Gas chromatography (GC) results displayed that the levels of butyrate and valerate in cecum varied depending on the type of DF diet. At the same time, propionate and acetate were comparable across all the DF diets. This suggests that while consuming a high-fibre diet is crucial for generating SCFAs and sustaining good health, the kind of DF ingested significantly impacts the creation of SCFAs, leading to differences in biomarkers that can affect neurodegenerative disorders in various ways [7]. A study on mice showed that consuming fructans can enhance butyrate production by the gut microbiota, which can help reduce the number of reactive astrocytes in the hippocampus [8]. Reactive astrocytes are believed to be more common in the early stages of AD. When APP/PS1 transgenic (Tg) male mice were fed this diet, their anxiety levels and memories improved. Mice fed a control diet without additional fibre had increased propionate levels, which were positively correlated with the number of reactive astrocytes in the hippocampal nucleus. This indicates that brain mitochondrial dysfunction contributes to the onset of neurodegenerative diseases. This is due to the high respiration rate, which leads to activated astrocytes. This, in turn, can lead to decreased energy production and energy synthesis. Studies also show that individuals with propionic acidaemia, a hereditary disorder in which the body cannot adequately metabolise specific components of proteins and lipids, have higher faecal propionate concentrations and are more likely to experience cognitive difficulties and other symptoms. Additionally, research suggests that children with autism spectrum disorders have higher propionate levels in their faeces than typically developing children. AD patients also have higher levels of acetate and propionate in their saliva, and healthy mice with transplanted microbiota from AD

patients have higher propionate levels in their faecal samples [8]. Numerous berries, including bilberry, lingonberry, and blackcurrant, have been shown to enhance spatial memory and increase gut flora diversity. This was demonstrated in a study on the effects of fruits, which are high in fibre, on cognitive outcomes in mice on high-fat and low-fat diets. It might be suggested that berries are beneficial for preventing neurodegenerative diseases due to this [9]. Additionally, research has found that depressed mice have lower levels of SCFA, particularly butyrate, which, when increased, can decrease depression and chronic stress in rodents. Recent research suggests that administering *Faecalibacterium prausnitzii*, a bacterium that produces butyrate through fibre fermentation, may alleviate depressive symptoms in mice [10]. Butyrate is effective in inhibiting histone deacetylases (HDACs), which are linked to depression in PD patients [11]. Additionally, high levels of HDACs are associated with neuropsychiatric disorders like schizophrenia and depression, making HDAC inhibitors a potential treatment for anxiety and fear-related cognitive disorders [12].

By analysing blood serum and plasma of mice fed different diets using gas chromatography (GC), the academic community can uncover significant revelations about the effects of diet on the production of SCFAs.

2. Aim of the thesis

The main goal of this thesis is to enhance our comprehension of how various diets impact the colonic production of SCFAs by microorganisms. This is accomplished by analysing the SCFAs profiles in blood samples through gas chromatography, as SCFAs not used by the microbiota or by the colon cells is absorbed into the blood stream. The research aims to investigate the microbial metabolites present in the blood serum of mice from two separate studies - one that focused on fruits and the other on processed oats. By comparing the diets within each study and between the two studies, we hope to gain insights into the effects of these diets. Additionally, we will conduct a literature study to uncover possible explanations of how dietary components can influence SCFAs profiles.

3. Materials and methods

3.1 Materials

3.1.1 Reagents and Solutions

Chemicals were collected from the chemical storage room. Analytical-reagent grade SCFAs were used to prepare the standard solutions, which were, in turn, diluted with water to yield seven target analytes. Acetic acid (99.5%) was obtained from VWR International (France). Propionic acid (99%) was procured from Janssen Chimica (Belgium). Valeric acid (99%) was obtained from Merck Schuchardt (Germany). Butyric acid (99%), iso-valeric acid (99%) and iso-butyric acid (99%) were procured from Sigma-Aldrich (Sweden). Trioctylphosphine oxide (TOPO) (99%) and dihexyl ether (97%) were used to prepare an organic membrane liquid obtained from Sigma-Aldrich (Sweden). Formic acid (99%) and HCl (37%) were obtained from VWR International (France). The GC column was cleaned using 12% (v/v) formic acid, and 2M HCl was used to acidify the serum. NaOH in a water solution (0.1M) was used as an acceptor and procured from VWR International (France). The internal standard (IS) was made by mixing 2-ethyl butyric acid (99%) from Sigma-Aldrich (Sweden) with 2M HCl. All of the water used for analytical procedures was Millipore water obtained by the Milli-Q® reagent water system (France) with a resistivity of 18.2 MΩ cm, meaning that it contains low concentrations of dissolved salts. Methanol, used as a solvent for gas chromatography, was obtained from VWR International (France).

3.1.2 Equipment

Gas chromatography was carried out using an Agilent 7683 N GC system equipped with a flame ionisation detector (FID) and an N10149 automatic liquid sampler. Inside a column for free fatty acid phase (DB-FFAP 125-3237, J&W Scientific, Agilent Technologies Inc., USA) made of a fused-silica capillary with a length of 30 m, internal diameter of 0.530 mm and a coated film of 0.50 μm was used. A hollow fibre tube made from polypropylene (PP) (Accurel PP 50/280) was obtained from Membrana GmbH (Wuppertal Germany) and cut into strands of circa 17 cm. It has an inner diameter of 280 μm, a wall thickness of 50 μm and a pore size of 0.1 μm. Household aluminium foil was cut into small pieces to keep the hollow fibre's single knot in place. Syringes from BD Micro-Fine (for U-100 insulin injection) were used to fill NaOH into the hollow fibre for extraction and to flush the NaOH from the hollow fibre into short thread vials (1.5 ml) inserted with pointy conical glass vials (Supelco) obtained from Sigma-Aldrich (Sweden). The syringes have a needle with an outer diameter of 0.30 mm, a length of 8 mm and can hold 0.3 ml. All data processing was executed with the help of HP ChemStation Plus software (B.01.03, Agilent).

3.2 Methods

3.2.1 Standard curve

First, a 1L stock solution was prepared from acetic (15.0125 g/L), propionic (1.1112 g/L), butyric (0.8811 g/L), iso-butyric (0.8811 g/L), iso-valeric (3.0630 g/L) and valeric acid (0.5107 g/L). This stock solution was then diluted seven times to lower the concentration of the analytes and to eliminate the possibility of interference from other substances. The following dilution factors were used: 50; 2.5; 1.3; 1.7; 2; 2.5; 4. The dilutions were then transferred to Eppendorf tubes and stored in the freezer at -20°C.

The standard dilutions were taken out of the freezer a day before GC analysis and kept in the fridge. On the analysis day, 100 µl of the standard dilution was transferred to the conical glass vials. Left-over standard dilutions were put back in the refrigerator and used for the next day. Analysis showed no difference between freshly thawed standard dilutions and those in the fridge for a week. All standard dilutions except the one with a dilution factor of 50 were utilised to obtain a standard curve. The chromatogram showed much noise for the samples to be first analysed, so it was decided to have three cleaning steps before analysing the first sample. First, the GC column was cleaned thrice using 12% (v/v) formic acid. Then, after every sixth sample, the column was cleaned once. Standard curves were obtained working according to the method of Zhao et al., 2006. A description of this procedure can be found in subparagraph: 2.2.4 Extraction technique.

3.2.2 Experimental design blood samples

The project included 32 blood samples collected from mice at the endpoint of the experiments 'Processed oats' and 'Fruit supplementation'. Authorised personnel has collected the blood, and blood serum and plasma was stored at -40 °C and -70°C until analysis. Briefly, in 'Processed oats', seven different groups of young male mice were fed standard chow (R36) supplemented by 30% of oat products, namely: bio-processed oat bran (BOB), and bio-processed oat bran made by using two different types of ultra pressure namely BOB1 and BOB2. The other three groups got either R36 supplemented with 30% bio-processed whole oats (BWO) or whole oats made using two different ultra-pressure types named BWO1 and BWO2.

The "Fruit supplement" study involved feeding different diets to groups of old male mice, including a standard chow diet (SDS), a high-fat (HF 60E% from fat) diet with and without fruit supplementation (HF+F), and a low-fat (LF 10%E from fat) diet with and without fruit supplementation (LF+F). In total, the blood plasma of 5 subjects in each treatment group of the fruit study has been analysed. A summary of the experimental groups can be found in **Table 1**, and more information on the composition of the diet can be found in Appendix **Table A1**.

Table 1: *Experimental design of studies*

	Fruit Study	**Oat Study**
Supplementation	20% fruits	30% oats products
Gender	Male	Male
Age (at the start of the study)	14 months old	2 months old
Duration of study	4 months	4 weeks
Blood fraction	Plasma	Serum

Before analysing the samples, a standard curve was created by analysing the stock solution dilutions. Afterwards, some serum samples were used to practise the extraction procedure to become acquainted with the technique and test different dilutions of serum samples as it became clear that there would not be an adequate amount of serum available from the fruit supplementation study. For this reason, the method was optimised. The samples from the oat study were pooled to test undiluted, 1:2 dilution and a 1:4 dilution of blood serum to water. Each treatment group consisted of 10 animals, so 50 μl of each subject was taken and pooled to a total of 500 μl, which was then used to test the different dilutions.

3.2.3 Sample preparation
The 1:4 dilution was chosen for the fruit study since the GC could detect SCFA with this dilution. First, the serum or plasma was diluted with water and treated with 2M HCl containing the IS. After that, the pH of the serum solution was measured to check if it was 2.0 or below. If this was not, 2M HCl was added to adjust the pH. The solution was then put into a 10 mL tube containing 3.0 ml of the diluted serum solution. These vials were then used during the extraction step.

3.2.4 Extraction technique
First, the fibre was cut into strands of roughly 17 cm and aluminium foil into small pieces. After that, the BD Micro-Fine syringe was used for flushing and injecting the fibre with a NaOH solution. To generate an organic liquid membrane, the fibre was submerged in TOPO fluid for two to three seconds to saturate the holes of the fibre. Then the fibre was flushed again and filled with the NaOH solution. The fibre was removed from the syringe, and a single knot was made. The knot was then covered with aluminium foil to prevent the knot from unravelling. The fibre was then submerged in water until all samples were prepared for shaking. Before that, a paper towel was used to softly dry the fibres' surface. The threads were put into vials containing the diluted serum and wholly immersed in the solution. One vial contained two strings of fibre (duplicates). The vials were then sealed and placed on a shaker (IKA Vibrax®) that vibrated for 16 hours at 350 revolutions per minute. This ensures that the fibre absorbs the SCFAs in the diluted serum solution. The fibres were removed from the vials after 16 hours and dried with a paper towel. After 16 hours, the threads were removed from the vials and dried with a paper towel. The aluminium foils were cut, and an air-filled BD Micro-Fine Syringe was

attached to the fibres to flush out the NaOH solution containing the absorbed analytes into glass vials. Afterwards, 10 µl of 1M HCl was added to the vials containing the acceptor/analyte solution for gas chromatography analysis.

3.2.5 Gas chromatography

The starting oven temperature was 100°C and maintained for 0.5 minutes for the cleaning steps. The temperature was then raised to 225°C by 40°C/min. The initial oven temperature for the samples was 90°C and held for 0.5 minutes. The temperature increased to 180°C at 8°C/min and held for 1 minute and was then raised to 200°C by 20°C/min and maintained for 5 min. The flame ionisation detector (FID) temperature was set to 250°C. The flow rates of air, hydrogen and nitrogen as make-up gas were 300, 30 and 45 ml/min. The injection volume for formic acid 12% (v/v) was 2 µl, and the injection volume for the samples was set at 1 µl. The running time for each sample was approximately 18 minutes.

3.2.6 Data analysis and statistics

First, the area of the peaks was calculated as follows: $\frac{A_{SCFA}}{A_{IS}}$. Where A_{SCFA} and A_{IS} are the areas under the curve. An example of a chromatogram result can be found in the appendix **Figure B1**. The order and retention time at which the SCFAs appear and spend in the column is as follows: acetic acid (~1.8 min), propionic acid (~2.4 min), iso-butyric acid (~2.6 min), butyric acid (~3.1 min), iso-valeric acid (3.4 min), valeric acid (~4.1 min), and the IS (~4.3 min). Since duplicates were performed, the average of these areas was taken. Line charts were created with that data and the concentration of SCFA and IS in the vials. Then the coefficient of determination (R^2) was determined, as well as the corresponding curve parameters (correlation coefficient (a) and origin (b)) to calculate the concentration of each SCFA in the samples. An example of these line charts can be found in Appendix **Figure B2-B7**. All statistical analysis was performed using GraphPad Prism software.

Fermentation indices were calculated according to Undseth et al., 2015 as it is believed that SCFA can have pro-inflammatory (fermentation index A) or anti-inflammatory levels (fermentation index B) [13]. The following formulas were used:

$$Fermentation\ index\ A = \frac{(acetic\ acid - propionic\ acid - butyric\ acid)}{total\ SCFAs}$$

$$Fermentation\ index\ B = iso\text{–}butyric\ acid + iso\text{–}valeric\ acid$$

4. Results and discussion

4.1 Fruit study

The analysis of the fruit study involved a one-way ANOVA of all five groups, which revealed that only valeric acid exhibited significance between STD feed and HF+F (as displayed in **Figure 1D).** Thus, to explore further possibilities, certain groups were excluded from the statistical analysis. Individual SCFAs and total SCFAs were subjected to the following one-way ANOVA tests STD feed versus HF versus HF+F, STD feed versus LF versus LF+F, STD feed versus HF versus LF, STD feed versus HF+F versus LF+F, and HF versus HF+F versus LF versus LF+F. Additionally, unpaired t-tests were used to determine whether different diets had a significant impact. These tests include STD feed versus HF+F, STD feed versus LF+F, STD feed versus LF, STD feed versus HF, LF versus HF, HF+F versus HF, LF+F versus LF, and LF+F versus HF+F. Propionic acid did not exhibit any significance in any of the above-mentioned tests, as shown in **Figure 1A**. As per prior research, these results are advantageous as propionic acid has been shown to be increased in the plasma and serum of PD patients, which in turn escalates the severity of motor symptoms [14,15]. However, research on SCFA levels and brain disorders presents inconsistent findings.

Upon observing the levels of different acids, it is apparent that all diets have higher concentrations of SCFAs compared to the chow diet (SDS). This is particularly interesting since the chow diet is mainly plant-based. A plant-based diet is characterised by high fibre content and low fat but a higher ratio of unsaturated to saturated fat than an omnivorous diet. In contrast, the other diets in this study contain animal-based products, namely casein and lard, in combination with soybean oil. Although the LF and LF+F diets mostly derive their fat content from soybean oil, they still contain small amounts of lard. Previous studies have shown that individuals who follow a plant-based diet tend to have higher levels of formic acid, acetate, propionate and butyrate in their serum than those who follow an omnivorous diet. This is due to a shift in microbiota, where positive correlations were found between plant-based characteristic bacteria and serum SCFAs [16]. People shifting from an omnivore diet to a Mediterranean diet, which is higher in vegetables, fruit, and unsaturated fat, showed increased SCFA levels [17]. Furthermore, another research found negative correlations between levels of acetate, propionate, iso-butyrate, and valerate with omega-3 polyunsaturated fatty acids and saturated fats [18], which contrasts with the current study. **Figure 1B** indicates a significant difference in SCFA concentration levels between the STD diet and LF and LF+F diet, even though soybean oil contains high levels of omega-3 polyunsaturated fatty acids. Similarly,

Figure 1C shows that the groups that consume more saturated fat have higher levels of butyric acid.

Carbohydrates, fibre, and energy intake were negatively correlated with iso-butyrate and iso-valerate levels, while a positive correlation was seen between carbohydrates and butyrate. This indicates that carbohydrates, fibre, and fats are the primary nutrients influencing faeces' SCFA levels [18]. This is also in contrast with our study (see **Figure 1B**), as the LF (+F) diet is at least three times higher in carbohydrates than the HF (+F) diet but still increases iso-butyric acid levels. The study on the differences between a vegetarian and Mediterranean diet also found that following a vegetarian diet increased iso-valeric and iso-butyric acid more than a Mediterranean diet consisting of meat. Nonetheless, another study associated the intake of meat and dairy products with increased production of these particular SCFAs [18]. In addition, our findings suggest that an animal-based diet increases iso-butyrate and iso-valerate levels (as seen in **Figure 1** and Appendix **Figure B8-C**), although not always significant. Conversely, a study on the difference between overweight and lean people found no correlation between faecal SCFA levels and fat intake [19]. According to Brinkworth et al., overweight participants experienced a decrease in faecal SCFA concentrations when following an energy-restricted high-fat diet compared to an energy-restricted low-fat diet. However, the latter study had extremely low carbohydrate and fibre intakes in the high-fat diet, making it difficult to compare the results [20]. On the other hand, a study in overweight individuals found that a high saturated fatty acid diet increased SCFA concentration. However, a high monounsaturated fat diet did not affect faecal SCFA. [21]

Lastly, in comparing LF vs LF+F and HF vs HF+F, no significant change was observed in SCFA concentration, as illustrated in **Figure 1** and Appendix **Figure 3**. This was also evident in a study examining the effect of lingonberry on caecum SCFAs. Mice fed insoluble lingonberry had lower total SCFAs, including acetate, propionate, and butyrate, in their caecum compared to a high-fat control containing cellulose. Conversely, the soluble lingonberry diet increased total SCFAs, acetate, propionate, and butyrate in the caecum of the mice compared to the high-fat control [22]. This indicates that the fruit fraction type significantly impacts SCFA production, as soluble fibres are digested by gut microbiota and produce postbiotics. In contrast, insoluble fibres are minimally fermented and are known as bulking fibres [1]. However, a study examining the effects of insoluble and soluble fractions in raspberry, bilberry, and black currant found higher concentrations of butyrate in the serum of rats fed with insoluble fractions [23]. The same applied to rats fed with passion fruit peel, containing high amounts of insoluble fibre. This increased acetic and butyric acid levels in faeces significantly [24]. Nevertheless, caecum SCFA levels were higher in rats fed with soluble fruit fractions. It was also observed that soluble bilberry fraction formed the highest amount of SCFAs

[23]. The current research suggests that supplementing fruits to an HF or LF diet does not impact SCFA levels, and higher SCFA levels could be due to the addition of cellulose to the diet, which is known to contain insoluble fibre. However, when scrutinizing the diet compositions of LF and HF compared to LF+F and HF+F, it can be seen that the diets supplemented with fruits contain only half of the cellulose found in the HF and LF diet (data not shown). Therefore, fruits may have an effect when added to an HF or LF diet, but this may not be detectable since cellulose was partially replaced with fruits to keep the amount of fibre in all diets relatively the same.

Although fruits are high in fibre, they are as well a good source of polyphenols, which have shown potential as prebiotic compounds. The exact process by which polyphenols increase the production of SCFA is not fully understood. However, they are believed to promote the growth of anaerobic microorganisms like *Lactobacillus*, *Lachnospiraceae*, and *Ruminococcaceae*, increasing SCFA production. This indicates that the fibre in berries and their polyphenols may play a role in SCFA production [25]. Other foods like extra virgin olive oil, dark chocolate, tea, red wine, coffee, and spices such as cloves, cinnamon, and turmeric contain polyphenols and have been researched for their potential therapeutic properties [26]. A study found that anthocyanin intervention improved the neuronal function decline caused by a high-fat diet in mice and increased the abundance of SCFA-producing bacteria such as *Faecalibaculum*, and especially *Roseburia* [27]. Finally, a study investigating anthocyanin content in various berries found that black currant and blackberry contain high amounts of this phenolic compound. In contrast, raspberry and red currant have lower amounts [28].
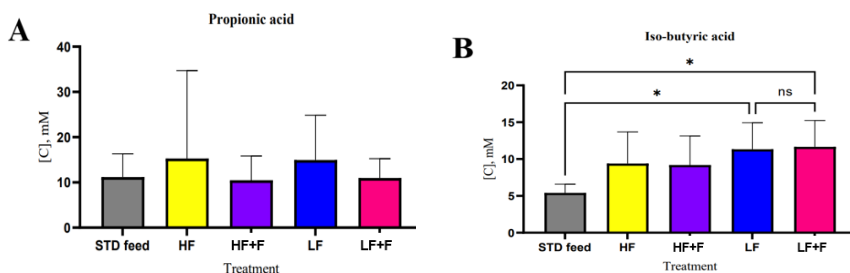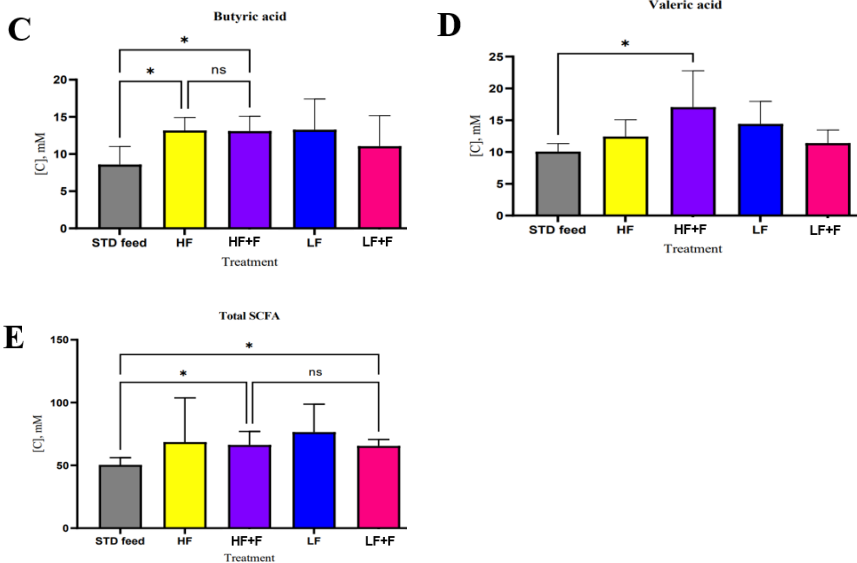
Figure 1: *Concentrations of SCFAs in plasma samples of mice on various diets supplemented with a fruit mixture.*

Concentrations (mM) of propionic acid (A), iso-butyric acid in STD feed versus LF versus LF+F (B), butyric acid in STD feed versus HF versus HF+F (C), valeric acid in STD feed versus HF+F (D), and total SCFAs in STD feed versus HF+F versus LF+F (E), in blood plasma samples in old mice on standard chow and HF, HF+F, LF and LF+F diets. Data represent estimated marginal mean ± SD for n=5 per diet group. Figure 1B-Ewere compared using one-way ANOVA and Tukey's multiple comparisons tests. Significant differences are denoted as *P<0.05.

According to Undseth et al. 2015 [13], the fermentation index A, which indicates the pro-inflammatory state, shows light variations among the groups. This data implies that all five diets are non-inflammatory and considered "safe" as excessive levels of SCFAs can be toxic for the epithelium, as shown in rat studies [29]. Other research has also linked high SCFA concentrations in faeces to obesity, gut permeability, and gut dysbiosis. However, there are contradicting studies and more analysis on faecal and circulating SCFA levels is necessary to determine the negative impact of high SCFA levels on health [30].

According to research by Undseth et al. in 2015 [13], the fermentation index B indicates an anti-inflammatory state. The STD feed has a slightly lower index compared to LF+F and LF feeds. This data suggests that all diets have anti-inflammatory

properties. However, recent studies suggest that iso-valerate and iso-butyrate, which are markers of protein fermentation, may have pro-inflammatory properties. This could lead to the production of harmful fermentation products for the colon epithelium. The study found that adults have higher levels of iso-valeric and iso-butyric acid compared to young children [31]. This implies that lower levels of these acids, as found in the standard diet, are more favourable. However, due to conflicting data, further research is necessary to make definitive conclusions in this area.

Table 2: *Fermentation indices of fruit study.*

	Fermentation Index A	**Fermentation Index B**
STD feed	-0.18	9.0
HF	-0.23	13.7
HF+F	-0.15	14.5
LF	-0.16	17.7
LF+F	-0.13	19.9

Fermentation indices were calculated from SCFA concentration (mM) in blood plasma. Data represented as a mean for n=5 per diet group.

4.2 Oat study

The section titled "3.2.2 Experimental Design Blood Samples" noted that the BOB and BWO groups were comprised of three different oat bran and whole oats fractions, each created using different levels of ultra pressure. Despite this variation, we found very little statistical difference when we conducted a one-way ANOVA and Tukey's multiple comparisons tests on these six groups in conjunction with standard chow (R36). As a result, we decided to combine BOB, BOB1, and BOB2 into a single category called "total BOB," and BWO, BWO1, and BWO2 into a single category called "total BWO." We then performed one-way ANOVA and Tukey's multiple comparison tests on all groups and unpaired t-tests on two groups (as shown in **Figure 2**). Our findings indicated that there was no significant difference between total BOB and total BWO, suggesting that the type of fraction used does not impact SCFAs serum levels, despite the assumption that a diet with BOB would increase SCFAs concentration more than a diet with BWO since oat bran contains more fibre than whole oats. This was also observed in a study investigating the effect of oat products on gut microbiota in obese rats. Although not significant, oat bran showed higher levels in colonic content of individual and total SCFA compared to oatmeal and oat flour, which is for this comparison translated to whole oats [32].

The R36 diet is a plant-based feed that aims to provide all the necessary nutrients for rodents. However, when examining **Figure 2**, it is evident that the total and individual SCFA serum levels in mice on the R36 diet are relatively low compared

to mice on an R36 diet supplemented with 30% BOB or BWO. **Figure 2** displays a significant impact on SCFA concentrations from the diet supplemented with BOB and BWO. Propionic and valeric acid did not exhibit any significance between all possible comparisons, including one-way-ANOVA, unpaired t-test of total BOB and BWO, total BOB and R36, and total BWO and R36. In addition, iso-valeric acid did not show significance between total BOB and R36.

The findings regarding acetate and butyrate are consistent with other studies. However, oat consumption has been observed to cause a significant increase in propionic acid levels. For instance, in a human study, it has been shown that the consumption of 80 grams of oats for 45 days resulted in higher levels of acetate and propionate in blood plasma [33]. Another study, which investigated the impact of various grains on rats following a high-fat diet, found no increase in colonic SCFAs after oat supplementation, except for butyric acid [34].

Previous research primarily focuses on the main SCFAs - acetate, propionate, and butyrate - and measures them in either faecal or colonic content samples. This could explain the variation in results, as SCFAs can induce their active uptake transporter on the intestinal epithelial wall. Therefore, faecal SCFAs may not accurately reflect colonic SCFA production from fermentation. For a better comprehension of the effects of diet on SCFA changes, serum, plasma and faecal samples should be collected for SCFA analysis [33].

As mentioned in section 4.1 ''fruit study'' do not only fibres influence SCFA production, but polyphenols are believed to have a prebiotic effect as well. Studies have revealed that oat products are high in polyphenols as well and contain comparable levels to the levels found in fruits and vegetables [35]. Perhaps combining oats and fruits could create synergy, resulting in a changing microbiota and higher SCFA production. But, this has to be explored further.
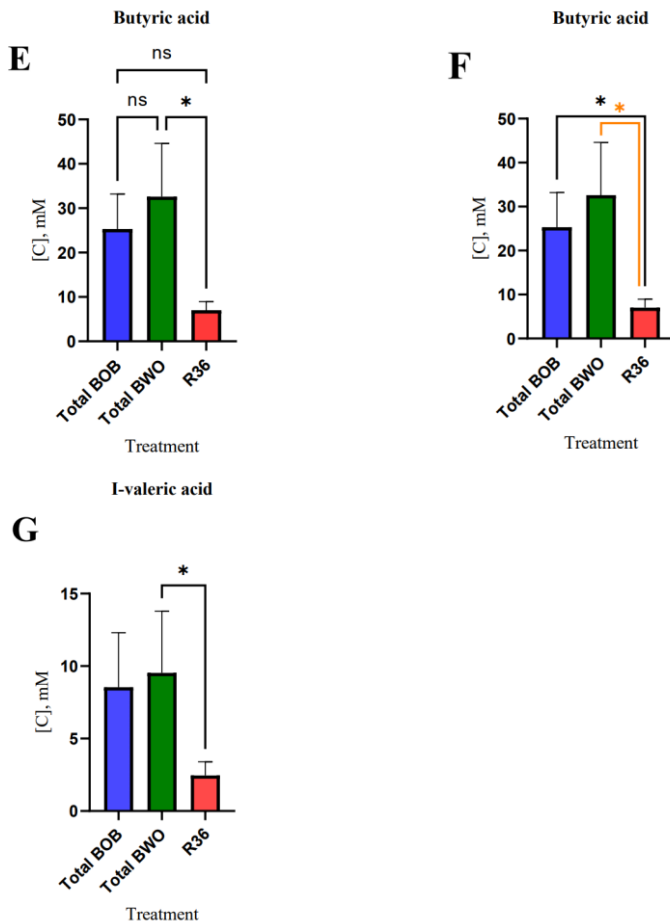
Total SCFAs

A

Acetic acid

B

I-butyric acid

C

I-butyric acid

D

23
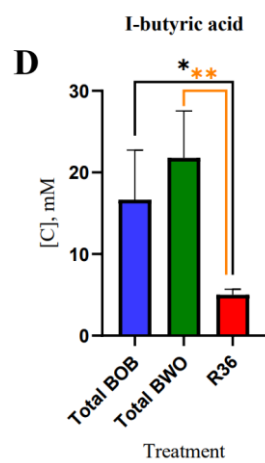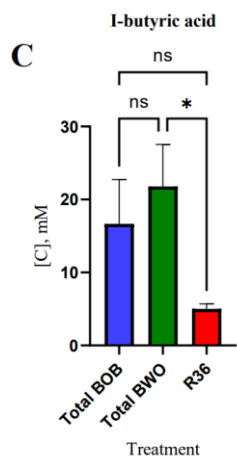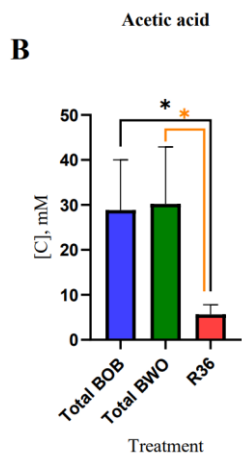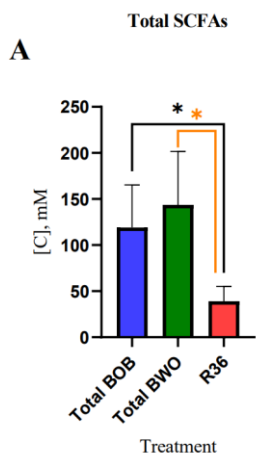
Figure 2: Concentrations of SCFAs in serum samples of mice on standard chow and different ultra-processed oat diets.

Concentrations (mM) of total SCFAs (A), acetic acid (B), iso-butyric acid (C), iso-butyric acid (D), butyric acid (E), butyric acid (F), and iso-valeric acid (G) in blood serum samples in young mice on standard chow and different ultra-processed oats. Data represent estimated marginal mean ± SD for n=10 per diet group. Figures 2C and 2E were compared using one-way ANOVA and Tukey's multiple comparisons tests. Figures 2A, 2B, 2D, 2F and 2G are compared by applying an unpaired t-test. Black lines show significance between R36 and total BOB, and orange lines show significance between R36 and total BWO in A, B, D and F. Significant differences are denoted as *P<0.05, **P<0.01.

The results of the fermentation index A suggest that there are no tendencies between the groups, indicating that the three diets are not inflammatory. However, the fermentation index B shows substantial variation between R36 and the two diets that are supplemented with oats. In the 'fruit study' section, it was noted that some studies have found iso-valeric and iso-butyric acid to be anti-inflammatory, while others have found them to be pro-inflammatory. If we consider these acids to have an anti-inflammatory effect, the addition of oats shows promising results, with higher levels observed. However, if we consider them to be pro-inflammatory, the supplementation of oats could have a negative impact on health.

Table 3: *Fermentation indices of oat study.*

	Fermentation Index A	**Fermentation Index B**
BOB	-0.21	25.2
BWO	-0.23	31.3
R36	-0.37	7.5

Fermentation indices were calculated from SCFA concentration (mM) in blood serum. Data represented as a mean for n=10 per diet group.

4.3 Comparison of oat diet and fruit diet

Interestingly, the results of a standard chow test conducted on male mice of different ages yielded unexpected findings. Surprisingly, the older mice had a higher serum concentration of SCFAs (as seen in **Table 4**), except for propionic acid, compared to the younger ones, contradicting what was initially expected. After conducting an unpaired t-test at a 95% confidence interval (CI) to compare the mean difference of total SCFAs in both groups fed with standard chow, there was no statistical significance, as shown in **Figure 3A**. However, when looking at the quantity of total SCFAs between the groups, it can be observed that the older group had higher levels of SCFAs. When analysing individual SCFAs, only acetic acid showed a significant difference between the two groups, as shown in **Figure 3B**. It was found that the concentration of acetic acid in the older group (5.66 ± 2.16 mM) was significantly higher than in the younger group (11.50 ± 2.6 mM) ($t(6) = 3.258$, $p = 0.0175$) with a difference of 5.88 mM (95% CI). Although some research has found similar results, elevated concentrations of acetic and propionic acid in the serum of older mice compared to young mice [36], most studies show the opposite.

Table 4: *Concentration of SCFAs in serum samples of mice in different age groups.*

SCFA	Age group	
	14 months -18 months	2 months - 3 months
Acetic acid	11.5 ± 2.59	5.66 ± 2.16
Propionic acid	11.17 ± 5.14	12.99 ± 6.25
Iso-butyric acid	5.41 ± 1.18	5.02 ± 0.68
Butyric acid	8.59 ± 2.40	7.04 ± 1.90
Iso-valeric acid	3.60 ± 2.22	2.46 ± 0.94
Valeric acid	10.07 ± 1.26	5.85 ± 4.10
Total SCFAs	50.34 ± 16.14	39.02 ± 15.99

Concentration (mM) of acetic, propionic, iso-butyric, butyric, iso-valeric, valeric and total SCFAs in blood plasma and serum samples in young and old male mice on standard chow. Values are represented as estimated marginal mean ± SD for n=5-10 per age group.
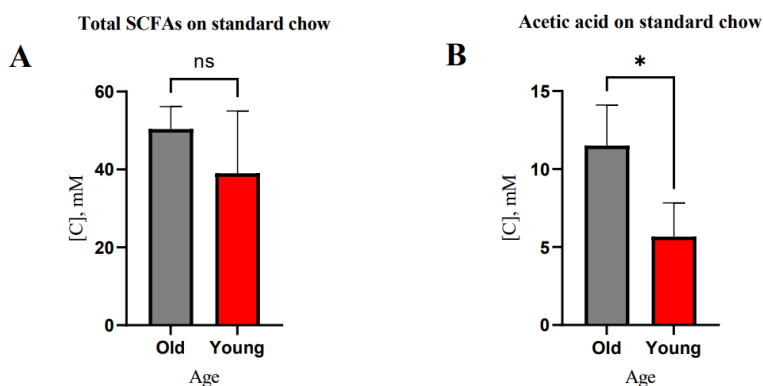

Figure 3: *Concentration of total SCFAs and acetic acid in serum samples of mice in different age groups.*

Concentration (mM) of total SCFAs (A) and acetic acid (B) in blood plasma and serum samples in young and old male mice on standard chow. Data represent estimated marginal mean ± SD for n=5-10 per age group and are compared by applying an unpaired t-test. *P<0.05.

Through literature research, it has been found that the concentration of SCFAs in human faecal samples tends to increase in adults below the age of 50 in comparison to those aged 80 years or above [37]. The same trend has also been observed in studies conducted on young and aged mice, as the younger mice showed a higher

concentration of SCFAs in their faecal samples [38]. Additionally, research on age-related changes in the gut of young and old mice has revealed that the Firmicutes/Bacteroidetes (F:B) ratio increases with age [39], as well as *Enterobacteriaceae*, while *Akkermansiaceae* and *Streptococcaceae* are reduced, which could be linked to SCFA production [40].

One possible explanation for this discrepancy is a slight variance in the standard feed used (as seen in **Table A1**). For instance, the protein sources differed between the fruit study (soy protein concentrate and whey powder) and the oat study (soy and potato protein). Additionally, the fruit study had a slightly higher percentage of grams of fibre and percentages of calories from carbohydrates compared to the oat study, which could have affected SCFA production. In contrast, the opposite was true for the percentage of protein and fat between the two studies.

The variance between the two studies could, according to previous research as well be related to the mice's EI and body mass index (BMI), as well as the makeup of the microbiome (as mentioned above), but this information has not been provided. Previous research on SCFA plasma levels in humans shows that those with a higher BMI and waist-to-height ratio (WHtR) tended to have higher levels of SCFAs in their plasma, particularly butyrate/iso-butyrate with BMI and valerate and total SCFAs with WHtR. Even individuals with abdominal obesity who consumed a moderate level of insoluble fibre had a higher abundance of butyrate/iso-butyrate and total SCFAs than those without abdominal obesity. Two theories suggest that people who struggle with overweight or obesity may have less efficient SCFA absorption, resulting in increased SCFA excretion or different ways of processing SCFA precursors. However, further studies on circulating and faecal SCFAs are needed to understand these hypotheses fully [41].

As stated in section 4.1, titled "Fruit Study," research indicates that young children have lower levels of iso-valeric and iso-butyric (fermentation index B) compared to adults [31]. **Table 5** also highlights a similar difference, albeit not large, between the oat and fruit study.

Table 5: Fermentation indexes of age study.

	Study	**Age**	**Fermentation Index A**	**Fermentation Index B**
STD feed	Fruit	14 months	-0.18	9.0
R36	Oat	2 months	-0.37	7.5

Fermentation indices were calculated from SCFA concentration (mM) in blood plasma and serum. Data represented as a mean for n=5-10 per diet group.

5. Conclusions and future perspectives

This research focused on investigating the influence of different diets on the production of SCFAs. The analysis of mice's dietary records and blood serum using gas chromatography provided valuable insights into the relationship between diet and SCFA profiles. Interestingly, the findings revealed that all types of diets in the oat and fruit study exhibited higher concentrations of SCFAs than the standard feed. Previous research suggests that plant-based diets lead to higher SCFA levels due to shifts in microbiota. However, the current study's findings contradict this, indicating inconsistencies in the relationship between SCFA levels and diets. Notably, the addition of fruits to a high-fat or low-fat diet did not significantly impact SCFA levels. Nevertheless, fruit consumption, along with its fibre and polyphenol content, is believed to promote the growth of specific beneficial bacteria, leading to increased SCFA production. Therefore, incorporating fruits into the diet may have potential benefits for maintaining gut health.

The study also highlighted the complexity of SCFA production, as different dietary components, such as carbohydrates, fibre and fats, demonstrated varying correlations with specific SCFAs. Contrary to some previous findings, this study observed that diets high in saturated fat and omega-3 polyunsaturated fatty acids increased acetate, iso-butyrate and valerate levels. These discrepancies emphasize the need for further research to understand the intricate interactions among dietary components, gut microbiota, and SCFA production.

Future research should focus on the correlations between SCFA levels in blood serum or plasma, and faeces to establish robust biomarkers that reflect colonic SCFA production. Furthermore, investigating the influence of specific dietary components, such as protein and fat sources and nutrient composition, on SCFA levels will provide valuable insights into the modulation of gut microbiota and its impact on overall health. This could pave the way for personalized nutrition strategies that harness the power of our gut microbiota to promote overall health. Additionally, more light should be shed on the pro-inflammatory and anti-inflammatory effects of SCFAs. Lastly, combining foods high in fibre and polyphenols could show interesting results and be brought to the market as functional foods.

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Appendix

A. Nutritional data

Table A1: *Diet composition*

	Fruit study					Oat study
	SDS	HF	HF+F	LF	LF+F	R36
Type of protein	Soy protein concentrate and whey powder	Casein	Casein	Casein	Casein	Soy and potato protein
Protein (kcal%)	14.4	18	18	18	18	18.5
Type of fat	Soybean oil	Lard and soybean oil	Lard and soybean oil	Soybean oil and lard	Soybean oil and lard	Vegetable oil
Fat (kcal%)	2.7	60	60	10	10	4
Type of nitrogen-free extract (NFE) / carbohydrates	Unspecified	Maltodextrin and sucrose	Maltodextrin	Corn starch, maltodextrin and sucrose	Corn starch and maltodextrin	Unspecified
Types of sugar	Unspecified	Sucrose	Fructose, glucose and sucrose	Sucrose	Fructose, glucose and sucrose	Unspecified
NFE / carbohydrates (kcal%)	62	21	21	72	72	56
Type of fibre	Mix of fibre	Cellulose	Cellulose	Cellulose	Cellulose	Mix of fibre
Fibre (gram%)	4.6	6.5	6.3	4.7	4.6	3.5

B. Figures
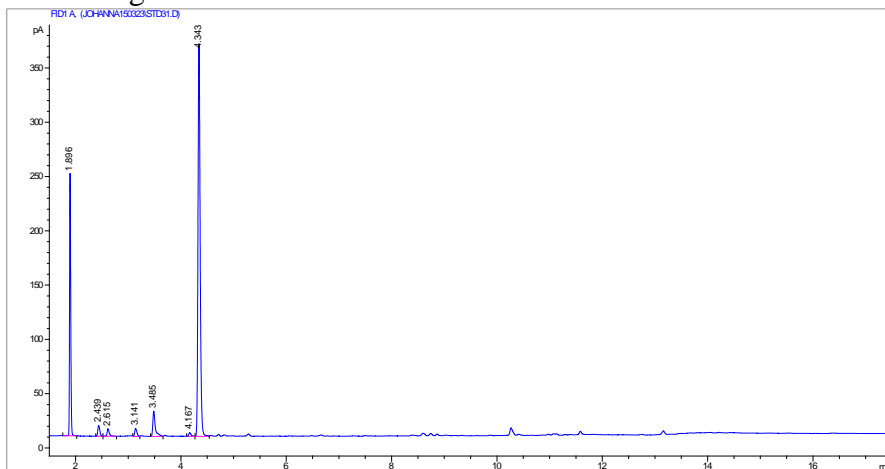
Figure B1: *Example of Standard curve preparation per each.*

Order and retention time of SCFAs and IS during GC analysis. Acetic acid (∼1.8 min), propionic acid (∼2.4 min), iso-butyric acid (∼2.6 min), butyric acid (∼3.1 min), iso-valeric acid (3.4 min), valeric acid (∼4.1 min), and the IS (∼4.3 min).
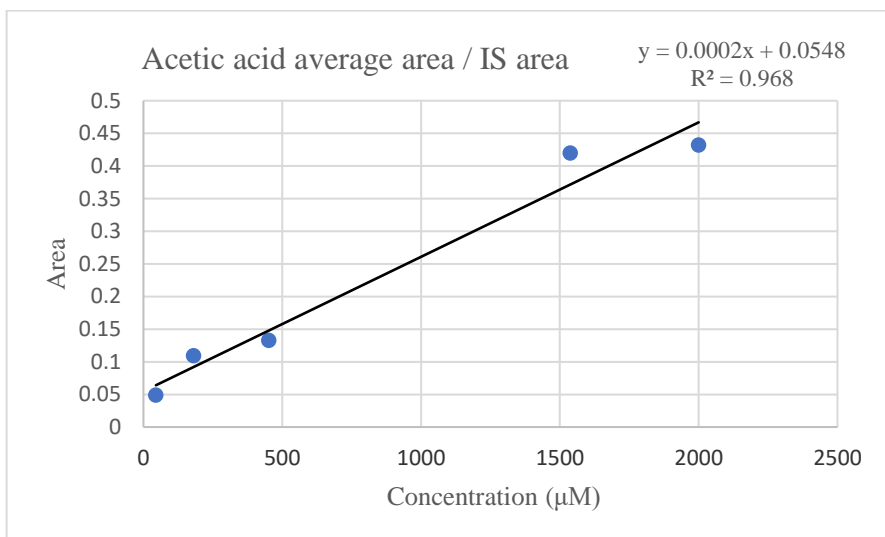

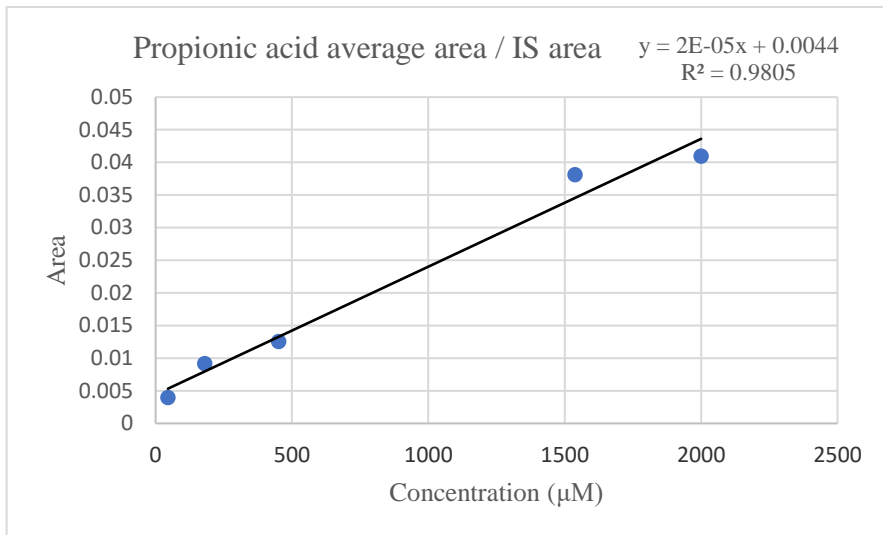***Figure B2:*** *Standard preparation for acetic acid.*
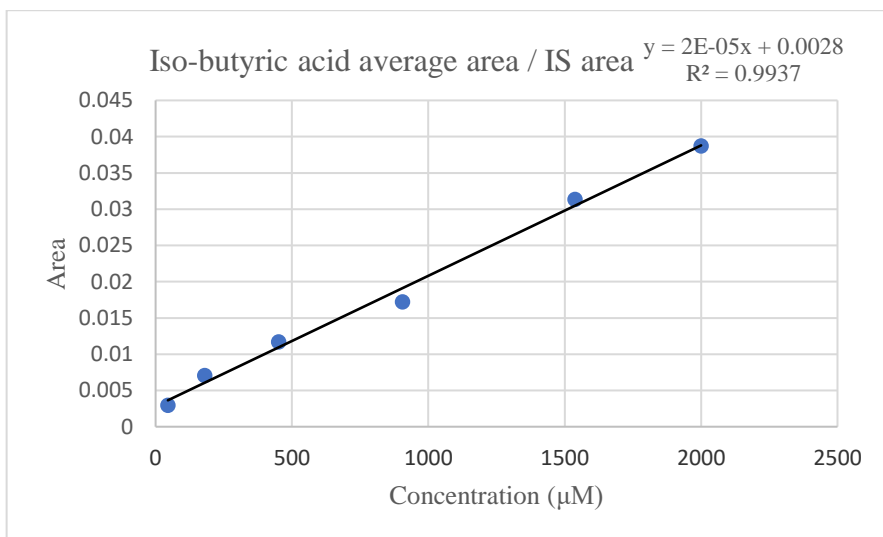
Figure B3: *Standard preparation for propionic acid.*

Figure B4: *Standard preparation for iso-butyric acid.*
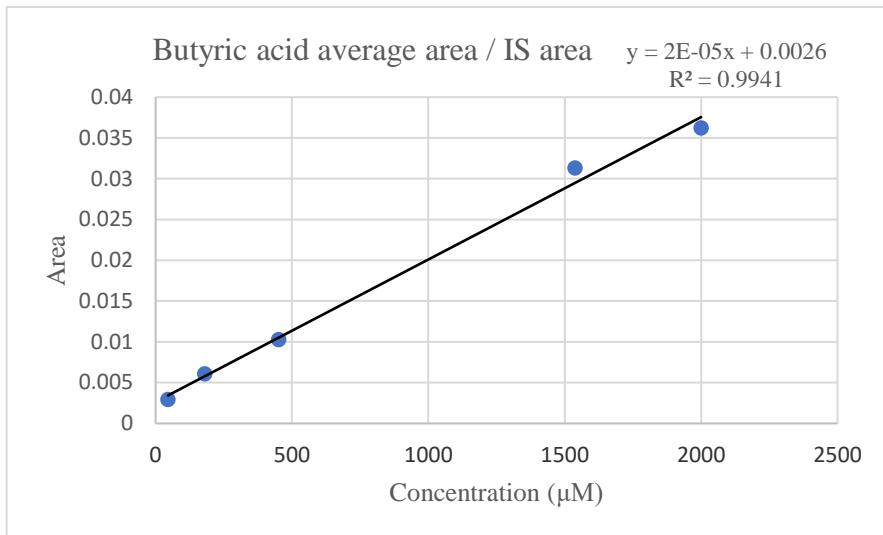
Figure B5: *Standard preparation for butyric acid.*
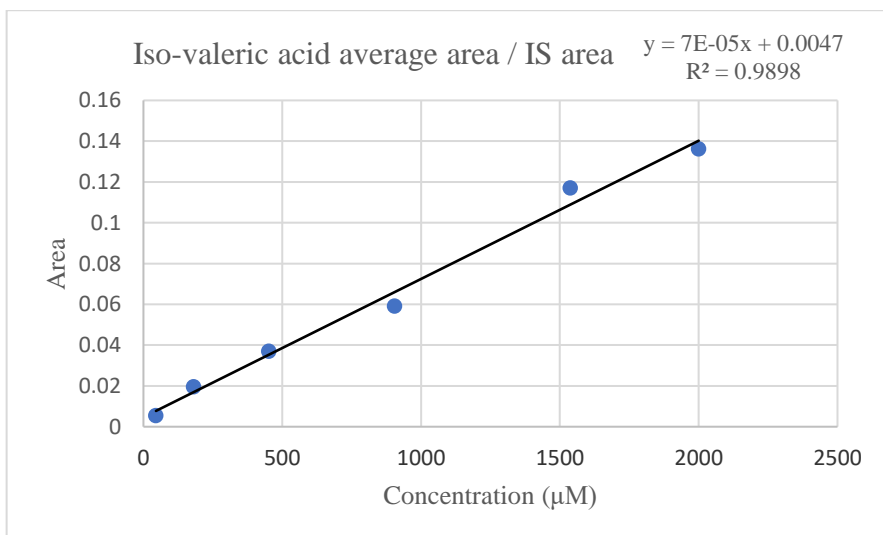
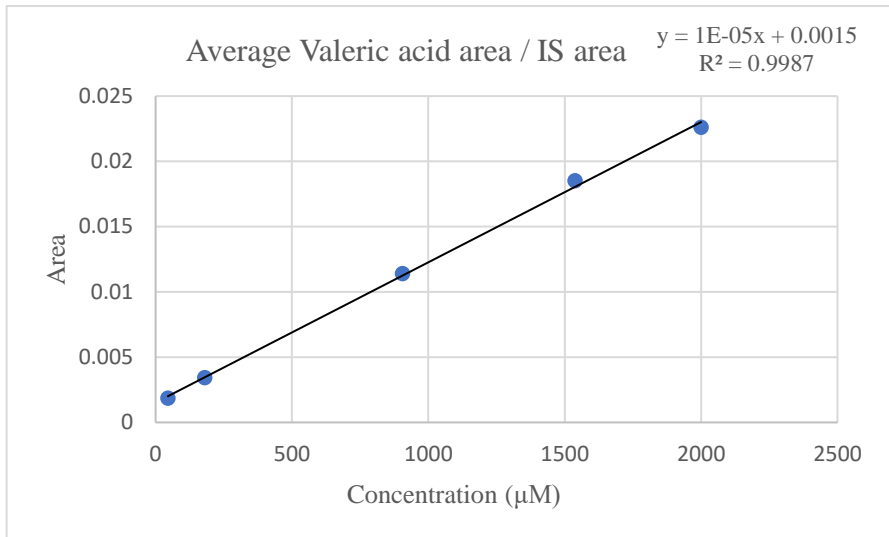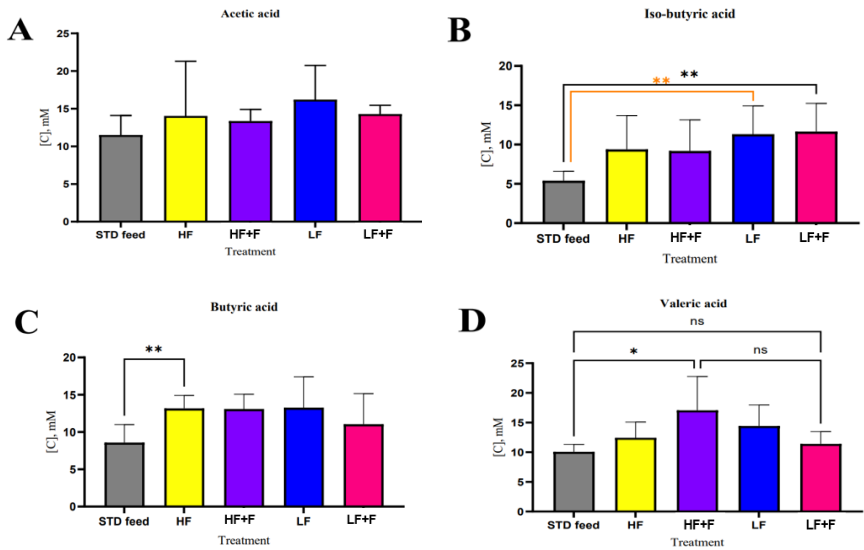**Figure B6:** *Standard preparation for iso-valeric acid.*

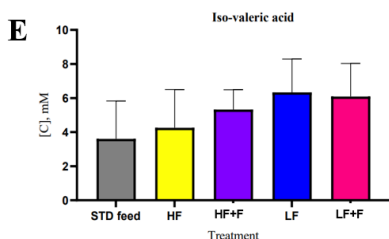*Figure B7: Standard preparation for valeric acid.*

Figure B8: *Concentrations of SCFAs in plasma samples of mice on various diets supplemented with a fruit mixture.*

Concentrations (mM) of acetic acid (A), iso-butyric acid in STD feed versus LF and STD versus LF+F (B), butyric acid in STD feed versus HF (C), valeric acid in STD feed versus HF+F versus LF+F (D), and iso-valeric acid (E) in blood plasma samples in old mice on standard chow and HF, HF+F, LF and LF+F diets. Data represent estimated marginal mean ± SD for n=5 per diet group. Figure B8-D was compared using one-way ANOVA and Tukey's multiple comparisons tests. Figures B8-B and C were compared by applying an unpaired t-test. Significant differences are denoted as *P<0.05, **P<0.01.