A Dissertation for the Degree of Master

Functional evaluation of *Fusarium venenatum*-based microbial protein using multi-omics analysis

Multi-omics 분석을 이용한 *Fusarium venenatum* 기반 미생물 단백질의 기능 평가

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Abstract

Functional evaluation of *Fusarium venenatum*-based microbial protein using multi-omics analysis

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These days, meat is the main source of protein for humans. However, meeting the global demand for meat is predicted hard in the future for several reasons such as the increasing global population and meat consumption, the low efficiency of converting feed to meat, the environmental burden, and greenhouse gases emission. Many food companies and researchers have sought alternative protein sources such as cultured meat, plant-based protein, and edible insect to supply protein sustainably for the future. Microbial protein which is made by fermentation of *Fusarium venenatum* is one of the candidates for alternative protein

sources. Therefore, we aimed to elucidate the functional evaluation of *F. venenatum*-based microbial protein as an alternative protein source using multi-omics analysis.

In the first study, we conducted experiments using *C. elegans*. We fed *Escherichia coli* OP50 (OP50) or *E. coli* OP50 with *F. venenatum* (F.V). In the lifespan and killing assay, F.V group showed improved longevity and innate immune response compared to OP50 group. To evaluate lipid quantification Nile red and Oil red o staining were performed and the result showed significantly reduced fat accumulation in F.V group compared to OP50 group. Thus, we performed a whole transcriptomic analysis to identify alteration at the molecular level by *F. venenatum*. The result showed notably downregulated gene expressions related to fat synthesis including *pod-2* and *fasn-1* and upregulated gene expressions related to the fat breakdown pathway in F.V group compared to OP50 group.

The second experiment was conducted to confirm the anti-obesity effect of *F. venenatum* in mice induced with a high-fat diet. Blood analysis showed that toxicity biomarkers were reduced and lipid profiles were improved in the groups orally administered with *F. venenatum* compared to the HFD group. Histological data revealed that groups fed with *F. venenatum* showed significantly reduced fat accumulation in both liver and adipose tissue. The metabolomic analysis showed the high concentration of primary bile acid and its prosecutor cholesterol in the fecal of groups fed with *F. venenatum* compared to other groups. This result implied that *F.*

venenatum entrapped the digestive enzyme including primary bile acid and cholesterol which may have led to a reduction in lipid digestion and consequently decrease in fat accumulation. In addition, the metagenomic analysis showed that *F. venenatum* altered the composition of microbial composition and increased the alpha diversity.

In conclusion, this series of functional studies reveal the possibility of *F. venenatum*-based microbial protein as a sustainable alternative protein. In addition, *F. venenatum*-based microbial protein reduced fat accumulation when fed to animals with metabolic diseases such as obesity. These results imply *F. venenatum*-based microbial protein may apply as an anti-obesity supplement.

Keyword : *Fusarium venenatum*, Microbial protein, Meat-alternative, Functional evaluation, Anti-obesity

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List of Abbreviations

Abbreviation	Name
SCP	Single cell protein
PDCAAs	Protein digestibility-corrected amino acid score
SCFAs	Short-chain fatty acids
MUFAs	Monounsaturated fatty acids
PUFAs	Polyunsaturated fatty acids
SFAs	Saturated fatty acids
GLP-1	Glucagon-like peptide-1
PYY	Peptide tyrosin-tyrosin
TC	Total cholesterol
HDL	High-density lipoprotein
LDL	Low-density lipoprotein
VLDL	Very low-density lipoprotein
TG	Triglycerides
C. elegans	*Caenorhabditis elegans*
NHR	Nuclear hormone receptor
SREBP	Sterol response element-binding protein

AMPK	AMP-activated kinase
BHI	Brain Heart Infusion
LB	Luria-Bertani
NGM	Nematode growth medium
OP50	*Escherichia coli* OP50
GC-MS	Gas Chromatography-Mass Spectrometry
SEM	Standard error of the mean
BCAAs	Branched-chain amino acids
OGTT	Oral glucose tolerance test
AST	Aspartate transaminase
ALT	Alanine transaminase
H&E	Hematoxylin and eosin

Chapter 1. Literature review

1.1. Conventional protein source

Protein is important in human life. Protein helps the body repair, regenerate cells, and build new structural and functional proteins such as enzymes and hormones. Also, it improves growth and development in children and adolescents (Bohrer, 2017). Adequate intake of dietary protein is important for humans in all stages of life. Protein undernutrition may lead to physical weakness, stunted growth, anemia, edema, vascular dysfunction, and impaired immunity. The protein consists of amino acids linked by peptide bonds. Dietary proteins are hydrolyzed by proteases and peptidases to amino acids, dipeptides and tripeptides in the gastrointestinal tract. It is crucial to consume a suitable amount of essential amino acids (Wu, 2016). Some amino acids can’t be synthesized in humans. In other words, humans must consume those amino acids through diet. Insufficient intake of individual essential amino acids may occur physical and biochemical issues (Reeds & Hutchens, 1994). Meat is one of the foods rich in high-quality protein and essential amino acids.

1.1.1. Conventional protein source: Meat

Meat is a conventional protein source for humans. It has been defined as "Skeletal muscle and its associated tissues (including connective tissues,

blood vessels, nerves, and bones) and edible remains, derived from mammals, avian, and aquatic species, especially from domesticated animals such as hogs, cattle, poultry, sheep and goat" (Kauffman, 2001; Seman et al., 2018). Meat is considered a highly nutritive food provides not only high-quality protein but also micronutrients such as iron, zinc, vitamin B and selenium (Godfray et al., 2018). Also, it contains all the essential amino acids, and their bioavailability is higher than other dietary sources (Cosgrove et al., 2005; Davey et al., 2003).

1.1.2. The difficulty of meeting the growing meat demand

The global population continues to increase and is prospected to increase to 9.2 billion by 2050 (Moreland et al., 2010). About 30% of human protein intake per day comes from livestock products, and in developed countries, about 50% comes from livestock products (Steinfeld et al., 2006). The world's average meat consumption per capita and total meat consumption is increasing due to the increase in the average individual income and global population (Godfray et al., 2018). To meet this demand, one-third of the earth's ice-free land is being used for growing livestock, and many forests are being destroyed to create pastures and arable land for raising livestock and producing their feed (Ramankutty & Foley, 1999; Steinfeld et al., 2006). Previous research expected if this current consumption pattern continues globally, in 2050, 1,250 million tonnes of meat and dairy need to be produced per year to meet the global demand

(Ritala et al., 2017). In other words, it will be difficult to keep up with the growing global meat demand because of the environmental impact and also the low efficiency of converting feed to meat. Due to the concern about this problem, attention on alternative protein sources is increasing to replace conventional protein source meat (Grasso et al., 2019). Many food companies and researchers are focusing on alternative protein source that can replace meat and provide protein more sustainably.

1.1.3. Obesity has become a global problem

Obesity is a global epidemic these days (Organization, 2000; Wang & Lobstein, 2006). Obesity has increased in many countries worldwide with more than 600 million adults reported as obese and more than 1.9 billion as overweight (Caballero, 2019; Malik et al., 2013). The population with obesity can easily be exposed to the risk of chronic diseases such as type II diabetes, hypertension, coronary heart disease and cancers (Aneja et al., 2004; Calle & Thun, 2004; Dixon, 2010; Hossain et al., 2007; KEYS, 1980; Salter, 2013). Obesity can result from an imbalance between energy intake and consumption (Peng et al., 2016). Despite the high nutritional value of meat, excessive consumption can induce overconsumption of energy and result in obesity (Salter, 2018). Previous research found a positive association between meat consumption and obesity among U.S. adults (Wang & Beydoun, 2009). In the face of the increasing global population, meat consumption, environmental burden and obese population, efforts are

being made to develop food that can play the role of an alternative protein source and substitute conventional protein source (Kumar et al., 2017; Smetana et al., 2015). For these reasons a variety of alternative protein sources has been developed and microbial protein which is made from the dried cells of microorganisms is one of them (De Steur, 2001; Nasseri et al., 2011; Peregrin, 2002; Sadler, 2004).

1.2. Microbial protein

In the early fifties, due to the increasing world shortage of protein, great efforts have been made to seek alternate protein sources. For this reason, a microbial protein referred to as single cell protein (SCP) was first mentioned in 1996 to describe protein production from biomass derived from different types of microorganisms such as yeast, bacteria, algae and fungi (Nasseri et al., 2011). Microbial protein has been considered an alternative protein source for food and feed with the interesting feature that it can be produced on a large scale (Sharif et al., 2021). Also, it has a good advantage of can be produced regardless of climate, soil status and less land used which can be provided sustainably (Hülsen et al., 2018).

1.2.1. Definition and benefit of microbial protein

Microbial protein, which is also referred to as SCP (Although some microorganisms such as fungi and algae are multicellular), is the edible

biomass of microorganisms. There are a few microorganisms that can be used to produce microbial proteins such as yeast, bacteria, algae and fungi (Nangul & Bhatia, 2013). Produced microbial proteins can be utilized for both animal feed and human food. Producing microbial proteins are known for several beneficial characteristics (Nasseri et al., 2011; Onyeaka et al., 2022; Sharif et al., 2021).

- Ease of fermentation and production
- Able to use a variety of substrates to grow
- High efficiency in substrate conversion
- High productivity due to fast growth rate
- Do not require broad land or a large amount of water
- Can be harvested independently with the weather
- Do not degrade environments or contribute to greenhouse gases and climate change

1.2.2. Different types of microorganisms for microbial protein

Lots of microorganisms, including yeast, bacteria, algae and fungi can be used to produce microbial protein. Microbial protein is known for its high nutritional value, including high protein, essential amino acids, vitamins, and lipid profile (Nasseri et al., 2011). The different compositions of the main types of microorganisms are shown in Table 1. To be used as SCP, candidate microorganisms should not produce toxic or carcinogenic compounds during fermentation.

Table 1. The compositions of the main types of microorganisms (% dry weight)

Composition	Fungi	Yeast	Bacteria	Algae
Protein	30-45	45-55	50-65	40-60
Fat	2-8	2-6	1-3	7-20
Ash	9-14	5-10	3-7	8-10
Nucleic acid	7-10	6-12	8-12	3-8

(Brinton & Warren, 1976)

1.2.2.1. Yeast

At first, yeast is a good microorganism to produce a microbial protein. It is known for a few advantages such as high lysine content, providing vitamin B group complex, and being able to grow at low pH (Nasseri et al., 2011; Ritala et al., 2017). However, there are disadvantages to using, including low growth rates, low methionine contents, high contents of nucleic acid, and low cell digestibility are hindered which can limit the nutritional value (Alvarez & Enriquez, 1988).

1.2.2.2. Bacteria

Bacteria are known for their high growth rate, high protein, and essential amino acid content (Ravindra, 2000). The content of methionine is up to 3.0% which is higher than the other microorganisms (Øverland et al., 2010). Also, it provides a vitamin B group complex. However, small cell size makes it difficult to harvest after fermentation and contains high nucleic acid compared to other microorganisms (Nasseri et al., 2011). Also, there is risk of food poisoning by the presence of toxins produced by some gram-negative bacteria (Onyeaka et al., 2022).

1.2.2.3. Algae

Algae is known for providing high protein contents and fats (including ω-3 fatty acids and carotenoids), vitamins A, B, C, and E, mineral salts, and chlorophyll (Sousa et al., 2008). Also, it is known for its low nucleic acid

content (Nasseri et al., 2011). However, it has disadvantages of using as food, such as having cellulosic cell walls which can hardly be digested by humans and other non-ruminants and having a high concentration of heavy metals (Becker, 2007).

1.2.2.4. Fungi

Fungi have the advantage of the ease of growing, and harvesting with their and low water activity and large size (Onyeaka et al., 2022). It is known for its good composition of amino acids, which can be favorably compared with the FAO guidelines. High in lysine and threonine content but relatively low methionine content, although they still meet the FAO/WHO recommendations (Ritala et al., 2017). It is also known for providing vitamins, especially vitamin B group complex including biotin, choline, folic acid, glutathione, niacin, pantothenic acid, pyridoxine, riboflavin, thiamine and 4-aminobenzoic acid (Onyeaka et al., 2022). The content of the cell wall is rich in chitin and glucan which can contribute as fiber to the diet (Wiebe, 2002). However, it has the disadvantage of a low growth rate, and some fungi produce mycotoxins (Nasseri et al., 2011; Onyeaka et al., 2022).

1.2.3. The benefit of using microbial protein on environment

The livestock industry provides a valuable protein source to humans. However, it has negative environmental impacts, such as deforestation,

emission of greenhouse gases, water use, industrial waste and eutrophication (Poore & Nemecek, 2018). This limit the growth of the livestock industry which is the main source of conventional protein for human. In this context, microbial protein got the attention about being a potential alternative protein source for humans and animals in the future. Microbial protein has less impact on the environment compared to the present livestock industry (Huang & Kinsella, 1986). Also, it is known for its ability to utilize agricultural waste, industrial waste, food waste and natural gas like methane as the substrate which is regarded as a pollutant (Ritala et al., 2017; Tropea et al., 2022). Using these wastes for fermenting microbial protein has two functions which are reducing the pollutants and producing edible protein. In the previous research, the microbial protein was produced from biowastes, by a combination of anaerobic digestion and thermochemical gasification. The biowastes were converted to substrates (e.g., H_2, CH_4, CO_2, NH_3,) which can be used by fermentation of microbial protein (Matassa et al., 2020). Also, there are some studies about producing microbial protein via fermentation of food waste, such as wheat bran (Yunus et al., 2015), orange and lemon peel (Milala et al., 2018), and whey (Schultz et al., 2006). According to previous research, replacing 20% of per-capita ruminant meat consumption with microbial protein can reduce annual deforestation, lower CO_2 emissions roughly in half, and reduce methane emissions by 2050 (Humpenöder et al., 2022). This result represented microbial protein can be a sustainable protein source that can guarantee future food safety by

upcycling the carbon and nutrient from waste and low emission of greenhouse gas. The methods for microbial protein production using various substrates are shown in Figure 1.

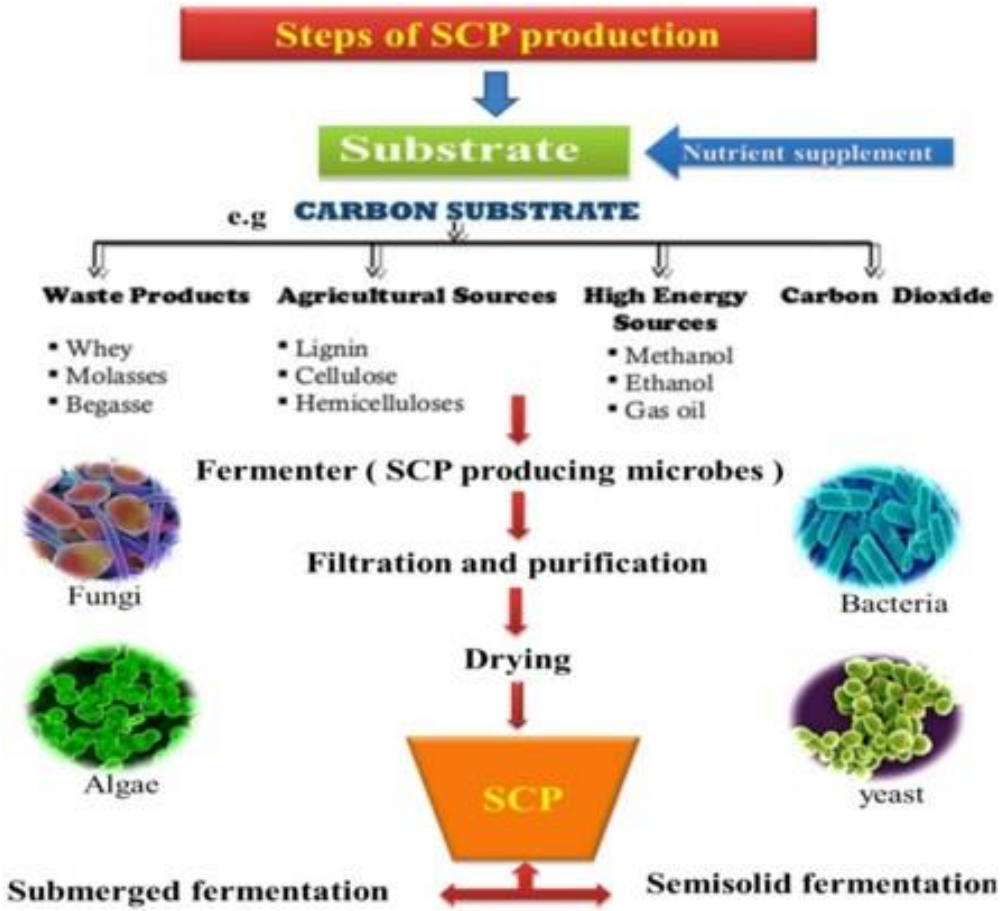


Figure 1. Method for producing the microbial protein with different types of substrates (Sharif et al., 2021).

1.3. Mycoprotein

Mycoprotein is a whole food source derived from the fermentation of fungi (Coelho et al., 2020). The conventional method of obtaining protein was feeding animals with carbohydrates and then getting the meat from their carcass, but protein from mycoprotein can be obtained by supplying carbohydrates to fungi (Finnigan, 2011). Less carbon, water, and land are used to obtain the same amount of protein in mycoprotein than animals (Nadathur et al., 2016; Ritchie et al., 2017). A comparison of greenhouse gas emissions and water use per from different protein sources is shown in Figure 2. Also, unlike animals, fungi can use agro-industrial wastes which include carbohydrate polymers such as cellulose, starch, proteins, lipids and other microelements (Yunus et al., 2015). In the previous research, some of the agro-industrial wastes were successfully used as substrates to produce mycoprotein, including pea-processing industry byproduct (Souza Filho et al., 2018), oats meal (Prakash et al., 2015), date juice (Hosseini & Khosravi-Darani, 2011), date waste (Reihani & Khosravi-Darani, 2018) and date sugar (Hosseini et al., 2009). This refers to mycoprotein produced by can be produced by a variety of inexpensive substrates, which can lead to low costs and high sustainability.

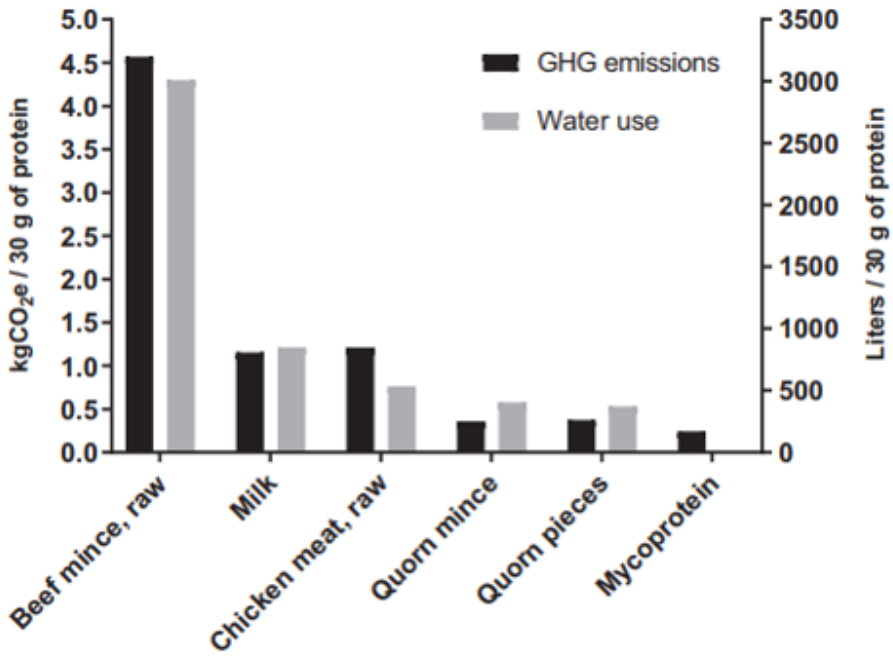


Figure 2. Comparison of greenhouse gas emissions and water use per production of 30g of protein from different protein sources (Coelho et al., 2020) **(kg CO_2e, kilograms carbon dioxide equivalent; GHG, greenhouse gas emissions).**

1.3.1. Mycoprotein: *Fusarium venenatum*

In the late 1960s, *Fusarium venenatum* A3/5 (KACC No 49797) was first discovered by Rank Hovis McDougall with the aim of finding the potential source of protein for human consumption (Finnigan et al., 2019). Among candidates, *F. venenatum* A3/5 showed a high level of protein, low odor, and low toxicity (King et al., 2018). To evaluate the safety of *F. venenatum* A3/5, a ten-years assessment program was conducted (Finnigan et al., 2019). To be used as food, mycoprotein should not contain mycotoxin. During the evaluation, some strains of *F. venenatum* produced toxins during fermentation. However, *F. venenatum* A3/5 did not produce any toxins under similar ferment conditions to others. In addition, it did not show any adverse effects on the immune or other symptoms with consumption (Finnigan et al., 2019). In 1985, the Ministry of Agriculture, Fisheries and Food, United Kingdom (MAFF, UK) approved the sale of mycoprotein derived from *F. venenatum* A3/5 in the United Kingdom (Finnigan et al., 2017). In 2002, it was notified as a generally recognized as safe (GRAS) strain by the Food and Drug Administration, United States (FDA, U.S.) (Bartholomai et al., 2022). After being accepted by FDA as GRAS in 2002, a mycoprotein product made by *F. venenatum* A3/5 has been sold in the United States and also sold in other countries worldwide (Finnigan et al., 2019).

1.3.2. Nutritional value of *Fusarium venenatum*

F. venenatum is known for its content of high-protein, high-fiber, low-

fat and relatively low-energy (Dunlop et al., 2017). *F. venenatum* is a high-quality protein source that can produce all essential amino acids and had a 0.996 score on a protein digestibility-corrected amino acid score (PDCAAS). It scored higher than chicken and beef and was very close to egg and milk which scored a maximum score of 1 (Derbyshire & Delange, 2021; Edwards & Cummings, 2010). Also, the previous study has demonstrated that the bioavailability of amino acids in *F. venentum* was similar to milk and higher than plant proteins (Coelho et al., 2021). The cell wall of *F. venenatum* comprises approximately one-third of chitins and two-thirds of β-glucan, suggesting that this can also be used as prebiotics in the intestine (Bottin et al., 2016; Finnigan, 2011). The previous study using *in-vitro* colonic models showed that this dietary fiber is fermentable and induces the production of short-chain fatty acids (SCFAs) such as acetate, propionate and butyrate (Harris et al., 2019). Previous research showed that intake of unsaturated fatty acids reduced the risk of cardiovascular diseases (Telle-Hansen et al., 2019). The fat composition of *F. venentum* showed high of monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs) but low in saturated fatty acids (SFAs) (Derbyshire & Ayoob, 2019). The PUFAs to SFAs ratio of beef was 0.1 (the ratio would reduce with the increase of fatness) at the same time, *F. venentaum* was 1.44 (Derbyshire & Delange, 2021). *F. venenatum* also provides micronutrients such as calcium, iron, manganese, sodium, phosphorus, selenium, zinc, vitamin B2, and vitamin D (Derbyshire & Delange, 2021).

1.3.3. Muscle development with *Fusarium venenatum*

To maintain and recondition skeletal muscle mass, an adequate amount of protein intake is necessary. The skeletal muscle mass is modulated through the balance of muscle protein synthesis and breakdown. Intake of protein increases the rate of muscle protein synthesis, mainly related to increased plasma amino acids, especially leucine (Rieu et al., 2006; Wall et al., 2013). Also, ingested protein induces the secretion of insulin which inhibits muscle protein breakdown (Louard et al., 1992). Therefore, these lead to anabolic states in the postprandial state and offset the muscle protein loss from the fasting state. *F. venenatum* is rich in protein and amino acids especially high in leucine (Coelho et al., 2020). The previous study with healthy young men who consumed protein-matched milk or *F. venenatum* (although approximately double mass and energy were consumed due to *F. venenatum* being a whole food source.) showed the equivalent of the bioavailability of essential amino acids and leucine in the hours after intake (Dunlop et al., 2017). These results showed that *F. venenatum* can help maintain and develop muscle. The effect of consuming *F.venenatum* on muscle synthesis is shown in Figure 3.

1.3.4. Antioxidant ability with *Fusarium venenatum*

A previous study revealed that *F. venenatum* contains a variety of unique metabolites such as flavonoids, hexadecane, phenolic compounds imidazole, and 2, 5 bis (1, 1-dimethylethyl) which are also known for

antioxidants and free-radical scavenging potential (Prakash & Namasivayam, 2014). According to the previous study, the intake of foods containing high flavonoids and phenolic compounds improved protective ability against the development of diabetes and cardiovascular diseases (Gorissen & Witard, 2018). The previous study to evaluate the antioxidant effects of *F. venenatum* showed that a variety of metabolites extracted from *F. venenatum* through acetonitrile and methanolic extraction showed high scavenging activity compared to standard ascorbic acid (Thomas et al., 2017). These results showed that *F. venenatum* could play the role of a natural antioxidant. The effect of consuming *F. venenatum* on antioxidant activity is shown in Figure 3.

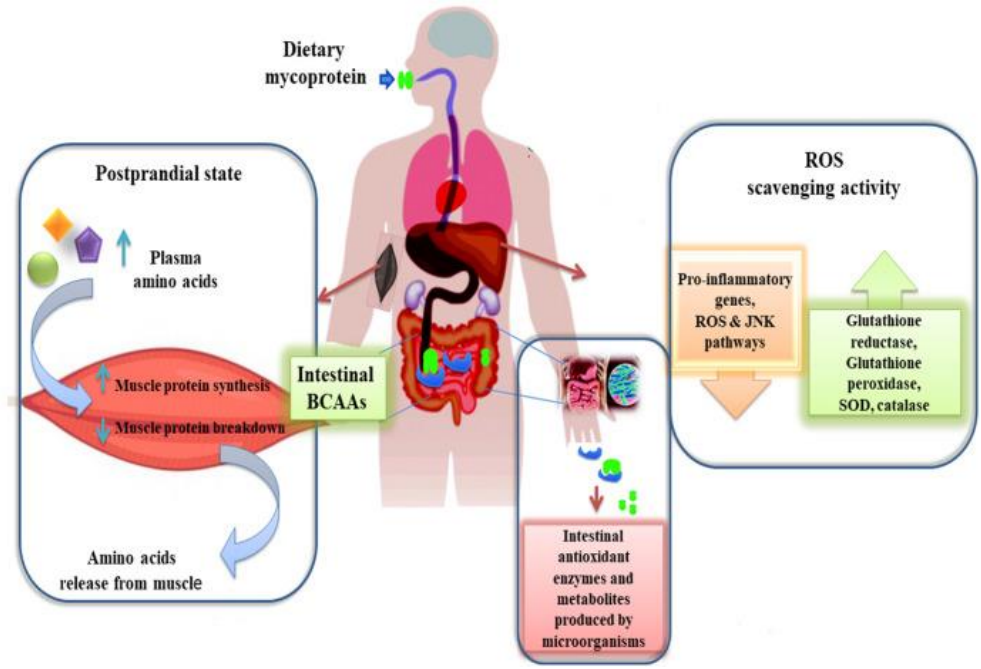


Figure 3. Impact of consuming *Fusarium venenatum* on muscle synthesis and antioxidant activity (Ahmad et al., 2022).

1.3.5. Improved lipid profile with *Fusarium venenatum*

The high fiber content of *F. venenatum* is known to help improve blood lipid levels and increase satiety (Turnbull et al., 1990; Williamson et al., 2006). According to the previous study, intake of *F. venenatum* which is rich in dietary fiber helped to sustain the blood insulin level and regulated the digestive system including delaying gastric emptying (Cherta-Murillo et al., 2020). The previous study on consuming isocaloric meals *ad libitum* containing *F. venenatum* or chicken in overweight volunteers showed that significant reduction in the energy intake and insulin concentrations in the group taking *F. venenatum* meal. However, there was no significant difference in appetite regulation hormone levels such as glucagon-like peptide-1 (GLP-1) and peptide tyrosine-tyrosine (PYY) (Bottin et al., 2016). According to previous studies, consuming *F. venenatum* increased high-density lipoprotein (HDL) and reduced low-density lipoprotein (LDL) (Turnbull et al., 1990). In the previous study, healthy adults were provided with a diet high in *F. venenatum* or fish and meat as a control diet. After 7 days of the experimental period the level of plasma total cholesterol (TC), low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL) were reduced in the group consumed the *F. venenatum* diet (Coelho et al., 2021). Study with healthy adults, consuming *F. venenatum* for six weeks significantly reduced the level of TC. Individuals with high TC at the baseline had a better reduction by *F. venenatum*. This result suggested that *F. venenatum* may be more useful to modulate blood TC levels in obese and

overweight subjects with type 2 diabetes and non-alcoholic fatty liver disease (Ruxton & McMillan, 2010). Studies with obese and overweight individuals showed that *F. venenatum* improved the levels of lipid biomarkers (Bottin et al., 2016; Dunlop et al., 2017). The *in-vivo* study using a Triton X-100-induced hyperlipidemic rat model showed intake of *F. venentuam* significantly reduced the plasma levels of TC, Triglycerides (TG), LDL and VLDL compared with control (Thomas et al., 2017). The *in-vitro* experiment using *F. venenatum* showed that the structure of *F. venenatum* may entrap digestive enzymes such as amylase, lipase and bile salts. This may lead to decreasing in starch hydrolysis and lipolysis (Colosimo et al., 2020). These results showed that *F. venenatum* could improve lipid profile. Overall, due to their high and good protein, high-fiber and low-fat, mycoproteins from *F. venenatum* are not only good candidates for meat substitutes but also can be considered as a functional food to treat obesity.

Chapter 2. Evaluation of the *Fusarium venenatum* in *Caenorhabditis elegans* using multi-omics analysis

2.1. Introduction

Protein is important for maintaining human life. It constitutes body structure and functional material such as enzymes, antibodies, and hormones which are necessary for a human being (Bohrer, 2017). In other words, humans have to consume an appropriate amount of protein to maintain a healthy life. Meat is a conventional protein source for humans. Meat is known for its high nutritional value, such as high quality of protein and amino acids. Also, it contains micronutrients, including iron, zinc, vitamin B, and selenium (Cosgrove et al., 2005; Davey et al., 2003; Godfray et al., 2018).

The global population continues to increase and is prospected to increase up to 9.2 billion by 2050 (Moreland et al., 2010). In 2050, to meet the demand for meat consumption, 1,250 tons of meat and dairy need to be produced per year (Ritala et al., 2017). Nowadays, one-third of the earth's ice-free land is utilized to raise livestock and many deforestations are being conducted to create pasture and arable land to raise livestock (Ramankutty

& Foley, 1999; Steinfeld et al., 2006). Due to global warming acceleration, greenhouse gas emissions, and animal welfare, it seems challenging to expand the livestock industry to meet the global demand in the future. In addition, it may be challenging to meet the demand due to the low efficiency of converting feed to meat. Therefore, developing alternative non-animal-derived protein sources is necessary for a nutritionally sustainable future.

These days obesity has become an epidemic (Organization, 2000; Wang & Lobstein, 2006). Worldwide obesity population has been increasing, with more than 600 million adults reported as obese and more than 1.9 billion as overweight (Caballero, 2019; Malik et al., 2013). Obese people can be easily exposed to several risks, such as type II diabetes, hypertension, coronary heart disease and cancers (Aneja et al., 2004; Calle & Thun, 2004; Dixon, 2010; Hossain et al., 2007; KEYS, 1980; Salter, 2013). The imbalance between energy intake and energy expenditure is known as causing obesity (Peng et al., 2016). Excessive consumption of meat which is a conventional protein source for humans, can induce an immoderate intake of energy and can lead to obesity (Salter, 2018). Previous research, proposed a positive correlation between meat consumption and obesity in U.S. adults (Wang & Beydoun, 2009). In the face of the increasing global population, meat consumption, environmental burden, and obese population, efforts are being made to develop food that can be supplied sustainably and play the role of an alternative protein source (Kumar et al., 2017; Smetana et al., 2015). Many companies and researchers have been developing different

types of alternative protein sources, and mycoprotein, made by the fermentation of fungi, is one of them (Nasseri et al., 2011; Peregrin, 2002).

Mycoprotein is known as a whole food source derived from the fermentation of fungi (Coelho et al., 2020). The method of getting protein from mycoprotein differs from getting protein from the conventional protein source meat. The method of getting protein from meat is feeding animals carbohydrates and then getting the meat from their carcass. However, protein from mycoprotein can be obtained by supplying carbohydrates to fungi (Finnigan, 2011). Compared to animals, mycoprotein needs less water and land to produce the same amount of protein. Also, fewer greenhouse gas emissions occur (Coelho et al., 2020; Nadathur et al., 2016; Ritchie et al., 2017). In addition, due to fungi's characteristics, various substrates can be used, including food byproducts which can lead to lower costs and increased sustainability (Hosseini & Khosravi-Darani, 2011; Hosseini et al., 2009; Prakash et al., 2015; Reihani & Khosravi-Darani, 2018; Souza Filho et al., 2018). *F. venenatum* contains high-protein, high-fiber, low-fat, and relatively low-energy (Dunlop et al., 2017). It can produce all essential amino acids and have 0.996 point in PDCAAS which is higher than chicken and beef (Derbyshire & Delange, 2021; Edwards & Cummings, 2010). The bioavailability of amino acids in *F. venentum* was higher than in plant proteins and close to milk (Coelho et al., 2021). *F. venenatum* also contains micronutrients such as calcium, iron, manganese, sodium, phosphorus, selenium, zinc, vitamin B2, and vitamin D (Derbyshire & Delange, 2021).

The nematode *Caenorhabditis elegans* (*C. elegans*) as an experimental model has a lot of advantages including cost-effectiveness, ease to handle, simple genetics, amenability to high-throughput screening and a transparent body (Backes et al., 2021). Although *C. elegans* and humans are evolutionarily different, many counterparts that play an important role in human metabolism came out to have a similar role in *C. elegans* metabolism (Wang et al., 2008). For example, fat and sugar transporters, nuclear hormone receptor (NHR) and sterol response element-binding protein (SREBP) transcriptional regulators, energy-sensing kinases such as AMP-activated kinase (AMPK) and TOR kinase and neuroendocrine regulators such as insulin and serotonin (Ashrafi, 2007). Although the fat regulation of *C. elegans* is different from that of mammals, the important metabolic pathways related to fat metabolism found in mammals are highly conserved in *C. elegans*, including fatty acid synthesis, elongation desaturation, and β-oxidation (Zheng & Greenway, 2012). For these reasons, *C. elegans* is known and used as an ideal surrogate animal model for research (Lin et al., 2019; C. Lin et al., 2020; Yue et al., 2019).

Currently, to prevent future protein deficiency in advance, many companies and researchers are seeking potential alternative protein sources to replace conventional protein source meat. In this study, we investigate whether *F. venenatum* can serve as an alternative protein source based on high-quality protein content. Also, we investigate the effect of *F.venenatum* on fat metabolism.

2.2. Materials and methods

2.2.1. Nutritive value

2.2.1.1. Nutritional composition

Fusarium venenatum is a filamentous fungus that can form mycoprotein (Coelho et al., 2020). The mycoprotein derived from the fermentation of *F. venenatum* is high in protein, high in fiber, low in fat, and low in energy content (Finnigan, 2011). *F. venenatum* KACC No.49797 (strain A3/5) was received from the National Institute of Agricultural Sciences (Korea).

2.2.1.2. Mycotoxin quantification assay

For mycotoxin quantification assay, zearalenone, fumonisin B1, fumonisin B2, and deoxynivalenol was quantified in this study. Briefly, zearalenone, fumonisin B1, and fumonisin B2 were quantified with HPLC-MS/MS methods. For extraction, 2~5 g of homogenized *Fusarium venenatum* was added to 20 ml of extracting solution (50% acetonitrile solution containing 0.1% of formic acid). The volume of this solution referred to as V1. After 30 min extracting period, it was centrifuged at 3700 G for 10 min. The extracted solution was filtered with filter paper (Whatman GF/A). The volume of filtered solution is referred to as V2. After that 3 ml of V2 solution was collected and water was added to reach 15 ml

total. This total volume of solution is referred to as V3. For Refinement, to activate the solid-phase extraction cartridge, 2 ml of Acetonitrile and 2 ml of water were dropped. 5 ml of V3 solution was dropped after. The amount of dropped solution of V3 referred to as V4. Subsequently, 2 ml of water and 2 ml of 10% of acetonitrile were dropped same speed as the previous one to remove all the remaining solutions in the solid-phase extraction cartridge. To dissolve, 2 ml of Acetonitrile solution containing 0.1% of formic acid and 4 ml of methanol were dropped and then dried with nitrogen at 50°C. 0.5~1 ml of 50% methanol solution containing 0.1% of formic acid was added to the dried material. The amount of added solution is referred to as V5. The solution was filtered with a filter (Whatman® membrane filters PTFE). LC-MS/MS was used for further determination. The separation was conducted using a C18 column (3 mm × 150 mm, 3 μm). The temperature of column was 40°C.

Amount of mycoprotein(μg/kg) = C * (V1 / S) * (V3 / V2) * (V5 / V4)

C: Content of mycotoxin obtained from the calibration curve (ng/mL)

S: Sample amount (g)

Deoxynivalenol was quantified with HPLC-UV method. For extraction, 20 g of homogenized sample was mixed with 100 ml of water and then homogenize for 5 min using homogenizer. The homogenized sample was centrifuged with 10000G for 20 min and the supernatant was filtered with

glass fiber filter (Whatman GF/A). For the refinement, 2ml of filtered solution was dropped for 1 drop per sec to the deoxynivalenol immunoaffinity column. 5 ml of water was dropped same speed as the previous one. 3 ml of acetonitrile was added to dissolve and the dissolved solution was dried with nitrogen at 50°C. The residue was dissolved in 1mL of mobile phase (acetonitrile:water 17:83, v/v). Before determination, the solution was filtered with a 0.45 μm membrane filter. LC-MS/MS was used for further determination. The separation was conducted using a C18 column (4.6mm × 250 mm, 5 μm).

Amount of mycoprotein(mg/kg) = C * V/S * D

C: Content of deoxynivalenol obtained from the calibration curve (μg/mL)

V: Final volume of test solution

S: Sample amount (g)

D: Dilution multiplies of test solution

2.2.2. Functional experiment using *C. elegans*

2.2.2.1. Bacterial strains and culture conditions

Salmonella Typhimurium SL1344 was grown at 37°C for 24 h in nutrient broth medium (BD Biosciences, Sparks, MD, USA). *Staphylococcus aureus* Newman and *Listeria monocytogenes* EGD-e were grown at 37°C for 24 h in brain heart infusion (BHI) broth medium (BD Biosciences, Sparks, MD, USA). *Escherichia coli* O157:H7 EDL933 and

Escherichia coli OP50 (OP50) were grown at 37°C for 24 h in Luria–Bertani (LB) broth medium (BD Biosciences, Sparks, MD, USA). To prepare live bacterial lawns for *C. elegans* feeding, bacteria were collected by centrifugation at 6000 rpm for 10 min. Then resuspended in a sterile M9 buffer. After repeating centrifugation and resuspending three times it was plated on nematode growth medium (NGM) plates.

2.2.2.2. *C. elegans* culture condition

The *C. elegans* strain *fer-15;ferm-1* mutant was used because they are unable to produce progeny at 25°C without alteration in the *C. elegans* phenotype (Park et al., 2018). Worms were routinely maintained on NGM agar using standard techniques (Brenner, 1974). Eggs were extracted in sodium hypochlorite–sodium hydroxide solution. Synchronized L1 worms were grown on NGM plates seeded with OP50 at a restrictive temperature (25°C) to obtain sterile L4/young adult worms.

2.2.2.3. *C. elegans* lifespan and killing assays

To evaluate whether *F. venenatum* effect on *C. elegans* lifespan and immune response against pathogenic bacteria, we established methods with slight modification with a previous study method (Kim et al., 2021; Park et al., 2018).

To perform a lifespan assay, L1 or L4 stage of *C. elegans* strain *fer-15;fem-1* were individually transferred with a platinum wire onto 35-mm-

diameter NGM agar plates with 100μL of OP50 (OD:5; ca. 8.0 x 10^9 CFU/mL) or OP50 (final concentration OD: 5) + *F. venenatum* (20 mg/ml). The selection of a concentration of *F. venenatum* (20 mg/ml) which we used in this study was based on the previous study (Colosimo et al., 2020). For lifespan assay using extracted protein, the protein was extracted using a sonicator (KUS-650, KSA). Extracted protein was prepared with three concentrations of 0.5 mg/ml, 0.75 mg/ml and 1 mg/ml. NGM agar plates with 100 μL of OP50 (OD:5; ca. 8.0 x 109 CFU/mL) or OP50 (final concentration OD: 5) + extracted proteins were prepared. For each lifespan assay, 90 *C. elegans* per treatment were assayed in three plates (30 worms per plate) and incubated at 25°C. The numbers of live *C. elegans* were counted daily and transferred to a new plate every 2 days. Determination of alive or dead *C. elegans* was conducted by touching gently with a platinum wire and the assay conducted until all *C. elegans* died.

To perform a killing assay, L4 stage *C. elegans* were placed onto 35-mm-diameter NGM agar plates with 100 μL of OP50 (OD: 5) or OP50 (Final concentration OD: 5) + *F. venenatum* (20 mg/ml). After preconditioning for 48 hours, transferred to NGM agar plates with pathogenic bacteria including *Salmonella* Typhimurium SL1344 (OD: 5), *Staphylococcus aureus* Newman (OD: 5), *Escherichia coli O157:H7 EDL933* (OD: 5), and *Listeria monocytogenes* EGD-e (OD: 5) then incubated at 25°C. For each killing assay, 90 *C. elegans* per treatment were assayed in three plates (30 worms per plate) and incubated at 25°C. Live *C.*

elegans were measured daily and transferred to a new plate every 2 days. *C. elegans* were gently touched with a platinum wire to determine between alive and dead. The assay was conducted until all *C. elegans* died.

2.2.2.4. *C. elegans* behavior and body size

Locomotive activity and body size was measured by using the Wormlab software (MBF Bioscience, Vermont, USA) as previously reported (Sun et al., 2016; Yue et al., 2019). Briefly, *C. elegans* were transferred to a fresh OP50 seeded low peptone NGM plate and allowed to acclimate for 10 min before filming. Each video was taken for 1 min for tracking analysis. Width, length, and locomotive activity (peristaltic speed (μm/s)) were measured. At least 10 worms in each group were measured and experiments were repeated three times. Pharynx pumping rate was measured to estimate food intake by using a stereomicroscope as previously described (Farias-Pereira et al., 2020). The pumping rate was monitored by counting the pharyngeal contraction for 30 sec. At least 10 worms in each group were measured and experiments were repeated three times.

2.2.3. Evaluation of fat accumulation in *C. elegans*

2.2.3.1. Nile red staining

Nile red staining was performed as in the scheme established in the previous study with slight modification (Escorcia et al., 2018). When *C. elegans* (*fer-15;fem-1*) reached L4 stage, transferred to plates with OP50

(OD: 5) or OP50 (Final concentration OD: 5) + *F. venenatum* (20 mg/ml) for 48 hours. After 48 hours, *C. elegans* were gathered with PBST (1x phosphate-buffered saline + 0.01% Triton X-100) into 1.5 mL microfuge tube. Then washed with PBST for twice and centrifuged 3500rpm 1 min to remove the supernatant. 500 µL of 40% isopropanol was added to the worm pellet and incubated at room temperature for 5 min. Centrifuged 3500rpm 1min to remove the supernatant. 600 µL of Nile red working solution (Add 6 µL of Nile red stock solution in 1 ml of 40% isopropanol) was added to each sample and inverted tubes three times to fully mix the Nile red working solution with *C. elegans*. The sample was rotated in the dark at room temperature for 2 hours. Then centrifuged 3500rpm 1 min and removed supernatant. 5 µL of *C. elegans* suspension was placed on a microscope slide with 2% agarose gel and put on the coverslip carefully to avoid any air bubbles and breaking *C. elegans* body. *C. elegans* was photographed with Olympus IX53 microscope (Olympus, Tokyo, Japan). At least 10 worms in each group were measured and experiments were triplicated. Images were measured by ImageJ software.

2.2.3.2. Oil red O staining

Oil red O staining was performed with a slight modification of the previous studies (Ramirez-Zacarias et al., 1992; Yen et al., 2010). *C. elegans* were exposed to OP50 (OD: 5) or OP50 (final concentration OD: 5) + *F. venenatum* (20 mg/ml) for 48 hours. After 48 hours exposures period, *C.*

elegans were stained with VitroView™ Oil Red O stain kit (Cat. NO VB-3007) with slight modification. *C. elegans* were gathered into a 1.5 mL microfuge tube and washed with PBS twice then centrifuged 3500rpm 1 min to remove the supernatant. 100 µl of 40% isopropanol was added to the worm pellet and incubated at room temperature for 3 min. After incubation, *C. elegans* were centrifuged at 3500rpm for 1 min then the supernatant was removed. 500 µl of pre-stain solution was added for 2 mins then the supernatant was removed after 3500rpm 1 min centrifugation. 500 µl of pre-warmed Oil Red O solution was added then incubated for 8-10 minutes in a 60ºC oven then the supernatant was removed after 3500rpm 1min centrifugation. 500 µl of pre-warmed differentiation solution was added then incubated for 2-5 minutes in 60ºC oven. After this procedure, *C. elegans* were washed twice then centrifuged 3500rpm 1 min to remove the supernatant. 5 µL of *C. elegans* suspension was placed on a microscope slide with 2% agarose gel and put on the coverslip carefully to avoid any air bubbles and breaking *C. elegans* body. *C. elegans* was photographed with Olympus IX53 microscope (Olympus, Tokyo, Japan). At least 10 worms in each group were measured and experiments were triplicated. Images were measured by ImageJ software.

2.2.4. Transcriptomic analysis

Transcriptomic analysis was performed with a slight modification of the previous study (Ryu et al., 2021). L4 stage *C. elegans* (*fer-15;fem-1*)

were fed with OP50 (OD: 5) or OP50 (final concentration OD: 5) + *F. venenatum* (20 mg/ml). Total RNA from worms was immediately extracted to examine gene expression in the host using Trizol reagent (Invitrogen, Carlsbad, CA, USA) and purified using a RNeasy Mini Kit (QIAGEN, Valencia, CA, USA) based on the manufacturer's instructions. For RNA-seq, a TruSeq RNA Sample Prep Kit v2 (Illumina, San Diego, CA, USA) was used based on the manual, and the cDNA library was made based on the basic protocol provided by Illumina. Libraries were then sequenced on an Illumina HiSeq 2000 platform with paired end read sequencing (2 × 150 bp). Using Trimmomatic 0.38 (Bolger et al., 2014), the adapter sequence, base quality less than 3 from the ends of the reads, bases not satisfying the window size = 4 and mean quality = 15 were removed. Then, trimmed data were generated, with reads shorter than 36 bp removed, and further analysis was performed based on high-quality reads. The index of the reference genome was generated using the Hisat2 v2.1.0 program (https://daehwankimlab.github.io/hisat2/ accessed on 4 November 2020) (Kim et al., 2015). Next, uniquely mapped reads were quantified with Subread/featureCounts version v1.5.1 (http://subread.sourceforge.net/ accessed on 4 November 2020) (Liao et al., 2014), using Ensembl version 82 transcriptome definitions. R package edgeR was used to analyze different expressions between different types of samples in generated data (Robinson et al., 2010). To define genes that are significantly different in expression, the threshold value of |log2-fold change > 1| and p-value < 0.05 were used.

To identify the function of differentially expressed genes, the DAVID online tool (Dennis et al., 2003) and Cytoscape (Shannon et al., 2003) were used.

2.2.5. Metabolomic analysis

Metabolomic analysis was conducted with a slight modification of a previous study. (Yoo et al., 2022). Briefly, L4 stage worms were fed with OP50 or OP50 (final concentration OD: 5) + *F. venenatum* (20 mg/ml) for 48 h and then washed with sterile deionized water to remove bacteria and fungi on their body. Then worms were mechanically disrupted using a pestle (Kontes Glass, Vineland, NJ, USA). After disruption, each sample was mixed with ice-cold methanol and vortexed vigorously on the ice for 1 min. The vortexed sample was centrifuged at 10,000×g for 10 min at 4°C. The supernatants were filtered with 0.2 μm polyvinylidene fluoride syringe filters (Whatman, Maidstone, UK) and vacuum dried. For gas chromatography-mass spectrometry (GC-MS) analysis, 30 μL of methoxyamine hydrochloride (Sigma-Aldrich, St. Louis, MO, USA) in pyridine (20 mg/mL), was added to each sample to dissolve. After dissolving, they were incubated at 30°C for 90 min to derivatize. Subsequently, for trimethylsilylation, each sample was added with 50 μL N,O-bis(trimethylsilyl)trifluoroacetamide (Sigma-Aldrich) and incubated at 60°C for 30 min. Then 10 μL of fluoranthene (Sigma-Aldrich) was added.

GC-MS analysis was performed using a TRACE™ 1310 Gas Chromatograph (Thermo Fisher Scientific, Waltham, MA, USA) with an

ISQ LT single quadrupole mass spectrometer (Thermo Fisher Scientific), and separation was conducted using a DB-5MS column (60 m × 0.25 mm, 0.25-um film thickness, Agilent, Santa Clara, CA, USA). The oven temperature was held at 50°C for 2 min, constantly increased at 5°C/min, held at 180°C for 8 min, gradually increased at 2.5°C/min to 325°C, and then held for 10 min. The sample was injected at 300°C, and helium was used as the carrier gas, with a flow rate of 1.5 mL/min and a split ratio of 1:60. For GC-MS detection, an electron ionization system with an ionization voltage of 70 eV has used and the temperature of the ion source was 270°C. The mass scan range was set at 30–450 (m/z), and the acquisition rate was 5 spectra/s. NIST Mass Spectral Search Program (version 2.0; National Institute of Standards and Technology [NIST], Gaithersburg, MD, USA) was used to identify detected metabolites. Further analyses were performed using MetaboAnalyst 5.0 (Pang et al., 2021).

2.2.6. Statistics

C. elegans lifespan and killing assay data were analyzed with the Kaplan-Meier method and graphed SigmaPlot 12.0 (Systat Software Inc.). Other data were statistically analyzed and graphed with using Prism 9 (Graphpad Software, USA). Statistical significance was considered when p value was below 0.05 (*), 0.01 (**), 0.001 (***), 0.0001 (****). All data are expressed as means ± the standard error of the mean (SEM).

2.3. Results

2.3.1. Nutritional value

2.3.1.1. Nutritional composition

Mycoprotein derived from *Fusarium venenatum* fermentation is known for its content high in protein, high in fiber, low in fat, and low in energy (Finnigan, 2011). The nutritional composition of *Fusarium venenatum* KACC No.49797 (strain A3/5) is shown in Table 2.

Table 2. Nutritional composition of *Fusarium venenatum* KACC No.49797 (strain A3/5)

Items	**Unit**	***F.venenatum* KACC No.49797**
Moisture	g/100g	0.00
Ash		3.24
Crude Protein		22.26
Crude Fat		20.24
Carbohydrate		54.26
Crude Fiber		45.64

2.3.1.2. Mycotoxin quantification assay

Fusarium species are known for producing mycotoxins such as fumonisin, zearalenone, and trichothecene (Ekwomadu et al., 2021). Once mycotoxin is produced, it remains in food and feed because it's chemically stable. Deoxynivalenol is one of the main toxins belonging to the trichothecene family. We performed a mycotoxins quantification assay to quantify mycotoxins, including fumonisin, zearalenone, and deoxynivalenol in *F. venenatum*. The result showed that *F. venenatum* produces low mycotoxins (Table 3).

Table 3. Mycotoxin content of *Fusarium venenatum* KACC No.49797 (strain A3/5)

Mycotoxin	Unit	Method	Limit of quantification	Result
Fumonisins B1	μg/kg	HPLC-MS/MS	4	8.60
Fumonisins B2	μg/kg	HPLC-MS/MS	4	Non-detected
Zearalenone	μg/kg	HPLC-MS/MS	5	Non-detected
Deoxynivalenol	mg/kg	HPLC/UV	0.2	Non-detected

2.3.2. Functional experiment using *C. elegans*

2.3.2.1. Lifespan assay

We first evaluated whether *F. venenatum* affects the lifespan in *C. elegans* L1 (Larval) and L4 (Young adult) stages. The group of *C. elegans* fed with OP50 is referred to as OP50 and the group of *C. elegans* fed with OP50+ *F. venenatum* (20 mg/ml) is referred to as F.V. F.V showed a significantly improved lifespan at both L1 and L4 stages compared with OP50 which was used as a control in this study (Figure 4A) ($p = 0.000$ for L1 stage and $p = 0.0001$ for L4 stage)

Next, to examine whether protein extracted from *F. venenatum* affects the lifespan of *C. elegans*, we extracted protein from *F. venenatum*. Protein was extracted with a sonicator and three different concentrations of extracted protein were prepared (0.5 mg/mL, 0.75 mg/mL, 1 mg/mL). Extracted proteins were fed to *C. elegans* with OP50. In this study, we confirmed that extracted protein concentrations of 0.75 mg/ml and 1mg/mL significantly enhanced the lifespan of *C. elegans* compared to OP50 ($p = 0.0000$ for both concentrations). However, the extracted protein concentration of 0.5 mg/mL had not shown any significant difference with OP50 (Figure 4B) ($p = 0.1500$). Therefore, these results indicated that intact *F. venentaum* and extracted protein concentrations of more than 0.75 mg/mL can significantly improve the lifespan of *C. elegans in vivo*.

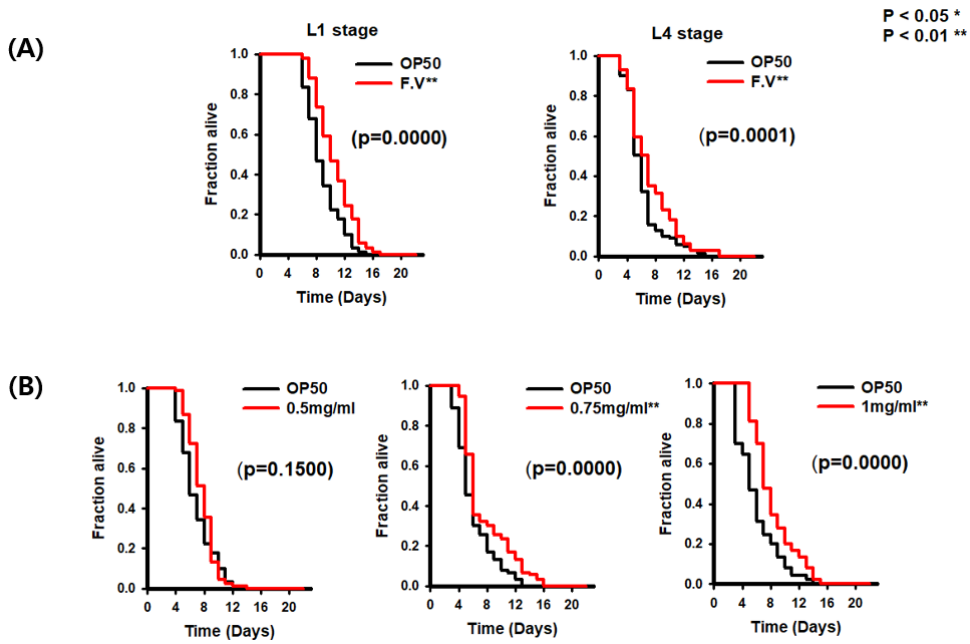


Figure 4. Lifespan assay of *Caenorhabditis elegans* fed with *Fusarium venenatum* and extracted protein.

(A) Enhanced lifespan of *C. elegans* via treating *F. venenatum* compared to *E. coli* OP50 starting from the L1 stage and L4 stage. Survival statistics compared to *C. elegans* fed with *E. coli* OP50: p = 0.0000 for *C. elegans* fed with *F. venenatum* starting from the L1 stage (left) and p = 0.0001 for starting from the L4 stage (right). (B) Improved lifespan of *C. elegans* via feeding extracted protein from *F. venenatum* compared to *E. coli* OP50. Survival statistics compared to *C. elegans* fed with *E. coli* OP50: p = 0.1500 for *C. elegans* fed with concentration 0.5 mg/ml (left), p = 0.0000 for concentration 0.75 mg/ml (middle) and p = 0.0000 for concentration 1 mg/ml (right) of extracted protein from *F. venenatum*. Statistical analysis was performed compared to OP50 using Kaplan-Meier method, and

differences were considered significant when p value was below 0.05 (*), 0.01 (**). OP50 group, received *E. coli* OP50; F.V group, received *E. coli* OP50 with *F. venenatum*; 0.5 mg/ml group, received *E. coli* OP50 with 0.5 mg/ml of extracted protein from *F. venenatum*; 0.75 mg/ml group, received *E. coli* OP50 with 0.75 mg/ml of extracted protein from *F. venenatum*; 1 mg/ml group, received *E. coli* OP50 with 1 mg/ml of extracted protein from *F. venenatum*.

2.3.2.2. Killing assay

A Killing assay was performed to evaluate whether *F. venenatum* affects the host immune response. After preconditioning for 48 hours, they were exposed to foodborne pathogenic bacteria. First, we used *Salmonella* Typhimurium SL1344 and *Escherichia coli* O157:H7 EDL933 as gram-negative pathogenic bacteria. F.V showed a significant protective effect against *S.* Typhimurium SL1344 ($p = 0.0048$) and *E. coli* O157:H7 EDL933 ($p = 0.0309$) compared to OP50 (Figure 5A, 5B). *Listeria monocytogenes* EGD-e and *Staphylococcus aureus* Newman was used as gram-positive bacteria. The viability of *C. elegans* in F.V was significantly enhanced compared to OP50 when *L. monocytogenes* EGD-e ($p = 0.0000$) and *S. aureus* Newman ($p = 0.0126$) were used as pathogenic bacteria (Figure 5C, 5D). Taken together, the results indicated that *F. venenatum* confers resistance to infection by gram-negative bacteria and gram-positive bacteria.

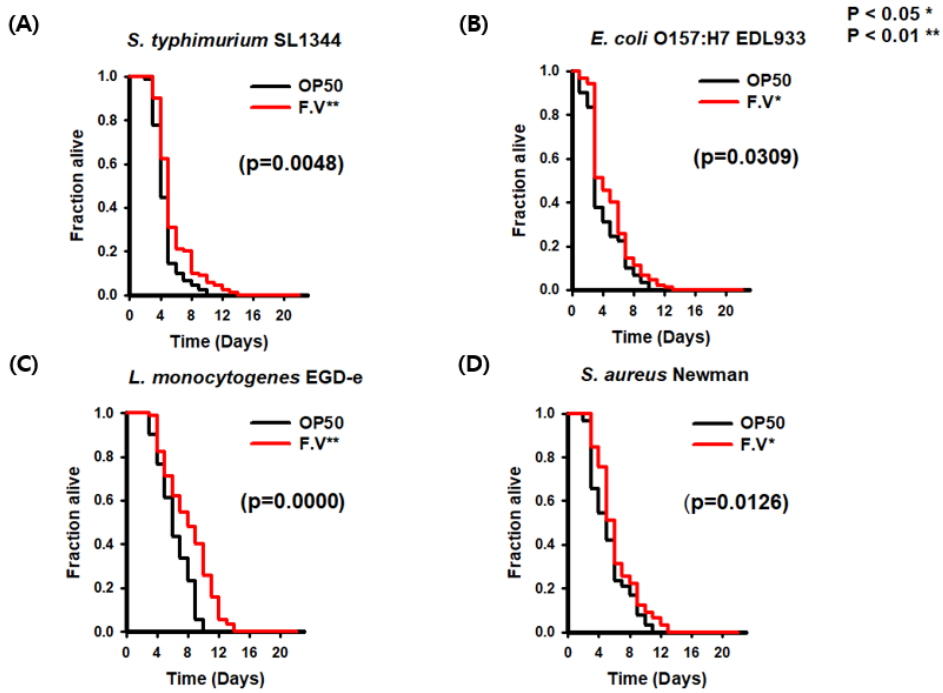


Figure 5. Killing assay of *Caenorhabditis elegans* fed with *Fusarium venenatum* with four-foodborne pathogenic bacteria (Two of each gram-negative and gram-positive bacteria)

(A, B) *C. elegans* fed with *F. venenatum* showed increased immune ability against gram-negative bacteria compared to *C. elegans* fed with *E. coli* OP50. Survival statistics compared to *C. elegans* fed with *E. coli* OP50: p = 0.0048 for *C. elegans* fed with *F. venenatum* against *Salmonella* Typhimurium SL1344 and p = 0.0309 for *C. elegans* fed with *F. venenatum* against *Escherichia coli* O157:H7 EDL933. (C, D) *C. elegans* fed with *F. venenatum* demonstrated enhanced resistance against gram-positive bacteria. Survival statistics compared to *C. elegans* fed with *E. coli* OP50: p = 0.0000 for *C. elegans* fed with *F. venenatum* against *Listeria monocytogenes* EGD-e

and p = 0.0126 for *C. elegans* fed *with F. venenatum* against *Staphylococcus aureus* Newman. Statistical analysis was performed compared to OP50 using Kaplan-Meier method, and differences were considered significant when p value was below 0.05 (*), 0.01 (**). OP50 group, received *E. coli* OP50; F.V group, received *E. coli* OP50 with *F. venenatum*.

2.3.2.3. Body size

Body size was measured to evaluate whether feeding *F. venenatum* changes the phenotype of *C. elegans*. After an exposure period of 48 hours, length and width were measured. The F.V showed significantly decreased length and width compared to OP50 (Figure 6A, 6B) ($p < 0.0001$ for length and $p < 0.0001$ for width). Taken together, *F. venenatum* reduced the body size, which may refer to reduced fat accumulation.

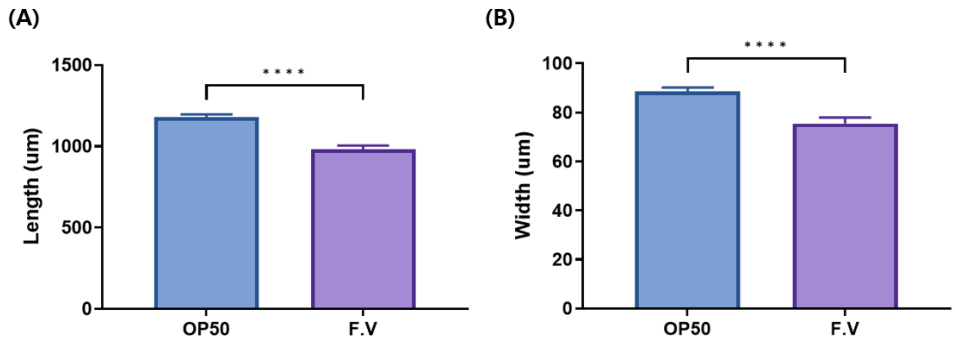


Figure 6. Length and width of *Caenorhabditis elegans* fed with *Fusarium venenatum*

(A) Length of *C. elegans* reduced by feeding *F. venenatum* compared to *E. coli* OP50 ($p < 0.0001$). (B) Width of *C. elegans* reduced by feeding *F. venenatum* compared to *E. coli* OP50 ($p < 0.0001$). Data are expressed as means ± standard errors. Statistical analysis was performed using t-test and differences were considered significant when p value was below 0.05 (*), 0.01 (**), 0.001 (***), 0.0001 (****). OP50 group, received *E. coli* OP50; F.V group, received *E. coli* OP50 with *F. venenatum*.

2.3.3. Evaluation of fat accumulation in *C. elegans*

2.3.3.1. Quantification of fat accumulation

To reveal the effect of *F. venenatum* on fat regulation, Nile red staining and Oil red O staining were used in this study. Lipid accumulation was examined after 48 hours exposure period. In Nile red staining, F.V showed significantly less fat accumulation compared to OP50 (Figure 7A, 7B) ($p < 0.0001$). A similar result was presented in Oil red O staining (Figure 7C, 7D) ($p = 0.0006$). In summary, these results indicated that *F. venenatum* reduce fat accumulation in *C. elegans.*

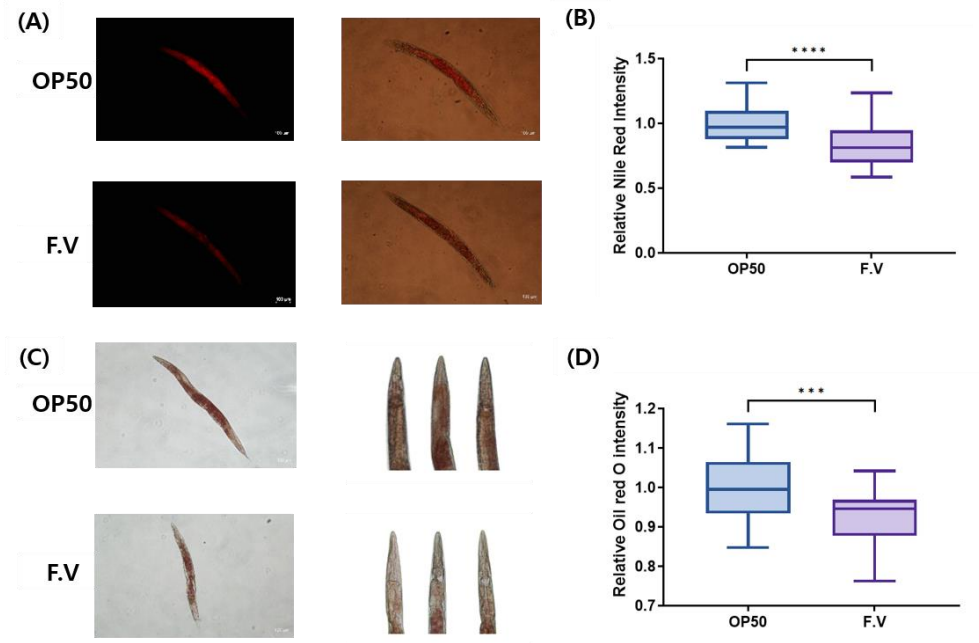


Figure 7. Fat measurement of *Caenorhabditis elegans* fed with *Fusarium venenatum* using Nile red staining and Oil red o staining

(A, B) Nile red staining showed *C. elegans* fed with *F. venenatum* reduced fat accumulation compared to *C. elegans* fed with *E. coli* OP50. (A) Nile red staining (left), Merged (right). (B) Quantification of Nile red intensity reduced in *C. elegans* fed with *F. venenatum* compared to those fed with *E. coli* OP50 ($p < 0.0001$). (C, D) Oil red o staining demonstrated that *C. elegans* fed with *F. venenatum* reduced fat accumulation compared to *C. elegans* fed with *E. coli* OP50. (C) whole-body image of Oil red o-stained *C. elegans* (left), Head image of Oil red o-stained *C. elegans* (right). (D) Quantification of Oil red o intensity reduced in *C. elegans* fed with *F. venenatum* compared to fed with *E. coli* OP50 ($p = 0.0006$). Data are expressed as Min to Max. Statistical analysis was performed using t-test and

differences were considered significant when p value was below 0.05 (*), 0.01 (**), 0.001 (***), 0.0001 (****). Microscope data are shown at x100 magnification. OP50 group, received *E. coli* OP50; F.V group, received *E. coli* OP50 with *F. venenatum*.

2.3.3.2. *C. elegans* behavior

Peristaltic speed (energy expenditure) and pumping rate (feed intake) were determined after 48 hours exposure period. Peristaltic speed and pumping rate were measured to confirm whether the reduced fat accumulation was due to increased energy expenditure or reduced feed intake, respectively. The result showed that there was no significant difference in peristaltic speed and pumping rate between OP50 and F.V (Figure 8A, 8B) ($p = 0.0967$ for peristaltic speed and $p = 0.0573$ for pumping rate). Taken together, these results suggested that reduced fat accumulation by *F. venenatum* was not likely due to the change in energy expenditure or feed intake, which may be related to another mechanism, such as altered fat metabolism.

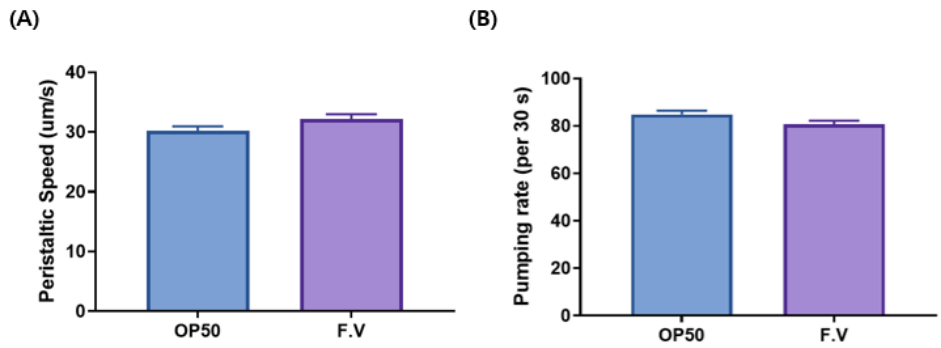


Figure 8. Peristaltic speed and pumping rate of *Caenorhabditis elegans* fed with *Fusarium venenatum*

(A) Peristaltic speed has no significant difference between *C. elegans* fed with *F. venenatum* or *E. coli* OP50 (p = 0.0967). (B) Pumping rate has no significant difference between *C. elegans* fed with *F. venenatum* or *E. coli* OP50 (p = 0.0573). Data are expressed as means ± standard errors. Statistical analysis was performed using t-test and differences were considered significant when p value was below 0.05 (*), 0.01 (**), 0.001 (***), 0.0001 (****). OP50 group, received *E. coli* OP50; F.V group, received *E. coli* OP50 with *F. venenatum*.

2.3.4. Transcriptomic analysis

2.3.4.1. Regulated gene related to fat metabolism

Due to no difference in energy expenditure and energy intake we performed whole-transcriptomic analysis to identify changed fat metabolism via feeding *F. venenatum* at the molecular level. Total RNA was extracted after 48 hours of the exposure period. We found many gene expressions related to fat metabolism were regulated by *F. venenatum.* The genes *pod-2* and *fasn-1* which are related to the fat synthesis pathway were downregulated with feeding *F. venenatum* (Figure 9A). On the other hand, a large portion of genes that are related to mitochondria and peroxisome's fatty acid breakdown pathway were significantly upregulated (Figure 9B). In summary, our results suggested that *F. venenatum* downregulates the fat synthesis pathway and upregulates the fat breakdown pathway which may result in the inhibition of fat storage.

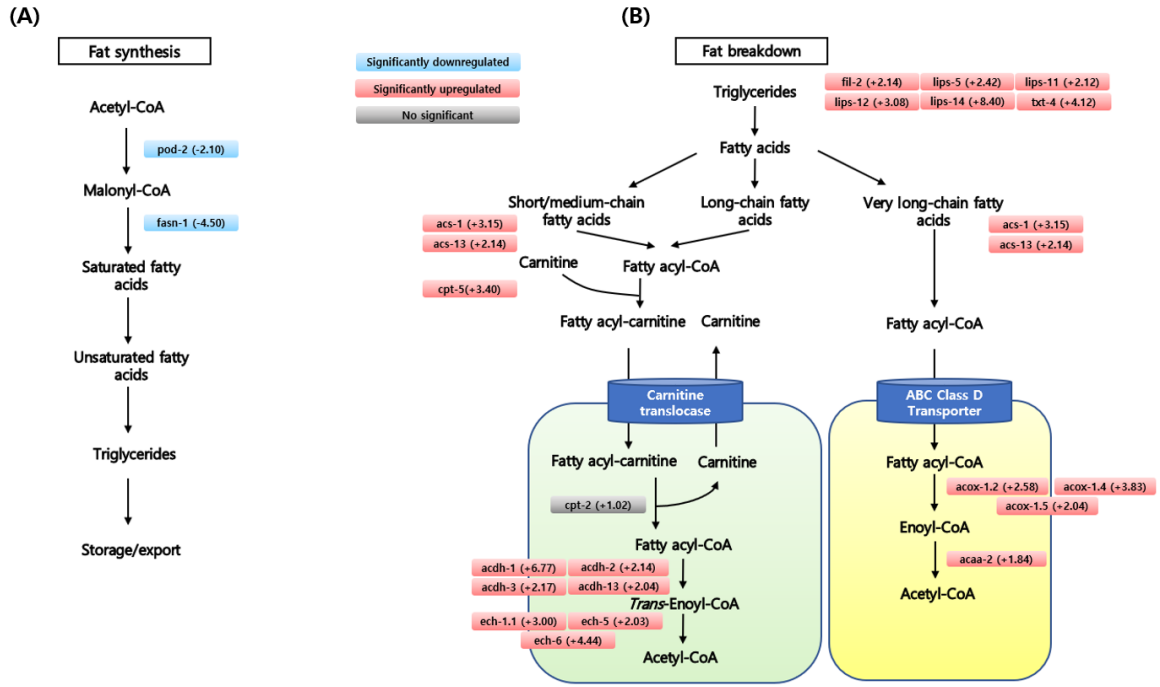


Figure 9. Regulated genes related to fat synthesis and breakdown pathway in *Caenorhabditis elegans* fed with *Fusarium venenatum*

(A) Significantly downregulated genes related to the fat synthesis pathway in F.V compared to OP50. (B) Significantly upregulated genes related to fat breakdown pathway in F.V compared to OP50. OP50 group, received *E. coli* OP50; F.V group, received *E. coli* OP50 with *F. venenatum*.

2.3.4.2. Visualized and analyzed gene network

Cytoscape is used to identify the function of differentially expressed genes (Shannon et al., 2003). We used significantly upregulated genes via feeding *F. venenatum* to map the upregulated pathway (Figure 10A). We found many pathways were upregulated via feeding *F. venenatum* including FoxO signaling pathway, tryptophan metabolism, and glutathione metabolism. We used significantly downregulated genes to map the downregulated pathway (Figure 10B). Fatty acid biosynthesis, cysteine and methionine metabolism, and Drug metabolism were downregulated via feeding *F. venenatum*. Taken together, altered gene expressions by *F. venenatum* are related to improvement in longevity and reduction of fat accumulation.

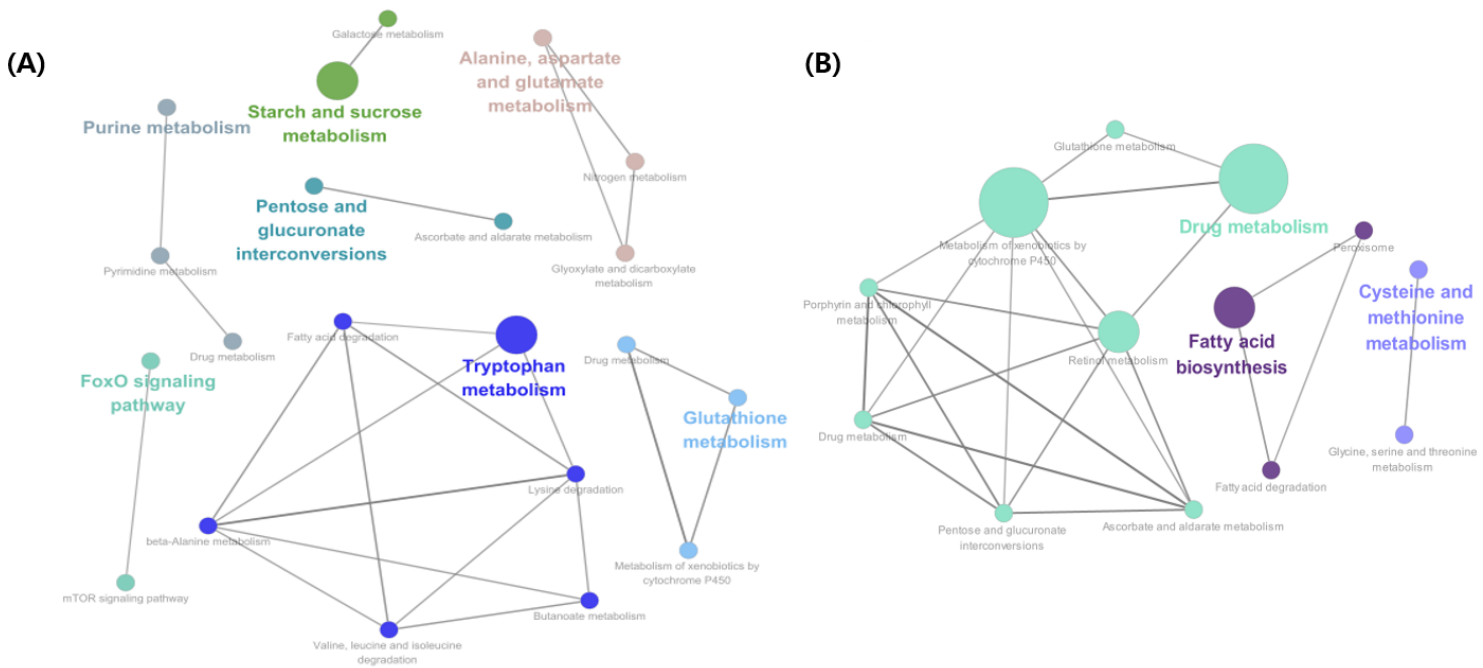


Figure 10. Visualization of upregulated and downregulated pathways in *Caenorhabditis elegans* fed with *Fusarium venenatum*

(A) Upregulated pathway via feeding *F. venenatum.* Cytoscape was performed using genes with a threshold value of fold change > 2 and p-value < 0.05 in F.V compared to OP50. (B) Downregulated pathway via feeding *F. venenatum.* Cytoscape was performed using genes with a threshold value of fold change < -3.5 and p-value < 0.05 in F.V compared to OP50. OP50 group, received *E. coli* OP50; F.V group, received *E. coli* OP50 with *F. venenatum*.

2.3.5. Metabolomic analysis

The metabolites were extracted from *C. elegans* after a preconditioning period of 48 hours. PLS-DA analysis showed differences in metabolite profiles between OP50 and F.V (Figure 11A). VIP scores indicate the most important variable in the PLS-DA. L-leucine which was higher in F.V compared to OP50 scored the highest VIP score among metabolites (Figure 11B). The metabolites with more than 2-fold changes between the two groups were shown in Figure 11C. Twenty-seven metabolites including d-xylose, 2-aminoadipic acid, alpha-Linolenic acid, nicotinic acid, and branched-chain amino acids (BCAAs) were upregulated, and two metabolites were downregulated with more than a 2-fold change in F.V.

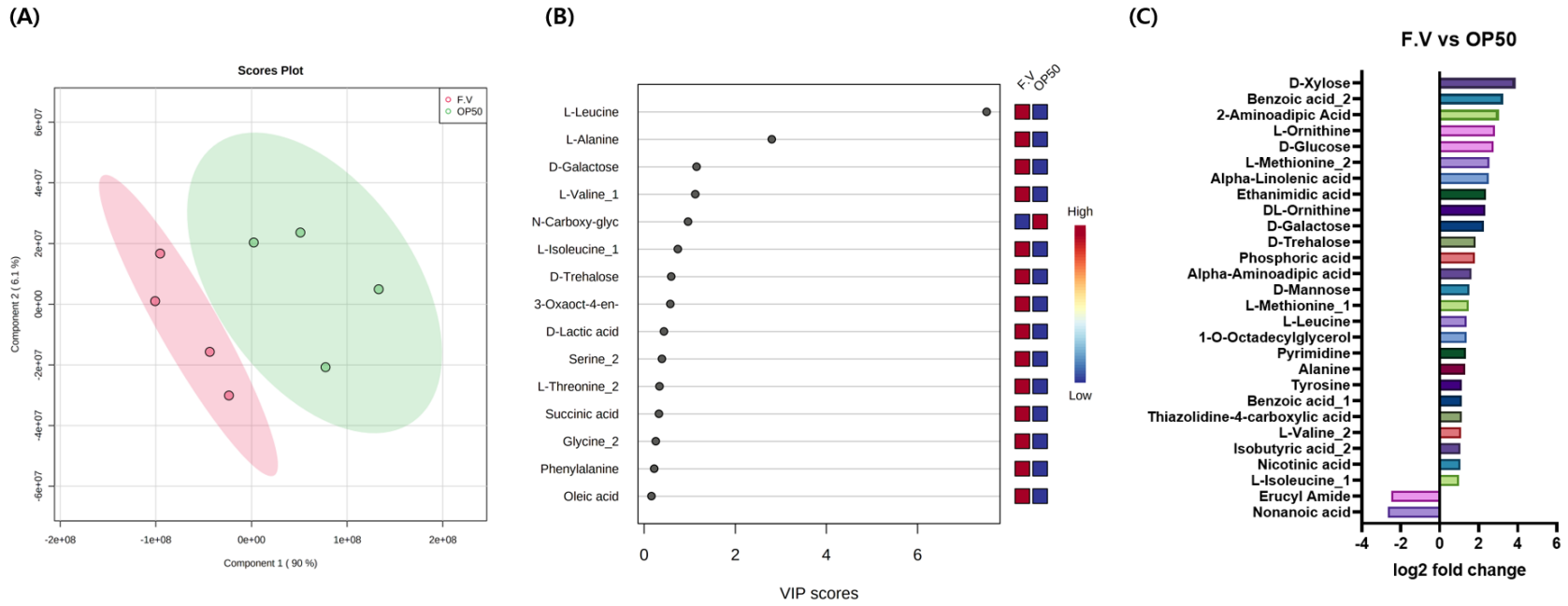


Figure 11. Changed metabolites profile in *Caenorhabditis elegans* fed with *Fusarium venenatum*

(A) PLS-DA analysis of metabolites composition between F.V and OP50. (B) VIP scores of PLS-DA. (C) Metabolites that were upregulated in F.V compared to OP50 with a threshold value of fold change > 2. OP50 group, received *E. coli* OP50; F.V group, received *E. coli* OP50 with *F. venenatum*.

2.4. Discussion

Due to the growing population, meat consumption, environmental pollution and the obese population finding new alternative protein sources which are healthy and can be supplied sustainably became the main interest for the future (Godfray et al., 2018; Moreland et al., 2010; Ramankutty & Foley, 1999). Due to its high-protein, high-fiber, low-fat, and low-energy contents, *F. venenatum* is attracting attention as an effective meat substitute and anti-obesity food (Dunlop et al., 2017; Moore & Chiu, 2001). Also, it is considered an almost complete protein with produces all essential amino acids and scored 0.996 in PDCAAS (Edwards & Cummings, 2010). We used *C. elegans* as a surrogate animal model to evaluate *F. venenatum* as an alternative protein source and anti-obesity ability. The *C. elegans* used in this study is known as a useful *in vivo* experimental model for fat metabolism research with lots of advantages including cost-effectiveness, transparent body, simple genetic, and fat metabolism found in mammals are highly conserved (Backes et al., 2021; C. Lin et al., 2020; Yue et al., 2019).

C. elegans is known as one of the suitable experimental model for aging research since their life cycle is short and provide a convenient methodology to examine at the molecular levels (Tissenbaum, 2015). Thus, we first evaluated whether *F. venenatum* and extracted protein can prolong the lifespan of *C. elegans*. Feeding *F. venenatum* extended longevity in both L1 and L4 stages of *C. elegans*. Also extracted protein from *F. venenatum*,

the concentration of 0.75 mg/ml and 1 mg/ml extended the lifespan of *C. elegans.* It indicated that *F. venenatum* and its contained proteins help prolong the longevity of *C. elegans*.

C. elegans has evolved to have an innate immune response to protect against infection by pathogenic bacteria (Kim et al., 2002). The pathogenic bacteria which can colonize and infect the intestine can affect the lifespan of *C. elegans* (Herndon et al., 2002). We performed the killing assay whether *F. venenatum* can improve the immune response of *C. elegans.* Preconditioning *C. elegans* with *F. venenatum* showed protective effects against both gram-negative (*Salmonella* Typhimurium SL1344 and *Escherichia coli* O157:H7 EDL933) and gram-positive (*Listeria monocytogenes* EGD-e and *Staphylococcus aureus* Newman) pathogenic bacteria. These results indicated *F. venenatum* may induce the immune response of *C. elegans* against food-borne pathogenic bacteria. The cell wall of *F. venenatum* is predominantly composed of glucosamine, chitin, and β-glucans (Finnigan, 2011). One of the possible reasons for improved immune response in *C. elegans* by *F. venenatum* is due to β-glucans in the cell wall. β-glucan is known as a natural compound with immune stimulatory and immunomodulatory (Geller et al., 2019; Murphy et al., 2010). We considered the improved innate immune response against pathogenic bacteria may be due to β-glucan in *F. venenatum.* Further studies are needed to confirm the relationship between improved immune response and β-glucan of *F. venenatum* in *C. elegans.* Also according to the previous study,

F. venenatum has antimicrobial effects against gram-positive bacteria (*Staphylococcus aureus* and *Enterococcus faecalis*) and gram-negative bacteria (*Escherichia coli* and *Klebsiella pneumonia*) (Mohammed et al., 2021). The previous study reported that extracts of *F. venenatum* with acetonitrile showed significant antimicrobial activity against *S. aureus* (Thomas et al., 2017). In addition, many studies revealed that *Fusarium* sp. can produce secondary metabolites which have antibacterial abilities such as antibiotic Y, beauvericin, enniatins, and fusaric acid (Meca et al., 2010; Son et al., 2008). These results may also indicate that *F. venenatum* itself may protect *C. elegans* from pathogenic bacteria in the intestine. Further studies are needed to elucidate whether *F. venenatum* may exert antimicrobial activity against pathogenic bacteria in the intestine.

Next, we measured the body size to evaluate whether *F. venenatum* may alter the phenotype of *C. elegans.* We confirmed the reduction of length and width which may reflect by reduced fat accumulation (Farias-Pereira et al., 2020). *C. elegans* is known as a suitable experimental model for studying lipid metabolism (Yen et al., 2010). Fat accumulation can easily be measured in *C. elegans* with Nile red staining and Oil red O staining (Escorcia et al., 2018; Shen, Kershaw, et al., 2018). Nile red staining method is known for measuring triglycerides (Gao et al., 2014) and the Oil red O staining method is known for assessing triglycerides and cholesterol (Ramirez-Zacarias et al., 1992). Triglycerides are known as the main fat storage molecules present in neutral lipid droplets in the *C. elegans* intestine

and skin (Watts & Ristow, 2017). Increased triglyceride implies fat accumulation in *C. elegans*. Due to these results, Nile red and Oil red O staining were conducted to evaluate the fat storage of *C. elegans*. The result of Nile red and Oil red O staining revealed that *F. venenatum* reduced the fat accumulation in *C. elegans*. This result was supported by a previous study *in vitro*, the structure of *F. venenatum* (20 mg/ml) reduced lipid digestion by binding to lipase and bile salts (Colosimo et al., 2020). Also, dietary fibers are known for reducing lipid digestion by inhibiting lipase activity (Balasubramaniam et al., 2013; Houghton et al., 2015). Further studies are needed to reveal *whether F. venenatum* may decrease lipid digestion in *C. elegans*. The other possible explanation is by β-glucans in *F. venenatum*. β-glucans in the cell wall may have helped lower the fat accumulation in *C. elegans*. In the previous study, β-glucans extraction from *Phellinus baumii*, significantly reduced fat accumulation in *C. elegans* (Kim & Lee, 2012). Also, β-glucans extraction from *Grifola frondosa* mushroom showed anti-obesity and anti-aging activity in *C. elegans* (He et al., 2017). In addition, *Prowashonupana barley* which contains high β-glucans reduced fat accumulation and improved and lifespan in *C. elegans* (Gao et al., 2015). Taken together, these results imply structure and composition of *F. venenatum* cell wall might have helped reduce fat accumulation in *C. elegans*.

Increased energy expenditure or decreased energy intake is known to result in decreased fat accumulation. (Peng et al., 2016; Sun et al., 2016;

Yue et al., 2019). To determine whether reduced fat accumulation by *F. venenatum* was due to increased energy expenditure or decreased feed intake the peristaltic speed and pumping rate was assessed. Results indicated that reduced fat accumulation was related to neither increased energy expenditure nor decreased feed intake. It may be due to alterations in molecular levels.

C. elegans has a known genetic sequence in which more than 65% of genes have homologous genes with humans. Also, the metabolic pathway related to fat metabolism found in mammals is highly conserved in *C. elegans.* Fat metabolism in *C. elegans* is regulated by many key transcriptional regulators related to regulatory pathways of lipogenesis, desaturation, and fatty acid oxidation (Zheng & Greenway, 2012). A previous study base on gene expression on fat storage in *C. elegans* reported numerous gene expressions can affect phenotypes (Mori et al., 2017). Recently whole transcriptomic analysis data has revealed differential gene expression in *C. elegans* during exposure to microbes or extracted material (Yu et al., 2021). Therefore whole-transcriptomic analysis was performed to identify differentially expressed genes related to fat metabolism via *F. venenatum.* We found out *F. venenatum* significantly downregulated genes related to fat synthesis and upregulated genes related to the fat breakdown pathway. In the fat synthesis pathway, acetyl-CoA which is the starter of the pathway is the most important substrate. In sequence, acetyl-CoA is carboxylated by *pod-2* to form malonyl-CoA then catalyzed by *fasn-1* to

form different lengths of fatty acids which are mainly palmitic acid (C16:0). After the desaturase or elongase of fatty acids, triglycerides are formed and stored (Ashrafi, 2007). In this study, *F. venenatum* significantly downregulated *pod-2* and *fasn-1* which agrees with previous studies that downregulated expression of *pod-2* and *fasn-1* led to reduced fat accumulation (Mejia-Martinez et al., 2017; Yang et al., 2022). In the fat breakdown pathway, triglycerides are broken down into glycerol and fatty acids. Then, the short, medium, and long-chain fatty acids are broken down in mitochondria and very-long-chain fatty acids are broken down in peroxisomes (Ashrafi, 2007; Shen, Yue, et al., 2018; Watts & Ristow, 2017). In this study, *F. venentaum* significantly upregulated the large portion of genes related to the fat breakdown pathway in both mitochondria and peroxisome which may result in reduced fat storage in *C. elegans.* It was consistent with previous studies that increased genes related to the fat breakdown pathways reduced fat accumulation (Huang et al., 2017; Y. Lin et al., 2020).

Cytoscape was used to determine significantly changed metabolic pathways via *F. venenatum* (Shannon et al., 2003). We used significantly upregulated genes to identify upregulated pathways. Interestingly, FoxO signaling pathway was upregulated which is known as increasing lifespan in *C. elegans* (Lin et al., 2001). In the previous study, mutations in daf-2/InR allowed daf-16/FoxO to enter the nucleus, where it promotes lifespan (Lin et al., 2001; Murphy, 2006). This suggested that upregulated daf-16/FoxO in

this study may be related to increased longevity of *C. elegans* by *F. venenatum*. In mammalian studies, the FoxO signaling pathway is known to increase mitochondrial metabolism, lipid oxidation and antioxidant response in adipocytes (Murphy, 2006). Also known as increasing lipid oxidation in myocytes and reducing lipogenesis in hepatocytes (Gross et al., 2008; Ioannilli et al., 2020). The tryptophan metabolism and fatty acid degradation were also upregulated via feeding *F. venenatum*. According to a previous study increased tryptophan levels lead to increased longevity in *C. elegans* (Edwards et al., 2015) In a mammalian study upregulated tryptophan metabolism resulted in increased fatty acid oxidation and lowered fat storage (Goodarzi et al., 2021; Ioannilli et al., 2020; Ruan et al., 2014). Also, we found that glutathione metabolism was upregulated. Glutathione is known as a cellular antioxidant that can neutralize reactive oxygen species and prolong lifespan (Gusarov et al., 2021). To confirm the downregulated metabolic pathway, significantly downregulated genes were used. The fatty acid biosynthesis pathway was downregulated via feeding *F. venenatum*. Also, we found cysteine and methionine metabolism was downregulated. In a previous study, the antidiabetic drug metformin increased lifespan by disrupting methionine metabolism in *C. elegans* (Cabreiro et al., 2013). A study using *Drosophila* showed that increased metabolism of cysteine or methionine failed to extend the lifespan (Massie & Williams, 1985). In mammals, with numerous epidemiologic data, upregulated cysteine metabolism was highly linked with increased body fat and the risk of obesity.

This may implicate downregulated cysteine metabolism can lead to the reduction of body fat (Elshorbagy et al., 2012). According to the previous study, reducing methionine metabolism led to the loss of weight, increased energy expenditure and improved glucose tolerance (Cooke et al., 2020). Taken together, altered gene expression by *F. venenatum* indicated increased longevity and reduction of fat accumulation.

GC-MS was performed to determine the changed metabolites via *F. venenatum* and the relationship between altered metabolites with fat metabolism. PLS-DA analysis showed altered in metabolite profiles with feeding *F. venenatum*. VIP scores which indicate the most important variable in the PLS-DA showed l-leucine which was high in the F.V group was the highest among metabolites. d-xylose was upregulated the most in *C. elegans* fed with *F. venenatum.* d-xylose is known as a monosaccharide sugar. In the previous study using mice, supplementing d-xylose showed reduced body weight, blood glucose level, lipid accumulation in the liver, adipose tissue weight, and expression of inflammation-related genes (Lim et al., 2015; Lim et al., 2018). 2-aminoadipic acid which is reported as enhancing lipolysis and browning of white adipose tissue via activating β3AR signaling in mice was upregulated via *F. venenatum* in *C. elegans* in this study (Xu et al., 2019). Alpha-linolenic acid was upregulated via *F. venenatum*. According to the previous study in *C. elegans* treatment of alpha-linolenic acid increased lifespan dose-dependently. Also, nhr-49 was activated by alpha-linolenic acid to stimulate the gene expression involved

in the β-oxidation of lipids. Nicotinic acid was significantly upregulated. In the previous study, in *C. elegans*, nicotinic acid and leucine synergized together to stimulate AMPK and Sirt1 signaling resulting in reduced fat accumulation and increased lifespan (Bruckbauer & Zemel, 2014). Three amino acids including leucine, isoleucine, and valine are known as BCAAs. BCAAs which were upregulated in F.V is known for playing a crucial role in Insulin/IGF-1 signaling pathway and increased BCAAs can lead to an extent in lifespan in *C. elegans* (Wang et al., 2018). In addition, Butyric acid which is commonly known as short chain fatty acid produced by the fermentation of fiber was upregulated at about 1.7 folds change in *C. elegans* fed with *F. venenatum* compared to OP50 (Data not shown). It may be due to the high content of fiber in *F. venenatum*. According to the previous study, supplementation of butyric acid significantly reduced fat accumulation in *C. elegans* (Zheng et al., 2010). Several studies using mice demonstrated that butyric acid which is produced by the fermentation of fiber reduces body fat accumulation, stimulates satiety hormones, improves insulin sensitivity, increases energy expenditure, and enhances mitochondria function (Gao et al., 2009; Keenan et al., 2006). These results were consistent with the present study that *F. venenatum* increased lifespan and reduced fat accumulation. In summary, feeding *F. venenatum* altered the metabolites composition in *C. elegans* and the altered metabolites were related with improve lifespan and reduce fat accumulation.

In conclusion, the present results suggest that *F. venenatum* can prolong

the lifespan, improve, immune response, and reduce fat accumulation in *C. elegans*. Moreover, *F. venenatum* influenced the gene expression of *C. elegans* which were reducing the fat synthesis pathway and increased the fat breakdown pathway. Lastly, *F. venenatum* altered the metabolite profile of *C. elegans*, and increased metabolites by *F. venenatum* were related to increasing lifespan and reducing fat accumulation. Taken together *F. venenatum* may play the role of an alternative protein source and anti-obesity diet.

Chapter 3. Evaluation of the anti-obesity effect of *Fusarium venenatum* in mice using multi-omics analysis

3.1. Introduction

These days, people are obtaining protein from a conventional protein source, meat. Meat is known for containing not only high-quality protein and amino acids but also micronutrients such as iron, zinc, vitamin B, and selenium (Cosgrove et al., 2005; Davey et al., 2003; Godfray et al., 2018). In 2050, 1,250 tons of meat and dairy are needed per year to meet the demand of the growing global population and increasing meat consumption (Godfray et al., 2018; Moreland et al., 2010; Ritala et al., 2017). However, due to enlarging deforestation, increasing greenhouse gas emissions, and global warming accelerated due to the livestock industry, it seems challenging to increase meat production to meet the global demand (Ramankutty & Foley, 1999; Steinfeld et al., 2006). Due to these reasons, finding alternative protein sources which are non-animal-derived is getting attention for a nutritionally sustainable future (Grasso et al., 2019).

Obesity has become an epidemic these days (Organization, 2000; Wang & Lobstein, 2006). According to the previous report, worldwide obesity

populations have been increasing in many countries across the world. More than 600 million adults are considered as obese and more than 1.9 are overweight (Caballero, 2019; Malik et al., 2013). Being obese has a high risk of chronic diseases including type II diabetes, hypertension, coronary heart diseases, and cancers (Aneja et al., 2004; Calle & Thun, 2004; Dixon, 2010; Hossain et al., 2007; KEYS, 1980; Salter, 2013). The previous study has reported that despite the high nutritive value of meat, excessive consumption of meat may induce an over-intake of energy and result in an imbalance between energy intake and energy consumption which can lead to obesity (Peng et al., 2016). Also, a previous study with U.S. adults found that there was a positive relationship between meat consumption and obesity (Wang & Beydoun, 2009). Due to these reasons, many efforts are being made by companies and researchers to develop a diet that can substitute conventional protein-source meat and treat obesity.

Mycoprotein is a whole food derived from the cultivation of fungi (Coelho et al., 2020). It was first mentioned in late 1960 with the effort of finding the potential source of protein for human consumption (Finnigan et al., 2019). Unlike the conventional method of gaining protein, which is feeding animals with carbohydrates and obtaining protein from their body mycoprotein can be gained by providing carbohydrates to fungi and gaining protein from the biomass (Finnigan, 2011). This refers to less carbon, water, and land are needed to produce the same amount of protein in mycoprotein than animals (Nadathur et al., 2016; Ritchie et al., 2017). Much fewer

greenhouse emissions occur from producing mycoprotein than from animals (Coelho et al., 2020). In addition, compared to plants it has the benefit of can be produced regardless of climate and soil characteristics (Hülsen et al., 2018). The fermented product of *F. venenatum* is one of the mycoproteins (Coelho et al., 2020). It is known for its contents of high-protein, high-fiber, and relatively low-energy (Dunlop et al., 2017). Also, it has low-fat content and a favorable fatty acid profile which is low content of saturated fat (Denny et al., 2008). The cell wall of *F. venenatum* comprises nearly one-third of chitins and two-thirds of β-glucan which are known to play the role of prebiotics in the intestine (Bottin et al., 2016; Finnigan, 2011). According to previous studies, *F. venenatum* which is rich in fiber helped to improve the blood lipid profile such as increased levels of plasma HDL, reduced LDL, and TC (Cherta-Murillo et al., 2020; Coelho et al., 2021; Thomas et al., 2017; Williamson et al., 2006). Also, it reduced energy intake but there was no significant difference in appetite regulation hormone level such as GLP-1 and PYY (Bottin et al., 2016). In addition, the *in-vitro* experiment showed that the structure of *F. venenatum* entrapped digestive enzymes such as amylase, lipase, and bile salts (Colosimo et al., 2020). These results indicated that *F. venenatum* is not only a good candidate for an alternative protein source but also can be considered as a functional food to treat obesity.

Mice have been utilized as biomedical research animal models due to their physiological, anatomical, and genetic similarity to humans. Also, mice

have been preferred as animal models because of their advantages including small size, short life cycle, and ease of maintenance (Bryda, 2013). High-fat diet treated C57BL/6 mouse is widely employed as an experimental animal model for obesity and diabetes studies (Jayaprakasam et al., 2006). The high-fat diet treated C57BL/6 mice showed significant lipid accumulation in liver and adipose tissue (Li et al., 2020). According to a previous study, male mouse tends to gain more body weight and fat weight than females (Nishikawa et al., 2007). This implies sex may impact mouse obesity development. In this study, only male C57BL/6 mice were used.

Recently, lots of efforts have been made to prepare for future protein deficiency in advance. Since obesity has become an epidemic these days a few studies have been done to treat obesity. Mycoprotein which is derived from fermentation is one of the alternative protein sources to supply protein sustainably for the future. Especially, mycoprotein derived from the cultivation of *F. venenatum* is known as a potential diet that can treat obesity. The aim of this study is to evaluate the anti-obesity ability of *F. venenatum* using C57BL/6 mice. In addition, we investigated the change in obesity-related indicators, intestinal metabolites, and microbiota composition.

3.2. Materials and methods

3.2.1. Animals, Diets and Experimental design

3.2.1.1. Animals and Diets

All experimental protocols and animals were approved by the Institutional Animal Care and Use Committee of Seoul National University (certificate SNU-220111-4). In the present study, 5-week-old male C57BL/6 mice (n = 25) were obtained from SamTako Bio (Korea). Then housed five per cage. A normal chow diet and sterile water were provided to mice *ad libitum* in standard laboratory condition (steady temperature, 23 ± 1℃; humidity, 55 ± 5%; and 12 h light/dark cycle). After one week of acclimation period without any intervention to adapt to a new environment, mice were divided into five groups of five animals each randomly depending on body weight. A normal control group was fed with a normal chow diet then the rest of the groups were fed with a 45 kcal% Fat rodent diet (D12451, Research Diets Inc., USA) as a high-fat diet.

3.2.1.2. Experimental design

Group 1, Normal control group (CON group), received no oral administration. Group 2, Negative control group (HFD group), received an oral administration of 200 μL of PBS every day. Groups 3 and 4, treatment groups (FL and FH group), received an oral administration of *F. venenatum*

(200 or 800 mg/kg) every day, respectively. The dose of *F. venentaum* was based on the previous study of *F. venenatum* effect on Antihyperlipidemic using rats as an animal model (Thomas et al., 2017). Group 5, Positive control group (POC group), received an oral administration of Simvastatin (40 mg/kg) every day. The body weight, feed intake and water intake were measured every week. All mice were humanely sacrificed after 12 weeks. The overall design of the experiment is shown in Figure 12.

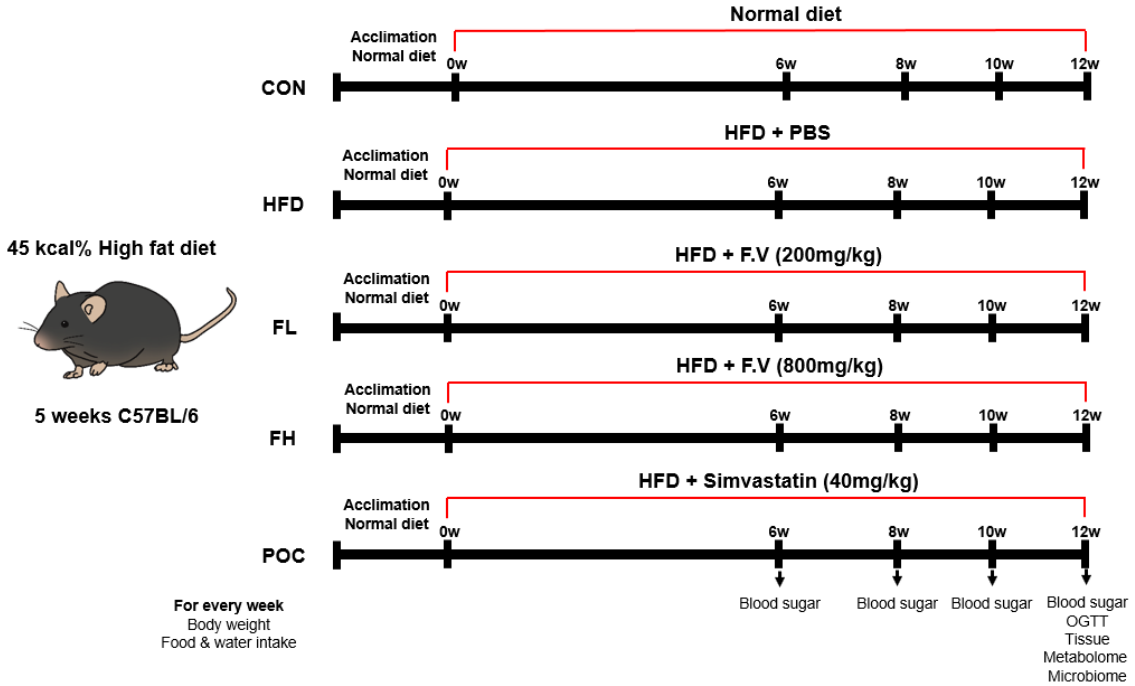


Figure 12. Design of experiment

CON group, the Normal control group, received no oral administration with a normal diet; HFD group, the Negative control group, received an oral administration of 200μL of PBS with a high-fat diet; FL group, the low-dose treatment group, received an oral administration of 200 mg/kg of *F. venenatum* with a high-fat diet; FH group, the high-dose treatment group, received an oral administration of 800 mg/kg of *F. venenatum* with a high-fat diet; POC group, the Positive control group, received an oral administration of 40 mg/kg of Simvastatin with a high-fat diet.

3.2.2. Blood analysis

3.2.2.1. Fasting blood glucose and oral glucose tolerance test

Mice were fasted for 16 h to measure fasting blood glucose. From week 6, fasting blood glucose was measured every two weeks. Oral glucose tolerance test **(**OGTT) was measured on week 12. Mice were fasted for 16 h before OGTT then D-glucose (2 g/kg of body weight) was gavaged. The blood samples were taken from the tip of the tail vein and then measure using an Accu-Chek glucose meter (Roche Diagnostics GmbH, Mannheim, Germany). Measurements were conducted at 0, 15, 30, 60, 90, and 120 min after the administration.

3.2.2.2. Lipid profile

The mice serum was collected at week 12. Aspartate transaminase (AST), alanine transaminase (ALT), TC, HDL, and TG were measured using a Fuji DRI-CHEM Clinical Chemistry Analyzer FDC 3500 (Fujifilm, Tokyo, Japan). LDL was calculated with the following formula.

$$LDL = TC - HDL - (TG/5)$$

3.2.2.3. Inflammatory cytokines

The serum from mice was collected to examine the serum levels of IL-1β and IL-10. They were measured using ELISA kits (IL-1β, Abcam, ab197742; IL-10, Abcam, ab255729). Experiments were performed according to the manufacturer's instructions. Colorimetric changes were

measured using a spectrophotometer (SpectraMax ABS Plus, San Jose, CA, USA) at 450 nm.

3.2.3. Histological analysis

Mesenteric fat and liver samples from each mouse were washed with sterilized PBS. Samples were fixed in 10 percent v/v formalin and then embedded in paraffin for staining with hematoxylin and eosin (H&E). The stained samples were observed with a KFBIO digital slide scanner (Konfoong bioinformation tech co., LTD.) SABIA software (EBIOGEN, Seoul, Korea) was used for the quantification assay.

3.2.4. Metabolomic analysis

Metabolomic analysis was performed with a slight modification of the previous study (Yoo et al., 2022). The fecal samples were collected and stored at -80°C until metabolomic analysis. Each fecal sample was weighed out and diluted with a methanol ratio of 1:3. Vortexed for 1 min and incubated on ice. After centrifugation at 10,000rpm for 10 min at 4°C, the upper layer of the supernatant was filtered with a 0.2 μm pore size polyvinylidene fluoride syringe filter (Whatman, Maidstone, England). Aliquots of 200 μL of the filtered supernatant were concentrated to dryness in a vacuum concentrator and stored at -80°C prior to derivatization and analysis by GC-MS. The extract was derivatized with 30 μL of a solution of 20 mg/mL methoxyamine hydrochloride in pyridine (Sigma, St. Louis, MO,

USA) at 30°C for 90 min, and 50 µL of N,O-Bis(trimethylsilyl)trifluoroacetamide (Sigma, St. Louis, MO, USA) was subsequently added at 60°C for 30 min. Fluoranthene was added to the extract as an internal standard.

The GC-MS analysis was performed using a Thermo Trace 1310 GC (Waltham, MA, USA) coupled to a Thermo ISQ LT single quadrupole mass spectrometer (Waltham, MA, USA). A DB-5MS column with 60 m length, 0.2 mm i.d. and 0.25 µm film thickness (Agilent, Santa Clara, CA, USA) was used for separation. For analysis, the sample was injected at 300 °C and a split ratio of 1:60 with 7.5 mL/min helium split flow. The metabolites were separated with 1.5 mL constant flow helium with an oven ramp of 50 °C (2 min hold) to 180 °C (8 min hold) at 5°C/min, to 210°C at 2.5 °C/min, and to 325°C (10 min hold) at 5 °C/min. The mass spectra were acquired in a scan range of 35-650 m/z at an acquisition rate of 5 spectra per sec. The ionization mode was subjected to electron impact, and the temperature for the ion source was set to 270°C. The spectra were processed by Thermo Xcalibur software using automated peak detection, and the metabolites were identified by matching the mass spectra and retention indices of the NIST Mass spectral search program (version 2.0, Gaithersburg, MD, USA). The metabolite data were then normalized based on the intensity of the fluoranthene internal standard. Further analyses were conducted using MetaboAnalyst 5.0 (Pang et al., 2021).

3.2.5. Metagenomic analysis

Metagenomic analysis was performed following the previous study methods (Kang et al., 2022). The fecal samples were collected on week12 and then aseptically homogenized and gDNA was extracted with the DNeasy PowerSoil Pro Kit (Qiagen, Hilden, Germany). The V4 region of the 16S rRNA genes was amplified (V4 amplicon primer sequences: forward, 5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGTGCCAGCMGCCGCGGTAA-3'; reverse, 5' -GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGGACTACHVGGGTWTCTAAT-3'), and the amplified DNA was sequenced using Illumina® iSeq 100 (Illumina, Inc. San Diego, CA, USA) following the manufacturer's manuscripts (Kazantseva et al., 2021). Further analysis were performed using mothur(Schloss et al., 2009) software and MicrobiomeAnalyst (Dhariwal et al., 2017).

3.2.6. Statistics

Data were statistically analyzed and graphed using Prism 9 (Graphpad Software, San Diego, CA, USA). Statistical significance was considered when p value was below 0.05 (*), 0.01 (**), 0.001 (***), 0.0001 (****). All data are expressed as means ± the standard error of the mean (SEM).

3.3. Results

3.3.1. Body weight, feed intake and water intake

The body weight, feed intake and water intake were measured to evaluate the alleviated effect of *F. venenatum* on high-fat diet-induced weight gain. After 12 weeks, the body weights of CON, HFD, FL, FH, and POC groups showed 29.28, 43.48, 35.92, 32.95, and 35.74 g, respectively (Figure 13A, 13B). Compared to the HFD group, CON, FL, FH, and POC groups showed significantly lower body weight ($p < 0.0001$, $p = 0.0074$, $p = 0.0005$, $p = 0.0060$ respectively). Individual daily body weight gain showed similar results (Figure 13C). In daily feed intake, the CON group showed significantly increased feed intake compared to other groups (Figure 13D). It may be due to the total calorie difference between normal chow and a high-fat diet. There was no significant difference in daily feed intake between HFD, FL, FH, and POC groups. The daily water intake showed similar results to daily feed intake (Figure 13E). The result showed that *F. venenatum* reduces body weight gain with no difference in feed and water intake.

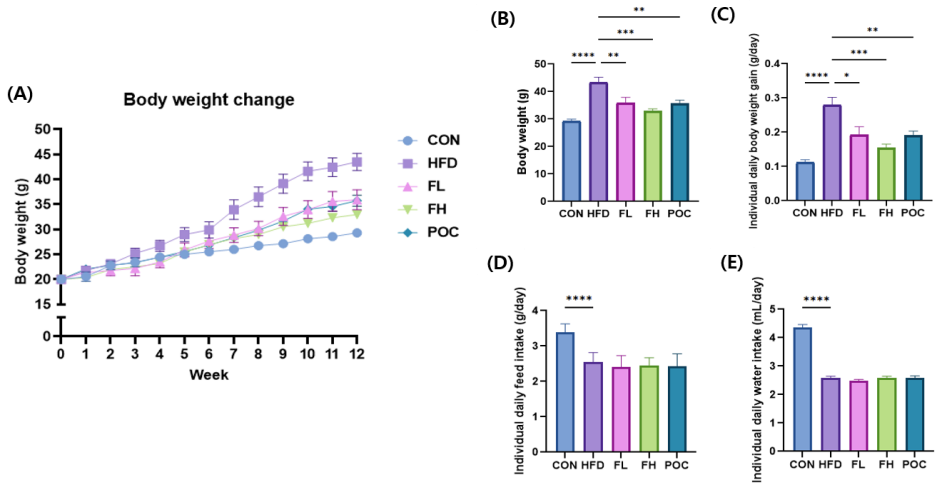


Figure 13. Changed body weight, feed intake, and water intake by feeding *Fusarium venenatum*

(A) Body weight change. (B) Body weight on week 12. (C) Individual daily body weight gain. (D) Individual daily feed intake. (E) Individual daily water intake. Data are expressed as means ± standard errors. Statistical analysis was performed using One-way ANOVA and differences were considered significant when p value was below 0.05 (*), 0.01 (**), 0.001 (***), 0.0001 (****). CON group, received no oral administration with a normal diet; HFD group, received an oral administration of 200 μL of PBS with a high-fat diet; FL group, received an oral administration of 200 mg/kg of *F. venenatum* with a high-fat diet; FH group, received an oral administration of 800 mg/kg of *F. venenatum* with a high-fat diet; POC group, received an oral administration of 40 mg/kg of Simvastatin with a high-fat diet.

3.3.2. Blood analysis

3.3.2.1. Blood glucose

Mice were fasted for 16 hours before confirming fasting blood glucose level. Fasting blood glucose was determined every 2 weeks starting from week 6 (Figure 14A). On week 6 CON and FH groups showed significantly reduced blood glucose levels at about 53% and 42% respectively compared to HFD groups (both $p < 0.0001$). There was no significant difference between NC and FH groups. Also, FL and POC groups showed lower blood glucose than the HFD group but had no significant difference. On week 8 CON, FL, and FH groups showed significantly lower blood glucose levels at about 53%, 20%, and 50% respectively compared to HFD groups ($p < 0.0001$, $p = 0.0069$, and $p < 0.0001$ respectively). But no significant difference between HFD and POC groups. Starting from week 10, every group including CON, FL, FH, and POC groups showed significantly lower glucose levels compared to the HFD group. On week 12, compared to the HFD group, groups CON, FL, FH, and POC showed significantly reduced blood glucose at about 54%, 27%, 59%, and 26% respectively (Figure 14B) ($p < 0.0001$, $p = 0.0002$, $p < 0.0001$, and $p = 0.0001$ respectively). The results revealed that *F. venenatum* reduced the increased fasting glucose levels induced by a high-fat diet

3.3.2.2. Oral glucose tolerance test (OGTT)

OGTT was performed on week 12 (Figure 14C). Mice were orally

gavaged with d-glucose (2 g/kg of body weight) after 16 hours of the fasting period. Blood glucose levels were measured at 0, 15, 30, 60, 90, and 120 min after the administration. Comparing the area under the curve of HFD with other groups, every group was significantly lower (Figure 14D). Among groups, the reduction degree was highest in CON with a degree of 50% ($p < 0.0001$) followed by FH with a degree of 45% ($p < 0.0001$), POC with a degree of 28% ($p < 0.0001$), and FL with the degree of 18% ($p = 0.0018$). The results confirmed that *F. venenatum* improves glucose tolerance in mice.

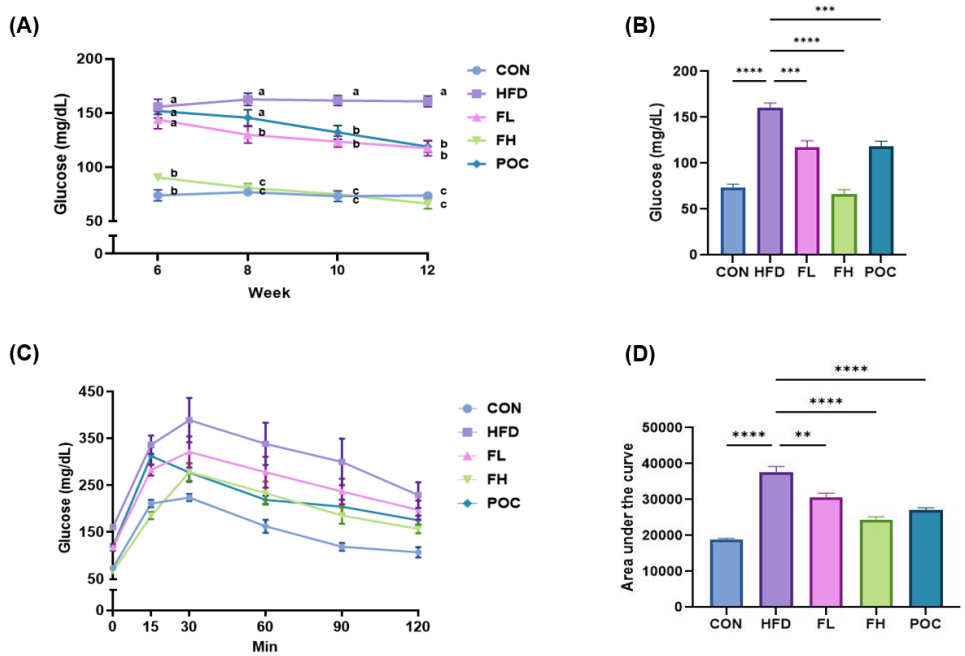


Figure 14. Altered blood glucose and oral glucose tolerance test (OGTT) by feeding *Fusarium venenatum*

(A) Fasting blood glucose level from week 6 to week 12. (B) Fasting blood glucose on week 12. (C) Blood glucose level on oral glucose tolerance test (OGTT). (D) Area under the curve. Data are expressed as means ± standard errors. Statistical analysis was performed using One-way ANOVA and differences were considered significant when p value was below 0.05 (*), 0.01 (**), 0.001 (***), 0.0001 (****). CON group, received no oral administration with a normal diet; HFD group, received an oral administration of 200 μL of PBS with a high-fat diet; FL group, received an oral administration of 200 mg/kg of *F. venenatum* with a high-fat diet; FH group, received an oral administration of 800 mg/kg of *F. venenatum* with a high-fat diet; POC group, received an oral administration of 40 mg/kg of Simvastatin with a high-fat diet.

3.3.2.3. Inflammatory cytokines

On week 12, inflammatory cytokines were measured using the serum. Pro-inflammatory cytokine IL-1β was significantly increased in the HFD group compared to the CON, FL, FH, and POC groups at about 45%, 53%, 43%, and 47% respectively (Figure 15A) ($p = 0.0337$, $p = 0.0129$, $p = 0.0440$, and $p = 0.0289$ respectively). There was no significant difference between CON, FL, FH, and POC groups. Anti-inflammatory cytokine IL-10 showed the lowest level in HFD group compared to other groups (Figure 15B). CON and FH groups were at significantly higher levels than HFD group at about 93% and 143% respectively ($p = 0.0043$ and $p = 0.0002$). There was no significant difference between CON and FH groups.

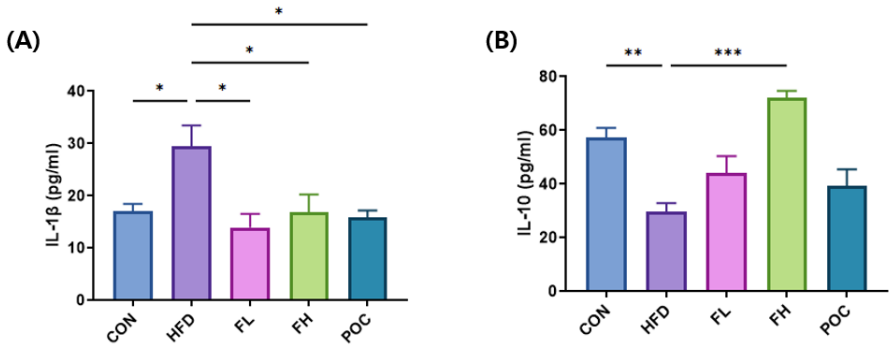


Figure 15. Effect of *Fusarium venenatum* on serum pro-inflammatory and anti-inflammatory cytokines

(A) Pro-inflammatory cytokine, IL-1β. (B) Anti-inflammatory cytokine, IL-10. Data are expressed as means ± standard errors. Statistical analysis was performed using One-way ANOVA and differences were considered significant when p value was below 0.05 (*), 0.01 (**), 0.001 (***), 0.0001 (****). CON group, received no oral administration with a normal diet; HFD group, received an oral administration of 200 μL of PBS with a high-fat diet; FL group, received an oral administration of 200 mg/kg of *F. venenatum* with a high-fat diet; FH group, received an oral administration of 800 mg/kg of *F. venenatum* with a high-fat diet; POC group, received an oral administration of 40 mg/kg of Simvastatin with a high-fat diet.

3.3.2.4. Lipid profile and liver toxicity biomarkers

Serums were collected on week 12 to evaluate lipid profiles and liver toxicity biomarkers. The HFD group showed a significantly higher level of TC compared to CON, FL, FH, and POC groups at about 48%, 22%, 44%, and 36% respectively (Figure 16A) ($p < 0.0001$, $p = 0.0020$, $p = 0.0001$, and $p = 0.0067$ respectively). There was no significant difference between FL, FH, and POC groups. Similar result was shown in TG (Figure 16B) (CON, 56%, $p < 0.0001$; FL, 31%, $p = 0.0017$; FH, 66%, $p < 0.0001$; POC, 44%, $p = 0.0050$ compared to HFD group). CON and FH groups had no significant difference. In HDL, CON group showed a significantly lower level than HFD group (Figure 16C) ($p = 0.0127$). There was no significant difference between HFD, FL, FH, and POC groups. In LDL, HFD group showed a significantly higher level compared to CON, FL, FH, and POC groups at about 67%, 41%, 64%, and 53% respectively (Figure 16D) ($p < 0.0001$, $p = 0.0014$, $p < 0.0001$, $p = 0.0019$ respectively). There was no significant difference between FL, FH, and POC groups. Also, no significance was shown between CON and FH groups. In AST and ALT, HFD group showed a significantly higher level compared to CON group at about 46% and 57% (Figure 16E and 16F) ($p = 0.0034$ for AST and $p = 0.0036$ for ALT). The HFD group showed the highest level of AST and ALT level compared to other groups. The results reveal that *F. venenatum* improve lipid profile in serum.

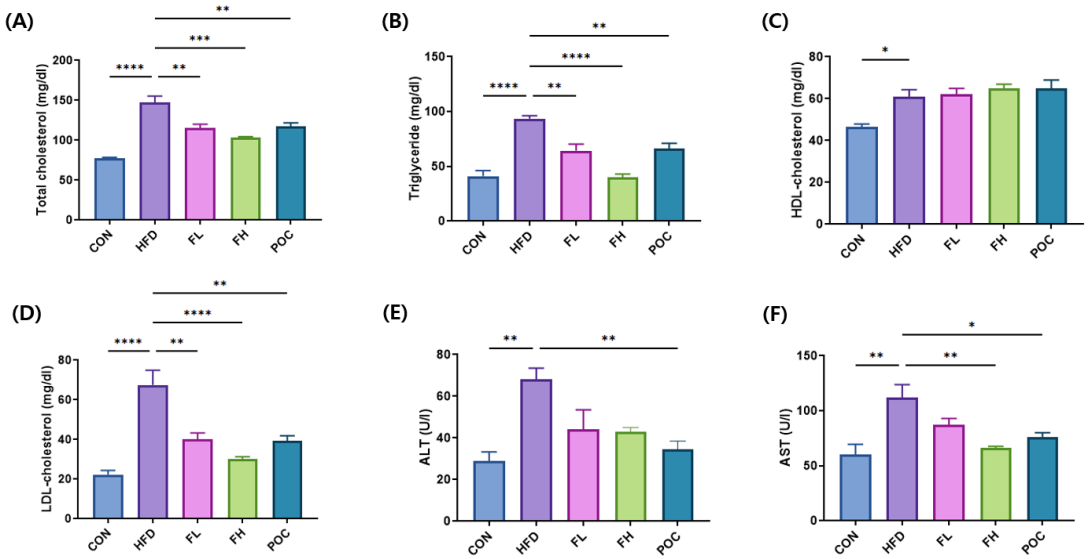


Figure 16. Altered serum lipid profile and liver toxicity biomarkers by feeding *Fusarium venenatum*

(A) Total cholesterol. (B) Triglyceride. (C) High-density lipoprotein-cholesterol. (D) Low-density lipoprotein-cholesterol. (E) Alanine transaminase. (F) Aspartate transaminase. Data are expressed as means ± standard errors. Statistical analysis was performed using One-way ANOVA and differences were considered significant when p value was below 0.05 (*), 0.01 (**), 0.001 (***), 0.0001 (****). CON group, received no oral administration with a normal diet; HFD group, received an oral administration of 200 μL of PBS with a high-fat diet; FL group, received an oral administration of 200 mg/kg of *F. venenatum* with a high-fat diet; FH group, received an oral administration of 800 mg/kg of *F. venenatum* with a high-fat diet; POC group, received an oral administration of 40 mg/kg of Simvastatin with a high-fat diet.

3.3.3. Histological analysis

3.3.3.1. Fat weight

After 12 weeks, to estimate fat accumulation, white fat mass was measured in three parts of adipose tissue (Perigonadal fat, Perirenal fat, and Mesenteric fat) (Figure 17). In perigonadal fat, CON group showed about 64% lower of fat mass compared to HFD group ($p < 0.0001$). Compared to HFD group, fat depot was significantly reduced in FL group at about 36%, FH group at about 50%, and the POC group at about 25% ($p < 0.0001$, $p < 0.0001$, $p = 0.0027$ respectively). Compared to POC group, fat mass was significantly lowered in FH group at about 25% ($p = 0.0042$). In perirenal fat, CON group showed about 65% lower of fat depot than HFD group ($p < 0.0001$). Compared to HFD group, fat accumulation was significantly lowered in FL group at about 30%, FH group at about 48%, and the POC group at about 27% ($p = 0.0002$, $p < 0.0001$, $p = 0.0007$ respectively). Compared to POC group, fat mass was significantly reduced in FH group at about 21% ($p = 0.0118$). In mesenteric fat, CON group showed about 65% lower of fat depot than HFD group ($p < 0.0001$). Compared to HFD group, fat accumulation was significantly reduced in FL group at about 48%, FH group at about 57%, and the POC group at about 37% ($p < 0.0001$, $p < 0.0001$, $p = 0.0002$ respectively).

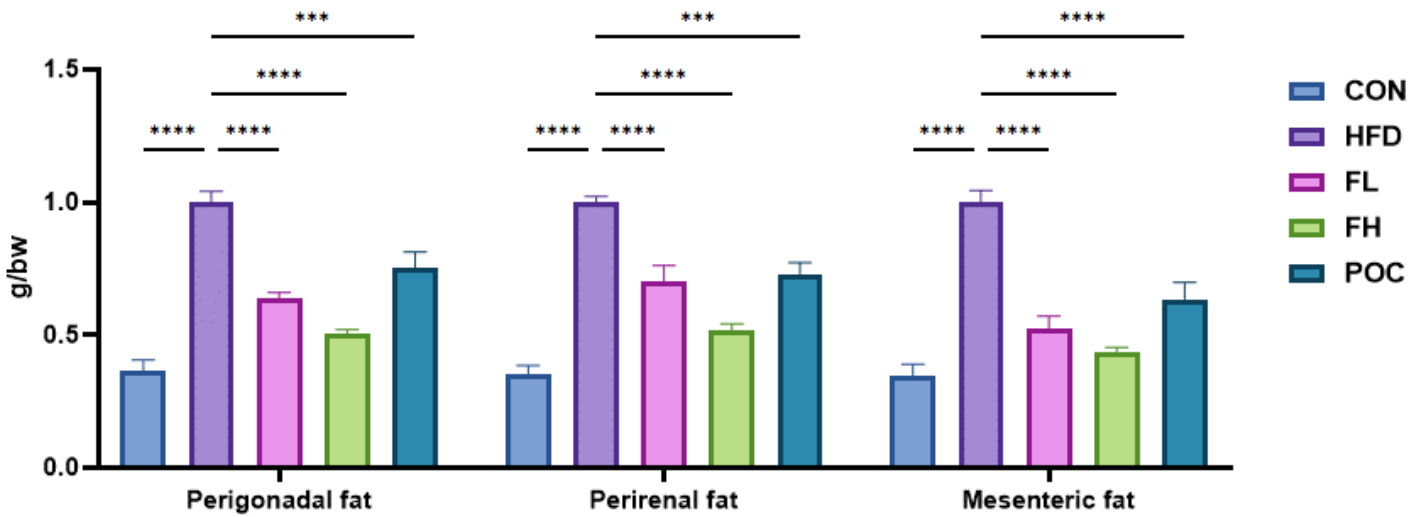
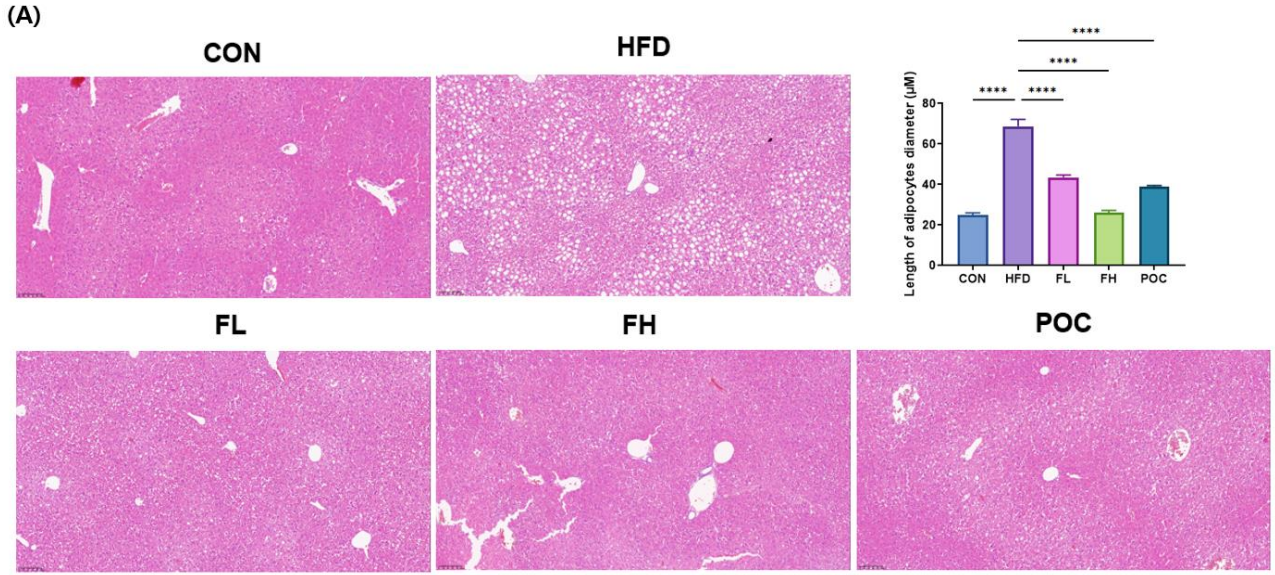


Figure 17. Assessment of fat storage reduction in white adipose tissue according to *Fusarium venenatum* feeding

Weight of three parts of fat including Perigonadal fat, Perirenal fat, and Mesenteric fat. Data are expressed as means ± standard errors. Statistical analysis was performed using One-way ANOVA and differences were considered significant when p value was below 0.05 (*), 0.01 (**), 0.001 (***), 0.0001 (****). CON group, received no oral administration with a normal diet; HFD group, received an oral administration of 200 μL of PBS with a high-fat diet; FL group, received an oral administration of 200 mg/kg of *F. venenatum* with a high-fat diet; FH group, received an oral administration of 800 mg/kg of *F. venenatum* with a high-fat diet; POC group, received an oral administration of 40 mg/kg of Simvastatin with a high-fat diet.

3.3.3.2. H&E analysis (Liver & Fat)

H&E analysis was performed using liver and mesenteric adipose tissue to evaluate the fat accumulation histologically. In Liver tissue, The CON, FL, FH, and POC groups showed significantly reduced length of adipocyte diameter compared to the HFD group at about 64, 43, 62, and 44 percent respectively (Figure 18A) ($p < 0.0001$, $p < 0.0001$, $p < 0.0001$, and $p < 0.0001$ respectively). CON and FH groups showed no significant difference ($p = 0.9900$). Mesenteric adipose tissue showed similar results (Figure 18B).


(A)
CON
HFD
FL
FH
POC
Length of adipocytes diameter (μM)
0
20
40
60
80
CON
HFD
FL
FH
POC
****
****
****
****

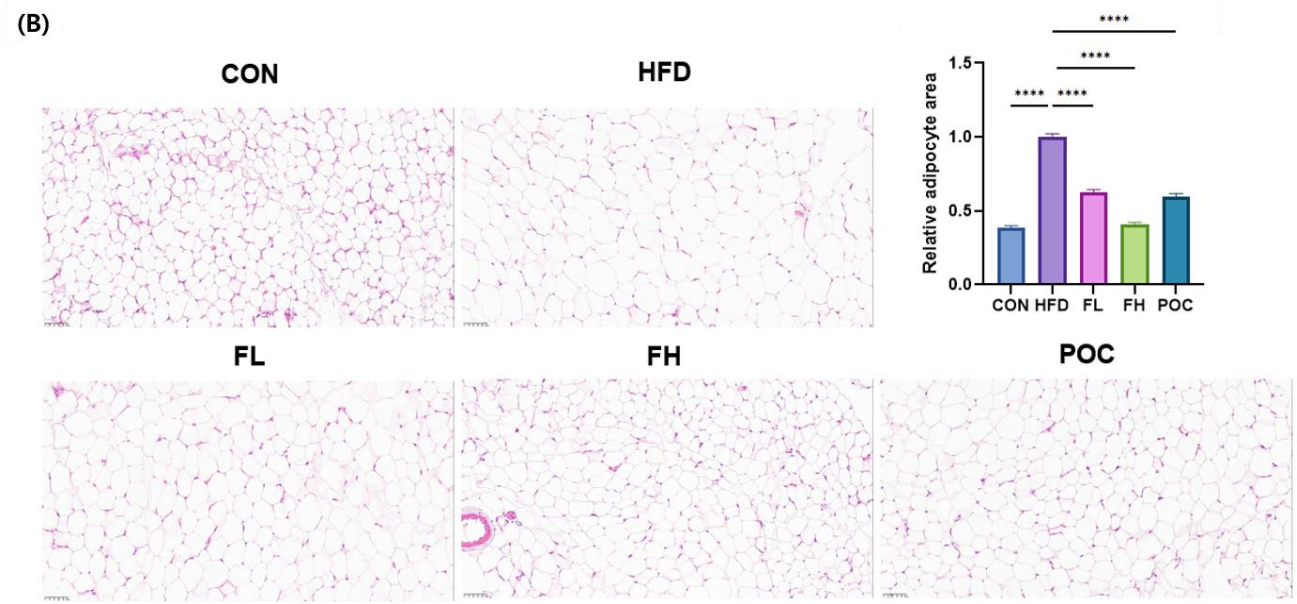
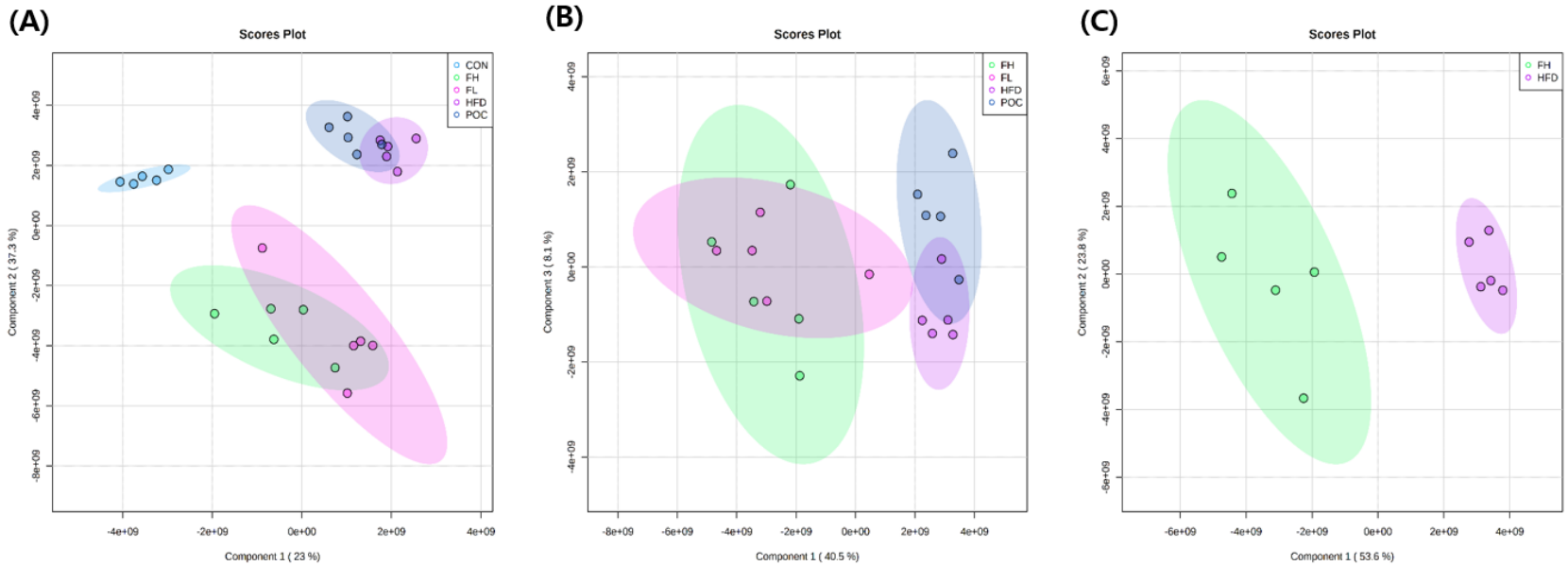


Figure 18. Evaluation of fat reduction in the liver and fat tissue by feeding *Fusarium venenatum* through H&E analysis
(A) Adipocyte morphology in Liver tissue. (B) Adipocyte morphology in Mesenteric adipose tissue. Data are expressed as means ± standard errors. Statistical analysis was performed using One-way ANOVA and differences were considered significant when p value was below 0.05 (*), 0.01 (**), 0.001 (***), 0.0001 (****). Liver and mesenteric adipose tissue morphology were shown at x20 and x40 magnification respectively. CON group, received no oral administration with a normal diet; HFD group, received an oral administration of 200 μL of PBS with a high-fat diet; FL group, received an oral administration of 200 mg/kg of *F. venenatum* with a high-fat diet; FH group, received an oral administration of 800 mg/kg of *F. venenatum* with a high-fat diet; POC group, received an oral administration of 40 mg/kg of Simvastatin with a high-fat diet.

3.3.4. Metabolomic analysis

Metabolomic analysis was performed to observe changes in metabolite composition among groups. The metabolites were extracted from the fecal samples on week12. PLS-DA analysis using all groups showed the different metabolite profiles between groups (Figure 19A). CON group which fed with a normal chow diet clustered itself and HFD and POC groups clustered together while FL and FL groups clustered together too. PLS-DA analysis using groups fed with a high-fat diet showed that HFD group was clustered with the POC group and FL with the FH group (Figure 19B). Heatmap was performed with the top 25 significantly changed metabolites in groups fed with a high-fat diet (Figure 19D). The heatmap showed metabolites including d-lactic acid, benzoic acid_1, primary bile acid, propionic acid_1, and d-(-)-fructose was high in both FL and FH groups compared to other groups. Also, we confirmed that the metabolites profile was different between HFD and FH groups (Figure 19C). To confirmed significantly changed metabolites between HFD and FH groups, Heatmap was performed with the top 25 significantly changed metabolites (Figure 19E). It showed that metabolites including primary bile acid, cholesterol, and propionic acid_1 were detected highly in the FH group fecal compared to the HFD group. In addition, the detection of Butyric acid_1 was significantly higher in the FH group compared to the HFD group (Data not shown).


(A)
Scores Plot
CON
FH
FL
HFD
POC
Component 2 (37.3 %)
Component 1 (23 %)
(B)
Scores Plot
FH
FL
HFD
POC
Component 3 (8.1 %)
Component 1 (40.5 %)
(C)
Scores Plot
FH
HFD
Component 2 (23.8 %)
Component 1 (53.6 %)

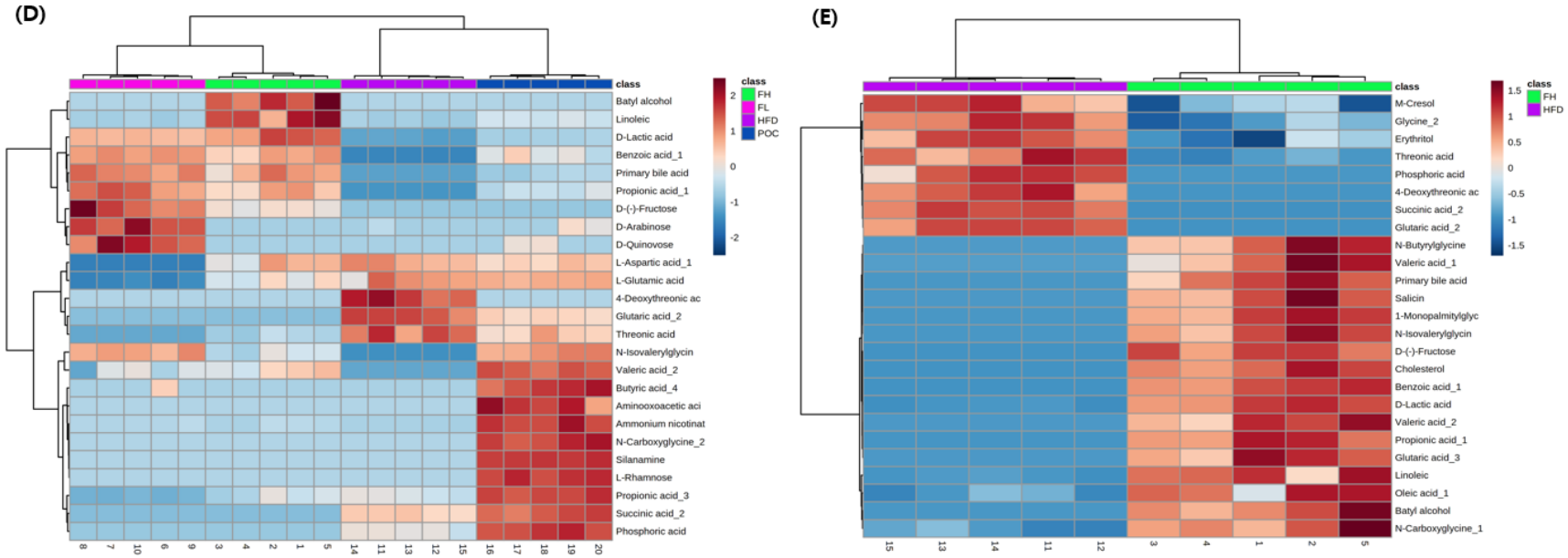


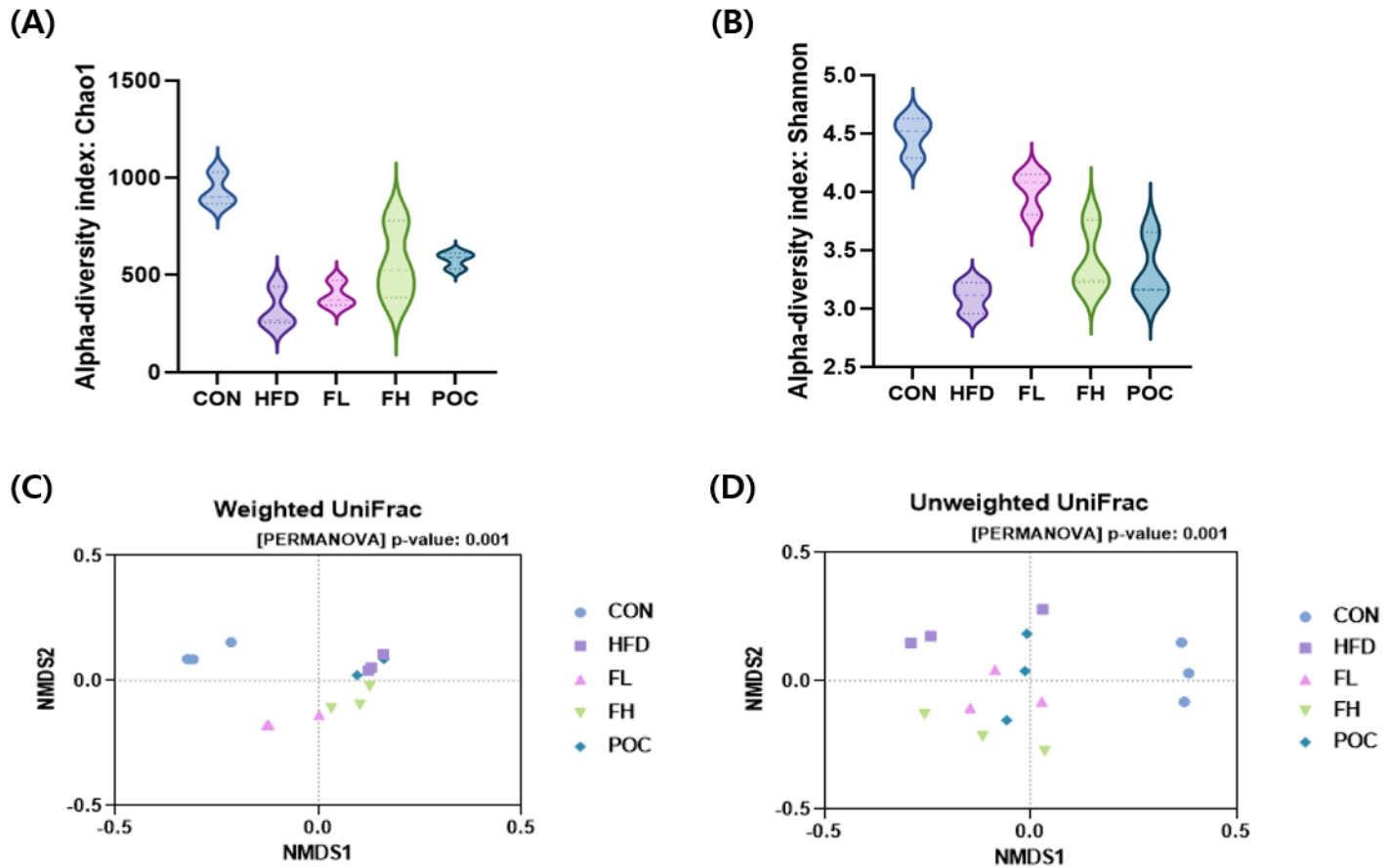
Figure 19. Evaluation of changes in fecal metabolite composition followed by *Fusarium venenatum* feeding

(A) PLS-DA analysis of metabolites composition among all groups. (B) PLS-DA analysis of metabolites composition among groups fed with a high-fat diet. (C) PLS-DA analysis of metabolites composition between HFD and FH groups. (D) Heatmap analysis with the top 25 changed metabolites among groups fed with a high-fat diet. (E) Heatmap analysis with the top 25 changed metabolites between HFD and FH groups. CON group, received no oral administration with a normal diet; HFD group, received an oral administration of 200 μL of PBS with a high-fat diet; FL group, received an oral administration of 200 mg/kg of *F. venenatum* with a high-fat diet; FH group, received an oral administration of 800 mg/kg of *F. venenatum* with a high-fat diet; POC group, received an oral administration of 40 mg/kg of Simvastatin with a high-fat diet.

3.3.5. Metagenomic analysis

Metagenomic analysis was performed using a week 12 fecal sample to evaluate the difference in microbiota compositions among groups. We measured the alpha diversity of the fecal samples using the Chao1 and Shannon index (Figure 20A, 20B). In the Chao1 index, the CON group was the highest and HFD was the lowest. The Shannon index showed similar results to the Chao index. The weighted and unweighted beta diversity data showed all samples from each group were clustered together (Figure 20C, 20D).

When we investigate the fecal microbiota composition at the phylum level, Firmicutes showed lower relative abundance in the HFD group compared to other groups. On the other hand, Bacteroidetes, Proteobacteria, and Fusobacteria were relatively high in abundance in the HFD group compared to other groups (Figure 20E). At the family level, *S24-7* and *Bacteroidaceae* were relatively high in abundance in the HFD group compared to others. On the contrary, *Lachnospiraceae* was relatively low in abundance in the HFD group compared to others (Figure 20F). At the genus level, *Lactobacillus* was relatively high abundance and *Clostridium* and *Coprococcus* were relatively low abundance in the HFD group compared to other groups (Figure 20G). Comparing groups fed with a high-fat diet showed *Oscillospira* was relatively high in FL, FH, and POC groups than HFD group.


(A)
Alpha-diversity index: Chao1
1500
1000
500
0
CON HFD FL FH POC
(B)
Alpha-diversity index: Shannon
5.0
4.5
4.0
3.5
3.0
2.5
CON HFD FL FH POC
(C)
Weighted UniFrac
[PERMANOVA] p-value: 0.001
NMDS2
NMDS1
0.5
0.0
-0.5
CON
HFD
FL
FH
POC
(D)
Unweighted UniFrac
[PERMANOVA] p-value: 0.001
NMDS2
NMDS1
CON
HFD
FL
FH
POC

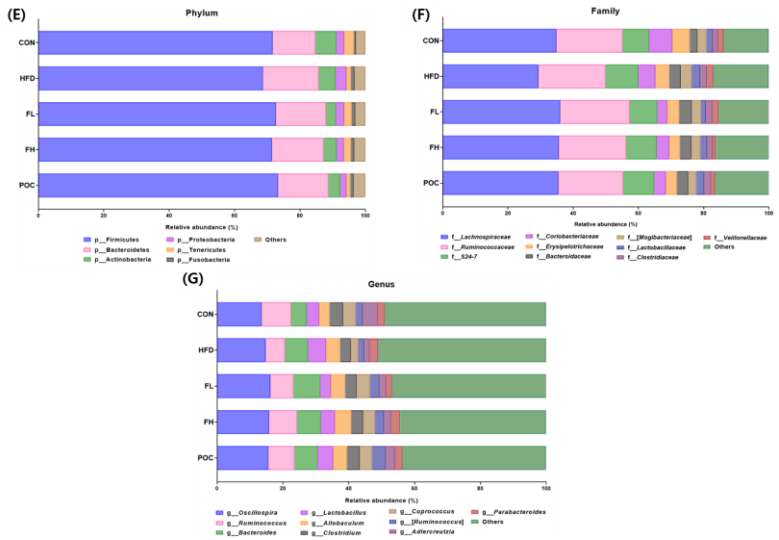


Figure 20. Assessment of changed fecal microbial diversity and composition by feeding *Fusarium venenatum*

(A) Alpha diversity (Chao index values) comparison of fecal microbiota among the groups. (B) Alpha diversity (Shannon index values) comparison of fecal microbiota among the groups. (C) PCoA plots based on weighted UniFrac distances of fecal microbiota. (D) PCoA plots based on unweighted UniFrac distances of fecal microbiota. Each plot represents each sample, and the axes represent the two dimensions that account for the highest amount of variance in the communities. (E) Microbial composition at the Phylum level. (F) Microbial composition at the Family level. (G) Microbial composition at the genus level. CON group, received no oral administration with a normal diet; HFD group, received an oral administration of 200 μL of PBS with a high-fat diet; FL group, received an oral administration of 200 mg/kg of *F. venenatum* with a high-fat diet; FH group, received an oral administration of 800 mg/kg of *F. venenatum* with a high-fat diet; POC group, received an oral administration of 40 mg/kg of Simvastatin with a high-fat diet.

3.4. Discussion

Meat is known for containing high-quality protein and amino acids (Cosgrove et al., 2005; Davey et al., 2003; Godfray et al., 2018). Therefore, it has been considered a conventional protein source for humans. Due to the growing global population and increasing meat consumption, many researchers predicted that 1,250 tons of meat and dairy might be needed per year in 2050 to meet global meat consumption (Godfray et al., 2018; Moreland et al., 2010; Ritala et al., 2017). Increasing the number of livestock and expanding the industry is inevitable to meet the demand of future consumption. However, it seemed challenging due to the enlargement of deforestation, increases in greenhouse gas emissions and acceleration of global warming by the livestock industry (Ramankutty & Foley, 1999; Steinfeld et al., 2006). Also, there was research about an increasing number of obese populations that have been linked to excessive consumption of meat (Peng et al., 2016; Wang & Beydoun, 2009). Due to these reasons finding an alternative protein source that can not only be provided nutritionally sustainably but also can help improve obesity has become an important problem.

Mycoprotein which is derived from the fermentation of fungi had attention as an alternative protein source. Especially mycoprotein derived from *F. venenatum* is considered a potential alternative protein source due to their high content of protein and fiber, and relatively low fat and energy

(Denny et al., 2008; Dunlop et al., 2017). Also, *F. venenatum* is known for producing all essential amino acids and the cell wall can play the role of prebiotics (Bottin et al., 2016; Edwards & Cummings, 2010; Finnigan, 2011). The aim of this study is to evaluate whether *F. venenatum* can not only sustainable protein source for the future but also treat obesity. C57BL/6 mice were used to assess the anti-obesity effect of *F. venenatum* using multi-omics analysis.

First, we evaluated the effect of *F. venenatum* on body weight. We fed mice a high-fat diet which is closely associated with weight gain. On week 12, we confirmed the body weight of groups fed with *F. venenatum* was significantly lower than the HFD group. The previous study suggested that *F.venenatum* may increase satiety due to fiber content (Denny et al., 2008). However, in this study, there was a slight reduction in feed intake in FL and FH groups compared to the HFD group, but no significant difference was shown. In addition, the previous study revealed that *F. venenatum* reduced energy intake but no significant difference in appetite regulation hormones such as GLP-1 and PYY (Bottin et al., 2016). Further studies are needed to evaluate the effect of *F. venenatum* on satiety.

Next, we examined the fasting blood glucose level and OGTT to evaluate whether *F. venenatum* can ameliorate hyperglycemia due to the administration of a high-fat diet which is also associated with type 2 diabetes and obesity. According to the previous study, long-term consumption of excessive calories via high-fat diet promotes *de novo*

lipogenesis which can lead to fat accumulation, especially in the liver and adipocytes. The increased fat in the liver and adipocyte can induce hyperglycemia and insulin resistance. Also, due to insulin being known for stimulating *de novo* lipogenesis, developed insulin resistance can make fat accumulation easier (Kim et al., 2017). In this study, the HFD groups showed hyperglycemia status through long-term feeding with a high-fat diet. However, FL and FH groups that fed with *F. venenatum* showed reduced glucose levels compared to the HFD group through the consumption of *F. venenatum*. This result suggests that long-term consumption of *F. venenatum* improves hyperglycemia and obesity status induced by a high-fat diet. According to the previous study, fiber can delay the passage of diet into the small intestine by reducing the rate of gastric emptying (Leclere et al., 1994). This can lead to the slow diffusion of glucose in the small intestine and reduce insulin secretion and the glycaemic response (Bornet et al., 2007; Edwards et al., 1988; Venn & Green, 2007). The glycaemic response of mycoprotein was conducted in a previous study by Turnbull and Ward (Turnbull & Ward, 1995). Briefly, participants received with test meal (mycoprotein) or a control meal, then an oral glucose tolerance test was conducted. The only difference between meals was a difference in dietary fiber content which was high in mycoprotein. Result showed that mycoprotein showed lower serum glucose and insulin response. So, the author mentioned that fiber content in mycoprotein may reduce postprandial glycemia and insulinemia. Mark reported a similar result that mycoprotein

reduced the postprandial blood concentrations of insulin (Marks et al., 2005). In agreement with the previous study, we speculate the fiber content in *F. venenatum* may reduce the rate of gastric emptying and glucose diffusion into the intestine which led to lowered glycaemic response and reduced secretion of insulin consequently ameliorating fat accumulation by a high-fat diet.

It is well known that the long-term consumption of a high-fat diet can induce fat accumulation, especially in the liver and adipocytes. In this study, the three parts of white adipose tissue were weighed including perigonadal fat, perirenal fat, and mesenteric fat to evaluate the effect of *F. venenatum* on high-fat diet induce fat accumulation. The result showed that groups fed with *F. venenatum* reduced the weight of white adipose tissue compared to the HFD group. Also, the histological analysis showed reduced fat depot in the liver and adipocytes in groups fed with *F. venenatum* compared to the HFD group. With no significant difference in feed intake between groups, we speculate the reduced fat depot might have occurred due to the slow absorption of glucose by *F. venenatum* which may have reduced the secretion of insulin. However, further study is needed to evaluate the change in serum insulin concentration. This result was in agreement with the previous study that mentioned that mycoprotein might be a useful diet to treat obesity and type 2 diabetes (Denny et al., 2008).

Serums were collected on week 12 to determine the effect of *F. venenatum* on inflammatory cytokines, liver toxicity biomarkers, and lipid

profile. According to the previous study, chronic low-grade inflammation is one of the characteristics of obesity (Gregor & Hotamisligil, 2011). The result showed that the mice fed with *F. venenatum* showed reduced pro-inflammatory cytokine and increased anti-inflammatory cytokine compared to the HFD group. ALT and AST which is considered liver toxicity biomarker were reduced in mice fed with *F. venenatum* compared to the HFD group. This result represented that *F. venenatum* has no toxical effect on the liver. On the lipid profile, previous studies showed that consuming *F. venenatum* improved the lipid profile compared to control diets such as meat, fish, and chicken (Bottin et al., 2016; Coelho et al., 2021; Turnbull et al., 1990). However, there was no study that fed *F. venenatum* along with control diets such as high-fat diets. Our study was conducted to evaluate whether *F. venenatum* can improve lipid profile when fed along with a high-fat diet. The result showed that TC, TG, and LDL were lowered in groups fed with *F. venenatum* compared to the HFD group. It was in agreement with the previous *in-vivo* study using a Triton X-100-induced hyperlipidemic rat by Thomas which feeding *F. venenatum* reduced TC, TG, and LDL (Thomas et al., 2017). However, the mechanism of reduced TC, TG, and LDL was not fully elucidated. The *in-vitro* study by Colosimo suggested that *F. venenatum* may lead to a reduction of hydrolysis and lipolysis by entrapping digestive enzymes such as amylase, lipase, and bile salts (Colosimo et al., 2020). In agreement with the previous study, we detected significantly high levels of primary bile acid from the feces of mice

fed with *F. venenatum* compared to other groups. This metabolomic analysis with fecal proved that *F. venenatum* may entrap the primary bile acid. Interestingly, we also detected cholesterol in the feces of mice fed with *F. venenatum*. This result demonstrated that *F. venenatum* may not only able to entrap digestive enzymes but also cholesterol which is a precursor of bile salts. Bile salts are known as synthesized from cholesterol in the liver and it is the primary route of cholesterol excretion from the body (Jones et al., 2013). Once bile salts are synthesized, it is stored in the gallbladder and released into the duodenum then play the functional role of promoting intestinal digestion and absorption of dietary fats and fat-soluble vitamins (Hofmann, 1990). The bile salts are efficiently conserved by enterohepatic recirculation. The majority of released bile salts are re-absorbed through active transport in the jejunum and ileum or passive diffusion in the small and large intestines (Roberts et al., 2002; Trauner & Boyer, 2003). Despite the reabsorb, approximately 4% of bile salts are lost through the feces in every bile acid circulation. Thus, *de novo* bile salts synthesis from cholesterol is consecutively proceeded to maintain a steady quantity of bile salts (Jones et al., 2013). There are several ways to reduce TC and LDL including statin (3-hydroxy3-methylglutaryl coenzyme A reductase inhibitors), fibrates, niacin, cholesterol absorption inhibitors, and bile acid sequestrants (Brautbar & Ballantyne, 2011). We speculated due to the loss of primary bile acid and dietary cholesterol by feeding *F. venenatum*, endogenous cholesterol may be used to synthesize bile salts to conserve

stable status. In this way, TC and LDL may consequently be reduced in serum. Also, the digestion of lipids may have been reduced due to entrapped digestive enzymes by *F. venenatum* which may lead to reduced TG. Further studies are needed to evaluate whether *F. venenatum* can entrap other digestive enzymes such as amylase and lipase *in vivo*.

The fiber content of *F. venenatum* is known for its prebiotic properties. SCFAs, mainly acetic acid, propionic acid, and butyric acids are the major metabolites produced through the fermentation of complex dietary carbohydrates by gut microbiota. A previous study performed *in vitro* batch fermentation assay to evaluate the SCFAs production from the fermentation of *F. venenatum*. The result showed that the fiber of *F. venenatum* appeared to promote the production of propionic acid and butyric acid at the expense of acetic acid (Harris et al., 2019). In agreement with the previous study, the metabolomic analysis showed FH group showed higher levels of propionic acid and butyric acid compared to the HFD group. According to the previous study, the high production of propionic acid and butyric acid is likely due to the content of β-glucan in *F. venenatum* (Hughes et al., 2008). The previous study reported that propionic acid and butyric acid but not acetic acid can cause weight loss by stimulating anorexigenic gut hormones and decreased feed intake (Chakraborti, 2015; Lin et al., 2012). Also, a previous study mentioned that unlike propionic acid and butyric acid, acetic acid has the obesogenic potential with the ability to act as a substrate for hepatic and adipocyte lipogenesis. On the contrary, propionic acid and

butyric acid have an anti-obesogenic potential (Chakraborti, 2015). A previous study revealed that SCFAs including propionic acid and butyric acid can reduce energy intake by stimulating the release of PYY and GLP-1 by activating the FFAR2 and FFAR3 receptors which are found in colonic enteroendocrine cells (Den Besten et al., 2013; Li et al., 2018; Tolhurst et al., 2012). Propionic acid has been reported to the inhibition ability of cholesterol synthesis which can antagonize the cholesterol increased by the action of acetic acid. Also, it can inhibit the expression of resistin in adipocytes (Chakraborti, 2015). Butyric acid has been reported it is the main energy source for colonocytes, increases intestinal barrier function, provides anti-inflammatory potential, increases mitochondrial activity, improves insulin sensitivity and prevent diet-induced obesity (Chakraborti, 2015). Also, it can increase energy expenditure through the activation of AMPK, up-regulating gene expression involved in lipolysis and fatty acid β-oxidation, and activating brown adipose tissue (Den Besten et al., 2015; Gao et al., 2009; Hong et al., 2016; Li et al., 2018). A previous study revealed that the production of butyric acid has the potential to mitigate obesity and related comorbidities (Brahe et al., 2013). Also, supplementation of butyrate showed the prevention of obesity in high-fat diet-treated animals (Fang et al., 2019; Pelgrim et al., 2017). Taken together propionic acid and butyric acid which were increased by feeding *F. venenatum* might prevent obesity by affecting energy metabolisms such as decreasing energy intake and/or increasing energy expenditure.

Metagenomics analysis was conducted with week 12 fecal samples to evaluate the difference in microbial diversity and composition between groups. The alpha diversity is known as representing the richness and evenness of microbes (Hagerty et al., 2020). The alpha diversity was significantly higher in the CON group compared to the HFD group, which was consistent with the previous research that obese individuals have lower alpha diversity than individuals in lean status (Andoh et al., 2016; Duan et al., 2021; Pinart et al., 2021; Tang et al., 2022). Comparing groups fed with a high-fat diet, FL, FH, and POC groups showed higher alpha diversity compared to the HFD group. It was in agreement with the previous study that obese and metabolically healthy people had a higher alpha diversity compared to obese and metabolically unhealthy people (Kim et al., 2020). Several studies have shown the correlation between increased alpha diversity with a high-fiber diet (Sidiropoulos et al., 2020; Xu & Knight, 2015). We speculated high fiber content of *F. venenatum* helped to improve the alpha diversity. Beta diversity refers to the differences in microbiota composition between samples. Weighted UniFrac (Quantitative metrics) use a percentage of microorganisms for calculation whereas unweighted (Qualitative metrics) only consider the presence or absence of microorganisms (Yu et al., 2020). The beta diversity result showed that the samples from each group were clustered together, which indicated that the treatments could induce to have a specific microbial composition.

At the phylum level, previous studies suggested that the ratio of the two

major bacterial phyla Firmicutes and Bacteroidetes is related to obesity states. According to previous studies increased levels of Firmicutes and decreased Bacteroidetes were found in the gut microbiota in the obese individual (Ley et al., 2005; Ley et al., 2006). However, there are several studies showed inverse results (Serino et al., 2012; Sidiropoulos et al., 2020). Therefore, with the rapid accumulation of data, there is an ongoing controversy that suggested there is no clear association between the abundance of Firmicutes and Bacteroidetes with obesity (Tseng & Wu, 2019). In this study, Firmicutes was a lower relative abundance and Bacteroidetes was a higher relative abundance in the HFD group compared to other groups. It was consistent with the previous study which fed a high-fat diet to genetically similar mice for 3 months to induce obesity. Comparing obese-induced mice and not induced mice, obese induce mice showed a low relative abundance of Firmicutes and a high relative abundance of Bacteroidetes in the microbial composition of their fecal (Serino et al., 2012). In addition, previous research described that the Western lifestyle, a low-fiber diet, has been associated with obesity, loss of bacterial diversity and increased relative abundance of Bacteroidetes. On the other hand, a non-Western lifestyle, a high-fiber diet, increased the relative abundance of Firmicutes (Hold, 2014; Sidiropoulos et al., 2020; Vangay et al., 2018; Yatsunenko et al., 2012). This result suggested that *F. venenatum* which is high fiber diet may have increased the relative abundance of Firmicutes and reduced the relative abundance of Bacteroidetes. The relative

abundance of Proteobacteria was reduced in CON, FL, FH and POC groups compared to the HFD group. Previous studies have described that an increase in the relative abundance of Proteobacteria is a signature of dysbiosis and dysbiosis has been linked with the status of obesity (Altomare et al., 2019; Shin et al., 2015; Vamanu & Rai, 2021). In addition, several metagenomic studies have demonstrated that a higher relative abundance of Proteobacteria was observed in obese mice (Crovesy et al., 2020; Kim et al., 2020). Taken together, we speculated that *F. venenatum* may have reduced the relative abundance of Proteobacteria which is a marker of dysbiosis and obesity status. Also consistent with previous studies, the relative abundance of Fusobacteria was highest in HFD group than in other groups (Andoh et al., 2016; Crovesy et al., 2020; Kim et al., 2020).

At the family level, the relative composition of *Lachnospiraceae* was reduced in HFD groups compared to other groups, which was consistent with the previous research that the abundance of *Lachnospiraceae* decreased in obese individuals (Duan et al., 2021; Menni et al., 2017; Serino et al., 2012). The family *Ruminococcaceae* has previously reported the ability to suppress obesity (Menni et al., 2017). In this study, *Ruminococcaceae* was relatively abundant in the group fed with *F. venenatum* compared to the HFD group. On the other hand, *S24-7* and *Bacteroidaceae* were higher in the HFD group compared to other groups. It agreed with the previous research that the relative abundance of *S24-7* and *Bacteroidaceae* increased in obese mice (Serino et al., 2012; Tang et al., 2022).

At the genus level, the relative abundance of *Oscillospira* was high in FL, FH, and POC groups compared to the HFD group when comparing groups fed with a high-fat diet. A previous study comparing obese people who are metabolically unhealthy or metabolically healthy showed that *Oscillospira* was high in metabolically healthy obese people (Kim et al., 2020). This result indicated that *Oscillospira* may relate to a good metabolic health condition in obese people. The relative abundance of *Lactobacillus* was higher in the HFD group compared to others. The previous study with obese people with dyslipidemia showed the enriched composition of *Lactobacillus* (Jin et al., 2021). The genus *Clostridium* and *Coprococcus* were relatively high in CON, FL, FH, and POC groups compared to the HFD group. The previous study revealed that *Clostridium* may be associated with the metabolically healthy condition in obese people (Kim et al., 2020). A previous study using fecal microbiota transplantation of wild douc fecal to germ-free mice showed that wild douc microbiota prevented gaining weight in both low and high-fiber diets. It suggested that the bacteria such as *Coprococcus* and *Clostridium* might have an effect in preventing fat accumulation in combination with a high-fiber diet. It agreed with the present study that *Clostridium* and *Coprococcus* were relatively high in groups fed with *F. venenatum* which is high in fiber compared to the HFD group. This result implied that the high fiber content of *F. venenatum* may help to grow specific genera such as *Clostridium* and *Coprococcus* which can help prevent weight gain.

In conclusion, *F. venenatum* significantly reduced the fat accumulation in mice fed with a high-fat diet to induce obesity. However, there was no significant difference in feed and water intake. We also confirmed the increased gastrocnemius muscle in mice fed with *F. venenatum* which suggested that *F. venenatum* may also relate to muscle mass development (Data not shown). Further study is needed to elucidate the impact of *F. venenatum* on the muscle development of mice. Also, *F. venenatum* improved hyperglycemia and lipid profile due to the administration of a high-fat diet. The metabolites data revealed that *F. venenatum* is able to entrap digestive enzymes such as primary bile acid and cholesterol which can lead to a reduction of lipid digestion. However further study is needed to demonstrate whether *F. venenatum* can entrap other digestive enzymes such as amylase and lipase. Metagenomic analysis showed that *F. venenatum* successfully improved the alpha diversity. In addition, it increased genera including *Clostridium* and *Coprococcus* which were suggested to effectively prevent fat accumulation in combination with a high-fiber diet. In summary, *F. venenatum* which is considered a sustainable protein source for the future can prevent obesity.

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Summary in Korean

Multi-omics 분석을 이용한 *Fusarium venenatum* 기반 미생물 단백질의 기능 평가

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단백질은 몸을 구성하는 기본 물질이며 인간이 삶을 영위하는 데 가장 중요한 물질이다. 특히, 고기는 대표적인 단백질 공급원으로 여겨지며 많은 사람들이 고기를 섭취하여 필요한 단백질을 보충하고 있다. 전 세계적인 인구 및 고기 섭취량 증가에 따라 증가하는 수요를 맞추기 위하여 사육 두수를 늘려야 한다. 하지만 현재 축산업으로 인해 발생하는 환경 문제들로 인해 사육 두수를 늘리는 것은 힘들 것으로 보이며 결과적으로 미래에는 고기의 수요를 맞추기 힘들 것으로 예상된다. 이에 따라 많은 연구진들이 미래의 지속적인 단백질 공급을 위하여 대체단백질들을 찾기 위해 노력하였으며 배양육, 식물성 단백질, 식용 곤충 등이 후보물질들로 대두되었다. 본 연구에서 사용

한 물질은 *Fusarium venenatum*을 배양하여 생산한 미생물 단백질로서 대체단백질 후보물질 중 하나이다. 본 연구는 멀티오믹스 분석 기술을 이용하여 *F. venenatum* 기반 미생물 단백질의 대체단백질원으로의 기능을 평가하기 위하여 진행되었다.

첫 번째 실험으로는 예쁜꼬마선충을 이용한 실험을 진행하였다. 예쁜꼬마선충에게 *Escherichia coli* OP50을 단독으로 먹인 그룹을 OP50 그룹이라 명명하고 *E. coli* OP50와 함께 *F. venenatum*을 먹인 그룹을 F.V 그룹이라 명명하였다. Lifespan assay와 Killing assay를 통해 F.V 그룹이 OP50 그룹에 비해 수명이 연장되고 면역력이 향상된 것을 확인하였다. 지방 축적양을 확인하기 위하여 Nile red와 Oil red o 염색 실험을 진행하였으며 이를 통해 F.V 그룹에서 OP50 그룹에 비해 지방 축적양이 감소한 것을 확인하였다. 또한 *F. venenatum*의 급여가 유전자 발현에 영향을 미치는지 확인하기 위하여 전사체 분석을 진행하였으며 본 연구에서 F.V에서 OP50에 비해 지방 합성과 관련된 유전자 *pod-2*와 *fasn-1*의 발현은 억제되었으며 반대로 지방 분해와 관련된 유전자들의 발현은 증가하였음을 확인하였다.

두 번째 실험은 고지방식이로 비만을 유도한 마우스에서 *F. venenatum*의 항비만 효과를 확인하기 위하여 진행되었다. 혈액분석 결과 *F. venenatum*을 경구투여한 그룹에서 HFD 그룹에 비해 간 독

성 바이오마커는 감소하고 지질 지표들은 개선된 것을 확인하였다. 조직학적 분석을 통해 *F. venenatum* 급여가 간과 지방조직의 지방 축적을 감소시킨 것을 확인하였다. 또한 대사체 분석을 통해 *F. venenatum*이 1차 담즙산과 콜레스테롤 같은 소화효소들과 결합하여 분변으로 배출시켰고 이를 통해 지방의 소화가 감소하여 지질 지표가 개선되고 지방 축적을 감소시키는 것으로 예측되었다. 추가적으로 장내미생물 분석을 통해 *F. venenatum*의 급여가 장내미생물총 구성을 변경시켰으며 알파 다양성을 증가시킨 것을 확인하였다.

결론적으로 본 연구에서 지속가능한 대체단백질로써 *F. venenatum* 기반 미생물 단백질의 가능성을 확인하였으며 또한, 비만과 같은 대사질환을 지닌 동물에 *F. venenatum* 기반 미생물 단백질을 급여하였을 시, 지방 축적 및 지방 생성 대사가 줄어드는 것을 확인한바 향후 기능성을 보유한 *F. venenatum* 기반 미생물단백질의 다양한 활용이 기대된다.