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Monascus pilosus SWM-008 red mold rice and its components, monascinol and monascin, reduce obesity in a high-fat diet-induced rat model through synergistic modulation of gut microbiota and anti-lipogenesis

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This study is the first to explore the effects of the novel yellow pigment monascinol (Msol) from red mold rice (RMR) on reducing body fat and to compare its effects with those of monascin (MS) and ankaflavin (AK). In a high-fat diet-induced rat model, different doses of RMR fermented rice (RL, RM, RH) and purified Msol, MS, and AK were administered over an 8-week period. The results showed that all treatment groups significantly reduced body weight and fat mass. Msol, in particular, activated acetyl-CoA carboxylase (ACC), inhibiting fatty acid synthesis and reducing triglyceride accumulation. All treatments suppressed the differentiation of preadipocytes into mature adipocytes by inhibiting CCAAT/enhancer-binding proteins β (C/EBPβ) and C/EBPα, as well as peroxisome proliferator-activated receptor γ (PPARγ). In the liver, RL, RM, RH, MS, and AK enhanced the expression of AMP-activated protein kinase (AMPK), ACC, peroxisome proliferator-activated receptor α (PPARα), and carnitine palmitoyl transferase-1α (CPT-1α), thereby promoting fatty acid metabolism. Additionally, RMR and its active components, MS and Msol, reduced body fat by modulating gut microbiota. These compounds significantly decreased the abundance of bacteria associated with fat storage, such as Oliverpabstia intestinalis, while increasing the abundance of bacteria linked to energy expenditure and lipid breakdown, such as Akkermansia muciniphila and Ruminococcus callidus. Moreover, MS and Msol upregulated proteins involved in fat degradation, such as UCP1, thereby enhancing fat burning and reducing fat accumulation. These regulatory effects suggest that Monascus and its components have potential in managing metabolic health and reducing obesity.

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1. Introduction

Obesity, a major global health issue with rising prevalence, has been linked to imbalances in gut microbiota, prompting increased interest in probiotics for gut health. Dysbiosis is associated with various diseases, including obesity, diabetes, asthma, atopic dermatitis, Alzheimer's, and Parkinson's disease.[1] Adipose tissue, composed mainly of adipocytes, is crucial for energy metabolism and is distributed as subcutaneous and visceral fat, making up about 80% and 5–20% of total body fat, respectively.[2,3] Excess energy intake enlarges adipocytes, affecting organs like the brain, liver, kidneys, heart, and muscles.[4] Understanding how gut microbiota influences adipose tissue is key to addressing obesity and related health issues.

Non-alcoholic steatotic liver disease (NAFLD), a major cause of liver disease worldwide, has a global prevalence of about 32%, with higher rates in males (40%) than females (26%). Regional differences exist due to factors like obesity rates and genetics, with prevalence exceeding 40% in the Americas and Southeast Asia. NAFLD prevalence has increased significantly over the past decades and is projected to rise further by 2030 if current trends continue.[5] Nutrition and dietary interventions are fundamental to managing NAFLD. Caloric restriction and

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adherence to a Mediterranean diet, low in added fructose and processed meats, have shown significant benefits. Intermittent fasting also holds promise for reducing liver fat and promoting ketogenesis, though further research is needed. Personalized diets, considering genetics and gut microbiota, may enhance intervention effectiveness.[6]

Adipocytes originate from preadipocytes through a process known as adipogenesis. During this process, preadipocytes differentiate into mature adipocytes by accumulating triglycerides (TG) and expressing specific proteins. The differentiation of preadipocytes is regulated by a network of transcription factors, including CCAAT/enhancer-binding proteins β (C/EBPβ), peroxisome proliferator-activated receptor γ (PPARγ), and CCAAT/enhancer-binding proteins α (C/EBPα). C/EBPβ initiates the expression of PPARγ and C/EBPα. Both PPARγ and C/EBPα then engage in a positive feedback loop to sustain the differentiated state of the cells, ensuring the development and function of mature adipocytes.[7] This regulatory mechanism is essential for maintaining adipose tissue homeostasis and its role in energy balance. Disruption in these processes can lead to metabolic disorders and affect overall health.

Gut bacteria can be broadly categorized into pathogenic and commensal types. Pathogenic bacteria, such as *Helicobacter pylori*, *Salmonella*, and *Shigella*, are known to cause diseases like ulcers, gastroenteritis, and diarrhea.[8–10] In contrast, commensal bacteria provide protective benefits, including resistance to pathogen invasion and enhanced immune responses, which can help reduce cancer risk.[11] The gut microbiota is a complex microbial ecosystem, primarily influenced by diet.[12] It plays a crucial role in fermenting food to produce short-chain fatty acids (SCFAs), which affect energy metabolism and fat storage. The two dominant bacterial phyla in the gut, *Firmicutes* and *Bacteroidetes*, account for approximately 85% of the microbiota. *Firmicutes* produce butyrate, which enhances insulin sensitivity, reduces inflammation, and regulates energy metabolism.[13] *Bacteroidetes* produce acetate and propionate; acetate influences fat storage and appetite, while propionate regulates appetite and fat synthesis.[14,15] These SCFAs are essential for maintaining gut health.

The gut microbiota significantly influences metabolic processes and energy balance, and imbalances can lead to conditions like obesity by affecting nutrient absorption, energy regulation, and fat storage.[16] Certain gut bacteria are associated with obesity: *Duncaniella*, *Bifidobacteria*, *Akkermansia*, *Bacteroidetes*, *Prevotella*, and *Christensenellaceae* are negatively correlated with obesity, while *Oscillibacte* is positively correlated, potentially contributing to inflammation and insulin resistance.[17–20] Maintaining a balanced gut microbiota is crucial for managing metabolism and preventing obesity, highlighting the importance of a healthy microbiome for overall health.

Monascinol, a secondary metabolite from the fungus *Monascus pilosus*, is gaining attention for its potential health benefits. It is produced during the fermentation of red mold rice (RMR) and affects lipid metabolism and liver health. Its complex polycyclic structure, with multiple hydroxyl groups similar to monascin, is key to its biological activity. Monascinol has shown promising effects in improving lipid profiles by lowering total cholesterol (TC) and triglycerides (TG). It also positively influences low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C). Additionally, it supports liver health by regulating fatty acid metabolism and reducing liver lipid content. Recent studies have confirmed these benefits and suggest potential applications in functional foods.[21] Although research on monascinol is still limited, early evidence points to further benefits, such as antioxidant and anti-cancer properties. These findings reinforce its promise as a functional ingredient in health supplements. Further research could reveal new therapeutic uses and strengthen its role in dietary health interventions.

This study investigates the anti-obesity effects of *Monascus pilosus* SWM-008 fermented rice, along with its active components monascinol (Msol), monascin (MS), and ankaflavin (AK), in high-fat diet-fed Sprague-Dawley rats over an eight-week period. The research focuses on their impact on body weight gain, food intake, adipose tissue weight, body fat percentage, and fat cell size, as well as lipolysis activity and lipid profiles (including hepatic, fecal, and blood lipids).

This study investigates the mechanisms through which the test treatments influence preadipocyte differentiation and lipid metabolism to better understand their potential anti-obesity effects. Specifically, the expression of key regulators of adipogenesis, including C/EBPβ, PPARγ, and C/EBPα, is analyzed to determine how the treatments affect the process of adipose development. These factors are critical in initiating and promoting the differentiation of preadipocytes into mature adipocytes, making them central to understanding adipogenesis.[22,23] Markers of lipid metabolism, such as p-AMPK, p-ACC, PPARα, CPT-1α, SIRT1, UCP-1, and FFAR2, are examined to evaluate how the treatments impact energy balance, fatty acid synthesis, oxidation, and thermogenesis. For instance, p-AMPK and p-ACC are pivotal for energy homeostasis and fatty acid metabolism, while UCP-1 is a key marker for thermogenic activity in brown and beige adipose tissue, indicating energy dissipation as heat.[24–26] Therefore, the study examines how these treatments influence key regulators of preadipocyte differentiation (C/EBPβ, PPARγ, C/EBPα) and lipid metabolism (p-AMPK, p-ACC, PPARα, CPT-1α, SIRT1, UCP-1, FFAR2). Additionally, the effects on gut microbiota will be analyzed to explore its potential role in mediating obesity-related outcomes. This comprehensive approach aims to provide new insights into the mechanisms by which these compounds regulate lipid metabolism and body fat, potentially offering novel strategies for managing obesity.

2. Materials and methods

2.1. Chemicals

The materials used in this study consisted of various chemicals, reagents, commercial kits, and specific substances.

Dimethyl sulfoxide (DMSO), cholesterol, methanol, and chloroform were obtained from Merck Co. (Darmstadt, Germany), Acros Organics (Waltham, MA, USA), and Avanter (Radnor, PA, USA). Hydrochloric acid (HCl) and sodium hydroxide (NaOH) were also sourced from Merck Co. (Darmstadt, Germany). Additional reagents, including *p*-nitrophenyl butyrate and Tween 20, were purchased from Merck Co. (Darmstadt, Germany), while the Folin–Ciocalteu reagent and heparin were supplied by Sigma Chemical Co. (St Louis, MO, USA).

For analytical measurements, total cholesterol (TC) and triglyceride (TG) assay kits were provided by Fortress Diagnostics (Antrim, UK), while glycerol assay kits were obtained from Randox Laboratories Ltd (Antrim, UK). The BCA protein assay kit was sourced from Merck (Kenilworth, NJ, USA). ELISA-related antibodies and proteins included p-AMPK antibody from Affinity (Cincinnati, OH, USA); AMPK protein and antibody, p-ACC antibody, ACC protein and antibody, and PPARα protein and antibody from Proteintech (Chicago, IL, USA); PPARγ protein and antibody from Sino Biological (Beijing, China); CPT-1α protein from MyBiosource (San Diego, CA, USA); and SIRT1 protein and antibody from Affinity (Cincinnati, OH, USA) and Sino Biological (Beijing, China). Additional antibodies included C/EBPβ protein and antibody from Proteintech (Chicago, IL, USA) and Servicebio (Wuhan, Hubei, China); GAPDH antibody from Servicebio (Wuhan, Hubei, China); UCP-1 protein from Abcam (Cambridge, UK); UCP-1 antibody from Affinity (Cincinnati, OH, USA); FFAR2 protein from Cloud-Clone (Katy, TX, USA); and FFAR2 antibody from Proteintech (Chicago, IL, USA). HRP-conjugated Affinipure Goat Anti-Rabbit IgG (H + L) and Goat Anti-Mouse IgG (H + L) were also procured from Proteintech (Chicago, IL, USA).

2.2. Animals and diets

Monascus pilosus SWM-008 fermented rice, along with the pure substances monascinol, monascin, and ankaflavin, used in this study were provided by Sun-Way Biotechnology Co., Ltd (Taipei, Taiwan). The fermented rice used contains no citrinin, with 1 g of SWM-008 fermented rice providing 3 mg of monascinol and 6 mg of monascin. The recommended daily intake for the SWM-008 fermented rice is 1 g.

2.3. Experimental dosage, animal housing, and procedures in a high-fat diet rat study

The animal experimentation followed a modified model based on the methods of.[27] Six-week-old male Sprague-Dawley (SD) rats were purchased from Lesco Biotechnology Co., Ltd (Taipei, Taiwan), and a total of 72 rats, with 8 rats per group, were used. The rats were housed in controlled conditions with 60% relative humidity, a temperature of 24 ± 1 °C, and a 12-hour light/dark cycle (8:00–20:00), with food and water provided *ad libitum*. After a 4-day acclimatization period, the rats were assigned to experimental groups and subjected to an 8-week study. Weekly measurements included food intake, water consumption, and body weight, and the rats were fed fixed amounts of experimental samples mixed with distilled water using a small drinking device. The dosages were determined based on our previous studies.[28] The selected doses align with effective dose ranges previously reported for red mold rice in metabolic health research. These doses were categorized into low, medium, and high levels to enable a comprehensive dose–response analysis. For the purified compounds (Msol, MS, AK), the dosages were designed to correspond to the concentrations of these compounds present in the selected RMR doses. The doses for the experimental substances were calculated based on an adult human weight of 60 kg. For the red mold rice groups, the low-dose (RL group) corresponded to 0.5 g day^{-1} for an adult, the medium-dose (RM group) to 1 g day^{-1}, and the high-dose (RH group) to 2 g day^{-1}. For monascinol, the doses were adjusted to correspond to 3 mg day^{-1} (Msol-M group) and 6 mg day^{-1} (Msol-H group) for adults. Similarly, monascin (MS-H group) and ankaflavin (AK-H group) were dosed at 6 mg day^{-1} for adults. Using a conversion factor of 6.2 based on body weight (0.5 kg for rats), the daily doses were calculated as follows: RL at 25.83 mg per rat, RM at 51.67 mg per rat, RH at 103.33 mg per rat, Msol-M at 0.16 mg per rat, Msol-H, MS-H, and AK-H at 0.31 mg per rat. The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Institutional Animal Care and Use Committee (IACUC) of the National Taitung University (approved no. NTTU 110007).

High-fat diet formulations were prepared by using 72.3% Chew diet #5001, a standard diet containing approximately 23–24% crude protein, 4–5% crude fat, 4–6% crude fiber, and 6–7% ash, with a caloric density of 3.4 kcal g^{-1}, providing balanced basal nutrition. For the high-fat diet (HF group), 26.7% butter powder was added as the primary source of saturated fat, along with 1% cholesterol to simulate a high-fat, high-cholesterol diet, resulting in an increased caloric density of 4.3 kcal g^{-1} compared to the normal diet (NOR group), which consisted of 100% Chew diet #5001. This formulation was designed to effectively induce metabolic alterations associated with high-fat diet consumption and serves as a reliable model for investigating diet-induced metabolic disorders. The experimental groups included NOR (normal diet), HF (high-fat diet), RL (low-dose *M. pilosus* SWM-008 fermented rice), RM (medium-dose *M. pilosus* SWM-008 fermented rice), RH (high-dose *M. pilosus* SWM-008 fermented rice), Msol-M (low-dose monascinol), Msol-H (high-dose monascinol), MS-H (high-dose monascin), and AK-H (high-dose ankaflavin). Each week, the amounts of experimental substances were adjusted based on body weight changes, and during the 8-week study, the rats were administered a fixed quantity of the experimental substance daily *via* gavage. Body weight changes were monitored weekly, and these changes were compared among groups at the end of the experiment. Additionally, food intake was recorded weekly, and feed efficiency was calculated at the end of the experiment using the formula: Feed efficiency = (weight gain/food intake) × 100%. Prior to sacrifice, rats were fasted for 16 hours and euthanized using carbon dioxide inhalation, with death confirmed by the absence of respiration and heartbeat. After euthanasia, blood and organs were collected, with

blood samples centrifuged at 12 000*g* for 15 minutes, and the upper serum transferred into 2 mL Eppendorf tubes for storage at −20 °C for future analysis. The liver, perirenal fat, and epididymal fat were cleaned with physiological saline, dried, and weighed. These tissues were then fixed in 10% formalin for subsequent histological staining, while the remaining liver and other tissues were washed with physiological saline and stored at −80 °C for further analysis.

2.4. Measurement of lipid concentrations in blood

Lipid concentrations in the blood, including total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C), were determined using a biochemical autoanalyzer (Beckman-700, Fullerton, CA, USA).

2.5. Analysis of liver and fecal lipids

For extraction, 0.1 g of liver tissue, fat tissue, or dried fecal powder was homogenized in 1 mL of chloroform–methanol (v/v = 2 : 1). After filtration, the filtrate, which contained the majority of lipids, was brought to 1 mL with chloroform–methanol (v/v = 2 : 1). The solution was centrifuged at 15 000*g* for 15 minutes. The upper layer was evaporated in a fume hood and then reconstituted in dimethyl sulfoxide (DMSO). Samples were stored at −20 °C for later analysis of TC and TG in liver tissue or feces.[29] TC and TG concentrations in liver tissue and fecal samples were analyzed using commercial biochemical reagents: TC was measured with BXC 0261 (Randox), and TG was measured with BXC 0271 (Randox). The procedures followed the manufacturer's instructions.

2.6. Histological evaluation and lipolytic activity analysis in adipose tissue

Fat tissue samples were fixed in 10% formaldehyde, dehydrated, and embedded in paraffin, with sections stained using hematoxylin and eosin (H&E) for histological analysis. The slides were examined under a microscope at 100× magnification to evaluate adipose tissue morphology. For adipocyte cross-sectional area calculations, sections of perirenal and epididymal fat were photographed, and the areas were measured using Image J software (National Institutes of Health, Bethesda, MD, USA) Individual adipocytes were delineated manually within the software, utilizing the scale bar for calibration. The cross-sectional areas were then calculated based on the calibrated magnification, ensuring precise and reproducible measurements.[30] Adipocyte numbers were estimated by homogenizing 0.1 g of fat tissue in 500 μL of chloroform–methanol (v/v = 2 : 1) followed by centrifugation at 12 000*g* for 10 minutes.[29] After evaporation of the upper layer, the residue was reconstituted in DMSO, and triglyceride concentration was measured using a commercial kit. Adipocyte numbers were calculated using the formula: Adipocyte number = lipid content/cell cross-sectional area × triolein density (0.915).[31]

For lipase activity measurement, 0.1 g of fat tissue was washed, dried, minced, and incubated with 1 mL KRB buffer at 37 °C for 1 hour. Glycerol concentration in the supernatant was determined using a commercial kit (GY105, Randox), and lipolysis efficiency was expressed as glycerol release per unit tissue weight.[32] Additionally, heparin-releasable LPL (HR-LPL) activity was measured by incubating fat tissue in KRB buffer containing 10 U mL^{-1} heparin at 37 °C for 1 hour. The supernatant was mixed with buffer and enzyme reaction solution, and the reaction was initiated by adding p-nitrophenyl butyrate (pNPB). After 10 minutes at 37 °C, the reaction was stopped, and the absorbance of the upper layer was measured at 400 nm to determine LPL activity.[33]

2.7. ELISA-based protein expression and quantification in liver and adipose tissues

After sacrifice, liver and fat tissues stored at −80 °C were thawed, and approximately 0.1 g of each tissue was homogenized in 500 μL of PBS (6.2 mM K_2HPO_4, 3 mM KH_2PO_4, pH 7.4). The homogenate was centrifuged at 15 000 rpm for 10 minutes at 4 °C, and the supernatant was stored at −80 °C for further analysis. Protein content was determined using a commercial bicinchoninic acid (BCA) protein assay kit. To prepare the color reagent, Reagents A (BCA) and B ($CuSO_4$) were mixed in a 50 : 1 ratio. Samples were then mixed with the color reagent in a 25 : 200 ratio, incubated at 37 °C for 30 minutes, and absorbance was measured at 562 nm using a spectrophotometer. Protein concentration was calculated from a standard curve.

2.8. Statistical analysis

All analyses, except for the fecal microbiota diversity analysis, were performed in triplicate, with results presented as mean ± standard deviation (SD). Statistical analysis was conducted using one-way ANOVA with SPSS 12.0, followed by Duncan's Multiple Range Test for inter-group comparisons. Statistical significance was set at $p < 0.05$.

2.9. Fecal sample collection and 16S rRNA gene amplification

Fecal samples were collected from all experimental groups before euthanasia to investigate gut microbiota changes following the administration of test materials. Samples were stored at −80 °C and sent to Turex Biotech Co., Ltd (Taipei, Taiwan) for further analysis. Total genomic DNA was extracted using the QIAamp PowerFecal DNA Kit (Qiagen Co, Hilden, Germany), and the DNA concentration was measured with a Qubit 4.0 fluorometer (Thermo Fisher Scientific Co., Waltham, MA, USA). The extracted DNA was adjusted to a concentration of 1 ng μL^{-1} for further analysis. Full-length 16S rRNA gene sequences (V1–V9 regions) were amplified using barcoded primers and KAPA HiFi HotStart ReadyMix (Roche Co, Basel, Switzerland) in a polymerase chain reaction (PCR). PCR products were resolved on a 1% agarose gel, and the main bands around 1500 bp were selected and purified using AMPure PB Beads for subsequent sequencing.

2.10. Bioinformatics analysis and gut microbiota profiling

Sequencing data were processed through rigorous quality control, where overlapping subreads were combined to gene-

rate consensus reads (CCS), and only high-quality sequences, known as HiFi reads (RQ > 20), were selected for further analysis. The DADA2 pipeline was used to generate Amplicon Sequence Variants (ASVs), which involved quality filtering, dereplication, and chimera removal. The resulting ASVs were compared to multiple databases, including NCBI, GreenGenes, SILVA, eHOMD, and UNITE, to assign species-level taxonomy and create an ASVs table for downstream analysis. Alpha diversity, representing species diversity within individual samples, was assessed using metrics such as Faith's Phylogenetic Diversity, the Simpson index, Menhinick richness, and Pielou's evenness. Group differences were analyzed using Kruskal–Wallis tests, and the results were visualized through various alpha diversity plots generated in R (v4.0.5). Beta diversity, which reflects the differences in species composition between groups, was examined using Partial Least Squares Discriminant Analysis (PLS-DA) plots, along with UniFrac distances and clustering trees generated through UPGMA (Unweighted Pair Group Method with Arithmetic Mean) analysis. Species composition differences between groups were analyzed using Welch's *t*-test, and results were visualized in bar charts. Additionally, metagenomeSeq analysis in R was performed across different taxonomic levels (Phylum, Class, Order, Family, Genus, Species), with p-values adjusted using the Benjamini and Hochberg False Discovery Rate (FDR) method to produce *q*-values. Species with statistically significant relative abundances ($p/q < 0.05$) were identified and plotted. Linear discriminant analysis (LDA) Effect Size (LEfSe) was applied to identify statistically significant biomarkers across groups, and species with significant abundance differences were visualized in a clustered heatmap. Finally, correlation analysis was performed using the linkET package in R, where heatmaps were generated to depict the associations between microbiota composition and physiological or biochemical data.

3. Results

3.1. Effects of Msol, MS, and AK on body weight, food intake, and feed efficiency

Male SD rats were randomly divided into nine groups with no significant differences in initial body weight ($p > 0.05$) (Table 1). After eight weeks of feeding a high-fat diet with different test substances, the HF group showed significantly higher body weight than the NOR group ($p < 0.05$), while groups fed with Msol-M, Msol-H, MS-H, and AK-H demonstrated a significant reduction in body weight ($p < 0.05$). Although RL, RM, and RH groups did not show a clear dose–response effect, RL and Msol-M showed a trend toward better less weight gain. The timeline of body weight changes across the experimental groups is presented in Fig. 1. The absence of a clear dose–response relationship in the RL, RM, and RH groups may be attributed to a plateau effect, where the medium dose (RM) was sufficient to elicit a near-maximal response, and higher doses (RH) did not result in additional significant effects. Moreover, the complex composition of red mold rice, which contains multiple bioactive compounds, might lead to non-linear interactions that obscure a straightforward dose–response pattern. Individual variability in metabolic responses among the animals may also play a role in this observation. These factors provide potential explanations for the observed lack of dose-dependent effects.

The HF group had lower food intake compared to the NOR group, likely due to the higher caloric density of the high-fat diet (NOR: 3.34 kcal g^{-1}; HF: 4.17 kcal g^{-1}). No significant reduction in food intake was observed in the RL, RM, RH, Msol-M, Msol-H, MS-H, and AK-H groups compared to the HF group ($p > 0.05$). Feed efficiency, calculated as [(weight change/total food intake) × 100%], was significantly higher in the HF group ($p < 0.05$), possibly due to the high caloric content. The RL, RH, Msol-M, Msol-H, MS-H, and AK-H groups significantly reduced feed efficiency compared to the HF group ($p < 0.05$),

Table 1 Effects of *M. pilosus* SWM-008 fermented rice, Msol, MS, and AK on body weight, food intake, total calorie intake, and feed efficiency in rats fed a high-fat diet

Groups	Initial body weight (g)	Final body weight (g)	Weight gain (g)	Food intake (g)	Caloric intake (kcal)	Feed efficiency (%)
NOR	296 ± 6^{a}	510 ± 27^{a}	214 ± 28^{a}	1490.3 ± 101.5^{a}	4998.4 ± 340.3^{a}	14.2 ± 1.0^{a}
HF	297 ± 9^{a}	585 ± 17^{c}	288 ± 19^{d}	1156.4 ± 42.3^{b}	4987.8 ± 182.4^{a}	24.2 ± 1.6^{d}
RL	289 ± 7^{a}	527 ± 23^{ab}	238 ± 19^{abc}	1097.2 ± 87.6^{b}	4732.1 ± 378.1^{a}	21.9 ± 1.5^{b}
RM	288 ± 8^{a}	553 ± 26^{b}	265 ± 25^{cd}	1115.8 ± 60.6^{b}	4812.5 ± 261.3^{a}	23.7 ± 1.3^{cd}
RH	290 ± 9^{a}	548 ± 21^{b}	258 ± 17^{c}	1141.8 ± 89.8^{b}	4924.4 ± 387.2^{a}	22.6 ± 0.6^{bc}
Msol-M	291 ± 10^{a}	512 ± 31^{a}	222 ± 39^{ab}	1116.8 ± 86.9^{b}	4816.6 ± 374.8^{a}	22.6 ± 2.2^{bc}
Msol-H	294 ± 9^{a}	534 ± 25^{ab}	240 ± 19^{abc}	1131.0 ± 58.9^{b}	4878.1 ± 254.3^{a}	22.2 ± 1.2^{bc}
MS-H	292 ± 8^{a}	542 ± 38^{b}	250 ± 32^{c}	1110.5 ± 84.7^{b}	4789.8 ± 365.4^{a}	22.5 ± 1.6^{bc}
AK-H	294 ± 8^{a}	539 ± 17^{ab}	245 ± 18^{bc}	1088.5 ± 54.7^{b}	4694.5 ± 235.8^{a}	22.5 ± 1.2^{bc}

Two groups of SD rats were fed a normal diet (NOR group) or a high fat diet (HF group) without the administration of test materials, respectively. The SD rats fed high fat diet, that were administrated with *M. pilosus* SWM-008 fermented rice of low doses (25.83 mg day^{-1} 500 g bw) (RL group), *M. pilosus* SWM-008 fermented rice of medium doses (51.67 mg day^{-1} 500 g bw) (RM group), *M. pilosus* SWM-008 fermented rice of high doses (103.33 mg day^{-1} 500 g bw) (RH group), Monascinol of medium doses (0.16 mg day^{-1} 500 g bw) (Msol-M group), Monascinol of high doses (0.31 mg day^{-1} 500 g bw) (Msol-H group), Monascin of high doses (0.31 mg day^{-1} 500 g bw) (MS-H group), Ankaflavin of high doses (0.31 mg day^{-1} 500 g bw) (AK-H group), respectively. Data are presented as means ± SD ($n = 8$). Mean values within each column with different superscripts are significant difference ($p < 0.05$).

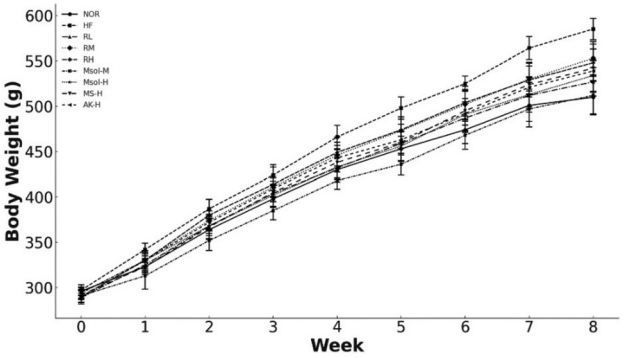

Fig. 1 Timeline of body weight changes in male SD rats fed with different test substances for eight weeks. Two groups of SD rats were fed a normal diet (NOR group) or a high fat diet (HF group) without the administration of test materials, respectively. The SD rats fed high fat diet, that were administrated with *M. pilosus* SWM-008 fermented rice of low doses (25.83 mg day^{-1} 500 g bw) (RL group), *M. pilosus* SWM-008 fermented rice of medium doses (51.67 mg day^{-1} 500 g bw) (RM group), *M. pilosus* SWM-008 fermented rice of high doses (103.33 mg day^{-1} 500 g bw) (RH group), Monascinol of medium doses (0.16 mg day^{-1} 500 g bw) (Msol-M group), Monascinol of high doses (0.31 mg day^{-1} 500 g bw) (Msol-H group), Monascin of high doses (0.31 mg day^{-1} 500 g bw) (MS-H group), Ankaflavin of high doses (0.31 mg day^{-1} 500 g bw) (AK-H group), respectively. Data are presented as means ± SD (n = 8).

suggesting that these substances may help regulate pathways to lower body fat beyond reducing food intake.

3.2. Influence of Msol, MS, and AK on liver weight and body fat composition

As shown in Table 2, a high-fat diet significantly increased liver weight, body fat weight, and body fat percentage in rats (p < 0.05). Compared to the HF group, feeding with RL, RH, Msol-M, Msol-H, MS-H, and AK-H effectively reduced liver weight, body fat weight, and body fat percentage. The Msol-M group showed the most significant reduction in liver weight by 25.9% (p < 0.05), while the MS-H group showed the greatest reduction in body fat weight by 36.5% and body fat percentage by 32.7% (p < 0.05). Besides a high-fat diet increased the cross-sectional area and cell number of perirenal and epididymal fat. Feeding with Msol-H and MS-H significantly reduced adipocyte cross-sectional area and cell number (p < 0.05), with Msol-H being the most effective. Histological staining of fat tissue (Fig. 2) revealed a marked increase in perirenal fat volume in the HF group compared to the NOR group. Feeding with RL, RM, RH, Msol-M, Msol-H, MS-H, and AK-H inhibited the increase in fat volume, with Msol-H and MS-H showing the most pronounced effects.

3.3. Msol, MS, and AK improve lipid metabolism and excretion

In Table 3, elevated serum TC and TG are linked to increased cardiovascular risk. After 8 weeks, the HF group showed significantly higher serum TC and TG levels than the NOR group (p < 0.05). RH reduced serum TC by 28% compared to HF (p < 0.05), while Msol-M, Msol-H, MS-H, and AK-H reduced TC by 13.7%, 13.3%, 10%, and 12.4%, respectively (p < 0.05). All test groups lowered serum TG levels compared to HF (p < 0.05), with RH and Msol-H showing the largest reductions at 26.1% and 27.5%, respectively (p < 0.05).

In the liver, the HF group had significantly higher TC and TG levels than NOR (p < 0.05). All test substances significantly reduced liver TC compared to HF (p < 0.05), with reductions of 32.5% (RL), 37.4% (RM), 38.2% (RH), 36.2% (Msol-M), 42.5% (Msol-H), 41.0% (MS-H), and 38.6% (AK-H), with no significant differences between groups (p > 0.05). For liver TG, RL, RM, and RH reduced levels by 56.0%, 55.6%, and 57.5%, respect-

Table 2 Effects of *M. pilosus* SWM-008 fermented rice, Msol, MS, and AK on liver weight, total body fat, body fat percentage, adipocyte cross-sectional area, and cell umber in rats fed a high-fat diet

					Perirenal fat pads		Epididymal fat pads	
Groups	Liver weight (g)	Liver weight /Body weight (%)	Total body fat (g)	Total body fat percentage (%)	Cell cross-sectional area (μm^2)	Cell number × 10^4	Cell cross-sectional area (μm2)	Cell number × 10^4
NOR	13.69 ± 0.81^{a}	2.71 ± 0.11^{a}	10.86 ± 1.84^{a}	2.12 ± 0.28^{a}	14 313 ± 1162^{a}	8.31 ± 0.99^{a}	15 240 ± 1230^{a}	7.81 ± 0.58^{a}
HF	22.72 ± 2.11^{d}	3.94 ± 0.40^{c}	25.57 ± 4.48^{c}	4.43 ± 0.70^{c}	25 955 ± 1146^{e}	18.94 ± 1.63^{b}	26 792 ± 1747^{d}	18.23 ± 1.06^{c}
RL	18.12 ± 2.00^{bc}	3.39 ± 0.24^{ab}	18.32 ± 1.94^{b}	3.36 ± 0.30^{b}	23 548 ± 2322^{d}	9.44 ± 1.67^{a}	19 829 ± 2223^{c}	10.92 ± 1.15^{b}
RM	19.16 ± 1.85^{c}	3.42 ± 0.26^{ab}	19.21 ± 4.00^{b}	3.41 ± 0.55^{b}	23 129 ± 2550^{d}	9.03 ± 1.17^{a}	18 998 ± 2230^{bc}	10.89 ± 1.61^{b}
RH	20.06 ± 1.39^{c}	3.66 ± 0.27^{bc}	18.82 ± 5.31^{b}	3.32 ± 0.71^{b}	21 744 ± 1654^{c}	8.91 ± 1.15^{a}	18 498 ± 2154^{bc}	10.74 ± 1.88^{b}
Msol-M	16.84 ± 2.31^{b}	3.28 ± 0.37^{a}	17.95 ± 3.55^{b}	3.35 ± 0.55^{b}	20 770 ± 2468^{c}	9.36 ± 1.28^{a}	18 899 ± 1938^{bc}	10.95 ± 2.23^{b}
Msol-H	18.96 ± 2.51^{bc}	3.51 ± 0.38^{ab}	17.27 ± 1.69^{b}	3.19 ± 0.46^{b}	16 885 ± 1810^{b}	8.88 ± 1.22^{a}	17 236 ± 1351^{b}	10.65 ± 1.20^{b}
MS-H	18.55 ± 2.14^{bc}	3.52 ± 0.29^{ab}	16.23 ± 2.96^{b}	2.98 ± 0.31^{b}	20 357 ± 1328^{c}	9.09 ± 1.23^{a}	17 773 ± 1839^{bc}	10.70 ± 2.19^{b}
AK-H	18.97 ± 2.06^{bc}	3.52 ± 0.31^{ab}	17.60 ± 3.36^{b}	3.16 ± 0.54^{b}	23 655 ± 2327^{d}	9.32 ± 2.11^{a}	18 839 ± 1574^{bc}	10.81 ± 1.69^{b}

Two groups of SD rats were fed a normal diet (NOR group) or a high fat diet (HF group) without the administration of test materials, respectively. The SD rats fed high fat diet, that were administrated with *M. pilosus* SWM-008 fermented rice of low doses (25.83 mg day^{-1} 500 g bw) (RL group), *M. pilosus* SWM-008 fermented rice of medium doses (51.67 mg day^{-1} 500 g bw) (RM group), *M. pilosus* SWM-008 fermented rice of high doses (103.33 mg day^{-1} 500 g bw) (RH group), Monascinol of medium doses (0.16 mg day^{-1} 500 g bw) (Msol-M group), Monascinol of high doses (0.31 mg day^{-1} 500 g bw) (Msol-H group), Monascin of high doses (0.31 mg day^{-1} 500 g bw) (MS-H group), Ankaflavin of high doses (0.31 mg day^{-1} 500 g bw) (AK-H group), respectively. Data are presented as means ± SD (n = 8). Mean values within each column with different superscripts are significant difference (p < 0.05).

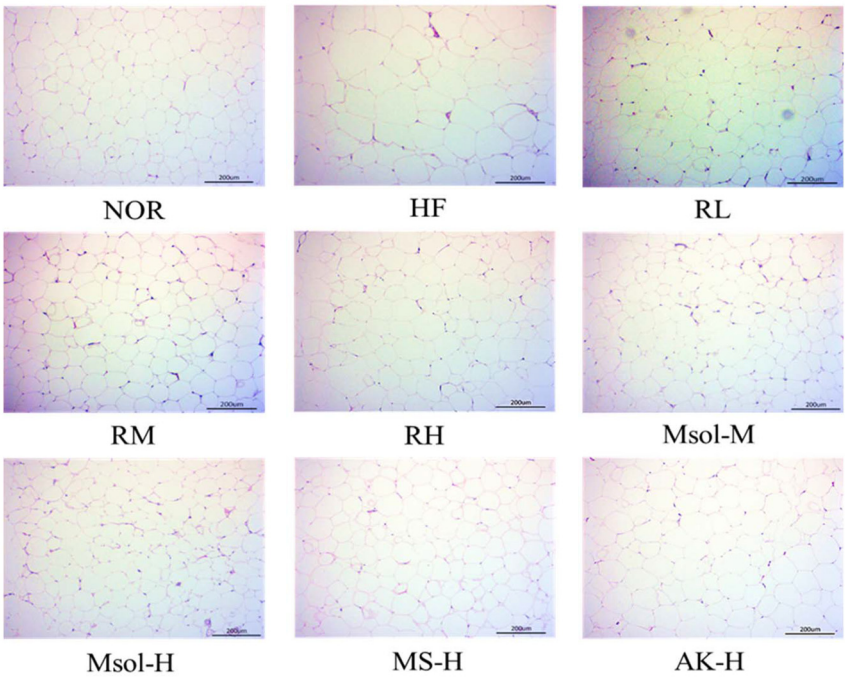

Fig. 2 Effects of with *M. pilosus* SWM-008 fermented rice, Msol, MS, and AK on the histopathological staining images of perirenal adipose tissue in rats fed a high-fat diet (100×). Two groups of SD rats were fed a normal diet (NOR group) or a high fat diet (HF group) without the administration of test materials, respectively. The SD rats fed high fat diet, that were administrated with *M. pilosus* SWM-008 fermented rice of low doses (25.83 mg day^{-1} 500 g bw) (RL group), *M. pilosus* SWM-008 fermented rice of medium doses (51.67 mg day^{-1} 500 g bw) (RM group), *M. pilosus* SWM-008 fermented rice of high doses (103.33 mg day^{-1} 500 g bw) (RH group), Monascinol of medium doses (0.16 mg day^{-1} 500 g bw) (Msol-M group), Monascinol of high doses (0.31 mg day^{-1} 500 g bw) (Msol-H group), Monascin of high doses (0.31 mg day^{-1} 500 g bw) (MS-H group), Ankaflavin of high doses (0.31 mg day^{-1} 500 g bw) (AK-H group), respectively.

Table 3 Effects of *M. pilosus* SWM-008 fermented rice, Msol, MS, and AK on TC and TG concentrations in the liver and feces, and lipase and HR-LPL activities in adipose tissue of rats fed a high-fat diet

	Liver		Feces		Adipose tissue	
Groups	TC (mg g^{-1})	TG (mg g^{-1})	TC (mg g^{-1})	TG (mg g^{-1})	Lipase activity (U L^{-1})	HR-LPL activity (U L^{-1})
NOR	6.25 ± 0.42 ^{c}	19.57 ± 4.04 ^{b}	15.93 ± 1.47 ^{a}	18.25 ± 3.25 ^{a}	4.93 ± 0.93 ^{d}	58.41 ± 4.64 ^{c}
HF	7.91 ± 0.42 ^{d}	40.14 ± 2.79 ^{c}	38.03 ± 2.33 ^{b}	26.45 ± 3.35 ^{b}	3.92 ± 0.25 ^{c}	74.82 ± 5.96 ^{d}
RL	5.34 ± 0.68 ^{b}	17.68 ± 2.77 ^{b}	53.97 ± 8.59 ^{d}	58.56 ± 5.41 ^{cd}	2.52 ± 0.22 ^{b}	56.74 ± 8.85 ^{c}
RM	4.95 ± 0.29 ^{ab}	17.84 ± 2.89 ^{b}	53.41 ± 7.14 ^{d}	60.37 ± 6.04 ^{d}	2.48 ± 0.24 ^{b}	44.21 ± 9.28 ^{b}
RH	4.89 ± 0.68 ^{ab}	17.06 ± 2.94 ^{b}	51.22 ± 4.13 ^{d}	63.88 ± 5.01 ^{d}	2.11 ± 0.28 ^{ab}	43.23 ± 11.61 ^{b}
Msol-M	5.05 ± 0.54 ^{ab}	14.40 ± 1.57 ^{a}	46.22 ± 4.72 ^{c}	61.27 ± 7.80 ^{d}	3.59 ± 0.37 ^{c}	37.21 ± 14.47 ^{ab}
Msol-H	4.55 ± 0.95 ^{a}	13.80 ± 0.94 ^{a}	44.45 ± 2.83 ^{c}	54.92 ± 3.97 ^{c}	1.73 ± 0.35 ^{a}	30.49 ± 10.89 ^{a}
MS-H	4.67 ± 0.89 ^{ab}	14.18 ± 1.85 ^{a}	44.64 ± 3.82 ^{c}	54.26 ± 7.06 ^{c}	2.49 ± 0.23 ^{b}	37.12 ± 15.92 ^{ab}
AK-H	4.86 ± 0.53 ^{ab}	13.84 ± 1.59 ^{a}	45.46 ± 4.27 ^{c}	54.92 ± 4.20 ^{c}	2.54 ± 0.21 ^{b}	37.42 ± 14.24 ^{ab}

Two groups of SD rats were fed a normal diet (NOR group) or a high fat diet (HF group) without the administration of test materials, respectively. The SD rats fed high fat diet, that were administrated with *M. pilosus* SWM-008 fermented rice of low doses (25.83 mg day^{-1} 500 g bw) (RL group), *M. pilosus* SWM-008 fermented rice of medium doses (51.67 mg day^{-1} 500 g bw) (RM group), *M. pilosus* SWM-008 fermented rice of high doses (103.33 mg day^{-1} 500 g bw) (RH group), Monascinol of medium doses (0.16 mg day^{-1} 500 g bw) (Msol-M group), Monascinol of high doses (0.31 mg day^{-1} 500 g bw) (Msol-H group), Monascin of high doses (0.31 mg day^{-1} 500 g bw) (MS-H group), Ankaflavin of high doses (0.31 mg day^{-1} 500 g bw) (AK-H group), respectively. Data are presented as means ± SD (n = 8). Mean values within each column with different superscripts are significant difference (p < 0.05).

ively, reaching levels comparable to NOR (p > 0.05). Msol-M, Msol-H, MS-H, and AK-H reduced TG by 64.1%, 65.6%, 64.7%, and 65.5%, respectively (p < 0.05).

All test substances significantly increased fecal TC excretion compared to HF (p < 0.05), with increases of 41.9% (RL), 40.6% (RM), and 34.7% (RH), and 21.5% (Msol-M), 16.9% (Msol-H), 17.4% (MS-H), and 19.5% (AK-H). For fecal TG excretion, RL, RM, and RH showed the largest increases at 121.4%, 128.2%, and 141.5%, respectively, while Msol-M, Msol-H, MS-H, and AK-H showed increases of 131.6%, 107.6%,

105.1%, and 107.6% ($p < 0.05$). These results suggest that RH, Msol, MS, and AK enhance lipid excretion through feces, contributing to their lipid-lowering effects. Future studies could investigate whether this enhanced lipid excretion is mediated by increased lipid transport to the intestinal lumen or reduced lipid absorption in the gastrointestinal tract, using targeted absorption and metabolism studies.

Lipase hydrolyzes fat into glycerol and fatty acids; an increase in lipase activity in adipose tissue is associated with reduced body fat. However, as shown in Table 3, the test substances did not significantly increase lipase activity compared to the HF group ($p > 0.05$); instead, they significantly decreased lipase activity ($p < 0.05$). This suggests that the test substances may inhibit fat formation rather than promote fat breakdown.

Lipoprotein lipase (LPL) facilitates the storage of free fatty acids in adipose tissue. A high-fat and high-carbohydrate diet can reduce LPL activity in skeletal muscle while increasing it in adipose tissue, promoting fat storage and leading to obesity. As shown in Table 3, the HF group had significantly higher HR-LPL activity compared to the NOR group ($p < 0.05$), indicating increased lipid storage in adipose tissue. After administration of the test substances, HR-LPL activity was significantly reduced by 24.2%, 40.9%, 42.2%, 50.3%, 50.4%, and 50.0% in the RL, RM, RH, Msol-M, MS-H, and AK-H groups, respectively ($p < 0.05$), with Msol-H showing the most significant reduction at 59.2% ($p < 0.05$). These findings suggest that Msol-H effectively reduces HR-LPL activity, thereby decreasing fat storage. While our study indicates reduced HR-LPL activity, it remains unclear whether this is due to a direct reduction in enzymatic activity or a decrease in HR-LPL protein concentration. Future studies using enzymatic assays and protein expression analysis (*e.g.*, western blot) are needed to determine the exact mechanism. Additionally, histological analysis of adipose tissue could provide further insights into the localization and activity of LPL.

3.4. Regulation of lipolysis and lipogenesis by Msol, MS, and AK

During the early stages of preadipocyte differentiation, transcription factors C/EBPβ and C/EBPδ are transiently expressed, inducing the expression of PPARγ and C/EBPα, which subsequently promote differentiation into mature adipocytes.[22,23] As shown in Fig. 3(A), a high-fat diet significantly increased C/EBPβ, PPARγ, and C/EBPα protein levels in perirenal fat in the HF group ($p < 0.05$). Administration of test substances reduced the expression of these proteins, with the MS-H group showing the most significant reduction in C/EBPβ expression ($p < 0.05$). The Msol-M, Msol-H, MS-H, and AK-H groups significantly decreased PPARγ expression ($p < 0.05$), while RH, Msol-H, and MS-H groups showed the greatest reductions in C/EBPα levels ($p < 0.05$). These findings suggest that MS-H is most effective in reducing preadipocyte differentiation into mature adipocytes, thus decreasing fat formation.

SIRT1 in adipocytes inhibits PPARγ, and its overexpression reduces fat formation. UCP-1 is found in brown and beige adipose tissue, where it is activated by β-adrenergic signaling to convert energy into heat.[25] As shown in Fig. 3(A), the RL, RM, RH, Msol-M, Msol-H, and MS-H groups significantly increased SIRT1 protein levels compared to the HF group ($p < 0.05$), with the Msol-H group showing the highest increase ($p < 0.05$). No significant difference was observed in the AK-H group compared to the HF group ($p > 0.05$). UCP-1 protein expression was significantly increased in the RH and Msol-H groups compared to the HF group ($p < 0.05$). FFAR2, which inhibits the breakdown of TG to FFA, was significantly decreased in the RH, Msol-H, MS-H, and AK-H groups compared to the HF group ($p < 0.05$). These results suggest that Msol-H effectively inhibits PPARγ to reduce fat formation, enhances UCP-1 for thermogenesis, and lowers FFAR2 to promote fatty acid breakdown.

Furthermore, adipose tissue and liver lipid metabolism exhibit complementary regulatory mechanisms under the influence of test compounds. In the liver, the expression of key markers involved in fatty acid synthesis and oxidation was analyzed. As shown in Fig. 3(B), a high-fat diet suppressed p-AMPK, p-ACC, PPARα, and CPT-1α expression ($p < 0.05$). Administration of the test substances generally increased the expression of p-AMPK, p-ACC, PPARα, and CPT-1α ($p < 0.05$). The MS-H and AK-H groups showed the greatest increase in AMPK phosphorylation compared to the HF group ($p < 0.05$). While no significant difference was observed between the Msol-M and Msol-H groups ($p > 0.05$), both significantly increased ACC phosphorylation compared to the HF group ($p < 0.05$), suggesting that Msol inhibits TG accumulation in the liver. The RL, RM, RH, MS-H, and AK-H groups significantly increased PPARα and CPT-1α expression compared to the HF group ($p < 0.05$), with the RH group showing the highest increase in CPT-1α expression ($p < 0.05$). Additionally, the specific modulation of these markers suggests that red mold rice enhances CPT-1α expression to promote fatty acid oxidation in the liver, while Msol-M and Msol-H enhance ACC phosphorylation to inhibit fatty acid synthesis, thereby reducing hepatic TG accumulation. Together, these findings provide a comprehensive view of how test substances regulate lipid metabolism across different tissues, contributing to their anti-obesity and metabolic health benefits.

3.5. Modulation of gut microbiota and its correlation with metabolic health by Msol, MS, and AK

This study investigated the effects of *M. pilosus* SWM-008 fermented rice and its active components MS, Msol, and AK on gut microbiota composition. As shown in Fig. 4(A), the results indicated significant changes in the abundance of gut bacteria. In the HF group, an increase in the abundance of *Blautia glucerasea* was observed. Similarly, the HF group also demonstrated an increased abundance of *Akkermansia muciniphila*. In contrast, cardiovascular-related bacteria such as *Eubacterium coprostanoligenes*, *Ruminococcus albus*, and *Ruminococcoides billi* were significantly reduced in the HF group. The RH, Msol, MS, and AK groups exhibited distinct effects on gut microbiota composition. In the RH group, bacteria such as

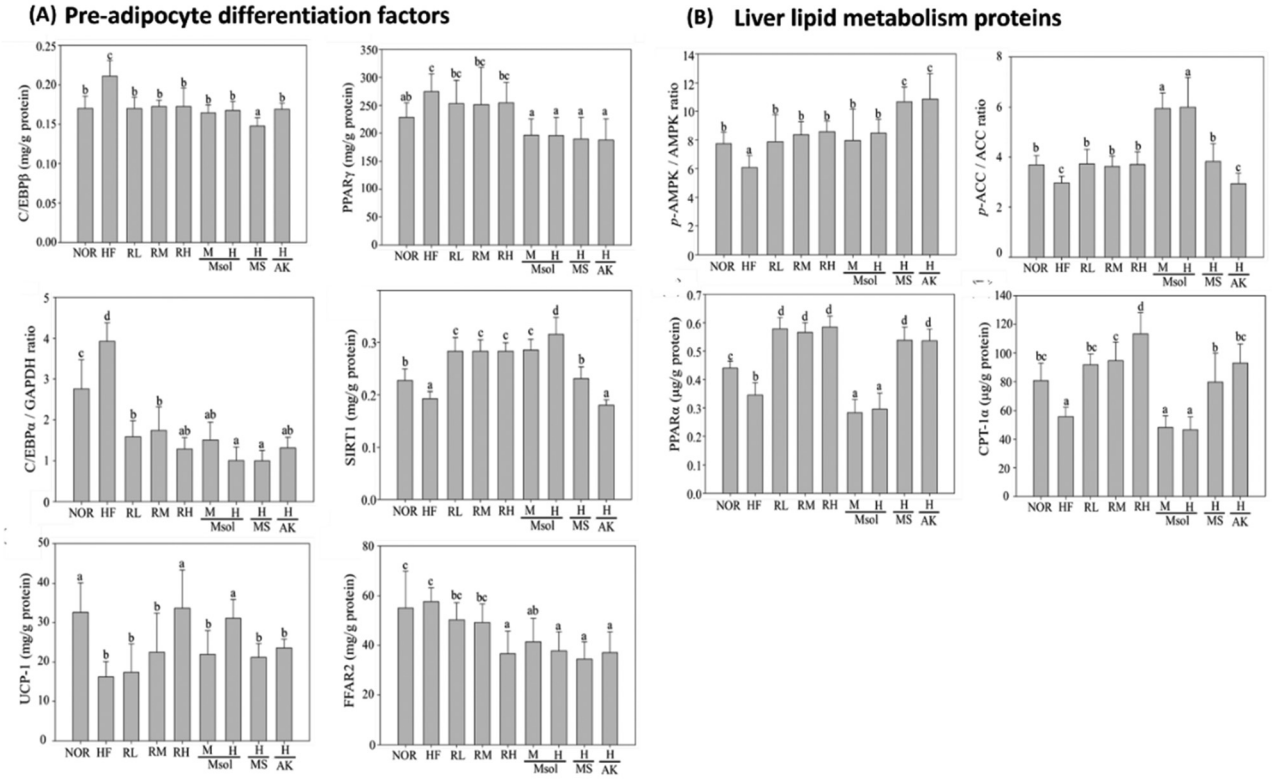

Fig. 3 Effects of *M. pilosus* SWM-008 fermented rice, Msol, MS, and AK on (A) pre-adipocyte differentiation factors in adipocytes and (B) liver lipid metabolism proteins in high-fat diet-fed male SD rats. Two groups of SD rats were fed a normal diet (NOR group) or a high fat diet (HF group) without the administration of test materials, respectively. The SD rats fed high fat diet, that were administrated with *M. pilosus* SWM-008 fermented rice of low doses (25.83 mg day^{-1} 500 g bw) (RL group), *M. pilosus* SWM-008 fermented rice of medium doses (51.67 mg day^{-1} 500 g bw) (RM group), *M. pilosus* SWM-008 fermented rice of high doses (103.33 mg day^{-1} 500 g bw) (RH group), Monascinol of medium doses (0.16 mg day^{-1} 500 g bw) (Msol-M group), Monascinol of high doses (0.31 mg day^{-1} 500 g bw) (Msol-H group), Monascin of high doses (0.31 mg day^{-1} 500 g bw) (MS-H group), Ankaflavin of high doses (0.31 mg day^{-1} 500 g bw) (AK-H group), respectively. Data were generated using enzyme-linked immunosorbent assay (ELISA) kits to quantify the expression levels of pre-adipocyte differentiation factors and liver lipid metabolism proteins. Results are presented as means ± SD (n = 8). Mean values within each column with different superscripts are significantly different ($p < 0.05$).

Negativibacillus massiliensis and *Lacrimispora aerotolerans* significantly increased. The Msol group showed an increase in *Bifidobacterium pseudolongum* subsp. *globosum*. The MS group demonstrated an increase in *Prevotella rara* and *Enterocloster bolteae*, while the AK group showed an increased abundance of *Akkermansia muciniphila*. These results suggest that different dietary treatments exert diverse impacts on gut microbiota composition.

The Firmicutes/Bacteroidetes (F/B) ratio is commonly used as an indicator of disease risk. Compared to the NOR group, the high-fat diet significantly reduced the relative abundance of Bacteroidetes, thereby increasing the F/B ratio, which is often associated with obesity and metabolic dysfunction. As shown in Fig. 4(B), administration of the test substances, including MS, Msol, and AK, led to a reduction in the F/B ratio compared to the HF group, indicating a potential ameliorative effect on the gut microbial imbalance induced by a high-fat diet. At the phylum level, five predominant bacterial phyla were detected: Firmicutes, Bacteroidetes, Verrucomicrobia, Actinobacteria, and Proteobacteria, with Firmicutes and Bacteroidetes together comprising over 90% of the total gut microbiota. At the species level, supplementation with MS, Msol, and AK led to significant changes in specific gut microbial populations, notably increasing the abundance of Akkermansia muciniphila and Bacteroides thetaiotaomicron, while decreasing the abundance of Romboutsia ilealis. These findings suggest that MS, Msol, and AK selectively modulate bacterial populations within the gut, which may contribute to improved metabolic health outcomes.

Alpha diversity refers to the diversity within an ecosystem or specific area and reflects the richness and evenness of sample composition. In gut microbiota analysis, the indices used include Simpson and Menhinick richness. The Simpson index measures diversity, where higher values indicate lower species diversity. Menhinick richness represents species richness, where higher values indicate greater richness. As shown in Fig. 4(C), the AK group significantly increased gut microbiota diversity and reduced phylogenetic distance compared to the HF group ($p < 0.05$) but significantly decreased species richness ($p < 0.05$). No significant changes in diversity and richness were observed in the other test substance groups compared to the HF group ($p > 0.05$).

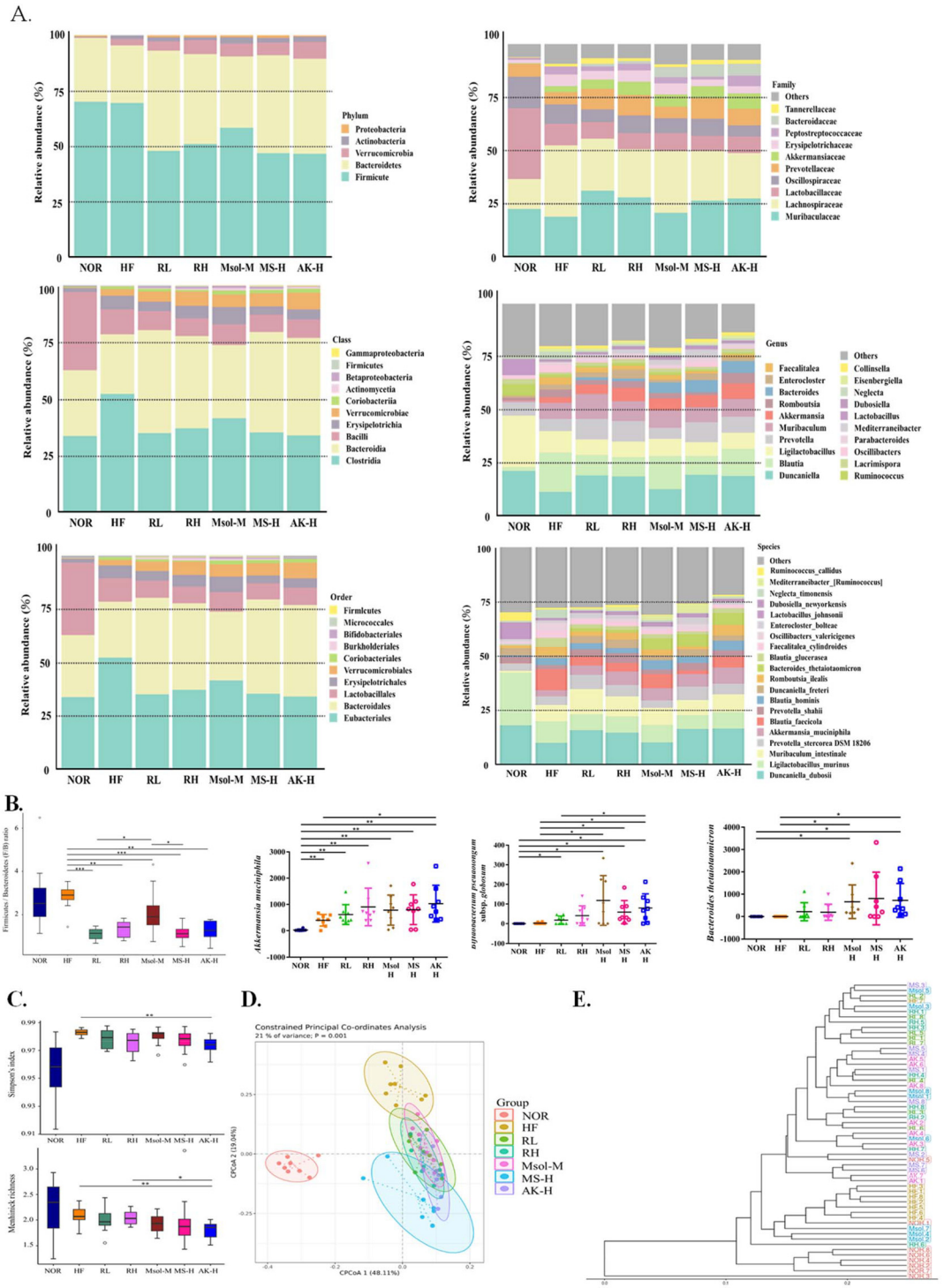

Fig. 4 Effects of *M. pilosus* SWM-008 fermented rice, Msol, MS, and AK on gut microbiota composition (A), alpha-diversity (B and C), beta-diversity (D), and species distribution (E) in rats fed a high-fat diet. Data are presented as means ± SD (n = 8). Data were assessed using Kruskal–Wallis test, mean values within each column with different superscripts are significant difference (*p < 0.05, **p < 0.01, ***p < 0.001). Extreme values for non-pairwise statistics are shown on the plot as open dots.

Beta diversity assesses differences in microbial community composition between samples or groups and serves as an indicator of similarity among gut microbiota compositions across groups. As shown in Fig. 4(D), the high-fat diet group (HF) was clearly separated from the NOR group, indicating significant differences in microbial composition. After administering RL, RH, Msol-M, MS-H, and AK-H, the gut microbiota composition of these groups was distinctly separated from the HF group,

suggesting that the test substances improved gut microbiota similarity. The UPGMA (Unweighted Paired-Group Method Using Arithmetic Means) analysis illustrates the phylogenetic relationships between microbial samples, with different groups represented by distinct colors. As shown in Fig. 4(E), the evolutionary distances between samples fed with test substances and those on a high-fat diet were markedly distinct, indicating low species similarity. Comparisons among the test substance groups showed closer distances between RL and RH groups, as well as between MS-H and AK-H groups, suggesting high species similarity within these pairs.

This study aimed to elucidate the effects of *M. pilosus* SWM-008 fermented rice and its active constituents—MS, Msol, and AK—on the composition of the gut microbiota. As illustrated in Fig. 5(A), the administration of these substances induced significant shifts in the abundance of key bacterial taxa. In the high-fat diet (HF) group, there was a notable increase in the abundance of *Blautia glucerasea* and *Akkermansia muciniphila*, while the levels of *Eubacterium coprostanoligenes*, *Ruminococcus albus*, and *Ruminococcoides billi* were significantly reduced. These results suggest that a high-fat diet exerts a distinct influence on microbial community dynamics, favoring some taxa while diminishing others. The RH, Msol, MS, and AK treatment groups exhibited differentiated impacts on the gut microbiota composition. In the RH group, the relative abundance of *Negativibacillus massiliensis* and *Lacrimispora aerotolerans* showed a significant increase, highlighting the potential modulatory effect of RH supplementation on gut microbial balance. Similarly, the Msol group was characterized by an elevated presence of *Bifidobacterium pseudolongum subsp. globosum*. In the MS group, a marked increase was observed in *Prevotella rara* and *Enterocloster bolteae*, whereas the AK group was associated with an increased abundance of *Akkermansia muciniphila*. These observations indicate that each treatment exerts a unique influence on microbial diversity and community composition.

SCFAs such as acetate, propionate, and butyrate are critical metabolites produced by gut bacteria and are central to maintaining gut and metabolic health. SCFAs play various roles, including modulating energy metabolism, immune function, and inflammation. Fig. 5(B) shows that the Msol-M group significantly reduced fecal acetate levels compared to the HF group ($p < 0.05$), suggesting a potential decrease in lipid accumulation. In contrast, RL, RH, MS-H, and AK-H groups showed no significant differences in fecal acetate levels ($p > 0.05$). Regarding fecal propionate levels, the RH group exhibited a significant increase ($p < 0.05$), indicating enhanced microbial fermentation activity, while RL, Msol-M, MS-H, and AK-H groups did not show significant changes compared to the HF group ($p > 0.05$). Furthermore, no significant differences were observed in fecal butyrate levels among the treatment groups and the HF group ($p > 0.05$), indicating that butyrate production was not markedly affected by the test substances.

HAllA (Hierarchical All-against-All Association) analysis was used to explore the correlations between gut microbiota and obesity in rats. As shown in Fig. 6(A), this study explores the impact of gut microbiota on body fat formation, weight gain, lipid metabolism, cardiovascular disease, and steatotic liver disease risk by analyzing correlations between microbial abundance and metabolic health indicators. Results reveal significant associations between gut microbiota and key pathways related to adipocyte differentiation, fatty acid metabolism, and energy regulation. *Ligilactobacillus murinus* and *Lactobacillus johnsonii* showed negative correlations with body fat ($p < 0.05$), suggesting a role in reducing fat formation through fatty acid metabolism. In contrast, *Blautia glucerasea* and *Oliverpabstia intestinalis* were positively correlated with body fat ($p < 0.01$), indicating promotion of fat synthesis and storage. *Ruminococcus callidus* showed negative correlations with body fat ($p < 0.05$), supporting its role in enhancing fatty acid metabolism. Weight gain was positively correlated with *Oliverpabstia intestinalis* and *Absicoccus porci* ($p < 0.05$), while *Akkermansia muciniphila* was negatively correlated with body fat and liver fat accumulation and positively associated with feed efficiency ($p < 0.05$), indicating potential benefits for reducing fat accumulation.

Lipase activity was positively associated with *Ruminococcus callidus* and *Vescimonas fastidiosa* ($p < 0.05$), suggesting enhanced fat breakdown, whereas *Akkermansia muciniphila* showed a negative correlation ($p < 0.05$). Transcription factors involved in adipocyte differentiation, such as C/EBPβ and C/EBPα/GAPDH, were correlated with *Ligilactobacillus murinus* and *Ruminococcus callidus* ($p < 0.05$), indicating a regulatory role in fat metabolism. SIRT1, a marker of lipid metabolism, was positively associated with *Prevotella stercorea* DSM 18206 and *Enterocloster bolteae* ($p < 0.05$). FFAR2, a fatty acid receptor, was positively correlated with *Duncaniella dubosii* ($p < 0.01$) and negatively with *Muribaculum gordoncarteri* ($p < 0.01$). p-AMPK/AMPK, an energy metabolism marker, was positively correlated with *Akkermansia muciniphila* and *Enterocloster bolteae* ($p < 0.01$), suggesting enhanced energy metabolism and reduced fat storage. From a cardiovascular perspective, serum TG are positively correlated with *Bacteroides thetaiotaomicron* and negatively with *Duncaniella dubosii* ($p < 0.05$). *Blautia glucerasea* shows a positive correlation with serum TG ($p < 0.05$), potentially elevating cardiovascular risk. Liver triglycerides (Liver TG) correlate positively with *Blautia glucerasea* and *Oliverpabstia intestinalis*, while *Akkermansia muciniphila* shows a negative correlation ($p < 0.05$). Overall, the study highlights the contrasting roles of gut microbiota in fat accumulation and metabolism. Specific strains like *Akkermansia muciniphila* and *Ruminococcus callidus* could help reduce fat storage, while others like *Blautia glucerasea* may promote it, suggesting potential microbiota-targeted strategies for managing metabolic diseases.

Fig. 6(B) systematically analyzed the correlations between gut microbiota composition, body fat formation, metabolic indicators, and related protein expressions, highlighting the significant role of gut microbiota in host energy metabolism and lipid accumulation. The abundance of *Oliverpabstia* and *Absicoccus* showed a positive correlation with feed efficiency

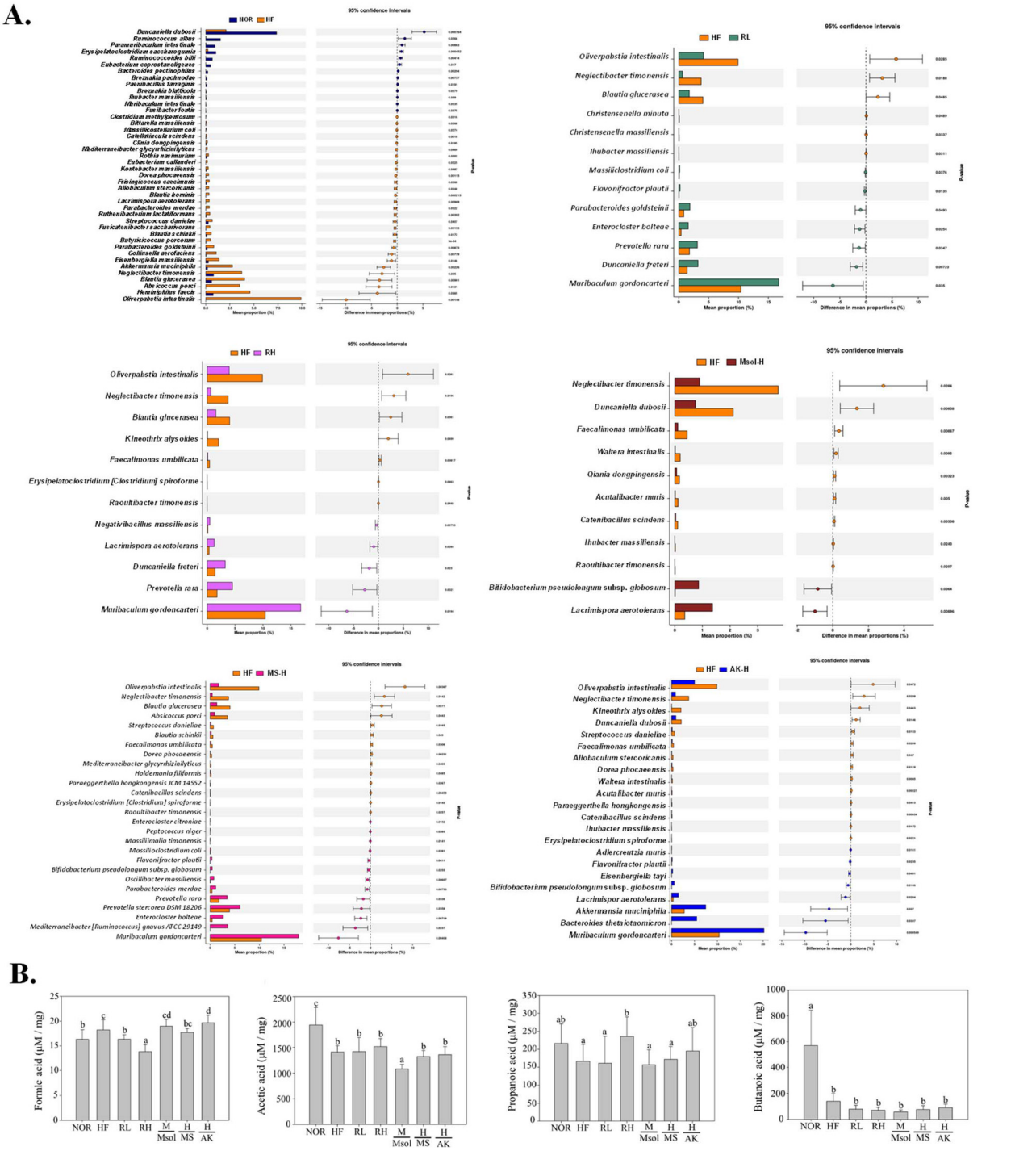

Fig. 5 Effects of *M. pilosus* SWM-008 fermented rice, Msol, MS, and AK on gut microbiota composition (A), and on short-chain fatty acids (B) in the gut microbiota of rats fed a high-fat diet. Data are presented as means ± SD (n = 8). Mean values within each column with different superscripts are significant difference ($p < 0.05$).

and body fat formation, suggesting that these bacteria may promote energy storage and fat synthesis. Conversely, these metabolic indicators were negatively correlated with the expression levels of FFAR2 and UCP1, indicating that these proteins are crucial in energy expenditure and fat breakdown. RMR and Msol significantly increased UCP1 expression, while RMR, MS, and AK significantly reduced the abundance of *Oliverpabstia*, suggesting that these components can modulate gut microbiota to reduce fat accumulation. In the HF group, fecal cholesterol and triglyceride levels were elevated; however,

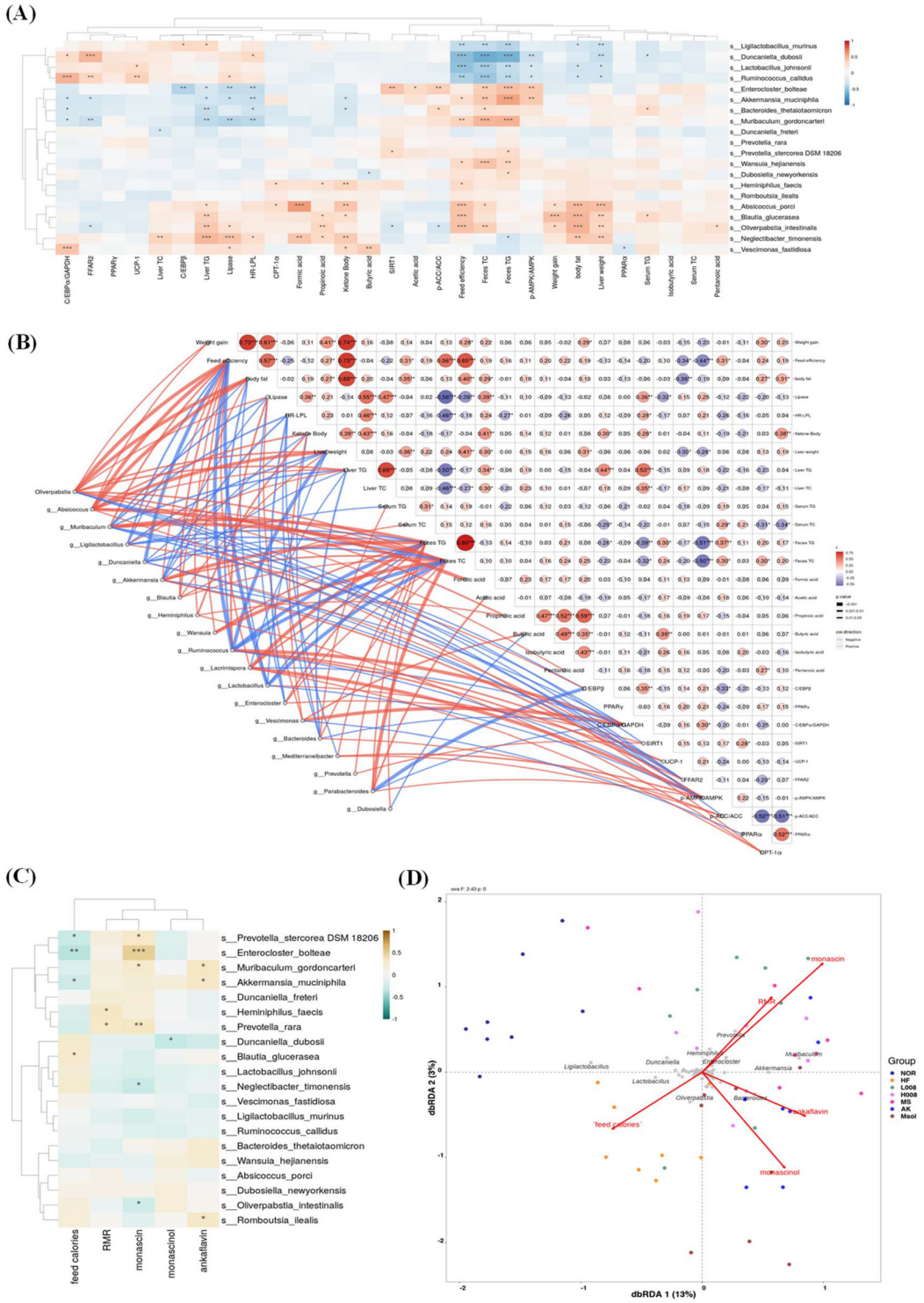

Fig. 6 Correlation analysis and redundancy analysis (RDA) of gut microbiota in rats fed a high-fat diet. (A) Heatmap displaying the correlation between the top 20 species and biochemicals factors. (B) Correlogram illustrating genus-function relationships, with red and blue lines indicating positive and negative correlations, respectively. (C) Heatmap showing the correlation between the top 20 genera and experimental factors. (D) Constrained Ordination Plot using Redundancy Analysis (RDA), integrating the top 20 genera, predicted metabolic pathways, and experimental factors. Pearson correlation and FDR-adjusted p-values were applied to assess statistical significance.

RMR and its active components promoted the excretion of these lipids, reducing lipid absorption and subsequently decreasing body fat content. Fecal cholesterol and triglycerides were negatively correlated with the expression of C/EBP-α and FFAR2 and positively correlated with the expression of SIRT1 and p-AMPK, indicating their roles in lipid metabolism and

energy regulation. Additionally, *Akkermansia* was positively correlated with fecal cholesterol and triglycerides, further emphasizing its impact on lipid metabolism. While there were fewer significant correlations between SCFAs and obesity-related protein expressions, *Oliverpabstia* showed positive correlations with propionic and valeric acids, and *Absicoccus* was positively correlated with formic acid, indicating potential roles for these bacteria in specific fatty acid metabolism. Overall, this study demonstrates that by modulating gut microbiota and metabolic pathways, RMR and its derivatives can effectively regulate lipid metabolism and reduce body fat accumulation, providing a potential approach for managing obesity and metabolic diseases.

In Fig. 6(C), integrating environmental factor analysis, this study reveals that certain gut microbiota are involved in metabolic and fat accumulation processes. *Prevotella stercorea* DSM 18206, *Enterocloster bolteae*, and *Akkermansia muciniphila* negatively correlated with feed calories, suggesting they may reduce energy intake or increase expenditure, whereas *Blautia glucerasea* positively correlated, indicating it promotes energy utilization. *Heminiphilus faecis* and *Prevotella rara* positively correlated with red mold rice (RMR), suggesting effects on energy metabolism or lipid pathways. Monascin increased *Prevotella stercorea* DSM 18206, *Enterocloster bolteae*, *Muribaculum gordoncarteri*, and *Prevotella rara* while decreasing *Neglectibacter timonensis* and *Oliverpabstia intestinalis*. Monascinol increased *Muribaculum gordoncarteri*, *Akkermansia muciniphila*, and *Romboutsia ilealis* but decreased *Duncaniella dubosii*, indicating distinct roles in metabolic regulation.

In Fig. 6(D), distance-based redundancy analysis (dbRDA) showed that environmental factors like feed calories, RMR, and its derivatives significantly influence gut microbiota composition and metabolic health. High feed calories increased *Blautia glucerasea*, linked to fat accumulation, and decreased *Prevotella stercorea* DSM 18206, *Enterocloster bolteae*, and *Akkermansia muciniphila*, showing a negative correlation with high caloric intake. RMR intake raised *Heminiphilus faecis* and *Prevotella rara*, potentially benefiting lipid metabolism. Monascin and monascinol selectively modulated gut bacteria, impacting lipid metabolism and health outcomes, suggesting that dietary components like RMR derivatives can alter microbiota composition and metabolic health.

4. Discussion

The study evaluated the anti-obesity effects of *M. pilosus* SWM-008 fermented rice, focusing on the active component Msol in high-fat diet-induced obese rats. The fermented rice, containing Msol, MS, and other metabolites, significantly reduced body weight, adipocyte size, hepatic lipid accumulation, and HR-LPL activity ($p < 0.05$). Msol showed strong anti-obesity effects, particularly against weight gain and lipid metabolism regulation, comparable to MS and more effective than AK. Although Msol was less effective than MS in reducing fat weight and body fat percentage, it still outperformed AK. No significant differences were found among Msol, MS, and AK in promoting fecal lipid excretion ($p > 0.05$). Overall, the findings suggest that *M. pilosus* SWM-008 fermented rice, rich in Msol and other bioactive compounds, is a potent multi-component treatment for reducing obesity parameters.

Obesity results from fat accumulation. The study showed that Msol reduces body weight, fat mass, adipocyte size, and hepatic lipid accumulation while inhibiting HR-LPL activity ($p < 0.05$). Lipase and HR-LPL activities are key indicators of lipid metabolism, related to lipid breakdown and storage, respectively. The lack of increase in lipase activity suggests that Msol primarily promotes hepatic lipid breakdown and inhibits fat storage rather than enhancing overall lipid breakdown. During adipocyte differentiation, preadipocytes transform into mature adipocytes through a cascade of transcription factors, starting with C/EBPβ inducing PPARγ, which subsequently induces C/EBPα.[23] Msol significantly downregulated the expression of C/EBPβ, PPARγ, and C/EBPα ($p < 0.05$), showing a more pronounced effect compared to MS and AK. Other proteins in adipose tissue, such as SIRT1, inhibit PPARγ to suppress adipocyte differentiation, while UCP-1 activation induces thermogenesis.[24,25] FFAR2 facilitates acetate and propionate transport to adipocytes, inhibiting triglyceride breakdown into free fatty acids.[14] Msol increased SIRT1 and UCP-1 expression and decreased FFAR2 expression ($p < 0.05$), showing stronger effects than MS and AK in reducing fat formation and promoting fatty acid breakdown. In the liver, Msol enhanced AMPK activity, leading to increased ACC phosphorylation, which inhibited fatty acid synthesis and reduced triglyceride (TG) accumulation. However, Msol had limited impact on increasing PPARα and CPT-1 activity, which are involved in β-oxidation. Thus, Msol mainly reduces TG accumulation without significantly enhancing fatty acid metabolism.

MS and Msol, with similar structures as secondary metabolites produced by *Monascus* species, both reduce body weight, feed efficiency, fat weight, body fat percentage, adipocyte size, cell number, hepatic lipid accumulation, and HR-LPL activity ($p < 0.05$). Despite these similarities, Msol shows a stronger effect in regulating adipose tissue by significantly increasing SIRT1 and UCP-1 expression and decreasing FFAR2 expression ($p < 0.05$), which more effectively inhibits preadipocyte differentiation and promotes fatty acid breakdown. While MS is more effective in promoting β-oxidation in the liver and enhancing fecal lipid excretion, Msol offers an advantage in preventing fat formation and enhancing lipid breakdown in adipose tissue, making it particularly effective for targeting fat storage and adipogenesis-related pathways in obesity management. *Monascus pilosus* SWM-008 fermented rice, containing MS, Msol, and other active metabolites, has synergistic anti-obesity effects. It reduces hepatic lipid accumulation and enhances fecal lipid excretion more effectively than the pure compounds. The fermented rice decreases adipocyte differentiation markers (C/EBPβ, PPARγ, C/EBPα), increases SIRT1 and UCP-1, and reduces FFAR2 ($p < 0.05$), enhancing lipid breakdown and thermogenesis. AMPK, ACC, and PPAR are closely interconnected in regulating energy and lipid metabolism,

forming a coordinated network. As an energy sensor, AMPK phosphorylates and inhibits ACC, reducing malonyl-CoA levels and promoting fatty acid oxidation. Additionally, AMPK modulates the PPAR family: it activates PPARα and PPARδ to enhance fatty acid oxidation and energy expenditure while suppressing PPARγ to reduce fat accumulation. This synergistic interaction effectively improves lipid metabolic imbalances and holds therapeutic potential for obesity, non-alcoholic steatotic liver disease, and other metabolic disorders.[26] The fermented rice also significantly boosts p-AMPK, p-ACC, PPARα, and CPT-1α, promoting β-oxidation and reducing lipid accumulation. Additionally, it lowers TC, TG, LDL-C, and raises HDL-C effectively, suggesting its potential as a functional food for obesity management.

Gut microbiota imbalance can contribute to obesity. As shown in Fig. 5, *M. pilosus* SWM-008 fermented rice, Msol, and MS did not significantly affect species diversity, richness, or evenness ($p > 0.05$). AK increased diversity but reduced richness ($p < 0.05$). Beta diversity analysis (Fig. 4) showed that *M. pilosus* SWM-008, Msol, MS, and AK improved gut microbiota similarity compared to the high-fat diet group, likely due to Msol and MS. NAFLD, closely linked to obesity but also observed in non-obese individuals, has been associated with distinct gut microbiota profiles. A study analyzing gut microbiota using 16S rRNA sequencing in 171 biopsy-confirmed Asian NAFLD patients and 31 non-NAFLD controls, with external validation in a Western cohort, identified significant microbial diversity changes correlated with fibrosis severity in non-obese individuals but not in obese individuals. Key microbial taxa, including Ruminococcaceae and Veillonellaceae, were associated with fibrosis severity in non-obese subjects. Additionally, elevated fecal bile acids and propionate levels were observed, especially in non-obese individuals with advanced fibrosis. Representative species from these taxa were further tested in three murine NAFLD models, demonstrating their role in exacerbating liver injury, highlighting the microbiota's involvement in NAFLD pathogenesis, particularly among non-obese individuals.[34]

Fig. 6 shows correlations between gut microbiota and obesity. *Akkermansia* and *Duncaniella* are negatively correlated with liver TC and TG but positively with fecal TC and TG, suggesting reduced liver lipid synthesis and increased fecal excretion. Conversely, Oscillibacter and Neglecta promote lipid synthesis, correlating positively with obesity. Previous studies have found that red mold rice increases the abundance of Prevotellaceae and Bacteroides and decreases Lachnospiraceae.[35] The Prevotellaceae family is positively associated with obesity and high dietary fiber intake,[36,37] while increased Bacteroides can reduce high-fat diet-induced obesity.[38] Lachnospiraceae is positively correlated with glucose and lipid metabolism, potentially inducing metabolic disorders.[39,40] In this study, a high-fat diet increases the abundance of *Oliverpabstia*, *Blautia*, *Neglectibacter*, and *Akkermansia* while reducing *Duncaniella* and *Ruminococcus*. Although *Oliverpabstia* has not been extensively studied, it can be inferred to have a positive correlation with obesity. The findings regarding Ruminococcaceae and Veillonellaceae align with prior evidence of their roles in lipid metabolism and fibrosis severity, particularly in non-obese NAFLD individuals, further underscoring the complex interactions between gut microbiota, obesity, and liver disease.[34]

Duncaniella, which may prevent hyperlipidemia and hyperglycemia,[41] is elevated by RL supplementation, suggesting a potential benefit for lipid and glucose profiles. *Prevotella*, linked to oxidative stress, inflammation, and type 2 diabetes, is reduced by RL and MS-H groups, indicating a beneficial effect in managing weight and inflammation.[35] However, the role of *Prevotella* can vary depending on microbiome context. *Muribaculum* produces SCFAs that promote lipolysis and fatty acid oxidation while inhibiting cholesterol synthesis.[31] Its abundance decreases in RL, RH, MS-H, and AK-H groups, suggesting mitigation of inflammation and prevention of weight gain. *Bifidobacterium*, known for its positive effects on lipids, blood pressure, and anti-obesity properties, is increased in the Msol-M group, indicating a beneficial modulation of gut flora. *Lacrimispora*, formerly *Clostridium* XIVa, is associated with liver lipid metabolism, anti-inflammatory effects, and gut barrier enhancement.[42]

The Msol-M group positively modulates *Lacrimispora*, suggesting a role in supporting liver health and enhancing gut integrity. *Mediterraneibacter*, linked to inflammation but also involved in preventing alcoholic steatotic liver disease, insulin secretion, and acetate production,[43] is modulated positively in the MS-H group, indicating potential benefits for liver health and insulin sensitivity. *Akkermansia*, known for reducing insulin resistance, serum lipids, body weight, and blood glucose levels[44–46] is positively influenced by RL, RH, Msol-M, MS-H, and AK-H, supporting its anti-inflammatory effects and improvement of gut barrier function, crucial in obesity and metabolic syndrome management. *Bacteroides*, which aids in lipid and glucose metabolism, enhancing gut barrier function, and promoting lipid metabolism,[47,48] is also positively modulated by AK-H, suggesting benefits in lipid and glucose homeostasis. Overall, RL, RH, Msol-M, MS-H, and AK-H show distinct effects on gut microbiota, highlighting their potential in improving gut health and managing obesity-related conditions. The specific modulation of these taxa could support targeted strategies for metabolic health through diet and supplementation.

A high-fat diet significantly alters gut microbiota, impacting metabolism and increasing inflammation. Genera such as *Collinsella*, *Parabacteroides*, and *Blautia* were elevated in the HF group, contributing to lipid metabolism disorders and inflammation. *Collinsella*, known for its pro-inflammatory properties, may promote cholesterol reabsorption and fat accumulation in the intestine.[49] Red mold rice and its active components, MS, Msol, and AK, demonstrated positive effects on gut microbiota. RL and MS increased *Prevotella* and *Muribaculum*, both associated with SCFA production, which supports lipid metabolism and helps reduce body fat.[36,37,50] Both MS and Msol groups also significantly increased the abundance of *Bifidobacterium*, a genus with well-documented

metabolic regulatory functions that enhances gut barrier function and reduces systemic inflammation, thereby improving metabolic health.[51] MS, Msol, and AK supplementation increased beneficial genera like *Bifidobacterium*, *Akkermansia*, *Bacteroides*, and *Muribaculum*, indicating improvements in gut health and potential anti-inflammatory effects. It is particularly important to note that while AK, MS, and Msol are all yellow pigments produced by *Monascus* species, the *M. pilosus* strain used in this study produces only MS and Msol but not AK. This study thus provides an opportunity to explore the functional differences among these pigments in modulating gut microbiota. Despite their shared origins, MS and Msol demonstrate distinct effects in promoting the growth of specific gut bacterial genera, suggesting unique mechanisms in regulating host metabolism. Therefore, AK, MS, and Msol may offer different applications for metabolic health management, warranting further research into their roles in host-gut microbiota interactions.

Studies have shown that the role of gut microbiota in obesity and high-fat diets can also be observed in humans. There are significant differences in the gut microbiota composition between obese and normal-weight individuals. The gut microbiota of obese individuals typically exhibits a higher Firmicutes/Bacteroidetes ratio, which may lead to increased energy extraction efficiency and accumulation of adipose tissue in the host.[52] Moreover, high-fat diets have been proven to induce changes in gut microbiota regardless of obesity status, leading to microbial imbalance in the gut, which may further promote the development of obesity.[53] Several studies have indicated that obese individuals have lower gut microbiota diversity and reduced levels of beneficial bacteria, such as *Akkermansia muciniphila* and *Faecalibacterium prausnitzii*, which are typically associated with a healthy metabolic state.[54] These findings suggest that, although the present study is based on animal models, similar changes in gut microbiota have also been observed in human studies. This further supports the potential regulatory role of gut microbiota in obesity and metabolic health and underscores its value as a potential target for obesity treatment.

This study has certain limitations that should be acknowledged. First, the use of only male rats limits the generalizability of the findings to female subjects, as sex-specific differences in metabolism and hormonal regulation may influence the efficacy of Msol, MS, and AK. Future studies involving female animals are necessary to better understand these potential variations. Second, the relatively short duration of this study (8 weeks) may not fully capture the long-term effects of the treatments on obesity and metabolic health. Longer intervention periods are critical to evaluate the sustainability of the observed effects and assess potential safety concerns. To

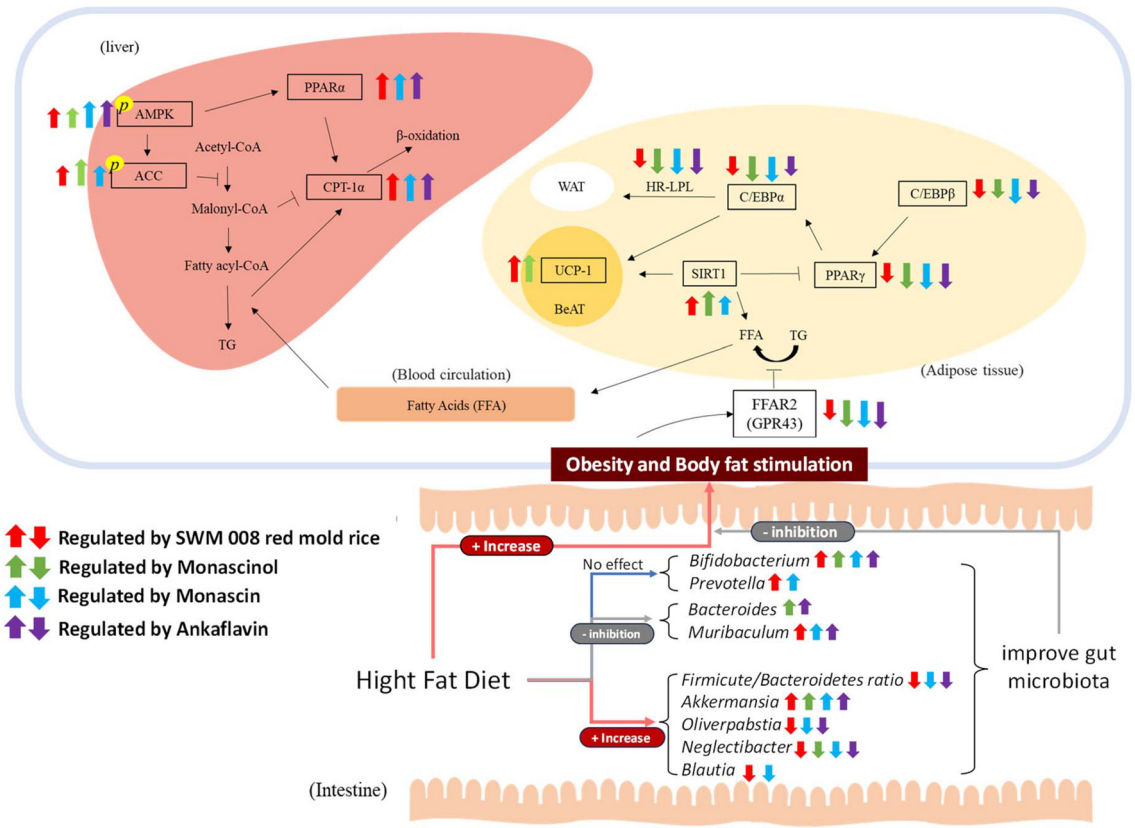

Fig. 7 Mechanism on the regulation of body fat formation and gut microbiota by *Monascus pilosus* SWM-008 fermented red mold rice and functional compounds.

address these limitations, future research directions could enhance the translational potential of these findings. Investigating the bioavailability and metabolism of Msol, MS, and AK is essential for translating these results into human dietary applications. Studies focusing on absorption, distribution, metabolism, and excretion (ADME) will provide valuable insights for dose optimization and functional food development. Additionally, exploring the potential synergistic effects of combining these compounds represents a promising avenue. The interaction of Msol, MS, and AK could amplify their anti-obesity effects and uncover novel mechanisms for managing metabolic health.

Moreover, future studies should include the analysis of brown adipose tissue (BAT) to evaluate its weight, thermogenic activity, and its contribution to the observed effects of the test compounds. As BAT plays a critical role in thermogenesis and metabolic regulation, understanding its involvement could further elucidate the mechanisms underlying the anti-obesity and metabolic benefits of these compounds. Incorporating such assessments will significantly enhance our understanding of the interplay between these bioactive compounds and adipose tissue thermogenesis. Additionally, our findings suggest that the test compounds may inhibit lipid formation rather than promote lipid breakdown, as evidenced by reduced HR-LPL activity and unaltered lipase activity in adipose tissue. Enzymes such as acetyl-CoA carboxylase (ACC), fatty acid synthase (FAS), and stearoyl-CoA desaturase-1 (SCD1), which are critical in lipid biosynthesis, could also contribute to these effects. Future research should investigate the activity and protein levels of these enzymes using techniques like western blot or enzymatic assays to better understand their roles in lipid formation and the potential mechanisms involved. Furthermore, while this study measured fecal levels of short-chain fatty acids (SCFAs) such as acetate, propionate, and butyrate, it did not analyze their levels in the blood. SCFAs are key metabolites produced by gut microbiota and have systemic effects on lipid metabolism, energy homeostasis, and inflammation. Future studies should include blood SCFA analysis to assess their systemic impact, particularly through the gut-liver and gut-brain axes. This would clarify how changes in SCFA levels contribute to the observed metabolic effects and provide a more comprehensive understanding of the test compounds' anti-obesity mechanisms.

These additional investigations would provide valuable insights into the anti-obesity effects of Msol, MS, and AK and further clarify their impact on lipid metabolism.

5. Conclusion

Obesity, driven by diet, lifestyle, and genetic factors, is linked to metabolic syndrome, including steatotic liver disease and type 2 diabetes. Red mold rice contains bioactive compounds that reduce fat accumulation. While MS and AK are well-known for these effects, this study is the first to examine the rolw of Msol in reducing body fat. Using a high-fat diet-induced obese rat model, the effects of *M. pilosus* SWM-008 fermented rice (RL, RM, RH) and purified Msol, MS, and AK were evaluated over 8 weeks. All treatments significantly reduced body weight and fat mass ($p < 0.05$). Msol effectively inhibited fatty acid synthesis by activating ACC and reducing TG accumulation, and also suppressed adipocyte differentiation by downregulating C/EBPβ, C/EBPα, and PPARγ. High-fat diets disrupt gut microbiota, but RMR and its components can modulate these changes by lowering the F/B ratio and increasing beneficial bacteria like *Akkermansia* and *Bifidobacterium*. This suggests RMR and its yellow pigments have potential to improve gut health. Additionally, RL, RM, RH, MS, and AK enhanced lipid breakdown and thermogenesis in adipose tissue by increasing SIRT1 and UCP-1 expression. Overall, the findings suggest that RMR and its components, especially Msol, are effective in managing obesity by reducing fat formation, enhancing lipid metabolism, and favorably altering gut microbiota (Fig. 7).

Author contributions

Chin-Feng Liu: investigation, writing, experiment design, supervision; Hui-Tzu Chuang: experiment design, animal test, sample analysis, writing. Chia-Shu Wang: sample preparation; Ya-Wen Hsu: methodology, validation, investigation. Tzu-Ming Pan: methodology, validation, supervision, review & editing. Chun-Lin Lee: conceptualization, investigation, resources, supervision, writing – review & editing. All authors have read and agreed to the published version of the manuscript.

Institutional review board statement

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Institutional Animal Care and Use Committee (IACUC) of the National Taitung University (approved no. NTTU 110007, 2019/12/29).

Data availability

All data included in this study are available upon request by contacting the corresponding author.

The data supporting this article have been included as part of the ESI.

Conflicts of interest

The authors declare no conflict of interest in this paper.

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