

ORIGINAL ARTICLE

# Mechanisms of combined deer antler polysaccharides and postbiotics supplementation for regulating obesity in mice

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## Popular scientific summary

- The combined use of antler polysaccharides and postbiotics has a significant effect on lipid regulation.
- Supplementation of antler polysaccharides and postbiotics can significantly inhibit the weight gain of obese mice, reduce serum total cholesterol, triglyceride and low-density lipoprotein levels, and significantly increase serum high-density lipoprotein levels.
- Supplementation of antler polysaccharides and postbiotics can improve liver lipid droplet accumulation and adipocyte hypertrophy.
- Supplementation of antler polysaccharides and postbiotics can regulate the expression of lipid synthesis genes sterol regulatory element binding protein 1, fatty acid synthase peroxisome proliferator-activated receptor  $\alpha$  and acyl-CoA oxidase 1.
- Supplementation of antler polysaccharides and postbiotics can regulate the expression of inflammation-related genes tumor necrosis factor- $\alpha$ , interleukin-6, and interleukin-1.

## Abstract

**Objective:** This study investigated the mechanisms related to lipid metabolism regulation after combined supplementation with deer antler polysaccharides and postbiotics.

**Methods:** Thirty-two male mice were divided into high-fat diet, HD + deer antler polysaccharides, HD + *Bacillus coagulans* postbiotics, and HD + deer antler polysaccharides + *B. coagulans* postbiotics groups. The diets contained 60% fat. After 9 weeks, the effects of deer antler polysaccharides and postbiotics on lipid metabolism were assessed through blood biochemical, histological tissue staining, and polymerase chain reaction analyses.

**Results:** Supplementation with deer antler polysaccharides and postbiotics significantly inhibited weight gain in obese mice, reduced serum total cholesterol, triglyceride, and low-density lipoprotein levels and markedly increased the serum high-density lipoprotein level. Additionally, hepatic lipid droplet accumulation and adipocyte hypertrophy improved. The expressions of the lipid synthesis genes, sterol regulatory element-binding protein 1 (i.e. *SREBP-1c*), and fatty acid synthase (i.e. *FAS*), significantly decreased, while peroxisome proliferator-activated receptor alpha (i.e. *PPAR- $\alpha$* ) and acyl-CoA oxidase 1 (i.e. *ACOX1*) expression significantly increased. The expressions of the inflammation-related genes, tumor necrosis factor-alpha (i.e. *TNF- $\alpha$* ), interleukin (*IL*)-6, and *IL-1* also significantly decreased.

**Conclusion:** Thus, combined deer antler polysaccharides and postbiotic supplementation regulated obesity in mice, potentially by modulating lipid synthesis and inflammation-related gene expression.

Keywords: deer antler polysaccharides; postbiotics; lipid metabolism; regulatory effects

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Obesity is a growing global health concern and a complex metabolic disorder characterized by excessive fat accumulation. Obesity substantially elevates the risk of developing several health conditions, such as cardiovascular diseases, type 2 diabetes, and certain types of cancer (1). The global burden of obesity has reached alarming levels, with recent studies indicating that approximately 39% of adults worldwide are overweight or obese (2). Obesity is primarily the result of an imbalance between energy intake and expenditure, often influenced by genetic, environmental, and behavioral factors. Diet, physical activity, and genetic predisposition play crucial roles in its development (3). Excessive calorie consumption, particularly of high-fat and high-sugar diets, coupled with a sedentary lifestyle, are key contributors to the obesity epidemic (4). In addition to the direct impact on physical health, obesity is also associated with alterations in endocrine functions, such as dysregulated adipokine production and insulin resistance (5). At the molecular level, obesity is characterized by altered lipid metabolism, chronic low-grade inflammation, and dysfunction in several key signaling pathways, including those involving insulin, leptin, and adiponectin. These disruptions contribute to a vicious cycle of metabolic dysregulation and inflammatory responses, further exacerbating obesity and its associated comorbidities (6, 7). Recent studies have focused on the molecular mechanisms underlying obesity, particularly the role of key regulatory genes (8, 9). Sterol regulatory element-binding protein 1c (*SREBP-1c*) and fatty acid synthase (*FAS*) enzymes are pivotal in lipogenesis, thus promoting fat storage and synthesis (10, 11). Conversely, peroxisome proliferator-activated receptor alpha (*PPAR- $\alpha$* ) and acyl-CoA oxidase 1 (*ACOX1*) play critical roles in fatty acid oxidation and energy expenditure (12, 13). Additionally, obesity is associated with a state of chronic low-grade inflammation, wherein pro-inflammatory cytokines, such as tumor necrosis factor-alpha (*TNF- $\alpha$* ), interleukin-6 (*IL-6*), and interleukin-1 (*IL-1*), are upregulated, further exacerbating metabolic dysregulation (14–16). Understanding these interactions is crucial for developing effective therapeutic strategies against obesity. Current strategies for managing obesity primarily involve lifestyle interventions, including dietary modifications and increased physical activity. However, these approaches are often challenging to implement and maintain in the long term (17). Pharmacological and surgical interventions are also available but are typically associated with various side effects and risks (18). As a result, there has been increasing interest in exploring alternative therapeutic strategies, including natural products and postbiotics, which may provide a safer and more sustainable option for obesity management (19, 20).

Deer antler velvet is a traditional Chinese medicinal product derived from the antlers of deer (21). It has been

utilized in various cultures, especially in East Asia, for centuries due to its purported health benefits. In recent years, scientific interest in deer antler velvet has grown, with studies investigating its bioactive components and their potential therapeutic applications in various physiological and pathological conditions, including obesity, inflammation, and immune dysfunction (22, 23). It contains various bioactive compounds, including polysaccharides, amino acids, and minerals (24). Consequently, it has been used for its purported health benefits, including anti-inflammatory and anti-obesity effects. One of the most well-documented effects of deer antler velvet is its anti-inflammatory properties. Studies have shown that DAV supplementation can reduce the levels of pro-inflammatory cytokines such as *TNF- $\alpha$* , *IL-1*, and *IL-6*, which are involved in the pathogenesis of various chronic diseases, including obesity (25, 26). This anti-inflammatory effect is thought to be mediated by the modulation of the *NF- $\kappa$ B* signaling pathway, a key regulator of inflammation (27). Research has also highlighted the potential of deer antler velvet in regulating lipid metabolism. Several studies have reported that DAV supplementation can decrease total cholesterol (TC), triglycerides (TG), and low-density lipoprotein cholesterol (LDL-C), while increasing high-density lipoprotein cholesterol (HDL-C), suggesting its beneficial role in managing dyslipidemia and preventing cardiovascular diseases (28). The underlying mechanisms may involve the modulation of key lipid metabolism-related enzymes and the improvement of lipid transport and storage in adipocytes. Recent studies have highlighted the potential of deer antler polysaccharides (DAPs) to modulate lipid metabolism and reduce body weight in animal models. DAPs have been shown to inhibit the expression of lipogenic genes, such as *SREBP-1c* and *FAS*, while promoting the expression of fatty acid oxidation-related genes, like *PPAR- $\alpha$*  and *ACOX1* (29). Furthermore, DAPs exhibit anti-inflammatory properties, which help reduce pro-inflammatory cytokines associated with obesity, making them a promising candidate for obesity management.

Postbiotics have also emerged as a novel approach to modulating metabolic health (30). Recent studies have demonstrated that postbiotics can improve gut microbiota composition, enhance gut barrier function, and regulate inflammatory responses, thereby benefitting obesity (31). Postbiotic administration has also been linked to improved insulin sensitivity and reduced body weight, emphasizing their role in the prevention and treatment of obesity-related metabolic disorders (32). *Bacillus coagulans* has been extensively studied for its spore-forming ability and lactic acid production capacity, and its probiotic characteristics have also been widely investigated. *B. coagulans* produces valuable metabolites, examples include lactic acid and polysaccharides, which are recognized for their health benefits, such as anti-inflammatory

properties, immune system modulation, and regulation of lipid metabolism. Recent studies have highlighted the potential of *B. coagulans* postbiotics for managing obesity (30). *B. coagulans* postbiotics can downregulate lipogenic genes, such as *SREBP-1c* and *FAS*, which are involved in fat storage, while upregulating genes, like *PPAR-α* and *ACOX1*, that promote fatty acid oxidation. This shift in gene expression helps reduce fat accumulation and enhance energy expenditure (33). Postbiotics derived from *B. coagulans* have been demonstrated to reduce the levels of pro-inflammatory cytokines, such as TNF- $\alpha$ , IL-6, and IL-1. Therefore, these postbiotics improve metabolic health by reducing chronic inflammation, significantly contributing to obesity-related complications (34).

The significance of combining DAPs with postbiotics lies in their complementary mechanisms of action. While DAPs directly influence lipid metabolism and inflammatory pathways, postbiotics enhance gut health and modulate systemic metabolic responses. This combination could synergistically inhibit weight gain, improve serum lipid profiles, and reduce inflammation in obesity models. By elucidating the mechanisms by which DAPs and postbiotics exert their effects, we aim to provide insights into potential therapeutic interventions for obesity. This study helps clarify the interplay between natural compounds and metabolic regulation and highlights the importance of integrating traditional medicine with modern nutritional science in the fight against obesity.

## Materials and methods

### DAPs preparation

Sika deer antler powder was mixed with water at a 1:10 ratio, and the solid residue was removed by filtration using 40 kHz ultrasound for 30 min. The filtrate was then concentrated under reduced pressure using a rotary evaporator to obtain a more concentrated polysaccharide solution. Subsequently, 70% (v/v) ethanol was introduced, and the mixture was kept at 4°C overnight. The resulting polysaccharide precipitate was collected through centrifugation, followed by freeze-drying to yield a stable powder suitable for long-term storage and subsequent analysis or application. The purity and content of DAPs were determined by phenol-sulfuric acid method.

### Preparation of *B. coagulans* MZY531 postbiotics

The *B. coagulans* MZY531 strain (Jilin Mingzhiyuan Biotechnology Co. Ltd., Changchun, China) was cultured on a Luria-Bertani agar plate, then transferred into glucose yeast extract peptone liquid medium and incubated at 50°C with shaking at 1,800 rpm for 24 h. Following centrifugation (3,000 rpm, 4°C, 10 min), the bacterial pellet was collected and resuspended in a sterile isotonic sodium

chloride solution, adjusting the bacterial concentration to  $1.0 \times 10^9$  CFU/mL. A 50 mL aliquot of this *B. coagulans* MZY531 suspension was subjected to ultrasonic disruption in an ice bath for 15 min at 800 W using an ultrasonic processor. The resulting liquid was then vacuum freeze-dried to obtain a concentrated post-biotic powder of *B. coagulans* MZY531.

### Animal model establishment

Thirty-two male ICR mice (Yisi Experimental Animal Technology Co., Ltd., Changchun, China) were housed in a controlled environment at  $23 \pm 2^\circ\text{C}$  with  $55 \pm 5\%$  relative humidity under a 12-h light/dark cycle for 1 week. After acclimatization, the mice were randomly assigned to four groups: high-fat diet (HD), high-fat diet with deer antler polysaccharides (HDAPs), high-fat diet with *B. coagulans* postbiotics (HBCP), and high-fat diet with both deer antler polysaccharides and *B. coagulans* postbiotics (HDBP). The diet consisted of 60% fat. During the study, the HD group administered water via gavage. After 9 weeks, the mice were fasted for 12 h before euthanasia. Blood was collected, centrifuged at 10,000 rpm for 5 min at 4°C to isolate serum, and liver and epididymal fat tissues were harvested. All samples were stored at  $-80^\circ\text{C}$  for subsequent analysis.

### Determination of biological parameters in serum

Total cholesterol (TC), triglyceride (TG), LDL-C, and HDL-C levels in the serum were measured using kits following the manufacturer's instructions (Jiancheng Bioengineering Institute, Nanjing, China).

### Histological section staining

Liver tissue and abdominal fat were preserved overnight in 10% formalin solution, then embedded in paraffin, and cut into 4- $\mu\text{m}$  thick sections. The sections were deparaffinized using xylene and ethanol and washed with deionized water for 5 min. Staining was performed using hematoxylin and eosin, and the sections were photographed using a microscope at  $200 \times$  magnification.

### Real-time fluorescent quantitative polymerase chain reaction for measuring liver lipogenesis and inflammation-related gene expression

Initially, 50 mg of liver tissue was homogenized in 500  $\mu\text{L}$  of TRIzol using a handheld homogenizer. After the addition of 200  $\mu\text{L}$  of chloroform, the mixture was vortexed for 30 s and centrifuged at 10,000 rpm for 15 min at 4°C. The supernatant was collected, and 500  $\mu\text{L}$  of isopropanol was introduced. The sample was centrifuged again at 10,000 rpm for 10 min at 4°C, and the lower phase was discarded. Subsequently, 75% ethanol was added, and the sample was centrifuged at 7,000 rpm for 5 min at 4°C. The ethanol was removed, and the RNA was dissolved

in RNA-free water for concentration determination. Complementary DNA was synthesized using a reverse transcription kit (TianGen Biotech, Beijing, China). A 20  $\mu$ L reaction mixture was prepared with the primers listed in Table 1. The PCR conditions were as follows: initial denaturation at 95°C, followed by 45 cycles of 95°C for 15 s, 60°C for 20 s, and 72°C for 35 s.

#### Statistical analysis

All data are presented as means  $\pm$  standard deviations. Statistical analysis was performed using SPSS (version 23.0; IBM Corp., Armonk, NY, USA). Duncan's multiple range test ( $P < 0.05$ ) was applied to identify significant differences between groups, with distinct letters (a, b, c) representing differences, where  $a > b > c$ .

**Table 1.** Primer sequence of the genes

Genes	Primer	Sequence(5'-3')
SREBP-1c	Sense	5'-AAGCAAATCACTGAAGGACCTGG-3'
	Anti-sense	5'-AAAGACAAGGGGCTACTCTGGGAG-3'
FAS	Sense	5'-AGGGGTCGACCTGGTCTCTCA-3'
	Anti-sense	5'-GCCATGCCAGAGGGTGGTT-3'
ACOX1	Sense	5'-TATTTCGGCTATGACTGGGCACA-3'
	Anti-sense	5'-GATGGATACTTTCTCGGCAGGA-3'
PPAR- $\alpha$	Sense	5'-GGATGTCACACAATGCAATTCGCT-3'
	Anti-sense	5'-TCACAGAACGGCTTCCTCAGTT-3'
TNF- $\alpha$	Sense	5'-ATGGCCCAGACCCTCACA-3'
	Anti-sense	5'-TTGCTACGACGTGGGCTACA-3'
IL-6	Sense	5'-GCTTAATTACACATGTTCTCTGGGAAA-3'
	Anti-sense	5'-CAAGTGCATCATCGTTGTTTCATAC-3'
IL-1	Sense	5'-GACCTCCAGGATGAGGACA-3'
	Anti-sense	5'-AGCTCATATGGGTCCGACAG-3'
$\beta$ -Activin	Sense	5'-AGCCTTCCTTCTGGGTATGG-3'
	Anti-sense	5'-CACTTGCGGTGCACGATGGAG-3'

## Results

#### Effects of DAPs and postbiotics on body weight

Food intake among the mice in each group did not differ throughout the experiment (Fig. 1A). In the first 3 weeks, body weight did not differ among the HD, HDAPs, and HBCP groups, but the body weight of the HDBP group was significantly lower than those of the other three groups. Starting from the fourth week, the body weights of the HDAPs, HBCP, and HDBP groups decreased significantly compared to the HD group; the HDBP group had the most significant decrease (Fig. 1B).

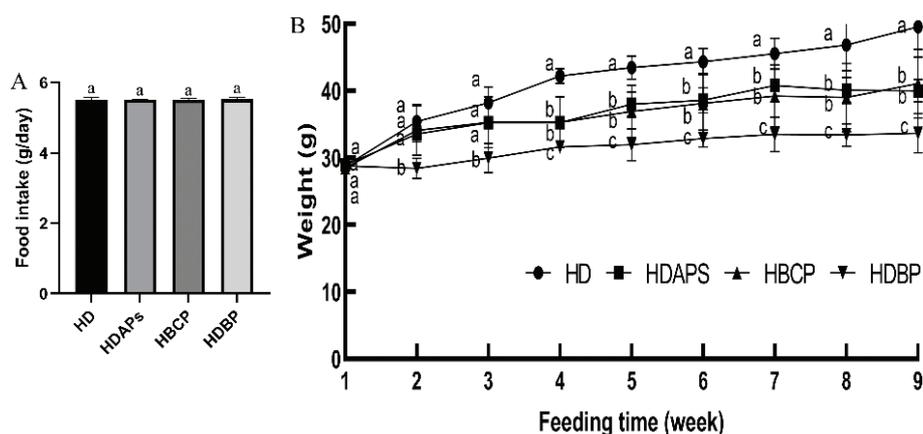
At 9 weeks, the weight in the HD group was significantly higher than that at the start. Furthermore, the HDBP group had the most significant decrease in weight compared to the HD group (Fig. 1B). Thus, the combined use of DAPs and postbiotics was more effective than DAPs or *B. coagulans* postbiotics alone.

#### Effects of DAPs and postbiotics on blood parameters

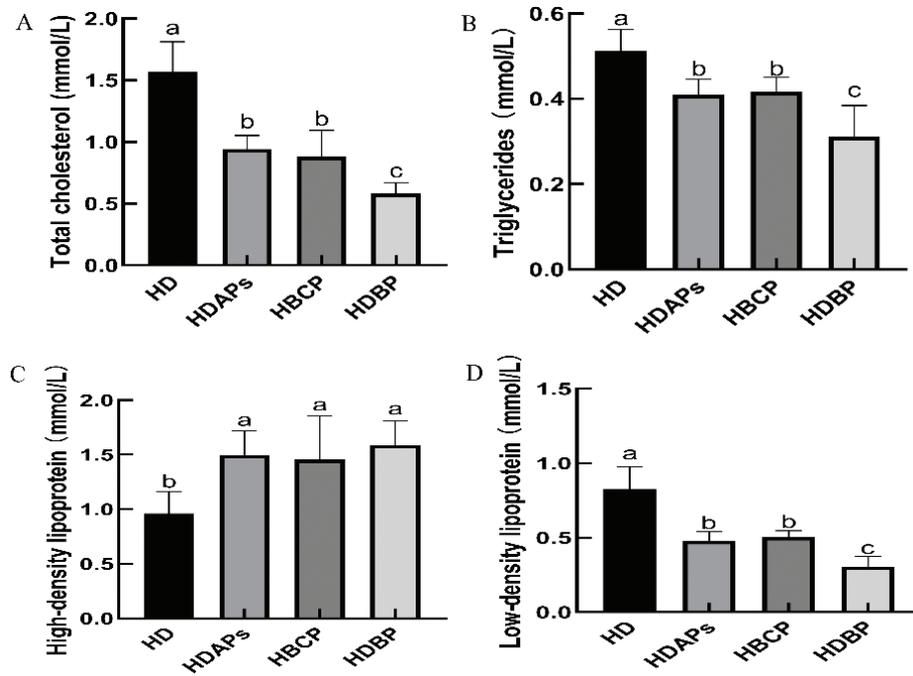
The levels of TC, TG, and LDL-C were significantly elevated, while the level of HDL-C was notably reduced in the HD group compared to the other three groups. This observation suggests that lipid metabolism disorders were present in mice (Fig. 2). In contrast, the HDBP group exhibited marked improvements, with significantly reduced levels of TC, TG, and LDL-C, and a substantial increase in HDL-C compared to the HD group.

#### Effects of DAPs and postbiotics on liver and adipose tissue

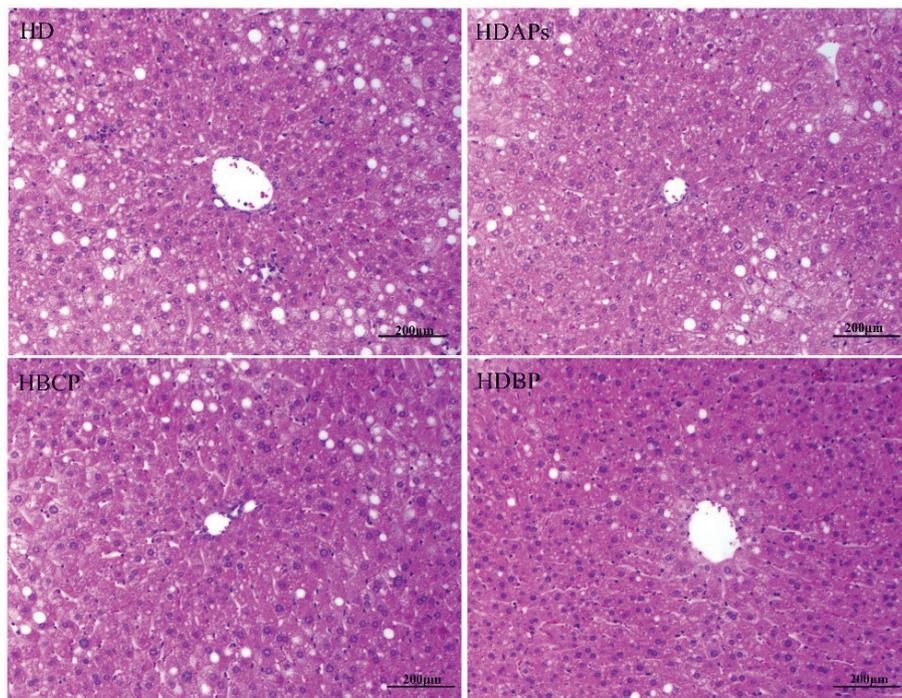
Considerable lipid droplet formation occurred in the liver tissue of mice from the HD group, whereas lipid droplet formation was markedly reduced in the HDBP group (Fig. 3). These results indicate that the combined use of DAPs and postbiotics inhibited lipid synthesis, reduced fat accumulation, and prevented abnormal enlargement



**Fig. 1.** Effect of DAPs and postbiotics on body weight gain in mice. (A) Food intake and (B) body weight. HD, high-fat diet; HDAPs, high-fat diet + deer antler polysaccharides; HBCP, high-fat diet + *Bacillus coagulans* postbiotics; HDBP, high-fat diet + deer antler polysaccharides + *B. coagulans* postbiotics. Duncan's multiple range test ( $P < 0.05$ ):  $a > b > c$ .



**Fig. 2.** Effects of DAPs and postbiotics on blood parameters of mice. (A) Level of total cholesterol, (B) level of triglyceride, (C) level of high-density lipoprotein, and (D) level of low-density lipoprotein. HD, high-fat diet; HDAPs, high-fat diet + deer antler polysaccharides; HBCP, high-fat diet + *Bacillus coagulans* postbiotics; HDBP, high-fat diet + deer antler polysaccharides + *B. coagulans* postbiotics. Duncan's multiple range test ( $P < 0.05$ ):  $a > b > c$ .



**Fig. 3.** Effect of DAPs and postbiotics on liver lipid droplet accumulation in mice. HD, high-fat diet; HDAPs, high-fat diet + deer antler polysaccharides; HBCP, high-fat diet + *Bacillus coagulans* postbiotics; HDBP, high-fat diet + deer antler polysaccharides + *B. coagulans* postbiotics.

of the liver. Moreover, the combined supplementation produced more considerable effects than the individual use of DAPs or postbiotics.

In the HD group, adipocytes in the abdominal adipose tissue were larger than those in the other groups and unequally sized; enlarged lipid droplets with signs of cell fusion were also observed, suggesting a tendency for giant cell formation. In contrast, following the combined supplementation of DAPs and postbiotics, the adipocytes in the HDBP group were considerably smaller and more uniformly sized than those in the HD group (Fig. 4).

#### Effects of DAPs and postbiotics on lipogenic and inflammatory gene expression

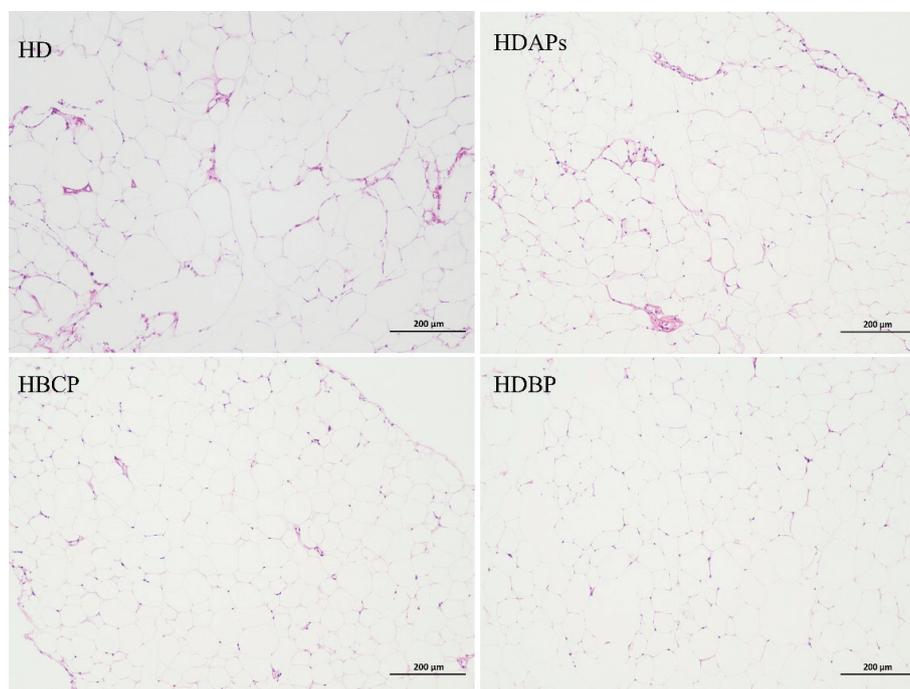
The expression levels of SREBP-1c and FAS were significantly reduced, whereas those of PPAR- $\alpha$  and ACOX1 were markedly increased in the HDBP group compared to the HD group. Furthermore, the expressions of inflammation-related genes, including TNF- $\alpha$ , IL-6, and IL-1, were significantly higher in the HDBP group than in the HD group. These results suggest that the combined supplementation of DAPs and postbiotics may have a beneficial effect on regulating both lipid metabolism and the expression of inflammation-related genes (Fig. 5).

#### Discussion

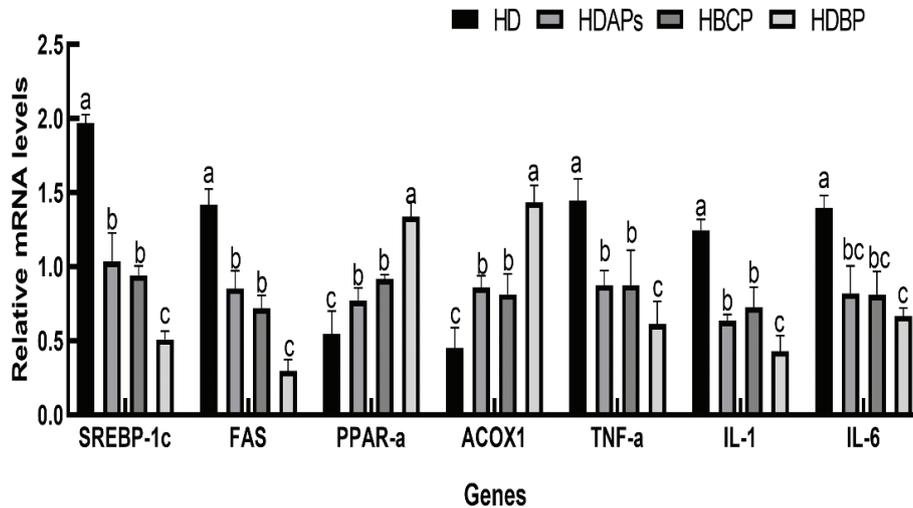
In this study, combined supplementation with DAPs and postbiotics effectively modulated serum lipid parameters.

DAPs may enhance lipid metabolism by improving hepatic function and promoting the expression of genes involved in fatty acid oxidation, thereby reducing lipid accumulation (35). Postbiotics can influence gut microbiota composition, improving intestinal barrier function and reducing systemic inflammation, which can lower cholesterol absorption and promote bile acid synthesis (36). The synergistic effect of these two components may create a favorable environment for lipid homeostasis and metabolic health. Previous studies have demonstrated that *B. coagulans* postbiotics elicit good regulatory effects on elevated serum lipid levels and liver steatosis (37). Our results are consistent with this, as we found that combined supplementation with DAPs and postbiotics significantly affected the regulation of serum lipids. In particular, the HDBP group exhibited significantly reduced levels of TC, TG, and LDL-C, along with a notable increase in HDL-C compared to the HD group. This combined supplementation method shows promises for regulating lipid profiles, potentially providing a novel approach to managing obesity and associated metabolic disorders.

*SREBP-1c* is an important regulatory gene for lipogenesis as its activation increases *FAS* expression, resulting in lipid accumulation (38, 39). PPAR- $\alpha$  is an important member of the nuclear receptor super transcription factor family that activates ACOX1, promoting fat oxidation in the body (40). Significantly increased *PPAR- $\alpha$*  and *ACOX1* expression suggests enhanced fatty acid



**Fig. 4.** Effect of DAPs and postbiotics on adipocytes of mice. HD, high-fat diet; HDAPs, high-fat diet + deer antler polysaccharides; HBCP, high-fat diet + *Bacillus coagulans* postbiotics; HDBP, high-fat diet + deer antler polysaccharides + *B. coagulans* postbiotics.



**Fig. 5.** Effect of DAPs and postbiotics on gene expression of lipid production and inflammation in mice. HD, high-fat diet; HDAPs, high-fat diet + deer antler polysaccharides; HBCP, high-fat diet + *Bacillus coagulans* postbiotics; HDBP, high-fat diet + deer antler polysaccharides + *B. coagulans* postbiotics. Duncan's multiple range test ( $P < 0.05$ ):  $a > b > c$ .

oxidation. PPAR- $\alpha$  plays a crucial role in lipid catabolism and energy expenditure, promoting the uptake and oxidation of fatty acids in the liver and muscle (41). *ACOX1* upregulation, an enzyme involved in the  $\beta$ -oxidation of fatty acids, further supports this notion. We observed decreased *SREBP-1c* and *FAS* levels in the HDBP group compared to the HD group, suggesting an inhibitory effect on lipogenesis. SREBP-1c is a critical transcription factor that regulates gene expression in fatty acid and triglyceride synthesis (10, 42). Reduced *SREBP-1c* expression implies that combined supplementation may hinder excessive fat accumulation by downregulating lipogenic pathways (43). In contrast, *PPAR- $\alpha$*  and *ACOX1* expressions increased significantly. Collectively, these findings suggest that DAPs and postbiotics act synergistically to promote lipid oxidation and limit lipid accumulation, counteracting obesity.

Additionally, we observed a significant decrease in the expression of inflammation-related genes, such as *TNF- $\alpha$* , *IL-6*, and *IL-1*, in the HDBP group compared to the HD group. Obesity is a chronic disease accompanied by inflammation, which in turn increases the risk of diseases such as diabetes and cancer (44). This finding is intriguing, as increased expression of these pro-inflammatory cytokines typically correlates with obesity and metabolic disorders. However, the role of inflammation in metabolic regulation is complex and context dependent (45, 46). Chronic low-grade inflammation is a hallmark of obesity, contributing to insulin resistance and metabolic dysregulation (47). Reduced expression of *TNF- $\alpha$*  and *IL-6* may reflect an adaptive response to improve inflammatory signaling pathways that could enhance lipid mobilization and energy expenditure. This result suggests that combining DAPs and postbiotics might promote a balanced

inflammatory response that supports metabolic health while also facilitating weight loss (48, 49). While the therapeutic potential of DAPs and postbiotics has been widely discussed, their safety and toxicological properties are equally important in assessing their suitability for long-term use. Studies on DAPs have shown that they are safe when taken at appropriate doses, with no significant acute toxicity observed in animal models (50). In addition, long-term studies of DAPs supplementation have shown no major adverse effects, indicating a good safety profile. Similarly, postbiotics, which are byproducts of probiotic microorganisms, have also been found to have a generally good safety profile. Studies have shown that postbiotics, such as microbial cell wall fragments, metabolites, and other bioactive components, are well tolerated in animal and human models (51). While the safety of velvet antler polysaccharides and postbiotics is well supported, more research is needed to evaluate the safety of their long-term use, especially in vulnerable populations such as those with compromised immune systems, and this work will also be the focus of our future research.

The interplay between lipid metabolism and inflammation is critical in understanding obesity and its associated complications. The dual effect of downregulating lipogenic genes while upregulating fatty acid oxidation markers, coupled with an increase in inflammatory markers, suggests a complex regulatory network where inflammation may serve both protective and detrimental roles. Modifying these pathways through dietary interventions, like DAPs and postbiotics, highlights the potential of these compounds in obesity management. Such combinations could be explored further in clinical settings by assessing improvements in lipid metabolism and possibly the inflammatory response. This study provides

new insights into the potential therapeutic benefits of combined supplementation of DAPs and postbiotics for regulating obesity in mice. By elucidating the synergistic effects of these compounds, the results suggest that DAP and postbiotics may be promising candidates for developing novel dietary strategies to combat obesity and its associated metabolic disorders. Furthermore, exploring the efficacy of these compounds in human populations provides a potential natural and safe alternative for the treatment of obesity.

### Conclusion

Combined supplementation with DAPs and postbiotics elicited regulatory effects in obese mice, reducing their body weight and improving serum TC, TG, HDL-C, and LDL-C levels. Supplementation also improved the accumulation of lipid droplets in the liver and fat hypertrophy. Finally, supplementation with DAPs and postbiotics significantly decreased *SREBP-1c* and *FAS* expression (lipid-forming genes), significantly increased *PPAR- $\alpha$*  and *ACOX1* expression, and significantly decreased *TNF- $\alpha$* , *IL-6*, and *IL-1* expression (inflammatory genes). This study was conducted over 9 weeks, which may not fully capture the long-term effects of DAPs and postbiotics on obesity and metabolic health. Longer-term studies are needed to assess the sustainability of the observed benefits and potential risks of prolonged supplementation. While the study provides valuable insights in a mouse model, the results need to be validated in human clinical trials. Differences in physiology between mice and humans may limit the direct applicability of these findings to human obesity management. Although key lipid metabolism and inflammation-related genes were assessed, other important pathways and factors involved in obesity, such as gut microbiota composition, insulin signaling, and adipokine levels, were not investigated in this study.

### Authors' contributions

Conceptualization, L.Y.Y. and J.T.L.; methodology, L.Y.Y. and J.T.L.; software, L.Y.Y.; validation, L.Y.Y., X.Y.T., and G.Q.Z.; formal analysis, J.T.L.; investigation, X.Y.T.; resources, X.X.; data curation, M. R. L.; writing – original draft preparation, L.Y.Y.; writing – review and editing, B.Z.; visualization, X.Y.T. and M. R. L.; supervision, B.Z.; project administration, B.Z.; funding acquisition, F.L.L. All authors have read and agreed to the published version of the manuscript.

### Conflict of interest and funding

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### Institutional review board statement

The Animal Welfare and Ethics Committee of Jilin Agricultural Science and Technology University approved the animal experiment (Approval No. 20221022).

### Informed consent statement

Not applicable.

### Data availability statement

The data presented in this study are available upon request from the corresponding author.

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