Journal of Functional Foods 121 (2024) 106398

Contents lists available at ScienceDirect

Journal of Functional Foods

journal homepage: www.elsevier.com/locate/jff

Cross-generational effects of dietary sea buckthorn on non-alcoholic fatty liver disease in offspring of obese female mice

Weipeng Zhang [a], Yonghua Shi [a], Yu Wang [a], Xuan Liu [a], Jianchen Yan [a], Haoran Chen [a], Junxing Zhao [a,b,*]

[a] College of Animal Sciences, Shanxi Agricultural University, Taigu 030801, PR China
[b] Shanxi Key Laboratory of Animal Genetics Resource Utilization and Breeding, Shanxi Agricultural University, Taigu 030801, PR China

ARTICLE INFO

Keywords:
Non-alcoholic fatty liver disease
Sea buckthorn
Maternal obesity
Glycolysis

ABSTRACT

Maternal obesity is a potential cause of non-alcoholic fatty liver disease (NAFLD) in the offspring. There is growing evidence that sea buckthorn plays an important role in protecting the liver. In this study, we established three dietary models of maternal to study the effects of different diets of maternal mice on the occurrence of NAFLD in their offspring and explored the underlying potential mechanisms. Studies have found that feeding sea buckthorn to maternal mice can improve liver steatosis and lipid deposition in the offspring. In addition, the expression of inflammation-related factors was reduced, the IκB/NF-κB pathway was inhibited, and inflammatory response was reduced in the offspring of maternal mice fed sea buckthorn. Furthermore, offspring exhibited increased antioxidant enzyme activity. Further, the results revealed that sea buckthorn inhibited glycolysis regulates AMPK/PKM2 activity, thereby reducing the occurrence of NAFLD. This study provides a novel strategy for treating NAFLD at the maternal level.

1. Introduction

Non-alcoholic fatty liver disease (NAFLD) is the most prevalent chronic liver disease globally, affecting approximately 25 % of adult population (Younossi et al., 2016). It is closely associated with metabolic syndrome components (Younossi et al., 2018), and is characterized by diffuse macrovesicular steatosis of hepatocytes as the main lesion. It progresses from simple hepatic steatosis to non-alcoholic steatohepatitis and can eventually develop into fibrosis or cirrhosis and lead to liver failure in some patients (Adams, 2005; Matteoni, 1999). NAFLD is strongly linked to obesity, diabetes, and insulin resistance, and poses significant health risks to humans. Its pathogenesis is complex and poorly understood, making it a challenge in modern medicine (Alexander et al., 2018).

NAFLD is a complex condition influenced by multiple factors, including genetic (Wardle et al., 2008) and environmental factors (He et al., 2009; Anstee and Day, 2013). Maternal obesity has been associated with the development of metabolic syndrome in the offspring (Boney et al., 2005; Hanson and Gluckman, 2014), and maternal lifestyle habits during pregnancy may play a role in the development of NAFLD (Alisi and Vajro, 2017). Animal experiments have confirmed that fetuses with intrauterine growth restriction (IUGR) due to prenatal hypoxia are more susceptible to NAFLD in adulthood (Cao et al., 2012). Research conducted on rodent models has shown that the offspring of mothers fed a high-fat diet (HFD) are more likely to develop NAFLD (Bruce et al., 2009; Mouralidarane et al., 2013; Qasem et al., 2010). These findings suggest that NAFLD may have fetal origin.

Sea buckthorn extract have been widely used to treat slow digestion, gastric dysfunction, cardiovascular problems, and liver damage (Chandra et al., 2018). Recently, it has gained worldwide attention owing to its medicinal and nutritional potential. Studies have shown that sea buckthorn seed oil has a strong inhibitory effect on oxidative damage in mice caused by CCl_4 (Ting et al., 2011). Sea buckthorn extract has been shown to normalize liver enzymes, serum bile acids, and immune system markers associated with liver inflammation and enhance immune function in mice (Zhang et al., 2021; Ze-Li Gao et al., 2003; Cheng, 1990). Although sea buckthorn extract exhibits liver-protectant effect, the underlying mechanism by which it prevents NAFLD remains unclear.

Pyruvate kinase M2 (PKM2) is a glycolytic enzyme primarily found in the brain, liver, and other tissues (Israelsen, 2013). As a transcriptional co-activator, PKM2 regulates glycolysis. Studies have shown that the expression of PKM2 is significantly increased in the livers of insulin-

* Corresponding author at: College of Animal Sciences, Shanxi Agricultural University, Taigu 030801, PR China
E-mail address: Junxzh@sxau.edu.cn (J. Zhao).

https://doi.org/10.1016/j.jff.2024.106398
Received 15 May 2024; Received in revised form 18 July 2024; Accepted 6 August 2024
Available online 11 August 2024

resistant animals, and PKM2 overexpression in HepG2 cells treated with palmitate can lead to massive lipid accumulation (Chen et al., 2017). Studies have confirmed significantly high expression of PKM2 in the liver tissues of NAFLD mice (Luca et al., 2018). Inhibition of PKM2-mediated glycolysis by kaempferol, a sea buckthorn extract, has been shown to reverse colitis (Wu et al., 2022). Therefore, our present research aims to investigate the potential of sea buckthorn consumption in improving liver metabolism and reducing the risk of NAFLD. Here, we evaluated the effects of maternal dietary sea buckthorn on hepatic lipid metabolism, inflammatory response, and oxidative stress in offspring, aiming to find a potential strategy for NAFLD alleviation.

2. Materials and methods

2.1. Care and use of animals

Twenty-four female mice (C57BL/6J, 8-week old) were randomly assigned to three diet groups: normal diet (chow group; D12450B, SPF Biotechnology Co., Ltd., Beijing, China), HFD (HFD group; 60 % energy from fat; D12492, SPF Biotechnology Co., Ltd.), and HFD containing 0.3 % (3 g/kg) freeze-dried sea buckthorn powder (HFDSB group; Xi'an Changyue Biological Technology, Co., Ltd., Xi'an, China). After ten weeks of feeding, female mice from the three groups were mated with male mice. Twelve male offspring were selected from each group and divided into two groups: normal diet and HFD. The mice were then raised for 15 weeks under controlled conditions, with a 12-hour light/dark cycle at 23 ± 2 °C with free access to food and water (Fig. 1). After 15 weeks, all offspring mice were fasted for 12 h, anaesthetized by inhalation of excess CO_2 and then euthanized by cervical dislocation, and whole liver was immediately isolated for subsequent experiments. All animal procedures were performed in accordance with the guidelines of the Animal Center of China and approved by the Institutional Animal Care and Use Committee of Shanxi Agricultural University (sxnd202028).

2.2. Hematoxylin and eosin staining

Liver samples were fixed in 4 % paraformaldehyde solution, dehydrated using xylene and graded alcohol, and embedded in paraffin after wax infiltration. The paraffin-embedded specimens were sectioned using a microtome (RM2265; Leica, Wetzlar, Germany) and stained with hematoxylin and eosin (H&E). Randomly selected fields of view were captured using a microscope (DMi8; Leica, Wetzlar, Germany), and the diameters were recorded.

2.3. Oil Red O staining

Fresh liver tissue was collected, fixed in 4 % paraformaldehyde, and immersed overnight in 30 % sucrose. The sample was placed in a mold and quickly frozen in optimal cutting temperature (OCT) embedding gel, and 6-μm thick frozen liver sections were cut using a cryomicrotome (Leica CM 3000, Wetzlar, Germany) at − 20 °C. The prepared frozen sections were fixed in 4 % paraformaldehyde for 10 min, soaked in 60 % isopropyl alcohol for 5 min, stained with working Oil Red O solution for 15 min, and immersed again in 60 % isopropyl alcohol. The sections were washed with deionized water, counterstained with hematoxylin for 1 min, soaked in deionized water again, and mounted with glycerol gelatin.

2.4. Enzyme-Linked immunosorbent assay

The liver sample was ground into a powder and accurately weighed. Physiological saline was added to the samples at a weight (g) to volume (mL) ratio of 1:9. Then, the homogenate was centrifuged for 10 min at 2500 rpm at 4 °C, and the supernatant was used for enzyme-linked immunosorbent assay (ELISA) (Wang et al., 2022). High-density lipoprotein cholesterol (HDL-C, A113-1–1), low-density lipoprotein cholesterol (LDL-C, A112-1–1), total cholesterol (TC, A111-1–1), triacylglycerol (TG, A110-1–1), catalase (CAT, A007-1–1), total superoxide dismutase (T-SOD, A001-3–1), total antioxidant capacity (T-AOC, A015-2–1), malondialdehyde (MDA, A003-1–2), reduced glutathione (GSH, A-006–2–1), and lactic acid (A019-2–1) in both the liver and serum were measured using their corresponding kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) following the manufacturer's manual. ELISA kits from Jiangsu Meimian Industrial Co., Ltd. were used to measure liver tumor necrosis factor (TNF)-α (919), interleukin (IL)-6 (14206), IL-10 (4502), and FAS (16703).

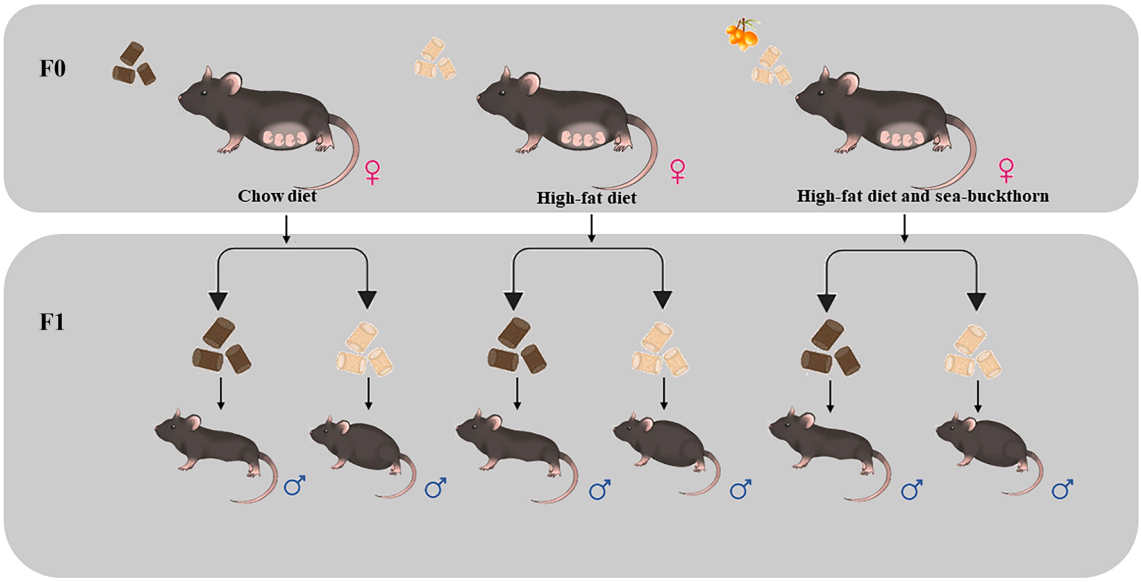

Fig. 1. Breeding program and experimental design. F0 female mice were randomly assigned to three diet groups: chow group, HFD group, and HFDSB group. After ten weeks of feeding, the female mice in the above three groups were mated with male mice. F1 generation male mice were divided into normal diet group and high-fat diet group and fed for 15 weeks.

2.5. Western blotting

To obtain total protein from the liver sample, mouse liver sample was ground in liquid nitrogen, and the sample powder was then homogenized in protein lysate (50 mM Tris-HCl pH 6.8, 2 % SDS, 10 % glycerol, 0.01 % bromophenol blue, 2 %β-Mercaptoethanol, 100 mM NaF, 0.1 % protease inhibitor aprotinin, 0.5 mM PMSF and 100 mM Na_3VO_4). After centrifugation, the supernatant was collected and used for protein separation by SDS-PAGE (80 V for 0.5 h, 120 V for 1.5 h). The separated proteins were transferred to polyvinylidene fluoride at 100 V and 4 °C for 1.5 h. The membrane was blocked with 5 % nonfat dry milk in PBS for 1 h and incubated sequentially with primary antibody (4 °C, overnight) and secondary antibody (room temperature, 1 h). Images were acquired using an Odyssey infrared imaging system (LI-COR Biosciences, Lincoln, NE, USA).

2.6. Quantitative Real-Time PCR (qPCR) analysis

Total RNA was extracted using TRIzol reagent (Sigma, St. Louis, MO, USA) according to the manufacturer's instructions. RNA quality was monitored using NanoDrop spectrophotometer (ND-2000, Thermo Fisher Scientific, Rockford, IL, USA). cDNA was synthesized using PrimeScript RT kits (Takara, Dalian, China), and qPCR was performed using SYBR Green fluorescent dye (Takara, Dalian, China) in a CFX Connect Real-time PCR Detection System (Bio-Rad, Hercules, CA, USA). The reaction steps were as follows: 95 °C for 30 s, 95 °C for 20 s, 60 °C for 30 s, 40 cycles. *18 s* was used as the housekeeping gene. The $2^{-\Delta\Delta CT}$ method was applied for the relative expression analysis of target genes. The primer sequences used for qPCR are listed in Table 1.

2.7. Antibodies

The antibodies against PPARγ (bs-4590R), C/EBPα (bs-1630R), IκB (bs-1287R), PKM2 (bs-0101R), AMPKα2 (bs-2771R), p-AMPKα2 (bs-4002R), and β-actin (bsm-33036 M) were obtained from Biosynthesis Biotechnology Co., Ltd. (Beijing, China). NF-κB (A19653) was purchased from ABclonal Technology Co., Ltd. (Wuhan, China). p-IκB (AF2002), p-NF-κB(AF3387), and p-PKM2 (DF2975) were purchased from Affinity Biosciences Co., Ltd. (Jiangsu, China). Goat anti-rabbit secondary antibody (926–32211) and anti-mouse secondary antibody (926–68070) were obtained from LI-COR Biosciences (Lincoln, NE, USA).

2.8. Statistical analysis

Statistical analyses were performed using GraphPad 9 software (Monrovia, CA, USA) with two-way analysis of variance (ANOVA). All data are expressed as mean ± standard error of the mean (SEM). $P<0.05$ was considered statistically significant (*$P<0.05$, **$P<0.01$).

Table 1
Primer sequences for quantitative real-time PCR.

Gene	Forward	Reverse
CD68	TGTCTGATCTTGCTAGGACCG	GAGAGTAACGGCCTTTTTGTGA
TNF-α	GCCAACGGCATGGATCTCAA	TAGCAAATCGGCTGACGGTG
IL-1β	TCGCAGCAGCACATCAACAA	TCCACGGGAAAGACACAGGT
IL-6	CCACTTCACAAGTCGGAGGC	TCTGCAAGTGCATCATCGTTGT
IL-4	AGTGAGCTCGTCTGTAGGGC	CAGGCATCGAAAAGCCCGAA
IL-10	TGGGTTGCCAAGCCTTATCG	TCAGCTTCTCACCCAGGGAA
PKM2	AGGACCTGAGATCCGAACTG	AGCCACAGGATGTTCTCGTC
HK2	GAGTTTGACCTGGATGTGGTTGC	CCTCCATGTAGCAGGCATTGCT
PFKM	ATGACCCATGAAGAGCACCA	GCACCGGTGAAGATACCAAC
PPARγ	GAGCACTTCACAAGAAATTACC	GAACTCCATAGTGGAAGCCT
FASN	CAAGCAGGCACACACAATGGAC	GACGCCAGTGTTCGTTCCTCG
C/EBPα	CCAAGAAGTCGGTGGACAAGA	CGGTCATTGTCACTGGTCAACT
18s	CGGCTACCACATCCAAGGAA	GCTGGAATTACCGCGGCT

3. Results

3.1. Effect of maternal dietary sea buckthorn supplementation on offspring liver tissue degeneration

Changes in the liver weight of offspring mice are shown in Fig. 2A. We found that the liver weight of the offspring of maternal mice receiving HFD was significantly higher than that of the offspring of maternal mice receiving normal diet ($P<0.05$). The liver weight of the offspring mice from maternal mice fed with sea buckthorn was significantly reduced ($P<0.05$). H&E staining of the liver (Fig. 2B) revealed extensive fatty infiltration in the liver of the offspring of obese mothers. In addition, we found that the liver degeneration was more obvious in the offspring of mice fed HFD, whereas the liver of the offspring of maternal mice fed sea buckthorn was obviously restored.

3.2. Maternal dietary sea buckthorn supplementation influences offspring liver lipid metabolism

To evaluate fat deposition in the liver, we performed Oil Red O staining and found that there was more lipid deposition and obvious lipid droplets in the liver tissue of the offspring of maternal mice in the HFD group, whereas the distribution of lipid droplets was significantly reduced in the HFDSB group (Fig. 3A). We then detected the expression of the adipogenic genes *C/EBPα* and *PPARγ* in the liver and found that HFD induced a significant increase in the transcription and protein levels of the above adipogenic genes in the liver ($P<0.01$; Fig. 3B and C). Supplementation with sea buckthorn significantly reversed this effect ($P<0.01$; Fig. 3B and C). Detection of the expression level and content of *FASN* mRNA in the liver revealed that FASN expression decreased in the offspring of maternal mice receiving sea buckthorn treatment ($P<0.01$; Fig. 3D and E). LDL-C, TG, and TC levels were significantly increased, whereas HDL-C levels were decreased in the liver of the offspring of maternal mice in the HFD group (Table 2). The results showed that dietary sea buckthorn supplementation in maternal mice affected the liver lipid metabolism in their offspring.

3.3. Effect of maternal dietary sea buckthorn supplementation on offspring liver antioxidant capacity

To assess hepatic oxidative stress, catalase (CAT), glutathione peroxidase (GSH-Px), total antioxidant capacity (T-AOC), superoxide dismutase (SOD) activities and malondialdehyde (MDA) content were determined. We found that CAT, GSH-Px, T-AOC, and SOD activities were significantly reduced and MDA level was significantly increased in the liver of the offspring of maternal mice in the HFD group (Table 3), and that the offspring showed more pronounced oxidative stress after HFD consumption; feeding sea buckthorn to female mice resulted in increased levels of CAT, GSH-Px, T-AOC, and SOD in the liver of the offspring, which attenuated the effects of NAFLD on the oxidative damage in the liver.

3.4. Effect of maternal dietary sea buckthorn supplementation on offspring liver inflammation

To investigate the effect of sea buckthorn on the regulation of hepatic inflammatory response in mice, we detected changes in the mRNA expression levels of the inflammation-related markers *TNF-α*, *CD68*, *IL-4*, *IL-6*, *IL-10*, and *IL-1β* in the liver. The expression of inflammatory factors in the offspring was attenuated by feeding sea buckthorn to maternal mice ($P<0.01$; Fig. 4A). Similarly, the expression levels of IL-6, IL-10, and TNF-α in the liver of the offspring of maternal mice fed HFD were higher than those fed normal diet, which was alleviated by sea buckthorn (Table 4). We also studied the NF-κB/IκB inflammatory pathway. The levels of IκB and NF-κB p65 phosphorylation were restored in the offspring of maternal mice fed sea buckthorn compared

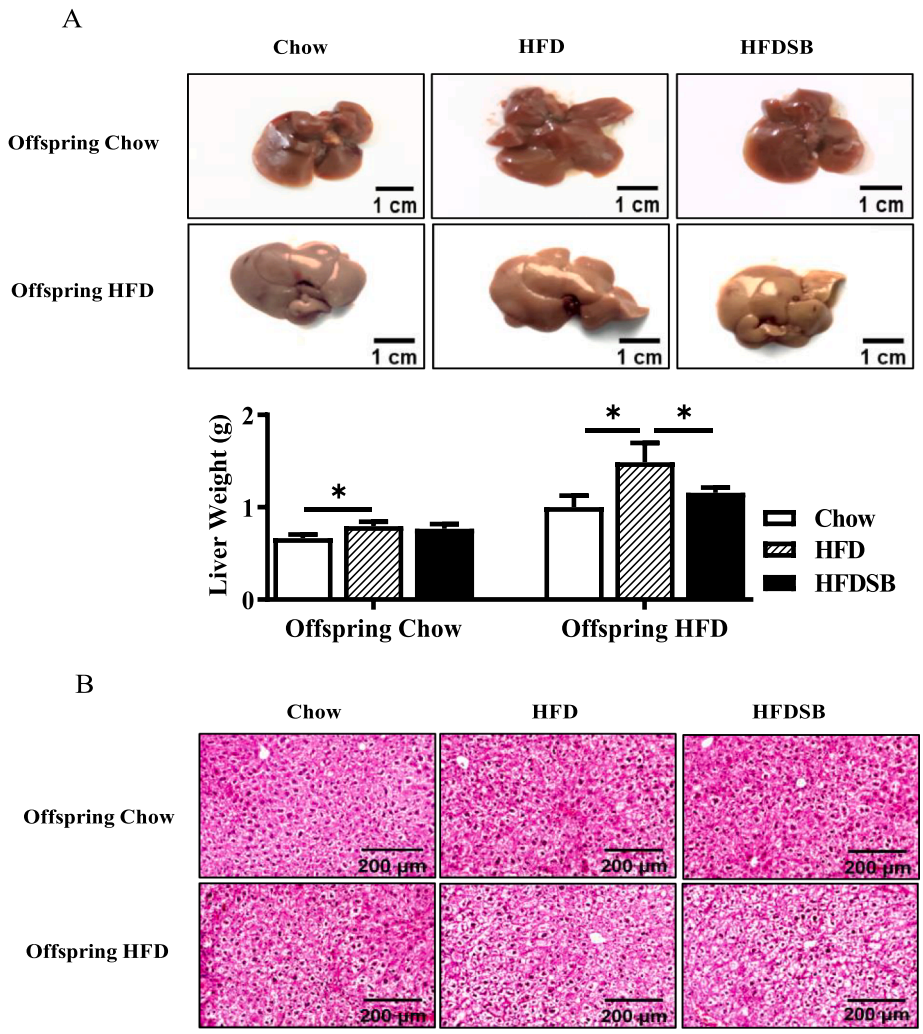

Fig. 2. Effect of dietary sea buckthorn to female mice on liver tissue degeneration in offspring (A) Images of liver and liver weight in different groups (scale = 1 cm). (B) H&E staining for liver sections (scale = 200 μm; mean ± SEM; n = 6 in each group; * $P<0.05$, ** $P<0.01$).

with that in the HFD group (Fig. 4B). This suggests that sea buckthorn alleviates inflammation by inhibiting the NF-κB/IκB pathway.

3.5. Maternal dietary sea buckthorn regulates AMPK/PKM2 activity in the liver of offspring

Since NAFLD is often accompanied by increased levels of glycolysis, we examined the expression of glycolysis-related genes and found that the expression of the glycolysis-related genes *PKM2*, *HK2*, and *PFKM* was restored in progeny mice after feeding sea buckthorn to maternal mice ($P<0.01$; Fig. 5A, B and C). Meanwhile, lactate being the final product of glycolysis, we found that the lactate content decreased in the liver of female mice after feeding sea buckthorn ($P<0.01$; Fig. 5D). AMPK is an important center for energy regulation; therefore, we detected the expression levels of AMPKα2/PKM2 using protein blotting. The results showed that compared with the high-fiber food group, feeding sea buckthorn could significantly increase the expression of p-AMPKα2 and reduce the expression of p-PKM2 ($P<0.01$; Fig. 5E). This effect was more pronounced in the offspring of maternal mice fed HFD. This suggests that sea buckthorn feeding may affect liver glycolysis by regulating the activity of AMPKα2/PKM2.

4. Discussion

NAFLD has become the most common chronic liver disease in recent years, affecting more than 30 % of the world's population and posing a significant threat to human health (Le et al., 2024). A study on NAFLD suggested that genetic factors play an essential role in the pathogenesis and progression of NAFLD. Epidemiological studies have also shown that NAFLD exhibits familial aggregation (Loomba and Sanyal, 2013) and that the heritability rate of liver fat is 39 %, indicating genetic susceptibility (Schwimmer et al., 2009). Presently, there is a trend to replace traditional therapies with medicinal plants, and their use in the treatment and prevention of diseases has attracted attention. For example, wolfberry polysaccharide can improve blood sugar homeostasis and intestinal barrier function in mice fed HFD (Zhou et al., 2023). Astragalus polysaccharide alleviates NAFLD in mice fed a HFD via taurohyodeoxycholic acid (THDCA) (Zheng et al., 2024). Sea buckthorn has a long history of being rich in nutritional value, containing most of the nutrients required by the human body (Pintea et al., 2005). In a recent study, sea buckthorn extract was reported to reduce weight, inhibit fat accumulation, and exert anti-inflammatory effects (Tanwar and Shweta, 2018). Therefore, investigation of the protective effects of sea buckthorn on the liver requires further research to confirm the biological effects of the constituent compounds.

The risk of NAFLD is transmitted to the offspring of obese mothers (Bruce et al., 2009). In the current study, we assessed the development and progression of NAFLD in mouse offspring and investigated the effects of maternal sea buckthorn diet supplementation on NAFLD in the offspring. We found that offspring fed a HFD had significant hepatic

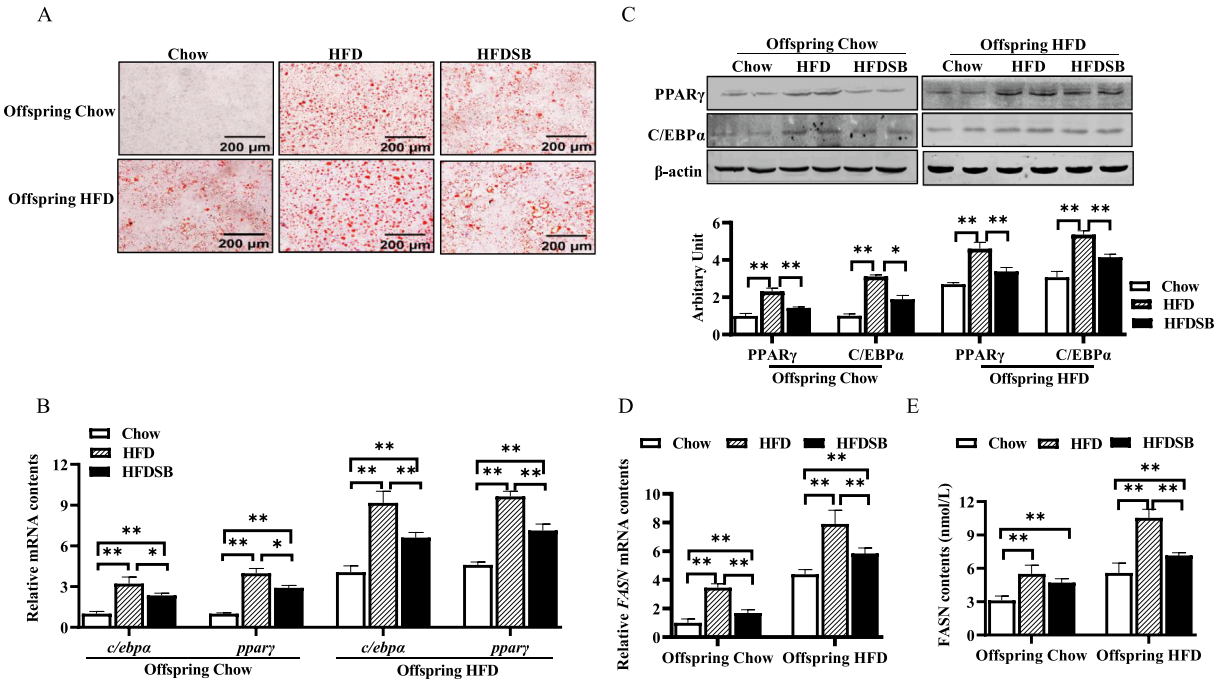

Fig. 3. Effects of dietary buckthorn in female mice on hepatic lipid metabolism of the offspring. (A) Representative Oil red O staining for liver sections. (B) mRNA levels of *c/ebpα* and *pparγ* in liver. (C) Protein contents of C/EBPα and PPARγ. (D) mRNA levels of *FASN*. (E) FASN levels in the Liver determined by ELISA (scale = 200 μm; mean ± SEM; n = 6 in each group; * $P<0.05$, ** $P<0.01$).

Table 2
Hepatic lipid index in the offspring mice.

Groups	TG (mM/gprot)	TC (mM/gprot)	HDL-C (mM/gprot)	LDL-C (mM/gprot)
Offspring Chow				
Chow	0.48±0.02^{a}	0.60±0.04^{a}	0.83±0.02^{a}	0.46±0.07^{a}
HFD	1.45±0.13^{b}	1.56±0.13^{b}	0.37±0.03^{b}	1.04±0.22^{b}
HFDSB	0.79±0.08^{c}	1.08±0.12^{c}	0.54±0.02^{c}	0.62±0.03^{c}
Offspring HFD				
Chow	1.54±0.13^{a}	1.67±0.12^{a}	0.53±0.06^{a}	1.54±0.09^{a}
HFD	4.03±0.35^{b}	3.64±0.23^{b}	0.23±0.02^{bb}	3.20 ±0.12^{b}
HFDSB	3.11±0.32^{c}	2.05±0.03^{c}	0.36±0.01^{c}	2.66±0.21^{c}

The data are presented as the mean ± ± SEM, n = 6 for each group. Values with different letters were significant difference ($P < 0.05$). T-CHO, Total cholesterol; TG, triglyceride; LDL, low density lipoproteins; HDL, high density lipoproteins.

Table 3
Hepatic antioxidant index in the offspring mice.

Groups	CAT (U/mgprot)	GSH-Px (μM/gprot)	T-AOC (U/mgprot)	MDA (mM/mgprot)	SOD (U/mgprot)
Offspring Chow					
Chow	48.17 ±0.67^{a}	42.18 ±5.00^{a}	1.56 ±0.12^{a}	3.39 ±0.22^{a}	257.66 ±4.07^{a}
HFD	14.80 ±0.97^{b}	15.59 ±0.69^{b}	0.79 ±0.10^{b}	7.23 ±0.33^{b}	197.2 ±2.16^{b}
HFDSB	30.79 ±1.16^{c}	30.97 ±0.24^{c}	1.04 ±0.02^{c}	4.62 ±0.30^{c}	224.44 ±3.32^{c}
Offspring HFD					
Chow	18.17 ±0.51^{a}	24.03 ±1.17^{a}	0.70 ±0.01^{a}	8.19 ±0.27^{a}	201.37 ±3.35^{a}
HFD	8.12 ±0.7^{b}	7.94 ±0.71^{b}	0.35 ±0.01^{b}	17.14 ±0.75^{b}	130.91 ±1.96^{b}
HFDSB	14.07 ±0.58^{c}	15.68 ±0.60^{c}	0.59 ±0.02^{c}	13.01 ±0.24^{c}	167.18 ±2.87^{c}

The data are presented as the mean ± ± SEM, n = 6 for each group. Values with different letters were significant difference ($P < 0.05$). CAT, Catalase; GSH, Glutathione peroxidase; T-AOC , Total antioxidant capacity; MDA, Malondialdehyde; SOD, Superoxide dismutase.

steatosis compared to those fed control diet. Importantly, the offspring of female mice in the HFD group developed NAFLD at a faster rate and had increased susceptibility to steatosis compared with the offspring of female mice in the other two dietary models. This finding is consistent with previous research (Mouralidarane et al., 2013). Simultaneously, we found that feeding sea buckthorn to female mice reversed this phenomenon, whereas the liver weight in the offspring of mice fed a HFD increased significantly. Liver section staining results also confirmed that the offspring of maternal mice fed HFD had obvious fat infiltration in the liver and were more susceptible to NAFLD, which is consistent with the findings of Oben et al. (Oben et al., 2010).

As NAFLD is mainly due to the accumulation of fat in the liver, we detected the expression of lipogenic genes in the liver. PPARγ is a vital regulator of adipogenesis (Nguyen et al., 2013). PPARγ and C/EBPα are essential for the activation of downstream target genes that initiate adipogenesis. In the current study, the liver expression of C/EBPα and PPARγ was suppressed as well as the hepatic lipid levels were improved in the offspring after feeding sea buckthorn to maternal mice. Therefore, dietary sea buckthorn supplementation in female mice prevented lipid accumulation by downregulating the expression of adipogenesis-related transcription factors and transmitting them to the offspring, thereby alleviating obesity and reducing the risk of NAFLD.

NAFLD is typically associated with oxidative stress and inflammatory responses, in addition to excessive fat deposition in hepatocytes (Luca et al., 2018). These mechanisms interact with the liver functions and affect the liver physiology. NAFLD causes an imbalance in the body's oxidative status, resulting in increased lipid peroxidation and oxidative stress (Sumida et al., 2013). T-AOC is a general term for antioxidant capacity. SOD scavenges superoxide anion free radicals that are harmful to the body (Ifeanyi, 2018). GSH-Px reduces toxic peroxides to non-toxic hydroxyl compounds, whereas CAT catalyzes the conversion of H_2O_2

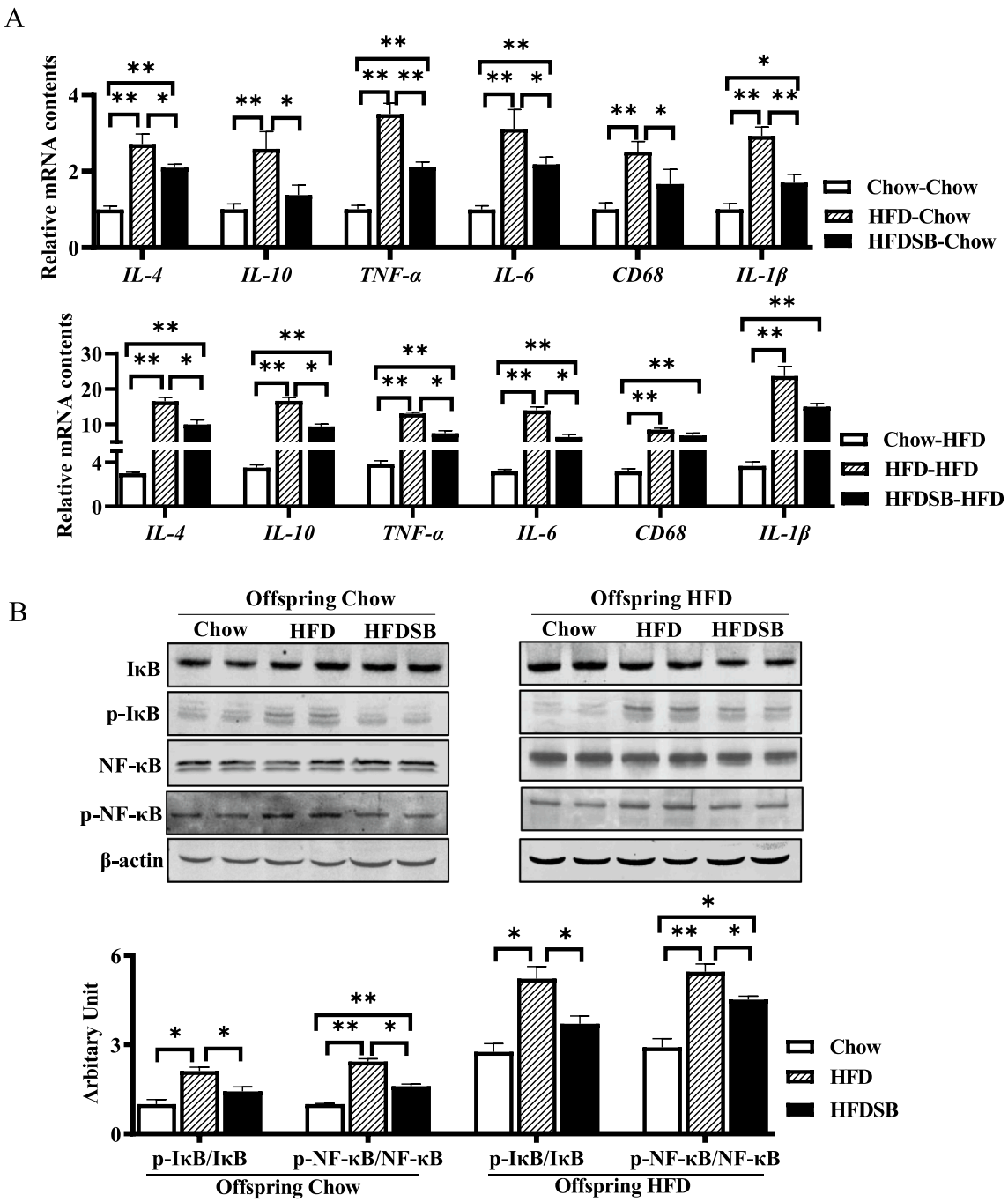

Fig. 4. Effect of dietary sea buckthorn to female mice on liver inflammation in offspring. (A) mRNA levels of *IL-4*, *IL-10*, *TNF-α*, *IL-6*, *CD68* and *IL-β* in liver. (B) Protein contents of IκB, p-IκB,NF-κB and p-NF-κB (mean ± SEM; n = 6 in each group; * $P<0.05$, ** $P<0.01$).

into water and oxygen (Shen et al., 2022). Antioxidant enzymes protect the body from toxic effects. We verified the effect on oxidative stress in the liver of the offspring, and found that the activity of hepatic antioxidant enzymes was significantly reduced in the offspring of female mice in the HFD group, leading to significant liver damage. When maternal mice were fed sea buckthorn, the activities of SOD, GSH-Px, CAT, and T-AOC increased, and the levels of antioxidant enzymes were restored in the offspring. These results indicate that sea buckthorn supplementation has significant benefits against oxidative stress in the liver.

A study has demonstrated that mice fed a high-fiber diet exhibit high levels of inflammation and are susceptible to NAFLD (Zhang et al., 2020), which is consistent with our findings. Following administration of sea buckthorn to maternal mice, the offspring showed an obvious decrease in inflammation and a notable decrease in the liver markers. Hyperactivation of the NF-κB pathway has also been reported in patients with NAFLD (Wang et al., 2020). NF-κB is a crucial transcriptional regulator of inflammatory response (Karin and Ben-Neriah, 2000; Ghosh and Karin, 2002). It is involved in the regulation of inflammatory signaling pathways in the liver. NF-κB is typically inactivated in the cytoplasm by binding to the inhibitory protein IκB to form a trimeric complex. Upon stimulation by pro-inflammatory factors, the IκB kinase (IKK) complex, which consists of the IKKα and IKKβ catalytic subunits and the IKKγ regulatory subunit, is activated and phosphorylates IκB. This phosphorylation causes the dissociation of NF-κB from the trimer, allowing free NF-κB dimer to enter the nucleus. Here, after feeding sea buckthorn fruit to female mice, the offspring liver showed low IκB

Table 4
Hepatic inflammatory factor levels in the offspring mice.

Groups	TNF-α (pg/mgprot)	IL-6 (pg/mgprot)	IL-10 (pg/mgprot)
Offspring Chow			
Chow	56.53±2.10[a]	4.89±0.43[a]	17.14±1.55[a]
HFD	139.38±5.62[b]	11.46±0.30[b]	45.60±2.93[b]
HFDSB	100.64±5.66[c]	7.76±0.59[c]	25.29±1.70[c]
Offspring HFD			
Chow	135.43±3.35[a]	12.09±1.08[a]	53.14±1.33[a]
HFD	356.38±5.56[b]	31.32±1.55[b]	126.26±2.99[b]
HFDSB	264.64±4.16[c]	19.93±0.34[c]	96.99±4.51[c]

The data are presented as the mean ± ± SEM, n = 6 for each group. Values with different letters were significant difference ($P < 0.05$). IL-6, interleukin 6; IL-10, interleukin 10; TNF-α, tumor necrosis factor α.

activity, further leading to a reduction in NF-κB. Therefore, feeding sea buckthorn to mothers can inhibit the activation of NF-κB inflammatory pathway and pass this effect to the offspring, thereby reducing the occurrence of liver inflammation.

Finally, we investigated the potential mechanisms by which sea buckthorn alleviates NAFLD. AMPK is an important kinase that regulates energy homeostasis in the body. It is the central regulator of metabolism in the body and maintains the physiological activity of cells. Studies have shown that palmitoleic acid in sea buckthorn fruit oil extract reduces liver cell damage via the AMPK/Akt pathway (Gao et al., 2020); palmitoleic acid itself is considered a potential nutrient against the development of NAFLD and alleviates the ability of HFD to reduce hepatic steatosis and inflammation in mice (Cruz et al., 2020), which is consistent with our previous findings. Some studies have reported enhanced glycolytic activity in patients with NAFLD and nonalcoholic steatohepatitis (NASH) (Ye et al., 2016; Wang et al., 2018). Glycolysis is the key process for energy production in almost all mammalian cells, and PKM2 plays a critical role in metabolic regulation by catalyzing the final step of glycolysis, the conversion of phosphoenolpyruvate to pyruvate and ATP (Walls et al., 2020; Li et al., 2021). Studies have shown that obesity is associated with NASH and that PKM2 is highly expressed in the liver tissues of NAFLD mice (Luca et al., 2018). These findings suggest that PKM2 plays a role in metabolic diseases. AMPKα2 and PKM2 are key molecules in the regulation of lipid metabolism in hepatocytes. Therefore, we speculate that the AMPKa/PKM2 metabolic sensing pathway plays a leading role in the ameliorative effect of sea buckthorn on NAFLD in mice. This study found that the mRNA levels of the three rate-limiting enzymes PKM2, HK2, and PFKM were significantly increased in the HFD group. We also found that the liver lactate content of mice in the HFD group was significantly increased, indicating that the occurrence of NAFLD causes enhanced glycolytic activity, which was significantly reduced after feeding sea buckthorn. Next, we found that the expression level of p-AMPKα2 in the liver tissues of mice after feeding sea buckthorn was significantly higher than that in the HFD group, and the activation of AMPKα2 depends on its phosphorylation, indicating that sea buckthorn can activate AMPKα2. To confirm our hypothesis, we analyzed PKM2 protein levels and found that sea buckthorn reduced p-PKM2 activity, thereby inhibiting the liver glycolytic pathway and alleviating liver damage caused by NAFLD. Taken together, these results support our hypotheses.

5. Conclusions

In summary, we evaluated the liver function of the offspring and found that maternal obesity impaired liver development in the offspring, increasing their susceptibility to NAFLD. When the mother was fed sea buckthorn, these effects were transferred to the offspring, resulting in a significant reduction in liver weight, alleviation of inflammation and oxidative stress, and inhibition of glycolysis, which may at least through the AMPK/PKM2 pathway, and thereby preventing NAFLD (Fig. 6). These findings confirm that the regulatory genes involved in glycolysis could serve as potential biomarkers and molecular targets for NAFLD. This study provides valuable insights into the prevention of NAFLD and promotes sea buckthorn as a functional food and nutritional supplement.

Author contributions

Conception and design of research: Junxing Zhao, Weipeng Zhang, and Yu Wang. Conduction of experiments: Yonghua Shi, and Xuan Liu. Data collection and analysis: Weipeng Zhang, Jianchen Yan, and Haoran Chen. Preparation of figures: Weipeng Zhang, Yonghua Shi, and Yu Wang. Writing – original draft: Weipeng Zhang, and Junxing Zhao. All authors read and approved the final manuscript. Funding acquisition: Junxing Zhao.

CRediT authorship contribution statement

Weipeng Zhang: Writing – original draft, Data curation, Conceptualization. **Yonghua Shi:** Methodology, Investigation. **Yu Wang:** Data

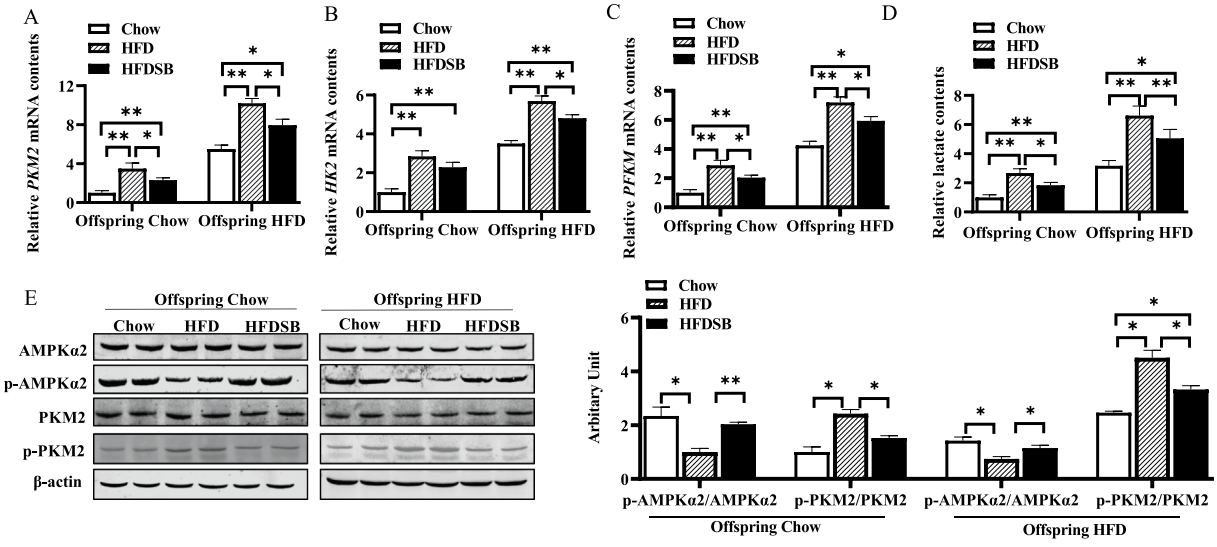

Fig. 5. Sea buckthorn inhibits aerobic glycolysis through the AMPK-PKM2 signalling pathway. Relative mRNA levels of (A) *PKM2*, (B) *HK2* and (C) *PFKM* in liver. (D) Lactate content in liver. (E) Protein contents of AMPKα2, p-AMPKα2, PKM2 and p-PKM2 (mean ± SEM; n = 6 in each group; *P<0.05, **P<0.01).

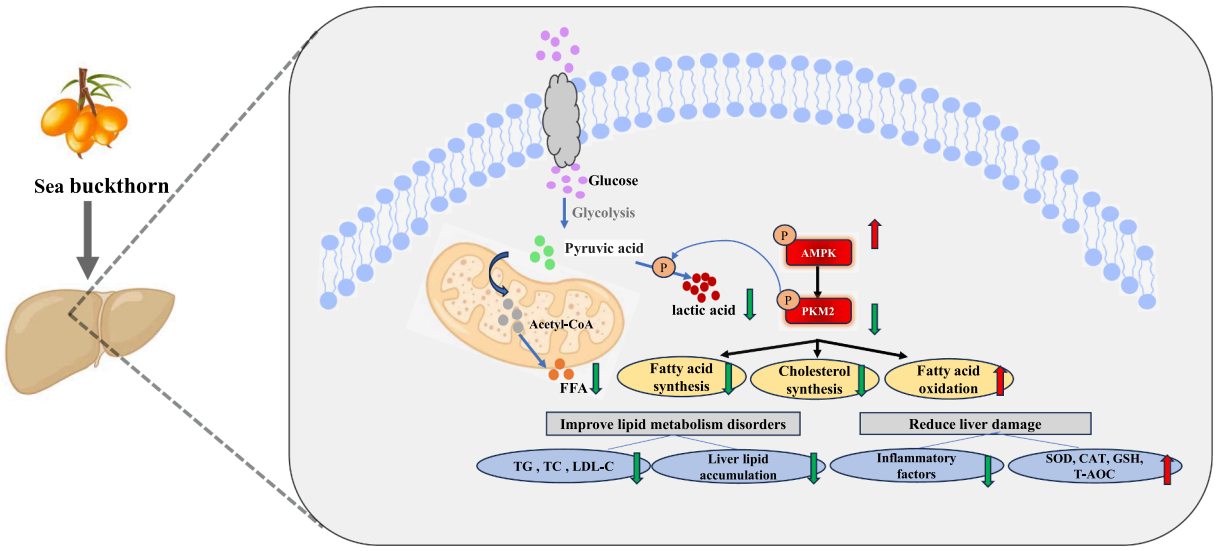

Fig. 6. Proposed mechanism of sea buckthorn alleviating NAFLD in mice.

curation, Conceptualization. **Xuan Liu:** Methodology, Investigation. **Jianchen Yan:** Methodology, Data curation. **Haoran Chen:** Methodology, Data curation. **Junxing Zhao:** Writing – review & editing, Project administration, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

This work was supported by the National Key R&D Program of China (2023YFD1301302) and the Distinguished and Excellent Young Scholar Cultivation Project of Shanxi Agricultural University (2022JQPYGC01).

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