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Original Communication

Lactoferrin Reduces Allergic Symptoms and Th2-Related Cytokines in a Juvenile Murine Model of Food Allergy

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Abstract

Background: Food allergies, particularly to peanuts and milk, are an increasing global health concern with few safe and effective interventions. Lactoferrin (LF), a multifunctional glycoprotein abundant in human milk, has immunomodulatory and gut-protective properties. However, the therapeutic potential of LF and the mechanism of action against specific food allergies remain incompletely understood. **Methods:** Five-week-old female BALB/c (Bagg ALBino c series) mice were sensitized intraperitoneally with peanut protein or whey protein combined with aluminum adjuvant to establish peanut or milk allergy models. Mice were then randomized into nine groups (n = 10): normal control, allergy model, dexamethasone positive control, and low-dose (0.1 mg/kg) or high-dose (1 mg/kg) LF intervention groups. After two weeks of oral LF administration, clinical symptoms, body weight changes, serum allergen-specific Immunoglobulin G1 [IgG1] and Immunoglobulin G2a [IgG2a] levels (Enzyme-Linked Immunosorbent Assay [ELISA]), splenic T helper 2 cell [Th2] cytokines (interleukin-5 [IL-5], interleukin-13 [IL-13]), immune organ indices, and jejunal and ileal histopathology were assessed. **Results:** LF intervention significantly reduced clinical allergy symptoms in both peanut- and milk-sensitized mice compared with model groups.In the milk allergy model, LF significantly reduced serum levels of both IgG2a and IgG1 (≥20 pg/mL reduction; $p < 0.05$). The reductions were statistically significant only in the milk model ($p < 0.05$). LF suppressed splenic IL-5 and IL-13 secretion (≥1 pg/mL reduction; $p < 0.01$). LF also mitigated allergy-induced elevation of the spleen index (≥1.5% reduction; $p < 0.05$) and improved intestinal villus structure, with reduced inflammatory infiltration. Notably, dose- and allergen-dependent effects were observed: low-dose LF (0.1 mg/kg) was more effective for milk allergy, while high-dose LF (1 mg/kg) was more effective for peanut allergy. **Conclusion:** Dietary LF supplementation effectively alleviates peanut and milk allergy responses in juvenile mice, as evidenced by reduced clinical symptoms, suppression of Th2-type cytokines (IL-5, IL-13) and total IgG subclasses, and protection of intestinal barrier integrity. These findings provide preclinical evidence supporting LF as a natural nutritional intervention strategy for managing food allergies in infants and young children. However, the precise immune mechanisms remains to be further elucidated.

Keywords: food allergy; Th2-type immune response; intestinal barrier; immunomodulation; lactoferrin

1. Introduction

Food allergy, an abnormal immune response to specific food proteins, has shown a rising incidence rate year by year, placing a heavy burden on patients' quality of life and the healthcare system, and has become a significant global public health challenge [1]. Data from the National Health and Nutrition Examination Survey (NHANES) and the Centers for Disease Control and Prevention (CDC) indicate that approximately 8% of children (about 1 in 13) and over 10% of adults in the United States report having food allergies [2]. More importantly, the prevalence of food allergies continues to increase. Their impact extends far beyond physiological reactions, deeply affecting patients' psychosocial well-being, family dynamics, socioeconomic status, and overall daily quality of life [3].

The most common food allergies are peanut and milk allergies, which affect many people worldwide. These conditions not only pose physiological risks but also impose significant psychological burdens on individuals. The main allergenic proteins in peanuts are Ara h2 and Ara h6 [4]; the main allergenic protein in milk is β-lactoglobulin [5]. However, there is currently no complete cure. The only approach is proactive prevention to alter disease progression and reduce overall impact.

Lactoferrin (LF), a naturally occurring immunomodulatory protein [6], has been studied for its potential to intervene in Immunoglobulin E [IgE]-mediated allergic signaling pathways. This research contributes to a deeper understanding of the mechanisms underlying allergy development and immune tolerance. Furthermore, compared to expensive and potentially side-effect-prone biological agents or oral immunotherapy, lactoferrin—as a natural food component or dietary supplement—is more cost-effective and generally more acceptable to the public [7].

A previous study demonstrated that lactoferrin can promote the secretion of T helper 1 cell [Th1]-type cy-

tokines, such as interleukin-12 (IL-12) and Interferon-gamma (IFN-γ), suppress Th2-type responses, such as IL-4 and IL-13, and enhance the function of regulatory T cells (Tregs) [8]. These effects are key to correcting the immune imbalance associated with allergies. Lactoferrin also promotes the growth of intestinal epithelial cells, helps maintain barrier integrity, selectively inhibits pathogenic bacteria, and encourages the growth of beneficial bacteria [9].

A clinical observational study investigated the association between breastfeeding and the risk of allergic diseases in children, with particular attention to the role of breast milk components such as lactoferrin [10]. A previous animal study demonstrated that oral lactoferrin administration can regulate Th1/Th2 cell balance in food-allergic mice, reducing serum IgE levels and modulating Th2-type cytokine production [11].

Therefore, investigating the inhibitory effects of lactoferrin on food allergies is supported by a solid scientific basis. This study investigates the inhibitory effect of lactoferrin on food allergies by establishing peanut allergy and cow's milk allergy mouse pup models. It aims to provide new insights for optimizing product formulations to suppress peanut and milk allergies in infants and young children, as well as offer technical support for the development of infant formula or other foods for special medical purposes.

2. Materials and Methods

2.1 Experimental Animals, Materials, and Instruments

Specific Pathogen Free [SPF]-grade 5-week-old BALB/c (Bagg ALBino c series) mice,average body weight 14 g, were used in this study. A total of 90 mice were provided by Sipeifu (Beijing) Biotechnology Co., Ltd. (License No.: SCXK (Jing) 2024-0001). The mice were housed in an SPF-grade animal facility with ad libitum access to food and water. The environmental conditions were maintained at a temperature of 20–24 °C, humidity of 40–70%, and a 12-hour light/dark cycle. This experiment was approved by the Animal Welfare and Ethical Committee of China Agricultural University (Approval No.: AW71305202-5-01).

Peanut protein powder (purity 99%), provided by Xintai Food Technology Co., Ltd.

Whey protein (purity 80%), provided by Hefei BASF Technology Co., Ltd.

Phosphate-buffered saline (PBS) buffer (1×, pH 7.2–7.5), produced by Wuhan Servicebio Technology Co., Ltd.

Alum adjuvant, produced by Thermo Fisher Scientific, USA.

Mouse Immunoglobulin G1 (IgG1), Immunoglobulin G2a (IgG2a), interleukin-5 (IL-5), and interleukin-13 (IL-13) Enzyme-Linked Immunosorbent Assay (ELISA) kits, produced by Beijing Borui Changyuan Technology Co., Ltd.

D-16C low-temperature high-speed centrifuge, produced by Sartorius, Germany.

DNM-9602 microplate reader, produced by Beijing Perlong New Technology Co., Ltd.

N·9548 grinding instrument, produced by Beijing Hede Technology Co., Ltd.

CO_2 euthanasia chamber (transparent, with ventilated lid), custom-made, China.

Medical-grade. CO_2 gas cylinder (purity ≥99.5%) with pressure regulator and precision flow meter, Radnor, Pennsylvania, USA.

Cervical dislocation forceps, Fine Science Tools (FST), Germany.

2.2 Methods

2.2.1 Protein Solution Preparation

Preparation of peanut protein solution: Defatted peanut protein powder and alum adjuvant (peanut protein:alum adjuvant = 2:1) were taken, an appropriate amount of PBS buffer (0.01 mol/L, pH 7.4) was added, and the mixture was thoroughly mixed to prepare a 10 mg/mL protein solution.

Preparation of whey protein solution: Whey protein powder and alum adjuvant (whey protein:alum adjuvant = 2:1) were taken, an appropriate amount of PBS buffer (0.01 mol/L, pH 7.4) was added, and the mixture was thoroughly mixed to prepare a 10 mg/mL protein solution.

The conventional dosage range for intraperitoneal injection in mice is 0.1–0.2 mL/10 g body weight, and for intragastric administration is 0.1–0.3 mL/10 g body weight. In this experiment, we estimated the mouse body weight to be approximately 20 g, and accordingly selected a dosage volume of 0.2 mL.

2.2.2 Experiment on the Inhibitory Effects of Peanut Allergy and Cow's Milk Allergy

2.2.2.1 Mouse Grouping and Treatment. After one week of acclimatization feeding, 90 five-week-old female BALB/c mice were randomly assigned into nine groups (n = 10) based on body weight: Normal Control (CON), Peanut Model (MOP), Milk Model (MOM), Positive Peanut Control (PPC), Positive Milk Control (PMC), Peanut Low-dose Lactoferrin (PLL), Peanut High-dose Lactoferrin (PLH), Milk Low-dose Lactoferrin (MLL), and Milk High-dose Lactoferrin (MLH).

Over the following two weeks:

(1) The CON group received an intraperitoneal injection of PBS solution (0.2 mL per mouse).

(2) The MOP, PPC, PLL, and PLH groups were intraperitoneally injected with peanut protein sensitization solution (0.2 mL per mouse; peanut protein:alum adjuvant = 2:1).

(3) The MOM, PMC, MLL, and MLH groups were intraperitoneally injected with whey protein sensitization so-

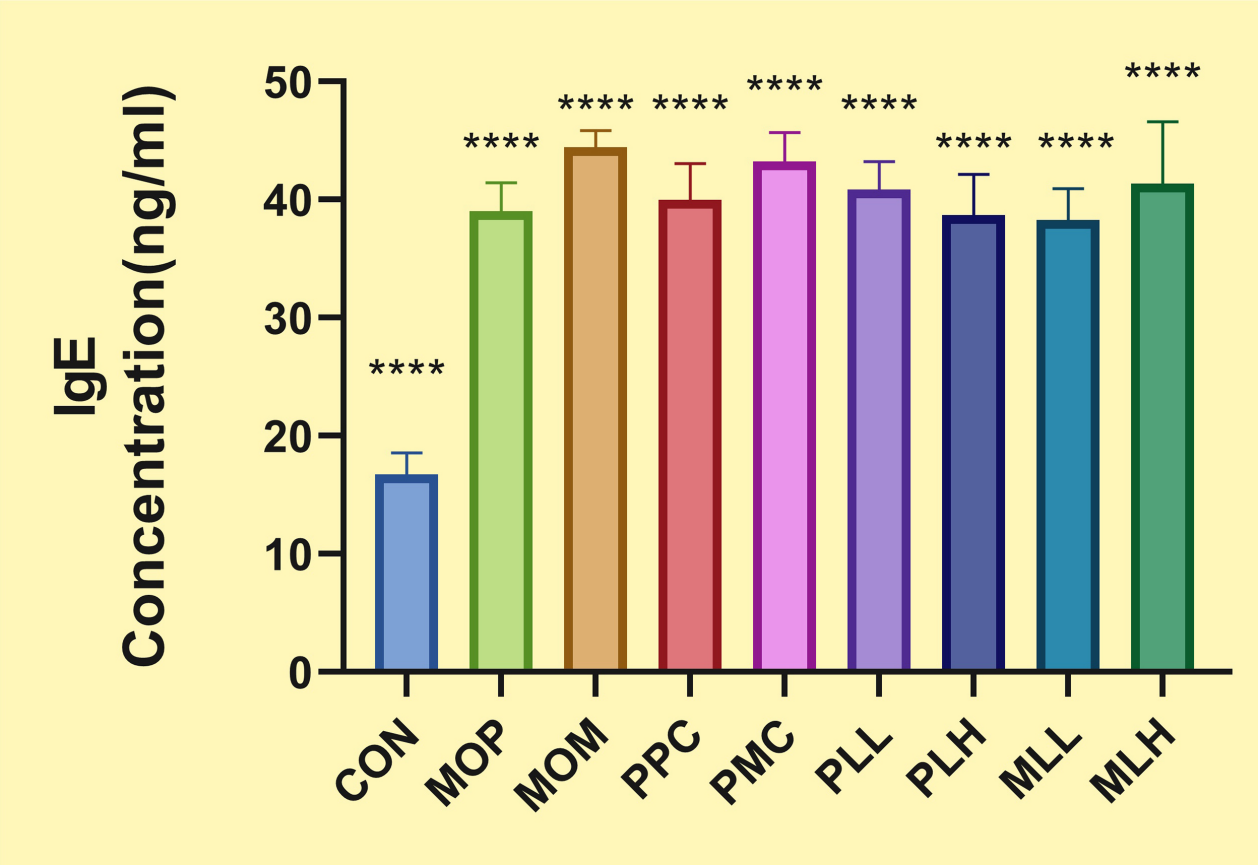

Fig. 1. Immunoglobulin E (IgE) levels in mice. Significance levels are indicated as compared with the control group or the corresponding model group. Compared with the control group, ****: $p < 0.0001$ (extremely significant). Abbreviations: CON, normal control; MOP, peanut model; MOM, milk model; PPC, positive peanut control; PMC, positive milk control; PLL, peanut low-dose lactoferrin; PLH, peanut high-dose lactoferrin; MLL, milk low-dose lactoferrin; MLH, milk high-dose lactoferrin.

lution (0.2 mL per mouse; whey protein:alum adjuvant = 2:1).

During this period, body weight was recorded every 2–3 days, and allergic symptoms such as diarrhea, skin itching, and respiratory distress were observed. Four weeks later, blood was collected from the inner canthus of the eye on the same day. Serum was separated, and IgE antibody levels were measured by ELISA solely to confirm successful sensitization (i.e., comparison between model groups and the normal control group). If IgE levels in the model groups were significantly higher than those in the control group, the allergic mouse models were considered successfully established. As shown in Fig. 1, compared with the CON group, the IgE levels in all other groups were significantly increased, indicating that the allergy model was successfully established. No post-intervention IgE measurement was performed because IgE has a relatively long half-life in mice (approximately 2–3 weeks), and a two-week lactoferrin intervention would be insufficient to detect a meaningful decline. Instead, we selected more dynamic indicators that directly reflect allergic inflammation—clinical symptoms, Th2 cytokines, and IgG subclasses—to evaluate the intervention effects.

Intervention procedure (Fig. 2)

After successful modeling, the following interventions were administered over the next two weeks:

(1) The CON and model groups (MOP, MOM) were fed a standard diet and orally administered an equivalent volume of PBS daily.

(2) The positive control groups (PPC, PMC) were fed a standard diet and orally administered dexamethasone (4 mg/kg body weight) daily.

(3) The low-dose LF groups (PLL, MLL) were fed a standard diet and orally administered LF (0.1 mg/kg body weight) daily.

(4) The high-dose LF groups (PLH, MLH) were fed a standard diet and orally administered LF (1 mg/kg body weight) daily.

All groups received oral administration once daily at approximately 11:00 AM for two consecutive weeks. Body weight, rectal temperature, and allergic symptoms were recorded weekly for each group. All data were measured in triplicate and the average value was calculated.

Based on the average body weight of mice in our laboratory (approximately 20 g), an administration dose of 1 mg/kg converts to approximately 20 μg per mouse. This

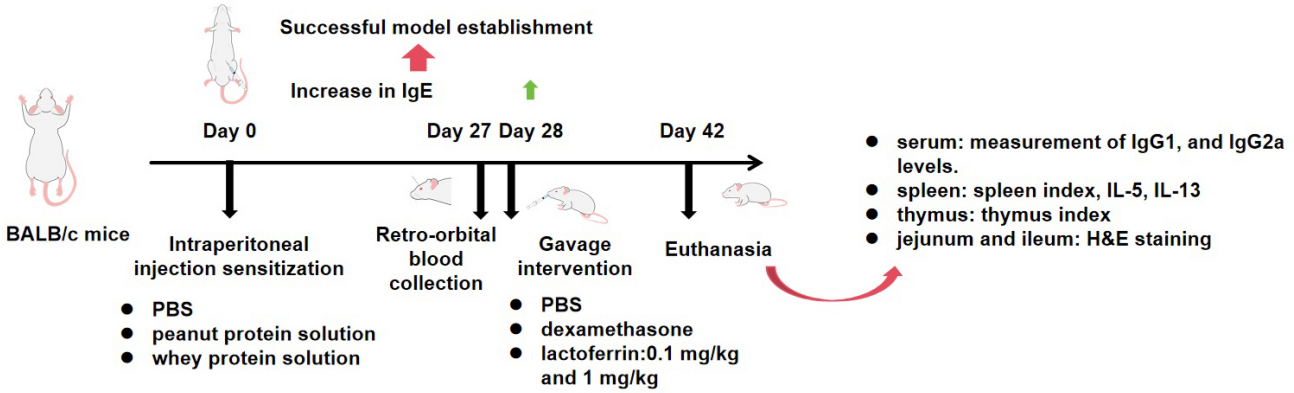

Fig. 2. A flowchart of the experimental procedures and interventions, primarily including experimental operations, intervention duration, and measurement indicators. Abbreviations: BALB/c, Bagg ALBino c series; PBS, phosphate-buffered saline;IgE, immunoglobulin E; IL-5, interleukin-5; IL-13, interleukin-13; IgG1, immunoglobulin G1; IgG2a, immunoglobulin G2a; H&E staining, hematoxylin-eosin staining.

dosage is considerably lower than the harmful dose of LF reported in the literature, indicating good safety in experimental animals. Furthermore, by including two dose groups (0.1 mg/kg and 1 mg/kg), this design facilitates observation of whether LF exhibits a non-linear dose-effect relationship under these experimental conditions, enabling a more refined capture of its potential low-dose biological activity.

2.2.2.2 Body Weight Measurement. Body weight was measured and recorded on days 0, 7, 14, 21, 28, 35, and 42. Measurements were taken three times at each time point, and the average value was calculated.

2.2.2.3 Determination of Immunological Organ Indices. On day 42, mice were euthanized by carbon dioxide (CO_2) inhalation followed by cervical dislocation, in accordance with the guidelines of the Laboratory Animal Welfare and Animal Experimental Ethics Review Committee of China Agricultural University and the AVMA Guidelines for the Euthanasia of Animals (2020 edition). Briefly, mice were placed in a transparent euthanasia chamber, and medical-grade CO_2 (purity ≥99.5%, from a compressed gas cylinder) was introduced at a flow rate that displaced 30–50% of the chamber volume per minute, regulated by a precision flow meter. After respiration ceased (approximately 5–6 minutes for adult mice), CO_2 flow was maintained for an additional 1 minute. The animals were then removed, and death was confirmed by cervical dislocation (performed by trained personnel, as mice weighed <200 g), followed by observation of fixed dilated pupils and absence of heartbeat. All animal procedures were approved by the Laboratory Animal Welfare and Animal Experimental Ethics Review Committee of China Agricultural University (Approval No. AW71305202-5-01).The thymus and spleen were removed and weighed using an analytical balance. The thymus index and spleen index were calculated according to the following formula:

Organ Index (%) = (Organ Mass / Body Weight) × 100.

2.2.2.4 Determination of Serum Specific Antibody Levels. Plasma samples from each group were allowed to stand at room temperature for 2–3 hours, then centrifuged at 4 °C and 3500 r/min to separate serum. The serum was aliquoted and stored at –20 °C for subsequent use. Measurement of IgG1 and IgG2a was performed according to the instructions provided with the ELISA kits.

2.2.2.5 Determination of Cytokine Levels in Spleen Tissue. On day 42, after euthanasia, spleens were extracted and placed in 1.8 mL cryotubes, then rapidly frozen in liquid nitrogen and stored at –80 °C. For analysis, an appropriate amount of physiological saline (saline-to-spleen ratio = 10:1) was added to fully submerge the spleen sample. The tissue was homogenized using a tissue grinder, centrifuged at 3000 r/min for 10 minutes, and the supernatant was collected for determination of IL-5 and IL-13 levels according to the ELISA kit instructions.

2.2.2.6 Observation of Histopathological Structure in Jejunum and Ileum. On day 42, after euthanasia, jejunum and ileum tissues were collected and fixed in paraformaldehyde solution. Samples were sent to Kangtai Medical Testing Service Hebei Co., Ltd. for sectioning. Hematoxylin-eosin (HE) staining was performed, and histopathological examination was conducted under an optical microscope.

2.3 Data Processing

Each sample was analyzed in triplicate. Experimental data were processed and analyzed using Microsoft Excel (version 2019, Microsoft Corp., Redmond, WA, USA) to generate standard curves. Subsequent statistical analysis and graphing were performed using GraphPad Prism (version 8.0.2, GraphPad Software Inc., San Diego,

CA, USA) and IBM SPSS Statistics (version 26.0, IBM Corp., Armonk, NY, USA). Two-way Analysis of Variance (ANOVA) and one-way ANOVA were employed to assess the significance of data differences. For multiple group comparisons, Dunnett's multiple comparison test was used to compare each group with the control group.

Results are expressed as mean ± SD, based on repeated measurements from four biological samples, each subjected to three determinations. Error bars represent the variability or uncertainty in the data. Significance levels are denoted as follows:

Compared with the control group, *: $p < 0.05$ (significant); **: $p < 0.01$ (more significant); ***: $p < 0.001$ (highly significant); ****: $p < 0.0001$ (extremely significant).

Compared with the corresponding model group, #: $p < 0.05$ (significant); ##: $p < 0.01$ (more significant); ###: $p < 0.001$ (highly significant); ####: $p < 0.0001$ (extremely significant).

3. Results

3.1 Effect of Lactoferrin on Body Weight Changes Induced by Peanut Allergy and Cow's Milk Allergy

Throughout the experimental process, changes in body weight were continuously monitored across all groups (Fig. 3A). Body weight is a critical and intuitive macroscopic indicator for assessing nutritional status, metabolic levels, and overall health. In experimental research, dynamic changes in body weight can sensitively reflect the physiological effects of interventions.

Overall, all mice showed an upward trend in body weight. After the allergy model was established on day 7, some mice experienced weight loss, consistent with allergic symptoms. Starting from day 21, mice in the positive milk control group exhibited a significant decline in body weight. By day 42, the body weight of mice in the peanut model group was significantly higher than that of the normal control group (Fig. 3A, $p < 0.001$).

3.2 The Effect of Lactoferrin on the Proliferation of Thymocytes and Splenocytes

Immune organ indices are essential metrics for assessing immune response vitality, reflecting the body's immune intensity and sensitively indicating the overall impact of external stimuli such as drugs, toxins, nutritional interventions, pathogen infections, or stress on the immune system.

The organ indices of the MOP and MOM groups were significantly higher than those of the CON group, indicating that peanut allergy and cow's milk allergy can cause inflammatory enlargement, affecting the growth and development of immune organs in mice (Fig. 3B,C). Compared with the MOP group, the spleen index of the PLH group showed a significant decrease ($p < 0.0001$; Fig. 3B), indicating that high-dose LF treatment can effectively suppress the excessive inflammatory response induced by peanut allergy, alleviate splenic inflammatory infiltration and congestive enlargement, and restore spleen weight toward physiological levels.

Compared with the MOM group, the spleen index of the MLL group decreased significantly ($p < 0.001$), and the thymus index of the MLL group also decreased significantly ($p < 0.05$; Fig. 3B,C). These results indicate that low-dose LF treatment can effectively suppress the excessive inflammatory response induced by cow's milk allergy, inhibit splenic and thymic inflammatory infiltration, and modulate the immune system.

3.3 Effect of Lactoferrin on Interleukin Secretion Levels

IgG is the most abundant antibody in serum and serves as the primary agent in humoral immune responses. It is further divided into different subtypes. IgG1 and IgG2a are critical indicators in mediating food allergies. In the peanut allergy model, compared with the CON group, serum total IgG1 levels in the MOP group showed an increasing trend, but the difference was not statistically significant (Fig. 3D). However, LF treatment (PLL and PLH) did not significantly alter IgG1 or IgG2a levels relative to the MOP group. Specifically, PLL slightly decreased while PLH slightly increased IgG1, but neither change reached statistical significance; for IgG2a, both PLL and PLH showed a decreasing trend without statistical significance ($p > 0.05$ for all comparisons, Fig. 3D,E).

In the cow's milk allergy model, compared with the CON group, serum total IgG1 levels in the MOM group were significantly elevated (Fig. 3D). Notably, compared with the MOM group, both MLL and MLH treatments significantly reduced serum total IgG1 ($p < 0.01$) and IgG2a ($p < 0.05$) levels (Fig. 3D,E). Taken together, the effect of reducing serum total IgG1 and IgG2a levels was statistically significant only in the cow's milk allergy model, while no significant reduction was observed in the peanut allergy model under the current experimental conditions.

Interleukin-5 (IL-5) and IL-13 are cytokines produced by Th2-type immune responses. They are important factors that induce B cells to produce allergen-specific IgE and IgG1. Compared with the MOP group, IL-5 levels in the PLL and PLH groups decreased extremely significantly ($p < 0.0001$; Fig. 3F), and IL-13 levels decreased significantly ($p < 0.05$; Fig. 3G). Compared with controls, the peanut-allergy group showed a modest, non-significant increase in IL-5 and IL-13. However, both low- and high-dose LF interventions significantly reduced these levels versus the allergy group, suggesting that LF effectively suppresses the elevation associated with peanut allergy.

Compared with the CON group, the MOM group showed decreased levels of IL-5 and IL-13 (Fig. 3F,G). Treatment with low-dose (MLL) and high-dose (MLH) lactoferrin further significantly reduced IL-5 levels compared with the MOM group ($p < 0.05$; Fig. 3F). Additionally, high-dose lactoferrin treatment (MLH) significantly

decreased IL-13 levels compared with the MOM group ($p < 0.001$; Fig. 3G). While the MLL group showed a significant decrease in IL-5 ($p < 0.05$), the MLH group achieved an extremely significant inhibition of IL-13 ($p < 0.001$).

3.4 Effect of Lactoferrin on Intestinal Villi Integrity

In the jejunal tissue sections, the jejunal villi of the CON group mice appeared thick and elongated, with relatively orderly alignment and shallow crypts. This indicates efficient digestion and nutrient absorption, providing a solid foundation for normal body weight gain and immune system development. These observations serve as the baseline for evaluating the severity of pathological changes in the other groups.

In contrast, compared with the CON group, the jejunal villi of the MOP and MOM group mice showed partial disruption (Fig. 4). Based on the section results, the jejunal tissue structure in the PLL, PLH, MLL, and MLH groups showed improvement, with reduced villous disruption, more orderly alignment, and partial restoration of villous length. This suggests that both low-dose and high-dose LF have a mitigating effect on peanut allergy and cow's milk allergy.

In the ileal tissue sections, the ileal villi of the CON group mice were slightly shorter and blunter compared to the jejunum but remained structurally intact (Fig. 4). The Peyer's patches (PPs) were small, located in the submucosa with clear boundaries, and showed no activation of germinal centers. In contrast, compared with the CON group, the ileal villi of the MOP and MOM group mice exhibited atrophy and shedding. Based on the section results, the ileal tissue structure in the PLL, PLH, MLL, and MLH groups showed improvement and appeared relatively intact.

4. Discussion

4.1 Lactoferrin Alleviates Clinical Symptoms and Immune Organ Hyperactivity in Food Allergy Models

Oral administration of lactoferrin (LF) significantly reduced allergy-associated clinical symptoms and normalized body weight trajectories in both peanut- and milk-sensitized juvenile mice. Body weight is a critical indicator of nutritional status and metabolic health. After sensitization, transient weight loss occurred, consistent with acute allergic responses. Notably, positive control groups receiving dexamethasone showed progressive weight decline beginning at day 21, particularly pronounced in the milk allergy model. This catabolic effect is due to dexamethasone, which promotes protein catabolism and increases fat breakdown, leading to weight loss and fat redistribution.

By day 42, peanut-allergic model mice had significantly higher body weight than healthy controls, suggesting immune-metabolic dysregulation characterized by abnormal fat accumulation. This likely results from chronic inflammation induced by peanut allergy, leading to altered energy storage and body composition. In contrast, the body weight of mice in the positive milk control group was significantly lower than that of the normal control group, reflecting the cumulative catabolic side effects of dexamethasone, which prevented weight recovery over time.

Mice in the lactoferrin-treated groups showed no significant difference in body weight compared with the normal control group. This indicates that LF can mitigate allergy-induced inflammation without causing the strong catabolic effects associated with dexamethasone. Studies suggest that intestinal inflammation reduces short-chain fatty acid synthesis, leading to energy metabolism disorders. As a dietary supplement with anti-inflammatory properties, LF helps maintain gut microbiota stability and enhances the mucosal barrier, thereby alleviating abnormal weight gain [12]. Consequently, mice receiving LF efficiently utilized ingested nutrients, avoiding the excessive weight gain seen in the peanut model and the weight loss observed in the positive control group. This safety advantage positions LF as a promising nutritional intervention for vulnerable pediatric populations.

Immune organ indices are crucial metrics for evaluating immune response vitality [13]. Allergy-induced immune organ hyperactivity was evidenced by significantly increased spleen and thymus indices in model groups. The thymus is the central site for T lymphocyte differentiation, development, and central tolerance induction, directly determining cellular immune function [14]. The spleen, as the largest peripheral secondary lymphoid organ, orchestrates humoral and cellular immune responses [15], with its enlargement reflecting allergen-driven lymphocyte proliferation and inflammatory infiltration [16]. LF intervention markedly reduced these indices, particularly the spleen index, indicating effective suppression of allergen-driven systemic inflammation.

Our findings differ from previous reports showing that LF increases spleen and thymus indices under non-allergic conditions. For example, Wei et al. [17] suggested that lactoferrin can significantly increase the spleen and thymus indices in mice. Hu et al. [18] proposed that lactoferrin can markedly elevate the spleen index in mice and enhance immunity within a certain dosage range. Khuyen et al. [19] reported that bovine lactoferrin enhanced immune function in rainbow trout fry, although no significant changes in spleen index were observed. We attribute this discrepancy to the fact that our model groups exhibited inflammatory enlargement of immune organs due to allergy, whereas previous studies examined immune enhancement in healthy animals. Furthermore, low-dose LF (0.1 mg/kg) was more effective in reducing spleen and thymus indices in the milk allergy model, while high-dose LF (1 mg/kg) showed greater effects in the peanut model. This dose- and allergen-dependent pattern suggests that the optimal LF dosage varies with allergen type, a finding with important implications for personalized nutritional strategies.

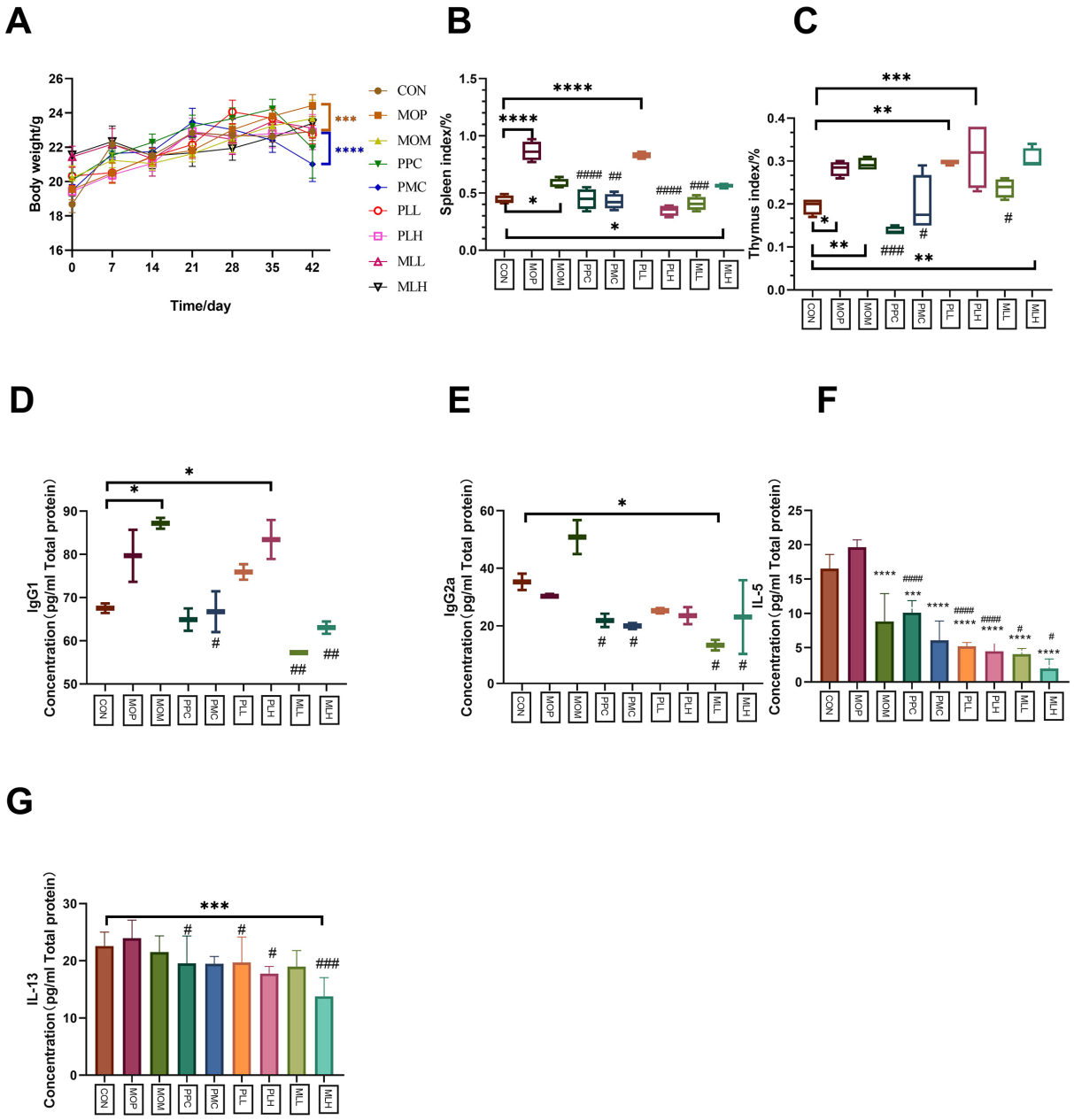

Fig. 3. The impact of lactoferrin on body weight, organ indices, and cytokine levels in mice. (A) Body weight change of young mice. (B) Spleen index of young mice. (C) Thymus index of young mice. (D) IgG1 level in the serum of young mice. (E) IgG2a level in the serum of young mice. (F) IL-5 level in the spleen of young mice. (G) IL-13 level in the spleen of young mice. Significance levels are indicated as compared with the control group or the corresponding model group. Compared with the control group, *: $p < 0.05$ (significant); **: $p < 0.01$ (more significant); ***: $p < 0.001$ (highly significant); ****: $p < 0.0001$ (extremely significant).Compared with the corresponding model group, #: $p < 0.05$ (significant); ##: $p < 0.01$ (moresignificant); ###: $p < 0.001$ (highly significant); ####: $p < 0.0001$ (extremely significant). Abbreviations: CON, normal control; MOP, peanut model; MOM, milk model; PPC, positive peanut control; PMC, positive milk control; PLL, peanut low-dose lactoferrin; PLH, peanut high-dose lactoferrin; MLL, milk low-dose lactoferrin; MLH, milk high-dose lactoferrin.

4.2 Lactoferrin Suppresses Th2-Dominant Immune Responses

Food allergy is fundamentally characterized by pathological Th2-polarized immune responses to dietary antigens, marked by elevated Th2 cytokines (IL-4, IL-5, IL-13) and increased production of IgG1. IgG1 [20] is a sensitizing antibody indicative of Th2-type immunity, mediating type I hypersensitivity reactions and reflecting the intensity

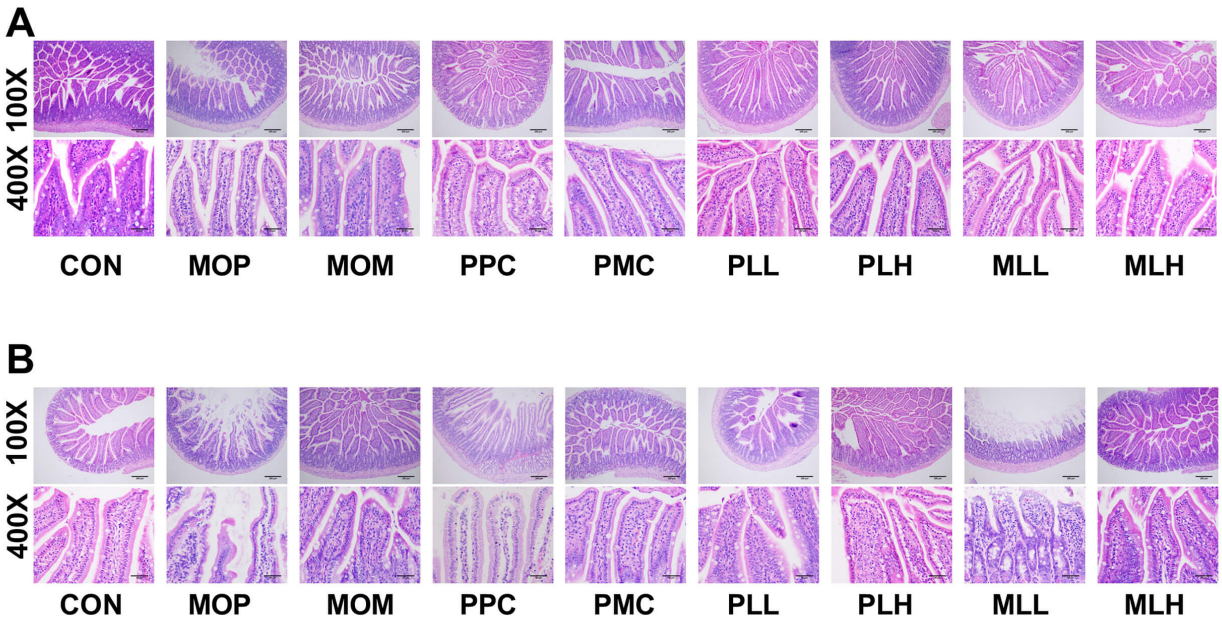

Fig. 4. Stained sections of jejunum and ileum of young mice at 100× and 400×. (A) Stained sections of jejunum of young mice at 100× and 400×. (B) Stained sections of ileum of young mice at 100× and 400×. Abbreviations: CON, normal control; MOP, peanut model; MOM, milk model; PPC, positive peanut control; PMC, positive milk control; PLL, peanut low-dose lactoferrin; PLH, peanut high-dose lactoferrin; MLL, milk low-dose lactoferrin; MLH, milk high-dose lactoferrin. Scale bar: 200 μm for images at 100× magnification; 50 μm for images at 400× magnification (applies to all images in Fig. 4). The HE staining images in Fig. 4 were captured at consistent magnifications within each row.

of sensitization [21]. IL-5 is a key cytokine for eosinophil differentiation and proliferation, mediating chronic inflammation and tissue damage [22]. IL-13 programs dendritic cells to drive Th2 differentiation and promotes B-cell production of allergen-specific IgE and IgG1 [23].

In this study, LF intervention significantly reduced splenic levels of IL-5 and IL-13, as well as serum concentrations of IgG1, in both peanut and milk allergy models. These results demonstrate that LF exerts its anti-allergic effects primarily by inhibiting the Th2 immune axis.

Lactoferrin achieves this modulation through multiple pathways. First, LF can directly act on innate immune cells such as dendritic cells (DCs) [24]. Research suggests that LF binds to specific receptors on DC surfaces, such as low-density lipoprotein receptor-related protein 1 (LRP1) [25], influencing their maturation and cytokine secretion profile, thereby predisposing them to induce immune tolerance rather than Th2 sensitization. These tolerogenic DCs promote the differentiation of naive T cells into regulatory T cells or inhibit their differentiation into pathogenic Th2 cells.

Second, LF is a potent inducer of Tregs [26]. Tregs suppress the activation and function of Th2 effector cells and allergen-specific B cells through cell-contact-dependent mechanisms or by secreting inhibitory cytokines such as IL-10 and TGF-β [27]. In this study, the reduced spleen index and decreased Th2 cytokines in LF-treated groups are consistent with enhanced Treg function, which inhibits abnormal lymphocyte proliferation and activation. Furthermore, LF's intrinsic iron-binding capacity contributes to its beneficial effects by chelating excess free iron, reducing oxidative stress [28], and creating a microenvironment unfavorable for Th2 cell activation and proliferation.

We did not report a reduction in serum total IgE after lactoferrin intervention because IgE was measured only at the end of sensitization (week 4) to confirm successful modeling, not as an endpoint for the two-week treatment. The half-life of mouse IgE (2–3 weeks) is comparable to or longer than the intervention period, making a measurable decline unlikely. In contrast, Th2 cytokines (IL-5, IL-13) and IgG subclasses have shorter turnover times and respond more rapidly to immunomodulation, making them suitable indicators of short-term effects. Therefore, the absence of IgE reduction does not contradict the anti-allergic conclusion; it reflects different kinetic profiles. Future studies with longer intervention periods are needed to assess the effect of lactoferrin on established IgE.

4.3 Allergen-Dependent Modulation of Th1-Associated Antibody Responses

A particularly intriguing finding is the differential effect of LF on IgG subclass responses across allergy mod-

els. IgG2a, which is typically associated with Th1-type immunity in mice, plays a protective role by neutralizing pathogens and reflecting the establishment of immune tolerance. In the cow's milk allergy model, LF significantly reduced serum levels of both IgG2a and the Th2-associated IgG1. In contrast, in the peanut allergy model, LF showed no significant effect on either antibody, although low-dose LF did produce a minor reduction in both IgG1 and IgG2a, and high-dose LF only affected IgG2a (all $p > 0.05$).

This discrepancy indicates that the immunomodulatory effect of LF on IgG subclasses is allergen-dependent. The reasons for this difference are not clear from the current data, but may be related to distinct allergen stability or inherent differences in the immune response to peanut versus milk proteins. Further studies are needed to elucidate the mechanisms underlying this allergen-specific effect.

4.4 Lactoferrin Protects Intestinal Barrier Integrity

Intestinal barrier dysfunction is a critical factor in both the initiation and perpetuation of food allergies [29]. The "leaky gut" hypothesis suggests that compromised epithelial integrity permits abnormal allergen translocation, triggering and sustaining allergic inflammation. The small intestine exhibits regional specialization: the jejunum is the primary site of allergen penetration [30] while the ileum, which contains Peyer's patches where most intestinal immune cells aggregate, serves as the command center for Th2 sensitization [31].

Histopathological examination showed that LF significantly ameliorated allergy-induced damage to jejunal and ileal architecture. Compared to model groups with villous atrophy, disruption, and inflammatory infiltration, LF-treated mice exhibited improved villous structure and reduced epithelial damage, particularly within Peyer's patches. This preservation of intestinal integrity provides compelling evidence that LF's immunomodulatory effects result in tangible physiological protection.

LF protects the intestinal barrier through complementary mechanisms [32]. First, systemic immunomodulation reduces Th2 cytokines such as IL-13, which directly compromise epithelial integrity. Second, LF directly promotes intestinal epithelial cell proliferation and migration via receptor-mediated signaling, accelerating mucosal repair. Third, LF modulates gut microbiota, inhibiting pathogens while promoting beneficial species that enhance barrier function through metabolites like short-chain fatty acids. Thus, LF exerts dual protective effects, simultaneously correcting systemic immune dysregulation and locally restoring intestinal barrier function.

4.5 Dose-Dependent and Allergen-Specific Effects of Lactoferrin

A notable finding is the differential dose-response between peanut and milk allergy models. Low-dose LF (0.1 mg/kg) was more effective in ameliorating milk allergy, particularly in immune organ indices, while high-dose LF (1 mg/kg) showed greater efficacy against peanut allergy. This pattern reflects differences in allergen properties and LF's concentration-dependent activities.

A previous study showed that LF can bind to high-affinity receptors such as CD14, thereby inhibiting inflammatory responses and preventing the activation of pro-inflammatory pathways, which contributes to localized anti-inflammatory and barrier-protective effects relevant to milk allergy, where inflammation is more confined to the intestinal mucosa [33]. Higher concentrations saturate additional receptors that mediate systemic immunomodulation, required to counter the robust Th2 response characteristic of peanut allergy. Additionally, major peanut allergens exhibit remarkable stability against digestion and efficient intestinal translocation [34], necessitating higher LF doses to effectively interrupt sensitization through allergen binding, barrier reinforcement, and immune modulation. These observations support the concept that optimal nutritional interventions should be tailored to specific allergens.

4.6 Limitations

This study has several strengths: a systematic comparison of LF effects on two major food allergens within a unified framework, identification of dose-dependent and allergen-specific response patterns, and integration of systemic immune markers with histopathological assessment of intestinal protection. These findings advance our understanding of LF's immunomodulatory mechanisms and provide preclinical evidence supporting its development for hypoallergenic infant formulas. Currently, a recent review [35] has highlighted the potential of lactoferrin in managing allergic airway diseases in children, while research on its preventive effects against food allergies remains relatively limited. Although the existing literature primarily focuses on airway diseases, discussions regarding LF's role in modulating immune responses and balancing immune pathways are highly valuable for understanding its potential mechanisms in food allergy.

However, some limitations warrant consideration. First, extrapolation of murine findings to humans requires caution. Second, this study did not directly examine Treg populations, gut microbiota composition, tight junction protein expression, or IgE dynamics. Some mechanistic interpretations discussed above are inferred from observed phenotypic changes and published literature and were not directly assessed in the present study. We will further explore and demonstrate the relevant underlying mechanisms in future studies.

Third, specific molecular signaling pathways remain to be elucidated. A growing body of evidence has shown that lactoferrin plays a role in various signaling pathways. For example, lactoferrin protects against lipopolysaccharide-induced intestinal inflammatory damage by regulating the NF-κB/PPAR signaling pathway [36];

it can also activate the PI3K/Akt signaling pathway to promote the proliferation of human intestinal epithelial cells [37]; additionally, it regulates MAPK signaling, reducing ferroptosis and preventing intestinal barrier damage [38]. In future research on signaling pathways, we should draw more on previous experience.

Fourth, a separate lactoferrin control group (i.e., normal animals receiving lactoferrin alone) was not included in the experimental design. Future studies should include a lactoferrin-only intervention group to more precisely quantify the relative contributions of its direct effects versus immunomodulatory effects and to further explore its molecular mechanisms.

Future research should use gene knockout models and multi-omics approaches to systematically clarify LF's mechanisms. Dose-optimization studies comparing preventive and therapeutic supplementation could identify optimal intervention windows. Clinical translation studies in high-risk infants are essential to evaluate safety and efficacy in humans. Despite these limitations, this study provides useful insights and supports LF as a safe, natural bioactive component for food allergy management.

5. Conclusion

This study systematically evaluated the interventional effects of lactoferrin using juvenile mouse models of peanut and cow's milk allergy. The key effects include suppressing Th2-biased immune responses, as evidenced by the strong inhibition of the key Th2 cytokines IL-5 and IL-13 in the spleen across both models, and an allergen-dependent reduction in serum levels of total IgG1 and IgG2a, which reached statistical significance in the cow's milk allergy model. Concurrently, LF intervention alleviated allergy-induced pathological spleen enlargement and significantly improved intestinal tissue structure, reducing villus damage and inflammatory infiltration. This demonstrates its dual efficacy in systemic immunomodulation and local intestinal barrier protection.

In summary, LF shows a clear inhibitory effect on peanut and milk allergies through multi-target actions, including suppressing Th2-biased immunity, downregulating specific antibody responses, and maintaining intestinal physical and immune barriers. This study deepens understanding of LF's immunomodulatory mechanisms and provides a solid scientific basis for its application as a safe, natural dietary ingredient in the development of functional foods, such as hypoallergenic infant formula, aimed at preventing or assisting in the management of common food allergies in infants and young children. Future research should further elucidate the molecular mechanisms underlying its allergen-dependent effects and advance clinical translation trials to verify its efficacy in humans.

Availability of Data and Materials

The datasets generated and analyzed during the *in vivo* experiments are not publicly available due to intellectual property restrictions by the funding agency, but are available from the corresponding authors upon reasonable request. Publicly available datasets were analyzed in this study.

Author Contributions

SL: data curation, formal analysis, funding acquisition, investigation, writing—original. QZ: writing, formal analysis, investigation. QW: investigation, formal analysis, investigation. HF: investigation, writing—review & editing, formal analysis. HL: formal analysis, validation. SL: investigation, data curation. TX: formal analysis, investigation. JX: methodology, investigation. XG, DL: conceptualization, funding acquisition, supervision, writing—review and editing. All authors contributed to the writing of the manuscript and critically reviewed its major academic content; all authors gave final approval of the version to be published and agreed to take responsibility for all aspects of the research, ensuring that any questions regarding accuracy or completeness are properly investigated and resolved.

Ethics Approval and Consent to Participate

This study was conducted in accordance with the guidelines of the Laboratory Animal Welfare and Animal Experimental Ethics Review Committee of China Agricultural University. The animal experimental protocol was approved by the Ethics Committee of China Agricultural University (Approval No. AW71305202-5-01). All animal procedures were performed in compliance with the relevant regulations and guidelines.

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Conflicts of Interest

The authors declare no conflicts of interest.

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