Food Science of Animal Products

Research Article

Immune-boosting effects of nutritional formulations containing acerola cherries extractive and lactoferrin

Haifu Jia[1,§], Yuhong Wang[1,§], Yueming Zhao[2,3], Ziyu Hu[1], Qingjing Liu[2,3], Yanmei Hou[2,3], Yujun Jiang[1], Qianyu Zhao[1] ✉, Chaoxin Man[1] ✉

[1] *Key Laboratory of Dairy Science, Ministry of Education, College of Food Science, Northeast Agricultural University, Harbin 150030, China*
[2] *Ausnutria Dairy (China) Co., Ltd., Changsha 410000, China*
[3] *Aunulife Biotechnology Co., Ltd., Changsha 410000, China*
[§] These authors contributed equally to this work.
✉ Address correspondence to Qianyu Zhao, 389832745@qq.com; Chaoxin Man, 87402577@qq.com

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Abstract: Reduced immunity can harm the health of the organism, and nowadays, improving immunity is getting more and more attention, so the nutrients with immune boosting function (acerola cherry, taurine, zinc gluconate, and lactoferrin) are compounded in the best ratio to develop a nutritional formula food, and evaluated by cellular immunity, humoral immunity, non-specific immunity. In this study, an immunocompromised mice model was established using cyclophosphamide (CTX), the ability and difference of different components to enhance the immunity of mice were determined by the gavage of different components. The results showed that the nutritional formula food could recover the body weight of immunocompromised mice, promote the development of immune organs in immunocompromised mice, enhance the delayed-type hypersensitivity (DTH) response, the ability to produce serum hemolysin and the phagocytosis of monocytes in immunocompromised mice, and increase the levels of immunoglobulin A (IgA), IgG and IgM in the serum of immunocompromised mice. It has proved that this nutritional formula food (containing acerola cherry, taurine, zinc gluconate, and lactoferrin) has synergistic effect and can work together on humoral immunity, cellular immunity and non-specific immunity to improve the immune resistance of mice, and has promising application.

Keywords: acerola cherry; lactoferrin; nutrition formulation; immunity

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1 Introduction

The immune system is the most important defense system of the organism, which specifically or non-specifically eliminates foreign substances that invade the organism and is also an important component in activating the immune response, conducting the immune reaction, and maintaining the effect of the corresponding immunity. The immune system protects the host from a large number of antigens, which are usually distributed in the environment or within body[1]. Immunity is a complex process that requires the close and orderly coordination of the components of the immune system. This process mainly includes a series of complex physiological processes, such as the orderly transmission of antigens, activation of cells, and formation of immune molecules. The orderly occurrence of these processes facilitates the effective genemiceion of immune responses, thus providing an orderly and effective regulation of the internal environment of the body and maintaining the homeostasis of the internal environment[2–3]. Reduced immunity will lead to a weakened protective function of the body, and can prevent the immune system from playing its normal protective role. It has been shown that nutritional intake and the strength of immune function are closely related, and balanced nutrition is an important prerequisite for improving the immunity of the body, such as lactoferrin can effectively influence and regulate certain aspects of immune function, mainly in the 3 major areas of humoral immunity, cellular immunity and non-specific immunity[4–7].

The acerola cherry is the fruit of the coniferous tree, also known as the Barbados cherry and the West Indian cherry. The acerola cherry is similar in size and shape to the standard cherry, but has 3 lobes. The acerola cherry is valued for its unique flavor and high vitamin C content[8], dietary fiber, ascorbic acid, and carotenoid components including lutein, α-carotene, and β-carotene[9–12]. Many of these ingredients have been shown to increase the body's anti-inflammatory response and are even more immune-boosting when consumed in conjunction with other bioactive substances[13]. It has been shown that consumption of acerola cherries significantly reduces the levels of the pro-inflammatory cytokines tumor necrosis factor α (TNF-α) in the liver in the presence of the damaging (toxic) effects of carbon tetrachloride on the liver[14]. Thus, the immunomodulatory effect of acerola cherry was confirmed. Lactoferrin, a non-heme iron-binding glycoprotein found in milk, contains about 700 amino acid residues and has a molar mass of about 80 kDa. lactoferrin has been proved to have a wide range of potentially beneficial biological activities, including antibacterial, anti-inflammatory and antitumor activities. Lactoferrin has also been reported to play a critical role in modulating the mammalian immune system, reducing gastrointestinal irritation, regulating iron metabolism and maintaining iron levels in the body[15]. Lactoferrin is able to accelerate the maturation of T cells and differentiate immature B cells[16], and increase natural killer cells in the lymphocyte population[17], and then regulate immune function. Lactoferrin

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administered at doses of 2.5, 5.0 and 10.0 mg/kg inhibited the activation of nuclear factor κB (NF-κB), TNF-α and interleukin (IL) 1β[18]. Immunomodulatory activity of lactoferrin may be due to its binding to endotoxin (lipopolysaccharide)[19]. Lactoferrin was found to attenuate lipopolysaccharide-induced inflammation by attenuating NF-κB/mitogen-activated protein kinase pathways, mitigating oxidative stress, and maintaining cellular barrier integrity, thus demonstrating a prominent role for lactoferrin in immunomodulation[20]. In addition, lactoferrin has therapeutic effects in neurodegenerative diseases. The neuroprotective effects of intranasal administration of lactoferrin were tested in a mouse model of Alzheimer's disease. Lactoferrin reduces β-amyloid deposition and ameliorates disease in individuals with Alzheimer's disease through activation of the ERK1/2-CREB and HIF-1α signalling pathways and AM10 expression in Alzheimer's disease[21]. It has been confirmed that lactoferrin as an immune adjuvant can complement *Bacillus* Calmette-Guerin (BCG) vaccination by stimulating the production of IL-12 by macrophages to enhance the delayed metabolic response and thus improve the efficiency of BCG vaccination; it can participate in tumor immunity by regulating cellular immunity such as T-lymphocytes. In addition, lactoferrin has a positive regulatory effect on immune repair[22]. Taurine is a semi-essential amino acid in animals, which exists in free form in the body and is widely distributed in various tissues and vital organs of animals. Due to the low activity of cysteine decarboxylase, the key enzyme for taurine synthesis in animals, taurine needs to be taken from outside in addition to its own synthesis in animals. Taurine is a rich source and has antioxidant, immunity-enhancing and anti-apoptotic functions[23]. In recent years, taurine has been widely researched in the medical field, mostly focusing on anti-cancer, anti-tumor, cardiovascular protection and other pharmacological effects[24–26]. $CD8^+$ T cells from cancer patients had high expression of *SLC6A6* during taurine uptake and decreased anti-tumour function of T cells after *SLC6A6* knockdown. RNA sequencing data and *in vitro* experiments showed that taurine enhanced T cell proliferation and function by enhancing oxidative phosphorylation and stimulating phospholipase C-γ1 (PLC-γ1)-mediated calcium and mitogen-activated protein kinase (MAPK) signalling pathways. The results suggest that taurine may improve the therapeutic effect of programmed death-1 inhibitors by modulating the function of $CD8^+$ T cells[27], highlights the beneficial effects of taurine on immune organs and immune cells. Zinc gluconate is the zinc salt of gluconic acid. As an organic zinc supplement, zinc gluconate is less irritating to the gastric mucosa, easily absorbed in the body, with high absorption mice and good solubility, and zinc gluconate can participate in the synthesis of nucleic acid and protein, enhance human immunity, and promote the growth and development of fetuses, infants and young children. In addition, zinc gluconate can enhance the phagocytosis of macrophages, promote the proliferation and differentiation of T cells and B cells, and enhance the activity of natural killer cells, thus improving the ability of immune cells to clear pathogens. Zinc gluconate is used in medicine and added to food as a nutritional supplement (zinc fortification) in the food industry[28–29]. However, it has been shown that after repeated intraperitoneal injections of 4 or 8 mg/kg zinc in rats, toxicity occurs in tissues and organs associated with the endocrine system[30].

In this study, BALB/c female mice were used as the study subjects, and the animal model of immune depression caused by cyclophosphamide (CTX) was used. The ability of different components of the samples to enhance immunity in mice was tested by gavage of acerola cherry concentrated powder, taurine and zinc gluconate, lactoferrin, the mixture of taurine, zinc gluconate and acerola cherry concentrated powder and the mixture of acerola cherry concentrated powder, taurine, zinc gluconate and lactoferrin, and the ability and difference of different components of the samples to enhance immunity in mice were determined.

2 Materials and methods

2.1 Reagents and chemicals

Nutritional formula food (including skimmed goat milk powder, whey protein concentrate powder, acerola cherry, taurine, zinc gluconate, and lactoferrin, etc.) was prepared by Aunulife Biotechnology Co., Ltd. under the brand name of Aunulife. Giemsa staining solution was provided by Beijing Solaibao Technology Co., Ltd. Dinitrofluorobenzene (DNFB), Hank's solution, and RPMI1640 culture solution were purchased from Sigma, USA. India ink, sheep red blood cells (SRBC), and chicken red blood cells were acquired by Shanghai Yuanye Biotechnology Co.

2.2 Equipments and instrument

PL2002 electronic balance purchased from Mettler Teledo, Switzerland. OptiClean-1300 vertical flow clean bench provided by Shanghai Likang Biomedical Technology Holdings Co., Ltd., China. IL fluorescence inverted microscope purchased from Leica, Germany. CO_2 incubator purchased from Thermo Fisher Scientific, USA. SPECTRAMAX full wavelength Enzyme Maker provided by Molecular Devices, USA.

2.3 Animal experiment grouping and treatment

A total of 147 BALB/c female mice (6 weeks old) were housed at (23 ± 3) °C and (55 ± 10)% relative humidity and maintained on a 12 h light-dark cycle. During the experiment, the bedding and water were changed twice a week, and the feed was changed every day to avoid fat oxidation and odor affecting the eating of the mice. Mice were acclimatized for one week and randomly divided into 7 groups (n = 21): blank group; model group; general food group: acerola cherry concentrated powder 0.508 3 mg/kg; nutritional fortification group: taurine 0.102 5 mg/kg, and zinc gluconate 0.143 5 mg/kg; lactoferrin group: lactoferrin 0.205 0 mg/kg; compound mixture group: taurine 0.016 0 mg/kg, zinc gluconate 0.022 0 mg/kg, and acerola cherry concentrated powder 0.782 0 mg/kg; end product group: nutritional formula food 32.8 mg/kg (including skimmed goat milk powder, whey protein concentrate and the above ingredients). After acclimatization feeding, except for the blank group, the other 6 groups were injected with CTX intraperitoneally at a dose of 80 mg/(kg·d) for 3 days at the beginning to establish a mouse immune deficiency model, and the blank group was injected with an equal volume of 0.9 g/100 mL NaCl. Each group was given subjects by gavage for 30 days continuously, and the blank group was given equal volume of physiological saline with the model group. At the end of the experiment, the mice were executed by cervical dislocation method to prepare for subsequent experiments (Figure 1).

2.4 Measurement of body weight and organ indices in mice

After 1 week of acclimatization feeding, mice were randomly grouped and initial body weights were recorded; body weights were measured and recorded at regular times each week for 30 days; final body weights were recorded before slaughter. After different samples were given by gavage, the mice were dislocated and executed, the thymus and spleen were removed on an ultra-clean

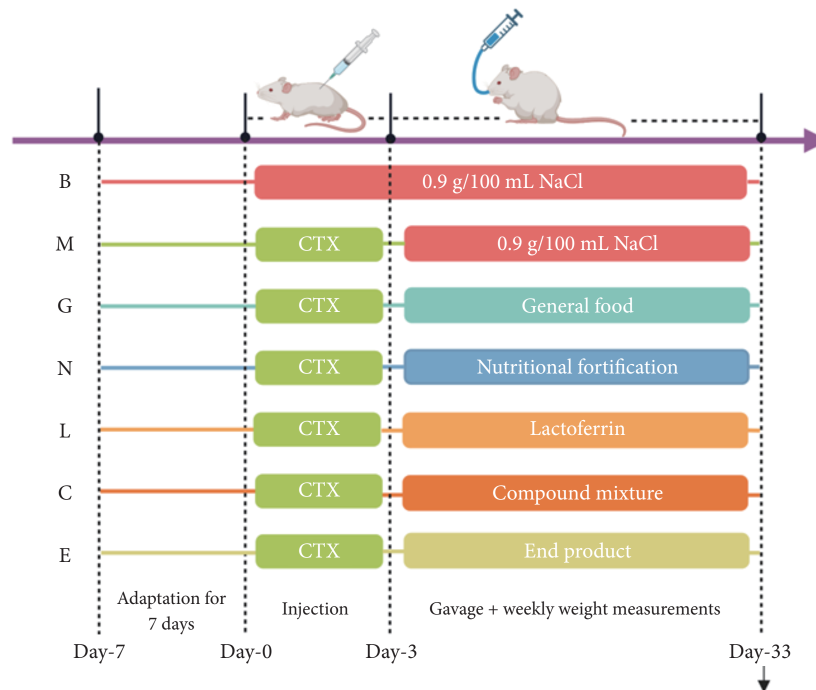

Figure 1 The animal experiment design. B, blank group; M, model group; G, general food group; N, nutritional fortification group; L, lactoferrin group; C, compound mixture group; E, end product group.

bench, weighed separately, and the organ indices were calculated for each group of mice by the following formulas (1)–(2):

$$\text{Thymus index (mg/g)} = \frac{\text{Weight of thymus (mg)}}{\text{Body weight (g)}} \quad (1)$$

$$\text{Spleen index (mg/g)} = \frac{\text{Weight of spleen (mg)}}{\text{Body weight (g)}} \quad (2)$$

2.5 Determination of delayed-type hypersensitivity (DTH)

2.5.1 Dinitrofluorobenzene-induced DTH in mice

Xylene was evenly applied to the front and back of the left ear of the mice to cause inflammation at 20 μL, and the blank group was left untreated. After 30 min, blood was taken from the eyes, the necks were broken and executed, the left and right ears of the mice were cut off, and the 2 circular ear pieces were punched at the same position with a punch, weighed, and the swelling mice of the ears was calculated by the following formula (3):

$$\text{Ear swelling rate (\%)} = \frac{\text{Weight of right ear piece (mg)} - \text{Weight of left ear piece (mg)}}{\text{Weight of left ear piece (mg)}} \quad (3)$$

2.5.2 SRBC induced DTH in mice

Each mouse was injected intraperitoneally with 0.2 mL of 2% (*V*/*V*) SRBC. Four days after sensitization, the thickness of the left hindfoot metatarsal was measured, and then 20 μL of 20% (*V*/*V*) SRBC was injected subcutaneously at the measurement site, and the thickness of the left hindfoot metatarsal was measured at 24 h after injection, and the mean value was taken 3 times at the same site.

2.6 Determination of serum hemolysin level

SRBC was prepared in physiological saline as 2% (*V*/*V*) cell suspension, and 0.2 mL was injected intraperitoneally into each mouse for immunization. After 4 days, the blood was removed from the eye and placed in a centrifuge tube for about 1 h. The coagulated blood was stripped from the wall of the tube to allow the serum to be fully precipitated, and the serum was collected by centrifugation at 2 000 r/min for 10 min. The serum was diluted with physiological saline, and the serum of different dilutions was placed in a microhemagglutination experimental plate, 100 μL per well, and then 100 μL of 0.5% (*V*/*V*) SRBC suspension was added, mixed well, loaded into a moist flat plate with a lid, and incubated at 37 °C for 3 h. The degree of agglutination of blood cells was observed microscopically.

Serum hemolysin level can be expressed by antibody level. The antibody level was calculated by the following equation (4):

$$\text{Antibody level} = S_1 + 2S_2 + \cdots nS_n \quad (4)$$

Where 1, 2, 3...... *n* represent the exponent of double dilution; *S* represents the level of agglutination degree.

2.7 Carbon contouring assay in mice

Diluted India ink (10 mL/kg) was injected from the tail vein of mice according to their body weight, and when the ink was injected, it was immediately timed. At 2 and 10 min after ink injection, 20 μL of blood was taken from the medial canthal plexus, respectively, and immediately added to 2 mL 0.1 g/100 mL Na_2CO_3 solution. The optical density (OD) value was measured at 600 nm with an enzyme marker, and Na_2CO_3 solution was used as a blank control. The mice were executed, the liver and spleen were taken, and the blood stains on the surface of the organs were blotted out with filter paper and weighed separately. The phagocytic index was calculated by the following equations (5)–(6):

$$\text{Contouring index } (k) = \frac{\lg OD_1 - \lg OD_2}{t_1 - t_2} \quad (5)$$

$$\text{Devouring index } (a) = \frac{\text{Body weight}}{\text{Weight of liver} + \text{Weight of spleen}} \times \sqrt[3]{k} \quad (6)$$

Where t_1 and t_2 represent different times (min); OD_1 represents OD of blood specimen at t_1; OD_2 represent OD of blood specimen at t_2.

2.8 Phagocytosis of chicken erythrocytes by mouse peritoneal macrophages

Activation of mouse macrophages: 4 days before the experiment, each mouse was injected intraperitoneally with 0.2 mL of 2% (V/V) pressed sheep blood erythrocytes. Mice were executed by cervical dislocation, and 4 mL of Hank's solution with calf serum was injected intraperitoneally per mouse, and the abdomen was gently rubbed 20 times to fully wash out the abdominal macrophages, then a small opening was cut in the abdominal wall, and 2 mL of the abdominal wash was aspirated in a test tube with a rubber-tipped pipette. Aspirate 0.5 mL of abdominal washings with a 1 mL sparger into a test tube containing 0.5 mL of 1% chicken blood erythrocyte suspension, and mix well. Aspirate 0.5 mL of the mixture with a syringe and add it to the agar ring of the slide. Place in the incubator and incubate for 20 min at 37 °C. After incubation, quickly wash off the unadhered cells with saline, fix in methanol solution for 1 min, and stain with Giemsa solution for 15 min. The cells were rinsed well with distilled water, air-dried, and the phagocytosis rate, i.e. the percentage of macrophages that phagocytosed chicken erythrocytes per 100 macrophages, was counted with a 40 × microscope.

2.9 Determination of cytokines in mice by enzyme-linked immunosorbent assay (ELISA)

After gavage of different products, blood was collected from the orbits of mice in each group under ether anesthesia, and the samples were centrifuged at 3 000 r/min for 10 min, and the serum was collected. The levels of immunoglobulin (Ig) A, IgG and IgM in each group of mice were detected by ELISA kits. The OD values at 450 nm were measured with an enzyme maker. The results were expressed as the concentration of cytokines per milliliter of mouse serum by the standard cytokines provided in the kit.

2.10 Statistical analysis

The data were analyzed using the statistical package SPSS 20.0 and plotted using Origin software version 8.6. All research results were expressed as the mean ± standard deviation. One-factor analysis of variance was used for multiple comparisons between groups, and the differences were considered statistically significant at $P < 0.05$.

3 Results and discussion

3.1 Effect on body weight and immune organ index in mice

In this study, mice were injected with CTX 80 mg/kg daily for 3 consecutive days to establish an immunosuppressed mouse model. After successful modeling, 6 groups of mice, except the blank group, were treated with different gavage for 30 consecutive days. The mice in each group grew and developed well during the experimental period (Figure 2A). It can be tentatively judged that this nutritional formula food is safe. The initial body weight values of the mice in all 7 groups at the beginning of the experiment were 17–18 g. The body weight of mice in the blank group increased and that of mice in the model group decreased significantly at the beginning of the second week. After gavage of the subjects, the body weight of mice in all experimental groups gradually increased with the increase of gavage time compared with mice in the model group, and after 4 weeks of gavage. The body weights of mice in all experimental groups were higher than those in the model group, and at the same time were close to those in the blank group, which indicated that the nutritional formula food could restore the body weight of immunocompromised mice to some extent.

Compared with the thymus index ((0.11 ± 0.02) mg/g) and spleen index ((0.21 ± 0.04) mg/g) of the model group, the thymus index ((0.20 ± 0.03) mg/g) and spleen index ((0.39 ± 0.02) mg/g) of the blank group were significantly higher ($P < 0.05$; Figures 2B and C), indicating that CTX was successful in modeling. Compared with the model group, the thymus index and spleen index increased in all groups after administration of gavage to the subjects, additionally, the thymus index ((0.18 ± 0.01) mg/g) and spleen index ((0.38 ± 0.03) mg/g) in the end product group were significantly higher than that of the model group ($P < 0.05$). This indicates that the nutritional formula food significantly promoted the development of spleen and thymus in mice, increased the immune response level of lymphocytes, and had immunomodulatory activity in immunocompromised mice.

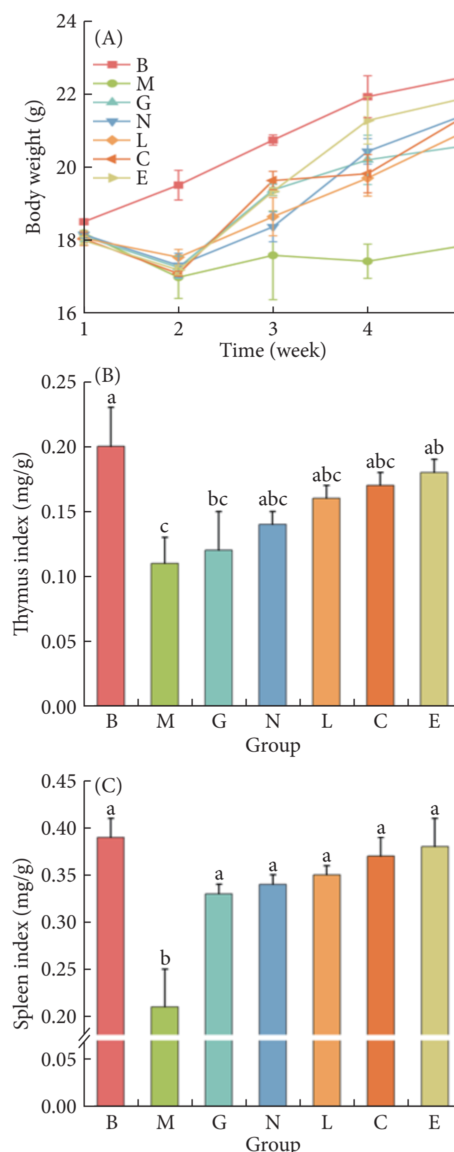

Figure 2 The changes of body weight and organ index in mice. (A) The body weight change in mice; (B) Thymus index; (C) Spleen index. B, blank group; M, model group; G, general food group; N, nutritional fortification group; L, lactoferrin group; C, compound mixture group; E, end product group. The results are expressed as the mean ± SD, n = 21. Different lowercase letters (a–c) indicate significant difference among groups ($P < 0.05$).

3.2 Effects on delayed allergic reactions in mice appearance

Mouse ear swelling response and mouse foot-plantar thickening response assay were used to evaluate the cellular immune function of mice. Compared with the blank group ((56.48 ± 9.97)%), mice in the model group ((20.28 ± 6.22)%) had significantly less ear swelling (P < 0.01; Figure 3A), indicating that CTX causes a significant decrease in the intensity of the DTH response and successful modeling; compared with the model group, the ear swelling rate increased in all groups after administration of gavage, with the end product group ((54.60 ± 12.79)%) caused a significant increase in ear swelling in the immunocompromised model mice (P < 0.01).

The difference in foot-plantar thickness before and after attack was lower in the model group mice ((0.31 ± 0.02) mm) compared with the blank group ((0.68 ± 0.03) mm; Figure 3B), and the difference was statistically significant (P < 0.000 1), indicating that CTX modeling was successful. Besides, the difference in foot-plantar thickness before and after attack were significantly higher in the other 5 experiment groups than in the model group (P < 0.05). In summary, it was shown that the nutritional formula food could increase the degree of DTH and enhance cellular immunity in immunocompromised mice.

3.3 Effect on serum hemolysin in mice

Serum hemolysin assay was used to evaluate the humoral immune function of mice. Compared with the blank group (31.67 ± 9.82), the serum hemolysin level of mice in the model group (7.00 ± 1.00) was significantly lower than that of the blank group (P < 0.001; Figure 3C), which proved that the mice were successfully modeled; compared with the model group, the serum hemolysin levels of mice in the 5 experiment groups were all higher than those in the model group, in addition, the lactoferrin group, the compound mixture group and the end product group were significantly different from the model group (P < 0.05). In conclusion, it was shown that the nutritional formula food had the ability to enhance the production of serum hemolysin in mice, induced B lymphocyte differentiation and proliferation, and enhanced the humoral immune function of mice.

3.4 Effects on monocyte-macrophage function in mice

The devouring index was significantly lower in the model group (4.47 ± 0.08) compared with the blank group (6.28 ± 0.15, P < 0.05; Table 1), indicating that CTX modeling was successful. Compared with the model group, the devouring index was higher in all groups given gavage, including the lactoferrin group (5.37 ± 0.36), compound mixture group (5.41 ± 0.18) and end product group (6.25 ± 0.49) with significant differences compared to the model group (P < 0.05).

Table 1 Effect of nutritional formula foods on the function of mouse monocyte-macrophage.

Group	Devouring index	Phagocytosis rate (%)
B	6.28 ± 0.15[a]	56.33 ± 7.02[a]
M	4.47 ± 0.08[d]	14.33 ± 3.21[d]
G	4.68 ± 0.15[cd]	21.00 ± 5.00[cd]
N	5.20 ± 0.09[cd]	29.33 ± 2.51[cd]
L	5.37 ± 0.36[c]	33.33 ± 5.03[bc]
C	5.41 ± 0.18[bc]	29.33 ± 2.51[cd]
E	6.25 ± 0.49[ab]	46.33 ± 7.02[ab]

Note: B, blank group; M, model group; G, general food group; N, nutritional fortification group; L, lactoferrin group; C, compound mixture group; E, end product group. All data are expressed as mean ± SD, n = 21. Different lowercase (a–d) letters in the same row indicate significant differences between groups (P < 0.05).

Compared with the blank group ((56.33 ± 7.02)%), the phagocytosis rate of the model group ((14.33 ± 3.21)%) was significantly lower (P < 0.05; Table 1), indicating the success of CTX modeling; compared with the model group, the phagocytosis rate of chicken erythrocytes by mouse macrophages given by gavage in each group was increased (P < 0.05). Compared with the model group, the phagocytosis rate of chicken erythrocytes were increased in all groups given by gavage, with significant differences in the lactoferrin group ((33.33 ± 5.03)%) and the end product group ((46.33 ± 7.02)%) compared with the model group (P < 0.05).

3.5 Effect on Ig content in mice

Compared with the blank group, IgA, IgG, and IgM in the model group were significantly lower (P < 0.05; Figures 4A–C), indicating that CTX was successful in modeling. Compared with the model group, the IgA, IgG and IgM in each group were increased after giving gavage, and the difference between the the end product group and the model group was significant (P < 0.05), the degree of effect on mouse IgM was as follows: the end product group > the compound mixture group > the lactoferrin group > the nutritional fortification group > the general food group, indicating that the nutritional formula food has a synergistic effect, which can increase the Ig content in the serum of immunocompromised mice, and can make the lymphocytes of the immune system of mice produce proteins, which are then converted into antibodies by the induction of antigens, with the function of improving immunity.

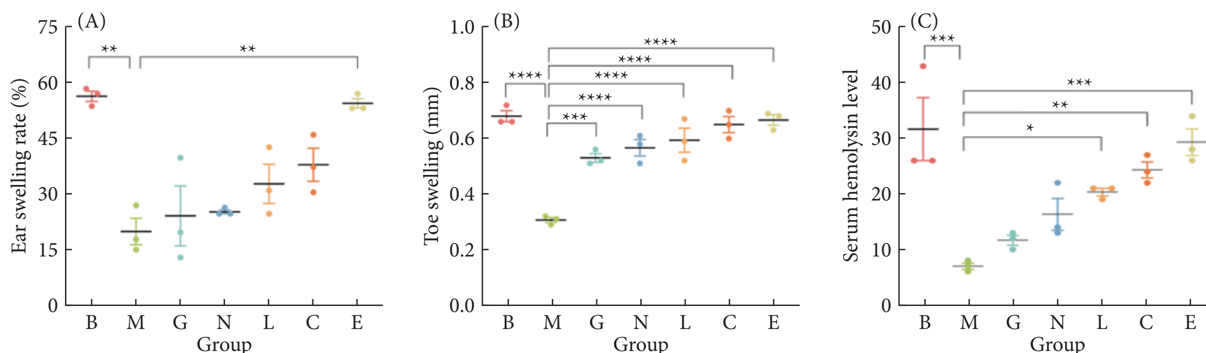

Figure 3 The comparison of specific immune indexes. (A) Ear swelling rate; (B) Toe swelling; (C) Changes of serum hemolysin levels. B, blank group; M, model group; G, general food group; N, nutritional fortification group; L, lactoferrin group; C, compound mixture group; E, end product group. * P < 0.05; ** P < 0.01; *** P < 0.001; **** P < 0.000 1 compare with model group.

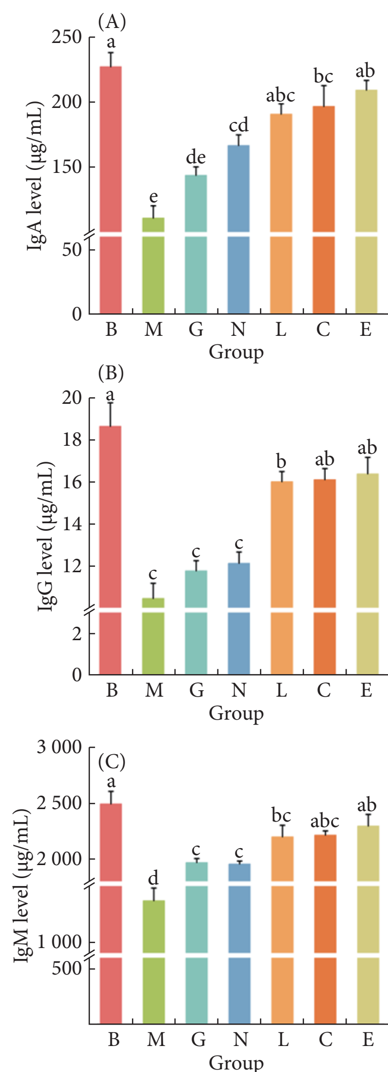

Figure 4 The changes in immunoglobulin (Ig) levels. (A) IgA, (B) IgG, and (C) IgM levels in mice. B, blank group; M, model group; G, general food group; N, nutritional fortification group; L, lactoferrin group; C, compound mixture group; E, end product group. The results are expressed as the mean ± SD, $n = 21$. Different lowercase letters (a–d) indicate significant difference among groups ($P < 0.05$).

4 Discussion

CTX can destroy the body's immune cells, initiate inflammatory and anti-inflammatory responses, and is often accompanied by gastrointestinal adverse effects, and is the most widely used model inhibitor of immunosuppression[31]. Thus, in this experiment, mice treated with CTX were used as an animal model of immunosuppression to demonstrate the immune enhancing effect of the nutritional formula food. The results showed that an immunosuppressive model in mice was successfully established. These experimental data are consistent with previous studies reported[32–33].

Body weight index is one of the most visual indicators of the health status of the body to a certain extent[34–35]. The results of this study revealed that the nutritional formula food was able to restore the body weight of immunocompromised mice to some extent. The thymus is the site of T-cell differentiation and maturation and has a regulatory role for both immune organs and immune cells. The spleen is the site of settlement of T and B cells, which produce various immune response responses[36]. The thymus and spleen are important immune organs and their organ indices reflect the immune function of the body. The value of the organ index is a visual indicator of non-specific immunity[37]. The spleen index and thymus index directly reflect the growth of the spleen and thymus, and indirectly reflect the level of immune response of lymphocytes[38]. The results of this study showed that the nutritional formula food could restore the thymus index and spleen index in immunocompromised mice and promote the development of immune organs.

To investigate the immunomodulatory mechanism of nutritional formula foods, this study evaluated specific and non-specific immunity. DTH in mice is a cellular immune response mediated by specifically sensitized T cells, and after sensitization, SRBC stimulates activation of T lymphocytes, which induces a local swelling response. Therefore, the degree of local tissue swelling due to the inflammatory response reflects the cellular immune function[39–40]. The results of this study showed that the nutritional formula food was able to increase the degree of DTH in immunocompromised mice, enhance the activity and proliferation of T-helper 1 cells, and enhance the antigen-specific DTH effect, and promote the proliferation of T lymphocytes in mice. SRBCs enter the body to stimulate the body to produce hemolysin, and the level of hemolysin can reflect the immune function of the body, and the level of hemolysin in the serum can reflect the proliferation and differentiation of the body's B cells and the ability to secrete antibodies into the body fluid[41–42]. The results of this study revealed that the nutritional formula food enhanced the ability of mice to produce serum hemolysin and improved humoral immune function in immunocompromised mice.

On the other hand, for non-specific immunity, the present study assessed the phagocytic capacity of monocyte-macrophage. The main purpose of phagocytosis is to avoid several diseases by cutting off foreign invaders as well as by eliminating dead, injured and malignant cells. Exogenously injected antigens, such as India ink, a preparation of colloidal carbon particles, are considered as foreign invasive particles and are removed by the reticuloendothelial system mainly through phagocytic processes in the liver and, to a lesser extent, in the spleen. Thus, the phagocytic index is closely related to phagocytosis[43–45]. Phagocytosis of exogenous stimuli by macrophages stimulates the innate immune response, and therefore macrophages are considered to be the primary target of most immunomodulatory agents[46]. The results of this study revealed that the nutritional formula food enhanced phagocytosis of monocytes-macrophages and had the ability to enhance non-specific immunity in immunocompromised mice.

In the body of an animal, a number of proteins protect the body from various infections and ensure a healthy immune system. The 2 main proteins in the blood are albumin and globulin. Albumin is often considered an important host defense against microbial pathogens, while globulins are the main building blocks for antibodies and other components of the immune system[47]. Therefore, in the event of immunodeficiency and malnutrition in the body, both albumin and globulin play an immune role in the body[48]. Igs are antigen recognition molecules derived from B cells, while antibodies are secreted forms of Igs, which consist of 2 pairs of identical heavy and light chains linked by disulfide bonds. There are 5 main types of antibodies: IgA, IgD, IgE, IgG and IgM[49]. The results of this study found that the nutritional formula food could increase the immunoglobulin level in immunocompromised mice, causing the immune system lymphocytes of immunocompromised mice to produce proteins, which were then converted into

antibodies by the induction of antigens, with the function of improving immunity, indicating that the nutritional formula food could repair and reverse the CTX-induced immunosuppression.

5 Conclusion

In this study, the nutritional formula food restored the body weight of immunocompromised mice to a certain extent and promoted the development of immune organs in immunocompromised mice; enhanced the DTH response and promoted lymphocyte proliferation in immunocompromised mice; enhanced the ability of immunocompromised mice to produce serum hemolysin and induced B lymphocyte differentiation and proliferation; enhanced the phagocytosis of monocytes-macrophages in immunocompromised mice; and increased the levels of IgA, IgG and IgM in the serum of immunocompromised mice. In summary, the nutritional formula food can work together on cellular immunity, humoral immunity, non-specific immunity and immunoglobulin to improve the immune resistance of mice, and acerola cherry, taurine and zinc gluconate showed synergistic effect, providing new ideas for the development of new functional foods with immune function.

Author contributions

Haifu Jia: Conceptualization; data curation; formal analysis; investigation; resources; validation; writing (original draft, review and editing). **Yuhong Wang:** Conceptualization; conceptualization; data curation; formal analysis; investigation; resources; validation; writing (original draft). **Yueming Zhao:** Visualization; supervision; validation. **Ziyu Hu:** Resources; validation; visualization. **Qingjing Liu:** Visualization; resources. **Yanmei Hou:** Supervision; validation. **Yujun Jiang:** Resources. **Qianyu Zhao:** Funding acquisition; Supervision. **Chaoxin Man:** Funding acquisition; supervision.

Conflict of interest

The authors declare no conflict of interest.

Ethical statement

This study was approved by the Laboratory Animal Welfare and Ethics Committee of Northeast Agricultural University. All animal experiments are approved by the Laboratory Animal Welfare and Ethics Committee of Northeast Agricultural University, License No. SCXK2020-0001 (Liaoning, China).

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