Immunomodulatory effects of chicken soups prepared with the native cage-free chickens and the commercial caged broilers

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ABSTRACT The objective of this study was to compare the immunomodulatory effects of the chicken soups prepared with the native free-range chickens and the commercial caged broilers in the immunosuppressive mice. The immunosuppressive mice model was established by the intra-peritoneal injection of 100 mg of cyclophosphamide (CTX) per kg body weight. The powders of Gushi Chicken Soup (GCS), Honglashan Chicken Soup (HCS), and Cobb Broiler Soup (CBS) were prepared by high-pressure stewing followed by spray drying. The chicken soups’ nutrient content and the effects of three chicken soups on the body weight, organ index, blood index, and serum cytokine and immunoglobulin contents in the immunosuppressive mice were determined. The three chicken soups promoted the recovery of immunosuppressive mice, but the expression mechanisms were different. The GCS was more effective than the HCS and CBS in restoring blood index, promoting cytokine secretion, and increasing immunoglobulin content (P < 0.05). The HCS stimulated the Th1-type immune response and promoted immunoglobulin secretion (P < 0.05), while the CBS increased the production of CD4+ and promoted the T-cell functions better than other soups (P < 0.05). Although soups from the native free-range chickens and the commercial caged broilers showed distinctly different mechanisms in promoting immunity, both could be used as potential immunomodulators.

Key words: chicken soup, native and broiler chickens, protein, immunity, blood indicators

INTRODUCTION

Compared with pork and beef, chicken meat is high in proteins with rich essential amino acids and low in fat and cholesterol contents (Fan et al., 2018). Numerous nutrients in chicken meat, including proteins, can be hydrolyzed and dissolved in water during the stewing process. Therefore, chicken soups are rich in collagen, peptides, carnosine, anserine, and taurine (Xiao et al., 2021). Besides the nutrients mentioned above, chicken soup is a good choice for special populations with dysphagia and neuromuscular diseases (Xing et al., 2022). In traditional Chinese food culture, chicken soup is used to prevent colds, relieve inflammation, and improve immunity. Saketkhoo et al. (1978) treated cold patients with chicken soup, hot water, or cold water and found that chicken soup relieved nasal congestion and runny nose better than other treatments. Renard et al. (2000) found that chicken soup inhibited the migration of neutrophils, thus showing anti-inflammatory activity. In recent years, increasing evidence suggested that functional foods from natural resources have high biological activities (Gao et al., 2021; Ratha et al., 2021), and chicken soup has been recommended to boost metabolism and fight against viruses (Renard et al., 2000).

China is rich in native chicken strains that are valuable natural resources. These indigenous native chickens are slow-growing varieties, while the commercial caged broilers are genetically improved and are fast-growing breeds. The increasing demand for chicken meat in China is mainly fulfilled by a few fast-growing commercial caged broiler strains, whereas the indigenous slow-growing Chinese native chicken's contribution is small. However, Chinese native chickens are highly preferred over commercial broilers by Chinese consumers because of their unique flavor and texture. Moreover, the traditional Chinese culture generally believes that the native Chinese chicken soups, represented by the free-range chicken soup, have stronger bioactive functions than the commercial broilers (Xiao et al., 2019). It has been reported that only slow-growing chicken varieties can
get the full benefits of a free-range rearing system because fast-growing varieties are characterized by a very low degree of adaptation (Branciari et al., 2009; Chen et al., 2013).

Numerous studies reported conflicting results about the effects of rearing systems on meat quality and nutrient content. Some studies reported that chicken meats produced from the free-range system have superior meat quality, immune-boosting effects, and nutrient content to the confinement systems (Jiang et al., 2011; Sun et al., 2013; Fu et al., 2015; Zheng et al., 2020). However, others report that the meats from the free-range system were inferior (Castellini et al., 2002; Mikulski et al., 2011) or not significantly different from those of the confinement systems (Chen et al., 2013). Xiao et al. (2021) reported that the content of water-soluble and low-molecular-weight compounds in broiler soup (Cobb broiler) was higher than those of the local Chinese chickens (Wudai and Cobb broiler chicken soup) and the local chicken soups in promoting immunity.

The Gushi and Honglashan chickens are 2 typical Chinese native chickens with a firm meat texture, predominantly used to prepare the chicken soup. Cobb chicken is the dominant commercial broiler chicken, mainly consumed and developed into processed meat products. The objective of this study was to determine the effect of the soups produced from the native free-range chickens and the caged commercial broiler chickens on the cellular and humoral immunities of the immunosuppressive mice. The results of this study would provide a scientific basis and rationale for consumers selecting the type of chickens in soup making.

## Materials and Methods

### Materials

Ten 2-year-old female free-range Gushi chickens were provided from Gushi County Sangao Co., Ltd (Xingyang, China), ten 2-year-old female free-range Honglashan chickens were provided from Qamdo Kanggong Poultry Technology Co., Ltd (Chengdu, China), and ten 6-week-old female caged Cobb broilers were provided from Chia Tai Food Co., Ltd (Wuhan, China). The cyclophosphamide (CTX) was obtained from Shanghai Yuanye Biotechnology Ltd (Shanghai, China). The enzyme-linked immunosorbent assay kits for the determinations of interleukin-2 (IL-2), IL-6, IL-10, tumor necrosis factor-α (TNF-α), interferon-γ (IFN-γ), immunoglobulin A (IgA), and IgG were purchased from Wuhan Enzyme-Free Biotechnology Co., Ltd. (Wuhan, China). A hematoxylin-eosin staining kit was purchased from Jiangsu Shitai Experimental Equipment Co., Ltd. (Haimen, China). Tris-Tricine-Sodium dodecyl sulfate-polyacrylamide gel electrophoresis kit was from Google Biology, Ltd (Wuhan, China).

### Preparation of Chicken Soup

After slaughtering and evisceration, 10 whole chickens were put in an electric pressure cooker (MY-YL50P701, Midea Co. Ltd., Foshan, China), and 3 volumes of water were added (w/v). The cooker's power was set at 65 kPa (114°C) for 20 min and then at 55 kPa (112°C) for 50 min. After cooking, the chicken soups were cooled to room temperature, put in a refrigerator at 4°C for 24 h, and removed the upper floating materials by filtering them through a cheesecloth. The chilled soups (Gushi chicken soup [GCS], Honglashan chicken soup [HCS], and Cobb broiler chicken soup [CBS]) were spray-dried using a spray dryer (SP-1500, Shanghai Shunyi Experimental Equipment Co., Ltd., China) with the inlet temperature of 170°C, outlet temperature 55°C and feeding rate at 13 mL/min, and stored at −80°C.

### Nutritional Composition and the Characteristics of Chicken Soup

#### Proximate Analysis

Proximate composition was determined according to the standard AOAC methods (2001). Protein content was determined by the Kjeldahl nitrogen determination method (conversion coefficient 6.25). Fat content was estimated by the Soxhlet extraction method with petroleum ether for 6 h. Ash content was determined by the high-temperature burning method in a muffle furnace. Total sugar was determined by the anthrone-sulfuric acid method.

#### Determination of Amino Acid Composition

The amino acids were determined using Sun et al. (2021) method with some modifications. The samples were hydrolyzed using 6 M hydrochloric acid in an oven at 110°C for 24 h, filtered through a 0.45 μm membrane, and dried in a vacuum desiccator. The dried sample was dissolved with 10 mL of sample loading buffer and analyzed using an automatic amino acid analyzer (A300, membra Pure GmbH, Beijing, China).

#### Determination of Fatty Acid Composition

The lipids in the samples were extracted using the method of Hara and Radin (1978). 2 mL of methylating reagent (5% sulfuric acid/methanol) was added to a test tube, capped tightly, and incubated in a water bath at 60°C for 40 min. After cooling to room temperature, 2 mL of hexane and 5 mL of water were added, mixed thoroughly, and left at room temperature overnight for phase separation. The analysis of fatty acid composition was performed with a gas chromatograph (8860, Agilent Technologies (Shanghai) Co., Ltd., Shanghai, China) equipped with an autosampler injector and flame ionization detector. A capillary column (DB-Fast FAME column, 30 m × 0.25 mm, 0.25 μm) was used. A splitless inlet was used to inject samples (1 μL) into the capillary column. Ramped oven temperature conditions (80°C for 0.5 min, increased to 165°C at 40°C/min held for 1 min, increased to 230°C at 4°C/min, and held for 7.5 min) were used. The temperatures of the inlet and detector were 250°C and 260°C, respectively. Helium was used as a carrier gas, and a constant column flow of 1.1 mL/min
was used. Flame ionization detector air, hydrogen, and make-up gas (helium) flows were 350 mL/min, 35 mL/min, and 43 mL/min, respectively. Fatty acids were identified by comparing them against the FAME standards. The compositions of fatty acids were reported as percentage composition of total lipids, and total peak area (pA*s) was used to calculate fatty acid composition.

**Animal Experiments**

**Establishment of Immunosuppressive Mice Model**

Seventy-five 6-week-old female BALB/c mice (animal certificate: SYXX 2020-0019) weighing 20.0 ± 2.0 g were purchased from the Animal Experiment Center of Huazhong Agricultural University (Wuhan, China). The Ethics committee approved the animal experiments. All animal procedures were performed following the Guidelines for the Care and Use of Laboratory Animals of Huazhong Agricultural University (Wuhan, China).

All animals were fed a regular chow diet and kept in an environment free of specific pathogens with a relative humidity of 60 to 70%, a temperature of 20 to 22°C, and a light/dark cycle of 12 h/12 h. The immunosuppressed model was established following Chen et al. (2020) with some modifications. After a week of acclimatization, the mice were randomly divided into 5 groups (15 mice/group): normal control (NC), model control (MC), GCS, HCS group, and CBS groups. To prepare the immunosuppressive model, the mice in the MC, GCS, HCS, and CBS groups were intraperitoneally injected with CTX (2 mL, 80 mg/kg BW) on days 1, 2, 3, 11, 19, and 27, while those in the NC group were treated with an equal volume of normal saline. During the experiment, the mice in the NC and MC groups were given 3 mL of normal saline, while those in the GCS, HCS and CBS groups were given 3 mL of the respective chicken soups by gavage at 800 mg/kg BW. The feeding trial was terminated on the 31st d, and the blood samples were collected on the 32nd d, 24 h after the last feeding. Before collecting the blood samples, the mice were anesthetized with ether. Blood samples were collected in glass tubes containing anticoagulants by orbit. The spleen and thymus were collected after sacrificing the mice using cervical dislocation. The detailed schedule is shown in Figure 1.

**Body Weight and Immune Organ Indices**

The weight of each mouse was recorded every three days. The spleen and thymus of the mice were separated and weighed after euthanizing. The immune organ index was calculated according to the following formula (Li et al., 2020):

\[
\text{Organ index (mg/g)} = \frac{\text{organ weight (mg)}}{\text{body weight (g)}}
\]

**Hematological Analysis**

The contents of white blood cells (WBC), lymph (Lymph), neutrophil (NEUT), red blood cells (RBC), platelets (PLT), and hemoglobin (HGB) were analyzed using an automatic hematology analyzer (BC-2800 VET, Mindray Co., LTD, China).

**Histological Observation**

Spleen and ileum samples were fixed in 4% paraformaldehyde for 24 h and then embedded in paraffin. The specimen was sliced (3-μm thick) using a digital slide scanner (Pannoramic, 3D HITECH, Hungary) and stained in the hematoxylin and eosin staining solution at room temperature for 10 min. The Case Viewer software (V2.3, Tangier Electronics Co., LTD, China) was used to collect the histological images for the histopathological assessment.

**Immunohistochemical Analysis**

The paraffin sections of the spleen were dewaxed by successively dipping in xylene, anhydrous ether, 85% alcohol, and 75% alcohol. Then, they were repaired in a hot water bath using sodium citrate buffer (pH 6.0, Wuhan Baiqian Biological Co., Ltd.) and EDTA-antigen retrieval solution (pH 9.0, Wuhan Baiqian Biological Co., Ltd., China). The spleen sections were incubated with diluted CD4 antibody (1:200, Abcam, Cambridge, UK) and CD8

![Diagram](attachment:figure1.png)

**Figure 1.** Flow chart of the experimental procedure. All mice were randomly classified into five groups (n=15) as follows: NC group (Normal control); MC group (Model control); GCS group (Gushi chicken soup); HCS group (Honglashan chicken soup); CBS group (Cobb broiler soup). The mice were sacrificed 24 h after the completion of administration.
antibody (1:200, Abcam, Cambridge, UK) at 4 °C overnight. Then biotin-labeled secondary antibodies were added to the slides for 50 min at room temperature, and then horseradish peroxidase (HRP)-labeled streptavidin was added. The slides were observed and recorded under a microscope after staining with a DAB kit (Jiangsu Shitai Experimental Equipment Co., Ltd, Nantong, China) and counterstaining with hematoxylin. 

**Cytokines and Immunoglobulins** A whole blood sample was refrigerated for 4 h and centrifuged at 1,409 g for 10 min. The upper serum was collected, and the levels of IgA, IgG, TNF-α, IFN-γ, IL-2, IL-6, and IL-10 were measured using the ELISA kits following the manufacturer’s instructions. The following formula was selected as the Th1/Th2 balance index (Neurath et al., 2002).

\[ \text{Th1/Th2} = \frac{(\text{TNF} - \alpha + \text{IFN} - \gamma)}{(\text{IL} - 6 + \text{IL} - 10)} \]

**Statistical Analysis**

All experimental data were expressed as the mean ± standard deviation. The effect of chicken soups on the measured variables was evaluated using an one-way analysis of variance (ANOVA). Duncan’s Multiple-Range test was used to determine the difference between treatments (P < 0.05). The differences between the CD4⁺ and CD8⁺ were analyzed using the Image-Pro Plus 6.0, and the mean integral optical density (IOD) value of the sections in each group was measured. All statistical analyses were conducted using the Origin 8.0 software (Origin Lab, MA).

**RESULTS AND DISCUSSION**

**Nutritional Composition of Chicken Soups**

The nutritional composition of the 3 chicken soups was shown in Table 1. The protein content of the GCS, HCS, and CBS was 76.21 ± 0.09%, 68.25 ± 0.18%, and 75.78 ± 1.05%, and the fat content was 1.68 ± 0.19%, 0.94 ± 0.26%, and 1.38 ± 0.03%, respectively. The fat content of the GCS was significantly higher than the HCS and CBS (P < 0.05). The HCS had significantly higher ash but lower fat content than the CBS (P < 0.05). In addition, the CBS had significantly higher total sugar contents than the HCS (P < 0.05). Lipids and proteins of meat could be altered by age (Xiao et al., 2019). An increase in age was usually accompanied by the accumulation of a higher content of intramuscular fat (Yu et al., 2020). Therefore, the difference in nutrient content in the three chicken soups may be related to age. Li et al. (2022) found that the chemical composition of chicken meat was different at different growth and development stages. Generally, stearic acid, L-histidine, and L-isoleucine showed an increasing trend with age, while L-aspartic acid decreased with age, consistent with our findings, as shown in Table S1 and Table S2.

**Table 1.** The proximate analysis of GCS, HCS, and CBS.

| Index (%) | GCS   | HCS   | CBS   |
|-----------|-------|-------|-------|
| Protein   | 76.21 ± 0.09ab | 68.25 ± 0.18bc | 75.78 ± 1.05bc |
| Fat       | 1.65 ± 0.19ab  | 0.94 ± 0.26bc  | 1.38 ± 0.03bc  |
| Ash       | 16.66 ± 0.3b   | 18.21 ± 0.26c  | 15.07 ± 0.22c  |
| Total sugar| 1.95 ± 0.11ab | 1.79 ± 0.13bc | 2.16 ± 0.14c  |

n = 3.

Abbreviations: CBS, Cobb broiler soup; GCS, Gushi chicken soup; HCS, Honglashan chicken soup.

Different letters in the same row represent significant differences (P < 0.05).

**Effects of Chicken Soups on the Body Weight and Organ Indices of Mice**

Body weight can reflect the immune status of the CTX-induced mice. As shown in Figure 2A, the body weight of the mice with the CTX treatment was significantly lower than the NC group. Among the immunosuppressive group of mice, the body weight of the GCS, HCS, and CBS groups was higher than the MC group. CTX is commonly used to treat tumors and various autoimmune diseases (Thone et al., 2008), but it also elicits side effects such as weight loss, inappetence, and thinning hair (Viaud et al., 2013). In this study, the body weight of mice in the MC group was the lowest, consistent with previous studies (Qi et al., 2018; Yun et al., 2021). The final average body weight of mice in the GCS, HCS, and CBS groups was higher than the MC group, indicating that three chicken soups ameliorated the CTX-induced damages and restored body weight.

The thymus and spleen indices are important indicators of immunology, which can reflect the nonspecific immunity of the body. As shown in Figure 2B, the thymus index of the MC group was significantly lower than the NC group (P < 0.05). Compared to the MC group, the thymus index was significantly higher in the GCS, HCS, and CBS groups (P < 0.05). Furthermore, the recovery effect of the GCS and CBS groups was better than the HCS group (P < 0.05). Figure 2C showed that the MC group had a significantly higher spleen index than the NC group (P < 0.05). The spleen index of the CTX-induced immunosuppressive mice was improved significantly by administering the GCS and CBS (P < 0.05). The thymus and spleen played necessary roles in immune response, and the decline of their organ indicators was a typical symptom of immunosuppression in mice (Wang et al., 2020a). The decreased thymus indices and the thymus atrophy in the MC group, as in the previous studies (Ding et al., 2019; Zhao et al., 2020a,b), indicated that the immunosuppressive model of mice was successfully established. The spleen index showed different changes according to the doses of CTX and physical conditions. Generally, the injection of CTX caused a decrease in the spleen index. However, some reports observed opposite results (Huang et al., 2020; Zhao et al., 2020a; Shen et al., 2021; Wang et al., 2021). Previous studies showed that inflammation due to high doses of CTX injection contributed to an enlargement of the spleen (Zhao et al., 2020a). Besides, extramedullary
hematopoiesis occurred in the spleen when the hematopoietic function of bone marrow was inhibited by CTX (Wang et al., 2009; Lee et al., 2021), increasing spleen index. Renard et al. (2000) found that chicken soup inhibited the migration of inflammatory cells, thus showing anti-inflammatory activity. Therefore, the GCS and CBS alleviated the inflammatory response, resulting in a significant decrease in the spleen index of the CTX-treated mice. The damage to the thymus and spleen caused by CTX was alleviated by administering the GCS, HSC, and CBS, and the improvement effect of the GCS and CBS was better than that of the HCS. Accumulated evidence suggested that Arg enhanced immunomodulatory effects in animals by improving the weight and functions of the thymus and spleen (Ochoa et al., 2001; Xu et al., 2018). The Arg content of the GCS and CBS was 3.8 g/100 g and 4.28 g/100 g, respectively, which was significantly higher than that of the CBS ($P < 0.05$, Table S1). Thus, the different effects of three chicken soups on the recovery of immune organs could be attributed to active compounds, such as amino acids, which possess immune regulatory activities.

**Effects of Chicken Soups on the Histomorphology of Mice**

The spleen is a center of lymphocyte differentiation, development, and maturation and the largest antibody-producing organ. Therefore, the spleen's functions are closely related to cellular immunity and humoral immunity. As shown in Figure 3A, the structure of the red pulp (red arrow) and white pulp (white arrow) of the spleen was normal, and lymphatic nodules were obvious in the NC group. In contrast, the spleen structure in the MC group was disordered, the red and white pulp boundaries were blurred, the number of lymphocytes decreased, and the number of macrophages increased. The changes observed in the CTX-treat mice were alleviated by the intraperitoneal injections of the 3 chicken soups. Especially, the spleen capsule was intact, and the germinal center converged in the GCS group. This result was consistent with Qi et al. (2018), who reported that the intake of bergenin reduced spleen damage in the immunosuppressive mice. The improvement effect of the 3 chicken soups on spleen tissues was similar to the spleen index.

The intestinal barrier function is a key component in preventing infection and inflammation. The effects of 3 chicken soups on the ileum of CTX-treated mice were shown in Figure 3B. The surface structure of the ileum in the NC group was clear, and the villi were intact and arranged neatly. However, the MC group showed obvious edema (black arrow) in the ileum and short villi with a loose structure and shallow crypt. These results indicated that CTX had a damaging effect on the ileum of the mice. The structural integrity of the ileal epithelium in mice was improved after administering the GCS,
Effects of Chicken Soups on the Blood Indices of Mice

Clinical blood parameters mainly reflect the pathological conditions through the indicators like WBC, RBC, HGB, and PLT (Xiong et al., 2017). WBC, composed of NEUT, Lymph, and monocytes, is a biomarker of immune activation and possible infection (Zhang et al., 2021). The RBC plays an important role in maintaining oxygenation and acid-base balance, recognizing antigens, clearing immune complexes, and promoting phagocytosis (Pretini et al., 2019). HGB is responsible for the transport of oxygen in the body, and the level of HGB can reflect physiological and neurodegenerative diseases (Russo et al., 2013). PLT not only controls thrombosis, promotes clotting, and repairs damaged wounds and blood vessels but also can prevent bacterial infections and resist inflammatory responses in mice (Hochstrasser, 2007).

The levels of WBC, Lymph, NEUT, RBC, HGB, and PLT in the MC group were significantly lower than those in the NC group (P < 0.05; Figures 4A–4F). The GCS and HCS groups had significantly higher WBC, Lymph, HGB, and PLT counts than the MC group (P < 0.05). The administration of CBS notably improved the Lymph and PLT counts (P < 0.05). Compared with the MC group, the number of PLT increased by 93.38%, 68.77%, and 72.18% in the GCS, HCS, and CBS groups, respectively, which were not significantly different from the NC group (P > 0.05), which agree with Li et al. (2020). This result indicated that three chicken soups promoted the recovery of bone marrow hematopoietic function and suggested that the decrease in spleen index was related to the inhibition of extramedullary hematopoiesis. Previous studies reported that unidentified factors in meat increased heme iron absorption and improved blood indicators (Miguel et al., 1968; Martínez-Torres and Layrisse, 1971; Hallberg et al., 2003). Thus, all 3 chicken soups could have restored the hematological markers by boosting iron absorption. However, the GCS and HCS groups showed significantly higher Lymph, RBC, and HGB counts than the CBS (P < 0.05). This result suggested that the GCS and HCS were better than the CBS in restoring immunosuppression by improving hematological parameters. Hunt (2005) reported that histidine residues combined with iron to form coordination compounds and promoted iron absorption. The histidine content of the GCS and HCS was 1.13 and 1.23 times higher than that of the CBS, respectively (P < 0.05, Table S1). Therefore, histidine could be related to the different recovery effects of the three chicken soups on the clinical blood parameters.

Effects of Chicken Soups on the Immunohistochemistry of Mice

The cellular immunity mediated by T lymphocytes is divided into CD4+ T and CD8+ T cells (Kim et al., 2016). The CD4+ is expressed mainly by the helper T cells, which secrete cytokines and aggregate immune cells at the response sites. The CD8+ T cells are mostly cytotoxic, specifically killing target cells (Kaech et al., 2002). The status of the immune system was influenced by the balance of the CD4+ and CD8+ T lymphocyte subsets. The imbalance of T lymphocyte subsets will lead to immune dysfunction (Wang et al., 2020a).

The immunohistochemical analyses of the spleen were used to show the effects of the 3 chicken soups on cellular immunity in the immunodeficient mice. As shown in 5A, the CD4+ expression level of splenocytes in the MC group was lower than that of the NC group, and the CD8+ expression presented an opposite trend. The data
in Figures 5B and 5C were obtained using IOD to quantify immunohistochemistry results. Compared with the NC group, the CTX-induced mice showed a significant decrease in the IOD values for CD4+ (P < 0.05) and a significant increase in the IOD values for CD8+ (P < 0.05). The treatment with the HCS and CBS enhanced the expression of CD4+ (P < 0.05). Compared with the MC group, decreases of CD8+ were observed in the GCS, HCS, and CBS groups (P < 0.05). This study demonstrated that the GCS, HCS, and CBS could regulate T cell differentiation to restore CTX-induced immune dysfunction. In addition, the CBS promoted CD4+ T lymphocyte differentiation more than the GCS (P < 0.05). Monk et al. (2013) reported an increase in the number of CD4+ T cells in the spleen of mice fed a fish oil-rich diet. Thus, we speculated that polyunsaturated fatty acids in the CBS played a significant role in promoting CD4+ T lymphocyte differentiation.

Figure 4. Effects of the chicken soups on the hematological parameters of the immunosuppressed mice. (A) WBC, (B) Lymph, (C) NEUT, (D) RBC, (E) HGB, (F) PLT. Data are shown as mean ± SD (n = 6). A–F Different letters within a Figure represent significant differences between groups (P < 0.05). Abbreviations: CBS, Cobb broiler soup; GCS, Gushi chicken soup; HCS, Honglashan chicken soup; MC, model control; NC, normal control.

Effects of Chicken Soups on the Serum Cytokines of Mice

Cytokines, synthesized and secreted by activated immune cells, are involved in immune response, inflammatory activation, hematopoietic function, and tissue repair by mediating information exchange and reciprocal regulation between immune cells. According to the lymphokines they produce, the CD4+ helper T cells are differentiated into Th1, Th2, Th17, and Treg (Zhu and Paul, 2010). Many studies showed that the differentiation of the Th1, Th2, Th17, and Treg cells plays an important role in regulating immune function (Lee, 2018; Chen et al., 2021). The Th1 cells are involved in the cellular immunity and the delayed-type hypersensitivity by producing the IL-2, TNF-α, and IFN-γ. The Th2 cells mediate humoral immunity and secrete the cytokines of IL-4, IL-6, and IL-10 (Neurath et al., 2002).
Th1/Th2 balance disruption increases the risk of many inflammatory and autoimmune diseases.

To further investigate the effects of 3 chicken soups on cytokines and their response mechanisms, the levels of IL-2, IL-6, IL-10, TNF-α, and IFN-γ were examined. The treatment with CTX significantly inhibited the secretion of IL-2, IL-6, IL-10, TNF-α, and IFN-γ compared with the NC group \((P < 0.05; \text{Figures } 6\text{A} - 6\text{E})\). After administration of the GCS, the levels of these cytokines in the immunosuppressive mice were significantly increased \((P < 0.05)\). Four cytokines (IL-2, IL-6, TNF-α, and IFN-γ) in the HCS and 3 cytokines (IL-2, TNF-α, and IFN-γ) in the CBS groups were significantly \((P < 0.05)\) higher than in the MC group. Following the significant variation in the cytokine, the ratio of Th1/Th2 cytokine was remarkably reduced by the CTX \((P < 0.05, \text{Figure } 6\text{F})\). The HCS group helped restore the Th1/Th2 balance. Wang et al. (2020b) also reported that Artesunate protected immunosuppressive mice by enhancing the release of inflammatory cytokines. This study suggested that the GCS had a better effect than the HCS and CBS in promoting cytokine secretion. Furthermore, the HCS effectively enhanced the secretion of Th1 cytokines (IFN-γ and TNF-α) and regulated the levels of Th2 cytokines (IL-6 and IL-10). Similar results also appeared with ovotransferrin, which enhanced the maturation of the intestinal dendritic cells and regulated the secretion of Th1 and Th2 cytokines (Zhu et al., 2018). Previous research showed that the immunomodulatory effects of active peptides were influenced by the hydrophobic and positively charged amino acids (Mercier et al., 2004; Jacquot et al., 2010; Wu et al., 2016). The difference in the content of hydrophobic and positively charged amino acids could be responsible for the differences in the immune activities of the three chicken soups (Table S1).

**Figure 5.** Effect of chicken soups on the expression \((\text{A})\) and IOD values \((\text{B})\) of the CD4⁺ and CD8⁺ in the spleen. The tissue slices were observed under a light microscope \((200 \times \text{magnification})\). Each group had 3 slices selected from 6 randomly selected visual fields on an immunohistochemical section, and 10 × 20 images were collected and processed using an image analysis software. Data are shown as mean ± SD \((n = 5)\).  \(^{\text{A–C}}\) Different letters within a Figure represent significant differences between different groups \((P < 0.05)\). Abbreviations: CBS, Cobb broiler soup; GCS, Gushi chicken soup; HCS, Honglashan chicken soup; MC, model control; NC, normal control.

**Effects of Chicken Soups on the Immunoglobulin of Mice**

Immunoglobulins are glycoproteins with antibody activities and are involved in humoral immunity. IgA
and IgG are important immunoglobulins of humoral immune responses. IgA is primarily synthesized by the plasma cells in mesenteric lymphoid tissues and is of great value for disease diagnosis. IgG is the most abundant and the only type of antibody that can cross the placenta, participate in complement activation and regulation, and neutralize toxins (Ohnuki et al., 2006). Numerous studies have shown that CTX inhibits the expression of Pax5 and Bcl6 genes, thereby suppressing the differentiation and proliferation of B lymphocytes and decreasing immunoglobulin levels (Huang et al., 2021; Zeng et al., 2021).

Figure 6. Effects of chicken soups on the serum cytokines in the immunosuppressed mice. (A) IL-2, (B) IL-6, (C) IL-10, (D) TNF-α, (E) IFN-γ, (F) Th1/Th2. Data are shown as mean ± SD (n = 6). A-F Different letters within a Figure represent significant differences between groups (P < 0.05). Abbreviations: CBS, Cobb broiler soup; GCS, Gushi chicken soup; HCS, Honglashan chicken soup; MC, model control; NC, normal control.

Figure 7 showed that the levels of IgA and IgG were significantly lower in the MC group compared to the NC group, 29.95% and 23.94%, respectively (P < 0.05). The IgA concentrations significantly increased in the immunosuppressive mice after treating with the GCS, HCS, and CBS (P < 0.05), and the IgA level in the GCS group reached normal levels. Similarly, the IgG levels in the GCS and HCS groups also increased to almost normal levels. The result indicated that the GCS, HCS, and CBS stimulated immunoglobulin production to a certain extent, thereby alleviating the immunosuppression. Growing studies found that methionine, threonine, and n-3 unsaturated fatty acids increased immunoglobulin levels and modulated humoral immunity (Bhargava et al., 1971; Zhu et al., 2019; Ma et al., 2022). The 3 chicken soups were rich in essential amino acids and polyunsaturated fatty acids, which might be related to their humoral immune-enhancing effects (Tables S1 and S2). In addition, it was found that the GCS and HCS promoted IgA and IgG secretion in the immunosuppressive mice, while the CBS increased only IgA content. Previous studies found that n-6 polyunsaturated fatty acid had adverse effects on immune functions (Calder et al., 2019; Dang et al., 2022). The n-6 polyunsaturated
fatty acid content in the GCS, HCS, and CBS was 2.53 mg/g, 2.25 mg/g, and 4.07 mg/g, respectively (P < 0.05, Table S2). The different immune effects of the 3 chicken soups could be related to the difference in n-6 polyunsaturated fatty acid content.

CONCLUSIONS

This study demonstrated that different chicken soup varieties alleviated immune suppression by improving peripheral blood levels, increasing the proportion of CD4+ T lymphocytes, and stimulating the secretion of immunoglobulin and cytokines. All 3 chicken soups showed immune-modulating activities, but their mechanisms were different: the GCS helped restore the peripheral blood indicators and promoted the secretion of IL-2, IL-6, IL-10, TNF-α, and IFN-γ, and increased the concentration of IgA and IgG. The HCS stimulated the Th1 immune response and promoted IgG secretion, stimulating the immune system. The CBS increased the ratio of CD4+ and promoted T cell functions. The GCS was superior to the HCS and CBS in promoting immune recovery in mice from the comprehensive analysis of cellular immunity and humoral immunity. These effects are probably attributed to the content and types of peptides in chicken soup. Further investigation on the signaling pathways and the active constituents related to different pharmacologic effects of chicken soups will be done in our following work. The relationship between the immune status of the 3 chicken strains and the content of active substances in their soups will also be investigated.

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Figure 7. Effects of chicken soups on the release of immunoglobulin in the immunosuppressed mice. (A) IgA. (B) IgG. Data are shown as mean ± SD (n = 6). A-BDifferent letters within a Figure represent significant differences between groups (P < 0.05). Abbreviations: CBS, Cobb broiler soup; GCS, Gushi chicken soup; HCS, Honglashan chicken soup; MC, model control; NC, normal control.

DISCLOSURES

We declare that the work described was original research that has not been published previously, and not under consideration for publication elsewhere. No conflict of interest exits in the submission of this manuscript, and manuscript is approved by all authors to be published.

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.psj.2022.102053.
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