

Qingru ZHANG, Guojun JIANG, Shupeng LI, Xiuhui ZHONG

Effect of a Chinese herbal formula *Astragalus* immunomodulator on immune function of chickens

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Abstract In order to investigate the immunomodulatory effects and the mechanism of a Chinese herbal medicine, *Zengmiansan* (*Astragalus* immunomodulator), on immune function of chickens, three hundred 1-day-old chickens were assigned randomly into 5 groups, i.e., the blank control group, the Chinese herbal medicine *Zengmiansan* (ZMS)-treated group, the cyclophosphamide group, the cyclophosphamide plus levamisole group, the cyclophosphamide plus ZMS group and the control group. All chickens were immunized with Lasota vaccine by nose-drip or eye-drop at the age of 6 days. Newcastle Disease (ND) antibody titers, growth indexes of the spleen, thymus and bursa of Fabricius, the concentrations of CD₄⁺ lymphocytes and CD₈⁺ lymphocytes in spleen, thymus and peripheral blood, and the apoptosis of splenocytes, thymocytes and bursa of Fabricius cells were observed at the ages of 14, 21, 28 and 35 days, respectively. Our results indicated that the NDV antibody titers of chickens in the Chinese herbal medicine ZMS-treated group at the ages of 14 and 21 days were significantly higher than that of the other groups ($P < 0.01$). The growth indexes of immune organs, the concentrations of CD₄⁺ lymphocytes and the ratio of CD₄⁺/CD₈⁺ T lymphocytes of chickens in the Chinese herbal medicine ZMS-treated group at the ages of 14, 21 and 28 d were significantly higher than those of the other groups ($P < 0.01$). The apoptotic splenocytes, thymocytes and bursa of Fabricius cells of chickens in the ZMS-treated group were significantly lower than the other groups ($P < 0.01$) at the ages of 14, 21 and 28 days.

Keywords chicken, Chinese herbal medicine, apoptosis, immune

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Qingru ZHANG, Guojun JIANG, Shupeng LI, Xiuhui ZHONG (✉)
College of Traditional Chinese Veterinary Medicine, Agricultural University of Hebei, Dingzhou 073000, China
E-mail: zhongxiuhui@hotmail.com

1 Introduction

Immunity failure in chickens occurs quite often because of pathogenic variations, irregular use of vaccines and lower immunologic responses of the birds (Xie and Song, 2005). In order to augment the immune effect of vaccines and to decrease their disease rate, it is important to enhance the immune ability of chickens by using immunopotentiators. Many medicines such as interferon, thymosin, levamisole, cytokine, etc., can enhance the immunity of chickens. However, the applications of immunopotentiators are found to have many shortcomings, such as higher cost, difficulties in getting the needed extracts, etc. A large number of studies indicate that some Chinese herbal medicines can modulate the immunity of chickens. Moreover, Chinese herbal medicines have a lot of positive effects including lower toxicities, fewer side effects, better natural products and less drug residues in animal products. Consequently, Chinese herbal medicines have become one of the main highlights in immunopotentiator studies of chickens (Zhang et al., 2003; Shu et al., 2005).

The herbal formula *Zengmiansan*, which was developed and prepared at the College of Traditional Chinese Veterinary Medicine, Agricultural University of Hebei, is a Chinese herbal medicine immunopotentiator. The present study was designed to investigate the effects and understand the mechanism of this Chinese herbal medicine formula on the immune functions of chickens.

2 Materials and methods

2.1 Preparation of Chinese herbal medicine

Zengmiansan was made up of *Radix astragali*, *Pimpinella thellungiana* Wolff, *Polygonum multiflorum* Thunb, *Dryopteris crassirhizoma* Nakai, *Artemisia annua* L., *Dichroa febrifuga* Lour and *Massa medicata* Fermentat. These herbs were mixed according to specific proportions

and decocted following the traditional procedures. The herbal soup was collected by filtering and simmering to the amount required, i.e., a one milliliter decoction containing one gram of dry herbs.

2.2 Animals and treatments

Three hundred 1-day-old Hyaline brown chickens were purchased from a chicken farm in Dingzhou city, China, and were divided randomly into 5 groups, with 60 chickens in each group: Group A (blank control group), Group B (Chinese herbal medicine *Zengmiansan* (ZMS) group), Group C (cyclophosphamide group (Cy, Jingsu Hengrui Medicine Co. Ltd., China)), Group D (Cy + levamisole group (LMS, Jingsu Huaiyin State-run Pharmaceutical Factory, China)), and Group E (ZMS + Cy group). All chickens were fed complete diets and were immunized with Lasota vaccine by nose-drip and eye-drop at the age of 6 days. From 7-days-old on, the chickens in Groups B and E were administered an oral gavage of herbal ZMS decoction at a dose of 2 mL per kilogram body weight daily for each chicken; the chickens in Group D received an oral gavage of LMS at a dose of 2 mL per kilogram body weight per day; the chickens in Groups A and C received an oral gavage of water at 2 mL per kilogram body weight daily. The trial was conducted for one consecutive week. The chickens in Groups C, D and E were given an intra-muscular injection (i.m.) of Cy at a dose of 80 mg·kg⁻¹ body weight at the ages of 10, 11, and 12 days. The chickens in Groups A and B were given an intra-muscular injection (i.m.) with ordinary saline at the same dose and at the ages of 10, 11, and 12 days for each chicken. All the chickens were fed in coops and were given free access to feeds and water, under the conditions of all day lighting and a proper temperature range adjusted with infrared ray light.

2.3 Growth indexes of spleen, thymus and bursa of Fabricius

The chickens in each group were weighted at the ages of 14, 21, 28 and 35 days, respectively. The chickens with body weights close to the mean value of the group were selected as experimental ones. There were 10 in each group. Two mL blood samples were taken by cardiopuncture before the birds were killed. The spleen, thymus and bursa of Fabricius were collected and cleansed with filter paper. The weight of each organ was weighted on an electron balance. The growth indexes of the spleen, thymus and the bursa of Fabricius were calculated with the following formula:

the growth index of spleen (or thymus or bursa of Fabricius) = the weight of spleen (or thymus or bursa of Fabricius) × 1000/body weight.

2.4 NDV antibody titer

The NDV antibody titers were measured with the β -trace method. The blood samples of five chickens from each group were picked randomly and serum samples were separated. The NDV antibody titers were examined on a 96-hole miniature reaction plate according to a red blood cell agglutination test and red blood cell agglutination inhibition test.

2.5 Concentration of CD₄⁺ and CD₈⁺ lymphocytes of spleen, thymus and bursa of Fabricius

Five chickens were picked randomly, and the spleen, thymus and bursa of Fabricius were collected. Single splenocytes, thymocytes and bursa of Fabricius cell suspensions were obtained using conventional practices. The cells were washed three times in cold phosphate-buffered saline (PBS) and cell concentration was adjusted to 1 × 10⁶ mL⁻¹, then Mouse anti-chicken phycoerythrin (PE)-conjugated CD₄⁺ or CD₈⁺ specific monoclonal antibodies (Mab, Southern Biotech Associates, Inc, USA) were added. The concentrations of CD₄⁺ and CD₈⁺ lymphocytes of the spleen, thymus and bursa of Fabricius were measured with FACScanto™ Flow cytometer (Becton & Dickinson Co., USA).

2.6 Apoptosis rate of splenocytes, thymocytes and bursa of Fabricius cells

Five chickens were picked randomly and the spleen, thymus and bursa of Fabricius were harvested. Single splenocytes, thymocytes and bursa of Fabricius cell suspensions were obtained using conventional methods. The apoptosis rates of the splenocytes, thymocytes and bursa of Fabricius cells were measured with FACScanto™ Flow cytometer.

2.7 Analysis of data

All data were analyzed with SPSS10.0 software. Statistical differences were conducted using the least significant difference method (LSD) ($P < 0.05$ was taken as significant).

3 Results

3.1 Effects of Chinese herbal medicine on growth indexes of spleen, thymus and bursa of Fabricius

The spleen, thymus and bursa of Fabricius growth indexes of the chickens in Group B at the age of 14, 21 and 28 days were significantly higher than those of the other groups ($P < 0.01$ or $P < 0.05$). The spleen and bursa of Fabricius

growth indexes of the chickens in Groups E and A on days 14, 21 and 28 were significantly higher than those in Group C ($P < 0.01$ or $P < 0.05$). The thymus growth indexes of the chickens in Group A on days 14 and 21 were significantly higher than those in Group C ($P < 0.05$). The thymus growth indexes of the chickens in Groups D and E on days 14 and 21 were significantly higher than those in Group C ($P < 0.05$). There was no significant difference in spleen and thymus growth indexes ($P > 0.05$) on day 35 among all the groups (Table 1).

3.2 Effects of Chinese herbal medicine on NDV antibody titers

The NDV antibody titers of the chickens in Group B on days 14, 21 and 28 were significantly higher than those of the other groups ($P < 0.01$). The NDV antibody titers of the chickens in Groups A, D and E on days 14 and 21 were significantly higher than those in Group C ($P < 0.01$). There was no significant difference in the NDV antibody titers ($P > 0.05$) on day 35 among all the groups (Table 2).

3.3 Effects of Chinese herbal medicine on the concentrations of CD₄⁺ lymphocytes and the ratio of CD₄⁺/CD₈⁺ lymphocytes

The concentrations of CD₄⁺ lymphocytes of the chickens' spleen and blood in Group B at the age of 14, 21, 28 and 35 days were significantly higher than those in the other groups ($P < 0.01$), and at 35-days old, the values were significantly higher than those in Group C ($P < 0.05$). The concentrations of CD₄⁺ lymphocytes of the chickens' thymus in Group B on the 14-, 21- and 28-day-old samples were significantly higher than those of the other groups ($P < 0.01$), with the values in Groups B and E at 21- and 28-days old significantly higher than those in Groups A, C and D. The concentrations of CD₈⁺ lymphocytes of the chickens' spleen and blood in Group B at the age of 14, 21 and 28 days were significantly lower than those of the other groups ($P < 0.05$ or $P < 0.01$), and the values at 35-days old were significantly lower compared with Group C ($P < 0.05$). However, the concentrations of CD₈⁺ lymphocytes

Table 1 Effects of Chinese herbal medicine on growth indexes of spleen, thymus and bursa of Fabricius

group	14-day-old	21-day-old	28-day-old	35-day-old
spleen				
A	1.16 ± 0.16 ^{Bb}	1.46 ± 0.18 ^{Bb}	1.74 ± 0.26 ^{Bb}	1.98 ± 0.32 ^a
B	1.42 ± 0.32 ^{Aa}	1.95 ± 0.17 ^{Aa}	2.31 ± 0.38 ^{Aa}	2.06 ± 0.28 ^a
C	0.74 ± 0.25 ^{Bc}	1.01 ± 0.28 ^{Bc}	1.49 ± 0.47 ^{BC}	1.91 ± 0.19 ^a
D	0.89 ± 0.17 ^{Bbc}	1.18 ± 0.38 ^{Bbc}	1.59 ± 0.36 ^{Bbc}	1.96 ± 0.27 ^a
E	1.01 ± 0.35 ^{Bb}	1.35 ± 0.08 ^{Bb}	1.66 ± 0.31 ^{Bb}	1.98 ± 0.14 ^a
thymus				
A	1.35 ± 0.16 ^{Bb}	3.00 ± 0.25 ^{Bb}	4.01 ± 0.72 ^{Bb}	5.22 ± 0.92 ^a
B	2.20 ± 0.12 ^{Aa}	4.06 ± 0.82 ^{Aa}	5.24 ± 1.29 ^{Aa}	5.39 ± 1.74 ^a
C	0.75 ± 0.19 ^{Bd}	2.21 ± 0.27 ^{Bd}	3.60 ± 0.56 ^{Bb}	4.91 ± 1.76 ^a
D	0.96 ± 0.26 ^{Bc}	2.66 ± 0.36 ^{Bc}	3.80 ± 1.61 ^{Bb}	5.01 ± 0.95 ^a
E	0.98 ± 0.28 ^{Bc}	2.72 ± 0.41 ^{Bc}	3.92 ± 0.89 ^{Bb}	5.12 ± 1.37 ^a
bursa of Fabricius				
A	2.57 ± 0.24 ^{Ab}	3.27 ± 0.41 ^{Ab}	4.01 ± 0.57 ^b	5.04 ± 0.69 ^a
B	2.97 ± 0.14 ^{Aa}	3.91 ± 0.29 ^{Aa}	4.97 ± 0.62 ^a	5.48 ± 0.91 ^a
C	0.90 ± 0.12 ^C	1.15 ± 0.17 ^C	2.71 ± 0.56 ^C	4.28 ± 0.29 ^a
D	0.98 ± 0.16 ^C	2.05 ± 0.22 ^{Bc}	3.02 ± 0.28 ^C	4.67 ± 0.63 ^a
E	1.73 ± 0.21 ^B	2.75 ± 0.34 ^{Bb}	3.56 ± 0.47 ^b	4.84 ± 0.75 ^a

Note: The difference between data with the same letter means no significance ($P > 0.05$). The difference between the data with different letters means significance ($P < 0.05$). The difference between the data with different capital letters means significance ($P < 0.01$). The same follows in the succeeding tables.

Table 2 Effects of Chinese herbal medicine on NDV antibody titers

group	14-day-old	21-day-old	28-day-old	35-day-old
A	4.70 ± 0.58 ^B	6.10 ± 0.35 ^B	5.20 ± 0.95 ^B	4.90 ± 0.60 ^a
B	5.70 ± 0.70 ^A	7.50 ± 0.55 ^A	6.30 ± 0.30 ^A	5.20 ± 0.55 ^a
C	4.20 ± 0.35 ^C	5.50 ± 0.70 ^C	5.10 ± 0.75 ^B	4.90 ± 0.65 ^a
D	4.70 ± 0.50 ^B	6.00 ± 0.75 ^B	5.20 ± 0.85 ^B	5.00 ± 0.90 ^a
E	4.90 ± 0.40 ^B	6.10 ± 0.90 ^B	5.40 ± 0.75 ^B	5.10 ± 0.70 ^a

of the chickens' thymus in Group B at the age of 14, 21 and 28 days were significantly higher than those in Groups A, C and D ($P < 0.01$). The ratio of CD_4^+/CD_8^+ lymphocytes of the chickens' spleen, thymus and blood in Group B at the age of 14, 21, 28 and 35 days were significantly higher than those in the other groups

($P < 0.01$), and the values on day 35 were significantly higher in comparison with Group C ($P < 0.05$). The ratio of CD_4^+/CD_8^+ T lymphocytes of the chickens' spleen, thymus and blood in Groups A and E on days 14, 21 and 28 were significantly higher than those of Group C ($P < 0.05$ or $P < 0.01$) (Tables 3, 4 and 5).

Table 3 Effects of Chinese herbal medicine on the concentrations of CD_4^+ lymphocytes

group	14-day-old	21-day-old	28-day-old	35-day-old
spleen				
A	15.41 ± 3.25 ^{Bb}	17.46 ± 5.27 ^B	16.78 ± 5.47 ^B	15.83 ± 4.28 ^b
B	20.68 ± 7.19 ^A	23.06 ± 7.34 ^A	20.29 ± 5.23 ^A	18.12 ± 5.45 ^a
C	10.24 ± 3.48 ^{Bc}	11.23 ± 3.25 ^C	13.83 ± 4.67 ^B	14.07 ± 4.57 ^b
D	10.52 ± 3.23 ^{Bc}	12.41 ± 3.67 ^C	14.22 ± 4.25 ^B	14.76 ± 5.19 ^b
E	13.25 ± 4.53 ^{Bb}	13.18 ± 4.19 ^C	15.09 ± 4.18 ^B	15.14 ± 2.96 ^b
thymus				
A	55.87 ± 6.25 ^B	60.71 ± 8.17 ^B	56.75 ± 6.82 ^B	53.56 ± 8.76 ^a
B	69.33 ± 7.89 ^A	81.29 ± 7.34 ^A	70.63 ± 7.18 ^A	59.42 ± 6.85 ^a
C	41.91 ± 5.48 ^C	58.05 ± 6.75 ^B	56.24 ± 4.67 ^B	55.12 ± 8.47 ^a
D	58.29 ± 9.17 ^B	63.78 ± 6.82 ^B	59.71 ± 6.39 ^B	55.87 ± 8.29 ^a
E	60.81 ± 3.46 ^B	76.89 ± 6.91 ^A	70.39 ± 5.79 ^A	52.70 ± 7.26 ^a
blood				
A	37.40 ± 5.16 ^B	40.63 ± 4.23 ^B	38.17 ± 4.72 ^B	39.49 ± 6.91 ^{ab}
B	45.68 ± 7.12 ^A	47.27 ± 6.37 ^A	41.89 ± 5.29 ^A	40.27 ± 5.78 ^a
C	30.24 ± 5.29 ^D	32.62 ± 5.27 ^D	33.53 ± 7.56 ^C	37.92 ± 4.71 ^b
D	32.52 ± 4.26 ^C	34.68 ± 3.36 ^C	36.83 ± 6.61 ^B	38.07 ± 4.46 ^{ab}
E	33.25 ± 4.28 ^C	35.74 ± 5.41 ^C	37.97 ± 7.59 ^B	38.89 ± 5.37 ^{ab}

Note: Figures in the table are the percentage of positive lymphocytes.

Table 4 Effects of Chinese herbal medicine on the concentrations of CD_8^+ lymphocytes

group	14-day-old	21-day-old	28-day-old	35-day-old
spleen				
A	48.85 ± 9.71 ^{Bb}	49.28 ± 11.63 ^B	47.11 ± 8.87 ^{Bc}	49.27 ± 8.29 ^a
B	42.09 ± 7.23 ^{Cc}	43.67 ± 8.28 ^C	43.91 ± 8.15 ^{Cd}	45.28 ± 9.43 ^b
C	55.88 ± 5.97 ^{Aa}	56.56 ± 10.08 ^A	53.51 ± 9.08 ^{Bb}	50.94 ± 9.47 ^a
D	53.38 ± 6.87 ^{Aa}	54.47 ± 9.37 ^A	62.28 ± 10.05 ^A	50.46 ± 7.17 ^a
E	50.51 ± 8.26 ^{Ab}	49.52 ± 6.82 ^B	49.01 ± 9.23 ^{Bc}	49.54 ± 7.17 ^a
thymus				
A	53.21 ± 7.18 ^A	50.22 ± 5.23 ^B	59.11 ± 6.97 ^B	59.46 ± 9.89 ^a
B	56.37 ± 4.53 ^A	61.46 ± 4.37 ^A	64.21 ± 8.15 ^B	62.31 ± 8.76 ^a
C	42.33 ± 5.28 ^B	51.86 ± 3.28 ^B	63.19 ± 11.08 ^B	64.71 ± 9.05 ^a
D	55.08 ± 4.76 ^A	52.80 ± 5.37 ^B	63.52 ± 10.75 ^B	64.48 ± 8.29 ^a
E	56.89 ± 3.79 ^A	63.02 ± 6.12 ^A	72.57 ± 9.16 ^A	59.91 ± 9.06 ^a
blood				
A	15.31 ± 3.16 ^{Bc}	16.08 ± 3.18 ^{Bc}	15.78 ± 2.26 ^{Ab}	16.28 ± 3.32 ^{ab}
B	14.25 ± 4.32 ^{Cc}	13.73 ± 2.17 ^C	14.13 ± 2.38 ^{Bc}	15.07 ± 4.28 ^b
C	19.69 ± 4.25 ^A	19.46 ± 5.28 ^A	17.37 ± 3.47 ^A	16.93 ± 4.19 ^a
D	17.67 ± 5.17 ^{Bb}	17.64 ± 4.38 ^{Bb}	16.63 ± 5.36 ^A	15.96 ± 5.27 ^{ab}
E	16.95 ± 3.33 ^{Bb}	17.08 ± 5.08 ^{Bb c}	16.32 ± 5.31 ^A	15.78 ± 4.14 ^{ab}

Note: Figures in the table are the percentage of positive lymphocytes.

Table 5 Effects of Chinese herbal medicine on the ratios of CD₄⁺/CD₈⁺ lymphocytes

group	14-day-old	21-day-old	28-day-old	35-day-old
spleen				
A	0.32 ± 0.09 ^B	0.35 ± 0.14 ^B	0.36 ± 0.11 ^B	0.32 ± 0.12 ^b
B	0.49 ± 0.07 ^A	0.53 ± 0.12 ^A	0.46 ± 0.10 ^A	0.40 ± 0.11 ^a
C	0.18 ± 0.08 ^C	0.20 ± 0.08 ^D	0.26 ± 0.09 ^{Cd}	0.28 ± 0.05 ^b
D	0.20 ± 0.11 ^C	0.23 ± 0.05 ^D	0.27 ± 0.08 ^{Cd}	0.29 ± 0.09 ^b
E	0.26 ± 0.08 ^B	0.27 ± 0.07 ^B	0.31 ± 0.08 ^{Cc}	0.31 ± 0.10 ^b
thymus				
A	1.05 ± 0.11 ^{Bca}	1.20 ± 0.13 ^B	0.96 ± 0.11 ^B	0.90 ± 0.1 ^{ab}
B	1.23 ± 0.09 ^A	1.32 ± 0.12 ^A	1.10 ± 0.13 ^A	0.95 ± 0.12 ^a
C	0.99 ± 0.11 ^{Cb}	1.12 ± 0.14 ^C	0.89 ± 0.10 ^{Cd}	0.85 ± 0.08 ^b
D	1.06 ± 0.13 ^{BCa}	1.21 ± 0.09 ^B	0.94 ± 0.12 ^{Cd}	0.87 ± 0.11 ^{ab}
E	1.07 ± 0.12 ^{BCa}	1.22 ± 0.10 ^B	0.97 ± 0.13 ^{Cc}	0.88 ± 0.09 ^{ab}
blood				
A	2.44 ± 0.87 ^B	2.53 ± 0.58 ^{Bb}	2.42 ± 0.68 ^{Bb}	2.43 ± 0.73 ^{ab}
B	3.21 ± 0.92 ^A	3.44 ± 1.21 ^A	2.96 ± 0.99 ^A	2.67 ± 0.79 ^a
C	1.54 ± 0.36 ^{Cd}	1.68 ± 0.59 ^{Cd}	1.93 ± 0.54 ^{Bc}	2.24 ± 0.95 ^b
D	1.84 ± 0.74 ^{Cc}	1.97 ± 0.62 ^{BCcd}	2.21 ± 0.76 ^{Bbc}	2.39 ± 0.57 ^{ab}
E	1.96 ± 0.38 ^{Cc}	2.09 ± 0.73 ^{Bc}	2.33 ± 0.83 ^{Bb}	2.46 ± 0.72 ^{ab}

3.4 Effects of Chinese herbal medicine on the apoptosis of splenocytes, thymocytes and bursa of Fabricius cells

The apoptosis of splenocytes, thymocytes and bursa of Fabricius cells of the chickens in Groups A and B on 14-, 21- and 28-day-old samples was significantly lower than

that in Groups C, D and E ($P < 0.01$). The apoptosis of splenocytes of the chickens in Group E on the 14- and 21-day-old samples were significantly lower than that of Groups C and D ($P < 0.05$). The apoptosis of splenocytes of the chickens in Group B on days 14 and 21 were significantly lower compared to Group A ($P < 0.05$) (Table 6).

Table 6 Effects of Chinese herbal medicine on the apoptosis of splenocytes, thymocytes and bursa of Fabricius cells

group	14-day-old	21-day-old	28-day-old	35-day-old
spleen				
A	0.43 ± 0.11 ^{Bb}	0.46 ± 0.05 ^{Bb}	0.45 ± 0.07 ^B	0.51 ± 0.09 ^b
B	0.29 ± 0.07 ^{Bc}	0.28 ± 0.08 ^{Bc}	0.42 ± 0.09 ^B	0.49 ± 0.11 ^b
C	3.30 ± 0.81 ^{Aa}	3.51 ± 0.73 ^{Aa}	1.55 ± 0.56 ^A	0.89 ± 0.21 ^a
D	2.93 ± 0.56 ^{Aa}	3.05 ± 1.06 ^{Aa}	1.16 ± 0.61 ^A	0.61 ± 0.16 ^b
E	2.05 ± 0.88 ^{Ab}	2.16 ± 0.91 ^{Ab}	0.89 ± 0.29 ^A	0.59 ± 0.07 ^b
thymus				
A	0.93 ± 0.16 ^B	1.38 ± 0.55 ^B	3.46 ± 1.72 ^B	3.42 ± 1.91 ^a
B	0.79 ± 0.12 ^B	1.12 ± 0.82 ^B	3.06 ± 1.29 ^B	3.13 ± 1.78 ^a
C	12.30 ± 4.19 ^{Aa}	13.51 ± 4.27 ^{Aa}	7.85 ± 3.56 ^A	4.49 ± 2.71 ^a
D	7.23 ± 2.26 ^{Ab}	9.15 ± 3.36 ^{Ab}	6.60 ± 3.61 ^A	3.78 ± 1.96 ^a
E	6.95 ± 3.28 ^{Ab}	8.96 ± 3.41 ^{Ab}	6.29 ± 2.89 ^A	3.65 ± 2.37 ^a
bursa of Fabricius				
A	0.53 ± 0.11 ^C	0.76 ± 0.05 ^C	0.85 ± 0.07 ^B	1.51 ± 0.09 ^a
B	0.39 ± 0.07 ^C	0.58 ± 0.08 ^C	0.82 ± 0.09 ^B	1.49 ± 0.11 ^a
C	7.30 ± 0.81 ^A	8.51 ± 0.73 ^A	4.25 ± 0.56 ^A	2.89 ± 0.21 ^a
D	5.93 ± 0.56 ^B	6.05 ± 1.06 ^B	3.16 ± 0.61 ^A	1.61 ± 0.16 ^a
E	4.05 ± 0.88 ^B	5.16 ± 0.91 ^B	2.89 ± 0.29 ^A	1.59 ± 0.07 ^a

Note: Figures in the table are the percentage of apoptotic lymphocytes.

4 Discussion

The spleen, thymus and bursa of Fabricius are important immune organs, so the immune functions of the body are closely related with the growth of these organs. The growth indexes of the spleen, thymus and the bursa of Fabricius are of great importance in evaluating the immunity of the body. Many studies have shown that *Radix Astragalus*, *Astragalus* polysaccharide (APS) and fleece flower polysaccharide can promote the growth of immune organs and enhance the growth indexes of immune organs (Li et al., 2004; Xu et al., 2005; Xu and Chen, 2006; An et al., 2007). Our results indicated that the Chinese herbal medicine ZMS could significantly enhance the growth indexes of the spleen, thymus and bursa of Fabricius of chickens. Furthermore, the Chinese herbal medicine may also resist the immunodepression caused by an immunity inhibitor, cyclophosphamide (Cy), and elevate the lowered immunity of chickens, an effect that is significantly better than the immunopotentiator, Levamisole (LMS), which is similar to the findings of other scholars (Wei et al., 2000).

In recent years, with the development of molecular biotechnology, apoptosis, or programmed cell death, has attracted much attention. The apoptosis of splenocytes, thymocytes and bursa of Fabricius cells can affect the development and immune function of the spleen, thymus and bursa of Fabricius. More and more studies have suggested that *Radix Astragalus*, *Astragalus* polysaccharide (APS) and *Polygonum multiflorum* can suppress the apoptotic process of splenocytes, thymocytes and bursa of Fabricius cells caused by dexamethasone (DXM) and Cy (Wei et al., 2000; Xia et al., 2001; Yang et al., 2001). *Astragalus* injection can affect the Fas/Fas-L mRNA content of ischemia/reperfusion injury (I/R). Young rats' spleen and nodi lymphatici mesenterici can decrease the apoptosis of macrophage (MΦ) and lymphocytes (Zhang et al., 2003). In our study, Chinese herbal medicine ZMS reduced the apoptosis of splenocytes, thymocytes and bursa of Fabricius cells caused by Cy, and enhanced the immunity of the birds. These results are similar with the findings of Wu et al. (2004).

Immunologic activity mainly depends on lymphocytes, including T lymphocytes and B lymphocytes. T lymphocytes consist of T helper cells/inducer T cells (Th/Ti) and suppressors T cells/ cytotoxic T cells (Ts/Tc), according to the function of the lymphocyte. But according to the cluster of differentiation (CD), T lymphocytes can be divided into CD₄⁺ and CD₈⁺ T cells. Among the T lymphocytes, Th/Ti belongs to CD₄⁺ T cell series, while Ts/Tc belongs to the CD₈⁺ T cells. Immunomodulation is mainly the function of CD₄⁺ T cells, which can promote the proliferation and differentiation of B lymphocyte, T lymphocyte and other immune cells. CD₄⁺ T cells can distinguish the cluster that contains MHC II and therefore are activated. The activated CD₄⁺ T cells can help B

lymphocytes differentiate and produce antibodies, induce the maturity of lymphocytes and promote the proliferation of cytotoxic T cells or killer T cells (Tc or Tk). However, the CD₈⁺ T cells are different from CD₄⁺ T cells, which have two functions serving as cytotoxic cells and as suppressors. On one hand, they can distinguish and clear the body cells which contain the MHC I cluster, and on the other hand, they can suppress the function of CD₄⁺ T cells; and, they suppress B lymphocyte producing antibodies and the proliferation and differentiation of other lymphocytes (Abbas et al., 1996). At a physiological state, it is important to keep a balance between the CD₄⁺ and CD₈⁺ T cells, and the immunologic reaction of the body is dependent on the promotion and suppression of CD₄⁺ and CD₈⁺ T cells on each other. The immune function will be disordered and thereafter cause diseases. If the amount and function of CD₄⁺ or CD₈⁺ T cells are abnormal, then the immune function of chickens will have close relations with the amount of CD₄⁺ and CD₈⁺ T cells and their proportion (Strong et al., 2001; Kamalavenkatesh et al., 2005). Many studies have shown that *Radix Astragalus* or *Astragalus* polysaccharide (APS) can increase the concentrations of CD₄⁺ lymphocytes and the CD₄⁺/CD₈⁺ ratio, and decrease the concentrations of CD₈⁺ lymphocytes (Zhang et al., 2003; Yuan et al., 2005; Cai et al., 2006). In our study, the Chinese herbal medicine ZMS significantly increased the concentrations of CD₄⁺ lymphocyte and CD₄⁺/CD₈⁺, and decreased the concentrations of CD₈⁺ lymphocyte of chickens. Furthermore, this Chinese herbal medicine preparation could resist the decrease in CD₄⁺ lymphocytes and CD₄⁺/CD₈⁺ caused by Cy. The results are in accord with the findings of many researchers (Chen et al., 2006; Zhu et al., 2006). This is probably the main reason why this Chinese herbal medicine can increase the NDV antibody titers of chickens.

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