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Effect of *Salmonella enteritidis* Infection on Immune Responses of Different Chicken Breeds

Li Zhengtian[1], Irfan Ahmed[2], Yuan Hemei[1], Dou Tengfei[3], Ge Changrong[3], Jia Junjing[3] and Xu Zhiqiang[3]*

[1]*School of Biological Resources and Food Engineering, Qujing Normal University, Qujing Yunnan, 655011, China*
[2]*Department of Animal Nutrition, Faculty of Veterinary and Animal Sciences, The Islamia University of Bahawalpur, Punjab, Pakistan*
[3]*College of Animal Science and Technology, Yunnan Agricultural University, Kunming, Yunnan, 650201, China*

Li Zhengtian and Irfan Ahmed contributed equally to this work.

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Growth performance, Daweishan miniature chicken, Broiler, *Salmonella enteritidis*, Immune organs weight, Immunoglobulins

ABSTRACT

Disease resistance traits of poultry are important economic traits in poultry production, and breeding for disease resistance is of great significance for poultry industry development. Keeping in view disease resistance traits, current study was performed to study immune response against *Salmonella* infection on 2-week-old local breed Daweishan mini chickens and Cobb broilers. The experimental chickens were raised to 2 weeks of age, 110 chickens from two chicken breeds with similar weights were randomly selected. The 50 chickens in the experimental group were treated with oral *Salmonella enteritidis* (1× 109 CFU/mL), 60 birds in the control group were fed the same dose of sterilized LB (Luria Broth) medium, and fasted and drank for 3 h before and after inoculation. Ten birds were selected from each group to slaughter on the day of 2 weeks of age (0 day post infection, dpi), 1 dpi, 2 dpi, 4 dpi, 6 dpi, and 8 dpi. The results showed that the live weight of the experimental group decreased compared with the control group after *Salmonella* infection. The spleen and thymus indexes of the Daweishan miniature chickens in the *Salmonella* infected group were significantly higher than those in the control group ($p<0.05$), while the spleen index and bursa of Fabricius index of the experimental group and the control group were higher than those of Cobb broilers at all time points after infection. The contents of IgG, IgA, IgM and complement C3 and C4 of Cobb broilers in *Salmonella* infected group and control group were higher ($p<0.05$) than those of Daweishan mini chickens. The results indicated that Daweishan miniature chickens and broilers had obvious genetic differences in immune performance to *Salmonella enteritidis*, and Daweishan miniature chickens had better resistance to *Salmonella enteritidis* than Cobb broilers.

INTRODUCTION

Salmonella enterica is a non-host-specific pathogen that not only causes diseases in livestock and poultry, but is also an important human food-borne pathogen. Poultry is one of the important ways for humans to become infected with *Salmonella enteritidis* through food (Bao *et al*., 2022; Tchoupou-Tchoupou *et al*., 2022). In past decades, with the increase in the intensification of the broiler breeding industry, *S. enteritidis* infections have become more and more serious, and most cases present systemic infections such as septicemia or fibrinous exudative inflammation, causing serious economic losses to the broiler industry. It also increases the risk of causing public health problems (Asheg *et al*., 2023; Rabie *et al*., 2023). Most human *Salmonella* infections are related to poultry-derived foods, such as eggs and meat (Burr *et al*., 2005). In the US, the annual cost of treating diseases caused by *Salmonella* and *Campylobacter* in poultry meat is approximately US 430 to 810 million, which is a huge loss (Bryan and Doyle, 1995) while in China 70%-80% of food poisoning is caused by *Salmonella* (Zhang *et al*., 2019).

Salmonella-infected chickens are an important source of foodborne Salmonellosis in humans (Song *et al*., 2024). Vaccination and precursory measures strategies require an understanding of the immune response, so it is

* Corresponding author: 791107187@qq.com
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important to understand mucosal immunity and response against bacterial infection. In current study, Yunnan local chickens breed daweishan mini chicken (a local breed that used for both meat and eggs) and the control Cobb broiler chicken as the research subjects. By comparing the development patterns of the immune organs of the two chicken breeds at different stages after *Salmonella* infection, blood immunoglobulin and complement levels. The purpose is to explore the differences in resistance of different chicken breeds to *Salmonella enterica*, laying foundation for further revealing the mechanism of action against *Salmonella enterica*, and provide a theoretical and experimental basis for research on disease-resistant breeding of local chickens in Yunnan.

MATERIALS AND METHODS

Experimental animals and strains

One day old daweishan mini and Cobb broilers chicks were selected for research from the chicken hatchery of Yunnan Agricultural University. The cotton swab method was used to detect *S. enteritidis*, and uninfected chickens were selected as experimental animals. The standard strain of *Salmonella enterica* (BNCC 103134) was purchased from Beina Chuanglian Biotechnology Co., Ltd.

Experimental design

The experimental chickens were raised using a grid-like mesh multi-layer cage management method. The diet was based on the NY/T 33-2004 (Chinese Chicken Feeding Standard, 2004) and the American NRC Broiler Chicken Feeding Standard (NRC, 1994). After adjustment, the diet for this experimental stage was designed. The two breeds of chickens were fed the same diet (powder). The diet composition is given in Table I.

When the experimental chickens were raised to 2 weeks of age, 110 chickens from two chicken breeds with similar weights were randomly selected, half male and half female. The 50 chickens in the experimental group were treated with oral *Salmonella enterica* (1×109 CFU/mL) (Barbosa *et al*., 2017), Sixty birds in the control group were fed the same dose of sterilized LB medium. Food and water were not provided for 3 h before and after inoculation. Ten chickens in each group were slaughtered on 2 weeks of age (0 day post infection, dpi), 1 dpi, 2 dpi, 4 dpi, 6 dpi, and 8 dpi.

Determination of live weight and immune organs indexes

The live weight of each chicken was weighed before slaughter, and the immune organs (thymus, spleen, bursa of Fabricius) of the test chickens were weighed immediately after slaughter, and the immune organs indexes was calculated by following formula.

Immune organ index = immune organ weight (g)/ live weight (g) × 100%

Table I. Diet composition and nutritional level.

Diet composition	**%**
Corn	64.70
Soybean meal	30.20
Soybean oil	1.10
Calcium hydrogen phosphate	1.50
Fine stone powder	0.70
Rubble powder	0.41
Methionine	0.08
Salt	0.35
Premix	1.00
Nutrient level	
Metabolizable energy/(MJ/kg)	12.13
Crude protein	24.03
Calcium	1.05
Total phosphorus	0.76
Available phosphorus	0.46
Lysine	1.22
Methionine	0.49
Methionine + Cystine	0.91

Note: The composition of composite premix is (converted to dietary content per kilogram): VA 15,000 U, VD_3 3,300 U, VE 62.5 mg, VK 3.6 mg, VB_1 3 mg, VB_2 9 mg, VB_6 6 mg, VB_{12} 0.03 mg, Niacin 60 mg, Calcium Pantothenate 18 mg, Folic Acid 1.5 mg, Biotin 0.36 mg, Choline Chloride 600 mg, Fe 80 mg, Cu 12 mg, Zn 75 mg, Mn 60 mg, I 0.35 mg, Se 0.15 mg, as well as antibacterial growth promoters, antioxidants, etc. Nutrient levels were calculated.

S. enteritidis *bacterial load detection*

After slaughtering the caecal contents of the experimental chickens were taken aseptically and a 10% (m/m) tissue homogenate was prepared. After diluting 10 times, 0.1 ml of the original solution and the dilutions of each concentration, was spread evenly on XLT-4 surface of the agar plate (Guangdong Huankai Microbiology Technology Co., Ltd.), was placed in a biochemical incubator at 37°C for 24 h. The 5 suspicious colonies were processed and the number of ST (lg CFU/g) after biochemical and serological identification were caculated.

Immunoglobulins and complement system

Blood was collected from daweishan mini chickens and Cobb broiler chickens at 0 dpi, 1 dpi, 2 dpi, 4 dpi, 6 dpi and 8 dpi, respectively, centrifuged at 3000 r/min at 4°C for 10 min, and the serum was separated to store at -20°C. Elisa kits produced by Jiangsu Baolai Biotechnology Co., Ltd. were used to measure complement C3, C4 and

immunoglobulin IgA, IgM, and IgY levels (Li *et al*., 2022). The detection instrument uses the Multiskan Sky-High full-wavelength microplate reader produced by Thermo Fisher Scientific Company of the United States.

Statistical analysis

Use Excel 2020 to initially organize the original data, and the results are expressed as mean ± standard error. SPSS 26.0 was used to conduct independent sample T test and one-way ANOVA and Duncan's method was used for multiple comparisons. Extremely significant differences were indicated by $p<0.01$ and significant difference was indicated by $p<0.05$.

RESULTS AND DISCUSSION

Growth performance

The study was aimed to evaluate the immune response differences between Yunnan local chicken breed and cobb broiler during early days of growth by immune organs and blood parameters. The changes in live weight of experimental chickens after *S. enteritidis* infection are shown in Table II. Data in table showed that due to the influence of *S. enteritidis* infection, the live weight of the daweishan mini chicken *Salmonella* infected group increased slowly at each time point after infection.

Table II. Effect of *S. enteritidis (S.E)* on live weight (g), spleen index (%), thymus index (%) and bursa index (%) of different chicken breeds.

Post-infection	Daweishan mini chicken		Cobb broiler	
	Control group	S.E infected group	Control group	S.E infected group
Live weight (g)				
0 Day	75.31±8.92^{b}	75.31±8.92	470.27±49.96^{b}	470.27±49.96
1st Day	80.34±12.31^{b}	79.67±11.20	496.75±51.67^{b}	482.30±50.35
2nd Day	87.25±14.02^{b}	82.25±11.34	530.25±65.04^{b}	495.50±53.25
4th Day	96.29±15.81^{ab}	87.02±12.03	565.57±67.85^{ab}	507.61±61.01
6th Day	103.61±15.13^{ab}	93.12±13.35	600.73±73.15$^{a\#}$	524.64±62.61
8th Day	112.57±16.75^{a*}	99.20±14.87	637.68±73.39$^{a\#}$	541.75±65.30
Spleen index (%)				
0 Day	0.21±0.04^{b}	0.21±0.04^{b}	0.09±0.03	0.09±0.03^{a}
1st Day	0.29±0.06^{ab}	0.50±0.01^{a*}	0.10±0.04	0.07±0.01^{ab}
2nd Day	0.25±0.05^{ab}	0.17±0.03^{b}	0.12±0.01	0.09±0.04^{a}
4th Day	0.32±0.04^{ab}	0.16±0.05^{b}	0.12±0.04$^{\#}$	0.05±0.02^{b}
6th Day	0.22±0.01^{b}	0.21±0.05^{b}	0.14±0.02$^{\#}$	0.03±0.02^{b}
8th Day	0.39±0.06^{a}	0.29±0.05^{b}	0.12±0.02$^{\#}$	0.05±0.02^{b}
Thymus index (%)				
0 Day	0.27±0.05^{b}	0.27±0.04^{b}	0.32±0.04	0.32±0.04^{b}
1st Day	0.31±0.04^{b}	0.52±0.06^{a*}	0.39±0.07	0.34±0.05^{b}
2nd Day	0.30±0.04^{b}	0.41±0.03^{ab}	0.44±0.03	0.39±0.05^{ab}
4th Day	0.40±0.07^{ab}	0.23±0.08^{b}	0.45±0.02	0.35±0.02^{b}
6th Day	0.38±0.05^{ab}	0.27±0.02^{b}	0.42±0.02	0.55±0.05$^{a\#}$
8th Day	0.52±0.05^{a*}	0.20±0.05^{b}	0.53±0.04$^{\#}$	0.34±0.07^{b}
Bursa index (%)				
0 Day	0.19±0.03^{b}	0.19±0.03^{b}	0.12±0.02	0.12±0.02^{b}
1st Day	0.26±0.03^{ab}	0.33±0.08^{a}	0.12±0.02	0.11±0.02^{b}
2nd Day	0.28±0.07^{ab}	0.27±0.04^{ab}	0.17±0.01	0.16±0.02^{ab}
4th Day	0.42±0.05^{a}	0.24±0.06^{ab}	0.18±0.03	0.12±0.03^{b}
6th Day	0.34±0.03^{ab}	0.19±0.02^{b}	0.17±0.05	0.19±0.01^{ab}
8th Day	0.39±0.02^{a}	0.39±0.04^{a}	0.17±0.02	0.30±0.03$^{a\#}$

Note: In the same row of the table above, comparison between daweishan mini chickens and Cobb broilers among different treatment groups, the significance of difference was indicated by */#, where */ # indicated significant difference ($P < 0.05$), and **/ ## indicated extremely significant difference ($P < 0.01$); In the same column, different lowercase letters of shoulder label indicated significant difference ($P < 0.05$), while no shoulder label or the same letter of shoulder label indicated no significant difference ($P > 0.05$), as shown in the following table.

After *Salmonella* infection, it multiplies in large quantities in the intestines, enters the bloodstream through the lymphatic system, and then multiplies in the spleen, cecum, bursa of Fabricius and other organs (Berndt *et al*., 2007). That causes systemic disease, with extensive inflammation and pathology in the liver, spleen and gastrointestinal tract leading to high mortality in chicks (Jones *et al*., 2007). The response of chickens to *Salmonell*a oral infection is characterized by a moderate cecal inflammatory response. This response is accompanied by increased leukocyte infiltration and expression of pro-inflammatory cytokines or immune effectors (such as inducible nitric oxide synthase) (Varmuzova *et al*., 2014), which may be related to the special physiological characteristics of the cecum (slow peristalsis, alkaline pH value, lack of digestive enzymes), making it the most vulnerable site for *Salmonella* infection. Feng (2016) found that when poultry is infected with *Salmonella*, it can cause disease in poultry and reduce the production performance of poultry by reducing feed intake. In addition, it can also reduce the fertilization rate, egg production rate and hatchability rate of adult chickens. Li (2014) artificially infected 12-days old white leghorn chickens with *S. E*. The live weight of the control group gradually increased, and the infection at 8th day after infection was significantly higher than that on days 0, 1, and 2 (p<0.05). The live weight of the *S.E* infected group at each time point after infection decreased compared to non-infected. Only on the 8th day after infection, the control group was significantly higher than the *S.E* infected group (p<0.05). In another report, FCR was increased in case of given *Salmonella* and heat stress to the chickens. It may be due to disruption of intestinal barriers when combination of *S. enteritidis* and heat stress were provided to the chickens which would allow migration of pathogenic bacteria through intestinal mucosa to spleen and an inflammatory infiltrate in the gut generated thus decreasing the growth performance parameters (Quinteiro-Filho *et al*., 2012). The reduction in growth performance has been allocated due to decrease feed intake and mucosa damage in the challenged birds. Compared with the control group, the live weight of the *S.E* infected group decreased to varying degrees, and was significantly lower than that of the control on the 6th and 8th days after infection group (p<0.05). As parallel to our study results. Feng (2016) results showed that after the chicks were infected with *S. E*, the body weight gain was reduced to varying degrees compared with the control group, and the thymus index and bursa index were reduced to varying degrees decreased, and the levels of serum mediators IFN-a, IL6 and TNF-a increased. In this experiment, 0 to 8 days after *S.E* infection, the live weight of the daweishan miniature chicken control group and *S.E* infected group increased by 49.4% and 31.8%, respectively, and the live weight of the cobb broiler control group and the *S.E* group increased by 35.6% and 31.8%, respectively. 15.2%. These findings are similar to results predicted by Shajia *et al*. (2023) who challenged commercial broiler chickens with *S. enteritidis* and found that after the challenge, the chickens generally showed depression, reduced feed intake, drooping wings, idleness, diarrhea, cold sensitivity, difficulty breathing and other clinical symptoms. Moreover, some researchers reported that bacteria addition has no significant effect on growth performance, including body weight (Kupryś-Caruk *et al*. 2018).

It can be seen that *S.E* infection hinders the growth and development of chickens to a certain extent and inhibits the increase in weight of chickens. This may be because *Salmonella* infection causes a decrease in feed intake of *S.E* infected chickens. The growth indicators of Cobb broilers were significantly lower than those of the control group at multiple time points, which indirectly revealed that Cobb broilers were more susceptible to foreign pathogenic microorganisms than daweishan mini chickens.

Immune organs indices

The changes in immune organs index including spleen, thymus and bursa of Fabricius index of test chickens after *S. enteritidis* infection are given in Table II. Visceral organs are the main place for digestion and absorption of various nutrients that poultry depends on for growth. Immune organs are functional organs of the body, and play a vital role in the growth and development of poultry (Mahmood *et al*., 2022). The weight of an immune organ is related to its function and the number of immune cells produced. The level of the organ index determines the degree of lymphocyte proliferation, and can roughly estimate the strength of the immune function (Saleem *et al*., 2022; Mohamed *et al*., 2023).

The spleen weight of *S.E* infected group was significantly higher than the control group on the first day after infection (p<0.05); while the *S.E* infected group of cobb broiler chickens had a lower immune organs index after *S. enteritidis* infection. The spleen index on the 4th, 6th and 8th days was significantly lower than that of the control group (p<0.05). Comparing different time points, the spleen index of the daweishan mini chicken and control group on the 8th day after *S. enteritidis* infection was significantly higher than that on the 0th and 6th day (p<0.05). The increase in weight index of spleen in broiler may be due to mitochondria swelling resulting in the organs edema or aggregation of the cytokines to enhance inflammation in infected groups of both chicken breeds (Dong *et al*., 2019).

Similar results were described by Wang *et al*. (2015) who inoculated Jining 100-day chickens with *S.E* and measured the immune organs index on the 1st, 3rd, 7th, 14th and 21st days after vaccination. The results showed that *S.E* infection can inhibit the increase in chicken weight non-significantly ($p>0.05$), while the impact on the spleen was mainly reflected on the 7th day after inoculation, and organ index decreased by 0.04% after *S.E* inoculation.

S.E infected group showed significantly ($p<0.05$) higher thymus index than control group on 1st and 2nd days of infection in daweishan mini chicken (Table II). While on 4th, 6th and 8th day, *S.E* infected group showed significantly ($p<0.05$) lower thymus index as compared to non- infected control group in daweishan mini chicken. The thymus index of the daweishan mini chicken control group on the 8th day after infection was significantly higher than on the 0th, 1st and 2nd days after infection ($p<0.05$). The changes in bursa of Fabricius index of experimental chickens after *S. enteritidis* infection are shown in Table II. The bursa index of the control group on day 4th and 8th after infection was significantly ($p<0.05$) higher than that on day 0th and 6th after *S.E* infection in daweishan mini chicken, and the difference between other time points was non-significant ($p>0.05$) at day 0. In contrast some studies mentioned that bursa weight decreases if overcrowding stress and infected with *S. enteritidis* in chickens but feed intake was increased (Gomes *et al*., 2014). The reduction of decreasing in the lymphoid organs in chickens related to the immunosuppression due to high stocking densities and *S. enteritidis*. The bursal index of the cobb broiler, control group was higher than the *S.E* infection group on the 1st, 2nd and 4th days after infection, but the difference was found non-significant ($p>0.05$).

On the 21st day after inoculation, the bursal index of the infected group increased significantly ($p<0.05$) compared with the control group. The thymus had little impact from bacteria before the 21st day after inoculation, but the organ index decreased significantly ($p<0.05$) on the 21st day after inoculation. Li (2014) artificially infected 12-days old white leghorn chickens with *S.E*. The results exhibited that spleen index increased after infection, and the infected group was higher than the control group, reaching a significant level at 24 h while thymus and bursa index were decreased. Our study results of daweishan mini chickens are generally consistent with the results of Wang *et al*. (2015), Li (2014) and Kamal *et al*. (2023). However, the changes in the bursa index of cobb broiler chickens are opposed to the above results, which may be due to different chickens with different immune potential against the *S.E* infection. It is related to the differentiation and development of B-lymphocytes in the bursa of Fabricius and the differences in the growth and apoptosis of immune cells (Sallam *et al*., 2023). The results of our study indirectly illustrate that although the mortality rate of *S.E* is low, causes the development of immune organs to be blocked, leading to the failure of later immunization and secondary diseases cause losses.

S. enteritidis *bacterial load*

The differences in *S. enteritidis* contents in the cecal contents of experimental chickens after *S.E* challenge are shown in Table III. After infected with *S. enteritidis*, the content of *S. enteritidis* in the cecum of Cobb broilers, gradually increased on days 1st, 2nd, 4th, and 6th after infection, and began to decrease on the 8th day significantly ($p<0.05$). However, smaller amounts of *S. enteritidis* were present in the cecal contents of daweishan mini chicken only on days 1st, 2nd, and 4th after infection. Okamura *et al*. (2012) described that after challenge of *S. enteritidis*, there was increase in no of bacteria but after salmonella flagella recombinant vaccination, there was significant decrease in bacterial load in liver and cecum. On days 1st, 2nd, and 4th after challenge, the content of *S. enteritidis* in the cecal contents of cobb broilers was significantly ($p<0.05$) higher than that of daweishan mini chickens. On days 6th and 8th after *S.E* challenge, the content of *S. enteritidis* in the cecum contents of cobb broilers was significantly higher than that of daweishan mini chickens ($p<0.05$). The *S. enteritidis* content was extremely significantly higher than that of daweishan miniature chickens ($p<0.01$) on days 6th and 8th. It can be seen that daweishan mini chickens are more resistant to *S. enteritidis* than cobb broiler.

Table III. Content of *S. enteritidis* in cecum contents after challenge (lg CFU/g)

	0 dpi	1 dpi	2 dpi	4 dpi	6 dpi	8 dpi
Cobb broiler	0	1.47 ± 0.12^{b}	1.89 ± 0.25^{b}	2.45 ± 0.39^{ab}	3.42 ± 0.87^{a}	3.16 ± 0.92^{a}
Daweishan mini	0	$0.23\pm0.04^{A*}$	$0.38\pm0.03^{A*}$	$0.27\pm0.02^{A*}$	0^{B**}	0^{B**}

Note: For the same column in the above table, the significance of the difference is indicated by *, where * means significant difference ($P<0.05$), ** means extremely significant difference ($P<0.01$); in the same row, different lowercase letters of shoulder label indicated significant difference ($P<0.05$), and different uppercase letters of shoulder label indicated extremely significant difference ($P<0.01$).

Immunoglobulins contents

Immunoglobulin refers to a globulin with antibody activity produced by B-cells that are converted into plasma cells after the immune system is stimulated by an antigen and can specifically bind to the corresponding antigen. It is the main substance that constitutes the body's humoral immunity. There are three confirmed immunoglobulins in poultry: IgY, IgM and IgA (Wu *et al*., 2021).

The changes in IgA, IgM and IgY content in the serum of experimental chickens after *S. enteritidis* infection are shown in Table IV. Among different breeds, the IgA content in the serum of the cobb broiler *S.E* infected group and the control group at each time point after infection was higher than the daweishan mini chicken. Within the same species, the experimental groups of daweishan mini chickens were higher than those in the control group to varying degrees, but the differences were non-significant (p>0.05); the overall IgA content of the experimental group showed an overall trend of increasing first and then decreasing, reaching the maximum on the 4th day after infection. The *S.E* group was significantly higher than the control group on days 2nd, 4th, and 6th after infection (p<0.05) in both breeds. It is believed that reduction in IgA level could impair the first defense mechanism used by host immune system against *S. enteritidis* invasion and also increasing corticosterone level due to heat stress (Quinteiro-Filho *et al*., 2012).

The *S.E* infected group showed higher IgM values than the control group at each time point after infection, but the difference was non-significant. The overall IgM content of the *S.E* group was higher than that of the control group. There was a trend of increase and then decrease, and was the highest on the 4th day after infection in cobb broiler. The 4th day after infection was significantly ($p<0.05$) higher than that on day 0 and day 1st *S.E* infected in Cobb broiler. Among all time points in the control group, the difference between groups is non-significant ($p>0.05$).

Table IV. Effect of *S. enteritidis* (*S.E*) infection on IgA content (ng/mL), IgM (mg/ml) and IgY (ng/ml) in different chicken breeds.

Post-infection	Daweishan mini chicken		Cobb broiler	
	Control group	S.E infected group	Control group	S.E infected group
IgA content (ng/mL)				
0 Day	203.75±18.52	203.75±18.52^{b}	228.11±20.82	228.11±20.82^{b}
1st Day	210.00±19.63	215.98±20.46^{ab}	236.60±21.93	262.58±22.46^{a}
2nd Day	208.00±20.46	223.00±21.41^{ab}	238.51±22.04	289.73±23.83$^{a\#}$
4th Day	217.00±21.79	232.05±26.95^{a}	239.54±23.06	288.27±24.19$^{a\#}$
6th Day	206.00±22.82	219.95±23.68^{ab}	246.45±23.17	285.74±25.97$^{a\#}$
8th Day	210.18±23.49	224.21±29.89^{ab}	259.95±24.07	272.56±24.31^{a}
IgM (mg/ml)				
0 Day	7.47±1.44	7.47±1.44	13.46±0.95	13.46±0.95^{b}
1st Day	6.04±0.96	7.11±1.43	14.93±0.97	15.94±0.22^{b}
2nd Day	5.82±0.72	5.24±1.05	16.47±0.73	17.18±0.15^{ab}
4th Day	6.14±1.23	6.29±1.04	17.36±0.82	18.38±0.78$^{a\#}$
6th Day	6.19±0.64	6.41±0.54	17.67±0.42	17.95±1.02^{ab}
8th Day	7.05±0.72	8.82±1.01	17.31±0.51	17.81±0.59^{ab}
IgY (ng/ml)				
0 Day	160.37±13.77	160.37±13.77^{b}	230.37±18.02^{b}	230.37±18.02^{Bb}
1st Day	186.36±12.28	191.11±11.75^{b}	237.09±17.12^{b}	242.55±16.78^{Bb}
2nd Day	187.75±17.19	219.46±11.23^{a}	247.28±19.41^{b}	280.04±13.69$^{a\#}$
4th Day	180.68±14.08	231.42±17.94^{a*}	230.96±15.78^{b}	274.57±17.18$^{a\#\#}$
6th Day	195.27±11.28	180.76±19.94^{ab}	237.79±15.72^{b}	324.54±18.33$^{Ab\#\#}$
8th Day	174.46±13.75	183.65±17.71^{ab}	277.43±16.53^{a}	337.47±13.96$^{Ab\#\#}$

Note: In the same row of the table above, comparison between daweishan mini chickens and cobb broilers among different treatment groups, the significance of difference was indicated by */#, where */# indicated significant difference (P < 0.05), and **/ ## indicated extremely significant difference (P < 0.01); In the same column, different lowercase letters of shoulder label indicated significant difference (P < 0.05), while no shoulder label or the same letter of shoulder label indicated no significant difference (P > 0.05), as shown in the following table.

Table V. Effect of *S. enteritidis (S.E.)* infection on C3 content (µg/L) and C4 content (µg/L) in different chicken breeds.

Post-infection	Daweishan mini chicken		Cobb broiler	
	Control group	S.E infected group	Control group	S.E infected group
C3 content (µg/L)				
0 Day	1146.26±50.38	1146.26±50.38^{b}	1472.45±68.45^{b}	1472.45±68.45^{b}
1st Day	1223.48±47.85	1275.22±67.85^{b}	1393.56±47.85^{b}	1448.96±77.82^{b}
2nd Day	1287.65±55.32	1366.34±69.42^{ab}	1466.44±55.32^{b}	1707.83±62.46$^{a\#}$
4th Day	1385.47±73.11	1589.34±75.82^{a*}	1574.49±43.11^{a}	1637.49±71.31^{a}
6th Day	1378.47±42.29	1386.72±49.27^{ab}	1502.63±62.29^{ab}	1560.57±52.28^{ab}
8th Day	1205.61±39.59	1245.97±68.51^{b}	1635.89±49.59^{a}	1672.68±48.76^{ab}
C4 content (µg/L)				
0 Day	907.07±63.33	907.07±63.33^{b}	1124.42±102.25	1124.42±102.25^{b}
1st Day	929.75±54.27	977.98±57.67^{b}	1164.82±116.05	1139.52±112.37^{b}
2nd Day	1007.37±65.41	1074.48±96.15^{ab}	1174.58±133.46	1257.64±98.87^{a}
4th Day	1070.85±54.11	1120.68±50.44^{a}	1121.38±137.29	1205.57±132.07^{ab}
6th Day	1011.67±57.47	1159.56±97.53^{a*}	1112.95±108.88	1211.41±130.71^{ab}
8th Day	1104.60±82.41	1293.64±95.04^{a*}	1161.98±156.27	1414.66±137.14$^{a\#}$

Note: In the same row of the table above, comparison between daweishan mini chickens and Cobb broilers among different treatment groups, the significance of difference was indicated by */#, where */ # indicated significant difference ($P < 0.05$), and **/ ## indicated extremely significant difference ($P < 0.01$); In the same column, different lowercase letters of shoulder label indicated significant difference ($P < 0.05$), while no shoulder label or the same letter of shoulder label indicated no significant difference ($P > 0.05$), as shown in the following table.

Among different breeds, the IgY content of the Cobb broiler test group and the control group at each time point after infection was higher than daweishan mini chickens. Within the same species, there are varying degrees of differences between the experimental group and the control group of daweishan mini chickens. On the 4th day after infection, the *Salmonella* infected group was significantly ($p<0.05$) higher than the control group. The 2nd and 4th days after infection were significantly higher than those on days 0 and 1 ($p<0.05$). The IgY content in the control group was lower than that on the 0th and 1st days ($P<0.05$). The decrease in the IgY levels might be related to the reduced immunological memory, thus increasing number of pathogen susceptibility. The IgY content of the *S.E* infected group in broiler on days 6th and 8th after infection was extremely significantly higher than that on days 0 and 1st ($p<0.01$), was significantly higher than that on days 2nd and 4th after infection ($p<0.05$).

Liu *et al*. (2015) studied the changes in some immune indicators and related genes of two newborn chicks with different genetic backgrounds after being inoculated with *Salmonella* and found that after challenge with *Salmonella*, the IgY, IL-6, and TNF-α of Beijing oil chickens. The serum medium level was significantly higher than that of white leghorn chicken. At 12 hpi, 24 hpi and 72 hpi, the difference in blood bacterial load among varieties was not significant. However, at 144 hpi, the bacterial load in blood of white leghorn chicken was significantly higher than that of Beijing you chicken. According to our study results, the *S.E* content in the cecal contents of cobb broiler chickens was significantly ($p<0.05$) higher than the daweishan mini chickens at 1 dpi, 2 dpi, and 4 dpi, and extremely significantly ($p<0.01$) higher than that of daweishan mini chickens at 6 dpi and 8 dpi. After two breeds of chickens were infected with *S.E* the contents of IgY, IgA, and IgM in the *S.E* infected group increased to varying degrees compared with the control group. Among them, the cobb broiler chicken reached a significant difference, while the large The IgA and IgM contents of the weishan mini chicken test group were not significantly different from those of the control group.

Complement system chicken breeds

The changes in C3 and C4 content in the serum of test chickens after *S. enteritidis* infection are shown in Table V. The complement system is widely involved in the body's antimicrobial defense response and immune response that mediate damaging responses in immune pathology. It is an effector system and effect amplification system with important biological significance in the body (Wang *et al*., 2024). Serum complement C3 and C4 are inherent components of the complement system that are important factors affecting the immune system of the animal body and play an important role in immune regulation, immune

pathology and immune defense (Keragala *et al*. 2018). Among different breeds, the C3 content of the cobb broiler infected group and the control group at each time point after infection was higher than that of the daweishan mini chicken. Within the same species, the infected group of daweishan miniature chickens was higher than the control group to varying degrees. On the 4th day after infection, the *S.E* group was significantly (p<0.05) higher than the control group. The C3 content of the experimental group increased first. The 4th day after infection was significantly (p<0.05) higher than that on days 0, 1st, and 8th after infection. While C4 content results showed that among different breeds. Within the same species, the *S.E* infected group of daweishan mini chickens increased to varying degrees compared with the control group. On the 6th and 8th days after infection, the *S.E* infected group was significantly higher than the control group (p<0.05). Regularly, the 4th, 6th, and 8th days after infection were significantly higher than those on days 0 and 1 (p<0.05), and the difference between the control group at each time point was non-significant; the cobb broiler chickens in the *S.E* infected group and the control group at each time point. The complement C3 and C4 contents of cobb broiler chickens were higher than those of daweishan mini chickens, and reached a significant level. It shows that the immunoglobulin content of daweishan mini chickens and cobb broilers has increased after being infected with *S. enteritidis*. It also indirectly shows that daweishan mini chickens can show strong disease resistance when infected by pathogenic microorganisms. Cobb broilers are more susceptible to infection by pathogenic microorganisms that is why; daweishan mini chickens have strong innate immunity.

CONCLUSION

S. enteritidis infection slowed down the growth rate of test chickens, increased the immune organ indexes such as spleen, thymus, and bursa of Fabricius, and increased the levels of IgA, IgM, IgY, and complement C3 and C4 in the blood. Many immune indicators were different within 1 dpi. It is suggested that 1 dpi may be a critical time point during early infection by *S. enteritidis*. In general, after infection with *S. enteritidis*, the immune response of cobb broilers was more extensive and more intense than that of daweishan mini chickens, confirming that daweishan mini chickens have stronger immunity to *S. enteritidis*.

DECLARATIONS

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IRB approval

The research was approved by Institutional Review Board.

Ethical statement

All procedures including rearing, sampling and culling of the birds were done according to the Yunnan Agricultural Animal Care and Use Committee.

Statement of conflict of interest

The authors have declared no conflict of interest.

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