The Journal of Agricultural Science

cambridge.org/ags

The effects of dietary soybean isoflavone on immunity in Chinese yellow-feathered broilers challenged with infectious bursal disease virus

S. Q. Jiang[1,*], Z. Y. Jiang[1,2], J. L. Chen[1,*], C. Zhu[2], P. Hong[1] and F. Chen[1]

[1]Guangdong Key Laboratory of Animal Breeding and Nutrition, Guangdong Public Laboratory of Animal Breeding and Nutrition, The Key Laboratory of Animal Nutrition and Feed Science in South China of Ministry of Agriculture, State Key Laboratory of Livestock and Poultry Breeding, Institute of Animal Science, Guangdong Academy of Agricultural Sciences, Guangzhou 510640, China and [2]Agro-biological Gene Research Center, Guangdong Academy of Agricultural Sciences, Guangzhou 510640, China

Animal Research Paper

*These authors contributed equally to this work.

Cite this article: Jiang S Q, Jiang Z Y, Chen J L, Zhu C, Hong P, Chen F (2018). The effects of dietary soybean isoflavone on immunity in Chinese yellow-feathered broilers challenged with infectious bursal disease virus. *The Journal of Agricultural Science* **156**, 832–838. https://doi.org/10.1017/S0021859618000758

Received: 20 July 2017
Revised: 5 December 2017
Accepted: 3 September 2018
First published online: 19 September 2018

Key words:
Chinese Yellow-feathered broilers; immunity; infectious bursal disease virus; lymphocyte proliferation; soybean isoflavone

Author for correspondence:
Z. Y. Jiang, E-mail: jiangz28@qq.com

Abstract

To investigate the effects of soybean isoflavone (SI) on immunity in infectious bursal disease virus (IBDV)-infected broilers, chicks were fed the same basal diet supplemented with 0 (non-infected control), 0 (infected control), 10, 20 or 40 mg/kg SI for 44 days. At 21 days old, chickens were inoculated with bursal infectious dose causing 50% morbidity of the IBDV BC 6/85 strain by the eye-drop and nasal route (except for non-infected controls). Results showed that, over 1–23 days post-infection (dpi), there was a significant interaction between SI supplementation level and time: high-level SI supplementation increased peripheral T lymphocyte proliferation, percentages of $CD3^+$, $CD4^+$ and $CD8^+$ T lymphocytes, $CD4^+$ to $CD8^+$ ratio, serum concentrations of IgA, IgM and IgG, and IBDV antibody titres. Except for serum IgA and IgM, these variables increased over time with far higher values at 23 dpi than earlier. Compared with non-infected controls, IBDV inoculation decreased peripheral T lymphocyte proliferation at 3 dpi, percentages of $CD3^+$, $CD4^+$ and $CD8^+$ T lymphocytes, and serum IgG, IgM concentration at 23 dpi, and increased IBDV antibody titres at 7, 15 and 23 dpi. Supplemental SI quadratically increased peripheral T lymphocyte proliferation, $CD4^+$ to $CD8^+$ ratio and serum IgA concentration at 3 dpi, percentages of $CD3^+$, $CD4^+$ and $CD8^+$ T lymphocytes at 3 and 23 dpi, and serum IgM concentration and IBDV antibody titres at 23 dpi. These results indicate that dietary SI improved cellular and humoral immunity of IBDV-infected birds and may enhance resistance of Yellow-feathered broilers to infectious diseases.

Introduction

In modern poultry husbandry, birds are raised intensively to maximize their productivity output, which makes them more susceptible to infectious diseases (Biggs, 1985). Infectious bursal disease (IBD) is a highly contagious, immunosuppressive viral disease of young chickens, caused by infectious bursal disease virus (IBDV) (Kibenge *et al*., 1988; Saif, 1991). Because of the resulting heavy mortality and economically detrimental loss of production, often as a result of the chickens' increased susceptibility to secondary infections and sub-optimal response to vaccinations, IBD has become a major problem in the poultry industry (Balamurugan and Kataria, 2006).

The most popular strategy for IBDV control is a passive immunization via an appropriate IBDV vaccine in order to achieve better antibody response (Fussell, 1998). However, vaccination has its own problems, which may lead to potential immunosuppression (Müller *et al*., 2003) or failure to respond to very virulent IBDV (van den Berg, 2000). Recently, several studies have revealed that the humoral immune response alone was not adequate in inducing protection against IBDV in chickens. Cell-mediated immune response, particularly T-cell involvement, plays the principal role in defence against IBDV and promotes viral clearance, even in the absence of antibody (Kim *et al*., 2000; Rautenschlein, 2002*a*, 2002*b*; Yeh *et al*., 2002). Moreover, cell-mediated immune response, especially involving T helper cells ($CD4^+$) and related cytokines, is actually involved in antibody production by activating and differentiating B lymphocytes into antibody-producing plasma cells (Raff, 1973; Tanimura and Sharma, 1997; Vervelde and Davison, 1997); thus, humoral immunity could improve as well.

No matter what type of immunity is responsible for defence against IBDV, the main purpose of improving it is to get better resistance to infection. As for animal production, safety and efficiency especially need to be taken into account. Among these, nutritional modulation of disease resistance is considered to be both a practical and efficient strategy in modern poultry production (Butcher and Miles, 2002; Abdukalykova *et al*., 2008). Improving the

CAMBRIDGE UNIVERSITY PRESS

humoral and cellular immune response through nutritional modulation may, therefore, be a worthy attempt to guard against IBDV infection.

Soybean isoflavone (SI) has been reported to produce immunomodulatory effects. Curran *et al.* (2004) reported that dietary soy isoflavones play a role in the modulation of cell-mediated immunity and type I inflammatory responses in response to bacterial infection. Daidzein enhances immune function in late-lactation cows under heat stress (Liu *et al.*, 2014). In addition, it has previously been found that the proportion of peripheral $CD4^+$ T lymphocytes in pigs increased when SI was supplemented appropriately (Cheng *et al.*, 2005).

According to the observations above, it was speculated that dietary SI may modulate the T-cell-mediated immune response, which could in turn fight against the IBDV infection and promote viral clearance. Additionally, humoral immunity of IBDV-infected birds could be enhanced when T-cell-mediated immune response are elevated. Therefore, the objectives of the present study were (a) to examine whether exposure to SI could improve T-cell-mediated and humoral immune response when birds are under IBDV infection; and (b) to elucidate whether dietary SI supplementation could benefit the development of T-cell-mediated and humoral adaptive immunity in IBDV-infected yellow-feathered broilers.

Materials and methods

Birds, virus and diets

The experimental protocol was reviewed and approved by the Institute of Animal Science, Guangdong Academy of Agricultural Sciences, China.

On the day of hatching, 200 male broiler chickens (*Lingnan* yellow-feathered broiler, a quality meat-type chicken, reaching market size at 63 days of age) were obtained from a commercial hatchery (Guangdong Wiz Agricultural Science and Technology Co., Guangzhou, P. R. China) and raised at a local facility under standard conditions, including routine vaccination against inactivated avian influenza virus (AIV) at day 10, with free access to water and feed. The strain of IBDV, BC 6/85, is a classic strain of virulent IBDV used as a standard challenge strain in China and was purchased from the China Institute of Veterinary Drug Control (Haidian District, Beijing). It had a titre of $10^5\times$ bursal infectious dose causing 100% morbidity per ml. Nutrient levels of the diets were based on the National Research Council (1994) recommended nutrient requirements of broiler chickens (Table 1).

Table 1. Ingredient and composition of the basal diets for Chinese yellow-feathered broilers at 1–21 and 22–44 days of age (as fed-basis)

Ingredients	Composition (g/kg)	
	1–21 days of age	22–44 days of age
Maize	584.0	608.0
Wheat bran	43.0	38.0
Fish meal	22.0	10.0
Soybean meal	264.0	220.0
Maize gluten meal	20.0	30.0
Soybean oil	13.0	29.0
Lysine	0.0	1.0
Methionine	1.0	0.8
Limestone	12.7	12.0
Dicalcium phosphate	15.1	14.5
Salt	2.5	2.5
Zeolite	12.7	24.2
Vitamin-mineral premix[a]	10.0	10.0
Total	1000.0	1000.0
Chemical composition[b]		
Metabolizable energy (MJ/kg)	12.13	12.55
Crude protein (g/kg)	199.3	187.4
Lysine (g/kg)	10.5	9.8
Methionine (g/kg)	4.6	4.0
Calcium (g/kg)	10.0	9.0
Non-phytate P (g/kg)	4.5	4.0

[a]Supplied per kilogram of diet: vitamin A, 14 700 IU; vitamin D_3, 3300 IU; vitamin E, 20 IU; vitamin K_3, 3.9 mg; vitamin B_1, 3 mg; vitamin B_2, 9.6 mg; vitamin B_6, 6 mg; vitamin B_{12}, 0.03 mg; nicotinic acid, 60 mg; pantothenic acid, 18 mg; folic acid, 1.5 mg; biotin, 0.36 mg; $FeSO_4{\cdot}7H_2O$, 80 mg; $CuSO_4{\cdot}5H_2O$, 8 mg; MnO, 80 mg; KI, 0.38 mg; $NaSeO_3$, 0.44 mg. The carrier was zeolite.
[b]Values were calculated from data provided by Feed Database in China (2016) except that crude protein was analysed.

Experimental design

On the first day of the experiment, 200 1-day-old yellow-feathered male broiler chickens were weighed and allotted randomly to five treatment groups, each consisting of four replicates of ten birds. Broilers were placed in floor pens (1×2 m). All birds were offered the same basal diet, supplemented with 0 (non-infected control), 0 (infected control), 10, 20 or 40 mg/kg SI (a synthetic SI, containing 98.5% glycitein, supplied by Newland Feed Science and Technology Co., Guangdong, China). These treatments are described as non-infected control, IBDV (0 SI), IBDV (10 SI), IBDV (20 SI) and IBDV (40 SI), respectively. At 21 days of age, chickens were inoculated with the bursal infectious dose causing 50% morbidity of the IBDV BC 6/85 strain by the eye-drop and nasal route, except for the non-infected control group. A pre-experiment had been conducted to titrate the optimal dose of the inoculation. By administering the chosen dose, visible pathological changes were visible on the bursa of Fabricius at 5 days post-infection (dpi) without evident morbidity or mortality. During the experiment, which lasted 44 days, infected and non-infected groups of chickens were housed in equivalent but separate places.

Sample collection

Eight broilers per treatment group (two birds per replicate) were selected randomly and bled into heparinized tubes (7 ml per bird) from a wing vein at 3, 7 and 23 dpi, for the examination of peripheral T lymphocyte proliferation and proportion of T lymphocyte sub-populations. At the same time, another 2 ml blood sample per bird was collected and allowed to clot in order to prepare serum for quantifying specific antibodies to the IBDV.

https://doi.org/10.1017/S0021859618000758 Published online by Cambridge University Press

Table 2. Effect of soybean isoflavone (SI) on peripheral T lymphocyte proliferation of Chinese yellow-feathered broilers challenged with infectious bursal disease virus (IBDV)[a]

T lymphocyte proliferation at three sampling days (proportion) (dpi)	Non-infected controls	IBDV infected and (dietary supplementation in mg/kg SI)				*P* values for SI level	
		IBDV (0 SI)	IBDV (10 SI)	IBDV (20 SI)	IBDV (40 SI)	Linear	Quadratic
3	0.36	0.21*	0.39	0.35	0.31	NS	0.004
7	0.37	0.30	0.30	0.35	0.36	NS	NS
23	0.59	0.48	0.51	0.50	0.54	NS	NS

[a]Peripheral T lymphocyte proliferation was evaluated at 3, 7 and 23 dpi. *P* values from ANOVA: SI supplementation < 0.001; dpi < 0.001; SI supplementation × dpi interaction = 0.001. Data (proportion of cells proliferating) are means, the SEM was 0.033 for $n = 4$, based on ANOVA error mean square.
*IBDV compared with non-infected controls by *t* test ($P < 0.05$).

T lymphocyte proliferation assay

Mitogenic responses of peripheral blood leukocytes, prepared as described previously by Lee *et al.* (1978), were quantified using an MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) assay. The heparinized whole blood samples were diluted with an equal volume of phosphate-buffered saline (PBS) and layered carefully on the surface of the lymphocyte separation medium. After 15 min centrifugation at 2100 rpm, a white cloudy band was observed in the lymphocyte separation solution interface. The lymphocyte band was collected and washed twice with RPMI-1640 media (Gibco Laboratories, Grand Island, NY, USA) without foetal bovine serum (FBS) (TBD, Tianjin, China). After centrifugation, the pellet was re-suspended in 10% FBS-RPMI-1640 media which contained 40 µl concanavalin A (ConA) (Sigma, St Louis, MO, USA) at a concentration of 1×10^7 cells/ml, and 200 µl per well was incubated in 96-well tissue culture plates. Each sample was seeded in eight wells. After 48 h of incubation at 37 °C under 5% CO_2, 25 µl of MTT (5 mg/ml) (Sigma) was added to each well. The plates were incubated for another 4 h, and then 100 µl of 10% sodium dodecyl sulphate (Sigma) was added to each well; the plates were finally shaken for 5 min to completely dissolve the precipitate. Light absorbance at 570 nm was measured with an ELISA plate reader (Spectramax M5, Molecular Devices, San Jose, CA, USA). Proliferation was expressed as the proportions of all cells that were proliferating.

Flow cytometric analysis of T lymphocyte sub-populations

As described above, single-cell suspensions of peripheral blood leukocytes were separated and re-suspended in PBS at a concentration of 1×10^7 cells/ml. Then 100 µl cell suspension was put into specific tubes and incubated with monoclonal antibodies, including fluorescein isothiocyanate-labelled mouse anti-chicken CD4, phycoerythrin-labelled mouse anti-chicken CD8α and spectral red-labelled mouse anti-chicken CD3 (Southern Biotechnology Associates Inc., Birmingham, AL, USA). Finally, samples were analysed using Cell Quest software on a BD FACSCalibur flow cytometer (Becton Dickinson Immunocytometry Systems, San Jose, CA, USA).

Determination of IgA, IgG, IgM and IBD antibody titres in serum

Serum IgA, IgG and IgM contents were measured with the chicken IgA, IgG, IgM ELISA kits (R&D Systems, Minneapolis, MN, USA) and the Spectramax automated ELISA reader. The antibody titre of IBDV was determined using ELISA kits (IDEXX Laboratories, Westbrooke, ME, USA) with serum diluted up to 500-fold with a sample diluent from the kit, and the optical densities were read at 650 nm in the Spectramax plate reader. Endpoint titres were calculated as described in the instructions. Titres >396 were considered as being positive and indicate previous vaccination or other exposure to IBD: titres <396 were considered to be negative.

Statistical analysis

Replicate was the experimental unit. The effects of SI supplementation level, dpi and their interaction, all considered to be fixed effects, were examined by two-way analysis of analysis (ANOVA) using the GLM procedures of SAS software (v9.2, SAS Institute, Cary, NC, USA). In the absence of SI, IBDV-infected and non-infected controls were compared by *t* tests. Orthogonal contrasts were used to evaluate linear and quadratic effects of SI supplementation. Significance was declared at $P < 0.05$. All results are expressed as means and the SEMs ($n = 4$) were derived from the error mean square of each ANOVA.

Results

T lymphocyte proliferation

The proliferation of peripheral T lymphocyte proliferation (Table 2) showed a significant ($P < 0.001$) interaction between dietary SI supplementation and time (dpi) but differences between supplemented diets were only apparent at 3 dpi. Proliferation was reduced ($P < 0.05$) in infected compared with non-infected birds. The response to SI supplementation was quadratic ($P = 0.004$) with minimal proliferation when birds were infected with IBDV alone and maximal when infection was combined with feeding the diet with 10 mg/kg SI.

T lymphocyte sub-populations

The percentages of peripheral T lymphocyte sub-populations are shown in Table 3. The proportions of $CD3^+$, $CD4^+$, $CD8^+$ T lymphocytes and ratio of $CD4^+$:$CD8^+$ T lymphocytes were all affected significantly ($P < 0.001$) by dietary SI supplementation level, dpi and interactions between the two main effects.

The proportions of $CD3^+$, $CD4^+$ and $CD8^+$ T lymphocytes in IBDV-infected birds all increased quadratically with increasing supplementation with SI at 7 and 23 dpi. Diets supplemented with SI increased the percentage of $CD3^+$ T lymphocytes; the response was quadratic with 20 mg/kg being maximal. At 23 dpi, there was a significant rise in the proportion of peripheral

https://doi.org/10.1017/S0021859618000758 Published online by Cambridge University Press

Table 3. Effect of soybean isoflavone (SI) on sub-populations of peripheral T lymphocytes (%) of Chinese yellow-feathered broilers challenged with infectious bursal disease virus (IBDV)[a]

Variable (SEM[b], $n = 4$)	Non-infected controls	IBDV infected and (dietary supplementation in mg/kg SI)				*P* values for SI level	
		IBDV (0 SI)	IBDV (10 SI)	IBDV (20 SI)	IBDV (40 SI)	Linear	Quadratic
$CD3^+$ T lymphocyte (%) SEM = (2.5) (dpi)							
3	12.0	9.5*	11	26	18	NS	<0.001
7	10.2	12	16	15	16	NS	NS
23	52	25*	38	59	57	NS	0.007
$CD4^+$ T lymphocyte (%) (SEM = 1.36) (dpi)							
3	6.0	7.5	7	17	13.0	NS	0.002
7	5.3	7	7.8	6.4	8.1	NS	NS
23	29	17*	23	35	32	NS	0.010
$CD8^+$ T lymphocyte (%) (SEM = 0.92) (dpi)							
3	3.0	3.5	3.2	5.5	5.8	NS	0.001
7	3.6	4.6	5.7	6.1	5.7	NS	NS
23	15	7.2*	11	15	12	NS	0.038
$CD4^+$:$CD8^+$ ratio (SEM = 0.20) (dpi)							
3	2.1	2.2	2.3	3.1	2.1	NS	<0.001
7	1.5	1.5	1.5	1.2	1.5	NS	NS
23	2.0	2.4	2.5	2.7	2.8	NS	NS

[a]Peripheral $CD3^+$, $CD4^+$ and $CD8^+$ T lymphocyte sub-populations (%) and $CD4^+$:$CD8^+$ ratio were examined at 3, 7 and 23 dpi. *P* values from each of 3 ANOVAs: SI supplementation < 0.001; dpi < 0.001; SI supplementation × dpi < 0.001. Data are means.
[b]SEMs are derived from ANOVA error mean square, for four replicates.
*IBDV compared with non-infected controls by *t* test ($P < 0.05$).

$CD3^+$ T lymphocytes compared with those at 3 or 7 dpi. Birds inoculated with IBDV had a significant decrease in the percentage of peripheral $CD3^+$ T lymphocytes compared with the non-infected birds, and infected birds receiving 20 or 40 mg/kg dietary SI had the same proportions as did non-infected controls. The percentages of peripheral $CD4^+$ and $CD8^+$ T lymphocyte sub-sets in those groups showed the same quadratic trends as for $CD3^+$ T lymphocytes; again, infected birds at 23 dpi were significantly lower than non-infected birds.

No differences were observed in the $CD4^+$:$CD8^+$ T lymphocyte ratio between the non-infected and IBDV-infected birds at any sampling time. There was a quadratic effect ($P < 0.001$) of dietary SI at 3 dpi with maximal response with 20 mg/kg SI.

IgA, IgG, IgM antibody response

As shown in Table 4, dietary SI supplementation level, dpi and the interactions significantly affected serum immunoglobulin concentrations in IBDV-infected birds ($P < 0.001$). Serum IgA concentration of birds increased quadratically at 3 dpi only ($P < 0.005$). There were no significant differences in serum IgG and IgM contents between non-infected controls and IBDV-infected birds at 3 and 7 dpi, but concentrations at 23 dpi were reduced in infected birds. At this time, IgG showed a linear response to dietary SI ($P < 0.001$) while there was a quadratic response in IgM ($P < 0.001$).

Infectious bursal disease virus antibody titres

Dietary SI supplementation level, dpi and the interactions significantly affected ($P < 0.001$) serum titres of IBDV antibody in IBDV-infected birds (Table 5). Using the typical threshold for 'positivity' in the kit used (396), non-infected birds remained negative throughout, and infected birds had positive titres by 7 dpi and continued to increase to 23 dpi. At 15 and 23 dpi, the effect of SI level on titres was quadratic ($P < 0.001$), highest responses occurred with 10 mg/kg SI.

Discussion

Cellular immunity may have an important role in defence against IBDV infection (Kim *et al.*, 2000; Rautenschlein, 2002*a*, 2002*b*; Yeh *et al.*, 2002). As the current results showed, the IBDV-infected broilers had a depressed T-cell mitogenic response, especially at 3 dpi. This may indicate an overall depression of T-cell function during viral infection (Sivanandan and Maheswaran, 1981). The degree of depression appeared to be more severe at 3 dpi, probably because of the elevated numbers of viral particles compared with the other sampling times.

Soybean isoflavone has been demonstrated to be an immunomudulator on cell-mediated immunity (Cooke *et al.*, 2006; Morimoto *et al.*, 2009; Belcavello *et al.*, 2012). The present results showed that adding SI to the diet increased peripheral T lymphocyte proliferation of infected birds at 3 dpi, reflecting enhanced

https://doi.org/10.1017/S0021859618000758 Published online by Cambridge University Press

Table 4. Effect of supplemental soybean isoflavone (SI) on serum IgA, IgG, IgM of Chinese yellow-feathered broilers infected with infectious bursal disease virus (IBDV)[a]

Ig class (SEM[b], $n = 4$)	Non-infected controls	IBDV infected and (dietary supplementation in mg/kg SI)				*P* values for SI level	
		IBDV (0 SI)	IBDV (10 SI)	IBDV (20 SI)	IBDV (40 SI)	Linear	Quadratic
IgA (μg/ml) (SEM = 2.46) (dpi)							
3	28	17	29	35	27	NS	0.004
7	32	26	25	14.5	18	NS	NS
23	20	20	16	16.7	20	NS	NS
IgG (μg/ml) (SEM = 2.74) (dpi)							
3	48	50	42	45	46	NS	NS
7	46	54	40	50	56	NS	NS
23	20	14.6*	18.4	22	34	<0.001	NS
IgM (μg/ml) (SEM = 4.30) (dpi)							
3	28	30	23	26	35	NS	NS
7	34	32	26	40	30	NS	NS
23	43	23*	55	47	49	NS	<0.001

[a]Serum IgA, IgG, IgM was measured at 3, 7 and 23 dpi. *P* values from each of three ANOVAs: SI supplementation < 0.001; dpi < 0.001; SI supplementation × dpi < 0.001. Data are means.
[b]SEMs are derived from ANOVA error mean square, for four replicates.
*IBDV compared with non-infected controls by *t* test ($P < 0.05$).

Table 5. Effect of supplemental soybean isoflavone (SI) on IBDV antibody titres of Chinese yellow-feathered broilers infected with infectious bursal disease virus (IBDV)[a]

IBDV antibody titres at five sampling days (dpi)	Non-infected controls	IBDV infected and (dietary supplementation in mg/kg SI)				*P* values for SI level	
		IBDV (0 SI)	IBDV (10 SI)	IBDV (20 SI)	IBDV (40 SI)	Linear	Quadratic
0	71	94	91	94	63	NS	NS
3	43	117	94	84	108	NS	NS
7	79	950***	1008	1191	1014	NS	NS
15	90	2115***	2909	1929	2013	NS	<0.001
23	59	3096***	3249	1965	2976	NS	<0.001

[a]IBDV antibody titres were measured at 0 (21-day-old before inoculation), 3, 7, 15 and 23 dpi. Titres >396 are considered to be positive, otherwise are considered to be negative. *P* values from ANOVA: SI supplementation < 0.001; dpi < 0.001; SI supplementation × dpi < 0.001. Results are means, the SEM was 51.76 for $n = 4$, based on ANOVA error mean square.
***IBDV compared with non-infected controls by *t* test ($P < 0.001$).

activation of lymphocytes at this acute stage of IBDV infection. The interaction between SI supplementation and dpi over 1–23 days period suggests that this positive effect of SI on peripheral T lymphocyte proliferation increased with time. A number of studies have reported that the effect of SI (or flavonoids generally) on lymphocyte proliferation was in a concentration or component-dependent manner (Wang *et al.*, 1997; Zhang *et al.*, 1997). In the current study, all concentrations of dietary SI improved lymphocyte proliferation, with lower concentration (10 or 20 mg/kg) being significant during early stages of infection and higher concentrations (20 or 40 mg/kg) significant later. Previous studies have reported that SI affects cellular function through diverse receptors and enzymes including acting as selective oestrogen receptor modulators (Donovan *et al.*, 2009). The SI used in the current study, glycitein, did not have oestrogen-like activity (unpublished data): it may exert a regulatory effect on cellular immunity in some other way, which should be examined in future studies.

The percentages of peripheral T lymphocyte sub-sets including $CD4^+$ and $CD8^+$ of IBDV-infected birds increased slightly at 3 and 7 dpi, which suggested a probable compensatory role of the thymus when direct cytolysis of B lymphocytes was induced by IBDV in the bursa of Fabricius (Sivanandan and Maheswaran, 1980). At 23 dpi, however, all of the examined peripheral T lymphocyte sub-sets ($CD3^+$, $CD4^+$, $CD8^+$) were reduced in IBDV-infected birds, while in the non-infected birds, T lymphocyte sub-sets increased gradually and reached a peak at this sampling time. The results suggested that IBDV induced immunosuppression of cellular immunity. The impaired T-cell function may be related to the direct lytic effect of IBDV on thymic cells (Sivanandan and Maheswaran, 1981).

In the present study, supplementation with SI increased the proportion of peripheral $CD3^+$ T lymphocytes, especially at 20 or 40 mg/kg levels at 3 and 23 dpi. At this time, the IBDV-infected birds with no dietary SI had the lowest proportion of $CD3^+$ T lymphocytes, while those on SI-supplemented diets

https://doi.org/10.1017/S0021859618000758 Published online by Cambridge University Press

had elevated proportions, even higher than that of the non-infected broilers. The same phenomenon was observed on $CD4^+$ and $CD8^+$ T lymphocytes. Most $CD4^+$ T lymphocytes are helper or inflammatory T cells when responding to a viral attack. The fact that $CD8^+$ T lymphocytes function as virus-specific cytotoxic T cells has shown to be important in the resolution of infection and elimination of viruses, both in chickens and in mammals (McNeal *et al*., 1995; Seo *et al*., 1997; Collisson *et al*., 2000). Zhang *et al*. (1997) reported that lymphocyte proportion of peripheral blood was increased in Swiss mice fed daidzein at high doses (20 and 40 mg/kg). Guo *et al*. (2002) found that exposure to genistein increased the number of splenic T cells and T-cell sub-sets in both male and female offspring of SD rats. Klein *et al*. (2002) demonstrated that early exposure to genistein had long-lasting effects on the immune systems of their male offspring by inducing higher percentage of $CD8^+$ T lymphocytes and total T lymphocytes in the spleen. Thus, the current study reflected that dietary supplementation of SI is beneficial in defence against IBDV infection and protecting T lymphocyte development from immunosuppression. The ratio of $CD4^+$ and $CD8^+$ T lymphocytes in the peripheral blood is an important indicator for evaluation of cellular immunity function. In the current study, a significant enhancing ratio of $CD4^+$ and $CD8^+$ lymphocytes was observed with 20 mg/kg SI at 3 dpi, which may indicate enhancement of T-cell-mediated immune response.

Humoral immunity is the primary mechanism of the protective immune response to IBDV (Kibenge *et al*., 1988) and an effective way to prevent reinfection (Powell, 1987). In the present study, SI addition at 10 mg/kg enhanced IBDV antibody titres at 15 and 23 dpi while 20 or 40 mg/kg SI had adverse effects. In order to further investigate the probable mechanism of SI on the production of specific antibody, titres of anti-AIV were measured at the same sampling times. There was no significant difference between SI-supplemented and non-supplemented birds in anti-AIV titres in response to routine vaccination (data not shown), which may indicate that the mechanism of SI for enhancement of antibody response is virus-specific, in this case against virulent IBDV.

A dramatic reduction in circulating IgM^+ B cells (Sharma *et al*., 2000) and complete lack of IgG (Saif, 1998) was caused by IBDV infection. The current data demonstrated that serum IgM and IgG concentrations decreased in IBDV-infected birds (no dietary SI) at 23 dpi compared with non-infected controls. Dietary SI prevented inhibition of humoral immunity as the contents of IgM and IgG in serum were elevated and returned to normal at 23 dpi. Thus, it is proposed that alleviation of immunosuppression of humoral immunity may be due largely to the enhanced T-cell-mediated immune response involved in IBDV clearance, which finally could reduce the lesions of immunoglobulin-producing cells in the bursa of Fabricius otherwise caused by IBDV. IgA is the predominant form of antibody in bodily secretions and plays an important role in defence against many pathogens. In the current study, IBDV infection had no effect on serum IgA concentration, but there was a clear early (3 dpi) increase in IgA content of IBDV-infected birds in birds fed diets supplemented with SI. This may indicate that dietary SI might enhance the humoral immune response to IBDV.

In conclusion, dietary glycitein, a soy isoflavone, increased peripheral T lymphocyte proliferation and T lymphocyte subpopulations as well as serum antibody production in birds challenged with IBDV. These results suggest that SI may play an immunoenhancing effect on both T-cell-mediated and humoral immunity in protecting against IBDV infection and inhibiting the immunosuppression caused by IBDV. It may be of value to supplement diets with SI to improve disease resistance of broilers.

Acknowledgements. W. Bruce Currie (Emeritus Professor, Cornell University, Ithaca, NY, USA) made suggestions on presentation.

Financial support. This work was funded by the grant agreement (8451064001000853) from Guangdong Provincial Natural Science Fund Committee, the earmarked fund for Modern Agro-industry Technology Research System (CARS-41) from Ministry of Agriculture, 'Twelve-Five' national science and technology support program (2014BAD13B02), and scientific and technological project (201804020091 and 2017B020202003) from Guangzhou science technology and innovation commission and Guangdong province science and technology department, P. R. China.

Conflict of interest. None.

Ethical standards. The experimental protocol was reviewed and approved by the Institute of Animal Science, Guangdong Academy of Agricultural Sciences, China.

References

Abdukalykova ST, Zhao X and Ruiz-Feria CA (2008) Arginine and vitamin E modulate the subpopulations of T lymphocytes in broiler chickens. *Poultry Science* **87**, 50–55.

Balamurugan V and Kataria JM (2006) Economically important non-oncogenic immunosuppressive viral diseases of chicken-current status. *Veterinary Research Communications* **30**, 541–566.

Belcavello L, Dutra JCV, de Freitas JV, Aranha IP, Batitucci M and do CP (2012) Mutagenicity of ipriflavone *in vivo* and *in vitro*. *Food and Chemical Toxicology* **50**, 996–1000.

Biggs PM (1985) Infectious animal disease and its control. *Philosophical Transactions of the Royal Society of London, Series B: Biological Sciences* **310**, 259–274.

Butcher GD and Miles RD (2002) *Interrelationship of Nutrition and Immunity*. VM139. Gainesvill, FL, USA: University of Florida. Available at http://edis.ifas.ufl.edu/VM104 (Accessed 9 January 2018).

Cheng ZG, Lin YC, Yu DQ, Jiang SQ, Zhou GL and Jiang ZY (2005) Effects of daidzein on growth performance and its potential mechanism in finishing pigs. *Acta Zoonutrimenta Sinica* **17**, 30–34.

Collisson EW, Pei JW, Dzielawa J and Seo SH (2000) Cytotoxic T lymphocytes are critical in the control of infectious bronchitis virus in poultry. *Developmental and Comparative Immunology* **24**, 187–200.

Cooke PS, Selvaraj V and Yellayi S (2006) Genistein, estrogen receptors, and the acquired immune response. *Journal of Nutrition* **136**, 704–708.

Curran EM, Judy BM, Newton LG, Lubahn DB, Rottinghaus GE, Macdonald RS, Franklin C and Estes DM (2004) Dietary soy phytoestrogens and ERα signalling modulate interferon gamma production in response to bacterial infection. *Clinical & Experimental Immunology* **135**, 219–225.

Donovan SM, Andres A, Mathai RA, Kuhlenschmidt TB and Kuhlenschmidt MS (2009) Soy formula and isoflavones and the developing intestine. *Nutrition Reviews* **67**, S192–S200.

Feed Database in China (2016) Tables of feed composition and nutritive values in China- fifteenth edition. *Chinese Feed* **21**, 33–43.

Fussell LW (1998) Poultry industry strategies for control of immunosuppressive diseases. *Poultry Science* **77**, 1193–1196.

Guo TL, White KL, Brown RD, Delclos KB, Newbold RR, Weis C, Germolec DR and McCay JA (2002) Genistein modulates splenic natural killer cell activity, antibody-forming cell response, and phenotypic marker expression in F0 and F1 generations of Sprague-Dawley rats. *Toxicology and Applied Pharmacology* **181**, 219–227.

Kibenge FS, Dhillon AS and Russell RG (1988) Biochemistry and immunology of infectious bursal disease virus. *Journal of General Virology* **69**, 1757–1775.

https://doi.org/10.1017/S0021859618000758 Published online by Cambridge University Press

Kim IJ, You SK, Kim H, Yeh H and Sharma JM (2000) Characteristics of bursal T lymphocytes induced by infectious bursal disease virus. *Journal of Virology* **74**, 8884–8892.

Klein SL, Wisniewski AB, Marson AL, Glass GE and Gearhart JP (2002) Early exposure to genistein exerts long-lasting effects on the endocrine and immune systems in rats. *Molecular Medicine* **8**, 742–749.

Lee LF, Sharma JM, Nazerian K and Witter RL (1978) Suppression and enhancement of mitogen response in chickens infected with Marek's disease virus and the herpes virus of turkeys. *Infection and Immunity* **21**, 474–479.

Liu DY, He SJ, Liu SQ, Tang YG, Jin EH, Chen HL, Li SH and Zhong LT (2014) Daidzein enhances immune function in late lactation cows under heat stress. *Animal Science Journal* **85**, 85–89.

McNeal MM, Barone KS, Rae MN and Ward RL (1995) Effector functions of antibody and $CD8^+$ cells in resolution of rotavirus infection and protection against reinfection. *Virology* **214**, 387–397.

Morimoto M, Watanabe T, Yamori M, Takebe M and Wakatsuki Y (2009) Isoflavones regulate innate immunity and inhibit experimental colitis. *Journal of Gastroenterology and Hepatology* **24**, 1123–1129.

Müller H, Islam MR and Raue R (2003) Research on infectious bursal disease – the past, the present and the future. *Veterinary Microbiology* **97**, 153–165.

National Research Council (1994) *Nutrient Requirements of Poultry*, 9th rev. Edn. Washington, DC, USA: National Academies Press.

Powell PC (1987) Immune mechanisms in infections of poultry. *Veterinary Immunology and Immunopathology* **15**, 87–113.

Raff MC (1973) T and B lymphocytes and immune responses. *Nature* **242**, 19–23.

Rautenschlein S, Yeh H and Sharma JM (2002*a*) The role of T cells in protection by an inactivated infectious bursal disease virus vaccine. *Veterinary Immunology and Immunopathology* **89**, 159–167.

Rautenschlein S, Yeh H, Njenga MK and Sharma JM (2002*b*) Role of intrabursal T cells in infectious bursal disease virus (IBDV) infection, T cells promote viral clearance but delay follicular recovery. *Archives of Virology* **147**, 285–304.

Saif YM (1991) Immunosuppression induced by infectious bursal disease virus. *Veterinary Immunology and Immunopathology* **30**, 45–50.

Saif YM (1998) Infectious bursal disease and hemorrhagic enteritis. *Poultry Science* **77**, 1186–1189.

Seo HS, Wang L, Smith R and Collisson EW (1997) The carboxyl-terminal 120-residue polypeptide of infectious bronchitis virus nucleocapsid induces cytotoxic T lymphocytes and protects chickens from acute infection. *Journal of Virology* **71**,7889–7894.

Sharma JM, Kim IJ, Rautenschlein S and Yeh H (2000) Infectious bursal disease virus of chickens: pathogenesis and immunosuppression. *Developmental and Comparative Immunology* **24**, 223–235.

Sivanandan V and Maheswaran SK (1980) Immune profile of infectious bursal disease. I. Effect of infectious bursal disease virus on peripheral blood T and B lymphocytes in chickens. *Avian Diseases* **24**, 715–725.

Sivanandan V and Maheswaran SK (1981) Immune profile of infectious bursal disease. III. Effect of infectious bursal disease virus on the lymphocyte responses to phytomitogens and on mixed lymphocyte reaction of chickens. *Avian Diseases* **25**, 112–120.

Tanimura N and Sharma JM (1997) Appearance of T cells in the bursa of Fabricius and cecal tonsils during the acute phase of infectious bursal disease virus infection in chickens. *Avian Diseases* **41**, 638–645.

van den Berg TP (2000) Acute infectious bursal disease in poultry: a review. *Avian Pathology* **29**, 175–194.

Vervelde L and Davison TF (1997) Comparison of the *in situ* changes in lymphoid cells during infection with infectious bursal disease virus in chickens of different ages. *Avian Pathology* **26**, 803–821.

Wang WQ, Higuchi CM and Zhang RQ (1997) Individual and combinatory effects of soy isoflavones on the *in vitro* potentiation of lymphocyte activation. *Nutrition and Cancer* **29**, 29–34.

Yeh H, Rautenschlein S and Sharma JM (2002) Protective immunity against infectious bursal disease virus in chickens in the absence of virus-specific antibodies. *Veterinary Immunology and Immunopathology* **89**, 149–158.

Zhang RQ, Li YP and Wang WQ (1997) Enhancement of immune function in mice fed high doses of soy daidzein. *Nutrition and Cancer* **29**, 24–28.