

## The effects of lipoic acid on soybean $\beta$ -conglycinin-induced anaphylactic reactions in a rat model

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The purpose of this study was to evaluate the effects of feeding a low dose of lipoic acid on attenuating soybean  $\beta$ -conglycinin-induced hypersensitivity using a rat model, with ovalbumin as the positive allergic control. Forty-eight recently weaned male Sprague-Dawley rats were assigned to four treatments and fed a cornstarch-casein-based diet either unsupplemented (Groups I, II and III) or supplemented with 25 mg/kg lipoic acid (Group IV). On days 1, 10, 17, and 24, Groups III and IV were sensitised with 20 mg  $\beta$ -conglycinin by means of intragastric gavage, while Group II was sensitised with 20 mg ovalbumin and Group I (control) with casein. On day 31, rats received a double dose of  $\beta$ -conglycinin, ovalbumin or casein, respectively. Ovalbumin-sensitised rats (Group II) and  $\beta$ -conglycinin-sensitised rats (Group III) demonstrated an increase in serum IgE and histamine release, but reduced growth performance compared to the control (Group I) ( $p < 0.05$ ). A low dose of lipoic acid had no effect on average weight gain, but increased villus height in the jejunum ( $p < 0.05$ ), while reducing serum  $\beta$ -conglycinin-specific IgE and histamine content in the jejunum. Moreover, lipoic acid supplementation did not significantly affect interferon- $\gamma$  or interleukin-4. Taken together, our results suggest that a low dose of lipoic acid could potentially be used as an immunomodulator to attenuate soybean  $\beta$ -conglycinin induced allergies.

**Keywords:** anaphylaxis; conglycinin; cytokines; histamine; IgE; lipoic acid; rats

### 1. Introduction

Due to their nutritional and functional properties, soybean proteins are major components of the diets of food-producing animals (De Schutter and Morris 1990) and are becoming increasingly important in the diet of humans (Friedman and Brandon 2001). However, soybean allergy has a significant impact on sensitive people, particularly infants and toddlers (Herian et al. 1990; Cantani and Lucenti 1997). Because of the high inclusion rates of soybean meal used in compound feeds for farm animals (such as pig, chicken, turkey, calves), soybean allergies were also often found in this connection. Weaned pigs and calves especially show significant soybean sensitivity (Li et al. 1990; Sun et al. 2008).

Together with well-balanced proteins, soybean also contains many anti-nutritional factors, including trypsin inhibitors, agglutinins, isoflavones, allergens, and alkaloids (Friedman and Brandon 2001). Among them,  $\beta$ -conglycinin, which

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accounts for about 30% of the total soybean proteins, was shown to be the major soybean allergen (Maruyama et al. 1998). It is well known as a major soybean storage protein, containing three different subunits,  $\alpha$ ,  $\alpha'$  and  $\beta$ , with molecular weights of 58–77, 58–83, and 42–53 kDa, respectively (Perez et al. 2000; Mujoo et al. 2003). An increasing number of studies suggest that  $\beta$ -conglycinin plays an important role in hypersensitive responses to food and feed (Xiang et al. 2002).

Recent research has focused on exploring the mechanisms of food-induced allergy and looking for methods to decrease immunoreactivity (Nowak-Węgrzyn 2003). An effective tool for the detection of  $\beta$ -conglycinin has been established via monoclonal antibody-based competitive ELISA (You et al. 2008). Approaches for preventing IgE-mediated food allergies such as the use of anti-IgE, probiotics and food allergy vaccines are not suitable for long-term and widespread application due to potential adverse side-effects (Laitinen and Isolauri 2005).

It is postulated that exogenous vitamin C can lower blood levels of histamine and thus help to reduce the severity of allergic reactions (Clemeston 1980). Our previous research suggested that a mega-dose of vitamin C (1000 mg/d) could be used to attenuate soybean allergies in pigs (Sun et al. 2009). However, it has also been proved that supplementation of long-term and mega-dose vitamin C has many adverse side-effects (Massey et al. 2005).

Lipoic acid, also called thioctic acid, widely exists in the cellular membranes and cytosol of both plants and animals (Packer et al. 1995). Similar to vitamin C, lipoic acid functions as an electron donor to protect the body from radicals and pollutants (Wada et al. 1997), and its activity is several-fold more powerful than vitamin C. Low doses of lipoic acid can attenuate oxidative damage *in vivo* (Gaetke and Chow 2003) and therefore protect humans from allergies, diabetes, high blood pressure and some other diseases, with few adverse side-effects (Roy et al. 1997; Ziegler and Gries 1997; Lam 2005).

The objective of this study was to investigate whether a low dose of lipoic acid can attenuate soybean  $\beta$ -conglycinin induced allergy more effectively. This was tested by using an experimental rat model for  $\beta$ -conglycinin anaphylaxis established by means of intragastric gavage with purified  $\beta$ -conglycinin.

## **2. Materials and methods**

### **2.1. Purified soybean $\beta$ -conglycinin**

Samples of purified  $\beta$ -conglycinin were kindly provided by Professor Shuntang Guo of the Food Institute of China Agricultural University. SDS-PAGE analysis confirmed that the samples contained more than 95%  $\beta$ -conglycinin (data not shown).

### **2.2. Animals, diets and experimental protocol**

All animals used in this experiment were maintained according to the guidelines of the China Agricultural University Animal Care and Use Ethics Committee (Beijing, China).

Forty-eight recently weaned male Sprague-Dawley rats (21 days old, BW  $35.0 \pm 2.69$  g) were purchased from the Institute of Genetics and Developmental Biology at the Chinese Academy of Science (Beijing, China). The rats were individually housed in polycarbonate cages with soft wood granulate floors.

The cages were located in a room with controlled lighting (12 h/d), temperature (22 ~ 25°C) and relative humidity (60 ~ 65%).

The rats were assigned to one of four treatments ( $n = 12$ ) and fed a casein-based diet either unsupplemented (Groups I, II and III) or supplemented with  $\alpha$ -lipoic acid (25 mg/kg, 98% purity, obtained from Healthjoy Chemical Company, Shanghai, China; Group IV). The basal diet was formulated according to the recommendations of the American Institute of Nutrition (Reeves 1997). All four diets were formulated to provide similar levels of crude protein, amino acids and energy (Table 1).

As shown in Table 2, rats in Groups III and IV were sensitised by intragastric gavage with 20 mg purified  $\beta$ -conglycinin (0.5 ml/rat of solution containing 40 mg/ml  $\beta$ -conglycinin dissolved in phosphate buffered saline with pH 7.4) on days 1, 10, 17, and 24. The amount of  $\beta$ -conglycinin was defined according to the

Table 1. Dietary composition and nutrient content of the basal diet.

| Ingredients [g/kg]       |       | Nutrient levels <sup>†</sup> |       |
|--------------------------|-------|------------------------------|-------|
| Cornstarch               | 481.4 | Gross energy [MJ/kg]         | 16.05 |
| Casein                   | 181.8 | Dry matter [%]               | 97.38 |
| Dextrinised cornstarch   | 90.9  | Ash [%]                      | 3.56  |
| Sucrose                  | 90.9  | Crude protein [%]            | 17.34 |
| Soybean oil              | 63.6  | Calcium [%]                  | 1.43  |
| Fibre*                   | 45.5  | Total phosphorus [%]         | 0.36  |
| Mineral mix <sup>#</sup> | 31.8  | Lysine [%]                   | 1.47  |
| Vitamin mix <sup>§</sup> | 9.1   | Methionine + cystine [%]     | 0.48  |
| L-cystine                | 2.7   | Threonine [%]                | 0.65  |
| Choline bitartrate       | 2.3   | Tryptophan [%]               | 0.18  |
| Tert-butylhydroquinone   | 0.01  |                              |       |

Notes: \*Cellulose acetate (Cellulose Fibers Company, Nantong, China). <sup>#</sup>Mineral mixture was prepared as AIN-93G [per 100 g of mixture]: CaCO<sub>3</sub>, 37 g; KH<sub>2</sub>PO<sub>4</sub>, 19.6 g; K<sub>3</sub>C<sub>6</sub>H<sub>5</sub>O<sub>7</sub> · H<sub>2</sub>O (K-citrate), 7.08 g; NaCl, 7.4 g; K<sub>2</sub>SO<sub>4</sub>, 4.66 g; MgO, 24 g; FeC<sub>6</sub>H<sub>5</sub>O<sub>7</sub> · H<sub>2</sub>O (Fe-citrate), 606 mg; ZnCO<sub>3</sub>, 165 mg; MnCO<sub>3</sub>, 63 mg; CuCO<sub>3</sub>Cu(OH)<sub>2</sub> · H<sub>2</sub>O, 32.4 mg; NaSiO<sub>3</sub> · 9H<sub>2</sub>O, 145 mg; CrK(SO<sub>4</sub>) · 12H<sub>2</sub>O, 27.5 mg; LiCl, 1.74 mg; H<sub>3</sub>BO<sub>3</sub>, 8.15 mg; NaF, 6.35 mg; NiCO<sub>3</sub> · 2Ni(OH)<sub>2</sub> · 4H<sub>2</sub>O, 3.06 mg; NH<sub>4</sub>VO<sub>3</sub>, 0.66 mg; Sucrose ad, 100 g. <sup>§</sup>Vitamin mixture was prepared as AIN-93 [per 100 g mixture]: Nicotinic acid, 300 mg; Ca-pantothenate, 160 mg; Pyridoxine hydrochloride, 70 mg; Thiamine hydrochloride, 60 mg; Riboflavin, 60 mg; Folic acid, 20 mg; D-biotin, 2.0 mg; Cyanocobalamin, 250 µg; All-trans-retinyl palmitate, 80 mg;  $\alpha$ -Tocopherol, 1500 mg; Cholecalciferol, 25 mg; Phylloquinone, 7.5 mg; Sucrose ad, 100 g. <sup>†</sup>Each value represents the mean of three analysed values.

Table 2. The experimental design.

|        | Group I<br>(Negative<br>control) | Group II<br>(Ovalbumin sensitised,<br>positive control) | Group III<br>( $\beta$ -conglycinin<br>sensitised) | Group IV<br>( $\beta$ -conglycinin sensitised +<br>lipoic acid treatment) |
|--------|----------------------------------|---------------------------------------------------------|----------------------------------------------------|---------------------------------------------------------------------------|
|        | Intragastric gavage              |                                                         |                                                    |                                                                           |
|        | Casein                           | Ovalbumin                                               | $\beta$ -conglycinin                               | $\beta$ -conglycinin                                                      |
| Day 1  | 20 mg                            | 20 mg                                                   | 20 mg                                              | 20 mg                                                                     |
| Day 10 | 20 mg                            | 20 mg                                                   | 20 mg                                              | 20 mg                                                                     |
| Day 17 | 20 mg                            | 20 mg                                                   | 20 mg                                              | 20 mg                                                                     |
| Day 24 | 20 mg                            | 20 mg                                                   | 20 mg                                              | 20 mg                                                                     |
| Day 31 | 40 mg                            | 40 mg                                                   | 40 mg                                              | 40 mg                                                                     |

previous investigations of Guo et al. (2007). Rats in Group II were sensitised with 20 mg of ovalbumin (0.5 ml/rat of a solution containing ovalbumin at 40 mg/ml, dissolved in phosphate buffered saline). The control group (Group I) was gavaged with 20 mg casein (0.5 ml/rat of a solution containing casein at 40 mg/ml dissolved in phosphate buffered saline) using the same treatment schedule. On day 31, rats received a double dose of  $\beta$ -conglycinin, ovalbumin or casein based on the recommendations of Fischer et al. (2005), respectively.

Water and feed were available *ad libitum* throughout the 31 day trial. Rats and feeders were individually weighed at the start and end of the trial (days 0 and 31) in order to calculate weight gain, feed intake and feed conversion ratio.

Blood was obtained from the tail vein of each rat using a 2 ml Clot Activator tube (Greiner Bio-One GmbH, Kremsmunster, Austria) on day 31, three hours after intragastric gavage. The blood was left to stand for 2 h in a 37°C incubator and then centrifuged at 3000 g in an Anke TGL-16B Centrifuge (Shanghai Anting Scientific Instruments, Shanghai, China) for 10 min at 4°C. The serum was immediately frozen and stored at -70°C for later analysis.

On day 32, all rats were sacrificed. The peritoneal cavity was opened and the spleen and small intestine were removed. The jejunum (the middle part of the whole jejunum) was quickly isolated and flushed with ice-cold saline to remove the digesta. The intestinal tissue was then stored in liquid nitrogen until analysis.

### 2.3. Observation of the small intestinal morphology

Intestinal segments were immediately removed from the jejunum (the middle part of the whole jejunum) and then flushed with physiological saline to remove the gut contents. One sample from each intestinal tissue, about 2 cm in length, was fixed in polyformaldehyde for the determination of intestinal morphology.

The samples of intestine were dehydrated and embedded in paraffin wax. They were then cut into 3  $\mu$ m slices and stained with haematoxylin and eosin before microscopic examination. The photographs (at  $\times 20$  magnification) of the small intestinal morphology were collected with an Axioskop-2 microscope (Olympus, Japan), a 1,300 by 1,030 pixel-cooled CCD camera (Princeton Instruments, Trenton, America) and an image-processing system (Visitron Systems, Puchheim, Germany). From each sample, sections that ran from the tip of the villus to the base of the crypt were identified. For each rat, the heights of the eight tallest well-oriented villi were measured from the tip to crypt mouth. The depths of the adjacent crypts were measured from crypt mouth to the base.

### 2.4. Determination of serum IgE antibody levels

Total serum IgE antibody was determined using a rat IgE Enzyme-Linked Immunosorbent Assay (ELISA) Kit according to the manufacturer's instructions (Biosource, Camarillo, CA, USA). The level of  $\beta$ -conglycinin-specific IgE was determined using an indirect ELISA as described by Helm et al. (2002) with slight modifications. Briefly, 96-well microtitre plates (Canda Jet Bio-Chemicals International, Guangzhou, China) were coated with  $\beta$ -conglycinin (5  $\mu$ g/ml) in carbonate buffer ( $\text{NaHCO}_3/\text{Na}_2\text{CO}_3$ , pH 9.6). Then, appropriately diluted rat serum was added and incubated for 1 h. Biotinylated anti-rat IgE and streptavidin-conjugated horseradish peroxidase (RapidBio Lab., Calabasas, CA, USA) were added and

incubated for an hour following each addition. O-phenylenediamine in substrate buffer was added and incubated at 37°C for 15 min in the dark. Prior to each addition, the plates were washed five times with PBST (0.05% Tween-20 in 0.01 mol/l phosphate buffered saline at pH 7.4). After adding 50  $\mu$ l stop buffer (2 mol/l H<sub>2</sub>SO<sub>4</sub>) to each well, the absorbance was measured at 450 nm and the data were expressed in optical density units.

### **2.5. Measurement of histamine levels and histamine release ratio**

Samples of small intestinal tissue, about 5 cm in length, were weighed and then frozen in liquid nitrogen. The samples were finely minced by syringe and lysed in lysis buffer (10 mM Hepes pH 7.4, 0.15 M NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100, 0.5% NP-40, 0.05% SDS with proteinase inhibitor cocktail freshly added) for 30 min on ice. The histamine content in the isolated supernatant was measured using a commercially available rat ELISA kit according to the introductions of the manufacturer (Biosource, Camarillo, CA, USA).

An additional tissue sample of the jejunum was obtained from the control rats for histamine release analysis. Intestinal mast cells were isolated and cultured as described by He et al. (1998). Purified mast cells were re-suspended in complete Hank's Balanced Salt Solution ( $1 \cdot 10^6$  cell/ml), and incubated in medium which was supplemented with serum obtained from the four treatment groups in 24-well microplates (Canda Jet Bio-Chemicals Int., Guangzhou, China) for 2 h. Then,  $\beta$ -conglycinin (Groups I, III and IV) or ovalbumin (Group II) was added to the suspension (final concentration of 100  $\mu$ g/ml) and cultured for another 1 h. The reaction was terminated by placing the plates on ice. The histamine content of both the cell pellet and the supernatant was measured. Residual histamine in the cell pellet was released by disrupting the cells in perchloric acid and centrifuging at 400 g for 5 min at 4°C. Histamine release ratio (HRR) was calculated as:

$$\text{HRR}[\%] = \left\{ \frac{\text{supernatant histamine}}{\text{supernatant histamine} + \text{cell pellet histamine}} \right\} \cdot 100,$$

where supernatant histamine and cell pellet histamine are given in  $\mu$ g/ml.

### **2.6. Spleen cell culture**

The spleen was aseptically removed from sacrificed rats. Spleen tissue was finely chopped with scissors into fragments, minced by syringe and washed twice with RPMI 1640 culture medium (HyClone Laboratories, Logan, UT, USA). After erythrocyte lysis, the splenocytes were detected by Trypan Blue Dye exclusion, counted to adjust the density of the cells to  $2 \cdot 10^6$  cells/ml, and re-suspended in complete RPMI 1640 medium supplemented with 10% foetal bovine serum. Spleen lymphocytes were used in the analysis of cytokine measurement.

### **2.7. Determination of cytokines by ELISA**

Spleen lymphocytes were prepared and suspended with a cell density of  $2 \cdot 10^6$  cells/ml as described above. A combination of 1 ml cell suspension and 1 ml of RPMI 1640 supplemented with concanavalin A (5  $\mu$ g/ml, Sigma, St Louis, MO, USA),

which served as the positive stimuli, was added into 24-well plates and incubated for 24 h at 37°C with 5% CO<sub>2</sub>. The cell culture supernatant was harvested and stored at –70°C for cytokine assay. The concentrations of interleukin-4 and interferon- $\gamma$  were determined using commercially available rat ELISA kits according to the introductions of the manufacturer (Biosource, Camarillo, CA, USA). Assays were performed in triplicate.

## 2.8. Statistical analysis

All data were analysed using the analysis of variance (ANOVA) procedure of the SAS system (Version 8.2, SAS Institute, Inc., Cary, NC, USA). Statistical significance was considered at  $p \leq 0.05$ .

## 3. Results

### 3.1. Growth performance

As shown in Table 3, rats sensitised with  $\beta$ -conglycinin (Group III) or ovalbumin (Group II) had significantly lower average daily gain than the control (Group I). Supplementation with lipoic acid (Group IV) did not improve the weight gain of rats sensitised with  $\beta$ -conglycinin. Feed intake and feed conversion ratio was not influenced by treatments.

### 3.2. Morphological structure of the small intestine

To investigate the effect of  $\alpha$ -lipoic acid on the  $\beta$ -conglycinin-induced modification of the small intestine, the morphological structure of the jejunum was observed. The results showed that the morphological structure of the jejunum was modified (Table 3). Compared to control animals, in the  $\beta$ -conglycinin-challenged Group III and in the ovalbumin-challenged Group II, the villus height was significantly decreased ( $p < 0.05$ ) and the crypt depth was increased ( $p < 0.05$ ). Supplementation with  $\alpha$ -lipoic acid can apparently improve the morphology of the small intestine when injured by  $\beta$ -conglycinin (increased villus height,  $p < 0.05$ ).

Table 3. Effects of  $\alpha$ -lipoic acid on growth performance and the morphological structure of the jejunum in  $\beta$ -conglycinin sensitised rats ( $n = 12$ ).

|                          | Group I<br>(Negative<br>control) | Group II<br>(Ovalbumin<br>sensitised) | Group III<br>( $\beta$ -conglycinin<br>sensitised) | Group IV<br>( $\beta$ -conglycinin<br>sensitised + lipoic acid) | SEM  | <i>p</i> -<br>value |
|--------------------------|----------------------------------|---------------------------------------|----------------------------------------------------|-----------------------------------------------------------------|------|---------------------|
| Performance              |                                  |                                       |                                                    |                                                                 |      |                     |
| BWG [g/d]                | 5.50 <sup>a</sup>                | 4.97 <sup>b</sup>                     | 4.95 <sup>b</sup>                                  | 5.26 <sup>ab</sup>                                              | 0.16 | 0.04                |
| Feed intake [g/d]        | 12.65                            | 11.63                                 | 11.64                                              | 12.27                                                           | 0.35 | 0.17                |
| Feed/gain ratio          | 2.30                             | 2.34                                  | 2.35                                               | 2.33                                                            | 0.02 | 0.10                |
| Jejunum                  |                                  |                                       |                                                    |                                                                 |      |                     |
| Villus height [ $\mu$ m] | 350.5 <sup>a</sup>               | 124.5 <sup>b</sup>                    | 154.6 <sup>b</sup>                                 | 293.8 <sup>a</sup>                                              | 32.2 | <0.01               |
| Crypt depth [ $\mu$ m]   | 88.4 <sup>a</sup>                | 138.4 <sup>b</sup>                    | 126.9 <sup>b</sup>                                 | 98.5 <sup>ab</sup>                                              | 15.7 | <0.01               |

Note: Mean values in the same row not sharing the same superscript letter are significantly different ( $p < 0.05$ ).

Table 4. Effects of  $\alpha$ -lipoic acid on serum IgE level, histamine content/release ratio and cytokine levels in  $\beta$ -conglycinin sensitised rats ( $n = 12$ ).

|                                                                  | Group I<br>(Negative<br>control) | Group II<br>(Ovalbumin<br>sensitised) | Group III<br>( $\beta$ -conglycinin<br>sensitised) | Group IV<br>( $\beta$ -conglycinin<br>sensitised + lipoic acid) | SEM   | <i>p</i> -<br>value |
|------------------------------------------------------------------|----------------------------------|---------------------------------------|----------------------------------------------------|-----------------------------------------------------------------|-------|---------------------|
| Total serum IgE<br>[ng/ml]                                       | 50.2 <sup>a</sup>                | 623.4 <sup>c</sup>                    | 450.3 <sup>b</sup>                                 | 330.8 <sup>b</sup>                                              | 89.4  | <0.01               |
| $\beta$ -conglycinin-specific<br>IgE level [OD <sub>450</sub> ]  | 0.272 <sup>a</sup>               | 0.275 <sup>a</sup>                    | 0.825 <sup>c</sup>                                 | 0.437 <sup>b</sup>                                              | 0.048 | <0.01               |
| Histamine content in<br>the jejunum [ $\mu$ g/g]                 | 7.15 <sup>a</sup>                | 13.44 <sup>c</sup>                    | 12.43 <sup>c</sup>                                 | 10.29 <sup>b</sup>                                              | 0.66  | <0.01               |
| Histamine release<br>ratio [%]                                   | 41.3 <sup>a</sup>                | 75.2 <sup>b</sup>                     | 74.2 <sup>b</sup>                                  | 60.3 <sup>b</sup>                                               | 5.64  | <0.01               |
| Cytokine levels in cultured splenocyte supernatant [ $\mu$ g/ml] |                                  |                                       |                                                    |                                                                 |       |                     |
| Interferon- $\gamma$                                             | 651.2 <sup>a</sup>               | 619.5 <sup>b</sup>                    | 620.1 <sup>b</sup>                                 | 640.0 <sup>ab</sup>                                             | 9.25  | 0.03                |
| Interleukin-4                                                    | 420.6 <sup>a</sup>               | 447.5 <sup>b</sup>                    | 444.8 <sup>b</sup>                                 | 432.6 <sup>ab</sup>                                             | 7.12  | 0.02                |

Notes: Means in the same row not sharing the same superscript letter are significantly different ( $p < 0.05$ ).

### 3.3. Total serum IgE and $\beta$ -conglycinin-specific IgE levels

To evaluate  $\beta$ -conglycinin-induced anaphylactic reactions and to explore the possible effects of lipoic acid on the development of allergy in sensitised rats, total IgE and  $\beta$ -conglycinin-specific IgE levels in the rat serum were determined. As shown in Table 4, on day 31 the total serum IgE and  $\beta$ -conglycinin-specific IgE concentrations were significantly elevated ( $p < 0.05$ ) in the  $\beta$ -conglycinin-treated Group III. Supplementation with a low dose of lipoic acid (Group IV) reduced serum  $\beta$ -conglycinin-specific IgE compared with the unsupplemented  $\beta$ -conglycinin-treated Group III ( $p < 0.05$ ).

### 3.4. Histamine content and histamine release ratio

The histamine content in the jejunum of the  $\beta$ -conglycinin-sensitised and ovalbumin-sensitised rats (Groups III and II) were significantly ( $p < 0.05$ ) higher compared to values of the control Group I (Table 4). However, treatment with lipoic acid (Group IV) significantly ( $p < 0.05$ ) lowered the histamine content compared with the untreated  $\beta$ -conglycinin-sensitised Group III.

Similar to the ovalbumin sensitised Group II, the histamine release ratio for the  $\beta$ -conglycinin sensitised Group III was significantly increased compared with values from the control group. Supplementation with lipoic acid (Group IV) did not suppress histamine release from mast cells significantly (Table 4).

### 3.5. Cytokine levels in the cultured splenocyte supernatant

To explore whether  $\beta$ -conglycinin treatment altered Th1/Th2 cytokine concentrations after sensitisation with  $\beta$ -conglycinin, interleukin-4 and interferon- $\gamma$  were measured in the cultured spleen cells. The results showed that interferon- $\gamma$  levels of rats sensitised with either  $\beta$ -conglycinin (Group III) or ovalbumin (Group II) were significantly lower, while interleukin-4 levels were higher, when compared with the control group (Table 4). Supplementation with lipoic acid (Group IV) did not significantly reverse the bias of interferon- $\gamma$  and interleukin-4 compared with the untreated sensitised Group III.

#### 4. Discussion

In the present study, the Sprague-Dawley rats were selected to establish a model for anaphylactic reactions induced by soybean  $\beta$ -conglycinin, with ovalbumin serving as the positive allergic control, which is well known as a source of food allergy (Diesner et al. 2008). Our results show that  $\beta$ -conglycinin-sensitised rats had reduced performance. Moreover, total serum IgE and  $\beta$ -conglycinin-specific IgE levels were significantly increased after sensitisation, which was associated with high histamine release in the rats from the sensitised group. This finding was similar to the results of the positive ovalbumin-allergic control, which indicated that a successful animal model for  $\beta$ -conglycinin sensitivity was established with the Sprague-Dawley rat. This model has many merits, including low costs and a short period of reproduction. In particular, it is good because high inclusion rates of soybean meal are used in compound feeds today and there are high physiological similarities between rats and farm animals (such as pig, chicken, turkey, calves). The rat model is validated for farm animals (Guo et al. 2007; Liu et al. 2008). A further advantage of this model is that it eliminates interference from other potential allergic components contained in soybean by using purified  $\beta$ -conglycinin.

Because of the widespread use of soybean in various food products (Friedman and Brandon 2001), the development of an effective method to reduce the allergenicity of soybean proteins would have major implications for the soybean industry. Therefore, the present study was also conducted to evaluate the effects of a low dose of lipoic acid on soybean  $\beta$ -conglycinin-induced food allergy in livestock.

Our results showed that feeding a low dose of lipoic acid to  $\beta$ -conglycinin-sensitised rats did not significantly attenuate the growth depression caused by  $\beta$ -conglycinin, but significantly improved the morphological structure of the small intestine (increased villus height,  $p < 0.05$ ). It appears that lipoic acid can suppress the production of serum  $\beta$ -conglycinin-specific IgE antibody, and reduce the histamine content in the jejunum, which suggest that lipoic acid inhibits the activation of cultured intestinal mast cells after  $\beta$ -conglycinin sensitisation. Based on this, it would appear that a low dose of lipoic acid has considerable potential to be used as an immunomodulator to prevent soybean  $\beta$ -conglycinin induced allergies.

In a previous study conducted in our laboratory using pigs, we showed that supplementation of a mega-dose of vitamin C attenuated glycinin-induced anaphylactic reactions to a certain degree (Sun et al. 2009). In the current study, the results show that a low dose of lipoic acid was also effective in suppressing the production of serum  $\beta$ -conglycinin-specific IgE antibody, inhibiting the activation of the cultured intestinal mast cells. We suggested that the novel additives, which function as an electron donor to protect the body from radicals and pollutants, could be effective immunomodulators that attenuate  $\beta$ -conglycinin-induced anaphylactic reactions even other food allergies. These findings indicate new approaches to reduce histamine release and prevent IgE-mediated food allergies.

In addition to being able to attenuate  $\beta$ -conglycinin induced allergy, recent studies have shown that lipoic acid can intervene in anaphylaxis (especially allergic asthma), contact dermatitis and early paediatric allergies in infants/children (Lam 2005; Bergqvist-Karlsson et al. 2006; Lee et al. 2008), as well as slow down the progression of some kinds of autoimmune diseases such as primary biliary cirrhosis

and collagen-induced arthritis (Bruggraber et al. 2003). However, the pathways and mechanisms of lipoic acid action that lead to these phenotypic features have not been fully elucidated.

To explore potential mechanisms, an analysis for cytokines in the cultured splenocyte supernatant was performed. It has been well documented that food-induced hypersensitivity is a predominantly Th2-type immune response (Van Wijk and Knippels 2007), in which the Th2-type representative cytokine interleukin-4 plays a key role in allergic inflammation by favouring the production of total IgE and antigen-specific IgE antibodies (Prescott and Hogan 2006). Besides, Snapper and Paul (1987) reported that interferon- $\gamma$  functioned reciprocally on the induction of IgE responses and histamine release, thus alleviating inflammation *in vivo*. However, our study indicated that lipoic acid supplementation did not significantly increase interferon- $\gamma$  or decrease interleukin-4, which suggested that the anti-allergy effect of lipoic acid might not be mediated by the reversing the bias of Th1/Th2 cells differentiated from T cells in the allergic subjects in response to  $\beta$ -conglycinin-induced anaphylaxis. Lee et al. (2008) found that lipoic acid inhibited tumour necrosis factor- $\alpha$  induced NF-kappa B activation, which suggested that the anti-histamine effect of lipoic acid might be mediated by another signal pathway, such as NF-kappa B.

In conclusion, it would appear that a low dose of lipoic acid has the potential to be used as an immunomodulator to prevent soybean  $\beta$ -conglycinin induced allergies. Further research on lipoic acid will be performed to elucidate the mechanism of the anti-anaphylaxis effects.

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