

Effects of β -conglycinin on growth performance, immunoglobulins and intestinal mucosal morphology in piglets

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One of the main causes of allergic reactions in young animals is β -conglycinin, an antigenic glycoprotein found in soya beans. Therefore, the objective of the study was to investigate the effects of a prior immunisation with β -conglycinin on growth performance, serum immunoglobulin levels and intestinal histology in piglets. Forty piglets (7 d of age) were randomly divided into four groups of ten piglets each. Piglets of Groups Im and Im+S were immunised twice by hypodermic injection with β -conglycinin at 500 μ g/kg body weight (BW) at day 7 and 21 of age. At day 23, Groups Im+S and S were intramuscularly injected with 5000 μ g β -conglycinin per kg BW. The piglets of Group C received a physiological saline solution by hypodermic injection. All piglets were weaned at the age of 23 d and blood samples were taken on days 7, 21 and 35. At the end of the trial, five piglets per group were slaughtered and the intestine was collected for evaluating mucosal histology. Compared to Group C, in Group S the average daily gain (ADG), feed intake and gain:feed ratio were decreased ($p < 0.01$), and serum levels of IgG and IgE were increased ($p < 0.01$). Furthermore, in this group the structure of duodenal and jejunal mucosa was severely damaged. But in Groups Im and Im+S the ADG was increased ($p < 0.05$), serum IgE levels were decreased ($p < 0.01$) and the intestinal mucosa was not damaged. The results suggest that prior immunisation with β -conglycinin can increase ADG and serum IgG levels and decrease serum IgE levels. Therefore, this method is also potentially able to protect the structural integrity of the intestinal mucosal epithelia and alleviate allergic reactions in piglets.

Keywords: allergens; histology; immunoglobulins; mucosa; performance; piglets; soya bean protein

1. Introduction

Soya beans have a high-quality protein and exhibit good nutritional, processing and functional properties. Therefore, it can be found in a wide range of food products (Golubovic et al. 2005; Sun et al. 2013). However, soya beans are also a dietary source of allergic reactions. Previous investigations revealed that soya bean protein can cause allergic reactions for instance in humans (Foucard and Malmheden Yman 1999; Niggemann et al. 1999), piglets (Li et al. 1990; Li, Nelssen, Reddy, Blecha, Klemm, Goodband, et al. 1991; Sun, Li, Dong, et al. 2008; Xu et al. 2010), calves (Lallès et al. 1996) and lambs (Ouédraogo et al. 1998). β -Conglycinin has been identified as one of the major allergenic proteins in soya beans, which is a primary storage protein in soya beans (Lallès et al. 1999). It was shown that β -conglycinin causes changes in intestinal

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morphology and disorders of immune function, resulting in growth depression and diarrhoea in young animals (Dréau et al. 1994; Qiao et al. 2003; Sun, Li, Li, et al. 2008; Hao et al. 2009). The antigen stimulates the immune system to generate secretory IgA and serum IgA, IgG, IgM and IgE (Dréau et al. 1995). In animals suffering from soya bean allergy mucosal lesions of the gastrointestinal tract are observed typically. Symptoms can be seen from abnormal bowel patterns, including accelerating crypt cell mitosis, plasma protein leakage into the intestine, goblet cell mucus exudation, intestinal villous atrophy, cell fusion and apoptosis due to allergy in piglets induced by β -conglycinin (Li, Nelssen, Reddy, Blecha, Klemm, Giesting, et al. 1991; Guo et al. 2007; Chen et al. 2011).

In order to eliminate the allergic potential of soya bean products, various methods were used to process soya beans, and even efforts were made to modify the gene structure of soya bean (Aoyama et al. 2001; Golubovic et al. 2005; Liu et al. 2010). So far, there are few reports about allergic reactions in piglets induced by soya bean antigen proteins which are alleviated with an immunising method in advance. With this method we attempted to evaluate the effects of β -conglycinin on potential immune protection, exploring a new way of preventing the allergic reaction induced by soya bean antigen protein in weaned piglets.

2. Material and methods

2.1. Characterisation of β -conglycinin

Purified β -conglycinin powder was kindly donated by Professor Shuntang Guo from the Food Institute of China Agricultural University (Patent number, 200,410,029,589.4, China). After lyophilisation, the protein content was determined and β -conglycinin purity was analysed according to a previous report (Hao et al. 2009). The purity of β -conglycinin was 90.0%.

2.2. Piglets and diets

Forty cross-bred piglets [(Duroc $\times$ Landrace) $\times$ Yorkshire] with an initial body weight (BW) of 2.98 ($\pm$ 0.07) kg were selected from four litters in the Antai Agricultural Group in Anhui (China) and all procedures were approved by the Anhui Agricultural University Animal Care and Use Committee. Ten piglets were kept in each pen, which was equipped with a feeder and a waterer to allow *ad libitum* access to water and feed. The piglets were housed in a mechanically ventilated nursery room with a lighting cycle of 12 h light and 12 h darkness, the temperature was maintained at 26–28°C and the relative humidity was set at 50–60%.

During the experiment the diets were provided in two phases, from day 7 to 22 and from day 23 to 35. The diets for the pre-weaning and post-weaning piglets (Table 1) were formulated to meet or exceed nutrient requirements suggested by the National Research Council (1998).

Feed consumption and BW of individual pigs were determined at weaning (23 d of age) and at the end of the trial (35 d of age) in order to calculate average daily gain (ADG), average daily feed intake (ADFI) and the gain:feed ratio ($G:F$).

Table 1. Ingredients and nutrient contents of experimental diets.

	Experimental diets	
	Pre-weaning (Day 7–22)	Post-weaning (Day 23–35)
Ingredients [%]		
Corn	–	60.85
Dried whole milk	46.00	–
Skimmed milk power	42.20	–
Soya bean meal (expanded) [†]	–	25.00
Whey powder	10.50	5.00
Fish meal	–	5.00
Calcium hydrogen phosphate	–	2.20
Limestone	–	0.69
Bran	–	0.37
Salt	0.30	0.25
Premix*	1.00	0.49
Choline chloride	–	0.15
Total	100.00	100.00
Chemical analysis [#]		
Crude protein [% of DM]	27.9	20.8
Calcium [% of DM]	1.04	0.64
Phosphorus [% of DM]	0.89	0.51
Lysine [% of DM]	1.61	1.06
DE [MJ/kg] (calculated)	14.5	13.5

Notes: [†]Expanded for eliminating the antigen proteins; *Provided per kg diet (pre-weaning/post-weaning): vitamin A, 7000/5250 IU; vitamin D₃, 2000/1050 IU; vitamin E, 10/4.5 IU; vitamin K₃, 2.2/31.2 mg; vitamin B₁, 2.375/0.375 mg; vitamin B₂, 4.8/1.8 mg; vitamin B₆, 0.15/0.15 mg; vitamin B₁₂, 17.5/7.5 µg; niacin, 16/6 mg; Ca-pantothenate, 5.75/3.75 mg; folic acid, 0.85/0.15 mg; biotin, 17.5/7.5 µg; lysine, 0.95/0.75 mg; antioxidant, 45/45 µg; enzyme preparation, 1100/1000 mg; flavour agents, 45/40 mg; sweet agents, 45/40 mg; neomycin, 0/20 mg; Cu, 15/15 mg; Fe, 144/144 mg; Zn, 110/110 mg; Mn, 20.18/10.18 mg; I, 0.4/0.4 mg; Se, 0.35/0.30 mg; [#]Analysed values.

2.3. Experimental design and sample collection

All the piglets (7 d of age) were randomly divided by weight and gender into four groups and ten piglets per group. The piglets of the control group (Group C) were injected with physiological saline solution by hypodermic injection. At the age of 7 d and 21 d, two further groups of piglets (Groups Im and Im+S) were immunised twice by hypodermic injection with the same dose of 500 µg β -conglycinin per kg BW (Xu et al. 2010). Furthermore, the piglets of Group Im+S received an intramuscular injection of β -conglycinin at 5000 µg/kg BW when they were 23 d old. The piglets of Group S were not immunised in advance and were directly sensitised with 5000 µg β -conglycinin per kg BW by intramuscular injection at the age of 23 d. All piglets were weaned at the age of 23 d.

From all piglets blood samples were collected via anterior vena cava on days 7, 21 and 35. The serum samples for analysis of immune function were obtained after centrifugation at 2000g for 15 min at 4°C and analysed immediately. At the end of the trial, five piglets from each group were slaughtered by an intracardiac injection of sodium pentobarbital (50 mg/kg BW) followed by jugular exsanguination. Standard histological procedure was performed as previously described with some slight modifications (Li et al. 1999). Briefly, longitudinal sections of duodenum, jejunum and ileum tissues (3 cm obtained from the proximal duodenum, middle jejunum and proximal ileum) were collected and flushed by

physiological saline solution to remove the gut contents. The intestinal tissues were then fixed in 3–5 volumes of 4% phosphate-buffered formaldehyde (pH 7.2) and embedded in paraffin.

2.4. Determination of serum IgA, IgM, IgG antibody levels

The serum IgA, IgM, IgG antibody levels were obtained by immunoglobulin A, M, G kit (Shanghai Fosun Long March Medical Science Co., Ltd) and measured by a semi-automatic biochemistry analyser (GF-D200, Shandong, China), according to the manufacturer's instructions.

2.5. Analysis of total IgE antibody levels by ELISA

The serum IgE antibody levels were determined by using the swine Enzyme-Linked Immunosorbent Assay Kit (ELISA kit; Rapidbio Company, CA, USA) and the ELISA reader was a microplate reader (BIO-RAD 680, Hercules, CA, USA) as described by the manufacturer.

2.6. Investigation of the intestinal mucosal histology

Different tissues prior to this fixed were examined by histological paraffin section. Six cross sections were obtained from each formalin-fixed segment and processed for histological examination using the standard haematoxylin and eosin method. Villus height and crypt depth were measured according to Wu et al. (1996) under a light microscope (CK-40, Olympus Co., Japan). Ten measurements of villus height and crypt depth were taken from each section on randomly selected microscopic fields. The histological analysis was performed by an investigator who was unaware of the origin of tissue sections.

2.7. Chemical analysis

Samples of all feeds were analysed for their nitrogen, calcium and total phosphorus contents using the methods of the AOAC (1990). Nitrogen was analysed using the Kjeldahl method (AOAC official method 988.05), calcium by titration with 0.1 N KMnO₄ (AOAC official method 927.02), and the total phosphorus was determined colourimetrically using a molybdovanadate reagent (AOAC official method 965.17). Lysine contents in feed were determined using high-performance liquid chromatography (Shimadzu LC 10 Liquid Chromatograph, Kyoto, Japan).

2.8. Statistical analysis

All data were analysed using the general linear model procedures of SAS system (SAS 2002; Version 9.1.3, SAS Institute, Inc., Cary, NC, USA). Data were presented as means $\pm$ standard deviation. The differences between the groups were examined using analysis of variance (ANOVA). Differences between means were considered statistically significant for values of $p < 0.05$. The statistical software Graph Pad Prism version 5.01 (Graph Pad Software, San Diego, California, USA) was used in all histograms.

Table 2. Growth performance of the experimental groups ($n = 10$).

	Experimental groups			
	Control (C)	Immunised (Im)	Immunised + sensitised (Im+S)	Sensitised (S)
Average daily gain [g/d]	320 ^{Ab}	362 ^{Aa}	353 ^{Aa}	191 ^B
Average daily feed intake [g/d]	461 ^A	489 ^A	492 ^A	370 ^B
Gain:feed [g/g]	0.694 ^a	0.740 ^a	0.717 ^a	0.516 ^b

Notes: Means not sharing the same superscript are significantly different. The indicated significance level of capital letters and lower case letters is $p < 0.01$ and $p < 0.05$, respectively.

3. Results

3.1. Growth performance

The effects of soya bean β -conglycinin on ADG, ADFI and $G:F$ are shown in Table 2. Compared with Group C, the ADG in Groups Im and Im+S was increased ($p < 0.05$), but ADG in Group S was significantly decreased ($p < 0.01$). In Group S, the ADFI and $G:F$ were significantly reduced compared with Group C ($p < 0.01$), while no differences were observed among Groups C, Im and Im+S.

3.2. IgA, IgM, IgG antibody levels

The immunoglobulin data are presented in Table 3. Compared with Group C, the serum IgG levels of piglets were significantly increased ($p < 0.01$) in Groups Im and Im+S on day 21 and 35. On day 35, in Group S the serum IgG levels were higher than in all other groups ($p < 0.01$). There were no significant differences in the IgA and IgM antibody levels among the groups throughout the experiment.

Table 3. Serum immunoglobulin levels of the experimental groups ($n = 10$).

		Experimental groups			
		Control (C)	Immunised (Im)	Immunised + sensitised (Im+S)	Sensitised (S)
IgG [g/l]	7	2.10 $\pm$ 0.21	2.21 $\pm$ 0.37	2.10 $\pm$ 0.21	2.28 $\pm$ 0.22
	21	1.96 $\pm$ 0.06 ^A	2.25 $\pm$ 0.10 ^B	2.23 $\pm$ 0.12 ^B	1.98 $\pm$ 0.06 ^A
	35	1.20 $\pm$ 0.04 ^A	1.55 $\pm$ 0.12 ^B	1.52 $\pm$ 0.09 ^B	1.75 $\pm$ 0.10 ^C
IgA [mg/l]	7	311 $\pm$ 38	313 $\pm$ 130	305 $\pm$ 164	313 $\pm$ 34
	21	313 $\pm$ 74	375 $\pm$ 72	373 $\pm$ 74	320 $\pm$ 74
	35	538 $\pm$ 43	560 $\pm$ 47	569 $\pm$ 49	602 $\pm$ 66
IgM [mg/l]	7	845 $\pm$ 99	896 $\pm$ 102	886 $\pm$ 118	814 $\pm$ 75
	21	482 $\pm$ 84	515 $\pm$ 99	510 $\pm$ 50	490 $\pm$ 56
	35	166 $\pm$ 28	183 $\pm$ 29	176 $\pm$ 15	184 $\pm$ 46

Note: Means not sharing the same superscript are significantly different at $p < 0.01$.

3.3. Total IgE antibody levels

At the age of 7 and 21 d, the IgE levels of experimental groups were not different (Figure 1). But on day 35, compared to Group C the serum IgE levels in Group S were significantly increased ($p < 0.01$) and in Groups Im and Im+S they were significantly decreased ($p < 0.01$). No significant differences were observed between Groups Im and Im+S.

3.4. Intestine mucosa epithelium histology

As shown in Figures 2, 3 and 4, the structure of intestinal mucosa was damaged at varying degrees, intestinal villus was shortened obviously, crypt was descended and lots of red cells and eosinophils existed in intestinal lamina propria in piglets from Group S. Intestinal villus was adhered to one another, mucosal epithelium was atrophied and a few red cells and eosinophils existed in intestinal lamina propria in Group C. Intestinal mucosal epithelium was clear and lined up in order, the structure of epithelium was intact and the nucleus was clear in Group Im+S.

To investigate the effect of β -conglycinin on intestinal mucosal reactions in piglets, the villous height and crypt depth were determined (Figures 5 and 6). Compared with Group C, in Groups Im and Im+S the villous height in duodenum and jejunum were enhanced ($p < 0.01$), and the crypt depth were reduced ($p < 0.01$). At the same time, in Group S the villous height in duodenum and jejunum was reduced ($p < 0.01$), and the crypt depth was enhanced ($p < 0.01$). In the ileum of piglets no significant differences between villous height and crypt depth were observed.

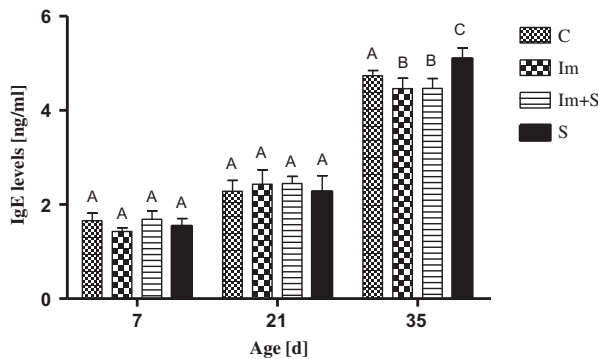


Figure 1. Total serum IgE levels (means $\pm$ SD; at the same time of age bars with different capital letters indicate significant differences at $p < 0.01$, $n = 10$).

Notes: C, Control group; Im, Immunised group; Im+S, Immunised and sensitised group; S, Sensitised group.

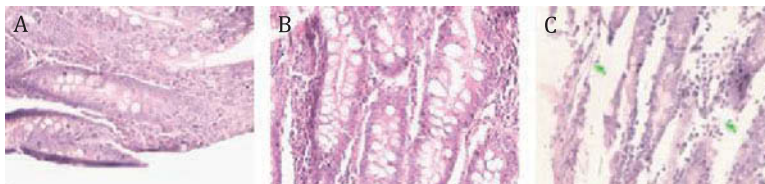


Figure 2. Histology of duodenal mucosal epithelium in experimental groups. HE 10×40 .

Notes: A. Group C: Duodenal villi are adhered to one another. B. Group Im+S: The structure of duodenal villi is intact and lots of goblet cells existed. C. Group S: duodenal villi are fractured and the number of goblet cells is reduced.

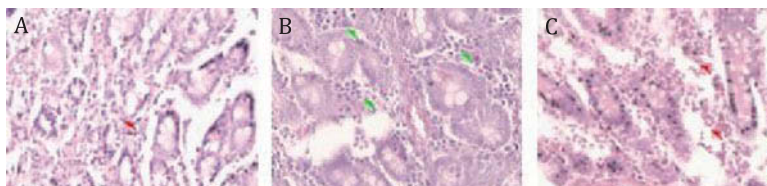


Figure 3. Histology of duodenal lamina propria in experimental groups. HE 10×40 .
Notes: A. Group C: There are a few eosinophils and lymphocytes in duodenal lamina propria. B. Group Im+S: A number of lymphocytes existed in duodenal lamina propria. C. Group S: There are lots of eosinophils in duodenal lamina propria.



Figure 4. Histology of jejunal mucosa in experimental groups. HE 10×40 .
Notes: A. Group C: The structure of jejunal villus is loose and the central lacteal is fuzziness; there are a lot of red cells in lamina propria. B. Group Im+S: The connective tissue and surface mast cells are tightly jointed in jejunal lamina propria and the central lacteal can be observed clearly. C. Group S: In jejunal lamina propria the structure of connective tissues is loose and even parts of tissues disappeared and lots of red cells exist.

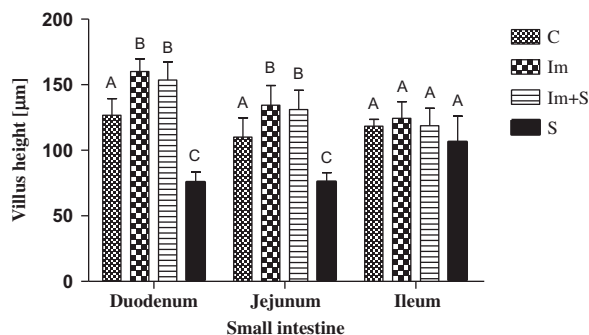


Figure 5. Comparisons of villus height in the small intestine (means $\pm$ SD; within an intestinal section bars with different capital letters indicate significant differences at $p < 0.01$, $n = 5$).
Notes: C, Control group; Im, Immunised group; Im+S, Immunised and sensitised group; S, Sensitised group.

4. Discussion

At present, the research on the effects of soya bean protein on the performance and immune function in animals has been intensified. It has been shown that high levels of soya bean protein (including β -conglycinin) depress growth, destroy the integrity of the intestine and suppress immune function in young animals (Lallès et al. 1996; Burrells

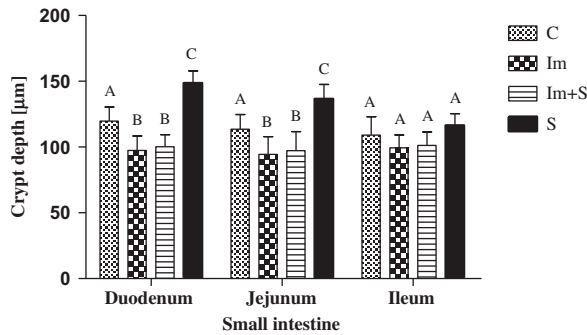


Figure 6. Comparisons of crypt depth in the small intestine (means $\pm$ SD; within an intestinal section bars with different capital letters indicate significant differences at $p < 0.01$, $n = 5$). Notes: C, Control group; Im, Immunised group; Im+S, Immunised and sensitised group; S, Sensitised group.

et al. 1999; Minehira et al. 2000). In this study, a swine model was established for inducing an allergic reaction to investigate the allergenic properties of soya bean proteins. It was found that in Group S the performance of piglets was significantly reduced. This effect referred to ADG, ADFI and $G:F$ as well. On the contrary, in Groups Im and Im+S the ADFI and $G:F$ were not decreased and ADG was significantly increased compared to the control. The results showed that high levels of β -conglycinin declined the growth performance of piglets, yet the immunisation of β -conglycinin in advance can alleviate allergic reactions effectively.

In the study of Dréau et al. (1995) it was found that the blood of their experimental piglets with diarrhoea contained high levels of anti-soya-bean-protein antibodies, mainly IgG. In the present study, the serum IgG antibody levels were significantly increased in 23-d-old piglets sensitised by β -conglycinin. This indicated that β -conglycinin was absorbed into the blood through the intestinal epithelial cell and produced large quantities of IgG antibody in the serum, and they played an important role in the systemic immune response, which had been proved in several previous studies (Helm et al. 2002; Gizzarelli et al. 2006).

Gastrointestinal food allergy is a food-stimulated IgE-mediated event. Soya bean-allergic patients often have increased IgE antibodies in the sera (Shibasaki et al. 1980; Burks et al. 1988). IgE also plays a critical role in most food-induced allergies (Ladics et al. 2003). In the presented experiment, in piglets of Group S which were directly injected with a high dose of β -conglycinin, the serum total IgE levels were significantly increased. This indicated that a high dose of β -conglycinin could enhance the secretion of serum IgE, which was consistent with results reported by Sun, Li, Li, et al. (2008). However, the serum IgE levels were significantly decreased in piglets immunised in advance by β -conglycinin. This reaction was probably due to the antigen stimulated body to produce specific protective antibody IgG, and IgG was combined with the inhibitory receptor, the IgE levels inhibited and eliminated the allergic reactions (Sun, Li, Li, et al. 2008).

The damaged integrity of the intestine is a common phenomenon in animals consuming soya bean proteins. Li et al. (1990), Li, Nelssen, Reddy, Blecha, Klemm, Goodband, et al. (1991) reported that pigs had lower villus height but greater crypt depth after the ingestion of soya bean protein. Dréau et al. (1994) observed an indication of epithelial cell hyperplasia in pigs fed with antigenic soya bean products. In our study, β -conglycinin

caused various degrees of damage to the morphological structure of intestinal villi in weaned piglets. Similar observations were obtained by Chen et al. (2011), where β -conglycinin could induce intestinal villous atrophy. These results indicated that the occurrence of food allergy may correlate with immune responses in the intestinal mucosa. However, after the piglets were immunised with β -conglycinin in advance, the structural integrity of the intestinal mucosa was effectively protected.

5. Conclusions

This study showed that a direct sensitisation by β -conglycinin caused a decrease of growth performance, an increase of serum IgG and IgE levels, and it damaged intestinal mucosal integrity in piglets. However, after prior immunisation of piglets with β -conglycinin the allergic reactions induced by β -conglycinin were reduced. This effect includes reduced serum IgE levels, improved ADG and a protection of the intestinal mucosa. Because studies on immunisation with soya bean antigen proteins to reduce their influence on weaning piglets are rare, the specific immune mechanism is not very clear and further explorations are needed.

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