Gastrointestinal Tissue Distribution of β-Conglycinin in Pigs at Different Growth Stages

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Abstract Soybean allergens may cross the gastrointestinal tissue and induce allergy, and their gastrointestinal tissue distribution could provide the basis of the allergic mechanism to some extent. But the relevant literatures for β-conglycinin are scarce. In the current study, the variation of β-conglycinin in gastrointestinal tissue *in vivo* was investigated using pigs as animal model in order to compare the distribution differences of β-conglycinin between the growth stages. Fifteen General No.1 barrows weaned on the 28th day were selected to carry out the animal experiments of three ages including weanling, growing and finishing stage. Pigs were fed diets with 4% purified β-conglycinin in experimental periods. The immunohistochemistry method was performed to detect the gastrointestinal distribution of β-conglycinin. The results indicated that there was a significant difference on the gastrointestinal mucosal distribution of β-conglycinin between pigs of different ages. The β-conglycinin went up from stomach to dedodenum, then slightly dropped until proximal-jejunum, and kept to rise until ileum for piglets ($P<0.001$). It from stomach to distal-jejunum increased slowly, but fell sharply for growers and finishers ($P<0.001$). The highest content of β-conglycinin was in the deodenum and ileum for piglets, and in distal-jejunum for growers and finishers ($P<0.05$). The β-conglycinin in intestinal villi and mucosa had similar distribution variation. The distribution of β-conglycinin in intestinal villi and crypt were not affected by growth phase.

Keywords: β-conglycinin, gastrointestinal tissue, pigs, growth stages, distribution

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1. Introduction

Soybean is a high-quality protein source for humans and animals. [1] However, the inflammatory allergenic components, also presented in soybean, have induced the serious clinical allergic symptoms ranging from skin, gastrointestinal distress to life-threatening asthma and death. [2,3,4,5,6] β-conglycinin, a primary storage protein accounting for about 30% of total storage protein in soybeans, [7] has been identified to bind IgE in soy allergies [8,9].

Studies on soybean allergy have been developed primarily in children and young animals.[4] Currently, some light had been shed on the sensitivity to soybean allergens during their different growth stages, which indicated that the immunoreactivity variation of allergens *in vivo* depends on the age. [10,11] Moreover, more mature animals did not show the allergic reaction to soybean. [12] So it is significant to compare the differences of sensitivity to β-conglycinin for humans and animals at different growth stages. Soybean allergens were digested

in the gastrointestinal tract, and absorbed through the gastrointestinal tissue, then induced allergic reactions. Their gastrointestinal tissue distribution could reflect the possibility of allergy occurrence to some extent, so it is vital to confirm the variation of this process. The gastrointestinal variation of immunoreactive β-conglycinin was explored in our previous reports using pigs as animal model. [10,13] The gastrointestinal tissue distribution of glycinin has also been investigated in our previous studies, [14] but β-conglycinin was still not developed.

In present study, the gastrointestinal tissue distribution of β-conglycinin was detected by the immunohischemical staining using pigs at different ages as animal model. The objective of the study was to compare the effects of growth stages on the gastrointestinal tissue distribution of β-conglycinin. Our work may provide further information on the lack of soybean allergy for adults and mature animals, which is an important implication on the mechanism of soybean-induced hypersensitivity.

2. Materials and Methods

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2.1. Preparation of β-conglycinin

Purified β-conglycinin suspension containing more than 85% β-conglycinin in diets were kindly donated by Professor Shuntang Guo at the Food Institute of China Agricultural University (Patent number, 200410029589.4, Beijing,China).

2.2. Production of Polyclonal Antibody

β-Conglycinin samples of over 95% purity as immunogens were isolated and purified from defatted soy flour by the method of Setsuko et al. (1987). [15] Polyclonal antibody against β-conglycinin was produced and purified as described in our previous study [13].

2.3. Animals, Diets and Experimental Design

Animals were maintained according to the rules of China Animal Care and use Committee. The experiment using pigs from the same herd was conducted in the Jilin University Experimental Pig Farm (Changchun, China). Fifteen healthy General No.1 barrows with an average initial body weight of 7.06± 0.5 kg, weaned on the 28th day, were randomly allotted to three groups, each group with five replicates. All pigs received diets without ingredients originating from soybean products in non-experimental periods; while the pigs received diets containing 4% purified β-conglycinin in experimental periods according to the report of Sun et al. (2008). [16] After 3 days of adaptation, the experimental periods of each group started, and lasted for 7 days. The experiment in each group was experienced from 30 to 37 days (weaning piglets), from 98 to 105 days (growing pigs) or from 168 to 175 days (finishing pigs), respectively. Pigs were fed supplements without leguminous products during the suckling period in order to adapt to this routine. The composition and nutrient content of the diets are shown in Table 1. The diets were formulated to meet NRC (1998) requirements on an isoenergetic and isonitrogenous basis.

Table 1. Ingredient composition and nutrient levels of the diets

Body weight of pigs (kg)	Experimental periods					Experimental periods		Experimental periods
	3-5	5-10	5-10	10-20	20-60	20-60	60-120	60-120
Ingredients(%)								
Maize	13.00	22.00	21.00	70.65	74.85	73.00	77.50	75.00
Fish meal	13.00	11.20	8.10	13.00	8.80	6.00	6.50	3.75
Corn gluten meal	—	—	—	9.00	9.00	9.50	6.50	3.80
Wheat bran	—	—	—	—	5.00	4.87	7.20	7.80
Whey powder	13.55	18.00	18.00	2.00	—	—	—	—
Milk powder	58.00	48.00	48.00	4.40	—	—	—	—
β-conglycinin	—	—	4.00	—	—	4.00	—	4.00
Corn oil	—	—	—	—	1.00	1.00	1.00	1.10
Salt	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Vitamin premix1	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Vitamin premix2	0.20	0.20	0.20	0.15	0.15	0.15	0.15	0.15
Limestone	0.10	0.30	0.40	0.40	0.70	0.85	0.70	0.85
$CaHPO_4$	1.17	—	—	—	—	0.10	—	1.10
L-Lysine	0.68	—	—	0.10	0.20	0.23	0.15	0.15
Nutrient contents3 (%)								
Crude protein	26.37	23.92	23.67	20.82	17.99	17.96	15.73	15.66
Digestive energy (MJ/kg)	17.23	16.91	16.81	14.38	14.09	14.00	14.01	13.90
Calcium	0.92	0.86	0.78	0.70	0.62	0.59	0.53	0.51
Phosphorus	0.75	0.83	0.76	0.66	0.55	0.50	0.50	0.45
Lysine	1.53	2.00	1.94	1.17	0.96	0.95	0.79	0.76

[1] Vitamin premixprovided per kilogram of complete diet: vitamin A, 45,000 IU; vitamin D3, 10,000 IU; vitamin E, 60 mg; vitamin K3, 5 mg; vitamin B1, 2mg; vitamin B2, 15 mg; vitamin B6, 6 mg; vitamin B12, 0.05 mg; folic acid, 0.5 mg; biotin, 0.1 mg; niacin, 40 mg; pantothenic acid, 25 mg..
[2] Mineral premix per kilogram of complete diet: Cu, 25 mg; Fe, 50 mg; Zn, 160 mg; Mn, 40 mg; Se, 0.3 mg; I, 0.3 mg.
[3] Calculated value.

2.4. Sample Collection and PREPARATION

Pigs were anesthetized with excess procaine and slaughtered 1 hour after the morning meal at the end of the experimental periods. The abdomen was opened and the gastrointestinal tract removed from the gastroesophageal junction to the distal end of the ileum. The tissue samples of 3 cm in length from the stomach, the mid-duodenum, the proximal-jejunum, the mid-jejunum, the distal-jejunum and the ileum were collected immediately. The 3-cm intestinal segments were washed in saline, fixed in formalin, embedded in paraffin. The sections were cut with five-μm thick and stained by the immunohistochemistry for microscopic examination.

2.5. Immunohistochemical Staining

To detect the gastrointestinal distribution of β-conglycinin, we applied the labeled Streptavidin-Peroxidase complex method using a commercially available kit (Ultrasensitive Kit, KIT9706, Maixin, Fuzhou, China) with the binding of anti-β-conglycinin polyclonal antibody. Paraffin sections were exposed to microwave pretreatment (in 10-mmol/L citrate buffer, pH 6 at 850W for 20 min). The 1.25μg mL^{-1} of anti-β-conglycinin polyclonal antibodies was the optimal concentration in order to reduce the nonspecific reaction. Sections were then counterstained with hematoxylin, dehydrated, cleared, and permanently mounted. Negative

control sections were produced by omission of the primary antibody.

2.6. β-Conglycinin Quantity in Tissue

The β-conglycinin in the gastro-intestinal tissue were quantified by measuring the Integrated optical density (IOD) of the immunoreactive staining. The specific brown staining was scanned. The IOD at the site of mucosa, villus and crypt of the different gastrointestinal tissue were estimated using Image-Pro Plus 5.0 software. The control sections resulted in negligible background staining (data not shown).

2.7. Statistical Analysis

All data were analyzed using the general linear model procedure of Statistical Package for Social Sciences version 11.5 (SPSS Inc., Chicago, USA). *P*-values≤0.05 were considered statistically significant. Duncan's multiple range test was employed to test the differences among the means.

3. Results

3.1. Location of β-conglycinin

Positive staining of β-Conglycinin was distributed in the mucosa of stomach, duodenum, jejunum and ileum (Figure 1). Much β-conglycinin was observed in mucous epithelium and lamina propria. And most of it was located in the intestinal villi, but less in the crypt, central lacteal and connective tissue. There was no positive staining found in negative control (photo not shown).
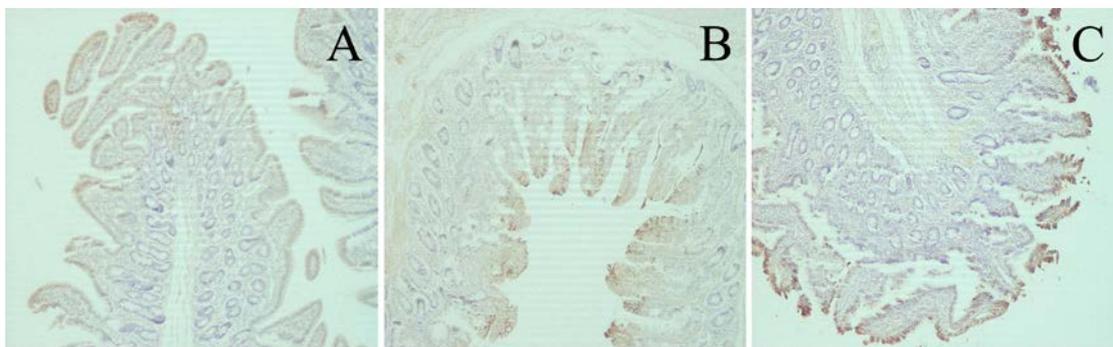

Figure 1. Location of β-Conglycinin (Arrows) in Distal- jejunum of Piglets (A), Growers (B) and Finishers (C) (80×)

3.2. Distribution of β-conglycinin in the Gastrointestinal Mucosa

The distribution of β-conglycinin in the gastrointestinal mucosa was significantly influenced by the growth phase (*P*<0.001) shown from Figure 2. The mucosal contents of β-conglycinin from proximal-jejunum to distal-jejunum increased slowly with the growth of age (*P*<0.001).

The mucosal content of β-conglycinin increased from stomach to duodenum then dropped and tended to go up but hardly changed between proximal-jejunum and distal-jejunum or between mid-jejunum and ileum in piglets (*P*<0.05). The mucosal content of β-conglycinin in growers and finishers was different from piglets, it increased slowly between the stomach and distal-jejunum but fell sharply in the ileum (*P*<0.05). Although the lowest content was in the stomach for pigs at different growth stages (*P*<0.05), the highest content of β-conglycinin in the gastrointestinal mucosa was in the duodenum and ileum for piglets and in distal-jejunum for growers and finishers (*P*<0.05).
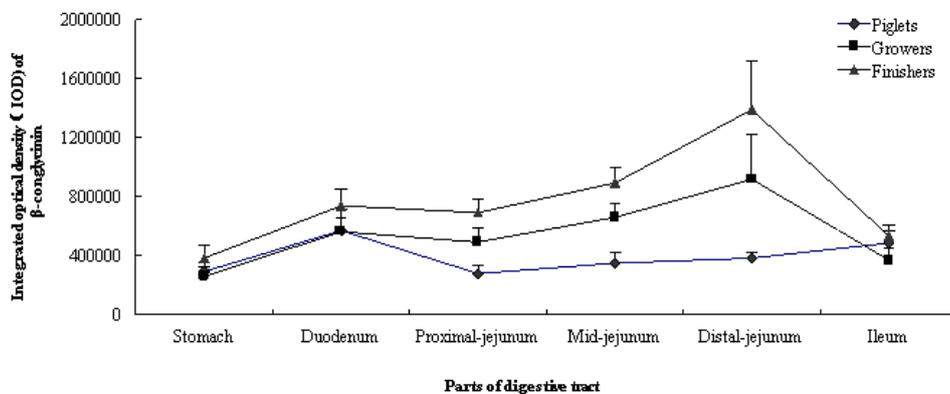

Figure 2. Distribution of β-Conglycinin in the different parts of gastrointestinal mucosa of piglets, growers and finishers

3.3. Distribution of β-conglycinin in the Small Intestinal Villi and Crypt

As presented in Figure 3, growth phase had no significant effects on distribution of β-conglycinin in intestinal villi (*P*=0.287). The distribution of β-conglycinin in intestinal villi of pigs at different growth phases was similar to that in intestinal mucosa. The β-conglycinin content in duodenum was more than jejunum and ileum for piglets (*P*<0.05), and it grew up slowly but did not result in a significant rise from proximal-jejunum

to distal-jejunum or from mid-jejunum to ileum ($P<0.05$). Different from piglets, the content of β-conglycinin for growers and finishers between the upper small intestine (duodenum and proximal-jejunum) showed no significant differences ($P>0.05$), and then increased steady until distal-jejunum but dropped drastically in the ileum ($P<0.05$).

Figure 4 showed that Growth phase and part of digestive tract had no significant effects on the content of β-conglycinin in the small intestinal crypt ($P>0.05$).
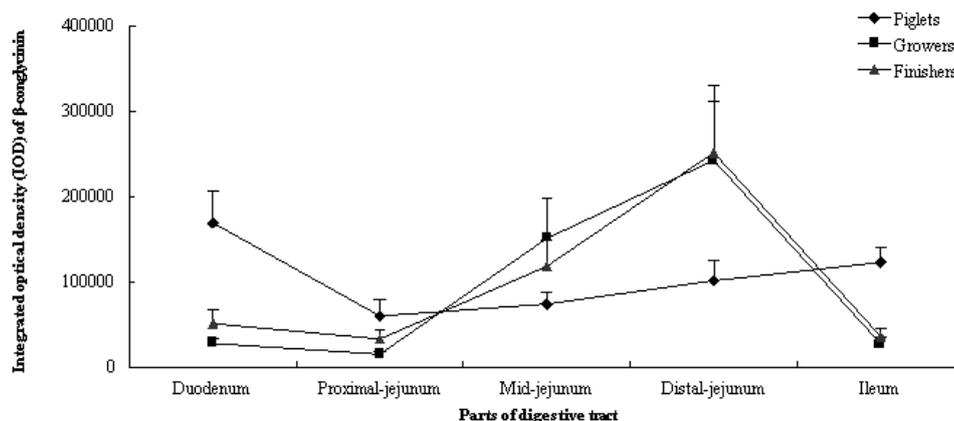

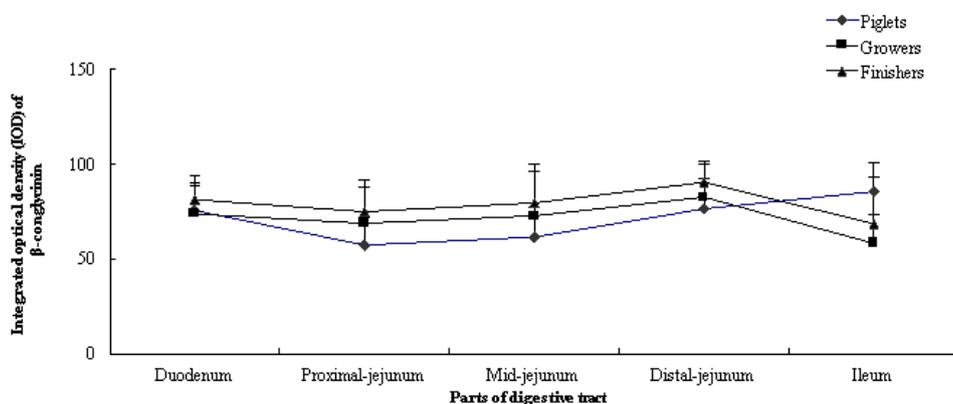**Figure 3.** Distribution of β-Conglycinin in the small intestinal villi of piglets, growers and finishers

Figure 4. Distribution of β-Conglycinin in the small intestinal crypt of piglets, growers and finishers

4. Discussion

A significant difference on the gastrointestinal mucosal distribution of β-conglycinin between pigs at different ages was found in this study, which mainly ascribe to their great discrepancy of digestive physiology. The lower gastroduodenal absorption of β-conglycinin for piglets might be caused by the immature digestive function, [17,19] the weaning stress [20,21] and the rapid stomach emptying time [17], and this status had been not changed until growers. With the increasing age, the mucosal distribution of β-conglycinin from jejunum to distal-jejunum increased slowly owing to the improved digestibility and absorbility of protein.

The data in this study indicated that the piglets presented a different distribution of β-conglycinin in the gastrointestinal mucosa compared with growers and finishers. Much β-conglycinin had occured in the ileum of piglets. It could be explained from two aspects. For one thing, the intestinal mast cells could be elevated by β-conglycinin [22,23] and activated by its specific IgE. This contributed to an excessive release of histamine, then might result in both the change of intestinal motility [24] and the occurrence of allergic symptoms. Since the mast cell numbers in the ileum were greater than in the proximal intestine (in the duodenum and jejunum) [16],

much release of histamine occurred in the distal intestine. Therefore, the β-conglycinin-induced allergy might enlarge the intestinal permeability, resulting in more β-conglycinin in the ileum. Moreover, many lymphoid patches are located in the distal-jejunum and the ileum, and the M cells, specialized epithelial cells, are found exclusively in the lymphoid follicle-associated epithelium (FAE) of Peyer's patches [25,26]. The M cells could transfer the allergens to the lymphoid cells of the *lamina propia*, and also disturb the permeability of the adjacent epithelium to allergic proteins. For another, the low mucosal contents of β-conglycinin in the ileum of grower-finisher pigs might be attributed to the little β-conglycinin in the ileal digesta.

Comparing with our previous report, [14] the gastrointestinal mucosal distribution variation of β-conglycinin was differed from glycinin. The present work clearly demonstrates the highest content of β-conglycinin in the gastrointestinal mucosa was in the duodenum and ileum for piglets, while glycinin was in the ileum. The stability to gastric digestion of β-conglycinin resulted in the higher protein residual in the stomach and the start of small intestine than glycinin, [10,27] which might give rise to a higher mucosal distribution of β-conglycinin in the duodenum compared with glycinin. In addition, the higher glycinin in the ileum than β-conglycinin could be explained by the limited hydrolysis of glycinin in the

small intestine due to the richness disulphide bonds and the hydrophobicity of basic polypeptides, [10] and this led to a increasing permeability for glycinin. The other distinction showed that the glycinin mucosal content of growers in the stomach and duodenum was higher than piglets, but no significant difference for β-conglycinin. Although the β-conglycinin from stomach to middle-jejunum tended to decreased with the growth of age, [10] the phenomenon of no distinct distribution between piglets and growers induced by β-conglycinin is non-existent to the best of our knowledge due to the relative scarcity of reports.

The villi could increase intestinal absorptive surface area, providing exceptionally efficient absorption of nutrients in the lumen. Therefore most of β-conglycinin was absorbed in the intestinal villi and the concentration of β-conglycinin in villi is far higher than that in crypt. So the β-conglycinin distribution variation of intestinal villi was similar to the intestinal mucosa.

5. Conclusion

The mucosal contents of β-conglycinin from stomach to distal-jejunum for pigs at different ages elevated slowly expect for piglets in the jejunum. The trend went up until ileum for piglets, but fell sharply for growers and finishers. The distribution variation of β-conglycinin in intestinal villi of pigs at different growth phases was similar to that in intestinal mucosa. The growth phase had no significant effects on distribution of β-conglycinin in intestinal villi and crypt.

Acknowledgments

Conflict of Interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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