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INFLUENCE OF GLYCININ AND B-CONGLYCININ OF SOYBEAN ON THE PROLIFERATION AND IMMUNE FUNCTION OF SUCKLING PIGLETS PERIPHERAL BLOOD MONONUCLEAR CELLS IN *IN VITRO* CULTURE

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ABSTRACT

This experiment studied the effect of different level of purified glycinin and β-conglycinin on proliferation and immune function of peripheral blood monomuclear cells (PBMC) of suckling pigs in vitro. The results indicated that the amount of cells in the group of glycinin and β-conglycinin increased gradually. The cell 3-(4,5)-dimethylthiahiazo (-z-yl)-3,5-di-phenytetrazolumromide (MTT) absorption at 570 nm wavelength was significantly affected by glycinin and β-conglycinin concentration (P<0..01). β-conglycinin had a significant positive correlation with interleukin -2, interleukin-6, interferon-γ and immune globulin A. In summary, glycinin and β-conglycinin could promote PBMC of suckling pigs to transform and proliferate *in vitro* culture, β-conglycinin could promote PBMC of suclking pigs to produce cytokine with IL-2, INF-γ, IL-6 and raise the level of immunoglobulin. The results showed that the antigen of β-conglycinin was stronger than that of glycinin.

Key Words: immune function, peripheral blood mononuclear cells, piglet, proliferation, soybean.

INTRODUCTION

Because of its well-balanced composition of amino acids and good physicochemical properties, soybeans are widely utilized in diets fed to humans and animals (Friesen *et al.*, 1993, Hancock *et al.*, 2000). However, soybean induced allergic symptoms have been recognized as a growing problem, especially in young animals, (Ballmer *et al.*, 2007, Friesen *et al.*, 1993) leading to intestinal injury (Barratt *et al*., 1978). Glycinin, also called 11S protein, accounts for more than 40% of the total soybean protein. It is composed of five subunits, each containing anacidic and a basic polypeptide linked by a disulfide bond (Golubovic *et al.*, 2005), β-Conglycinin(7S protein) is a trimeric glycoprotein with a molecular weight of 150–210 kDa. Its subunits, a (57–76 kDa), a' (57–83 kDa), and β(42–53 kDa) (Garcia *et al*. 1997) can be combined forming homotrimers or heterotrimers (L'Hocine *et al*. 2007). Available evidence suggests that glycinin contributes to a series of allergic reactions, damage to intestinal morphology, disorder of immune function, growth depression and diarrhea in piglets (Dreau *et al*., 1994, Li *et al*., 1990, Li *et al*., 1991). At the same time, β-conglycinin plays important roles in hypersensitive responses. When calves and piglets are fed β-conglycinin, transient hypersensitivity usually develops (Lalle`s *et al*., 1996, L'Hocine *et al*., 2007). Therefore, the use of soybean and soybean products containing β-conglycinin bears certain risks in feed of animals.

A Peripheral Blood Mononuclear Cell (PBMC) is a blood cell which have a round nucleus. To date, there is insufficient evidence linking soybean allergy with the alteration in PBMC numbers and immune function. Therefore, we hypothesized that there may be allergic reactions after added soybean glycinin and β-conglycinin.We studied the effect of purified glycinin and β-conglycinin on PBMC of suckling pigs *in vitro*. We also determined the effects of various dosages of glycinin and β-conglycinin on proliferation, cytokines and immunoglobulin levels, which are essential criteria to explore the effect of glycinin and β-conglycinin induced hypersensitivity in human and animals.

MATERIALS AND METHODS

Experimental animals: Four clinically healthy piglets, 21-day old, (Duroc×Landrace×Yorkshire) were obtained from the National Experimental Teaching Demonstration Center of Animal Science, Ya'an, China as blood donors. All animal protocols were approved by the Institutional Animal Care and Use Committee of Sichuan Agricultural University (Ya'an, China).

Experimental design: The study used a 2 × 6-test design that tested for 2 factors: the glycinin group and the β-conglycinin group. The 6 levels were 1-6 and 1-1,1-2,1-3,1-4,1-5 (According to the concentration of extract added: 0, 0.5 mg/ml,1.25 mg/ml,2.5mg/ml, 5 mg/ml, and 10 mg/ml). For each level of 12 to repeat and

repeated each of 1 well.

The extraction of glycinin and β-conglycinin: The extraction method was according to read Wang *et al*., (2006). Purity coefficient of glycinin and β-conglycinin was 93.8 and 67.2 percents

The isolation and culture of PBMC: PBMCs were isolated by using Lymphocyte Separation Kits which was purchased from the Institute of Biomedical Engineering Chinese Academy of Medical Science. The collected cells were counted and resuspended in a RPMI-1640 medium medium supplemented with 10% fetal bovine serum, L-glutamine, and antibiotics. The concentration of the cells was adjusted to 5×10^5 per ml. The suspension of the lymphocytes was seeded in 24 well flat-bottomed tissue culture plates containing different level of glycinin and β-conglycinin per well. Conconavalin A (Con. A) solution at a final concentration of 5 μg/ml/well was added immediately before incubation at 37°C in a humidified 5% CO_2 atmosphere for 72 h.

Determination: After the induction training, 100 μl of 0.5 g L^{-1} MTT solution was added to wells. Cells were incubated at 37 °C for 4h to allow colour development and thereafter, 200 μl dimethyl sulfoxide was added to the wells. Plates were incubated overnight at 37 °C for 30min to solubilize the formazan products. Absorbances were measured at the wavelength of 570 nm.

Different concentrations of Glycinin and β-conglycinin were induction trained for 72h in incubator with 37 °C, 5% CO_2, collect cells and culture solution in every well for the test IL-2, IL-4, IL-6, INF-γ, IgG, IgM, and IgA concentration. IL-2, IL-6 used radioimmunoassay, IL-4, INF-γ used ELISA method, IgG, IgM, and IgA test by using Immunoturbidimetric assay method in accordance with the kit instructions.

Statistical analysis: All data were analyzed by one-way analysis of variance with a statistical analysis software program (SAS version 9.1, SAS Institute, Cary, NC), and values were expressed as means ± SD. Group differences resulting in P values of less than or equal to 0.05(0.01) were considered to be statistically significant(very significant).

RESULTS

THE MTT OD value. Table-1, 2 shows that the MTT OD value was increased in all groups in a dose-dependent manner.

The cytokines and immunoglobulins. The results showed that IL-2 concentration increase when glycinin and β-conglycinin supplementation increased among all groups. IL-6 and INF-γ concentration had no difference (P> 0.05) among all glycinin groups except for the 2-6 group (Table 3).

The effects of different doses of glycinin and β-conglycinin on IgM, IgG and IgA levels in the medium are summarized in Table 4. It was recorded that the IgA in group 2-6 were significantly higher (P<0.01) than those in other groups. There were no differences (P>0.05) in the IgG among all groups.

DISCUSSION

The present study utilized a PBMC model to elucidate the effects of soybean glycinin and β-conglycinin induced allergy which is an important concern related to both animal nutrition and human health. To date, there have been many studies using young pigs as an animal model to investigate the effects of soybean proteins (Feng *et al*., 2007). Consistent with those reports, our study demonstrated that β -conglycinin could promote PBMC growth in order to produce more cytokine and raise the level of certain kinds of immunoglobulins. This experiment on the determination of cytokines showed that IL-2 concentration became higher with the increase in the concentration of antigen protein and IL-2 played the most important role in inducing proliferation (promoting cycle phase of T cell into the S phase from Gl phase) and differentiation of T lymphocytoma cells in an autocrine manner. Wilkinson and other scientists found in their studies that IL-2 could stimulate proliferation of lymphocytoma cells and increase secretion function of lymphocytoma cells, which is due to IL-2 induced by antigen promoting cell proliferation (Wilkinson and Newman 1994). The experiment also found that IL-6 increased, and IL-6 could induce IL-2R expression, and further induce IL-2 production, so as to promote T cell proliferation.

Normal immune function in the body depends on the interaction among a variety of immune cells. Helper T cell (Th) based on CD4 T cells secrete cytokines is divided into two different types of Th1 and Th2 cells. Th1 cells mainly secrete IL-2, IFN-γ, and so on, and have a major role in cell-mediated immunity and the immune response, participate in delayed-type hypersensitivity. Th2 cells secrete IL-10, IL-6, IL-4, and so on, are the main players in Humoral immune and allergic inflammation (Economides *et al*., 2003). In our experiment, β-conglycinin with 5-10 mg ml^{-1} can significantly promote the production of inflammatory cytokines. It can be inferred that β－conglycinin is stronger than that of glycinin. When adding isolated soy protein source or purified β-conglycinin to the piglet feed, the cells will lead to inflammatory cytokines, so it will cause allergic inflammation occcur on post-weaning, and Allergic-type mediated by the T cell (Miller *et al*., 1984).

Table 1. The influence of soybean glycinin extract on the proliferation of Pig peripheral blood lymphocytoma cells in vitro culture

Treatment group	concentration gradient of glycinin (mg/ml)	MTT OD value
1-1	0	0.13 ± 0.01^{Aa}
1-2	0.5	0.14 ± 0.03^{Aa}
1-3	1.25	0.20 ± 0.01^{Aa}
1-4	2.5	0.21 ± 0.05^{Aa}
1-5	5	0.35 ± 0.07^{Aab}
1-6	10	0.69 ± 0.07^{B}

The difference between data with different capital letters was significant among groups (P<0.01), and the difference between data with different small letters was significant among groups (P<0.05) and the same letters was not significant (P>0.05).

Table 2. The influences of soybean β-conglycinin extract on the proliferation of pig peripheral blood lymphocytoma cells in vitro culture

Treatment group	concentration gradient of β-conglycinin (mg/ml)	MTT OD value
2-1	0	0.13 ± 0.01^{A}
2-2	0.5	0.16 ± 0.04^{A}
2-3	1.25	0.29 ± 0.06^{A}
2-4	2.5	0.41 ± 0.05^{AB}
2-5	5	0.96 ± 0.08^{C}
2-6	10	1.70 ± 0.07^{C}

The difference between data with different capital letters was significant among groups (P<0.01), and the difference between data with different small letters was significant among groups (P<0.05) and the same letters was not significant (P>0.05).

Table 3. The influence of soybean glycinin and β-conglycinin extract on IL-2、IL-6 and IFN-γ of medium

Item compared	Group number	The concentration of extract (mg/ml)	IL-2 (ng/ml)	IL-6 (pg/ml)	IFN-γ(pg/ml)
compare	1-1	0	0.63 ± 0.31^{A}	157.96 ± 12.46^{A}	11.07 ± 2.50^{A}
Glycinin group	1-2	0.5	0.65 ± 0.24^{A}	148.61 ± 18.44^{A}	15.40 ± 1.33^{A}
	1-3	1.25	0.64 ± 0.29^{A}	166.48 ± 18.04^{A}	15.26 ± 1.29^{A}
	1-4	2.5	0.67 ± 0.05^{A}	141.73 ± 22.85^{A}	15.37 ± 0.81^{A}
	1-5	5	0.92 ± 0.22^{A}	156.18 ± 31.39^{A}	11.21 ± 1.40^{A}
	1-6	10	1.34 ± 0.42^{B}	169.92 ± 24.77^{A}	11.57 ± 1.19^{A}
β-conglycinin group	2-2	0.5	0.67 ± 0.13^{A}	103.90 ± 14.24^{A}	8.64 ± 1.55^{A}
	2-3	1.25	0.71 ± 0.26^{A}	103.45 ± 15.44^{A}	9.44 ± 1.62^{A}
	2-4	2.5	1.36 ± 0.29^{AB}	121.39 ± 18.86^{A}	12.72 ± 1.39^{A}
	2-5	5	2.24 ± 0.63^{B}	172.16 ± 18.18^{A}	48.35 ± 1.12^{B}
	2-6	10	3.93 ± 0.08^{C}	295.01 ± 12.13^{B}	87.18 ± 1.73^{C}

The difference between data with different capital letters was significant among groups (P<0.01), and the same letters was not significant (P>0.05).

Table 4. The influence of soybean glycinin and β-conglycinin extract on IgM、IgG and IgA of medium

Item compared	Group Number	The concentration of extract (Mg/ml)	IgM (g/L)	Ig G (g/L)	Ig A (g/L)
compare	1-1	0	0.7252 ± 0.0843^{A}	3.66±29.87	1.25 ± 0.88^{A}
Glycinin group	1-2	0.5	1.26 ± 0.19^{B}	3.57±0.14	1.76 ± 0.23^{AB}
	1-3	1.25	1.46 ± 0.15^{B}	3.44±0.21	3.23 ± 0.38^{B}
	1-4	2.5	1.84 ± 0.11^{BC}	3.49±0.32	3.28 ± 0.76^{B}
	1-5	5	1.92 ± 0.07^{C}	3.85±0.16	3.18 ± 0.46^{B}
	1-6	10	2.21 ± 0.30^{C}	3.88±0.24	3.23 ± 0.07^{B}
β-conglycinin group	2-2	0.5	1.34 ± 0.08^{B}	3.53±0.58	2.77 ± 0.38^{B}
	2-3	1.25	1.43 ± 0.40^{B}	3.86±0.78	3.15 ± 0.19^{B}
	2-4	2.5	1.83 ± 0.09^{B}	3.22±0.42	3.15 ± 0.19^{B}
	2-5	5	1.71 ± 0.02^{B}	3.77±0.50	3.58 ± 0.42^{C}
	2-6	10	2.87 ± 0.11^{C}	3.96±0.44	4.86 ± 0.07^{D}

The difference between data with different capital letters was significant among groups (P<.01), and the same letters was not significant (P>0.05).

Serum immunoglobulin (IgG, IgA, and IgM) levels reflect the humoral immune function in the body. The results of this experiment show that the influence of glycinin extract on IgM, IgG, and IgA of culture medium had no significant difference (P> 0.05) and the influence of β-conglycinin extract on IgM and IgG of culture medium had no significant difference (P> 0.05), but the IgA showed a gradual upward trend, and the IgA produced the most with 10 mg ml^{-1} β-globulin. In this experiment, the antibodies IgM, IgG, and IgA production were not significantly affected by glycinin, but the level of IgA was significantly raised by β-conglycinin. So it

could be inferred that β-conglycinin with the stronger allergens than glycinin, and further validated that the antigen of β-conglycinin was stronger than that of glycinin.

CONCLUSIONS: Glycinin and β-conglycinin could promote peripheral blood lymphocyte of suckling pigs to transform and proliferate in vitro culture, and influence β-conglycinin that is stronger than that of glycinin. β-conglycinin could promote peripheral blood lymphocyte of suckling pigs to produce cytokine with IL-2、INF-γ、IL-6 and it was more evident at 5-10 mg ml^{-1} β-conglycinin could promote peripheral blood lymphocyte of suckling pigs to produce a specific IgA, it got up to the largest at 5 mg ml^{-1}. Antigen of β-conglycinin was stronger than that of glycinin .

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