Identification of epitopes of the A1aBx and A5A4B3 subunits of glycinin antigenic in three animal species

Earl Taliercioa*, Telisa Lovelessa and Marc J. Turanob

aUSDA/ARS, Raleigh, NC, USA; bNorth Carolina Sea Grant, North Carolina State University, Raleigh, NC, USA

(Received 27 September 2013; accepted 17 March 2014)

Soybean meal is commonly added to a variety of animal feeds to supplement protein sources and to optimise growth. While soybean protein is a valuable food supplement it has been recognised as an important food allergen. The soybean seed storage protein, glycinin, has been identified as an allergen. A tiled peptide array of the A1aBx and A5A4B3 subunits of glycinin was screened to identify the epitopes that bind antibodies from multiple species. We have identified four regions in these two glycinin subunits that are antigenic in most or all of the species tested. One region is implicated in an allergic response in dogs by the dog’s ability to bind IgE. Three regions overlap or abut regions that are similar to allergenic epitopes in peanut. It will be critical to identify immunogenic regions able to cause allergies to soy in order to prioritise them for mitigation.

Keywords: allergen; dog; hybrid striped bass; IgE; IgG; rabbit

Introduction

Protein from soybean is commonly used to supplement food for humans and feed for livestock and pets. Over 90 million metric tonnes (valued at almost $39 billion) of soybean meal were harvested in 2010. Of that total, about 24 million metric tonnes of soybean meal went to the feed industry (The American Soybean Association, 2011). While soybean protein is a valuable food supplement, it has been shown to be allergenic in multiple species, including humans. The ‘Food Allergen Labeling and Consumer Protection Act of 2004 (Public Law 108-282, Title II)’ requires that the content of eight common food allergens, including soy, be listed on food labels to warn allergic individuals. At least 16 soybean proteins are known to cause allergies in humans, causing symptoms such as skin rash, gastrointestinal problems, rhinitis, asthma, hypotension and anaphylaxis (Burney et al., 2010; Holzhauser et al., 2009; Joseph, Hymowitz, Schmidt, & Herman, 2006). Among the best characterised soybean protein allergens are the seed storage proteins (SSPs), representing 40–60% of all proteins present in soybean meal. Conglycinin and glycycin are two SSPs in soybeans that are known to cause an allergic response. Conglycinin is a 7S trimer composed of different combinations of β, α and α′ subunits, all of which have been reported to be allergenic in humans (Thanh & Shibasaki, 1978). Glycinin is an 11S hexamer represented by five proteins divided into two groups based on similarity (Nielsen et al., 1989; Utsumi, Kohno, Mori, &
Kito, 1987). In this study group 1 is represented by A1aBx and group 2 is represented by A5A4B3. Each glycinin subunit is composed of an acidic and basic region (Badley et al., 1975; Nielsen et al., 1989). Detailed epitope mapping of the soybean SSP subunits has identified specific regions that are antigenic in pigs and/or humans (Beardslee, Zeece, Sarath, & Markwell, 2000; Helm et al., 2000; Krishnan, Kim, Jang, & Kerley, 2009; Taliercio & Kim, 2013).

Allergic responses to soybeans vary by species. The degree and type of response can affect the growth of livestock. Soybean proteins included in the diet of newly weaned pigs resulted in a growth lag and changes to intestinal villi associated with malabsorption (Helm et al., 2000; Li et al., 1990; Li, Nelssen, Reddy, Blecha, Klemm, & Giesting, et al., 1991; Li, Nelssen, Reddy, Blecha, Klemm, Goodband, 1991). The presence of IgG against soybean proteins in the serum of pigs that are fed soybean meal indicated that the growth lag is associated with an immune response to soy proteins. Allergies to soy protein have been reported for a variety of species that include dogs and rabbits. Allergenic responses have been observed in dogs when exposed to soy protein, which have exhibited many of the same symptoms reported for humans (Olivry & Bizikova, 2010; Ricci, Hammerberg, Paps, Contiero, & Jackson, 2010). Similarly, increased soybean meal usage in fish diets (primarily salmonid species) has been shown to cause intestinal enteritis, negatively impacting growth (Baeverfjord & Krogdahl, 1996; Heikkinen et al., 2006). Understanding potential allergic responses to soy protein is essential to optimising soy levels in the diet and minimising risks to production in the form of reduced growth.

The goal of this research was to identify epitopes of soybean glycinin that are antigenic in multiple species. In this study we identify sera from dogs, rabbits and hybrid striped bass (HSB; *Morone chrysops × M. saxatilis*) that have antibodies against glycinin. We have identified four epitopes that were antigenic in multiple species. Variants of these epitopes may be available in the *Glycine* germplasm or created in mutant populations. Such variants may provide improved soy protein for food and feed, and might help determine the role these epitopes play in the immune response to food.

**Materials and methods**

**Animal**

For this study 5 ml of sera was obtained from SPF New Zealand white rabbits older than 12 weeks, purchased from Thermofisher (Waltham, MA), and fed a proprietary diet that included soy protein. Rabbit sera were diluted 1/300 to screen enzyme-linked immunosorbent assay (ELISA) plates. About 1 ml of archived sera from Maltese × beagle dogs with a genetic predisposition to develop adverse food reactions were used in these studies. The dogs were housed in the laboratory research facility at the North Carolina State University College of Veterinary Medicine, and were under the care of the laboratory animal research unit. The dogs were fed a commercially available diet that included soy protein. Dog sera were diluted 1/100 to screen ELISA plates for proteins and peptides that were recognised by IgG antibodies, and, 1/10 dilutions were used to screen ELISA plates for IgE antibodies. The dog sera were kindly provided by Dr Bruce Hammerberg. HSB were tank-reared at the North Carolina State University Marine Aquaculture Research Center in Smyrna, NC, under IACUC protocol 09-147. Fish were reared for 12 weeks and fed one of four test diets that included 0–50% soybean meal. After 12 weeks, approximately 1 ml of blood was drawn from sample fish. HSB sera were diluted 1/75 to 1/300 for ELISA assays.
**ELISA of Glycinin**

Glycinin was purified by the method of Kwanyuen and 10 pg per well was dried onto an ELISA plate at 37°C (Kwanyuen, Wilson, & Burton, 1998). The ELISA was performed as described in Taliercio and Kim (2013). HRP-labelled species-specific antibodies against rabbit IgG, dog IgG, dog IgE were used to identify which sera had antibodies against glycinin on the ELISA plates. HRP-labelled antibodies were obtained from KPL (Gaithersberg, MD), Abserotec (Raleigh, NC) or Sigma (St. Louis, MO). The HSB sera with IgM antibodies against glycinin were identified by using an unlabeled goat antibody against HSB IgM, kindly provided by Dr RA Shelby (ret.), and an HRP-labelled rabbit anti-goat-IgG antibody to detect the positive sera. The unlabeled second antibody did not react with soy proteins significantly above background in the absence of primary serum (data not shown).

**Analysis of peptide library**

A set of peptides was designed to represent the glycinin protein. The series of peptides were each 16 amino acids long with an 11-amino-acid overlap (Mimotopes, Raleigh, NC). Detailed information about the peptides used in this study is shown in Supplementary data 1. The first peptide from the amino end of the protein was biotinylated at the carboxyl end. The remaining peptides had a biotinylated amino end extension of gly-ser-gly-ser (GSGS). Peptides on the array were numbered consecutively, beginning with the A1aBx protein acidic region (peptide1) and A5A4B3 protein acidic regions (peptide 92). The peptides were dissolved in 40% acetonitrile and diluted with PBST-azide [PBS pH 7.2 (7.5 mM dibasic sodium phosphate, 10 mM monobasic sodium phosphate and 0.58 M sodium chloride), 0.01% Tween 20, and 0.1% sodium azide]. One nanogram per well of each peptide was incubated for 1 hr on a streptavidin coated 96 well Nunc Maxisorp (cat#442404) microtiter plate that was blocked for 1 hr with 200 µl of blocking solution. Each plate included a positive and negative control peptide provided by the manufacturer of the peptide library. Each well was rinsed four times with 250 µl of PBST and dried at 37°C for 1 hr. Plates were stored in a refrigerated desiccator for up to two weeks. Peptide plates were immuno-stained for 16 hr with 100 µl/well of animal sera diluted in blocking buffer as described above. The wells were rinsed four times with 250 µl of PBST. For HSB sera and for dog sera IgE detection, 100 µl of the unlabeled second antibody was added to the wells and incubated for 16 hr, and rinsed four times in PBST buffer. A 1/2000 to 1/8000 dilution of the appropriate HRP-labelled antibody was added to each well for a 1-hr incubation and rinsed four times with 250 µl of blocking solution. The colorimetric substrates 2,2’-Azinobis [3-ethylbenzothiazoline-6-sulfonic acid]-diammonium salt or 3,3’,5,5’-tetramethylbenzidine from Kirkegaard & Perry Laboratories (KPL), Inc (Gaithersburg, MD) were used to quantify the HRP-labelled antibodies bound to the samples by reading the absorbance on a model 680 microplate reader (Bio-Rad) or Victor 31420 multi-label counter (Perkin Elmer) following the manufacturer’s instructions. The positive and negative control peptide coated wells were incubated with serum provided by Mimotopes and stained as expected (data not shown). The 1.5× the ELISA value of the negative control was subtracted as background. Controls lacking primary sera were tested for all reagents to confirm that all antibodies binding the target peptide are derived from the primary sera. After initial screening of dog sera putative positives were rechecked in triplicate and a contiguous non-reacting peptide was included in triplicate on the same plate. Screening of rabbit sera and HSB were performed in 1, 2 or 3 replications using the complete peptide library. Averages and standard deviations were calculated with Microsoft EXCEL (Redmond, WA).
Results

Identification of antigenic epitopes of glycinin in rabbits

Sera from 29 rabbits were screened for the presence of IgG that bind glycinin (Table 1). Most sera tested were above background (ELISA without primary serum), suggesting that glycinin was antigenic in most rabbits. ELISA of the peptide array representing the A1aBx and A5A4B3 subunits of glycinin was used to identify the antigenic epitopes that were recognised by IgG antibodies from selected rabbit sera. Fifteen sera included IgG that bound epitopes of the A1aBx or A5A4B3 subunits of glycinin (Figure 1). Sera that were positive for IgG that bind glycinin but failed to react with a peptide on the array may recognise non-linear epitopes or other glycinin proteins not represented on the array. The ELISA values on the peptide array ranged from 2.6 absorbance units to 0.18 absorbance units above background. The absorbance data are available as Supplementary data 2.

Identification of antigenic epitopes of glycinin in HSB

Sera from 20 HSB were screened for the presence of IgM antibodies against intact glycinin, (Table 2). Fish that were fed diets that did not include soy protein did not have

| Sera     | Average of three replications | SD  |
|----------|------------------------------|-----|
| PA4800   | 0.08                         | 0.01|
| PA4801   | 0.30                         | 0.02|
| PA4802   | 0.18                         | 0.01|
| PA4803   | 0.12                         | 0.01|
| PA4804   | 0.68                         | 0.04|
| PA4805   | 0.06                         | 0.01|
| PA4806   | 0.29                         | 0.05|
| PA4807   | 0.11                         | 0.01|
| PA4808   | 0.87                         | 0.06|
| PA4809   | 0.10                         | 0.01|
| PA4810   | 0.10                         | 0.01|
| PA4811   | 0.09                         | 0.01|
| PA4812   | 0.11                         | 0.00|
| PA4813   | 0.13                         | 0.00|
| PA4814   | 0.16                         | 0.01|
| PA4815   | 0.12                         | 0.01|
| PA4816   | 0.46                         | 0.01|
| PA4817   | 0.26                         | 0.00|
| PA4818   | 0.44                         | 0.02|
| PA4819   | 0.21                         | 0.02|
| PA4820   | 0.09                         | 0.00|
| PA4821   | 0.20                         | 0.01|
| PA4822   | 0.10                         | 0.01|
| PA4823   | 0.15                         | 0.03|
| PA5150   | 0.07                         | 0.00|
| PA5131   | 0.08                         | 0.00|
| PA5144   | 0.07                         | 0.01|
| PA4580   | 0.12                         | 0.00|
| PA4591   | 0.19                         | 0.00|

*aUsed on peptide array.
detectable levels of antibodies against glycbin as expected (sera 1–3, Table 2). Sera from 12 fish with detectable levels of anti-glycinin IgM and sera from 2 fish that were not tested for anti-glycinin IgM were assayed on the peptide array. The number of fish with IgM binding a given peptide are shown in Figure 2. The ELISA values on the peptide array ranges from 0.05 to 0.55 absorbance units above background and the ELISA values

Table 2. ELISA for glycginin using HSB sera.

| Sera   | Average of three replications | SD  |
|--------|------------------------------|-----|
| HSB-1  | 0.00                         | 0.00|
| HSB-2  | 0.04                         | 0.00|
| HSB-3  | 0.02                         | 0.01|
| HSB-5a | 0.27                         | 0.01|
| HSB-17a| 0.30                         | 0.02|
| HSB-19a| 0.24                         | 0.01|
| HSB-20 | 0.10                         | 0.02|
| HSB-23a| 0.24                         | 0.01|
| HSB-26a| 0.25                         | 0.04|
| HSB-27a| 0.29                         | 0.01|
| HSB-29 | 0.06                         | 0.00|
| HSB-78 | 0.14                         | 0.01|
| HSB-111| 0.12                         | 0.01|
| HSB-113| 0.20                         | 0.03|
| HSB-114a| 0.13                        | 0.01|
| HSB-115a| 0.12                        | 0.00|
| HSB-117| 0.23                         | 0.03|
| HSB-119| 0.09                         | 0.01|
| HSB-122a| 0.13                        | 0.00|
| HSB-124a| 0.16                        | 0.03|

*aUsed on protein array.

Figure 1. The number of rabbit sera that immuno-stain peptides representing A1aBx and A5A4B3. IgG-binding epitope profiles of acidic portion of A1aBx (1–56), basic portion of A1aBx (57–91), acidic portion of A5A4B3 (92–161) and basic portion of A5A4B3 (162–196).
Identification of antigenic epitopes of glycinin in dog

Six sera from hyper-allergenic dogs were used to screen the tiled array representing the A1aBx and A5A4B3 subunits of glycinin for epitopes that were bound by IgG antibodies (data not shown). These sera recognised a total of 11 peptides that were rescreened for their ability to bind dog IgG in triplicate (Table 3). Although robust second antibodies for detection of rabbit IgE were not available and precluded the identification of epitopes bound by rabbit IgE, these reagents were available for detection of dog IgE. We did not have enough sera to screen the entire peptide array for epitopes bound by IgE antibodies. However, we were able to determine that three peptides that were recognised by IgG antibodies in the sera of the dogs Blk23 and Dakota also were bound by IgE antibodies (Table 3), highlighted values in Table 3.

Discussion

A tiled peptide array was used for ELISA to identify epitopes of the A1aBx and A5A4B3 subunits of glycinin that are immunogenic in dogs, rabbits and HSB. We can estimate the size of the immunogenic epitopes knowing that the peptides on the array overlap by 11 amino acids and assuming these epitopes bind a single IgG in a given serum. We calculate antibodies that bind two consecutive peptides recognize epitopes that are 170 between 11 and 7 peptides long. Similarly, antibodies that bind three consecutive peptides recognize epitopes that are less than seven peptides long.

To gain an overview of the antigenicity of glycinin, the most antigenic regions are indicated on an alignment of the acidic (Figure 3) and basic (Figure 4) parts of the A1aBx
Table 3. ELISA of selected peptides with dog sera.

| Dog sera | Peptide | Average | SD  | Peptide | One replication | Average | SD  | Peptide | Average | SD  | Peptide | Average | SD  |
|----------|---------|---------|-----|---------|---------------|---------|-----|---------|---------|-----|---------|---------|-----|
| Aba5     | 20      | 0.65    | 0.05| 21      | 0.35          | 0.19    | 0.01| 30      | 0.19    | 0.01|
| Aba5     | 43      | 0.17    | 0.02| 45      | 0.09          | 0.24    | 0.01| 43      | 0.19    | 0.02|
| Aba5     | 44      | 0.35    | 0.02| 44      | 0.24          | 0.19    | 0.01| 44      | 0.19    | 0.01|
| Blk23    | 91      | 0.75    | 0.05| 92      | 0.27          | 0.66    | 0.04| 85      | 0.21    | 0.02|
| Dakota   | 69      | 0.29    | 0.03| 71      | 0.18          | 0.25    | 0.01| 76      | 0.13    | 0.01|
| Dakota   | 70      | 0.30    | 0.08| 70      | 0.26          | 0.15    | 0.01| 76      | 0.13    | 0.01|
| Dakota   | 121     | 0.21    | 0.05| 122     | 0.11          | 0.18    | 0.00| 107     | 0.12    | 0.00|
| Victoria | 122     | 0.27    | 0.02| 114     | 0.16          | 0.21    | 0.01| 114     | 0.20    | 0.00|
| Ernest   | 22      | 0.82    | 0.03| 69      | 0.25          | 0.13    | 0.01| 30      | 0.27    | 0.02|
| Ernest   | 117     | 0.64    | 0.08| 128     | 0.21          | 0.15    | 0.01| 107     | 0.13    | 0.00|
| Red      | 172     | 0.80    | 0.08| 186     | 0.17          | 0.14    | 0.01| 165     | 0.10    | 0.00|

Note: Average and SD of three replications unless otherwise noted.
Figure 3. Alignment of A1aBx and A5A4B3 acidic regions showing antigenic epitopes in three animal species. Identical amino acids between the proteins are shaded. Epitopes antigenic in pigs are underlined. Amino acids that are identical to antigenic epitopes of the peanut Ara h 3 protein are bold. Peptides antigenic in three or more individual rabbits and HSB are shown. Peptides antigenic in any dog are shown. Boxes indicate conserved regions of both A1aBx and A5A4B3 that bind antibodies from multiple species.
and A5A4B3 glycinin subunits. The alignment of the acidic parts of these two subunits shows three conserved regions that bind antibodies from multiple species (regions 1–3 in Figure 3) in both A1aBx and A5A4B3. Region 3 overlaps a region identified as antigenic in pigs. These A1aBx and A5A4B3 proteins are similar to the Ara h 3 protein from peanut that has been shown to bind IgE in individuals allergic to peanut (Rabjohn et al., 2018).
Of the four antigenic epitopes identified in peanut (shown in bold on Figure 3), three overlap regions in soybean that are immunogenic in multiple species. The overlap of these regions with a known peanut allergen further supports a role for these regions in soybean allergenicity. Figure 4 shows that the alignment of the basic parts of these two subunits that share one conserved region that binds antibodies from multiple species (region 1 in Figure 4). This region abuts a pig antigenic site (rabbit IgG binding sites overlap the pig antigen) and is the only region that binds dog IgE. Binding of dog IgE is the best evidence we present that implicates glycinin in an allergic response, though non-IgE-mediated response to these proteins cannot be ruled out (Olivry & Bizikova, 2010; Ricci et al., 2010). We also note that the peptide of acidic portion of A5A4B3 between region 2 and region 3 binds antibodies from multiple species unlike the similar peptide in A1aBx, suggesting there may be some inherent differences in immunogenicity of glycinin subunits which could be useful mitigating allergenicity of soybeans (Figure 3). Similarly, there is a region of the basic portion of A1aBx (starting at LKSQQ) that binds antibodies from all species tested that is not shared with A5A4B3 (Figure 4). It is unlikely that variants of all potential antigenic sites could be identified and bred into one plant, making the identification of antigenic regions involved in allergic responses in multiple species particularly important. Therefore less allergenic variants of region 1 of the basic part of A1aBx may improve soy as a protein supplement by making it less allergenic in multiple species. It is unknown if the antigenic regions of the acid portion of the A1aBx and A5A4B3 subunits that bound dog IgG but not dog IgE are antigenic without being allergenic. If these regions are antigenic without being allergic, the role these proteins play in immune response to food is an important question for further research.

Supplemental data
Supplemental data for this article can be accessed here.

References
Badley, R. A., Atkinson, D., Hauser, H., Oldani, D., Green, J. P., & Stubbs, J. M. (1975). The structure, physical and chemical properties of the soy bean protein glycinin. Biochimica et Biophysica Acta (BBA) – Protein Structure, 412, 214–228. doi:10.1016/0005-2795(75)90036-7
Baeverfjord, G., & Krogdahl, A. (1996). Development and regression of soybean meal induced enteritis in Atlantic salmon, Salmo salar L., distal intestine: A comparison with the intestines of fasted fish. Journal of Fish Diseases, 19, 375–387. doi:10.1111/j.1365-2761.1996.tb00376.x
Beardslee, T. A., Zeece, M. G., Sarath, G., & Markwell, J. P. (2000). Soybean glycinin G1 acidic chain shares IgE epitopes with peanut allergen Ara h 3. International Archive of Allergy and Immunology, 123, 299–307. doi:10.1159/000053642
Burney, P., Summers, C., Chinn, S., Hooper, R., Van Ree, R., & Lidholm, J. (2010). Prevalence and distribution of sensitization to foods in the European Community Respiratory Health Survey: A EuroPrevall analysis. Allergy, 65, 1182–1188.
Heikkinen, J., Vielma, J., Kemiläinen, O., Tiitola, M., Eskelinen, P., Kiuru, T., … von Wright, A. (2006). Effects of soybean meal based diet on growth performance, gut histopathology and intestinal microbiota of juvenile rainbow trout (Oncorhynchus mykiss). Aquaculture, 261, 259–268. doi:10.1016/j.aquaculture.2006.07.012
Helm, R. M., Cockrell, G., Connaughton, C., Sampson, H. A., Bannon, G. A., Beilinson, V., … Burks, A. W. (2000). A soybean G2 glycinin allergen. International Archives of Allergy and Immunology, 123, 205–212. doi:10.1159/000024445
Holzhauser, T., Wackermann, O., Ballmer-Weber, B. K., Bindslev-Jensen, C., Scibilia, J., Perono-Garoffo, L., … Vieths, S. (2009). Soybean (Glycine max) allergy in Europe: Gly m 5
(ß-conglycinin) and Gly m 6 (glycinin) are potential diagnostic markers for severe allergic reactions to soy. *Journal of Allergy and Clinical Immunology*, 123, 452–458. doi:10.1016/j.jaci.2008.09.034

Joseph, L. M., Hymowitz, T., Schmidt, M. A., & Herman, E. M. (2006). Evaluation of glycine germplasm for nulls of the immunodominant allergen P34/Gly m Bd 30k. *Crop Science*, 46, 1755–1763. doi:10.2135/cropsci2005.12-0500

Krishnan, H. B., Kim, W.-S., Jang, S., & Kerley, M. S. (2009). All three subunits of soybean b-conglycinin are potential food allergens. *Journal of Agricultural and Food Chemistry* 57, 938–943. doi:10.1021/jf802451g

Kwanyuen, P., Wilson, R. F., & Burton, J. W. (1998). Soybean protein quality. In S. S. Losceoglu & R. F. Wilson (Eds.), *The proceedings of the World Conference on Oilseed and Edible Oils Processing* (pp. 284–289). Champaign, IL: AOAC Press.

Li, D. F., Nelssen, J. L., Reddy, P. G., Blecha, F., Hancock, J. D., Allee, G. L., … Klemm, R. D. (1990). Transient hypersensitivity to soybean meal in the early-weaned pig. *Journal of Animal Science*, 68, 1790–1799.

Li, D. F., Nelssen, J. L., Reddy, P. G., Blecha, F., Klemm, R. D., Giesting, D. W., … Goodband, R. D. (1991). Measuring suitability of soybean products for early-weaned pigs with immunological criteria. *Journal of Animal Science*, 69, 3299–3307.

Li, D. F., Nelssen, J. L., Reddy, P. G., Blecha, F., Klemm, R., & Goodband, R. D. (1991). Interrelationship between hypersensitivity to soybean proteins and growth performance in early-weaned pigs. *Journal of Animal Science*, 69, 4062–4069.

Nielsen, N. C., Dickinson, C. D., Cho, T. J., Thanh, V. H., Scallon, B. J., Fischer, R. L., … Goldberg, R. B. (1989). Characterization of the glycinin gene family in soybean. *Plant Cell*, 1, 313–328.

Olivry, T., & Bizikova, P. (2010). A systematic review of the evidence of reduced allergenicity and clinical benefit of food hydrolysates in dogs with cutaneous adverse food reactions. *Veterinary Dermatology*, 21(1), 32–41. doi:10.1111/j.1365-3164.2009.00761.x

Rabjohn, P., Helm, E. M., Stanley, J. S., West, C. M., Sampson, H. A., Burks, A. W., & Bannon, G. A. (1999). Molecular cloning and epitope analysis of the peanut allergen Ara h 3. *Journal of Clinical Investigation*, 103, 535–542. doi:10.1172/JCI5349

Ricci, R., Hammerberg, B., Paps, J., Contiero, B., & Jackson, H. (2010). A comparison of the clinical manifestations of feeding whole and hydrolysed chicken to dogs with hypersensitivity to the native protein. *Veterinary Dermatology*, 21, 358–366.

Taliercio, E., Kim, S. W. (2013). Epitopes from two soybean glycinin subunits are antigenic in pigs. *Journal of the Science of Food and Agriculture*, 93, 2927–2932. doi:10.1002/jsfa.6113

The American Soybean Association. (2011). Soy stat. St Louis, MO: Author. Retrieved from http://www.soystats.com/

Utsumi, S., Kohno, M., Mori, T., & Kito, M. (1987). An alternate cDNA encoding glycinin A1aBx subunit. *Journal of Agricultural and Food Chemistry*, 35, 210–214. doi:10.1021/jf00074a011

Thanh, Vu Huu, & Shibasaki, K. (1978). Major proteins of soybean seeds. Subunit structure of β-conglycinin. *Journal of Agricultural and Food Chemistry*, 26, 692–695. doi:10.1021/jf60217a026

Xiang, P., Beardslee, T. A., Zeece, M. G., Markwell, J., & Sarath, G. (2002). Identification and analysis of a conserved immunoglobulin E-binding epitope in soybean G1a and G2a and peanut Ara h 3 glycinins. *Archives of Biochemistry and Biophysics*, 408(1), 51–57. doi:10.1016/S0003-9861(02)00534-9