Advances in Research on Anti-nutritional Effects and Detection Techniques of Glycinin (Postprint)

Authors: Song Qinglong, Yuan Xiang, Zhang Haiyan, Qiao Shiyan

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Abstract

Soybean globulin is one of the most thermally stable antigenic proteins and also the primary component in soybeans that induces allergic reactions and diarrhea in animals. This article focuses on elaborating the anti-nutritional effects of soybean globulin on piglets, calves, fish, and other animals, as well as its underlying mechanisms. Additionally, it analyzes methods for detecting soybean globulin in soybeans and their by-products, with particular emphasis on the latest research advances in enzyme-linked immunosorbent assay (ELISA), aiming to provide valuable references for the development and utilization of soybeans and their by-products.

Full Text

Preamble

Progress in Anti-Nutritional Action of Glycinin and Its Detection Technology

SONG Qinglong[1], YUAN Xiang[1], ZHANG Haiyan[1], QIAO Shiyan[2]*

[1]Beijing Longkefangzhou Bio-Engineering Technology Co., Ltd., Beijing 100193, China
[2]National Feed Engineering Technology Research Center, Beijing 100193, China

Abstract: Glycinin is one of the most thermally stable soybean antigenic proteins and the primary component responsible for allergic reactions and diarrhea in animals. This paper elaborates on the anti-nutritional effects and mechanisms of glycinin in piglets, calves, and fish, analyzes methods for detecting glycinin in soybean and its by-products, and highlights recent advances in enzyme-linked immunosorbent assay (ELISA) methodology, aiming to provide references for the development and utilization of soybean products.

Keywords: glycinin; anti-nutritional effect; detection method; application

Classification Codes: S816

Dietary allergic reactions frequently occur in livestock production. Numerous studies have found that adding raw soybean as a protein source to the diets of young animals causes intestinal mucosal hyperplasia and swelling, allergic diarrhea, growth inhibition, and even death in piglets and calves [1-4]. Research has confirmed that glycinin is one of the most immunogenic factors among soybean antigenic proteins, accounting for approximately 40% of total protein in soybean seeds. Glycinin is a polymer composed of six monomeric subunits (A–S–S–B) with a molecular weight of 320–360 kDa. Each subunit consists of an acidic polypeptide (A) and a basic polypeptide (B) linked by disulfide bonds (S–S) [5]. Glycinin exhibits strong thermal stability, and conventional heat treatment has limited effect on its inactivation. Therefore, understanding the anti-nutritional effects, mechanisms, and detection methods of glycinin is significant for guiding animal production.

1 Anti-Nutritional Effects and Mechanisms of Glycinin

1.1 Effects on Piglets

Studies have shown that glycinin primarily causes allergic reactions in young animals such as piglets, calves, and infants [5-6]. Liu Xin [6] demonstrated in mouse experiments that low to medium doses of glycinin readily induce immediate-type hypersensitivity reactions in BALB/c mice, which are immune responses mediated by both Th1 and Th2 T lymphocytes but dominated by the Th2 type. The study also revealed that functional deficiency of T regulatory cell subsets in the intestinal mucosa and their secreted inhibitory cytokines is the primary cause of allergic reactions. Sun Peng [7] found in piglet studies that glycinin-induced allergic reactions in piglets are Th2-type immune responses mediated by immunoglobulin E (IgE). Allergic piglets exhibited positive skin test reactions, elevated IgE antibody concentrations in serum and intestinal homogenates, increased levels of glycinin-specific immunoglobulin G1 (IgG1) and Th2-type cytokines interleukin-4 and interleukin-10 in serum, increased numbers of small intestinal mast cells, and enhanced histamine release, resulting in reduced growth performance and allergic diarrhea.

Wang [8] investigated the metabolic pattern of glycinin in the digestive system and found that its immunoreactivity gradually decreases from the stomach to the small intestine, with only 5.5% of activity remaining in the ileum. Glycinin-specific binding antigenic proteins were primarily distributed in the gastric mucosa, small intestinal villi, crypts, and mesenteric lymph nodes. These findings further confirm that feeding diets containing glycinin to weaned piglets causes dramatic reductions in villus height, severe villus shedding, lymphocyte hyperplasia in the intestinal mucosa, and accelerated mitosis in crypt cells [2,9]. Recent studies have shown that nutritional interventions can alleviate glycinin-induced sensitization. Antioxidants such as vitamin C [10], lipoic acid [11], and grape seed procyanidins [12] can effectively mitigate diarrhea caused by wean-

ing stress associated with glycinin by modulating the balance between Th1 and Th2 helper T lymphocytes and reducing glycinin-specific IgE production and histamine release.

1.2 Effects on Calves

Research on the effects of glycinin on calf performance and mechanisms has been limited in recent years. Early studies indicated that calves fed glycinin exhibited small intestinal villus atrophy, crypt cell hyperplasia, reduced xylose absorption, and consequently decreased performance [13-15]. Glycinin also weakened gastric contractility while enhancing intestinal contractility, significantly shortening digesta transit time and reducing nutrient digestibility [16-17]. The proposed mechanism involves intact glycinin molecules being directly absorbed into the blood and lymphatic circulation after consumption, triggering specific antibody-mediated Type I allergic reactions and lymphocyte-mediated delayed-type hypersensitivity reactions, thereby damaging the digestive system, impairing nutrient digestion and absorption, and reducing performance [18].

1.3 Effects on Fish

Research on glycinin in aquatic animals, particularly fish, has concentrated primarily on the past decade. Current evidence suggests that glycinin affects fish growth performance through two main mechanisms. First, glycinin induces intestinal allergic reactions in fish, causing intestinal tissue damage and structural changes that impair nutrient digestion and absorption, thereby reducing growth performance. Rumsey et al. [19] reported that a dietary glycinin content of 58.8 mg/g altered intestinal structure and reduced growth performance in rainbow trout. Baeverfjord et al. [20] observed that feeding soybean meal to Atlantic salmon reduced posterior intestinal single and composite fold heights by 44.3% and 21.7%, respectively. Refstie et al. [21] and Ksudhik et al. [22] added glycinin to the diets of rainbow trout and European sea bass, respectively, and found shortened mucosal folds in the posterior intestinal epithelium, deepened and widened lamina propria, and inflammatory responses in the intestinal mucosa. Wu et al. [23] investigated the effects of glycinin on growth and intestinal tissue in fish with different feeding habits and found that fold heights in the mid- and posterior intestine of carp and African catfish, as well as the anterior, mid-, and posterior intestine of grass carp, were significantly lower in the glycinin-supplemented groups. Villi in the anterior and posterior intestine of carp, the posterior intestine of African catfish, and the anterior intestine of grass carp showed varying degrees of damage, with loose and fractured lamina propria tissue.

Second, dietary glycinin supplementation reduces intestinal digestive enzyme activity, affecting fish growth performance. Wu et al. [24] reported that adding glycinin at 30 g/kg significantly decreased protease activity in the posterior and mid-intestine of juvenile carp, while supplementation at 60 g/kg significantly reduced protease activity in young carp without significantly affecting amylase

activity. Sun Ling [25] found that feeding carnivorous African catfish diets containing glycinin significantly reduced protease activity in the stomach, anterior intestine, and hepatopancreas, as well as amylase activity in the anterior and posterior intestine. Krogdahi et al. [26] demonstrated that feeding rainbow trout soybean meal-based diets reduced activities of brush border enzymes, maltase, alkaline phosphatase, lactase, and sucrase in the mid- and posterior intestine epithelium to varying degrees. In summary, glycinin exerts adverse effects on fish production performance, though the underlying mechanisms and potential impacts on other aquatic species require extensive further investigation.

2 ELISA Detection and Application of Glycinin

Currently, common methods for glycinin detection include sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), high-performance liquid chromatography (HPLC), ELISA, immunoblotting, and immunohistochemistry. Among these, HPLC (including chromatography-mass spectrometry), immunoblotting, and immunohistochemistry are cumbersome, costly, and rarely used in practical production. SDS-PAGE provides qualitative and semi-quantitative detection, estimating protein content based on the intensity of characteristic bands after staining. Huang et al. [27] used SDS-PAGE to analyze the relative contents of 11S (primarily glycinin) and 7S (primarily -conglycinin) fractions and their subunit compositions in 828 batches of Chinese soybean varieties, finding that 11S content ranged from 37.57% to 81.42% of the two globulin fractions, with an average of 60.30%, and 11S/7S ratios ranging from 0.60 to 4.38, averaging 1.56. Guan et al. [28] analyzed 175 Chinese cultivated soybeans and reported 11S/7S ratios of 0.77–4.67, averaging 1.8, with regional variations: northern spring soybeans showed consistent distribution with the overall trend, Huang-Huai summer soybeans concentrated at 1.6–1.8, southern spring soybeans mostly below 1.4, and southern summer soybeans mostly at 2.0–2.2, indicating significant variation in glycinin content among soybeans from different cultivation regions. Ma et al. [29] analyzed 706 Chinese soybean germplasm resources and similarly found rich genetic variation in the relative contents of 7S and 11S fractions and their subunits. While SDS-PAGE offers advantages of simple operation and low cost, its primary limitation is the inability to distinguish whether antigenic proteins are active, resulting in imprecise quantification. This deficiency hinders both accurate quality monitoring of soybean products by manufacturers and rational evaluation and selection by feed and farming enterprises.

ELISA is a high-throughput detection method characterized by high sensitivity, strong specificity, rapidity, simplicity, and ease of operation. Peng et al. [30] purified glycinin to 95% purity using acid precipitation and affinity chromatography, immunized rabbits to obtain polyclonal antibodies, and established an indirect competitive ELISA method with a linear detection range of 10–100 g/mL. Han [31] obtained glycinin at 90.1% purity, prepared polyclonal antibodies in rabbits, and developed a double-antibody sandwich ELISA for glycinin detec-

tion with a linear range of 2.5–5,000 ng/mL and sensitivity of 2.5 ng/mL. Ma et al. [32] immunized mice with glycinin of >92% purity to produce monoclonal antibody 4B2, establishing a competitive ELISA method with a linear range of 0.3–11.2 g/mL and detection limit of 0.3 ng/mL. Bu et al. [33] prepared polyclonal antibodies in rabbits using purified glycinin and established an indirect competitive ELISA with a linear range of 0.01–0.05 g/mL. Chen [34] prepared both rabbit polyclonal and mouse monoclonal antibodies using manually purified glycinin (>85% purity) and developed a double-antibody sandwich ELISA with a linear range of 3–200 ng/mL and sensitivity of 1.63 ng/mL. Zhang [35] established a direct ELISA method using self-prepared rabbit polyclonal antibodies as the primary antibody and horseradish peroxidase-labeled anti-rabbit immunoglobulin G (IgG) as the enzyme-conjugated secondary antibody, achieving a sensitivity of 10 ng/mL. With advances in protein purification and antibody technology, ELISA offers a cost-effective, accurate, and rapid method for detecting glycinin and other antigenic proteins in soybean products, representing an excellent option for practical production.

Currently, commonly used soybean processing products in China' s feed industry include extruded soybean, soybean meal, dehulled soybean meal, peeled and extruded soybean meal, fermented soybean meal, soybean protein isolate, and soybean protein concentrate. The authors have summarized recent experimental studies using ELISA to detect glycinin content in soybean and its by-products. As shown in , soybean processing to produce meal does not substantially alter glycinin content based on either mean values or ranges. In contrast, deep-processed products such as fermented soybean meal, extruded soybean, soybean protein concentrate, and soybean protein isolate show substantial reductions in glycinin content. These results indicate that conventional processing methods (e.g., pressing) cannot effectively reduce glycinin content, while deep processing treatments such as biological fermentation and high temperature can decrease but not completely eliminate it. However, high temperature significantly affects key nutrients in soybean products. Therefore, developing processing technologies that preserve nutritional content while reducing antigenic proteins like glycinin represents an important challenge for animal nutrition researchers.

Soy protein constitutes approximately 40% of soybean seeds, and purified 11S glycinin represents the largest monomeric component of soy protein, accounting for 25%–35% of total seed protein [18]. Based on this, the theoretical glycinin content in raw soybean is approximately 100–140 mg/g. As shown in , the measured glycinin content in 899 raw soybean samples closely matches this theoretical value, demonstrating that ELISA is an accurate method that reliably reflects glycinin content in soybean and its products.

Glycinin, as a major heat-resistant antinutritional factor in soybean, adversely affects animals (particularly young animals) fed soybean and its by-products. While numerous studies have examined its impact on piglet and calf performance, further research is needed to determine how to more accurately use feed ingredients with varying glycinin contents for precision feeding to minimize neg-

ative effects. Although relatively more research has been conducted on aquatic animals, studies are needed on the effects and mechanisms of glycinin on different fish species to enable precise application.

ELISA offers advantages of simple operation, rapid and accurate results, and low cost, making it a feasible method for determining glycinin content in soybean and its by-products. However, due to the diversity and complex composition of soybean by-products, different sample preparation methods must be investigated to avoid interference from impurities. Additionally, since glycinin comprises six subunits and certain subunits may be missing in some soybean varieties, more monoclonal antibodies need to be prepared to improve detection accuracy.

As a substance produced by legumes to resist adverse environmental conditions, glycinin possesses both nutritional value and pronounced antinutritional effects. Therefore, further investigation is needed on how to maintain protein nutrition while appropriately reducing antigenic protein content in soybean and its by-products. Since soybean antinutritional factors significantly affect the growth performance of livestock, poultry, and aquatic animals (especially young animals), further research and promotion by nutritionists are required to determine whether glycinin and other antinutritional factors can serve as quality evaluation indicators for soybean and its by-products.

Note: Figure translations are in progress. See original paper for figures.