Regulatory Toxicology and Pharmacology 89 (2017) 240–243

Contents lists available at ScienceDirect

Regulatory Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/yrtph

Allergenic potential of novel proteins – What can we learn from animal production?

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ARTICLE INFO

Article history:
Received 20 March 2017
Received in revised form
21 July 2017
Accepted 23 July 2017
Available online 24 July 2017

Keywords:
Allergenicity
Novel proteins
In vitro assays
Animal nutrition
Risk assessment

ABSTRACT

Currently, risk assessment of the allergenic potential of novel proteins relies heavily on evaluating protein digestibility under normal conditions based on the theory that allergens are more resistant to gastrointestinal digestion than non-allergens. There is also proposed guidance for expanded in vitro digestibility assay conditions to include vulnerable sub-populations. One of the underlying rationales for the expanded guidance is that current in vitro assays do not accurately replicate the range of physiological conditions. Animal scientists have long sought to predict protein and amino acid digestibility for precision nutrition. Monogastric production animals, especially swine, have gastrointestinal systems similar to humans, and evaluating potential allergen digestibility in this context may be beneficial. Currently, there is no compelling evidence that the mechanisms sometimes postulated to be associated with allergenic sensitization, e.g. antacid modification of stomach pH, are valid among production animals. Furthermore, examples are provided where non-biologically representative assays are better at predicting protein and amino acid digestibility compared with those designed to mimic in vivo conditions. Greater emphasis should be made to align in vitro assessments with in vivo data.

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1. Introduction

In what is considered a seminal study, Astwood et al. (1996) reported that allergenic proteins were more resistant to proteolytic digestion than non-allergenic proteins. Since then, resistance to digestion in the gastrointestinal (GI) tract has been considered a common property of food allergens. However, when additional allergens and non-allergens were selected controlling for protein structural family, resistance to digestion was not a differentiator (Fu et al., 2002; Herman et al., 2006a, 2007; Schnell and Herman, 2009; Bøgh and Madsen, 2016). Furthermore, some proteins that are considered to be allergenic have been shown to be readily degraded by the GI tract, and rapidly degraded in pepsin assays, e.g. Ara h 3 in peanuts (van Boxtel et al., 2008). Sensitization can, therefore, occur by routes other than post-gastric, as is observed in oral allergy syndrome. Thus, serious doubt has been cast as to whether resistance to digestion is sufficient to differentiate between an allergenic protein and a non-allergenic protein.

The European Food Safety Authority (EFSA) has recently proposed expanded guidance for the generation of in vitro data to aid in the risk assessment of novel genetically modified proteins for allergenicity (EFSA, 2016). Briefly, it is proposed that a two-step in vitro digestion assay be used to determine the extent of protein digestion. A range of conditions is recommended to reflect the range of gastrointestinal conditions found in the human population, including weakened digestive function. The FAO/WHO decision tree, however, advocates for assessment of comparative resistance to pepsin alone with the understanding that human digestion is highly variable and the assay is not a perfect indicator of allergenicity (Taylor, 2002). EFSA's review of the literature regarding in vitro protein digestion assays, although extensive, would benefit from expanding the scope of in vitro digestibility work that is assessed. If assessment of *resistance* of proteins to digestion is critical, then it would be worthwhile to review the literature for *susceptibility* of proteins to digestion, vis a vis, nutrient availability in animal nutrition. There have been extensive efforts over the last several decades to accurately predict protein and amino acid digestibility, and other metrics of protein availability,

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http://dx.doi.org/10.1016/j.yrtph.2017.07.024
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for animal production nutrition (Stein et al., 2007). The main advantage of reviewing the literature from an animal production perspective is the wealth of in vivo protein and amino acid digestibility data available across several different feedstuffs. Swine have long been used as models for the human gastrointestinal tract and are sensitive to many of the same allergens as humans (Rupa et al., 2009).

It is from this perspective that we add to the discourse regarding two underpinnings of the allergenicity guidance. Namely, (1) alteration of protein digestibility by external modifiers and (2), in vitro assays as a predictor of in vivo digestibility.

2. Alterations to gastric pH and protein digestibility

The pH of the gastric phase has received much attention in relation to food allergy since it is believed that impaired digestion may result in the stability of allergenic proteins and in sensitization (Astwood et al., 1996). Researchers point to an increased incidence of food allergies among users of antacid medications and theorize that such medications increase gastric pH and therefore impair the ability of pepsin to digest allergenic proteins (Untersmayr et al., 2003; Untersmayr and Jensen-Jarolim, 2008; Pali-Schöll and Jensen-Jarolim, 2011).

Gastric pH is indeed low, with fasting pH being <3 and <1 among some individuals. Yet, pH increases dramatically during a meal, with gastric pH rising to 3 < pH < 5 (Clark et al., 1993; Gardner et al., 2002; Kong and Singh, 2008). This is also true in swine (Chiang et al., 2008). This increase in gastric pH is partially due to the buffering capacity of the meal, and therefore, the extent of the pH rise is dependent on the matrix components of the meal. This pH rise is necessary to continue acid secretion and pepsinogen production from parietal cells and prevent negative feedback (Di Mario and Goni, 2014). The gastric pH begins to drop as the food bolus begins to move into the small intestine and returns to its fasting pH during gastric emptying. The return to a low pH then indicates that gastric acid secretion can cease. Thus, when food is present, pepsin is functioning under sub-optimal pH. This is not to understate the importance of low pH to the denaturation of proteins and activation of pepsin (Herman et al., 2006b). While the optimal pH for pepsin is often believed to be 2.0, the optimal pH for digestion actually differs depending on what substrate is being digested, the source organism from which the pepsin was collected (Piper and Fenton, 1965; Tanaka and Yada, 1996; Yoshimasu et al., 2002), the individual from which it was collected, and even the condition within an individual (Roberts, 2006). Furthermore, the peptides produced by digestions using porcine and human pepsin can differ (Ulleberg et al., 2011). Thus, the effects of pH on the human digestion of any given protein using porcine pepsin as a surrogate for human pepsin (as is common in in vitro assays) may not, even qualitatively, mimic the situation in humans.

The active ingredient in most popular over the counter antacids is calcium carbonate, and calcium carbonate is also one of the most used calcium sources for production animals. Elevated calcium intake among production animals may be similar to human intake of acid neutralizing products. Recent evidence in swine has indicated that high calcium intake depresses growth (González-Vega et al., 2016; Merriman et al., 2016). It has been known for some time that acid-base balance affects growth performance. Such growth depression may be mediated through depressed nutrient digestion and availability arising from elevated gastric pH. However, Merriman et al. (2016) indicates that feed efficiency (and, therefore, nutrient availability) was not impacted. Instead feed intake was depressed with high (~2× observed requirement) calcium intake. González-Vega et al., 2016 observed reductions in feed efficiency, but only when phosphorus intake was low. When phosphorus intake was increased (another critical buffer), no difference in feed efficiency was observed. Patience et al. (1986) fed increasing amounts of sodium bicarbonate or potassium bicarbonate and observed no alteration to nitrogen, lysine, or tryptophan digestibility. Thus, evidence suggests that nutrient availability remains the same.

Weanling pigs often suffer from allergic reactions and growth depression when soybean is fed (Engle, 1994; Coffey et al., 2000). These symptoms are mitigated as the pig ages, thus, sensitivity is believed to arise due to the immature gut of the weanling pig. Given that the gastric pH of a weanling pig is often >5, it is certainly plausible that a high pH is limiting enzymatic activity. However, the predominant protease during weaning is not pepsin, but rather chymosin, an enzyme that plays a critical role in milk clotting (Rezaei et al., 2013). It is not until the ~5th week of age that pepsin becomes the predominant protease. Thus, one might ask: even if pH were lowered, would there be additional protein degradation? Would any version of a pepsin assay have accurately segregated digestible and indigestible proteins in the weanling pig? The results of administering acidifiers in animal production would appear to indicate that the hypothesis that pH is the main driver may hold merit. The primary aim of acidifiers is to reduce gastric pH and allow pepsin to operate in a more favorable environment, thus improving protein digestion. The use of acidifiers does indeed improve protein availability and growth performance. However, the results of a meta-analysis have indicated that there is no alteration to gastric pH in swine when an acidifier is fed, including in weanling pigs (Tung and Pettigrew, 2006; Kil et al., 2011; Suiryanrayna and Ramana, 2015). These observations indicate that pepsin is likely no more active when an acidifier is fed than when it is omitted, and a mechanism other than increased enzyme efficacy is at play, e.g. reduced pathogen load in an acidic environment.

Currently, the pathway to allergenicity proposed by some researchers is not fully supported by observations in similar scenarios in animal production. Both immature and mature pigs appear to be quite resistant to pH changes due to external acid modifiers, and there is no clear linkage between their intake and reduced digestive capacity. Even among dyspeptic human individuals, it does not appear likely that enzyme efficacy is impaired through changes to gastric pH. However, inherently reduced digestive capacity, as in weanling pigs, may predispose animals to sensitization, and this is likely a more critical contributor to allergen susceptibility. Under this scenario, examination of pH in in vitro models may prove fruitless as predictors of determining the allergenic potential of novel proteins.

3. In vitro models of protein and amino acid digestibility in production animals

A number of factors may affect protein digestibility in the gastrointestinal tract and these may be of importance when designing an in vitro model (Swaisgood and Catignani, 2001). Fiber, by forming complexes with or surrounding proteins, or by influencing gut transit time, may impact the extent of protein digestion. Likewise, fiber may also increase secretion of mucin, and thereby increase the endogenous loss of amino acids from the intestinal tract (Mathai et al., 2016). The presence of other matrix components may also stimulate/depress the production of other gastrointestinal enzymes that breakdown elements preventing action by proteolytic enzymes. Anti-nutritive factors such as phytate and tannins may bind proteins and amino acids and prevent their availability; others such as trypsin inhibitor prevent action by proteolytic enzymes. Processing factors such as cooking also affect protein digestion. Ultimately, the net total of all these effects dictates the extent of protein hydrolysis. To replicate the net total

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