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Determining the safety of enzymes used in animal feed

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ABSTRACT

The purpose of this paper is to provide guidance for evaluating the safety of enzyme preparations used in animal feed. Feed enzymes are typically added to animal feed to increase nutrient bioavailability by acting on feed components prior to or after consumption, i.e., within the gastrointestinal tract. In contrast, food processing enzymes are generally used during processing and then inactivated or removed prior to consumption. The enzymes used in both applications are almost always impure mixtures of active enzyme and other metabolites from the production strain, hence similar safety evaluation procedures for both are warranted. We propose that the primary consideration should be the safety of the production strain and that the decision tree mechanism developed previously for food processing enzymes (Pariza and Johnson, 2001) is appropriate for determining the safety of feed enzymes. Thoroughly characterized non-pathogenic, non-toxigenic microbial strains with a history of safe use in enzyme manufacture are also logical candidates for generating safe strain lineages, from which additional strains may be derived via genetic modification by traditional and non-traditional strategies. For new feed enzyme products derived from a safe strain lineage, it is important to ensure a sufficiently high safety margin for the intended use, and that the product complies with appropriate specifications for chemical and microbial contamination.

1. Introduction

The purpose of this paper is to provide guidance for evaluating the safety of enzyme preparations used in animal feed. It is intended to address safety issues that are global in nature. The underlying scientific concepts have been considered at length in a previous paper dealing with food processing enzymes (Pariza and Johnson, 2001) and will not be restated here. However, the decision tree that was developed from these concepts in the previous paper is herein proposed for use in determining the safety of enzymes used in animal feeds. Regulatory issues that are specific to individual countries and/or locales are beyond the scope of our analysis.

Modern animal agriculture depends on the use of feeds that are consistent in quality and high in nutritive value. However, the complex plant materials that are economically favored for use as feed ingredients, such as coarsely processed grains (e.g., rolled corn) and high-fiber feedstuffs (e.g., small cereal grains, forages, and crop residues), have nutritive components that are resistant to endogenously-produced digestive enzymes (autoenzymes). Some feed components also have anti-nutritive effects, for example phytate, which reduces bioavailability of certain minerals, and oligosaccharides and other soluble carbohydrates that increase digesta viscosity and reduce nutrient absorption. Hence, extracting the maximal nutritive value from such complex feedstuffs typically requires supplementation of autoenzymatic activity with alloenzymatic activity (i.e., exogenously produced digestive enzymes from non-host sources) (Klasing, 1998; Bedford, 1996).

Ruminant animals (e.g., cattle and sheep) have the advantage of alloenzymatic digestion provided by rumen microflora, which enables ruminants to obtain nutrients from complex feed matrices that are not made available through autoenzymatic digestion. Accordingly, ruminants are able to subsist on diets comprised, for example, entirely of forages. Pigs, chickens and other monogastric animals lack the alloenzymes from rumen microflora, so for these species to derive optimal nutrient benefit from complex feed matrices it is necessary to provide added enzyme supplementation not available from resident intestinal microflora. Enzymes provided by supplementation may act during feed processing, while feed is present in storage and feeder bins, and following ingestion by acting within the digestive system itself.

The primary objective of using feed enzymes is to enhance availability of nutrients in the feed. However, grains such as wheat and corn display a significant degree of genetic variability in nutrient availability, and the extent to which nutrient availability from grains is improved with enzymes depends on the intrinsic digestibility of the substrate. Nutrient availability is generally more improved with enzymes for diets containing grain of low intrinsic digestibility than for diets containing grain of high intrinsic digest-

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ibility. Hence, a secondary benefit from enzyme supplementation is a reduction in variability in nutrient availability from feed ingredients, and enzymes can be strategically employed to enhance uniformity of animal performance (e.g., daily growth rate, egg production, or milk production) from such intrinsically variable feed ingredients. Minimizing the variability also aids in determining the required level of micronutrient supplementation to sustain maximum animal performance. The improved ability to pinpoint micronutrient supplementation level further enhances overall production efficiency, reduces cost of animal protein production, and reduces the environmental impact of animal agriculture. For a more in-depth discussion of the above aspects, see the review by Bedford (2000).

Table 1 lists enzymes commonly used in animal feed. As indicated, the objective of most applications is to improve feed efficiency for monogastric animals. However, ruminant species also benefit from added enzymes, such as phytase. The addition of phytase to feed improves phosphorus utilization in both ruminant (Kincaid et al., 2005; Knowlton et al., 2007) and monogastric animals (Pallauf and Rimbach, 1997), reducing the need for supplemental inorganic phosphate and the environmental problems that arise from organic phosphate excretion. In addition, ruminant livestock may also benefit from the use of added enzymes that optimize digestion (i.e., enhance nutrient availability) of high-fiber cereal grains such as barley, and high-fiber forages such as alfalfa, grass hay and corn silage (e.g., Beauchemin et al., 1995, 1997; Colombatto et al., 2003).

Feed enzymes are typically added to animal feed with the intent of aiding feed digestion and nutrient bioavailability within the animal's digestive system, although some enzymatic predigestion of the feed substrate may also occur during storage depending on the exposure time prior to ingestion of the feed by the animal. Hence, it is of general importance that the enzymes (1) are not inactivated prior to ingestion, and (2) that they function under the conditions of the gastrointestinal (GI) tract. Because many animal feeds are steam conditioned, pelleted, or extruded during manufacture, feed enzymes that are not able to withstand the high temperature and pressure during these processes must be applied to the feed after it has been processed (i.e., post-pellet application).

Enzyme encapsulation procedures may be utilized to stabilize an enzyme for feed processing, and then after ingestion to release the enzyme within the GI tract. Feed enzymes must also be able to function within the GI tract under conditions that include low pH, endogenous proteases, and in the case of ruminants, the microbial enzymes produced during rumen fermentation.

2. Historical context

Since the 1920s, researchers have observed beneficial effects from enzyme supplementation of poultry feeds, especially those feeds that contain grains with high-fiber content such as barley, oats, and rye (Burnett, 1962; Fry et al., 1958; Hastings, 1946; Jensen et al., 1957; Moran and McGinnis, 1968; Pettersson et al., 1990). Phytase was first added as a liquid to soybean meal in a chick study in 1968 (Nelson et al., 1968a,b).

In the mid-1980s, the addition of enzymes to animal feeds was expanded in areas where the supply of highly digestible ingredients, such as corn, was limited. The first enzyme products were cost-prohibitive to widespread commercial use, but incremental improvements in enzyme fermentation, recovery and processing, coupled most recently with breakthrough technologies (e.g., genetic engineering), have drastically reduced the cost of commercial enzyme preparations. van Beilen and Li (2002) estimated that by the end of the 20th century, 65% of poultry diets and 10% of swine diets contained phytases and/or carbohydrases.

Potentially useful enzymes are present in animal tissue, plants and microbes. In practice, it was found that some of these were not efficient vehicles for the commercial production of enzymes due to limitations in expression level and enzyme recovery and/or stability. With the development of recombinant DNA (rDNA) technology, enzyme manufacturers were able to isolate genes and express the desired enzyme activities in hosts (microorganisms or plants) that are more suitable for efficient enzyme production and recovery. These technological improvements led to the development of safe host systems that are currently in use, including strains of *Bacillus subtilis* and *Bacillus amyloliquefaciens* (de Boer and Diderichsen, 1991), *Bacillus licheniformis* (de Boer et al., 1994), *Aspergillus oryzae* (Barbesgaard et al., 1992), *Aspergillus niger* (Schuster et al., 2002; van Dijck et al., 2003), *Kluyveromyces lactis* (Bonekamp and Oosterom, 1994), and *Trichoderma reesei* (Nevalainen et al., 1994; Olempska-Beer et al., 2006). Following completion of fermentation, the microorganisms are inactivated and/or separated from the enzymes, and the enzymes further processed into safe and stable enzyme preparations for commercial use.

Feed enzymes are produced today in accordance with cGMP by contained, pure-culture fermentation of microbial strains that have been documented to be safe, and enzyme preparations are manufactured to comply with the chemical and microbiological purity standards established by FAO/WHO (JECFA, 2001), the Food Chemicals Codex (FCC, 2008). Although neither organization has direct regulatory authority, their general recommended standards for contaminant limits in food enzymes are considered in the formal approval process for animal feed enzymes by some authorities (see, for example, the enzyme marketing documentation document in the Official Publication of the American Association of Feed Control Officials (AAFCO, 2008)). Furthermore, enzyme preparations are manufactured to comply with specific regulatory requirements established by regional/national authorities in the EU, the US, and elsewhere.

All raw materials used in the fermentation, recovery and formulation stages of the enzyme manufacturing process must be safe and suitable for their intended use. Raw materials are Generally Recognized As Safe (GRAS) or otherwise deemed acceptable for use in feed. All raw materials should meet appropriate specifications and be inspected for quality before use. If the raw material is from an approved supplier, the quality inspection consists of a review of the manufacturer's certificate of analysis to assure conformance with specifications, along with a visual inspection or identification test. In other cases, raw materials are sampled by the quality control department of the enzyme manufacturer and subjected to the appropriate analyses to ensure their conformance to specifications.

3. Safety evaluation: Feed enzymes versus enzymes used in food processing

Feed enzymes are added to animal feed with the intent of increasing nutrient bioavailability. This objective is accomplished primarily by aiding digestion of the feed within the animal's digestive system, but enzymatic action may also occur during feed processing, transportation and storage in feed bins, prior to ingestion. In contrast, food processing enzymes are typically added with the intent of modifying a food component prior to final preparatory steps, during which the enzyme is typically denatured or destroyed, and are therefore, classified as processing aids because they have no function in the final product. While the difference in purpose and the resulting processing implications between feed enzymes and food processing enzymes represent important distinctions in intended use, the scientific principles that underlie food processing enzyme safety assessments can still be applied to the evaluation of feed enzyme safety.