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Safety of Enzymes Used in the Manufacture of Ethanol from Grains with Use of Co-Products as Animal Feed

Over the past few years, fuel ethanol production from grains has become a major business of national importance, resulting in increased availability of distillers' grains (DG) for use in animal feed. A natural question is whether the processing aids used in ethanol manufacture are safe for the animals fed the DG co-product. This issue is discussed below for enzymes, which are used as processing aids in biofuels manufacture to maximize generation of sugars that serve as substrate for fermentation to ethanol.

Enzymes used as processing aids in ethanol manufacture are nutritionally inconsequential and present little to no safety concern for either animals consuming DG or humans consuming animal products as:

1) enzymes have a long history of safe use in food and animal feed
2) extensive safety data on food and feed enzymes has been collected as part of enzyme GRAS determinations and feed enzyme approvals
3) enzymes are used at very low levels in ethanol manufacture
4) enzymes are inactivated and denatured in the process and any residual enzyme protein is not of any significance in the distillers' grains

Enzyme preparations used for biofuels manufacture are similar to those used in production of various foods including bread and beverages. One could ask whether the safety status of enzymes used in fuel ethanol production would be any different from that of enzymes used in near-identical processes for the production of food products such as beer or alcoholic beverages. During the alcohol recovery process which includes distillation at elevated temperatures, the enzymes are completely inactivated and denatured; becoming part of the protein fraction of the by-product, distillers' grains. Because enzymes are potent catalysts, very small amounts are needed in the process and typically less than 100 ppm is used. Consequently very little enzyme derived protein is present in the distillers' grains.

Based on over 40 years of safe commercial use in food and animal feed, enzymes can be categorized as 'non-toxic'[1] and 'intrinsically safe' proteins[2,3]. The large number of safety studies performed as part of numerous regulatory approvals of food enzymes around the world, substantiate that enzymes are not mutagenic or clastogenic nor are they oral, reproductive or developmental toxins.

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[1] Reed, G, 1975. Enzymes in Food Processing, second ed. AcademicPress, New York, pp. 549–554.
[2] Olempska-Beer, ZS et al. , 2006. *Regul. Toxicol. Pharmacol*. 45: 144–158.
[3] Noordervliet, PF, and DA Toet, 1987. Safety in enzyme technology, Biotechnology, vol. 7A. VCH Verlagsgesellschaft, Weinheim, Germany, pp. 711–741.

Like enzymes used in food processing and animal feed, enzymes used in biofuels manufacture are produced in well-controlled, submerged fermentations by well-characterized non-pathogenic and non-toxigenic bacteria and fungi. The enzyme production organisms generally have a well-established history of safe use and/or have undergone extensive safety evaluations as part of GRAS determinations prior to being employed for the production of food and feed enzymes [4,5]. The fermentation media components are of the appropriate purity for the intended use of the enzyme. A typical enzyme preparation contains the enzyme protein (e.g., glucoamylase, protease, etc.), non-enzyme components from the fermentation (peptides, amino acids, carbohydrates, lipids, salts), and formulation ingredients to standardize and maintain activity and/or improve handling. The formulation ingredients are carefully selected and established to be safe for the intended use. Enzyme preparations do not contain viable production microorganisms.

By-products from other fermentation processes to manufacture certain pharmaceutical and food ingredients have been fed to livestock for several decades without incident. These fermentation by-products, like distillers' grains, contain the remnants of the production microorganism, enzymes and spent biomass from the process.

Due to the catalytic nature of enzymes, they are used at very low levels in the ethanol process, typically in the range of 25 to 100 ppm active enzyme. During the ethanol process enzymes are denatured by high temperature and/or low pH during liquefaction and saccharification, and high temperature and lack of substrate during distillation, evaporation of syrup, and drying of DG. Further, the extent to which Distillers Grains are included in animal feed rations is self-limiting due to their incomplete nutrient profile. The maximum practical inclusion rate of DG in animal feed is 50%, as diets with more than 50% distiller's grains from any source would result in suboptimal animal performance and growers' profitability[6,7]. All of these factors result in very low exposure of enzyme protein to the animals.

When all these facts are taken into consideration, it is evident that enzymes used for biofuels manufacture and the resultant distillers' grains do not present any risks to livestock or the human food chain. Therefore, enzymes for biofuels production are safe for their intended use.

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[4] Pariza, MW and EA Johnson, 2001. *Regul. Toxicol. Pharmacol.* 33: 173–186.

[5] Pariza, MW and M Cook, 2010. Determining the Safety of Enzymes Used in Animal Feed. Regul. Toxicol. Pharmacol. 56: 332–342.

[6] Babcock, BA, JD Hayes, and JD Lawrence, 2008. Using Distillers Grains in the U.S. and International Livestock and Poultry Industries. Midwest Agribusiness Trade Research and Information Center. Accessible at: http://www.matric.iastate.edu/DGbook/

[7] http://www.farmfoundation.org/news/articlefiles/949-shurson-nollpaper11-28-05.pdf