The regulatory environment

by Betty Pendleton

The regulatory environment for direct-fed microorganisms, enzymes and silage inoculants has been a topic of discussion over the years. In the 1970s, guidelines were established for evaluating silage ingredients. In the late 1980s, a regulatory scheme for direct-fed microbial products was devised. The 1990s saw the development of a regulatory scheme for enzymes in animal feeds.

The regulation of direct-fed microorganisms and silage inoculants has long been a major topic of discussion between regulators and industry. In the 1970s, state and federal regulators, academia and industry worked together to establish recommended guidelines for evaluating silage ingredients. Several years ago, state and federal regulators along with industry developed a regulatory scheme that would allow continued marketing of live organisms as direct-fed products. More recently, a regulatory scheme has been finalized for the use of enzymes in animal feeds.

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REGULATORY STATUS OF DIRECT-FED MICROBIALS

Direct-fed microorganisms have been used in animal feeds for many years. This use has increased dramatically in the last decade. Previously, in order for direct-fed microbial products to be marketed, they had to be labeled in accordance with the Association of American Feed Control Officials (AAFCO) regulations for commercial feeds. For many years, these products were labeled as nutritional products with guarantees for protein, fat and fiber. This was the only legal way that these products could be sold. Obviously, these nutrient guarantees were not pertinent to the product's purpose. Industry and regulators searched for better label guarantees to describe direct-fed microbial products in a more meaningful manner.

In 1987, the Food & Drug Administration expressed concern about the use of live microorganisms in animal feeds. FDA stated that "FDA plans to charge products as unapproved new animal drugs if the labeling bears therapeutic claims, or if it claims to establish viable bacterial colonies in the intestines or claims to be composed of live organisms." FDA was concerned about the types and diversity of organisms being used as well as the practice of fermenting beyond optimal growth phase. They also questioned effects of metabolites that were possibly produced by long-term fermentation.

FDA, at the same time, recognized that many of these same organisms had been routinely used as food ingredients (i.e. cheeses, sausages, fluid milk products, yogurt, flavors, etc.).

FDA, AAFCO and the National Feed Ingredients Assn. (NFIA) (now merged with the American Feed Industry Assn. [AFIA]) worked together to develop a mutually acceptable regulatory scheme that would allow continued marketing of live organisms as direct-fed products. Industry's goal was to keep the subject of purpose statements separate from drug claims. Industry did not want these products classified as drugs and did not believe that they should be considered drugs based on their composition.

FDA requested that NFIA/AFIA serve as a clearinghouse to gather data that would provide the agency reassurance as to safety for microorganisms presently used in direct-fed products.

As a result of many discussions, the following generic content statement was developed by industry, FDA and AAFCO: "Contains a source of live (viable), naturally occurring microorganisms." Industry submitted names of organisms presently being used in direct-fed products along with data demonstrating safety and/or history of use for each organism. The list of microorganisms demonstrated some genetic diversity of the category. However, background information on each microorganism demonstrated there was uniformity among application of these organisms. While diverse genetically, these organisms are similar in their long history of use as well as their ubiquity within common ecosystems. FDA accepted the entire list of organisms (42) as being appropriate for use in

TABLE 1. Microorganisms found to be appropriate for use in animal feeds

Aspergillus niger	*Lactobacillus curvatus*
Aspergillus oryzae	*Lactobacillus delbruekii*
Bacillus coagulans	*Lactobacillus farciminis* (swine only)
Bacillus lentus	*Lactobacillus fermentum*
Bacillus licheniformis	*Lactobacillus helveticus*
Bacillus pumilus	*Lactobacillus lactis*
Bacillus subtilis	*Lactobacillus plantarum*
Bacteroides amylophilus	*Lactobacillus reuterii*
Bacteroides capillosus	*Leuconostoc mesenteroides*
Bacteroides ruminocola	*Pediococcus acidilacticii*
Bacteroides suis	*Pediococcus cerevisiae (damnosus)*
Bifidobacterium adolescentis	*Pediococcus pentosaceus*
Bifidobacterium animalis	*Propionibacterium acidpropionici* (cattle only)
Bifidobacterium bifidum	*Propionibacterium freudenreichii*
Bifidobacterium infantis	*Propionibacterium shermanii*
Bifidobacterium longum	*Saccharomyces cerevisiae*
Bifidobacterium thermophilum	**Enterococcus cremoris*
Lactobacillus acidophilus	**Enterococcus diacetylactis*
Lactobacillus brevis	**Enterococcus faecium*
Lactobacillus buchneri (cattle only)	**Enterococcus intermedius*
Lactobacillus bulgaricus	**Enterococcus lactis*
Lactobacillus casei	**Enterococcus thermophilus*
Lactobacillus cellobiosus	*Yeast*

*Formerly cataloged as *Streptococcus*

animal feeds.

To address FDA's concerns about the optimal fermentation period for these organisms, industry developed information on a generic manufacturing process demonstrating the use of control points by the industry. While individual manufacturers employ proprietary techniques for optimizing these processes in their manufacturing operation, the generic process information described the basic technology employed by manufacturers. The manufacturing process flow diagram illustrated steps required to produce and harvest microorganisms. Specific control points vary, but are performed by all manufacturers to ensure viability, identity and purity. The final microbial product has been tested for the presence of undesirable contaminating organisms. These control steps can be performed at any time during the process based upon the manufacturer's determination of applicability.

FDA published Policy Guideline, Guide 7126.41 and AAFCO adopted a policy statement delineating the regulatory scheme and requirements. All phases of the new requirements have been implemented and complied with by industry. Any company wanting to obtain a claim or a statement going beyond the approved generic content statement can work directly with FDA to determine requirements for such an approval. Such an approval would be obtained under the food additive petition approval system.

In most cases, adding an additional organism to the approved list requires an informal approval petition from FDA or possibly a food additive petition.

Table 1 contains a list of microorganisms found to be appropriate for use in animal feeds by FDA and as published in the *Official Publication* of AAFCO.

The following is an example of the required label for direct-fed microbial products:

Net Weight Shown On Bag

Blue Bird Direct-Fed Product

Contains a source of live (viable), naturally occurring microorganisms

GUARANTEED ANALYSIS:
Lactic acid bacteria.....200 billion CFU/lb. (list each organism in order of predominance) OR
Lactobacillus acidophilus.....10 billion CFU/lb.

INGREDIENTS: (Each ingredient must be specifically named in accordance with names and definitions adopted by AAFCO. Collective terms as approved by AAFCO may be used where applicable.)

DIRECTIONS FOR USE: (Directions for use and guaranteed analysis must be stated in the same units.)

Manufactured by:
Company Name
Address

In addition to information required in Trade Memorandum T-3-141, Single Ingredient Feed Evaluation Requirements and T-3-142, Specialty Product Registration Requirements, if applicable, refer to Trade Memorandum T-3-159 and the registration checklists RC-11 and RC-13. Both the microbial strain and the microbial product must be registered.

More detailed requirements are in Trade Memorandum T-3-143.

REGULATORY STATUS OF ENZYMES

Effective Jan. 1, 1998, all enzyme products labeled for use in animal feeds must have the enzymatic activity and the source organism(s) listed in the AAFCO *Official Publication*, under 30.1 Enzymes/ Source Organisms Acceptable for Use in Animal Feeds (Table 2). Any product not covered by Table 30.1 must comply with the Enzyme Marketing Coordination document listed in the *Official Publication*, which lists the information needed to be submitted to FDA for approval and addition to 30.1.

All products claiming or implying to be a source of enzymes must ensure that only those source organisms (column 2) and enzymatic activity (column 1) listed in Table 30.1 are being used.

Typical substrates (column 3) list many of the substrates on which the enzyme activity occurs. This list is not all-inclusive, but is meant as a guide only. Function (column 4) was established by submission of data by industry to justify this language to be used as the purpose statement to be used on the label.

Current Supported Use (column 5) was established to allow additional label statements as a result of the submission of supporting data. Industry representatives continue to negotiate additional statements and required data needed to substantiate.

The Enzyme Marketing Coordination document (see p. 53) should be used when developing information to request the addition of a new enzymatic activity or source organism be added to Table 30.1

Effective Jan. 1, 1998, all labels for products claiming or implying to be a source of enzymes must be in compliance with the new regulatory scheme. All AAFCO nutritional labeling requirements may also apply.

The following is an example of the required label for enzyme products:

Blue Bird Enzyme Product

Contains a source of (enzymatic activity) which can (function and/or current supported use statement (add species if applicable by AAFCO general labeling rules).

GUARANTEED ANALYSIS:
Alpha-amylase (*B. subtilis*), not less than.13,500 BAU/lb.
(The source organism[s] listed in order of predominance)
(Should be expressed in meaningful terms. The chosen units shall correspond with those present in the direction for use section or as determined by the analytical methods)

INGREDIENTS: (List in order of predominance and each ingredient must be specifically named in accordance with names and definitions adopted by AAFCO. Collective terms as approved by AAFCO may be used where applicable. An example for an enzyme ingredient would be "liquid *Bacillus subtilis* fermentation product." The enzymatic activity is not to be listed in the ingredient section.)

DIRECTIONS FOR USE. (Add all information needed by the consumer to appropriately use the product)
Cautions/warning statement - if needed
Expiration date - (if company determines one is needed)
Manufactured by
Company Name
Address
Net weight: 50 lb. (22.7 kg)

The Canadian requirements for the use of enzymes in feed are delineated in Trade Memorandum T-3-148, Regulatory Status of Enzyme-Bearing Products. All currently approved enzyme sources are listed in Schedule IV.

When an ingredient list is shown on any product label, the enzyme-bearing ingredient(s) (i.e., fermentation product from which the enzyme activity is derived) rather than the enzyme derived (e.g., cellulase) will be listed.

When a product is marketed for its enzyme content, guarantees for the enzyme activity will have to be made on the label. Such products will be registered as "enzyme sources."

Products bearing enzyme guarantees on the label are not exempted from registration, as no nutrient ranges were established in Table 4 of the Feeds Regulations. To support registration, companies will be required to submit in addition to the standard registration documents, sample of product, the product formulation, the description of the analytical methods for determination of the enzyme activity guarantee(s) and a certifi-

cation of analysis from three lots showing that, using the analytical methodology submitted, the product meets the guarantees shown on the labels and on expiration date.

The labeling statement "This product may cause dermal and respiratory irritation and/or sensitively. Appropriate protection equipment must be worn during handling" must be on labels or data justifying not using the statement must be submitted.

In addition, refer to registration checklist RC-14.

It has been reported that FDA will begin requiring a food additive petition to be submitted for any new submissions for a genetically modified enzyme.

REGULATORY STATUS OF SILAGE PRODUCTS

Labeling of products for addition to ensiled crops (silage additives) is regulated by AAFCO. Silage is not considered a commercial feed; however, products manufactured, distributed and intended for addition to silage are considered to be commercial feeds.

All ingredients used as silage additives (fermentation aids) must be established as safe based on their regulatory status. Products used for pesticidal effect fall under the jurisdiction of the Environmental Protection Agency. Silage products must be labeled with a guaranteed analysis, such as bacterial and/or enzymatic activity. AAFCO, FDA, academia and NFIA worked together in the 1970s to establish recommended guidelines for evaluating silage ingredients.

Recommended guidelines and research protocols for evaluating silage ingredients were accepted for publication as a progress report by AAFCO board of directors. Part of the report centered on minimum research information needed to make claims in three areas: (1) conservation and retention of nutrients during ensiling; (2) improved nutritive value of the ensiled forage crop, and (3) improved animal performance upon feeding the ensiled crop. FDA stated that the third claim area, improved animal performance, would require FDA development of any criteria for obtaining such an approval. The first two claims listed were accepted by AAFCO. The NFIA committee and cooperating groups formulated this information as a committee report as a result of objectives to:

- Review and update scientific literature in the field of ensiling;
- Develop proper labeling for silage ingredients;
- Establish official definitions for silage ingredients;
- Recommend analytical procedures for silage ingredients as well as methods for determining the quality of silage;
- Develop guidelines to determine the effect of silage ingredients on the conservation of nutrients during silage fermentation;
- Suggest research guidelines for determining improved nutritive value of the ensiling process, and
- Establish research guidelines for determining improved animal performance upon feeding an ensiled crop.

A literature search for compilation and publication of data on silage fermentation and its control was authorized by NFIA. The NFIA *Fermentation of Silage — A Review* was a result of those discussions and still used by the industry. Another excellent publication put out by NFIA is *Field Guide for Hay & Silage Management in North America*.

MEFTC continues to work on the development of analytical methods for silage products.

However, many applicable analytical methods do exist and are published in the *AFIA Laboratory Methods Compendium*; by the Association of Analytical Chemists, the American Association of Textile Chemists and Colorists, the American Association of Cereal Chemists, the United Stated Pharmacopeia, the National Academy of Sciences in the FCC, and in various academic journals such as Journal of Bacteriology, Journal of Agricultural & Biological Chemistry and Journal of Applied & Environmental Microbiology.

The following is an example of the required label for silage products:

Net weight Shown on Bag

Blue Bird Silage Inoculant

For conservation and retention of nutrients during ensiling

GUARANTEED ANALYSIS: Lactic Acid Bacteria, not less than..100 million CFU/lb.

INGREDIENTS: (Each ingredient must be specifically named in accordance with the names and definitions adopted by AAFCO. Collective terms as approved by AAFCO may be used where applicable.)

DIRECTIONS FOR USE: The directions for use and the guaranteed analysis must be stated in the same units.)

Manufactured by:
Company Name
Address

In Canada, the registration requirements for forage additives are covered under Trade Memorandum T-3-122, *Registration of Forage Additive Products*. Also refer to registration checklist RC-7.

Forage additives were classified in three categories:

- Nutritive additives such as molasses, grains, whey, urea, etc.;
- Preservatives, such as organic acids (and derivatives), mineral acids, antioxidants, etc., and
- Non-nutritive additives, such as enzymes, yeast and bacterial cultures, etc.

Forage additives are considered specialty products under the feeds regulations.

The product formulation must be submitted. The registration application must also be accompanied by a submission containing satisfactory evidence to substantiate at least one nutritional claim for the product. Nutritional claims must relate to the conservation, retention or preservation of recognized nutritional elements such as dry matter, protein, fiber-bound protein, vitamins, etc.

Claims relating to chemical or physical attributes of the forage (e.g., pH, temperature, mold count, etc.) are not acceptable as primary nutritional claims but may be accepted as secondary claims on the label provided at least one nutritional claim has been substantiated. For products considered as nutritive additives (Category 1), scientific data is not required since the guaranteed analysis stated on the product label substantiates its nutritional purpose.

Testing of forage additive products for efficacy must be performed under conditions similar to Canadian conditions. The experiments must be performed at a recognized research establishment, under the supervision of qualified research personnel. The research design must facilitate statistical analysis and results thereof be analyzed by appropriate statistical methods, all of which is submitted for evaluation.

For inoculant-type products, the company must also clearly describe the species of bacteria used and submit an analytical report with procedure for the identification and quantification of each bacterial strain. Submissions must include information demonstrating that the purity and origin of the bacterial strain(s) are acceptable under the Animal Disease & Protection Act & Regulations.

The type of guarantees that must be shown on the product label depend on the type of product, but can be summarized as:

- Nutritive additives: Nutrient guarantees reflecting significant nutrient additions to the forage;
- Preservatives: Guarantee for the preservative agent, and
- Non-nutritive additives: Guarantee for the active ingredient (e.g., enzyme activity, viable lactic acid-producing bacteria, etc.) ■

TABLE 2a. Enzymes/source organisms acceptable for use in animal feeds[1]

In the case of microbial enzymes it is understood that they are produced from nonpathogenic and nontoxigenie strains

Classification/name	Source organism	Typical substrate[2]	Function	Current supported use
Carbohydrates				
alpha-Amylase	Animal pancreatic tissue *Aspergillus niger*, var. *Aspergillus oryzae*, var. *Bacillus amyloliquefaciens* *Bacillus lentus* *Bacillus licheniformis* *Bacillus licheniformis* containing a *Bacillus stearothermophilus* gene for alpha-Amylase *Bacillus stearothermophilus* *Bacillus subtilis* containing a *Bacillus megaterium* gene for alpha-Amylase *Bacillus subtilis* containing a *Bacillus stearothermophilus* gene for alpha-Amylase *Bacillus subtilis*, var. Barley malt *Rhizopus niveus* *Rhizopus oryzae*, var.	Corn silage, corn, feed meal, corn gluten feed, soybean meal wheat, wheat middlings, wheat feed meal, barley, grain sorghum pea, oat, tapioca, millet, rice, rice feed meal	Hydrolizes starch	
Maltogenic alpha-Amylase	*Bacillus subtilis* containing a *Bacillus stearothermophilus* gene for Maltogenic alpha-Amylase	See alpha-Amylase	Hydrolyzes starch with production of maltose	
beta-Amylase	Barley malt	See alpha-Amylase	Hydrolyzes starch with production of maltose	
Cellulase	*Aspergillus niger*, var. *Humicola insolens* *Trichoderma longibrachiatum* (formerly *reesei* or *viride*)	Corn, barley, wheat, wheat bran, rye, grain sorghum	Breaks down cellulose	
alpha-Galactosidase	*Aspergillus niger*, var. *Morteirella vinaceae* var. *Saccharomyces* sp.	Sweet lupin, soybean meal	Hydrolyzes oligosaccharides	
beta-Glucanase	*Aspergillus niger*, var. *Aspergillus aculeatus* *Bacillus lentus* *Bacillus subtilis*, var. *Humicola insolens* *Penicillium funiculosum* *Trichoderma longibrachiatum* (formerly *reesei* or *viride*)	Wheat, barley, canola meal, wheat byproduct, oat groats, rye, triticale, grain sorghum	Hydrolysis of B-glucans, a type of non-starch polysaccharide	Reduction of digesta viscosity with barley based poultry diets, reduces soluble non-starch polysaccharides in digesta
beta-Glucosidase	*Aspergillus niger*, var.	Plant cell wall constituents	Hydrolyzes cellulose degradation products to glucose	
Glucoamylase (Amyloglucosidase)	*Aspergillus niger*, var. *Aspergillus oryzae*, var. *Rhizopus niveus* *Rhizopus oryzae*, var.	See alpha-Amylase	Hydrolyzes starch with production of glucose	
Hemicellulase	*Aspergillus aculeatus* *Aspergillus niger*, var. *Bacillus lentus* *Bacillus subtilis*, var. *Humicola insolens* *Trichoderma longibrachiatum* (formerly *reesei* or *viride*)	Corn, soybean meal, guar meal, barley, rye, grain sorghum, wheat, oats, peas, lentils	Breaks down hemicellulose	Reduction in stickiness of excreta in poultry fed guar meal
Invertase	*Aspergillus niger*, var. *Saccharomyces* sp.	Sucrose containing products and byproducts	Hydrolyzes sucrose to glucose and fructose	
Lactase	*Aspergillus niger*, var. *Aspergillus oryzae*, var. *Candida pseudotropicalis* *Kluyveromyces marxianis* var. lactis (formerly *Saccharomyces* sp.)	Lactose containing products and byproducts	Hydrolyzes lactose to glucose and galactose	
beta-Mannanase	*Aspergillus niger*, var. *Bacillus lentus* *Trichoderma longibrachiatum* (formerly *reesei* or *viride*)	Corn, soybean meal, guar meal, copra meal	Hydrolyzes B-mannans, a type of hemicellulose	Reduction in stickiness of excreta in poultry fed guar meal
Pectinase	*Aspergillus aculeatus* *Aspergillus niger*, var. *Rhizopus oryzae*	Corn, wheat	Breaks down pectin	
Pullulanase	*Bacillus acidopullulyticus* *Bacillus licheniformis* containing a *Bacillus deramificans* gene for pullulanase	See alpha-Amylase	Hydrolyzes starch	
Xylanase	*Aspergillus niger*, var. *Aspergillus oryzae* expressing a *Thermomyces lanuginosus* xylanase gene, *Bacillus lentus* *Bacillus subtilis* var. *Humicola insolens* *Penicillium funiculosum* *Trichoderma longibrachiatum* (formerly *reesei* or *viride*)	Corn, barley, rye, wheat, grain sorghum, triticale, oats	Hydrolyzes xylans, a component of hemicelluose	Reduction of digesta viscosity with poultry diets

[1]Source: Table 30.1 from AFFCO's 2002 *Official Publication*
[2]This list is to provide guidance and is not all inclusive

TABLE 2b. Enzymes/source organisms acceptable for use in animal feeds[1]

In the case of microbial enzymes it is understood that they are produced from nonpathogenic and nontoxigenie strains

Classification/name	Source organism	Typical substrate[2]	Function	Current supported use
Lipases				
Lipase	Animal pancreatic tissue	Plant and animal sources of fats and oils	Hydrolyzes triglycerides	
	Aspergillus niger, var.			
	Aspergillus oryzae, var.			
	Candida rugosa (formerly *cylindracea*)			
	Edible forestomach of calves, kids and lambs			
	Rhizomucor(*Mucor-*) *miehei*			
	Rhizopus oryzae			
Proteases				
Bromelain	Pineapples - stem, fruit	Pant and animal proteins	Hydrolyzes proteins	
Ficin	Figs	Plant and animal proteins	Hydrolyzes proteins	
Keratinase	*Bacillus licheniformis*	Plant and animal proteins	Hydrolyzes proteins	
Papain	Papaya	Plant and animal proteins	Hydrolyzes proteins	
Pepsin	Porcine or other animal stomachs	Plant and animal proteins	Hydrolyzes proteins	
Protease (general)	*Aspergillus niger*, var.	Plant and animal proteins	Hydrolyzes proteins	
	Aspergillus oryzae, var.			
	Bacillus amyloliquefaciens			
	Bacillus licheniformis			
	Bacillus subtilis, var.			
	Bacillus subtilis containing a *Bacillus amyloliquefaciens* gene for protease	Plant proteins	Hydrolyzes proteins	
Trypsin	Animal pancreatic tissue	Plant and animal proteins	Hydrolyses proteins	
Oxidoreductases				
Catalase	*Aspergillus niger*, var.	Hydrogen peroxide	Produces water and oxygen from hydrogen	
	Micrococcus lysodeikticus			
Glucose oxidase	*Aspergillus niger*, var.	Glucose	Degrades glucose to hydrogen peroxide and gluconic acid	
PHOSPHATES				
Phytase	*Aspergillus niger*, var.	Corn, soybean meal, Sunflower meal, hominy, tapioca, plant byproducts	Hydrolyzes phytate	Increases the digestibility of phytin-bound phosphorus in swine and poultry diets
	Aspergillus oryzae, var.			
	Phytase canola (*Brassica napus* expressing the *Aspergillus niger* phytase gene)			
	Aspergillus oryzae expressing the *Peniphora lycii* phytase gene			
	Schizosaccharomyces pombe expressing an *Escherichia coli* strain B phytase gene			
	Penicillium funiculosum			

[1]Source: Table 30.1 from AFFCO's 2002 *Official Publication*
[2]This list is to provide guidance and is not all inclusive

Enzyme Marketing Coordination from AAFCO *Official Publication*

NOTE: Sponsors of new enzyme/source organisms shall fully comply with this document by Jan. 1, 1998.

BACKGROUND

Enzymes are organic catalysts that affect the rate at which chemical reactions occur for specific substrates, including foods. AAFCO Policy Statement 7 describes the current sources of enzymes permitted in animal feeds. Rennet and papain are listed as GRAS under 21 CFR 582. All other enzyme materials to be used in animal feeds require a Food Additive regulation unless they are determined to be GRAS. The Center for Food Safety & Applied Nutrition (CFSAN) has published regulations for some enzyme preparations for use in human nutrition as secondary direct food additives under 21 CFR 173 and as GRAS food substances in 21 CFR 184. However, these applications are not directly transferable to animal use.

DEFINITIONS

The terms presented below are to clarify this document and do not represent nomenclature utilized by all enzyme manufacturers.

Enzyme. A protein made up of amino acids or their derivatives, which catalyzes a defined chemical reaction. Required cofactors should be considered an integral part of the enzyme.

Note: All other organic catalysts are excluded from consideration under this marketing coordination scheme.

Source organism. The organism that actually produces the enzyme(s).

Manufacturer. The firm or individual that actually produces the enzyme from the source organism.

Sponsor. The firm or individual that proposes adding an enzyme/source organism to the list published in the AAFCO *Official Publication* (*Official Publication*).

Enzyme preparation. A partially purified, unstandardized mixture of the enzyme(s) of interest and residues from the source organism. Enzyme preparations are not intended for sale or distribution for direct use on animal feed products without undergoing further processing.

Enzyme containing material. A material which is manufactured from the enzyme preparation, but is not necessarily, the final enzyme product. This material, if used in product development dials, must be substantially similar to the proposed product.

Enzyme product. A processed, standardized enzyme-containing material which has been produced with the intention of being sold for use on animal feed and feed ingredients. Examples of enzyme products would include feed grain treatments, commercial premixes and ready-to-use or apply materials.

Enzyme substrate. The material or substance which is acted upon catalytically by the enzyme.

Enzymatic activity (unit of). The catalytic activity required to convert a given amount of assay substrate to a given amount of product per unit time under the standard conditions set forth in the assay procedure.

REGULATORY APPROACH

The U.S. Food & Drug Administration considers all feed enzymes to be either food additives or GRAS substances as defined by the Federal Food, Drug & Cosmetic Act. However, the FDA plans at the present time to utilize regulatory discretion in the regulation of feed enzymes that present no safety concerns. A food additive petition will not be required for many products. However, if the agency has concerns about an enzyme/source organism, a formal food additive petition may be required.

This document, written jointly by the AAFCO, FDA, Agriculture & Agri-Food Canada and industry, describes the information which may be necessary for confirmation of the suitability of an enzyme/source organism for inclusion in the *Official Publication*. Issuance of a favorable informal opinion by the FDA may provide the safety and functionality substantiation necessary for AAFCO to adopt an official definition for a feed enzyme/source organism. All marketed enzymes must meet at least one of the following criteria: (1) be published in the *Official Publication*; (2) be the subject of a Food Additive regulation under 21 CFR 573; (3) be affirmed as GRAS; (4) be GRAS, or (5) be the subject of an informal no objection letter from the FDA (will be published in the next *Official Publication*). If an enzyme is published in the *Code of Federal Regulations* as an approved food additive it will also be included in the *Official Publication*. It should be noted that publication of an enzyme/source organism in the *Official Publication* does not remove a firm's responsibility of complying with applicable Canadian regulations.

The sponsor of an unpublished enzyme/source organism is to provide information which addresses issues of safety, functionality, labeling and manufacturing. The request for review should be sent to the designated AAFCO contact. The supporting information should be sent to the Division of Animal Feeds, Center for Veterinary Medicine, FDA. FDA will be asked to evaluate the information and determine its adequacy. If FDA determines that the enzyme/source organism does not require an approved food additive petition to ensure its safe use, AAFCO will be asked to propose a new or modify an existing definition under which the enzyme/source organism would be published in the *Official Publication*. Any restrictions on claims and use conditions will be addressed by the FDA in its statements to AAFCO and the sponsor. The official definition will include: trivial name and/or International Union of Biochemistry (IUB) name, if available; enzyme classification; source organism, and substrate(s).

In the information package, the sponsor may include material from the literature or current research. International data are acceptable provided conditions of testing simulate practices in this country. Supporting empirical information should be summarized and appropriate statistical analysis applied. Information that must be submitted by the sponsor includes: the sponsor's name and address, the enzyme, its proposed use and source organism. If any material written in a foreign language is included, a complete translation must be provided.

An appropriate section of the *Official Publication* is to be reserved for listing an enzyme and its source organism(s). After FDA evaluation of the information submitted for a new enzyme/source organism, a letter will be sent to the designated AAFCO contact. A copy of the letter will also be sent to the sponsor. Both the States and FDA will monitor the industry for compliance.

The following specific areas must be addressed by the sponsor:

Enzyme identity. The enzyme present in the enzyme preparation or product is to be identified and activity determined. The enzyme preparation or product is to be shown to contain no viable source organisms above an appropriate background. A suggested maximum is 1×10^4 colony forming units (CFU/g) of the source organism. If the source organism is published in the *Official Publication* under definition 36.14, there shall be no restriction on source organism numbers.

Identity information should include the following:

(A) Active enzyme substance — should be identified, preferably using the nomenclature system developed by the IUB. Specific terminology, such as phytase, pectinase, amylase or glucanase, are preferred.

(B) Enzyme substrate the specific substance on which the enzyme acts should be identified. General terminology such as carbohydrate, fiber, lipids and protein are acceptable; however, specific terminology such as starch, cellulose, phytin and lactose are preferred.

(C) Reaction products — the primary resultant product(s) from the enzyme substrate reaction should be identified to the extent that it is practical.

(D) Site of enzyme activity — the site of

activity is recognized to be on the feed/ingesta. Any other statement regarding site of activity is subject to FDA review.

Bioengineered sources of enzymes. A source organism may be bioengineered using recombinant deoxyribonucleic acid (rDNA) technology. This type of technology is defined as "any method by which DNA is manipulated *in vitro* and introduced into the source organism." Initially, use of bioengineered source organisms will be handled on a case-by-case basis. If the structure/amino acid sequence of an enzyme has not been significantly affected by changes in the genome of the source organism, it is not anticipated that additional requirements will be imposed for inclusion in the *Official Publication*. However, if a source organism has been modified by rDNA techniques to contain an antibiotic resistance gene, then the enzyme product should contain no detectable, viable source organisms and no transformable antibiotic resistance DNA.

Safety — animal/human/environment. Safety is the overriding issue with food and food ingredients and thus, for enzyme/source materials for animal use. Initial questions will reside around whether the enzyme preparation has adverse effects on either the animal, the environment, or humans via edible products from animals fed the enzyme.

Animal safety. Enzymes, as defined in this document, are amino acid-based catalysts used at low levels to alter animal feedstuffs. Because of this basic structure, it is reasonable to assume that these molecules will be digested in the gastro-intestinal tract, as would any other protein. Since an enzyme will be broken down into its constituent amino acids and cofactors and thus, be indistinguishable from other food molecules, the potential for residues in edible animal tissues appears minimal. Thus, the only other major factor which may raise a safety concern is the possible presence of compounds produced by or derived from the source organism. Pariza and Foster (1983)[1] have developed a set of guidelines to assess the safety of enzymes used in food processing. These guidelines address the safety of the source organism and the enzyme itself. Enzyme preparations that meet or surpass the criteria proposed by Pariza and Foster for human food should be safe for use in animal feed when utilized at the low levels normally employed for these catalysts.

Alternatively, the sponsor can provide data demonstrating no adverse effects when the most sensitive target animal is fed at least five times the maximum supplementation level for a period of 90 days or 50% of the species normal growing period, whichever is less. The species will be determined by product labeling and/or manufacturer suggestions.

Enzyme sponsors should also address the presence of enzymatic cofactors in the enzyme preparation. The presence of cofactors, such as the vitamins or nicotinamide adenine dinucleotide (NAD) is not of concern, but should be reported. If the enzyme requires potentially toxic cofactors, such as selenium or molybdenum, the submission should indicate the identity and amount of the cofactor.

Enzymes produced using current good manufacturing practices from food animals, edible and non-toxic plants or non-toxigenic and nonpathogenic microorganisms that do not produce antibiotics, should be safe for consumption at the low levels one would normally expect to encounter in animal feeds. In addition, the enzyme preparation should comply with the chemical and microbiological purity standards established by the Joint FAO/WHO Expert Committee on Food Additives[2] and the Food Chemical Codex.[3]

Carriers, diluents and processing aids used in the production of enzyme preparations and products must be substances that are acceptable for feed usage. If an enzyme preparation or product is standardized or diluted with feed-grade material, then applicable chemical and microbiological standards for the feed material will apply.

Human safety. Enzymes, as defined in this document, are amino acid-based catalysts used at low levels to alter animal feedstuffs. Because of this basic structure, it is reasonable to assume that these molecules will be digested in the gastro-intestinal tract, as would any other protein. However, it is the responsibility of the sponsor to provide appropriate data to assure human safety. Enzymes used in animal feed that pass the safety assessment proposed by Pariza and Foster should raise no human safety concerns.

If the Pariza and Foster decision tree is not used to evaluate the enzyme preparation, the sponsor must provide information regarding the fate of the enzyme in the target animal. If it cannot be assured that the enzyme is broken down to non-toxic metabolites, it may be necessary to quantify the amount of residue and identify safety concerns for these molecules. If an enzyme preparation is currently approved for addition to or conditioning of human foods, human safety data may not be required. However, human food use must be substantiated and a statement of similar/identical usage will be required. If human food safety is an issue, a food additive petition under 21 CFR 570 will be required.

Environmental safety. Information is required on each enzyme/source organism to assure that it does not adversely affect the environment. Information showing that the enzyme is composed of or broken down to normal non-toxic degradation products in the digestive tract of supplemented animals would be adequate to answer questions of environmental safety. If degradation metabolites have an unusual chemical composition not normally present in foods, it may be necessary to demonstrate metabolite safety for non-target species that may be exposed to target animal wastes. Environmental safety concerns could also be allayed if it could be demonstrated that the same or similar enzymes in approximately the same concentrations are excreted by free living organisms in a similar environment.

Functionality. The functionality of the enzyme itself must be documented. Either *in vivo* or *in vitro* data are acceptable to demonstrate enzyme functionality. The functionality statement associated with an enzyme/source organism combination will be determined by the data submitted under this proposal. The chosen research approach, either *in vivo* or *in vitro*, should answer questions relating to the amount of enzyme material necessary to have the intended effect and the use conditions (restrictions) for the enzyme. All experimental protocols should be described as would be required for publication in a peer-review journal. The procedure used to determine enzyme functionality should be described in detail. If functionality is determined by end product measurements, assay sensitivity and cross-reactivity to other constituents/contaminants should be discussed. Functionality data must substantiate the proposed label. Animal experiments demonstrating enzyme functionality are highly recommended. Trial design should ensure that statistical analysis of experimental data is possible. The number of trials should be adequate to document enzyme functionality under field conditions. Indicators of enzyme functionality could include increased nutrient digestibility and/or increased free nutrient levels. Sponsors should note, however, that label/advertising claims for improved animal performance or health will cause an enzyme to be classified as a new animal drug.

Functionality can also be addressed using *in vitro* studies with either complete feeds, feed ingredients or feed substrates being utilized as the enzyme substrate. Experimental design and the accompanying statistical analysis must be adequate to support enzyme functionality under field conditions. Dependent on the reaction catalyzed, the sponsor may wish to measure the disappearance of undegraded feed substrate or the appearance of enzyme reaction products. This approach directly measures the enzymatic digestion of foodstuffs when compared to a similarly treated control sample. Either experimental approach is acceptable. However, the sponsor must explain how observed *in vitro* effects translate to practical functionality of the enzyme on feed or feed ingredients.

Factors that should be explained in detail in the submission include the apparatus/reagents/protocol used to conduct all functionality experiments. Buffer solutions should be selected so as to provide appropriate pH environments similar to those in which the enzyme product is expected to be used. All control (untreated) samples of feedstuffs shall be treated identically to the enzyme samples, except for the addition of the enzyme. Incubation temperatures for the digestion period should not exceed the range of temperatures normally encountered under practical conditions for enzyme use. The enzyme containing material may be either research or technical grade, but must be similar to that which will be used commercially.

Complete feeds, feed ingredients or feed substrates obtained from a feed ingredient, can be used to simulate the feed to which the enzyme will be applied. These experimental substrates should be similar in analysis and in physical/chemical treatment to the feed which the enzyme will be used for in commercial situations. No less than five samples of each grain per treatment should be used in the trial. Use of feed, feed ingredients or substrates containing grain from several different lots (origins) would be desirable. However, the experimental design should ensure that lots (origins) are not confounded with enzyme treatment, i.e., all of lot 1 treated with the enzyme, while all control samples came from a different lot.

Enzyme functionality tested by *in vitro* activity on feed

- Collect samples of typical target feed or feed ingredient
- Treat with candidate enzyme mixture for a given period of time at appropriate pH and temperature
- Analyze samples for increased levels of breakdown products or decreased concentrations of targeted substrate
- Compare results with untreated control samples
- Enzyme treatments that result in significantly altered concentrations of targeted substrate or breakdown products are judged to be utilitarian for practical application

Quantification of enzyme product. Methodology is needed to measure the amount of activity of the enzyme product in its marketed concentrated (premix) form. Activity should be expressed as micromoles (moles) of released catalytic product per minute per gram of market product or in other standardized units. It is the responsibility of the sponsor to provide this methodology along with supporting information about its specificity, sensitivity and accuracy.

(A) Assay methodology
  1. Enzyme product; and/or
  2. Finished feed

(B) Specificity/sensitivity
  1. Two-external laboratory validation; or
  2. AOAC International validation, which can include the short form, or
  3. Other recognized methods

Labeling. The label should: describe the enzyme source (specific microbial or other source) that is recognized by FDA/AAFCO as safe and useful for the intended purpose; have a full listing of ingredients in order of preponderance; have a guaranteed analysis that is stated in meaningful terms; show a net quantity of product; contain warning and caution statements as needed; not have therapeutic or production claims; allow product identification by means such as lot numbers, expiration dates or another appropriate method of identification, and provide information on product storage, if necessary.

The product should be labeled in accordance with AAFCO and federal regulations. The label will include a guarantee of enzyme activity(ies) expressed in appropriate units. Clear directions for use that are reasonably certain to be followed in practice must be included, as should any known product limitations, such as ineffectiveness on specific forages. Adequate directions for use to enable the user to achieve the functionality of the enzyme(s) should be included, such as the feed ingredient(s) that the enzyme(s) acts on, the amount of product necessary to produce the intended effect and the length of time required to achieve this effect. If environmental factors, such as feed pH or moisture, or mechanical processing methods, like pelleting or extrusion, affect enzyme activity, these restrictions should also be noted on the label. Draft labeling should be included in the initial request of the sponsor.

The label must contain the following sections:

- Name of product.
- Functionality statement. "Contains a source of (enzymatic activity) which can (function and/or current supported use as stated in Section 30.1)." (statement based on information present in submission)
- Guaranteed analyses. (See AAFCO regulations 3 and 4.)

Enzymatic guarantees shall be expressed in appropriate units using either metric or avoirdupois measurements. The chosen units shall correspond with those present in the Use section. The source organism for each type of enzymatic activity shall be specified, such as: Protease (*Bacillus subtilis*) 5.5 mg amino acids liberated per minute per milligram.

If two sources have the same type of activity, they shall be listed in order of predominance based on the amount of enzymatic activity provided. However, the order of the ingredients in the Ingredients section is still determined by the amount (weight) of the different materials in the product.

- Ingredients. (listed in order of predominance by weight)
- Directions for use. Use instructions shall clearly state amount of enzyme required to achieve intended effect and other necessary information required for enzyme functionality.
- Caution/warning statements. (when required)
- Quantity statement.

Manufacturing. The sponsor is to provide information on the manufacture of the enzyme or quality controls (specifications) on the enzyme. The quality controls on the raw materials, on the manufacturing process/conditions, and on the enzyme product are to be presented. Appropriate information on product stability, labeling restrictions and special marketing controls are to be provided.

REFERENCES

(1) Pariza, M.W. and E.M. Foster. 1983. Determining the safety of enzyme used in food processing. Journal of Food Protection. 46(5): 453-468.

(2) Joint FAO/WHO Expert Committee on Food Additives. 1990. General specifications for enzyme preparations used in food processing. Food and Nutrition Paper No. 49. Pages 80-03.

(3) Anonymous. 1980. Food Chemical Codex. Page 107. National Academy Press: Washington, D.C. ■