Yeast and Their Uses in Feed



Mika Alewynse, PhD **Division of Animal Feeds** Center for Veterinary Medicine Presented at the April 2010 AAFCO Feed Administrators Seminar, Hickory Knob State Park, South Carolina

Ingredient Definitions

Where are they in the AAFCO Official Publication?

- Yeast
 - Section 96
- Fermentation products
 - Section 36
- Various other sections
 - Distillers and miscellaneous products, for example



Tentative Definitions

- If have enforcement discretion letter, are fine with CVM
 - Ingredient name establishes common or usual name
- AAFCO OP has Model Regulation 6(f)
 - Tentative definitions for ingredient shall not be used until adopted as official, unless no official definition exists or the ingredient has a common acceptable name that requires no definition, (i.e. sugar).
- Some firms try to use this clause to say no definition is required
- If no other definition exists, a tentative definition can be used
- What does your state law say?

Appropriate Definitions

How do I know the appropriate definition?

 Yeast are described using the section 96, Yeast

 Bacterial and mold ingredients are described using section 36, Fermentation Products

And never do the two mix.....

Appropriate Definitions (cont)

So, how do I tell what is what?

- Look at the scientific name of the organism
- Look at where the organism is listed
 - Types of yeast are identified in section 96, Yeast
 - Bacteria and molds are identified in section 36, Fermentation Products
 - Exceptions

Appropriate Definitions (cont)

Exceptions

- Direct-fed Microorganisms, definition 36.14
 - Yeast (as defined <u>elsewhere</u>)
 - Elsewhere is in section 96, Yeast

– Enzymes

- Microbial enzyme sources should be listed using section 36, Fermentation products definitions
 - Alpha-galactosidase
 - Invertase
 - Lactase
 - Lipase



Appropriate Definitions (cont)

I know the section, what's next?
Like all ingredients, read the definitions closely!
The text in the definition is your friend!



Yeast Definitions

- Identify what is in the ingredient and its microbial origin
- Only a few organisms
 - Saccharomyces cerevisiae
 - Saccharomyces boulardii reclassified as S. cerevisiae
 - Label must use Saccharomyces cerevisiae
 - Kluyveromyces marxianus
 - Candida utilis, formerly Torula
- Two broad categories
 Whole cells and solubles

Whole cells - with and without media

- Dead "non-fermentative"
 - 96.1 Primary dried yeast
 - 96.3 Irradiated dried yeast
 - Source of vitamin D
 - 96.7 Candida (Torula) dried yeast
 - 96.5 Grain distillers dried yeast
 - T96.12 Hydrolyzed yeast
- Viable "direct-fed microbial" definitions
 - 96.2 Active dry yeast
 - 96.8 Yeast culture



Brewers Yeast – "non-fermentative" Obtained from the production of beer and ale Separate definition for distillers yeast, 96.5 – 96.4 Brewers dried yeast – 96.10 Brewers liquid yeast Solubles - 96.9 Molasses yeast condensed solubles Liquid fermentation media – T96.11 Yeast extract Cell contents of yeast

96.8 Yeast culture* is the dried product composed of yeast (Saccharomyces cerevisiae and/or Kluyveromyces marxianus) and the media on which it was grown, dried in such a manner as to preserve the fermenting activity of the yeast. The media must be stated on the label.

*Note: No reference to media in main ingredient listing is required when yeast culture forms a component of a proprietary mixed feed.

- Grown on ground corn, hominy feed

- When is media listing required?
- Must be on single ingredient feed but could be listed on mixed feed label

Definitions are old and need updating

- Novel yeast species should not be marketed under these definitions as there are concerns
 - Particularly from ethanol production
 - Pathogenicity and potential for toxin production
- Botanical classification Saccharomyces is too broad
- Asking for comments about proposed yeast culture definition and other yeast species with history of use in feed

Questions

Images courtesy of The Microbe World at <u>www.edu.pe.ca/southernkings/microclass.htm</u>

Fermentation Products in Feed



Mika Alewynse, PhD Division of Animal Feeds Center for Veterinary Medicine

Presented at the April 2010 AAFCO Feed Administrators Seminar, Hickory Knob State Park, South Carolina

Fermentation Product Definitions

- Section 36 in the OP
- Identify what is in the ingredient and its microbial origin
- Most are byproducts from other processes
 - Check that substance of interest is still there
- Three broad categories
 - Whole cells, solubles, mixtures
- Terminology
 - Mycelium composed of cells from a mold
 - Biomass composed of cells from a bacteria
 - Water soluble exactly that
 - Broth growth media, generally from mold fermentation

36.2 Extracted _____ presscake
 36.13 Extracted _____ (presscake, meal or pellets)___

- Both composed of mycelium (whole organism)
- Obtained from an organism
 - Penicillium or Streptomyces
- Obtained from a particular production practice
 - Citric acid fermentation
 - Ask about whether organism historically used for this purpose
 - Firms developing novel organisms and may take advantage of lack of identity

- 36.9 Undried extracted ______ solids and fermentation solubles
 - Composed of mycelium (whole organism) and broth
 - Obtained from an organism
 - Penicillium or Streptomyces
 - Obtained from a particular production practice
 - Citric acid fermentation
 - Mixture of things

- Composed of extracted broth (substances must be soluble to be in a broth)
- Obtained from an organism
 - Penicillium or Streptomyces
- Obtained from a particular production practice
 - Citric acid fermentation

- 36.6 Dried ______ fermentation extract
- 36.7 Dried ______ fermentation solubles
 - Both composed of water soluble materials, not particles
 - <u>Only</u> for production of enzymes
 - Refer back to Table 30.1 in section 30 Enzymes for suitable organisms
 - If these ingredients are being marketed as a source of something other than an enzyme, look closely at the composition

- 36.16 Dried L-lysine fermentation product
- 36.17 Liquid L-lysine fermentation product
 - Both composed of biomass (bacterial cells) and L-lysine
 - Only acceptable organism is Corynebacterium glutamicum
 - Only as source of L-lysine

- 36.11 Dried ______ fermentation product
 36.12 Liquid ______ fermentation product
 Very general definitions
 Widely used in feed for multitude of purposes
 Including forage and grain inoculants
 Can be composed of whole organism and/or fermentation media/broth
 - Obtained from a listed organism

- 36.14 Direct-fed microorganisms (DFM)
 - Listed in ingredients list of label using
 - 36.11 Dried ______ fermentation product
 - 36.12 Liquid _____ fermentation product or occasionally as
 - a mycelium definition
 - Many extraction processes kill the organism
 - Cannot be a solubles definition
 - Solubles do not contain whole microbial cell

What are DFM Products?

Contain living (viable) microorganisms
 Two main categories

 Feed inoculants
 Intended to affect composition and/or digestibility of feed component
 May or may not be viable when consumed by animal
 Examples

 Inoculants for silage and high moisture grain

What are DFM Products? (cont)

 DFM products are intended to provide living (viable) microorganisms for addition to animal feed

– Why do the firms want to do that?

- Scientific literature suggests beneficial effects in animals
- Mirrors consumer interest in "natural" foods

New Organisms

Applicable to DFMs and feed inoculants
 Obtain AAFCO ingredient definition

- No history of unsafe use / inhabitant of GI tract
- Pathogenicity
- No commercial production of antibiotics
- Manufacturing data
- Information on the intended use

Microorganisms

 Accepted organisms listed in Fermentation products definition 36.14
 Unique label aspects – organisms are listed using 2 definitions

 The Genus species as listed in 36.14
 The type of fermentation product

Inoculants and the OP

Model regulations

 Guarantees - regulation 4(g)

 Statement for uniform interpretation and policy #23

 Registration and labeling of silage additive products
 Guarantees, directions for use, suitable ingredients

DFMs and the OP

Model regulations

 Acceptability and content - regulation 9(b)(4)
 Guarantees - regulation 4(g)

 Pet & specialty pet food regulations

 Guarantees - regulation PF4(h)

DFM Labels

DFM labels are not unique
 Everything that is needed for a "regular" feed label is also needed for DFMs

So, what should be there?

- If marketed as DFM, regulation 9(b)411 requires the statement
 - "contains a source of live (viable) naturally occurring microorganisms"

Complete Label

- Name
- Purpose (content) statement
- Guarantees
- Ingredient list
- Detailed use directions
- Caution/ warning statements
- Manufacturer/ distributor identifier
- Firm name and location
- Net content

Problem Areas

- What organisms
 Ingredient list
- > Use directions
- Guarantees



"Use by" dates (not mandated)

What Organisms to Use?

- Accepted organisms are listed in definition 36.14
 - Includes bacteria and fungi as listed
 - Yeast (as defined elsewhere)
- Yeast are defined in Section 96
 - Two yeast organisms are acceptable
 - Saccharomyces cerevisiae
 - Kluyveromyces marxianus
 - Candida (Torula) definition is not for viable organisms

What Organisms to Use? (cont)

What about microbes in the Enzyme table, 30.1?

- There is some overlap, but the organism must be in definition 36.14
- Why is the list so short?
 - Living microbes grow and reproduce
 - Concerns about toxin production and ability to cause human and animal illnesses

Ingredient List

Do not list just the name
 Aspergillus niger

- Use Fermentation definitions from Section 36
 - Liquid Lactobacillus plantarum fermentation product
 - Dried Aspergillus oryzae fermentation product

Potential Definitions

- Which fermentation ingredient?
- Look at the definitions
 - 36.11 Dried _____ Fermentation Product

is the product derived by culturing _____ on appropriate nutrient media for the production of one or more of the following: enzymes, fermentation substances, or other microbial metabolites, and dried in accordance with approved methods and good manufacturing practices. Protein, amino acids, fat, fiber, **cell count**, enzyme activity or nutrient metabolite level shall be guaranteed where applicable...

36.12 Liquid _____ Fermentation Product

is the liquid product derived by culturing or fermenting _____ on appropriate liquid nutrient media for the production of one or more of the following: enzymes, fermentation substances, or other microbial metabolites, and stabilized by approved methods in accordance with good manufacturing practices. Percent solids, **cell count**, enzyme activity or nutrient metabolite level shall be guaranteed where applicable...

Potential Definitions (cont)

 For definitions 36.11 and 36.12, fill in the blank with organism from 36.14
 Dried <u>Bacillus subtilis</u> fermentation product

If the definition states "extract," "extracted," or "solubles," probably not suitable for viable product
Yeast in a DFM product

Yeast are different!

- Do not use Fermentation definitions
 - Dried Saccharomyces cerevisiae fermentation product
- Use yeast definitions in Section 96
- The yeast must be alive, so firms should check with their supplier
 - Active dry yeast, 96.2
 - Yeast culture, 96.8
- If the definition states "non-fermentative," yeast are dead and it is not a DFM

Use Directions

Remember the end user
 Include avoirdupois units

 If different species or animal classes require different amounts of microbes, state that

Use rates often differ among species

Guarantees

Regulation 4(g)

- Guarantees for microorganisms shall be stated in colony forming units per gram (CFU/g) when directions are for using the product in grams, or in colony forming units per pound (CFU/lb) when directions are for using the product in pounds.
- A parenthetical statement following the guarantee shall list each species in order of predominance.

Either avoirdupois or metric, correspond to the use directions
 Include microorganisms

 Not required that individual species be listed separately

 Include colony forming units

 Not cell count



What is in the guarantee? Bacillus subtilis.....1 million CFU/ g

Add 1 kg (1000 g) to each ton of complete feed

– Microorganism – B. subtilis

- Units 1 million CFU/ g
 - in metric since use directions are metric

Total lactic acid bacteria......1 x 10⁶ CFU/ lb (Lactobacillus plantarum, Lactobacillus acidophilus, and Enterococcus faecium)

Feed 2 ounces per head per day

- Bacterial type Total lactic acid bacteria
 - Cannot include yeast in this guarantee as yeast are not bacteria
- Units 1 million CFU/ lb
- Microorganisms L. plantarum, L. acidophilus, E. faecium

Lactobacillus plantarum1 x 10⁴ CFU/ lb Lactobacillus acidophilus1 x 10⁴ CFU/ lb Enterococcus faecium1 x 10⁶ CFU/ lb

Feed 2 ounces per head per day

- Microorganisms L. plantarum, L. acidophilus, E. faecium
- Units 10^4 and 10^6 CFU/ lb
 - in avoirdupois since use directions are avoirdupois
- Guarantees can be long, especially if there are multiple organisms

Other Issues

"Use by" dates not required

- Why include them?
 - DFM must contain living microorganisms
 - Everything dies even with preservatives
- Use of "use by" dates prevents questions concerning guarantees with an out-of-date product

Heat

- Many organisms adversely affected by heat
 - Pelleting
 - Storage

DFM Claims

- Must be based on scientific data in animal species of interest with the specific microbial species
 - "Yeast supplementation can aid in maintaining cellulolytic bacteria population in the rumen of animals fed diets containing greater than 50% concentrate"
- CPG Sec. 689.100 Direct-Fed Microbial Products <u>http://www.fda.gov/ICECI/ComplianceManuals/CompliancePolicyGuidan</u> <u>ceManual/ucm074707.htm</u>
- CVM Policy On Competitive Exclusion Products <u>http://www.fda.gov/AnimalVeterinary/NewsEvents/CVMUpdates/ucm12</u> <u>7971.htm</u>

DFM Claims (cont)

- Use of "probiotic" or "prebiotic" on the label now acceptable
- Guidance for Industry Complementary and Alternative Medicine Products and their Regulation by the Food and Drug Administration -

http://www.fda.gov/RegulatoryInformation/Guidances/ucm144657.htm

 "Probiotics may be regulated as dietary supplements, foods, or drugs under the Act, depending on the product's intended use"

Issues for Microbes

Taxonomy changes
 Production of antimicrobials
 Nontraditional genus species
 Bioengineered organisms

 Food Additive approval needed

 Pathogenicity and toxin testing

 Different animals, different susceptibilities

Mikrobial Good Stuff

Contains a source of live (viable) naturally occurring microorganisms

Guaranteed Analysis	
Lactobacillus plantarum (min.)	1 x 10 ⁵ CFU/ lb
Lactobacillus acidophilus (min.)	1 x 10 ⁵ CFU/ lb
Enterococcus faecium (min.)	1 x 10 ⁶ CFU/ Ib
or	

Lactic acid bacteria (min.) 1.2 x 10⁶ CFU/ lb (Enterococcus faecium, Lactobacillus plantarum, Lactobacillus acidophilus)

Ingredients: Ground limestone, dried Enterococcus faecium fermentation product, dried Lactobacillus plantarum fermentation product, dried Lactobacillus acidophilus fermentation product, sodium sorbate (preservative), mineral oil

Directions for Use Feed 2 ounces per cow per day Feed 0.5 oz per pig per day

Store at room temperature and in a dry place Use within 12 months of manufacture

Manufactured by Mika Industries, 7519 Stand Place, Rockville, MD 20855

Net Wt 44 lb (20 kg)

Manufactured 4/12/10

Questions?

Pictures courtesy of Tree of Life (www.tolweb.org/Ascomycota)

Enzymes Used in Feed





Mika Alewynse, PhD Division of Animal Feeds Center for Veterinary Medicine

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Enzymes and the CFR

Approved ingredients listed in: – Title 21 Code of Federal Regulations **582.1585** Papain **582.1685** Rennet ■ 573.130 Aminoglycoside 3'phosphotransferase II for use in bioengineered oilseed rape, tomato, cotton Nothing unique in CFR for enzymes Novel enzymes Food additive regulation

Enzymes and the OP

- Accepted enzyme/ source organism combinations listed in Table 30.1
- Unique aspect Enzyme Marketing Coordination document
- Model regulations
 - Acceptability regulation 9(b)(5)
 - Purpose regulation 3(a)(3)(VI)
 - Guarantees regulation 4(h)
- Pet & specialty pet food regulations
 - Guarantees regulation PF4(h)

Enzyme Specific Uses

Why are there so many different enzymes used in feed?

- Added to affect processing of ingredient
 amylase
- Added to alter digestibility of feed ingredient
 - phytase
- Added to alter characteristics of digesta
 - xylanase

Enzyme Specific Uses (cont)

- There is no such thing as a generic animal feed
 - Multitude of species
 - Companion animals, traditional livestock, nontraditional species (fish, llamas, ostriches)
 - All stages of an animal's lifespan
 - Neonate, growing, mature, breeding, elderly (primarily companion animals)
- Current supported use statements are animal species specific
 - Reduction of digesta viscosity with poultry diets
- Functionality statements are not
 - Hydrolyzes phytate

Enzyme Specific Uses (cont)

- Plants and byproducts are large proportion of many feeds
- Often contain undesirable components
 - Phytate, reduces phosphorus availability
 - Stachyose and raffinose, less available sources of energy

Enzyme Labels

Where are the enzyme regulations
What should be on the label
Trouble spots



Complete Label

- Name
- Purpose statement
- Guarantees
- Ingredient list
- Detailed use directions
- Caution/ warning statements
- Manufacturer/ distributor identifier
- Firm name and location
- Net content

Problem Areas for Labels

Ingredient list
Use directions
Guarantees
"Use by" dates (not mandated)

Ingredient List

- No
 - Phytase
 - Phytase from Aspergillus niger
 - Aspergillus niger fermentation extract (phytase)
- Yes
 - Aspergillus niger fermentation extract
- If product contains a fermentation ingredient
 - Use definition from Section 36, Fermentation Products

Ingredient List (cont)

Can I use an organism listed in 36.14, the Direct-fed Microorganisms, for an enzyme product?

An enzyme and the organism that produces it are linked, as indicated in Table 30.1

Cannot mix and match

Potential Definitions

Which fermentation ingredient?Look at the definitions

- 36.6 Dried _____ Fermentation Extract

is the dried product resulting from extracting and precipitating by means of non-aqueous solvents or other suitable means, the water soluble materials from a fermentation conducted for maximum production of enzymes using a non-pathogenic strain of the microorganism in accordance with good manufacturing practices.

– 36.7 Dried _____ Fermentation Solubles

is the dried material resulting from drying the water soluble materials after separation of suspended solids from a fermentation conducted for maximum production of enzymes using a non-pathogenic strain of the microorganism in accordance with good manufacturing practices.

Potential Definitions (cont)

– 36.11 Dried _____ Fermentation Product

is the product derived by culturing _____ on appropriate nutrient media for the production of one or more of the following: **enzymes**, fermentation substances, or other microbial metabolites, and dried in accordance with approved methods and good manufacturing practices. Protein, amino acids, fat, fiber, cell count, enzyme activity or nutrient metabolite level shall be guaranteed where applicable...

– 36.12 Liquid _____ Fermentation Product

is the liquid product derived by culturing or fermenting _____ on appropriate liquid nutrient media for the production of one or more of the following: **enzymes**, fermentation substances, or other microbial metabolites, and stabilized by approved methods in accordance with good manufacturing practices. Percent solids, cell count, enzyme activity or nutrient metabolite level shall be guaranteed where applicable...

Potential Definitions (cont)

What about enzymes obtained from plants or animals?

- What is the common and usual name of the "ingredient?"
 - Dried pineapple
 - Fig extract
 - Dried pancreas
 - Phytase canola

Use Directions

Remember the end user

- Include avoirdupois units
- If different species or animal classes require different amounts, label should state that
 - Phytase use rates often differ among layers, broilers, turkeys, and swine

Those @*!! Guarantees

Regulation 4(h)

 Guarantees for enzymes shall be stated in units of enzymatic activity per unit weight or volume, consistent with label directions. The source organism for each type of enzymatic activity shall be specified, such as: Protease (Bacillus subtilis) 5.5 mg amino acids liberated/min./milligram. If two or more sources have the same type of activity, they shall be listed in order of predominance based on the amount of enzymatic activity provided.

Enzyme Marketing Coordination document

- Either avoirdupois or metric, correspond to the use directions
- Include source organism
- Include units
- List sources by contribution of enzymatic activity

What is in the guarantee?
Protease (Bacillus subtilis) 5.5 mg amino acids liberated/ minute/ gram

- Protease type of enzyme activity
- B. subtilis source organism
- mg amino acids liberated/ minute/ g unit of enzymatic activity

Guarantees can be long

- Protease (Bacillus subtilis) 5.5 mg amino acids liberated/ minute/ gram
- Firms can split and can explain units elsewhere on label
 - Protease (Bacillus subtilis) 5.5 units*/ g
 - 1 unit of protease activity liberates 1 mg amino acid from casein/ minute
 - Should mention assay conditions, i.e., pH, temperature

Most problematic are the units
 What is there?

 mg amino acids liberated/ minute/ g
 mg amino acids liberated – what the enzyme does
 minute – time unit

– g – unit of enzyme product

Units are <u>enzyme specific</u> – what enzyme does

Units are also <u>assay specific</u> for a particular type of activity



Guarantees - Enzyme Specific

mg amino acids liberated/ minute
Look at Function in Table 30.1

Phytase – hydrolyzes phytate
Protease – hydrolyzes proteins
Lipase – hydrolyzes triglycerides (fat)
Cellulase – breaks down cellulose

Guarantees - Assay Specific

- Units are also <u>assay specific</u> for a particular type of activity
- Few standard assays
- Assay can measure 1 of 2 things
 - Change in substrate
 - Decrease in protein concentration
 - mg casein hydrolyzed/ minute
 - Change in end product
 - Increase in amino acid levels
 - mg amino acids liberated/ minute
Guarantees-Assay Specific (cont)

What to choose?

- Pick easiest to measure, generally, breakdown product
 - Protease increase in amino acid concentrations
 - Phytase increase in amount of free phosphorus
 - Amylase increase in sugar levels

Guarantees (cont)

Are there any Standards?
– AOAC
– Food Chemical Codex



"Use by" Dates

Not required

Why include them?

 Enzymes are proteins and activity depends on protein structure

 Protein structure degrades with time even with preservatives

Inclusion could prevent questions concerning guarantees with an out-ofdate product

Mikazyme

Contains a source of phytase, derived from Aspergillus oryzae Phytase increases the digestibility of phytin-bound phosphorus in swine and poultry diets

Guaranteed Analysis

Phytase (A. oryzae) min.2500 FTU/ gOne FTU is the amount of phytase which liberates 1 micromole of phosphorus per
minute from sodium phytate at pH 5.5 and 37 C (under conditions of the assay)

Ingredients: Sodium sulfate, ..., ..., hydrogenated vegetable oil, starch, dried Aspergillus oryzae fermentation extract

Directions for Use (mixing directions)

For swine add 200-400 g/ton complete feed to provide 500 FTU/ kg feed For broilers add 200 – 400 g/ton complete feed to provide 500 FTU/ kg feed For layers add 100-200 g/ton of complete feed to provide 250 FTU/ kg feed

Store at room temperature and in a dry place Use within 12 months of manufacture

Warning (human health concern) May cause eye, skin, and respiratory irritation Inhalation may cause allergic reaction in sensitized individuals

Manufactured by Mika Industries, 7519 Stand Place, Rockville, MD 20855

Net Wt 44 lb (20 kg)

Manufactured 4/12/10

Mikazyme's Swine Feed with Phytase

For starter pigs weighing 11 to 44 lb Phytase increases the digestibility of phytin-bound phosphorus in swine and poultry diets

20%
1.2%
500 FTU/ kg

Ingredients: Corn, ..., ..., dried Aspergillus oryzae fermentation extract, cobalt carbonate, calcium iodate, sodium selenite

Feeding Directions

Feed as the complete ration to starter pigs weighing 11 to 44 lbs

*One FTU is the amount of phytase which liberates 1 micromole of phosphorus per minute from sodium phytate at pH 5.5 and 37 C (under conditions of the assay)

Manufactured by Blue Bird Feed Mill, City, State Zip

Net Wt 50 lb (22.67 kg)

Questions?

Images courtesy of <u>www-news.uchicago.edu</u>, www.wpi.edul, www.chemlin.net

Other Questions

Spirulina - blue-green algae

- Falls under limited use ingredients for species whose nutritional requirements are not well understood
- Accepted for use in ornamental (aquarium) fish diets only
- Not acceptable in other animal diets
 - No data and information submitted to support these other uses
- Testing is necessary, generally for microbiological, heavy metal, and chemical contaminants
- Acacia petals (Acacia nilotica)

Accepted for use in cockatiel bird diets only

Other Claims

- Must be based on scientific data in animal species of interest with the specific species
- Must relate back to the nutritional content of product
 - Nutritive value, aroma, flavor
- These statements are probably OK
 - Supports the Digestive System
 - Supports the Immune System
- These claims are not for a microbial product
 - Promotes Healthy Shiny Hair Coat
 - Acceptable for a product with vitamin E or essential fatty acids
 - Promotes Vitality in older horses