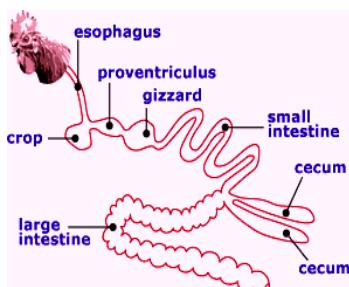


ZYMEX

ENZYMES WITH DIRECT FED ENZYME SECRETING MICROBES

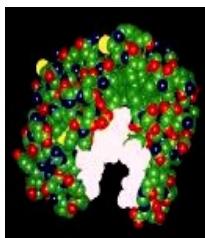
INTRODUCTION

Improved animal health and performance always has been the goal of people associated with livestock production. Consequently, any feedstuff, feed additive, drug or other compound that is capable of enhancing animal health or performance will interest producers, veterinarians, and animal nutritionists. Several compounds have been used to improve animal performance either by manipulation of the rumen environment (e.g., sodium bicarbonate) or by directly altering the composition and metabolic activities of rumen microorganisms (e.g., ionophores).



Digestion is the process of breaking down large, complex molecules, as provided by the birds' feed, into smaller components that can be absorbed into the portal blood system. The process involves changes in both physical and chemical structures of most dietary components. Poultry feeds consist of a complex array of particles differing not only in chemical composition, but also in size, hardness, solubility and ionic characteristics.

Under ideal conditions, this array of particles and chemicals with different characteristics degrade slowly in a step-wise manner as feed passes from the mouth to the large intestine. Particle breakdown is a constant process, although the gizzard provides the major site of this activity.



Enzymes are largely responsible for molecular degradation, although their pH greatly influences their efficacy. When digestion is reduced, there will be reduced bird growth and/or increased feed intake. Indigestion may also cause problems with manure/litter management, because non-digested residues in the large intestine often adsorb more water or produce feces that are more viscous.

Animal feed contains Cereals and Cakes.

Cell walls of cereals are primarily composed of carbohydrate complexes referred to as Non Starch Polysaccharides (NSP).

ANFs present in these NSP (like β -glucans and arabinoxylans) are non digestible and form high-molecular-weight viscous aggregates in the gastrointestinal tract.

They

- Affect the digestive enzymes.
- Cause endogenous losses
- Reduce the rate of passage.

- Stimulates pathogenic microbial proliferation.

Enzymes such as xylanase or β -glucanase into diets having ANF can effectively decrease viscosity and consequently reduce the anti nutritional effect of NSP. Cellulase, β -Glucanase, Xylanase, and Pectinase can degrade plant origin cell wall polymers. Amylase can increase the gut absorption levels of starches. Hemi Cellulase can degrade the difficult fiber. Protease can degrade the proteins. Lipase can degrade the lipids. Phytase helps in solubilising the phyto phosphorous. Tannases can degrade the plant toxins.

G.I Tract Region	Enzyme (or secretion)	Substrate	End Product	pH
Mouth	Saliva	Lubricates and softens food		
Crop	Mucus	Lubricates and softens food		4.5
Stomach (Gizzard and proventriculus)	HCl	Lowers stomach pH		2.5
	Pepsin	Protein	Polypeptides	
Duodenum	Trypsin, Chymotrypsin and Elastases	Proteins, Peptones and Peptides	Peptones, Peptides and amino acids	6 to 6.8
	Carboxy-peptidases	Peptides	Peptides and amino acids	
	Collagenase	Collagen	Peptides	
Jejunum	Peptidases	Peptides	Dipeptides and amino acids	5.8 to 6.6
	Polynucleotidase	Nucleic acids	Mono-nucleotides	

Posttranslational glycosylation has been reported to protect enzymes from deactivation caused by high temperatures and proteinases (Olsen and Thomsen, 1991).

All enzyme feed additives are considered either food additives or GRAS substances

The activity of enzymes retains more than 95% when stored at the temperature of 25 Deg C upto 3 months

PROTEINS:

Protein and amino acid availability are of greatest concern in animal and vegetable protein ingredients. Protein content and availability from cereals and their by-products seem to be more consistent and little affected by processing conditions.

Feedstuff	C. Protein (%)	Digestibility (%)			
		C. Protein	Lys	Met	Cys
Vegetable sources (cereals)					
Yellow maize	8	82 - 86	81	91	85
Wheat	12	78 - 82	81	87	87
Barley	10	70 - 82	78	79	81
Sorghum	10	67 - 72	78	89	83
Vegetable sources (oil seed meals)					
Peanut meals	49	88 - 91	83	88	78
Soybean meals	46	83 - 87	91	92	82
Cottonseed meal	43	61 - 76	67	73	73
Animal sources					
Blood meal	88	82-92	86	91	76
Fish meal	66	86 - 90	88	92	73
Meat meal	60	75 - 80	79	85	58
Feather meal	87	36 - 77	66	76	59

FATS:

G.I Tract Region	Enzyme (or secretion)	Substrate	End Product	pH
Mouth	Saliva	Lubricates and softens food		
Crop	Mucus	Lubricates and softens food		4.5
Stomach (Gizzard and	HCl	Lowers stomach pH		2.5

proventiculus)	Lipase	Fats	Fatty acids, mono-glycerides and glycerol	
Duodenum and Jejunum	Bile	Fats	Emulsification	
	Lipase	Fats	Fatty acids, mono-glycerides and glycerol	5.8
	Cholesterol esterase	Fatty acid - cholesterol esters	Fatty acid, cholesterol	to 6.6

Fat Type/Age	Digestibility		Metabolizable energy (kcal/kg)	
	0-21d	>21d	0-21d	>21 d
Tallow	80	86	7400	8000
Poultry Fat	88	97	8200	9000
Fish oil	92	97	8600	9000
Vegetable oil	95	99	8800	9200
Coconut oil	70	84	6500	7800
Palm oil	77	86	7200	8000
Vegetable soapstock	84	87	7800	8100
Restaurant grease	87	96	8100	8900

Cellulase

Breaks down cellulose and chitin

(chitin is cellulose like fiber found in the cell wall of candida).

Cellulases acts on cellulose molecules by hydrolysing the beta-1, 4 glycosidic linkages.

They largely produces cellobiose, which can ultimately yield glucose units, depending on the characteristic of the enzyme.

It helps free nutrients in both fruits and vegetables.

Measured in CU (Cellulase Units).

Activity of Enzyme:

One activity u/mg (u/ml) of cellulase is defined as that quantity of enzyme that can liberate 1g glucose in one minute at pH of 4.8.

Standard Product: 8000 u/g

Use the preparation between pH of 4.0~5.0,

Generally speaking, adding the preparation 50g to 1t dry materials will play well.

Protease:

Bonds with alpha 2-macroglobulin to support immune function when taken on an empty stomach.

Protease is responsible for digesting proteins in food, which is probably one of the most difficult substances to metabolize. Because of this, protease is considered to be one of the most important enzymes that we have. If the digestive process is incomplete, undigested protein can wind up in the circulatory system, as well as in other parts of the body.

When protease is present in higher quantities, it can help to clean up the body by removing the unwanted protein from the circulatory system. This will help to clean up the blood stream, and restore the energy and balance.

One of the tricks of an invading organism is to wrap itself in a large protein shell that the body would view as being "normal". Large amounts of protease can help remove this protein shell, and allow the body's defense mechanisms to go into action. With the protective barrier down, the immune system can step in and destroy the invading organism.

Protease refers to a group of enzymes whose catalytic function is to hydrolyze (breakdown) peptide bonds of proteins. They are also called proteolytic enzymes or proteinases. Proteases differ in their ability to hydrolyze various peptide bonds. Each type of protease has a specific kind of peptide bonds it breaks. Examples of proteases include: fungal protease, pepsin, trypsin, chymotrypsin, papain, bromelain, and subtilisin.

Proteolytic enzymes are very important in digestion as they breakdown the protein foods to liberate the amino acids needed by the body. Additionally, proteolytic enzymes have been used for a long time in various forms of therapy. Their use in medicine is gaining more and more attention as several clinical studies are indicating their benefits in oncology, inflammatory conditions and immune regulation.

Contrary to old beliefs, several studies have shown that orally ingested enzymes can bypass the conditions of the GI tract and be absorbed into the blood stream while still maintaining their enzymatic activity. Commercially, proteases are produced in highly controlled aseptic conditions for food supplementation and systemic enzyme therapy. The organisms most often used are Aspergillus niger and oryzae.
Measured in HUT (Hemoglobin Units in a Tyrosine Base).

Lipase

Lipase is an enzyme necessary for the absorption and digestion of nutrients in the intestines. This digestive enzyme is responsible for breaking down lipids (fats), in particular triglycerides, which are fatty substances in the body that come from fat in the diet. Once broken down into smaller components, triglycerides are more easily absorbed in the intestines. Lipase is primarily produced in the pancreas but is also produced in the mouth and stomach. Most people produce sufficient amounts of pancreatic lipase.

Along with lipase, the pancreas secretes insulin and glucagon, hormones that the body needs to break down sugar in the bloodstream. Other pancreatic enzymes include amylase, which breaks down amylose (a form of starch) into its sugar building blocks, and protease, which breaks down protein into single amino acids.

Source:

Lipase, monoacylglycerol:

Penicillium camembertii

Lipase, triacylglycerol:

Aspergillus niger

Aspergillus oryzae

Aspergillus oryzae, containing the gene for Lipase, triacylglycerol isolated from *Fusarium oxysporum*

Aspergillus oryzae, containing the gene for Lipase, triacylglycerol isolated from *Humicola lanuginosa*

Aspergillus oryzae, containing the gene for Lipase, triacylglycerol isolated from *Rhizomucor miehei*

Rhizopus arrhizus

Rhizomucor miehei

Rhizophus niveus

Rhizophus oryzae

Uses

In general, lipase supplements are thought to help the body absorb food more easily, keeping nutrients at appropriate, healthy levels throughout the body. Studies suggest that they may also be helpful for the following conditions:

Celiac Disease

Pancreatic enzymes have been most studied as part of the treatment for celiac disease. Celiac disease is a condition in which dietary gluten causes damage to the intestinal tract. Symptoms include abdominal pain, weight loss, and fatigue. People with celiac disease must consume a life-long gluten-free diet. Lipase, along with other pancreatic enzymes, may help in the treatment of this condition by enhancing the benefit of a gluten-free diet. In a study of 40 children with celiac disease, for example, those who received pancreatic enzyme therapy (including lipase) demonstrated a modest increase in weight compared to those who received placebo. The improvement in weight occurred within the first month of use; taking the pancreatic enzyme supplements for an additional month did not lead to more weight gain.

Indigestion

In a small study including 18 subjects, supplements containing lipase and other pancreatic enzymes were found to reduce bloating, gas, and fullness following a high-fat meal. Given that these symptoms are commonly associated with irritable bowel syndrome, some with this condition may experience improvement with use of pancreatic enzymes.

Other

Although scientific evidence is lacking, lipase has been used by trained clinicians to treat food allergies, cystic fibrosis, and autoimmune disorders, such as rheumatoid arthritis and lupus.

Dietary Sources

Lipase is produced primarily in the pancreas and is not found in food.

Available Forms

Lipase supplements are usually derived from animal enzymes, although plant sources of lipase and other digestive enzymes have become increasingly popular. Lipase may be taken in combination with protease and amylase enzymes.

Pectinase

Breaks down carbohydrates, such as pectin found in many fruits and vegetables.

Measured in AJDU

Activity of Enzyme: One activity u/mg(u/ml) of pectinase is defined as that quantity of enzyme that can liberate 1 galacturonic acid in one minute at pH of 3.5,

Standard pectinase :1200000 u/ml min

Optimum pH of 3.2~5.0,

The activity of enzymes retains more than 95% when stored at the temperature of 25 Deg C; upto 3 months.

Xylanase

Xylanase (EC 3.2.1.8) is the name given to a class of enzymes which degrade the linear polysaccharide beta-1,4-xylan into xylose, thus breaking down hemicellulose, which is a major component of the cell wall of plants.

As such, it plays a major role in the digestive system of herbivorous micro-organisms (mammals, conversely, do not produce xylanase).

Additionally, xylanases are present in fungi for the degradation of plant matter into usable nutrients.

Commercial applications for xylanase include the chlorine-free bleaching of wood pulp in the papermaking process, and the increased digestibility of silage (in this aspect, it is also used for fermentative composting).

In the future, xylanase may be used for the production of biofuel from unusable plant material.

DIRECT FED MICROBES

Many studies have reported the effective usefulness of DFM in

- Detoxifying
- Enhancing the immunity System,
- Improving F C R
- Reducing the Diarrhoea,

Organism	End Products or Potential Use
<i>Bacillus subtilis</i>	amylase, protease
<i>Bifidobacterium bifidum</i>	ureases, lactic acid, formic acid
<i>L. lactis</i>	amylase, hydrogen peroxide, proteases
<i>Lactobacillus acidophilus</i>	lactic acid, acidophilin, glycosidases
<i>Pediococcus acidilactici</i>	pediocin (bacteriocin)
<i>Propionibacteria</i> sp.	Ruminal lactate utilizer, propionate producer
<i>Propionibacterium thoenii</i>	propionicin PLG-1 (bacteriocin)
<i>Bacillus polymyxa</i>	Polymixin B Antifungal Peptide

COMPOSITION OF ENZYMIX

Aamylase	325 UI/g
Cellulase	200 UI/g
Lipase	250 UI/g
Protease	300 UI/g
DFM	< 1000 Million CFU/g

SALIENT FEATURES OF ZYMEX

- Higher quality end product from cleaner eggs or reduced carcass downgrades
- Improved environment
- improves the digestibility of the feed
- Lower feed costs
- More uniform pigs and birds
- Proven value in antibiotic growth promoter free nutrition
- Provides feed manufacturing with the opportunity to choose low cost feeding stuff to replace high cost feeding stuff.
- Provides feed manufacturing with the opportunity to reduce feed costs

SUGGESTED LEVEL OF INCLUSION:

Generally speaking, adding ZYMEX75- 150 g to 1M T dry materials will play well. But you should reconfirmed suitable dosage depending on your bench-scale experiment results.

OTHER MATTERS OF IMPORTANCE

Use the preparation between pH of 3.8~5.0,

The activity of enzymes retains more than 95% when stored at the temperature of 25 Deg C upto 3 months

WITHDRAWAL PERIOD

Not necessary

STORAGE:

All Enzymes and DFM should be kept away from moisture, excess heat, and light

Ref:

1. Ahmed, F. E. 2003. Genetically modified probiotics in foods. *Trends Biotechnol.* 21:491-497.
2. Applied and Environmental Microbiology, November 2005, p. 6769-6775, Vol. 71, No. 110099-2240/05/\$08.00+0 doi:10.1128/AEM.71.11.6769-6775.2005
3. Baran, M., and V. Kmet. 1987. Effect of pectinase on rumen fermentation in sheep and lambs. *Arch. Anim. Nutr.* Berlin. 7/8:643.
4. Beauchemin, K. A., W. Z. Yang, and L. M. Rode. 1999. Effects of grain source and enzyme additive on site and extent of nutrient digestion in dairy cows. *J. Dairy Sci.* 82:378-390.
5. Beauchemin, K., A., and L. M. Rode. 1996. Use of feed enzymes in ruminant nutrition. Proc. of the Canadian Society of Animal Science Annual Meeting, Lethbridge, Alberta. Pp 103-140.
6. Beauchemin, K., A., L. M. Rode, and V.J.H. Sewalt. 1995. Fibrolytic enzymes increase fiber digestibility and growth rate of steers fed dry forages. *Can. J. Anim. Sci.* 75:641-644.
7. Bedford, M. R., and H. Schulze. 1998. Exogenous enzymes for pigs and poultry. *Nutr. Res. Rev.* 11:91-114.[CrossRef]
8. Beecham, T. J., J. V. Chambers, and M. D. Cunningham. 1977. Influence of *Lactobacillus acidophilus* on performance of young dairy calves. *J. Dairy Sci.* 60(Suppl. 1):74. (Abstract)
9. Beharka, A. A., T. G. Nagaraja, and J. L. Morrill. 1991. Performance and ruminal development of young calves fed diets with *Aspergillus oryzae* fermentation extracts. *J. Dairy Sci.* 74:4326-4336.
10. Berkow R, ed. *The Merck Manual of Medical Information*. Home Ed. Whitehouse Station, NJ: Merck Research Laboratories; 1997.
11. Bruce, B. B., S. E. Gilliland, L. J. Bush, and T. E. Staley. 1979. Influence of feeding cells of *Lactobacillus acidophilus* on the fecal flora of young dairy calves. *Oklahoma Anim. Sci. Res. Rep.* 207.
12. Carroccio A, Iacono G, Montalto G, et al. Pancreatic enzyme therapy in childhood celiac disease. A double-blind prospective randomized study. *Dig Dis Sci.* 1995;40(12):2555-2560.
13. Cheng, K. J., S. S. Lee, H. D. Bae, and J. K. Ha. 1999. Industrial applications of rumen microbes. *Asian-Australas. J. Anim. Sci.* 12:84-92.
14. Choct, A., and G. Annison. 1992. Anti-nutritive effect of wheat pentosans in broiler-chickens: role of viscosity and gut microflora. *Br. J. Poult. Sci.* 33:821-834.
15. Dawson, K. A., and D. M. Hopkins. 1991. Differential effects of live yeast on the cellulolytic activities of anaerobic ruminal bacteria. *J. Anim. Sci.* 69(Suppl. 1):531. (Abstract)
16. Dawson, K. A., K. E. Neuman, and J. A. Boling. 1990. Effects of microbial supplements containing yeast and lactobacilli on roughage-fed ruminal microbial activities. *J. Anim. Sci.* 68:3392-3398.
17. Ehrmann, M. A., P. Kurzak, J. Bauer, and R. F. Vogel. 2002. Characterization of lactobacilli towards their use as probiotic adjuncts in poultry. *J. Appl. Microbiol.* 92:966-975.[CrossRef] [Medline]
18. Englyst, H. N., S. A. Bingham, S. A. Runswick, E. Collinson, and J. H. Cummings. 1989. Dietary fibre (non-starch polysaccharides) in cereal products. *J. Hum. Nutr. Diet.* 2:253-271.
19. Feng, P., C. W. Hunt, W. E. Julien, K. Dickinson, and T. Moen. 1992. Effect of enzyme addition on in situ and in vitro degradation of mature cool-season grass forage. *J. Anim. Sci.* 70(Suppl. 1):309. (Abstract)
20. Fuller, R. 1989. Probiotics in man and animals. *J. Appl. Bact.* 66: 365-378
21. Gusils, C., O. Oppezzo, R. Pizarro, and S. Gonzalez. 2003. Adhesion of probiotic lactobacilli to chick intestinal mucus. *Can. J. Microbiol.* 49: 472-478.[CrossRef][Medline]
22. Heck AM; Yanovski JA; Calis KA. Orlistat, a new lipase inhibitor for the management of obesity. *Pharmacother* . 2000 Mar;20(3):270-279.
23. Hirstov, A., T. A. McAllister, and K. J. Cheng. 1998. Effect of dietary or abomasal supplementation of exogenous polysaccharide-degrading enzymes on rumen fermentation and nutrient digestibility. *J. Anim. Sci.* 76:3146-3156.

24. Hutchenson, D. P., N. A. Cole, W. Keaton, G. Graham, R. Dunlap, and K. Pitman. 1980. The use of living, nonfreeze-dried *Lactobacillus acidophilus* culture for receiving feedlot calves. *Proc. West. Sec. Amer. Soc. Anim. Sci.* 31:213. (Abstract)
25. Jaquette, R. D., R. J. Dennis, J. A. Coalson, D. R. Ware, E. T. Manfredi, and P. L. Read. 1988. Effect of feeding viable *Lactobacillus acidophilus* (BT1386) on the performance of lactating dairy cows. *J. Dairy Sci.* 71(Suppl. 1):219. (Abstract)
26. Jenny, B. F., H. J. Vandijk, and J. A. Collins. 1991. Performance and fecal flora of calves fed a *Bacillus subtilis* concentrate. *J. Dairy Sci.* 74:1968-1973.
27. Jin, L. Z., Y. W. Ho, N. Abdullah, and S. Jalaludin. 1998. Acid and bile tolerance of *Lactobacillus* isolated from chicken intestine. *Lett. Appl. Microbiol.* 27:183-185.[CrossRef][Medline]
28. Kerovuo, J., and S. Tynkkynen. 2000. Expression of *Bacillus subtilis* phytase in *Lactobacillus plantarum* 755. *Lett. Appl. Microbiol.* 30:325-329.
29. Konig, J., R. Grasser, H. Pikor, and K. Vogel. 2002. Determination of xylanase, β -glucanase, and cellulase activity. *Anal. Bioanal. Chem.* 374: 80-87.[CrossRef][Medline]
30. Kopenecy, J., M. Marounek, and K. Holub. 1987. Testing the suitability of the addition of *Trichoderma viride* cellulases to feed rations for ruminants. *Zivocisna Vyroba.* 32:587.
31. Kung, L., Jr., and A. O. Hession. 1995. Altering rumen fermentation by microbial inoculation with lactate-utilizing microorganisms. *J. Anim. Sci.* 73:250-256.
32. Kung, L., Jr., E. M. Kreck, R. S. Tung, A. O. Hession, A. C. Sheperd, M. A. Cohen, H. E. Swain, and J.A.Z. Leedle. 1996. Effects of a live yeast culture and enzymes on in vitro ruminal fermentation and milk production of dairy cows. *J. Dairy Sci.* 80:2045-2051.
33. Martin, S. A., and D. J. Nisbet. 1992. Effect of direct-fed microbials on rumen microbial fermentation. *J. Dairy Sci.* 75:1736-1744.
34. Mathlouthi, N., M. A. Mohamed, and M. Larbier. 2003. Effect of enzyme preparation containing xylanase and β -glucanase on performance of laying hens fed wheat/barley- or maize/soybean meal-based diets. *Br. Poult. Sci.* 44:60-66.[CrossRef][Medline]
35. Newbold, C. J. 1995a. Microbial feed additives for ruminants. In: *Biotechnology in Animal Feeds and Animal Feeding.* R. J. Wallace and A. Chesson (Eds.). VCH. New York. Pp. 259-278.
36. Newbold, C. J., R. J. Wallace, and F. M. McIntosh. 1996. Mode of action of the yeast *Saccharomyces cerevisiae* as a feed additive for ruminants. *Brit. J. Nutr.* 76:249.
37. Newbold, C. J., R. J. Wallace, X. B. Chen, and F. McIntosh. 1995b. Different strains of *Saccharomyces cerevisiae* differ in their effects on ruminal bacterial numbers in vitro and in sheep. *J. Anim. Sci.* 73:1811-1818.
38. Orr, C. L., D. R. Ware, E. T. Manfredi, and D. P. Hutchenson. 1988. The effect of continuous feeding of *Lactobacillus acidophilus* strain BT1386 on gain and feed efficiency of feeder calves. *J. Anim. Sci.* 66(Suppl. 1): 460. (Abstract)
39. Patterson, J. A., and K. M. Burkholder. 2003. Application of prebiotics and probiotics in poultry production. *Poult. Sci.* 82:627-631.[Abstract/Free Full Text]
40. Physicians' Desk Reference . 55th ed. Montvale, NJ: Medical Economics Company, Inc.; 2001.
41. Reid, G., and R. Friendship. 2002. Alternatives to antibiotic use; probiotics for the gut. *Anim. Biotechnol.* 13:97-112.[CrossRef][Medline]
42. Rode, L. M., W. Z. Yanf, and K. A. Beauchemin. 1999. Fibrolytic enzyme supplements for dairy cows in early lactation. *J. Dairy Sci.* 82:2121-2126.
43. Rojas, M., and P. L. Conway. 2001. A dot blot assay for adhesive components relative to probiotics in microbial growth in biofilms. *Methods Enzymol.* 336:389-402.[Medline]
44. Roos, S., and H. Jonsson. 2002. A high-molecular-mass cell-surface protein from *Lactobacillus reuteri* 1063 adheres to mucus components. *Microbiology* 148:433-442.[Abstract/Free Full Text]
45. Savage, D. C. 1987. Microorganisms associated with epithelial surfaces and the stability of the indigenous gastrointestinal microflora. *Die Nahrung.* 5-6:383.
46. Scheirlinck, T., J. Mahillion, H. Joos, P. Dhaese, and F. Michiels. 1990. Integration and expression of -amylase and endoglucanase genes in the *Lactobacillus plantarum* chromosome. *Appl. Environ. Microbiol.* 55:2130-2137.
47. Selinger, L. B., C. W. Forsberg, and K.-J. Cheng. 1996. The rumen: a unique source of enzymes for enhancing livestock production. *Anaerobe* 2:263-284.
48. Shils ME, Olson JA, Shike M, eds. *Modern Nutrition in Health and Disease* . 9th ed. Philadelphia, Pa.: Lea and Febiger; 1999
49. Suarez F, Levitt MD, Adshead J, Barkin JS. Pancreatic supplements reduce symptomatic response of healthy subjects to a high fat meal. *Dig Dis Sci.* 1999;44(7):1317-1321.
50. Treacher, R. J. and C. W. Hunt. 1996. Recent developments in feed enzymes for ruminants. *Proc. Pacific Northwest Nutrition Conference.* Seattle, WA.

51. Tricarico, J. M., and K. A. Dawson. 1999. Effects of defined xylanase and cellulase enzyme preparations on digestive processes of ruminal microbial cultures. *J. Dairy Sci.* 77(Suppl. 1):252. (Abstract)
52. Vandevorde, L., H. Christianens, and W. Verstraete. 1991. In vitro appraisal of the probiotic value of intestinal lactobacilli. *World. J. Microbiol. In addition, Biotechnol.* 7:587-592.
53. Varel,V.H., K. K. Kreikemeier, H.J.G. Jung, and R. D. Hatfield. 1993. In vitro stimulation of forage fiber degradation by ruminal microorganisms with *Aspergillus oryzae* fermentation extract. *Appl. Environ. Microbiol.* 59:3171-3176.
54. William V Dashek (1997). Methods in Plant Biochemistry and Molecular Biology. CRC Press. ISBN 0-8493-9480-5. p. 313 Google Print reference "Xylans can be hydrolyzed by beta-xylanase"
55. Yang, W. Z., K. A. Beauchemin, and L. M. Rode. 1999. Effects of an enzyme feed additive on extent of digestion and milk production of lactating dairy cows. *J. Dairy Sci.* 82:391-403.
56. Yanovski SZ, Yanovski JA. Obesity. *N Engl J Med* . 2002; 346:591-602.