FEED ENERGY COMPANY

Effects of Exogenous Feed Enzymes on Dietary Energy Availability[1]

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Introduction

Enzymes are proteins and are present in all living cells, where they facilitate and/or regulate the numerous chemical reactions taking place. Enzymes act by recognizing and binding to its substrate (i.e., the molecule on which it acts) and usually only act on only one (1) specific substrate. Depending on the type of enzyme, it will then break specific bonds in the substrate, release the now cleaved substrate molecule, and be ready to bind to another molecule (Figure 1).

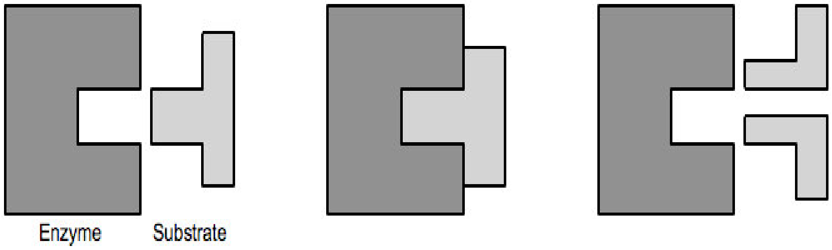

Figure 1. An enzyme recognizes its substrate and binds to, cleaves, and releases the now cleaved substrate.

All enzymes are made from one or more ‘strings’ of amino acids that take a specific three-dimensional (3-D) form. This 3-D conformation is important for the enzyme to be able to recognize the molecule on which it acts (i.e., its substrate). Excessive heat causes irreversible changes in the 3-D structure (i.e., the proteins denature) with a loss in function as a result. Feed processing (pelleting, extruding, etc.) causes heat from steam or friction and may damage any feed enzymes included in the diets. Thus, depending on the heat stability of a specific commercial feed enzyme, feed enzymes are added either before or after feed processing (the latter by spraying a enzyme solution onto the pellets). Enzymes work in an aqueous environment (i.e., not in the dry feed, but in the stomach and/or small intestines of the animal) and their activity is dependent on temperature and acidity (pH). Enzymes have an optimal temperature and pH at which they work best. Thus some enzymes will work primarily in the stomach (low pH, acidic), whereas others work in the small intestines (pH around 7, neutral).

Enzymes are often named after their substrate, adding -‘ase’ to the name. Hence, the enzyme that cleaves phytic acid (phytate) is called phytase. Enzymes can be produced and extracted from bacteria, fungi, and plants, so different types of enzymes that act on the same substrate may be commercially available and have different physical properties and efficiencies. If enzymes are added to a feed, they are termed ‘exogenous’ to differentiate them from the endogenous enzymes produced by the animal itself.

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Phytases

Phosphorus is a mineral required by all animals for maintenance, growth, and production. Most feed ingredients of plant origin contain sufficient phosphorus to meet the animals' daily phosphorus requirement; however, a large proportion of the phosphorus is contained in phytate (Figure 2). Because animals do not have a digestive enzyme capable of releasing phosphorus from phytate (i.e., the enzyme, phytase), the phytate-phosphorus is not digested and instead excreted in the feces. In order to meet the animals' daily phosphorus requirement, inorganic phosphorus (typically dicalcium phosphate) is therefore added to the diet to supplement the otherwise phosphorus-deficient diets. By adding the enzyme phytase, (some of) the phosphorus is released from the phytate molecule, yielding phosphate (HPO_4^{2-} or $H_2PO_4^-$), which can be absorbed by the animal. Hence, not only does dietary inclusion of phytase lower the need for dietary additions of (expensive) inorganic phosphorus, it also (with correct feed formulation) decreases phosphorus excretion. Because of the latter effect, the use of phytase is mandatory in some states and countries. It is thought that the rumen microbes produce sufficient phytase to release all the phytate-phosphorus in the feed consumed by ruminant animals—exogenous phytase is therefore not added to ruminant diets.

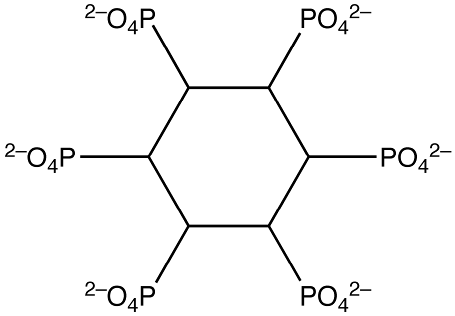

Figure 2. Some, but not necessarily all, the phosphorus in phytate (pictured) may be released by the enzyme phytase. Pigs and poultry do not secrete phytase, making the phosphorus contained in phytate unavailable for maintenance, growth, and production. Moreover, due to its negative charges, phytate may bind other nutrients, such as minerals or perhaps even amino acids, in turn making them unavailable to the animal.

While phytase is added to diets primarily to improve phosphorus availability, it has been suggested that phytase also improves availability of both dietary amino acids and energy. The increase in dietary energy content may be as much as 2%, corresponding to 30 kcal/lb diet (Table 1) (Namkung and Leeson, 1999). However, this secondary effect of phytase is inconsistent (Biehl and Baker, 1997; Snow et al., 2003; Juanpere et al., 2005; Liao et al., 2005a; Liao et al., 2005b). From the data in Table 1, it appears that phytase may (!) liberate small amounts of energy from corn-based diets, but not from wheat and barley-based diets. This difference may be because, unlike wheat, corn has a low 'natural' content of phytase and therefore benefits more from inclusion of exogenous phytase. Juanpere et al. (2005) suggested that the small amounts of energy liberated by phytase may be caused by an increased digestibility of dietary amino acids when phytase is added, in effect resulting in an oversupply of amino acids in relation to the animals' needs. These (excess) amino acids are then broken down and used by the animal as a source of energy, potentially explaining the small gain in dietary energy content when exogenous phytase is used. The same argument can be used for phytase releasing small amounts of otherwise indigestible carbohydrates bound to the phytate molecule. The product literature for Natuphos® (the only literature I could find that listed an energy value) suggests an inherent energy content of the phytase product to be used in diet formulation (i.e., in the feed formulation matrix). This energy may at least partially come from the carrier (e.g., wheat middlings) in the enzyme product.

Table 1. Effect of exogenous phytase on dietary energy content in different species of animals.

Phytase	Dose[1]	Energy measure[2]	Increase in energy content[3]	Diet[4]	Reference[5]
Broilers					
Natuphos®	1,200	TME$_n$	13 kcal/lb (1.2%), not significant	Cornstarch, SBM	(Biehl and Baker, 1997)
Natuphos®	1,149	AME$_n$	30 kcal/lb (2.3%), significant	Corn, SBM	(Namkung and Leeson, 1999)
Unnamed phytase	500	AME	26 kcal/lb (1.9%), significant	Corn, SBM	(Juanpere et al., 2005)
Unnamed phytase	500	AME$_n$	20 kcal/lb (1.6%), not significant	Corn, SBM	(Juanpere et al., 2005)
Phyzyme™ XP	1,200	IDE	–9 kcal/lb (–0.8%), not significant	Corn, rye, SBM	(Cowieson and Adeola, 2005)
Unnamed phytase	500	AME	–14 kcal/lb (–1.0%), not significant	Wheat, SBM	(Juanpere et al., 2005)
Unnamed phytase	500	AME$_n$	–16 kcal/lb (–1.2%), not significant	Wheat, SBM	(Juanpere et al., 2005)
Unnamed phytase	500	AME	–12 kcal/lb (–0.4%), not significant	Barley, SBM	(Juanpere et al., 2005)
Unnamed phytase	500	AME$_n$	–17 kcal/lb (–0.6%), not significant	Barley, SBM	(Juanpere et al., 2005)
Natuphos®	5,000	AME$_n$	159 kcal/lb (10.9%), not significant	Peanut meal alone	(Driver et al., 2006)
Laying hens					
Natuphos®	300	AME	5 kcal/lb (0.3%), not significant	Corn, SBM	(Snow et al., 2003)
Natuphos®	300	AME	32 kcal/lb (2.3 %), not significant	Corn, SBM, MBM	(Snow et al., 2003)
Natuphos®	300	AME	50 kcal/lb (4.0 %), not significant	Corn, SBM, WM	(Snow et al., 2003)
Phyzyme 5000G	700	AME	86 kcal/lb (6.5%), significant	Wheat, SBM, CM	(Silversides et al., 2006)
Phyzyme 5000G	700	AME	77 kcal/lb (5.8%), significant	Wheat, SBM, CM	(Silversides et al., 2006)
Phyzyme 5000G	700	AME	–54 kcal/lb (4.0%), significant	Wheat, SBM, CM	(Silversides et al., 2006)
Pigs					
Natuphos®	1,000	DE	16 kcal/lb (1.0%), not significant	Corn, SBM	(Liao et al., 2005b)
Natuphos®	1,000	DE	12 kcal/lb (0.7%), not significant	Wheat, SBM	(Liao et al., 2005b)
Natuphos®	1,000	DE	13 kcal/lb (0.8%), not significant	Wheat, SBM, CM	(Liao et al., 2005b)
Natuphos®	1,000	DE	4 kcal/lb (0.2%), not significant	Barley, peas, CM	(Liao et al., 2005b)

[1] Units/kg diet. One (1) phytase unit is defined as the amount of enzyme activity that liberates 1 mmol of inorganic phosphorus per minute from a 0.5 mM Na-phytate solution at pH 5.5 and 37°C.

[2] AME, apparent metabolizable energy; AME$_n$, nitrogen-corrected apparent metabolizable energy; DE, digestible energy; IDE, ileal digestible energy; TME$_n$, nitrogen-corrected true metabolizable energy.

[3] Compared with control diet (no added phytase).

[4] Main dietary ingredients (CM, canola meal; MBM, meat and bone meal; SBM, soybean meal; WM, wheat middlings).

[5] The list only includes experiments in which the effect of exogenous carbohydrases on dietary energy was specifically investigated.

Lipases and emulsifiers

Lipids are organic molecules that are soluble in non-polar solvents (e.g., hexane, chloroform, diethyl ether). Although many different types of compounds can be classified as lipids (e.g., waxes, fat-soluble vitamins, pigments, cholesterol), the following discussion will pertain only to the energy yielding lipids (i.e., triacylglycerols and free fatty acids). Lipids contain 2.25 times as much energy as carbohydrates (starch) and proteins, and are therefore an important ingredient in most animal diets. In addition, some lipids are dietary essential, meaning that they are needed for various metabolic functions in the body, but cannot be synthesized by the body fast enough or in sufficient amounts—they must therefore be consumed. Depending on the species of animal, linoleic acid is considered dietary essential, as are α-linolenic acid and arachidonic acid. However, arachidonic acid can be made from linoleic acid in sufficient amounts to satisfy the requirement in most animals (the cat is an exception) if the dietary content of linoleic acid is sufficient. Therefore, only linoleic acid is considered in formulation of diets for poultry and pigs. Most feed ingredients contain lipids (e.g., corn contains about 3.5% lipid, about half of which is linoleic acid), but supplemental lipids (e.g., vegetable oil, acidulated soybean oil, choice white grease, etc.) are often added to animal diets to increase the contents of energy and/or linoleic acid.

Because lipids are not water-soluble and the digesta is water based, lipids must be emulsified prior to absorption. Bile salts, made in the liver and secreted into the small intestine, acts as an emulsifier, enabling digestion by lipases. Although some species secrete a lipase into the saliva, pancreatic lipases, secreted into the small intestine, is responsible for the vast majority of lipid digestion. Most lipids are digested efficiently without the addition of exogenous lipases. However, the production and secretion of endogenous bile salts and lipases are relatively low in immature animals (e.g., newly hatched chicks and poults as well as newly weaned pigs), suggesting that exogenous bile salts and/or lipases may have an effect in diets for those animals (Atteh and Leeson, 1985). However, a recent study by Meng et al. (2004) found no effect on broilers' growth performance, fat digestibility, or energy utilization when lipase was added to diets supplemented with either corn oil or tallow.

Proteases

Proteins are large organic molecules consisting of amino acids bound together with peptide bonds. These amino acid chains may be hundreds of amino acids long, and twist into 3-D conformations held together by hydrogen and sulfur bonds. Often, more than one chain of peptide-bound amino acids is necessary to form the final, active protein. The amino acid sequence and the 3-D conformation is important for the protein's function, which can be structural (e.g., skeletal muscle) or regulatory (e.g., enzymes, hormones). Proteases are enzymes that cleave the peptide bonds between two amino acids; some proteases recognize specific amino acids and cleave the peptide bonds near those amino acids, other proteases are nonspecific and cleave near the end of the amino acid 'string.'

Although livestock and poultry are able to digest the majority of dietary protein, approximately 10% on average of dietary protein escape the small intestine undigested. Hence, addition of exogenous proteases may increase the animals' utilization of dietary protein. Improved protein digestibility has been reported when proteases were added to the diet (Hong et al., 2002; Omogbenigun et al., 2004), but inclusion of proteases probably does not improve energy digestibility or availability (unless the additionally digested amino acids are broken down for energy rather than being used for protein synthesis).

Carbohydrases

Carbohydrates are organic molecules produced by plants through photosynthesis. Carbohydrates are classified as monosaccharides (e.g., glucose, fructose, mannose, arabinose, etc.), disaccharides (e.g., lactose, maltose), and polysaccharides (e.g., starch, cellulose, stachyose). Only few monosaccharides (simple sugars) are found in feed ingredients—most are either disaccharides (e.g., lactose found in milk products) or polysaccharides (e.g., starch), the latter consisting of multiple monosaccharides bound together to form straight or branched chains.

The majority of carbohydrates found in feed ingredients can be divided into two groups based on the animal's ability to digest them: Starch and non-starch polysaccharides (NSP). Whereas starch is readily digested by animals, the carbohydrates classified as NSP are not. The NSP group includes cellulose, hemicellulose, gums, β-glucans, pectins, and others that often serve a structural function in the plant (and therefore is associated with the cell wall). Previously, NSP were termed 'fiber' or 'complex carbohydrates,' loosely defined as indigestible carbohydrates and quantified somewhat inadequately in feed ingredients as crude fiber, acid detergent fiber (ADF), and neutral detergent fiber (NDF).

Animals lack the ability to digest NSP because they do not produce and secrete the specific enzymes (carbohydrases) needed to break the bonds between the individual monosaccharides. Not only are the NSP themselves indigestible (and therefore contain no usable energy for the animal, diluting the dietary energy content), but they may also prevent digestion of otherwise digestible carbohydrates (and other nutrients) in part by encapsulation, thereby preventing physical access by digestive enzymes. Removing or degrading the fiber in a feed ingredient (e.g., hulled vs. hulless oats) will improve feed utilization (energy content) to some degree, but of equal—if not higher—importance is the physical effects NSP has on the viscosity of the digesta. The digestion of nutrients relies on the unimpeded movement and mixing of digestive enzymes and digesta throughout the gastro-intestinal tract. In solution, NSP react with water and aggregate into large mesh-like structures, increasing the viscosity of the digesta and interfering with the physical contact among digestive enzymes, feed components, and the intestinal wall (where nutrients are absorbed). Mature animals can compensate for the reduced digestion and absorption by producing more of the digestive enzymes, but this capacity is limited especially in young, immature animals (chicks, poults, and piglets). Moreover, the water-rich, viscous digesta tends to produce wet, sticky manure with associated litter management and manure-handling problems. Thus, carbohydrases are added to feed mainly to disrupt the viscous mesh-like structure (complete destruction of the mesh or digestion of the NSP is not necessary), in effect decreasing the viscosity of the digesta, and improving the dry matter digestibility, energy availability, and excreta consistency. Although some of the monosaccharides in the NSP may become available for energy to the animal, the main action of the carbohydrases is to decrease digesta viscosity. Hence, depending on the NSP content or types in a feed, carbohydrases may improve the availability of energy and other nutrients, such as amino acids, starch, lipids, and minerals.

Compared with corn, the dietary inclusion rates of small cereal grains (e.g., barley, oats, wheat, and rye) have traditionally been limited due to their relatively high contents of NSP and associated increased viscosity of the digesta and stickiness of the excreta. When carbohydrases are added to small cereal grain–based diets, improvements in nutrient digestibility, energy availability, and excreta consistency are typically observed—the use of exogenous carbohydrases are therefore fairly common in diets based on wheat and barley (only little oats and rye is fed) (Table 2). Recently, there has been interest in improving corn and soybean meal–based diets through the use of feed enzymes. Whereas the content of NSP in corn is relatively low, depending on growing conditions, variety, and processing, soybean meal contains relatively large amounts of NSP, including β-mannans. As a result, addition of carbohydrases to soybean meal–containing diets may improve nutrient digestibility and energy availability (Table 2).

Table 2. Effect of exogenous carbohydrases on dietary energy content in different species of animals.

Enzyme	Energy Measure[1]	Increase in energy content[2]	Diet[3]	Reference[4]
Broilers				
Mixture	AME_n	48 kcal/lb (3.3%), significant	Corn	(Meng and Slominski, 2005)
Hemicell®	ME_n	30 kcal/lb (2.2%), not significant	Corn, SBM	(Daskiran et al., 2004)
Hemicell®	NE_{gain}	31 kcal/lb (5.2%), significant	Corn, SBM	(Daskiran et al., 2004)
Mixture	AME_n	33 kcal/lb (2.3%), significant	Corn, SBM	(Meng and Slominski, 2005)
Avizyme® 1505	IDE	97 kcal/lb (7.9%), significant	Corn, rye, SBM	(Cowieson and Adeola, 2005)
Mixture	AME_n	20 kcal/lb (1.4%), not significant	Corn, CM	(Meng and Slominski, 2005)
Mixture	AME_n	18 kcal/lb (1.3%), not significant	Corn, peas	(Meng and Slominski, 2005)
Mixture	AME_n	22 kcal/lb (1.4%), not significant	Wheat, SBM	(Cowieson et al., 2003)
Mixture	AME	15 kcal/lb (1.1%), not significant	Wheat, SBM	(Wang et al., 2005)
Mixture	AME_n	14 kcal/lb (1.0%), significant	Wheat, SBM, peas	(Cowieson et al., 2003)
Mixture	AME_n	50 kcal/lb (3.8%), significant	Wheat, SBM, CM, peas	(Meng et al., 2005)
Mixture	AME_n	45 kcal/lb (3.3%), significant	Wheat, SBM, CM, peas	(Meng et al., 2004)
Laying hens				
Avizyme® 1500	AME_n	–8 kcal/lb (–0.6%), not significant	Corn, SBM	(Scheideler et al., 2005)
Avizyme® 2300	AME	–132 kcal/lb (–9.2%), significant	Wheat, SBM, CM	(Silversides et al., 2006)
Avizyme® 2300	AME	–36 kcal/lb (–2.8%), significant	Wheat, SBM, CM	(Silversides et al., 2006)
Avizyme® 2300	AME	0 kcal/lb (0.0%), not significant	Wheat, SBM, CM	(Silversides et al., 2006)
Roxazyme® G	AMEn	81 kcal/lb (6.2%), significant	Wheat, rye, SBM	(Pan et al., 1998)
Pigs				
Hemicell®	ME	45 kcal/lb (estimated)	Corn, SBM, DW	(Pettey et al., 2002)

[1] AME, apparent metabolizable energy; AME_n, nitrogen-corrected apparent metabolizable energy; IDE, ileal digestible energy; ME, metabolizable energy; ME_n, nitrogen-corrected metabolizable energy; NE_{gain}, net energy for gain.

[2] Compared with control diet (no added carbohydrase).

[3] Main dietary ingredients (CM, canola meal; DW, dried whey; SBM, soybean meal).

[4] The list only includes experiments in which the effect of exogenous carbohydrases on dietary energy was specifically investigated.

Summary

Of the various commercially available exogenous enzymes, only carbohydrases have a real potential for increasing a given diet's energy content. These enzymes increase the dietary energy content, not by making (more) starch and NSP available for absorption, but by reducing the NSP-induced viscosity of the digesta, in turn improving general nutrient digestibility and energy availability. It should be mentioned that the data on the efficacy of carbohydrases are somewhat inconsistent (Table 2), and depends in part on the type of carbohydrase (or carbohydrase mixture), the dose, and especially on the NSP type and content of individual feed ingredients (which may vary depending on the plants' growth conditions, etc.). Hence, it is difficult to assign a specific energy value to a given carbohydrase. In corn and soybean meal–based diets, inclusion of carbohydrases may liberate around 30 kcal of energy per pound of diet (Meng and Slominski, 2005). In other words, when including carbohydrases in a corn and soybean meal–based diet, one can reduce the energy content by 30 kcal/lb from, say, 1,250 kcal/lb to 1,220 kcal/lb and still expect similar performance as that of a diet containing 1,250 kcal/lb. The dietary energy content can be reduced by replacing parts of the supplemental fat (e.g., vegetable oil blends, choice white grease, etc) with corn (Table 3). Note that when carbohydrases are used to increase the dietary energy content—in effect replacing some of the supplemental fat—the linoleic acid[2] content is significantly reduced. This effect of carbohydrase is especially important for laying hens because they require higher amounts of linoleic acid in the diet than other species of poultry; Mannion et al. (1992) demonstrated that the egg weights increased when linoleic acid was supplied in excess of the NRC (1994) recommended content of 1% of the diet. To maximize egg weights, diets should contain more than 3.5% linoleic acid for hens 42–46 weeks of age and at least 2% linoleic acid for laying hens of 62–66 weeks of age (Mannion et al., 1992). Even if a high–linoleic acid supplemental fat source is included in an carbohydrase-containing diet, the resultant dietary linoleic acid content is sub-optimal (Table 3).

Table 3. Examples of diet formulation with and without carbohydrases, assuming carbohydrases will ‘release’ 30 kcal energy per pound of complete diet.

Item	**Laying hens**		**Turkey finisher**		**Broiler finisher**	
	–	**+**	**–**	**+**	**–**	**+**
Carbohydrase, lb	–	1.5	–	1.5	–	1.5
Corn, lb	1,218.6	1,241.3	1,308.3	1,331.0	1,185.5	1,208.2
Soybean meal (48% CP), lb	370.0	370.0	356.0	356.0	650.0	650.0
Meat and bone meal (50% CP), lb	170.0	170.0	155.0	155.0	–	–
Limestone, lb	166.4	166.4	4.8	4.8	26.6	26.6
Supplemental fat (AV3900 Layer),[1] lb	41.4	17.2	145.2	121.0	73.2	49.0
DL-Methionine, lb	5.6	5.6	1.8	1.8	3.8	3.8
Dicalcium phosphate (18.5% P), lb	–	–	–	–	31.0	31.0
Salt, lb	8.0	8.0	8.0	8.0	9.0	9.0
Trace mineral and vitamin mix, lb	20.0	20.0	20.0	20.0	20.0	20.0
Antibiotic, lb	–	–	0.9	0.9	0.9	0.9
Total, lb	2,000.0	2,000.0	2,000.0	2,000.0	2,000.0	2,000.0
Metabolizable energy, kcal/lb	1,293	1,263[2]	1,551	1,521[2]	1,406	1,376[2]
Metabolizable energy, kcal/kg	2,850	2,784[2]	3,420	3,354[2]	3,100	3,034[2]
Linoleic acid, %	2.48	1.90[3]	5.17	4.59	3.26	2.68
Crude protein, %	17.70	17.82	18.07	18.16	19.92	20.01
Lysine, %	0.90	0.90	0.90	0.90	1.10	1.10
Methionine+cystine, %	0.80	0.80	0.68	0.68	0.79	0.79
Calcium, total, %	4.06	4.06	0.95	0.95	0.95	0.95
Phosphorus, non-phytate, %	0.47	0.47	0.49	0.49	0.41	0.41

[1] Contains minimum 50% linoleic acid.

[2] The carbohydrase will ‘release’ 30 kcal of metabolizable energy per pound of diet (66 kcal/kg) from the diet, bringing the dietary energy content on par with the corresponding non-carbohydrase diet.

[3] If the supplemental fat only contains 5% linoleic acid (as opposed to the 50% linoleic acid content in AV3900 Layer), the dietary linoleic acid content is 1.51%.

Given the variable effects of carbohydrases (Table 2), special care must be taken to ensure that a carbohydrase-containing diet does not become deficient in essential nutrients and/or energy. The use of exogenous carbohydrases reduces the need for supplemental fat as a source of energy (Table 3), but it is often overlooked that the supplemental fat also supplies essential nutrients (e.g., linoleic acid) and antioxidants that help stabilize and preserve the quality of the diet. Therefore, the choice of supplemental fat becomes especially important in carbohydrase-containing diets (that are formulated with low levels of supplemental fat): The fat must contain sufficient linoleic acid to ensure dietary adequacy, it must be stable to prevent oxidation and destruction of linoleic acid (and other fatty acids and even vitamins from the vitamin premix), and it must be consistent from batch to batch to ensure reliable and constant nutrient delivery and quality.

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[1] Mention of trade names is for clarity only and does not imply endorsement.

[2] Linoleic acid is a dietary essential fatty acid that must be provided in the diet for poultry and pigs. According to the National Research Council, poultry diets must contain a minimum of 20 lb/ton of pure linoleic acid, whereas pig diets must contain a minimum of 2 lb/ton of pure linoleic acid to avoid a linoleic acid–deficiency (NRC 1994, 1998). Note that the NRC-recommended nutrient levels may not result in optimal or maximal production; the NRC recommendations only serve as a guideline to avoid deficiencies.

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