

# How Do We Evaluate Peroxidation of Feed Fats?

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## Take Home Message

A better understanding of methods to assess quality and feeding value of various energy ingredients such as feed fats and oils is needed because of variability in composition and quality, as well as in availability and cost. Commonly used lipid quality measures such as MIU, titer, free fatty acid content are used to insure that the lipid products meet trading specifications, but provide limited information regarding their feeding value, and provide essentially no information regarding the degree of lipid peroxidation of a given lipid source. Methods specific for evaluating lipid peroxidative stability can be divided into indicative and predictive tests. Indicative tests are used to measure the presence of peroxidation products in lipids and include: peroxide value (PV), thiobarbituric acid reactive substances (TBARS), anisidine value (AnV), conjugated dienes, hexanal value, 2, 4-decadienal (DDE), and 4-hydroxynonenal (HNE). Predictive tests measure the stability or susceptibility of lipids to oxidation and include active oxygen method (AOM), oxygen stability index (OSI), and oxygen bomb method (OBM). In predictive tests, the lipid is subjected to specific accelerated peroxidation conditions and a peroxidation endpoint is defined to determine the degree of peroxidation damage. However, assessment of the degree of lipid peroxidation is complex because the process consists of three phases: (1) an initiation phase which involves the formation of free lipid radicals and hydroperoxides as primary reaction products, (2) a propagation phase where hydroperoxides formed are decomposed into secondary peroxidation products, and (3) a termination phase involving the formation of tertiary peroxidation products. No individual indicative or predictive test appears to provide a complete assessment of the degree of lipid peroxidation. Therefore, to accurately analyze the amount of lipid damage caused by peroxidation, it may be advantageous to determine the degree of lipid peroxidation at several time points during the peroxidation process using more than one test. Results from a recent study conducted by our group (Liu et al., 2012) showed no effect of amount of lipid peroxidation (slow or rapid oxidation) in corn oil, canola oil, poultry fat, and tallow on DE or ME content or on apparent total tract digestibility of dry matter, GE, ether extract, nitrogen, carbon, and sulfur when fed to nursery pigs. Although responses among studies are inconsistent, recent studies have shown that feeding peroxidized lipids can reduce growth performance of pigs. Determining metabolic oxidation status is complex and involves several indicators such as serum TBARS, PPAR $\alpha$  activation in the liver, and measures of gut barrier function. Based on inconsistent responses among studies, it is unclear if antioxidants are necessary additions to lipids used in animal feed in order to maintain optimal nutritional value, or if their addition to swine diets are beneficial in overcoming a metabolic oxidative challenge.

## Is Lipid Peroxidation in Feed Fats Worth Consideration?

Energy is the most expensive nutritional component in animal feeds. In addition, the availability of high energy feed ingredients is decreasing primarily due to the demands for biofuel production. Reduced availability along with record high costs of feed ingredients is causing nutritionists to find ways to obtain the most nutritional and economic value from the feedstuffs used in commercial animal feeds. Therefore, more accuracy is needed to assess quality and feeding value of various high energy ingredients, such as vegetable oils, animal fats, animal-vegetable blends, and used oils from restaurants.



World production of vegetable oils has increased dramatically during the past 20 years with approximately 286 billion pounds produced annually. Palm oil represents about 30% of the world's total vegetable oil production, followed by soybean oil (28%), rapeseed/canola oil (15%), and sunflower oil (9%), with other vegetable oils accounting for less than 20% of the market (Figure 1). Consumption of edible vegetable oils is dominated by its use in the food industry (approximately 80% of the total use), but its growth has also been stimulated by industrial uses, such as for biodiesel production. Although smaller in magnitude, animal fats obtained from the rendering industry are important components in animal feeds and represent approximately 11 billion pounds annually. The most common sources of animal fats include: inedible tallow (36% of U.S. rendered fats), edible tallow (17%), poultry fat (11%), and lard (3%). In addition, yellow grease (i.e., recycled vegetable oils from restaurants) is also considered part of the rendering industry, and provides a substantial quantity of lipids (14%) to the animal industry. Like vegetable oils, animal fats can also be utilized in the production of biodiesel, thereby affecting availability and cost for use in animal feeds.

Feed lipids vary considerably in fatty acid composition, energy content, quality, and price. Although a variety of measures have been used to define lipid quality such as color, fatty acid profile, free fatty acid content, degree of unsaturation or saturation (iodine value, titer), saponification value, and impurities including moisture, insolubles, and unsaponifiables, very little is known about the effects of lipid peroxidation on energy value as well as swine health and performance. Therefore, the purpose of this review is to describe what is known about measuring lipid peroxidation in feed lipids, determination of metabolic oxidation status, and the impact of feeding peroxidized lipids on swine health, performance, and pork quality. Specific questions include:

1. Are we using the correct measures to accurately assess lipid quality?
2. Does lipid peroxidation affect energy content and dietary nutrient utilization?
3. Does lipid peroxidation affect swine health and performance?
4. How do we determine metabolic oxidation status of pigs?
5. Are antioxidants necessary?

### **Are We Using the Correct Measures to Accurately Assess Lipid Quality?**

Lipids used in animal feeds vary considerably in color, fatty acid profile, free fatty acid content, degree of unsaturation or saturation (iodine value, titer), saponification value, and impurities including moisture, insolubles, and unsaponifiables (Table 1). These indices are generally used to define quality or ensure that the lipid products meet trading specifications, but provide limited information regarding their feeding value. Furthermore, these quality measures provide no information regarding the degree of lipid peroxidation of a given lipid source. Therefore, additional measurements are required to assess lipid peroxidation.

Lipids produced from various processes are often heated for a considerable length of time at high temperatures (Frankel et al., 1984). Lipids with significant amounts of unsaturated fatty acids are thermally sensitive and unstable which can lead to extensive lipid peroxidation (Sherwin, 1978; Linfield et al., 1985). Heating lipids may also generate toxic lipid peroxidation products (Lin et al., 1989; Frankel, 1998; Adam et al., 2008) and may deplete endogenous antioxidants (Seppanen and Csallany, 2002). The presence of high amounts of oxidized lipids in the diet raises the levels of free radicals, aldehydes, and other oxidized metabolites that are toxic to animals. These secondary lipid peroxidation products are highly reactive and potentially cause damage to lipids, proteins, and nucleic acids, and thus, impair animal growth performance (Logani and Davies, 1979; Comporti, 1993). Thus, it is important to understand the degree of lipid peroxidation of lipid sources when evaluating lipid quality.



Worldwide vegetable oil production

Figure 1. Vegetable oil production.

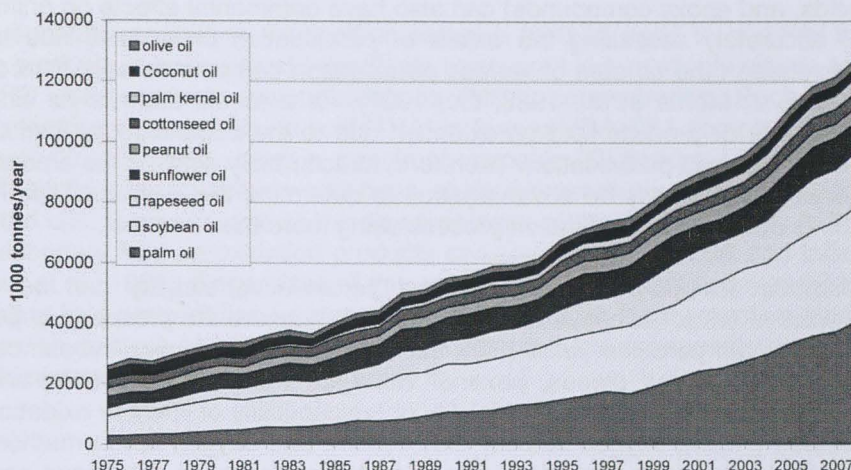


Table 1. Lipid quality indices.

Color	Quantified relative to the Fat Analysis Committee (FAC) standard, ranging from 1 (light) to 45 (dark).
Fatty acid profile	Relative amounts of individual fatty acids in a sample.
Free fatty acids	Amount of fatty acids not bound to the glycerol backbone in a triglyceride.
Insolubles	Amount of sediment in a sample. For example, fiber, hair, hide, bone, or soil.
Iodine value	Measure of chemical unsaturation, expressed as grams of iodine absorbed by 100 g of fat.
Moisture	Amount of moisture in a sample.
Saponification value	An estimate of the mean molecular weight of the constituent fatty acids in a sample, defined as milligrams of KOH required to saponify 1 g of fat. The higher the SV, the lower the mean chain length.
Titer	The solidification point of fatty acids in lipids, an important characteristic in producing soaps or fatty acids.
Total fatty acids	The total of both free fatty acids and fatty acids combined with glycerol.
Unsaponifiabiles	A measure of material in the lipid that will not saponify (form a soap) when mixed with caustic soda (NaOH or KOH). Examples include: sterols, hydrocarbons, pigments, fatty alcohols, and vitamins.

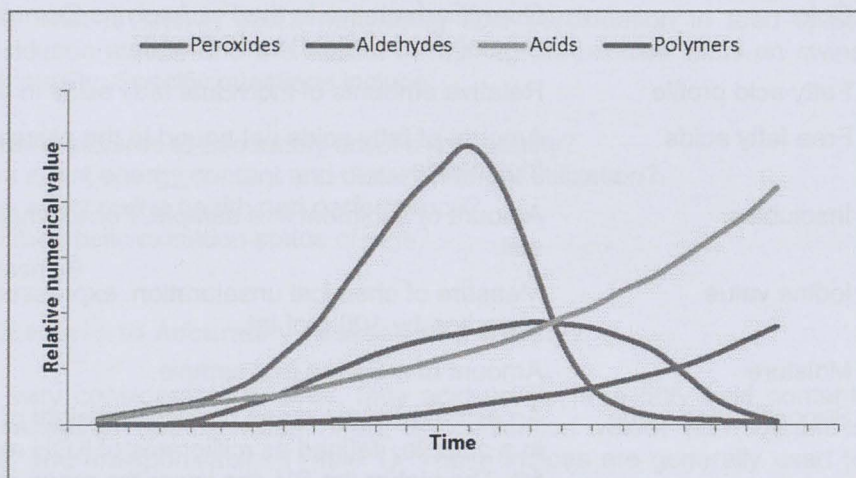
Lipid peroxidation is a complex process that is affected by several factors including degree of saturation, temperature, oxygen, heavy metals, undissociated salts, water, and other nonlipidic compounds. Assessment of the degree of lipid peroxidation is also complex because the process consists of three phases: (1) an initiation phase which involves the formation of free lipid radicals and hydroperoxides as primary reaction products, (2) a propagation phase where hydroperoxides formed are decomposed into secondary peroxidation products, and (3) a termination phase involving the formation of tertiary peroxidation products (Gutteridge, 1995; Young and McEneny, 2001). Lipid hydroperoxides initially formed during the lipid peroxidation process not only have the potential to impact lipid quality, and therefore on animal health and performance, but the formation of secondary



and tertiary peroxidation products (aldehydes, ketones, alcohols, hydrocarbons, volatile organic acids, and epoxy compounds) can also have detrimental effects on animal productivity. The difficulty of accurately assessing the extent of peroxidation in lipids is due to the rate of increase and decrease in the amount of various peroxidation compounds over time during each of these phases (Figure 2; Morita et al., 1983; Liu, 1997). As a result, there is no single method that adequately describes or predicts lipid peroxidation due to the complexity of lipid composition and the phases involved in lipid peroxidation. Therefore, to accurately analyze the amount of lipid damage caused by peroxidation, it may be advantageous to determine the degree of lipid peroxidation at several time points during the peroxidation process using more than one test.

Methods specific for evaluating lipid peroxidative stability can be divided into indicative and predictive tests. Indicative tests are used to measure the presence of peroxidation products in lipids and include: peroxide value (PV), thiobarbituric acid reactive substances (TBARS), anisidine value (AnV), conjugated dienes, hexanal value, 2, 4-decadienal (DDE), and 4-hydroxynonenal (HNE). Predictive tests measure the stability or susceptibility of lipids to oxidation and include active oxygen method (AOM), oxygen stability index (OSI), and oxygen bomb method (OBM). In predictive tests, the lipid is subjected to specific accelerated peroxidation conditions and a peroxidation endpoint is defined to determine the degree of peroxidation damage. One advantage of the predictive tests is that they have the ability to assess the relative effectiveness of different antioxidants.

**Figure 2.** Relative production of various lipid peroxidation compounds over time.



## Indicative tests

### *Peroxide value*

Peroxide value has been commonly used to evaluate lipids used in animal feeds for many years. Peroxide value measures the concentration of primary lipid peroxidation products, including peroxides and hydroperoxides, formed during the initial phase of lipid peroxidation. Peroxide value may provide useful information for predicting animal growth performance when lipids of various degrees of peroxidation are fed because peroxides and hydroperoxides can be toxic (Kaneko et al., 1988). However, although PV is one of the most widely used indicators of lipid peroxidation, it may provide misleading results. A high PV indicates high concentrations of peroxides and hydroperoxides present in a lipid which can be indicative of extensive lipid damage, but a moderate or low PV may indicate less peroxidative damage or may be due to the decomposition of hydroperoxides previously formed during the initiation phase because hydroperoxides are unstable when exposed to high temperatures (Frankel, 1998). Therefore, other peroxidation measures such as TBARS and p-anisidine value have been developed to quantify secondary peroxidation products and when used in conjunction with PV, will provide more accurate measures of the degree of lipid peroxidation.



### ***Thiobarbituric acid reactive substances***

Thiobarbituric acid reactive substances analysis is another common method used to quantify the secondary lipid peroxidation products in animal feed ingredients because it is a relatively simple and rapid procedure. The TBARS assay detects malondialdehyde (MDA) concentration. Each MDA molecule can react with 2 molecules of thiobarbituric acid (TBA) to form a MDA-TBA complex, which has a pink pigment and can be quantified using a spectrophotometer (Gutteridge, 1981). The cytotoxic and mutagenic effect of malondialdehyde has been studied extensively and it has been shown to be present in heated oils. However, one of the disadvantages of the TBARS assay is that other carbonyl-containing secondary lipid peroxidation products can also react with TBA and inflate the TBARS value (Esterbauer et al., 1991). Regardless of this possibility, the TBARS assay provides useful information for evaluating lipid peroxidation because malondialdehyde is highly reactive, and it provides a more accurate measurement of secondary peroxidation products produced during the propagation phase of peroxidation, which are not measured by PV.

### ***Anisidine value***

Anisidine value (AnV) is a measurement of the amount of high molecular weight saturated and unsaturated aldehydes in lipids. This assay involved the reaction between p-anisidine and aldehydes in acetic acid which generates a yellowish color and can be detected and quantified spectrophotometrically at 350 nm. Color intensity depends on the amount of aldehydes as well as on their structure (e.g. diunsaturated aldehydes have more intense color than monounsaturated and saturated aldehydes). However, like the PV assay, only one measurement of AnV may not provide reliable information of the degree of peroxidation for lipids exposed to extreme peroxidation conditions because AnV measurements follow a bell shaped curve as a function of peroxidation time (DeRouchey et al., 2004).

### ***Conjugated dienes***

Conjugated dienes are initial lipid peroxidation products and produced during the formation of hydroperoxides from unsaturated fatty acids. Conjugated dienes have been used to evaluate lipid peroxidation beginning in the 1960's (Antolovich et al., 2002), and exhibit an intense absorption at 234 nm (ultraviolet) which theoretically, is proportional to the formation of primary peroxidation products in lipids. Good correlations between conjugated dienes and PV have been found (Shahidi et al, 1994; Wanasundara et al., 1995). The advantages of using UV detection of conjugated dienes to predict lipid peroxidation are that it is simple, fast, and only small amounts of sample are required. However, this method can be less sensitive compared to direct PV analysis (Gordon, 2001; Antolovich et al., 2002). Furthermore, the presence of other compounds such as carotenoids, also have absorptivity in the same spectrum range and thereby lead to inaccurate results (Shahidi and Wanasundara, 2002). Therefore, caution must be taken when interpreting lipid peroxidation results using the conjugated dienes assay.

### ***Hexanal value***

Hexanal is one of the major secondary lipid peroxidation products generated during the termination phase of peroxidation of linoleic acid (C18:2 n-6) and other n-6 fatty acids. Hexanal is well recognized as a good indicator of lipid peroxidation (Ha et al., 2011), because the hexanal content is directly proportional to off-flavors caused by lipid peroxidation, and can be easily detected due to its low odor threshold (Shahidi and Pegg, 1994; van Ruth et al., 2000; Kalua et al., 2007). However, because hexanal is volatile at high temperatures, a low hexanal value does not necessarily indicate a low level of peroxidation in lipids because hexanal may have been lost depending on when the assay is performed in the peroxidation process.



### ***2,4-decadienal (DDE)***

The compound DDE is a byproduct derived from peroxidized linoleic acid during storage or heating (National Toxicology Program, 1993). Compared to the PV, Anv, and TBARS analyses, the DDE assay is more complicated and expensive requiring gas chromatography and mass spectrophotometry (Matthews et al., 1971; Selke and Frankel, 1987; Lasekan and Abbas, 2010). Low levels of DDE generate a deep-fried flavor, but excessive amounts of this specific dienaldehyde induce many undesirable effects, including peroxidative stress and proinflammatory reactions in human lung cells (Chang et al., 2005), cellular toxicity in liver and kidney tissues (Hageman et al., 1991), cellular proliferation in gastrointestinal epithelial cells (National Toxicology Program, 1993), and carcinogenic effects in the gastrointestinal tract (Hageman et al., 1991). Thus, with respect to the considerable negative impact of DDE on animal health, quantification of DDE may provide valuable information regarding the quality of dietary lipids. However, to date, no research has been conducted to investigate the effect of DDE on growth performance and health status in pigs.

### ***4-Hydroxynonenal (HNE)***

4-hydroxynonenal is one of the  $\alpha$ ,  $\beta$ -unsaturated lipophilic aldehydes formed during lipid peroxidation of n-6 polyunsaturated fatty acids, such as arachidonic or linoleic acid. This compound is well known for its cytotoxic and mutagenic effects (Witz, 1989; Esterbauer et al., 1991). Similar to the assay of DDE, measurement of HNE is complicated and expensive (Zanardi et al., 2002; Fitzmaurice et al., 2006). The reactive groups (an aldehyde, a double-bond at carbon 2, and a hydroxy group at carbon 4) of HNE make it a good marker of lipid peroxidation (Zarkovic, 2003). Furthermore, previous studies demonstrated that the consumption of fat sources containing 4-HNE are likely to increase the oxidative load in the mammalian metabolic system because 4-HNE readily conjugates to glutathione, thus depleting this important antioxidant (Seppanen and Csallany, 2002; Uchida, 2003). Treating cells directly with 4-HNE increases the activation of stress pathways (Biasi et al., 2006; Yun et al., 2009) and alters immune signaling pathways. However, to date, no information regarding the effect of HNE on growth performance and health status in pigs has been reported.

### ***Triacylglycerol dimers and polymers***

The amount of dimers and polymers formed during lipid peroxidation increases as heating time is increased (Sanchez-Muniz et al., 1993; Takeoka et al., 1997). However, limited information is available regarding measurement and applicability of the compounds for determining the quality and feeding value of lipids used in animal feeds. Therefore, to achieve a more complete peroxidation assessment of lipids that have been subjected to extreme peroxidation, future research should focus on developing methods to quantify triacylglycerol dimers or triacylglycerol polymers.

## **Predictive tests**

### ***Active oxygen method (AOM)***

The active oxygen method is a predictive method where purified air is bubbled through a lipid sample held at 97.8°C, and the PV of the lipid is determined at regular intervals. Active oxygen method is defined as the time required to reach a PV of 100 mEq/kg lipid (recorded in hours), or the PV of the lipid is measured at a predetermined amount of time, 20 hours (recorded as meq/kg lipid). By using AOM, the degree of lipid peroxidation can be determined much more accurately than a single PV assay because the PV are plotted over time and the PV concentration represents a bell shaped curve. The more stable the fat, the longer it will take to reach 100 mEq/kg lipid, or the lower the PV will be at the predetermined amount of time. The AOM method is included in compendia published by AOCS (Method Cd 12-57), however, the AOM method is labor-intensive and time-consuming (Jebe et al., 1993). Furthermore, because the AOM results are determined by measuring



unstable hydroperoxides, another disadvantage of the AOM test is its low reproducibility (Jebe et al., 1993).

### ***Oxygen stability index (OSI)***

Oxygen stability index is another predictive measure of lipid peroxidation potential and is similar to the AOM method in theory. In the OSI test, air passes through the lipid solution under a specific temperature, whereupon volatile acids that are decomposed from artificial lipid peroxidation are driven out by the air and subsequently dissolved in the water which increases its conductivity. Conductivity of the water is measured constantly and the OSI value is defined as the hours required for the rate of conductivity to reach a predetermined value. Advantages of the OSI method compared to the AOM method are that multiple samples can be analyzed easily and simultaneously, and the results from OSI tests are highly reproducible because the volatile acids are relative stable tertiary peroxidation products compared to hydroperoxides (Jebe et al., 1993; Mendez, 1996). Like AOM, the OSI test provides information regarding the changing trends in the concentration of volatile lipid peroxidation products by constantly monitoring conductivity. Therefore, OSI can provide a better evaluation of the degree of lipid peroxidation than a single indicative assay. However, because the accuracy of the OSI test depends on monitoring conductivity by quantification of the volatile fatty acids, it has similar disadvantages to using AnV, TBARS, HNE, and hexanal due to the potential loss of volatile fatty acids derived from lipid peroxidation before the OSI test is conducted.

### ***Oxygen bomb method (OBM)***

The oxygen bomb method is frequently used to evaluate the oxidative stability of food products or lipid extracts. Compared to AOM and OSI, OBM is not restricted to analyze pure oils and fats and is frequently used to assess the oxidative stability of food products and feed products (Gearhart et al. 1957, Inglis and Willington 1976, Shermer and Giesen 1997). The oxygen bomb machine includes a stainless steel container (bomb) connected to a pressure recorder. In the oxygen bomb method, a weighed sample is inserted into the bomb filled with a specific amount of oxygen. The change of oxygen pressure is constantly recorded during lipid peroxidation process and requires incorporating different amounts of oxygen into the analyzed samples. The amount of oxygen used is proportional to the degree of lipid peroxidation, and can be measured by monitoring the reduction of the oxygen pressure. As a result, the amount of peroxidation products can be calculated by recording the decrease in oxygen pressure in the bomb. Lipids in feed or food products that have a fast and high amount of oxygen uptake are more prone to peroxidative degradation (Gearhart et al. 1957; Blankens et al. 1973). The susceptibility to peroxidation of products in the oxygen bomb apparatus is mainly based on their total fat content.

### ***Correlation among indicative and predictive methods***

We recently completed an evaluation of the correlations among various indicative and predictive assays for assessing peroxidation in 4 lipids, each with 3 degrees of peroxidation (Table 2 and 3). Lipid sources were: corn oil (CN), canola oil (CA), poultry fat, and tallow. Peroxidation levels were: original lipids (OL), slow-oxidized lipids (SO), and rapid-oxidized lipids (RO). To produce peroxidized lipids, OL were either heated at 95°C for 72 h to produce SO, or heated at 185°C for 7 h to produce RO. Five indicative measurements (PV, AnV, TBARS, hexanal, HNE, and DDE) and 2 predictive tests (AOM and OSI) were performed to quantify the degree of peroxidation of the subsequent 12 lipids. Results showed that a high PV accurately indicated a high degree of lipid peroxidation, but a moderate or low PV may be misleading due to the unstable characteristics of hydroperoxides as indicated by the unchanged PV of rapidly oxidized CN and CA compared to their original state (OL). However, additional tests which measure secondary peroxidation products such as AnV, TBARS, hexanal, HNE, and DDE may provide a better indication of lipid peroxidation than PV for lipids subjected to a high degree of peroxidation. Similar to PV analysis, these tests may also not provide



irrefutable information regarding the extent of peroxidation due to the volatile characteristics of secondary peroxidation products and the ever changing stage of lipid peroxidation. For the predictive tests, AOM accurately reflected the increased lipid peroxidation caused by SO and RO as indicated by the increased AOM value in CN and CA, but not in poultry fat and tallow, which indicates a potential disadvantage of the AOM test. Oxidative stability index successfully showed the increased lipid peroxidation caused by SO and RO in all lipids, but it also has disadvantages similar to AnV, TBARS, hexanal, DDE, and HNE because OSI is directly dependent on the quantification of volatile secondary peroxidation products. These results indicate that lipid peroxidation measurements should be determined at appropriate time intervals by more than one test and include different types of peroxidation products simultaneously for accurate determination of peroxidation damage in lipids.

### **Does Lipid Peroxidation Affect Energy Content and Dietary Nutrient Utilization?**

A recent examination of lipids obtained from a local feed mill showed a range in total MIU from 0.8 to 3.7%, AOM from 8.0 to 332 hours, IV from 66.3 to 84.0 g/100 g lipid, PV from 0.4 to 7.3 mEq/kg, and FFA from 5.8 to 51.6%. Consequently, there appears to be a wide range in composition and quality of lipids being fed to livestock. Unfortunately little is known about each quality indices on the ability of the animal to utilize the lipid source for energy. Leeson et al. (1997) showed no impact of lipid rancidity on turkey performance. In contrast, Cabel et al. (1988) and Dibner et al. (1996) reported decreased broiler performance with an increase in fat rancidity. In addition, broilers fed oxidized vegetable oil had reduced fat and energy retention (Engberg et al., 1996). A similar discrepancy has been noted in swine. Fernández-Dueñas et al. (2008) reported no effect of oxidized canola oil or tallow on pig performance, while (Derouchey et al. (2004) reported that increasing the rancidity of choice white grease (PV of 105 mEq/kg equating to 6.3 mEq/kg diet) decreased feed intake, but fatty acid digestibility was not affected. Yuan et al. (2007) reported decreased digestibility of dry matter, crude protein, fat, and ME in nursery pigs fed oxidized fish oil. In addition to supplementing lipids by themselves, various animal and vegetable protein meals (i.e., meat and bone meal, dried distillers grains with solubles) also contain moderate amounts of lipids, and since these feedstuffs are heat processed, the lipids in these products may also be susceptible to oxidation (Song et al., 2011). To date, however, the data are inconclusive on the level of lipid oxidization in these feedstuffs and on subsequent animal productivity. Carpenter et al. (1966) and L'Estrange et al. (1967) fed growing pigs 10% meat meal with a peroxide value of 210 mEq/kg (resulting in 3.5 mEq/kg diet) and reported no difference in performance compared to pigs fed the same diet containing unoxidized lipids. In contrast, Fernández-Dueñas (2009) and Harrell et al. (2010) reported that diets containing DDGS or oxidized corn oil resulted in depressed pig performance. Results from a recent study conducted by our group (Liu et al., 2012) showed no effect of amount of lipid peroxidation (slow or rapid oxidation) on DE or ME content of corn oil, canola oil, poultry fat, and tallow in nursery pigs (Table 4). Furthermore, degree of lipid peroxidation had no effect on apparent total tract digestibility of dry matter, GE, ether extract, nitrogen, carbon, or sulfur (Table 5).



**Table 2.** Characteristics of the experimental lipids<sup>1</sup>.

	Corn oil			Canola oil			Poultry fat			Tallow		
	OL	SO	RO	OL	SO	RO	OL	SO	RO	OL	SO	RO
Crude fat, %	99.34	99.36	99.26	99.16	99.50	99.26	95.52	96.42	98.23	98.04	98.68	99.02
Free fatty acids, %	0.28	0.48	0.65	0.36	0.57	0.58	3.62	3.65	3.17	1.99	3.10	2.28
Total MIU <sup>2</sup>	1.00	1.02	1.22	1.01	0.89	0.96	2.24	1.01	1.23	0.78	0.60	0.64
Moisture, %	0.06	0.00	0.06	0.08	0.00	0.00	0.19	0.02	0.07	0.15	0.10	0.07
Insolubles, %	0.02	0.04	0.08	0.02	0.02	0.02	1.08	0.08	0.22	0.22	0.16	0.23
Unsaponifiables, %	0.92	0.98	1.06	0.91	0.87	0.94	0.97	0.93	0.94	0.41	0.34	0.34
<b>Fatty acids, %</b>												
Myristic (14:0)	0.06	0.06	0.07	0.08	0.09	0.08	0.63	0.63	0.65	3.03	3.21	3.29
Palmitic (16:0)	10.76	11.90	12.11	3.95	4.39	4.43	24.69	24.49	24.68	24.50	24.68	25.94
Palmitoleic (16:1)	0.10	0.10	0.12	0.22	0.23	0.23	7.11	7.39	7.19	2.55	2.71	2.55
Stearic (18:0)	1.71	1.91	1.93	1.78	1.93	1.95	5.93	5.62	5.80	21.59	20.00	21.97
Oleic (18:1)	27.70	29.84	29.80	64.57	65.47	66.82	38.07	39.16	39.20	32.03	33.48	30.62
Linoleic (18:2)	57.18	52.73	52.32	17.90	16.51	15.93	18.50	17.59	17.10	2.80	1.83	1.84
Linolenic (18:3)	0.79	0.62	0.63	7.09	5.73	5.01	0.77	0.67	0.69	0.22	0.12	0.11
U:S <sup>3</sup>	6.85	6.01	5.87	15.45	13.72	13.62	2.06	2.11	2.06	0.77	0.80	0.69
Iodine value <sup>4</sup>	125	119	118	105	100	98	73	73	72	35	35	32
Vitamin E, IU/g	0.40	< 0.10	< 0.10	0.29	< 0.10	< 0.10	< 0.10	< 0.10	< 0.10	< 0.10	< 0.10	< 0.10
<b>Oxidation products</b>												
PV <sup>5</sup> , mEq/kg	1	151	2	1	239	12	1	57	2	1	29	3
p-Anisidine <sup>6</sup>	< 1	61.4	142.9	1	37.0	154.8	3	88	22	4	120	19
TBARS <sup>7</sup> , μmol/kg	16	225	119	45	968	622	79	151	58	58	61	41
Hexanal, mg/kg	< 1	390	83	1	180	59	3	88	22	4	120	19
2, 4-decadienal, ppm	72	3728	1345	7	1091	511	30	442	169	47	261	125
HNE <sup>8</sup> , μmol/kg	0	194	594	0	105	221	0	2	0	0	13	6
AOM <sup>9</sup> , mEq/kg	103	575	528	112	419	533	4	298	5	< 2	6	446
OSI <sup>10</sup> , h	8.4	< 1.0	< 1.0	9.2	< 1.0	< 1.0	24.6	< 1.0	< 1.0	12.1	< 1.0	< 1.0

<sup>1</sup> OL: Lipids were stored as received without antioxidants or heating; SO, lipids heated for 72 h at 95°C with constant compressed air flow rate at 12 L/min; RO, lipids heated for 7 h at 185°C with constant compressed air flow rate at 12 L/min.

<sup>2</sup> Total of moisture, insolubles, and unsaponifiables.

<sup>3</sup> Unsaturated to saturated fatty acid ratio.

<sup>4</sup> Iodine value was calculated as iodine value = (C16:1) × 0.95 + (C18:1) × 0.86 + (C18:2) × 1.732 + (C18:3) × 2.616 (Method Cd 1-25; AOCS, 1998).

<sup>5</sup> PV = peroxide value

<sup>6</sup> There is no unit for p-anisidine value.

<sup>7</sup> TBARS = thiobarbituric acid reactive substances.

<sup>8</sup> HNE = 4-hydroxynonenal.

<sup>9</sup> AOM = active oxygen method measured as the peroxide value at 20 h of oxidation.

<sup>10</sup> OSI = Oxygen stability index.



**Table 3.** Correlation matrix among lipid composition and various peroxidation measures<sup>1</sup>.

	CF	FFA	MIU	Mo	In	Usap	Myr	Pal	Pmo	Ste	Ole	Lin	Linol	US	IV	VE	PV	AnV	TBARS	Hex	DDE	HNE	AOM	OSI	
CF	1.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
FFA	-0.81 0.01	1.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
MIU	-0.66 0.02	NS	1.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mo	-0.57 0.05	0.50 0.10	NS	1.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
In	-0.77 0.01	0.60 0.04	0.80 0.01	0.77 0.01	1.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Usap	NS	NS	0.58 0.05	NS	NS	1.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Myr	NS	NS	NS	NS	NS	-0.97 0.01	1.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pal	-0.64 0.03	0.89 0.01	NS	0.57 0.05	0.51 0.09	-0.52 0.08	0.69 0.01	1.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pmo	-0.86 0.01	0.93 0.01	NS	NS	0.60 0.04	NS	NS	0.77 0.01	1.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ste	NS	NS	NS	NS	NS	-0.96 0.01	0.99 0.01	0.71 0.01	NS	1.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ole	NS	NS	NS	NS	NS	NS	NS	-0.66 0.02	NS	NS	1.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lin	NS	-0.56 0.06	NS	NS	NS	0.68 0.02	-0.68 0.02	NS	NS	-0.68 0.01	NS	1.0	-	-	-	-	-	-	-	-	-	-	-	-	-
Linol	NS	-0.54 0.07	NS	NS	NS	NS	NS	-0.80 0.01	NS	-0.49 0.10	0.95 0.01	NS	1.0	-	-	-	-	-	-	-	-	-	-	-	-
US	0.52 0.09	-0.76 0.01	NS	-0.51 0.09	NS	NS	-0.65 0.02	-0.96 0.01	-0.63 0.03	-0.67 0.02	0.83 0.01	NS	0.94 0.01	1.0	-	-	-	-	-	-	-	-	-	-	-
IV	NS	-0.72 0.01	NS	-0.51 0.09	NS	0.85 0.04	-0.92 0.01	-0.79 0.01	NS	-0.93 0.01	NS	0.85 0.01	NS	0.66 0.02	1.0	-	-	-	-	-	-	-	-	-	-
VE	NS	-0.47 0.01	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	1.0	-	-	-	-	-	-	-	-	-
PV	NS	NS	NS	-0.57 0.05	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	1.0	-	-	-	-	-	-	-	-
AnV	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	1.0	-	-	-	-	-	-	-
TBARS	NS	NS	NS	-0.58 0.05	NS	NS	NS	-0.59 0.04	NS	NS	0.70 0.01	NS	0.60 0.04	0.62 0.03	NS	NS	0.75 0.01	NS	1.0	-	-	-	-	-	-
Hex	NS	NS	NS	-0.57 0.06	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0.50 0.10	NS	0.76 0.01	NS	NS	1.0	-	-	-	-	-
DDE	NS	NS	NS	-0.53 0.08	NS	NS	NS	NS	NS	NS	NS	0.56 0.06	NS	NS	NS	NS	0.61 0.04	NS	NS	0.94 0.01	1.0	-	-	-	-
HNE	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0.54 0.07	NS	NS	NS	NS	NS	0.67 0.02	NS	NS	0.49 0.10	1.0	-	-	-
AOM	NS	-0.51 0.09	NS	-0.75 0.01	NS	NS	NS	NS	-0.50 0.10	NS	NS	NS	NS	NS	NS	NS	NS	0.53 0.08	0.51 0.09	0.57 0.06	0.65 0.02	0.66 0.02	1.0	-	-
OSI	-0.60 0.04	NS	0.70 0.01	0.81 0.01	0.78 0.01	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	-0.57 0.05	NS	NS	NS	NS	-0.58 0.05	1.0	

<sup>1</sup> Abbreviations: CF = crude fat, FFA = free fatty acids, MIU = moisture, insolubles, and unsaponifiables, Mo = moisture, In = insolubles, Usap = unsaponifiables, Myr = myristic acid, Pal = palmitic acid, Pmo = palmitoleic acid, Ste = stearic acid, Ole = oleic acid, Lin = linoleic acid, Linol = linolenic acid, US = unsaturated:saturated ratio, IV = iodine value, VE = vitamin E, PV = peroxide value, AnV = p-ansidine value, TBARS = thiobarbituric acid reactive substances, Hex = hexanal, DDE = 2, 4-decadienal, HNE = 4-hydroxy nonenal, AOM = active oxygen method, and OSI = oxidative stability index. Top value represents correlation (r value) and bottom value represents significance (P value). If no value is given, it was not found to be different at  $P \leq 0.10$  and listed as NS = non-significant.



**Table 4.** Effect of thermally-oxidized vegetable oils and animals fats on lipid energy values for growing pigs<sup>1</sup>.

	Control diet	Corn oil			Canola oil			Poultry fat			Tallow			SEM	<i>P</i> value <sup>3</sup>		
		OL	SO	RO	OL	SO	RO	OL	SO	RO	OL	SO	RO		S	O	S×O
Obs <sup>2</sup>	6	9	9	9	8	8	8	8	8	8	9	8	8	--	--	--	--
Energy content, kcal/kg as-fed basis																	
GE	3,813	9,435	9,434	9,328	9,454	9,362	9,401	9,386	9,348	9,356	9,412	9,337	9,352	--	--	--	--
DE	3,293	8,846	8,682	8,668	8,867	8,648	8,725	8,519	8,274	8,511	8,316	8,168	8,296	268	0.08	0.60	0.99
ME	3,173	8,522	8,417	8,429	8,551	8,371	8,436	8,324	7,960	8,217	8,033	7,891	8,009	287	0.12	0.63	0.98

<sup>1</sup> Values are least squares mean (for control n = 6, for lipids diets n = 8 or 9); OL: lipids were stored as received without antioxidants or heating; SO: lipids heated for 72 h at 95°C with constant compressed air flow rate at 12 L/min; RO: lipids heated for 7 h at 185°C with constant compressed air flow rate at 12 L/min. Data for the Control represents the control diet while data for all lipid sources represents the energy of the lipid itself.

<sup>2</sup> Number of observations per treatment.

<sup>3</sup> S, lipid source; O, oxidation level; S×O, lipid source by oxidation level interaction.

**Table 5.** Apparent total tract digestibility (ATTD) of DM, GE, ether extract (EE), nitrogen (N), carbon (C), and sulfur (S), and N retention (NR) of diets (as-fed basis)<sup>1</sup>.

	Control	Corn oil			Canola oil			Poultry fat			Tallow			SEM	<i>P</i> value <sup>3</sup>			
		OL	SO	RO	OL	SO	RO	OL	SO	RO	OL	SO	RO		S	O	S×O	C vs L
Obs <sup>2</sup>	6	9	9	9	8	8	8	8	8	8	9	8	8	--	--	--	--	--
ATTD, %																		
DM	86.78	88.66	88.42	88.76	88.76	88.21	87.35	87.35	88.00	87.51	86.83	86.66	86.21	0.74	< 0.01	0.42	0.91	0.29
GE	86.35	88.92	88.59	88.84	89.35	88.67	87.69	88.44	87.56	87.81	86.83	86.53	86.15	0.78	< 0.01	0.23	0.91	0.06
EE	21.50	83.34	85.02	82.82	84.94	83.55	80.96	83.59	82.27	82.49	80.04	78.33	80.19	1.50	< 0.01	0.23	0.24	< 0.01
N	86.81	88.73	89.34	89.39	88.63	89.24	88.47	87.76	86.59	87.86	85.57	86.46	85.83	1.30	< 0.01	0.90	0.81	0.83
C	87.34	89.41	89.07	89.39	89.86	89.28	88.20	89.01	88.34	88.33	87.56	87.43	86.79	2.30	< 0.01	0.22	0.88	0.13
S	73.16	72.24	73.61	71.20	72.50	69.11	71.71	71.28	71.63	71.49	72.34	72.18	71.4	2.30	0.90	0.91	0.93	0.58
NR, % <sup>4</sup>	62.08	64.14	65.66	64.95	64.81	62.76	63.07	64.29	60.05	63.17	56.32	60.44	58.20	4.00	0.05	0.99	0.89	0.95

<sup>1</sup> Values are least squares mean (for control n = 6, for lipids diets n = 8 or 9); OL: lipids were stored as received without antioxidants or heating; SO: lipids heated for 72 h at 95°C with constant compressed air flow rate at 12 L/min; RO: lipids heated for 7 h at 185°C with constant compressed air flow rate at 12 L/min.

<sup>2</sup> Number of observations per treatment.

<sup>3</sup> S, lipid source; O, oxidation level; S × O, lipid source by oxidation level interaction; C vs L, control versus lipids.

<sup>4</sup> Nitrogen retention as a percentage of N intake.



## Does Lipid Peroxidation Affect Swine Health and Performance?

Growth suppression from feeding peroxidized lipids has been documented in several different animal species. In rats, Kimura et al. (1984) reported that feeding oxidized soybean oil impaired growth performance and induced diarrhea. Alexander et al. (1987) and Behniwal et al. (1993) also reported that rats fed diets containing oxidized corn or peanut oil had impaired growth rate. Similarly in chickens, feeding heated sunflower oil (Sheehy et al., 1994), oxidized rapeseed-soybean oil (Engberg et al., 1996), or oxidized poultry fat (Cabel et al., 1988; Dibner et al., 1996) reduced growth. In pigs, DeRouchey et al. (2004) reported that growth rate decreased when feeding diets containing choice white grease with increasing levels of rancidity. Feeding oxidized corn oil has also been shown to reduce growth performance in pigs (Fernández-Dueñas, 2009; Harrell et al., 2010). In contrast, other researchers reported no differences in growth rate and feed intake when poultry or swine were fed diets containing oxidized lipids (Sheehy et al., 1994; Mitchaothai et al., 2007; Fernández-Dueñas et al., 2008). The reasons for a lack of a negative growth response in these studies is unclear, but may be related to insufficient dietary oxidative challenge as measured by peroxide value in the lipids or diet. DeRouchey et al. (2004) suggested that there may be a peroxide value threshold ( $> 2.4$  meq/kg) for lipid peroxidation above which growth performance of pigs is decreased. Results from a recent study conducted by our group (Liu et al., 2012) showed that feeding rapidly oxidized lipids (corn and canola oil as well as poultry fat and tallow heated at  $185^{\circ}\text{C}$  for 7 h) to nursery pigs for 28 days resulted in a trend for reduced ADFI and a reduction in ADG compared to pigs fed non-oxidized lipids. These results indicate that lipid peroxidation can negatively impact pig growth performance.

## How Do We Determine Metabolic Oxidation Status of Pigs?

Metabolic oxidation status can be described as the physiological balance between the production of lipid peroxidation products and the pig's endogenous antioxidant ability to control the deleterious effects of peroxidation. Therefore, feeding peroxidized lipids to pig may increase the burden of detoxifying peroxidation products by the pig. Unfortunately, there is no single measurement or index that completely characterizes metabolic oxidation status of pigs, but several indicators have been commonly used.

Metabolic oxidation status in animals has been characterized by measuring TBARS and concentrations of antioxidants in blood and tissues. Engberg et al. (1996) observed that plasma concentrations of TBARS were higher, and  $\alpha$ -tocopherol concentrations were lower in broilers fed 11% oxidized vegetable oils (9% rapeseed oil, 2% soybean oil; PV = 156 meq/kg oil) with a dietary PV of 17.6 meq/kg feed. Similarly in swine, feeding oxidized corn oil (PV = 180 meq/kg oil) with dietary PV of 9 meq/kg feed increased plasma TBARS, and decreased  $\alpha$ -tocopherol concentrations in plasma and liver (Fernández-Dueñas, 2009). These results were confirmed in a recent study by Liu et al. (2012) where feeding slow and rapid oxidized lipids to nursery pigs increased serum TBARS concentration. However, Fernández-Dueñas et al. (2008) showed that feeding 5% oxidized canola oil (PV = 7.98 meq/kg oil) with dietary PV = 0.4 meq/kg feed did not change plasma TBARS. Therefore, using these measurement criteria, lack of an increase in plasma TBARS may be due to the insufficient dietary oxidative challenge (using PV as an indicator of peroxidation in oil and feed), and there may be a threshold level above which feeding peroxidized lipids can elevate metabolic oxidation status of pigs.

Changes in gene regulation also indicate alterations in lipid metabolism when animals are fed peroxidized lipids. Feeding thermally oxidized oil/fat to rats (Koch et al., 2008; Sulzle et al., 2004) and pigs (Luci et al., 2007; Liu et al., 2012) altered *in vivo* lipid metabolism by activating the peroxisome proliferator-activated receptor  $\alpha$  (PPAR $\alpha$ ) via up-regulation of some target genes in PPAR $\alpha$ , such as acyl CoA oxidase, catalase, and carnitine palmitoyltransferase-1. The transcription factor PPAR $\alpha$  controls the expression of fatty acid oxidative metabolism in many aspects, including



fatty acid uptake through membranes, fatty acid activation, intracellular fatty acid trafficking, fatty acid oxidation, ketogenesis, and triglyceride storage and lipolysis (Cabrero et al., 2001). Some mechanisms regarding these regulatory roles of PPAR $\alpha$  in lipid metabolism have been studied, while most of them are still unknown. However, results from a recent study conducted by our group (Liu et al., 2012) showed that pigs fed thermally oxidized lipids had increased activation of PPAR $\alpha$  in the liver, indicating alterations in fatty acid metabolism.

Changes in gut barrier function may be another indicator of metabolic oxidation status. The intestine is exposed to an unlimited number and variety of intraluminal agents including foreign antigens, microorganisms, and their toxins, which are derived from ingested food, xenobiotics, bacteria, and viruses (Walker and Sanderson, 1992). Some of these antigens pose no threat to the host, while others are harmful and may lead to disorders affecting the nervous and immune systems. The intestinal barrier consists of an intrinsic layer, including epithelial cells and tight junctions, and an extrinsic layer, which is comprised of bacteria and a coating of mucus with high concentrations of secretory IgA (Walker and Sanderson, 1992). Proper intestinal barrier function is essential for maintaining optimal health and balance throughout the body because it is the first line of defense against an increasingly toxic environment. Intestinal barrier function can be measured using the probes of mannitol and lactulose. This dual sugar test was first introduced by Menzies (1974) and is based on the fact that orally administered mannitol, a monosaccharide, will be passively absorbed through the intestinal epithelium, while lactulose, a disaccharide, normally does not pass the intestinal epithelium through the paracellular route unless the barrier function is compromised. Another method that has been used to determine gut barrier function *in vivo* is through the measurement of the circulating endotoxin concentrations in the blood (Sharma et al., 2007). Endotoxins are the complex lipopolysaccharide components of the outer cell wall membrane of Gram-negative bacteria, and are considered to be a major virulence factors that are responsible for the lethal effects and clinical manifestations of diseases in humans and animals (Swain et al., 2008). Normally, Kupffer cells in the liver detoxify endotoxins by phagocytosis (Rao, 2009). When the flux of endotoxins overwhelms the phagocytotic capacity of Kupffer cells, the endotoxins spill into the systemic circulation. It has been shown that impaired gut barrier function is associated with increased bacterial translocation and endotoxin absorption (Walker and Sanderson, 1992). Based on this theory, elevated endotoxin concentration in the blood has been used to indicate impaired gut barrier function in some studies (Sharma et al., 2007; Rao, 2009).

Dibner and Knight (2008) stated that oxidative stress in the gastrointestinal system, regardless of its cause, is associated with loss of barrier function due to the denaturation of the protein component of the enterocyte junctional complexes (Musch et al., 2006), which results in failure of both cell-cell adhesion and apical membrane integrity (Blikslager et al., 2007). Additionally, intestinal epithelial cells contain relatively high concentrations of PUFA, which are particularly effective in enhancing intestinal epithelia barrier integrity by improving natural resistance (Willemsen et al., 2008). It has been shown that long chain PUFA are susceptible to lipid peroxidation (Tappel, 1962). Oxidation of PUFA present in intestinal epithelial cell membranes may lead to cell injury, and thus, impair epithelial barrier function due to the disruption of the normal membrane structure and function (Lauridsen et al., 1999). In a human study, lipid hydroperoxides were found to increase intestinal cell apoptosis (Wang et al., 2000), suggesting that dietary oxidants could impair the integrity of the intestinal epithelium. However, little research has been conducted to evaluate the effects of feeding oxidized lipids to animals on gut barrier function. In a recent study by our group (Liu et al., 2012), feeding oxidized corn oil, canola oil, beef tallow, and poultry fat to nursery pigs showed no effect on intestinal barrier function as measured by the lactulose-mannitol protocol.

### **Are Antioxidants Necessary?**

Antioxidant chemistry is a complex field of science and this subject has been extensively reviewed by Wanasundara and Shahidi (2005) and Frankel (2007). Addition of antioxidants (i.e., butylated



hydroxyanisole, butylated hydroxytoluene, tocopherol, and ethoxyquin) has been evaluated in humans, rodents, and livestock, but their impact on animal physiological and performance parameters has been inconsistent (Fernández-Dueñas, 2009). Dibner et al. (1996) reported reduced feed efficiency in broilers fed oxidized poultry fat compared to birds fed unoxidized poultry fat, with the addition of ethoxyquin improving feed efficiency—regardless of lipid oxidation level. Likewise, supplementation of additional antioxidants improved growth performance in pigs fed diets containing DDGS or oxidized corn oil (Fernández-Dueñas, 2009; Harrell et al., 2010). In contrast, others have shown that supplementation of antioxidants have no effect on growth performance in animals under dietary oxidative stress (Wang et al., 1997; Anjum et al., 2002; Fernández-Dueñas et al., 2008; Song et al., 2012). Based on these inconsistent responses, it is unclear if antioxidants are necessary additions to lipids used in animal feed in order to maintain optimal nutritional value, or if their addition to swine diets are beneficial in overcoming a metabolic oxidative challenge.

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## NOTES

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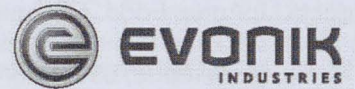
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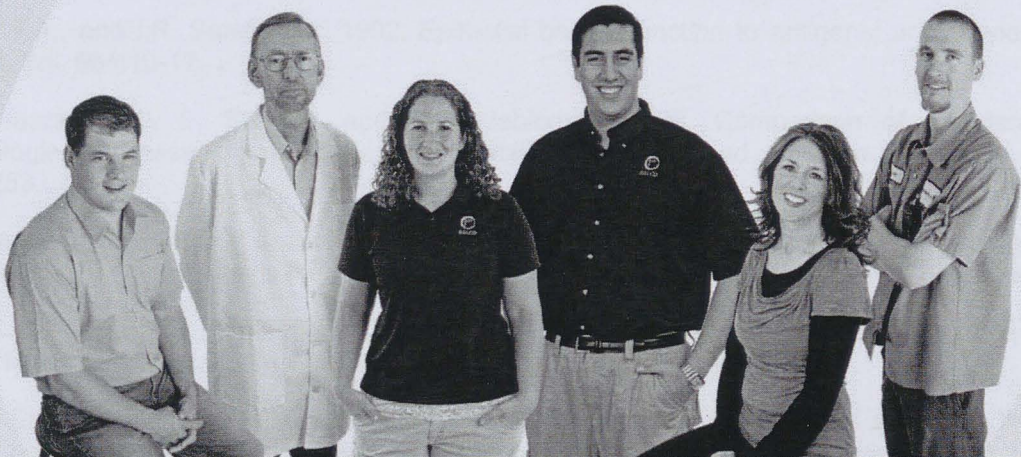
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