Evaluation of lipid peroxidation level in corn dried distillers grains with solubles

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ABSTRACT: Lipid peroxidation in feed can negatively affect animal health, growth performance, and meat quality. The objective of this study was to determine the lipid peroxidation level in corn dried distillers grains with solubles (DDGS) samples from 31 U.S. ethanol plants and compare results with a corn sample obtained from a corn processing plant. Lipids from each sample were extracted with hexane and analyzed for peroxide value (PV) and thiobarbituric acid reactive substances (TBARS). Peroxide values of DDGS samples ranged from 4.2 to 84.1 milliequivalents (meq)/kg oil (CV = 97.5%). The greatest PV among DDGS samples was 27 times greater than that of the corn sample (3.1 meq/kg oil). The TBARS values for DDGS samples ranged from 1.0 to 5.2 ng malondialdehyde (MDA) equivalents/mg oil (CV = 43.6%). The DDGS sample with the greatest TBARS value was 25 times greater than that of the corn sample (0.2 ng MDA equivalents/mg oil). Color of DDGS samples was measured by Minolta L*, a*, and b*, corresponding to the degree of lightness, redness, and yellowness, respectively. Correlations between PV, TBARS, and color were determined. Values of PV and TBARS were correlated positively ($r = 0.81$; $P < 0.001$). Both TBARS and PV were correlated negatively with L* ($r = –0.73$; $P < 0.001$, and $r = –0.63$; $P < 0.001$, respectively) and b* ($r = –0.67$; $P < 0.001$, and $r = –0.57$; $P < 0.001$, respectively), which suggests that darker and less yellow-colored DDGS samples were more likely to have a greater lipid peroxidation level, as measured by TBARS and PV, compared with lighter or more yellow-colored DDGS samples. However, a* was not correlated with either PV ($P = 0.97$) or TBARS ($P = 0.66$). These results indicate that color can be a preliminary indicator of lipid peroxidation level in DDGS, but a more reliable assessment of peroxidation level is achieved by measuring PV and TBARS.

Key words: color, dried distillers grains with solubles, lipid peroxidation, peroxide value, thiobarbituric acid reactive substances

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INTRODUCTION

Corn dried distillers grains with solubles (**DDGS**) are commonly added to commercial swine feeds. However, results from some studies have shown that reductions in growth performance may occur when high dietary levels (20 to 30%) of DDGS are fed to growing-finishing pigs (Whitney et al., 2006; Linneen et al., 2008; Stein and Shurson, 2009). Although several potential factors (i.e., low net energy levels, poor AA balance and digestibility, and excess dietary CP) have been suggested as potential contributors to inconsistent growth responses when some DDGS sources are fed to swine (Stein and Shurson, 2009; Xu et al., 2010), the presence of peroxidized lipids in DDGS may also be a contributing factor.

Lipid peroxidation is a free-radical chain reaction that produces peroxidized lipids and a series of toxic aldehydes (Blokhina et al., 2003). Lipids in corn DDGS are composed largely of PUFA, particularly linoleic acid (NRC, 1998), which are highly susceptible to peroxidation (Frankel et al., 1984; Linfield et al., 1985). Large quantities of secondary lipid peroxidation products, such as aldehydes, carbonyls and ketones, are produced when lipids are heated at relatively high temperatures (Esterbauer et al., 1991). The DDGS are dried at temperatures as high as 500°C (Rosentrater et al., 2012). Therefore, corn DDGS may be prone to significant lipid peroxidation.

Peroxidized lipids in animal feed can negatively affect animal health, growth performance, and meat

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quality (Miller and Brzezinska-Slebodzinska, 1993; Pfalzgraf et al., 1995). However, no studies have been published regarding lipid peroxidation levels in corn DDGS. Therefore, the objective of this study was to evaluate lipid peroxidation levels in corn DDGS samples from 31 U.S. ethanol plants, using peroxide value (**PV**) and thiobarbituric acid reactive substances (**TBARS**) as indicators (Shahidi and Zhong, 2005), and to determine correlations between PV and TBARS, and their association with DDGS color.

MATERIALS AND METHODS

Corn, Dried Distillers Grains with Solubles Samples, and Chemicals

Samples of freshly produced DDGS were obtained from 31 corn ethanol plants located in 9 states (IA, IL, IN, MI, MN, ND, OH, SD, and WI) in Midwest United States, between June 2010 and August 2010. A corn sample representing a common commercial variety was obtained from the feed mill storage bin at the University of Minnesota Southern Research and Outreach Center (Waseca, MN) in August 2010. All samples were stored at –20°C on receipt.

Chemical reagents, including n-hexane, glacial acetic, chloroform, potassium iodide, and sodium thiosulfate (**$Na_2S_2O_3$**), were obtained from Fisher Scientific (Pittsburgh, PA). Reagents including malondialdehyde (**MDA**), trichloroacetic acid (**TCA**), 2-thiobarbituric acid (**TBA**), and hydrochloric acid (**HCl**) were purchased from Sigma-Aldrich (St. Louis, MO).

Oil Extraction from Corn and Dried Distillers Grains with Solubles

Lipids from the corn and 31 DDGS samples were extracted with n-hexane after a modified procedure, as described by Seppanen and Csallany (2004). Specifically, 20 g of corn and each DDGS sample were weighed and ground for 3 min to a fine powder, and transferred to a 250-mL Erlenmeyer (Fisher Scientific, Pittsburgh, PA) flask containing 100 mL n-hexane. The corn and DDGS samples were mixed with n-hexane at room temperature for 15 min by stirring and the mixture was filtered via Whatman #1 filter paper (Piscataway, NJ) to collect the hexane solution containing lipids into a 250-mL, round-bottom flask. This step was repeated 2 times by adding 50 mL n-hexane each time. Therefore, by mixing and filtering 3 times, lipids from corn and each DDGS sample were extracted into 200 mL of hexane solution. Lipids were obtained after the hexane solution was evaporated by mildly heating at 40°C for ~5 min. All lipid samples extracted from corn and the 31 DDGS sources were stored at –20°C until further analyses of lipid peroxidation was conducted.

Measurement of Lipid Peroxidation Level in Oils Extracted from Corn and Dried Distillers Grains with Solubles

To determine the lipid peroxidation level, PV was measured in lipids extracted from corn and 31 DDGS samples after the official method described by Association of Official Analytical Chemistry (AOAC, 2005; Method 965.33). In general, 160 mg lipid extract was weighed into a 125-mL Erlenmeyer flask. A PV solution (15 mL) containing 9 mL glacial acetic, 6 mL chloroform, 1 mL saturated aqueous potassium iodide solution, and 15 mL deionized water were added to the flask and mixed for 1 min. A 4×10^{-4} N $Na_2S_2O_3$ solution was used to titrate until the yellow color disappeared. The PV of the lipid was calculated using this equation: PV = [volume of $Na_2S_2O_3$ (mL) × (4×10^{-4}) × 1,000]/weight of lipid (g). Because PV measures the concentration of hydroperoxides and peroxides, it was expressed as milliequivalents of peroxide (**meq**)/kg oil. All samples and standards were conducted in duplicate in 1 batch, with the intra-assay CV of 4.2%.

Thiobarbituric acid reactive substances were measured in lipids extracted from corn and 31 DDGS samples, using the method described by Buege and Aust (1978). Briefly, 200 μL of lipid samples and standards of MDA were mixed with TBARS solution including 15% (wt/vol) TCA, 0.375% wt/vol TBA, and 0.25 N HCl. The mixtures were heated in a boiling water bath for 15 min, followed by centrifugation at 3,000 × *g* for 15 min at 4°C. The supernatant was removed and read at 532 nm, using a spectrophotometer (SpectraMax 250, Molecular Device, Sunnyvale, CA). The TBARS results were expressed as ng MDA equivalents/mg oil. This assay was conducted in 4 batches with triplicate samples and standards. The intra-assay CV was 5.8% and the interassay CV was 4.9%.

Measurement of Color in Corn and Dried Distillers Grains with Solubles

Color of corn and 31 DDGS samples was measured, using a MiniScan XE Plus portable colorimeter (Model 45/O-S, Hunter Associates Laboratory, Reston, VA), and expressed using 3 parameters developed by the Commission Internationale d'Eclairage in Vienna, Austria. In the 3 parameter color scale, L* indicates lightness of color (L* = 0 represents black and L* = 100 represents white), a* indicates color position between red and green (negative values indicate green and positive values indicate red), and b* indicates color position between yellow and blue (negative values indicate blue

Table 1. Measurements of lipid peroxidation in oil extracted from corn dried distillers grains with solubles (DDGS) and DDGS color

		DDGS[1]				
Item	Corn	Average	Median	Minimum value	Maximum value	CV, %
PV,[2] meq/kg oil	3.1	13.9	11.7	4.2	84.1	97.5
TBARS,[2] ng MDA equivalents/mg oil	0.2	1.9	1.7	1.0	5.2	43.6
Color						
L*[3]	83.9	54.1	54.9	45.2	58.1	4.6
a*[4]	2.6	10.9	10.8	9.3	12.4	7.2
b*[5]	20.0	37.3	37.5	26.6	42.7	8.8

[1]Data from 31 DDGS sources.

[2]PV = peroxide value; TBARS = thiobarbituric acid reactive substances.

[3]L* = a greater value indicates a lighter color.

[4]a* = negative values indicate green and positive values indicate red; a greater positive value indicates a more reddish color.

[5]b* = negative values indicate blue and positive values indicate yellow; a greater positive value indicates a more yellowish color.

and positive values indicate yellow). Each sample was measured in triplicate and the intra-assay CV for L*, a*, and b* was 1.7%, 6.8%, and 3.3%, respectively.

Statistical Analysis

Data were analyzed using the CORR procedure (SAS Inst. Inc., Cary, NC) to determine the correlation between PV, TBARS, and color of DDGS. Pearson's Correlation Coefficient (r) with a P-value was reported to indicate the correlation between these parameters. The significance level chosen was $\alpha = 0.05$. Correlations were considered significant if $P < 0.05$, whereas values between $0.05 \leq P \leq 0.10$ were considered statistical trends.

RESULTS

Peroxide values and TBARS values varied among DDGS sources (Table 1). The PV of DDGS samples ranged from 4.2 to 84.1 meq/kg oil with a median value of 11.7 meq/kg oil and CV of 97.5%. The TBARS values of DDGS samples ranged from 1.0 to 5.2 ng MDA equivalents/mg oil with a median value of 1.7 ng MDA equivalents/mg oil and CV of 43.6%. Color of corn and DDGS samples was measured by Minolta L*, a*, and b*, corresponding to the degree of lightness, redness, and yellowness, respectively (Table 1). Compared with corn (L* = 83.9; a* = 2.6; b* = 20.0), DDGS color is darker (mean value of L* = 54.1), more red (mean value of a* = 10.9), and more yellow (mean value of b* = 37.3). Furthermore, the coefficient of variation for color was less than that of lipid peroxidation among DDGS sources (CV of L*, a*, and b* = 4.6%, 7.2%, 8.8%, respectively).

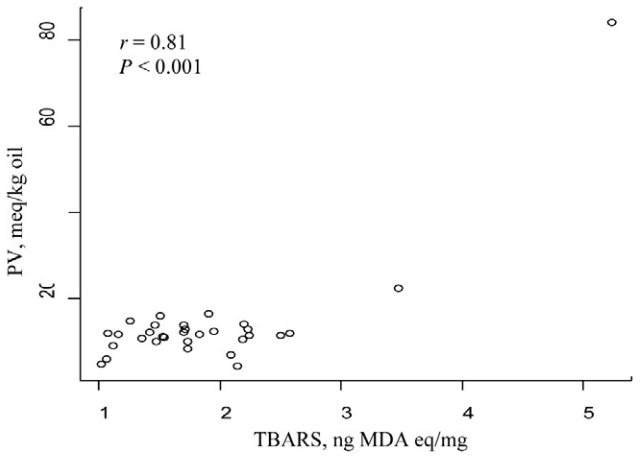

Figure 1. Correlation between peroxide value (PV) and thiobarbituric acid reactive substances (TBARS) in corn dried distillers grains with solubles; MDA = malondialdehyde.

Values of PV and TBARS were positively correlated ($r = 0.81$; $P < 0.001$; Fig. 1). Both TBARS and PV were correlated negatively with L* ($r = –0.73$; $P < 0.001$, and $r = –0.63$; $P < 0.001$, respectively) and b*($r = –0.67$; $P < 0.001$, and $r = –0.57$; $P < 0.001$, respectively, Fig. 2). However, a* was not correlated with either PV ($P = 0.97$) or TBARS ($P = 0.66$).

DISCUSSION

Several measurements have been developed to determine peroxidation level in lipids (Shahidi and Zhong, 2005). However, each peroxidation measure has limitations and measure different peroxidation compounds. Currently, there are no guidelines or standard methods used in the feed industry to assess lipid peroxidation of feed ingredients. Furthermore, none of these methods have been used to evaluate lipid peroxidation level in DDGS. Peroxide value is 1 of the most widely used indicators of lipid peroxidation in the food, feed, and rendering industries, because it is a relatively simple procedure with low cost (Shahidi and Zhong, 2005). Peroxide value measures the concentrations of peroxides and hydroperoxides formed during the initial stages of lipid peroxidation, which is a dynamic process occurring at a rapid rate (Palmquist and Jenkins, 2003). Hydroperoxides generated by lipid peroxidation begin to decompose as soon as they are formed (Seppanen, 2005). As a result, the change in PV with increasing lipid peroxidation level is not linear but follows a bell-shaped curve. A high PV indicates a high lipid peroxidation status, whereas a moderate value may be due to low lipid peroxidation level or the depletion of hydroperoxides after reaching high concentrations. Therefore, using PV as the only indicator of lipid peroxidation may not accurately reflect the degree of peroxidation and other measurements should

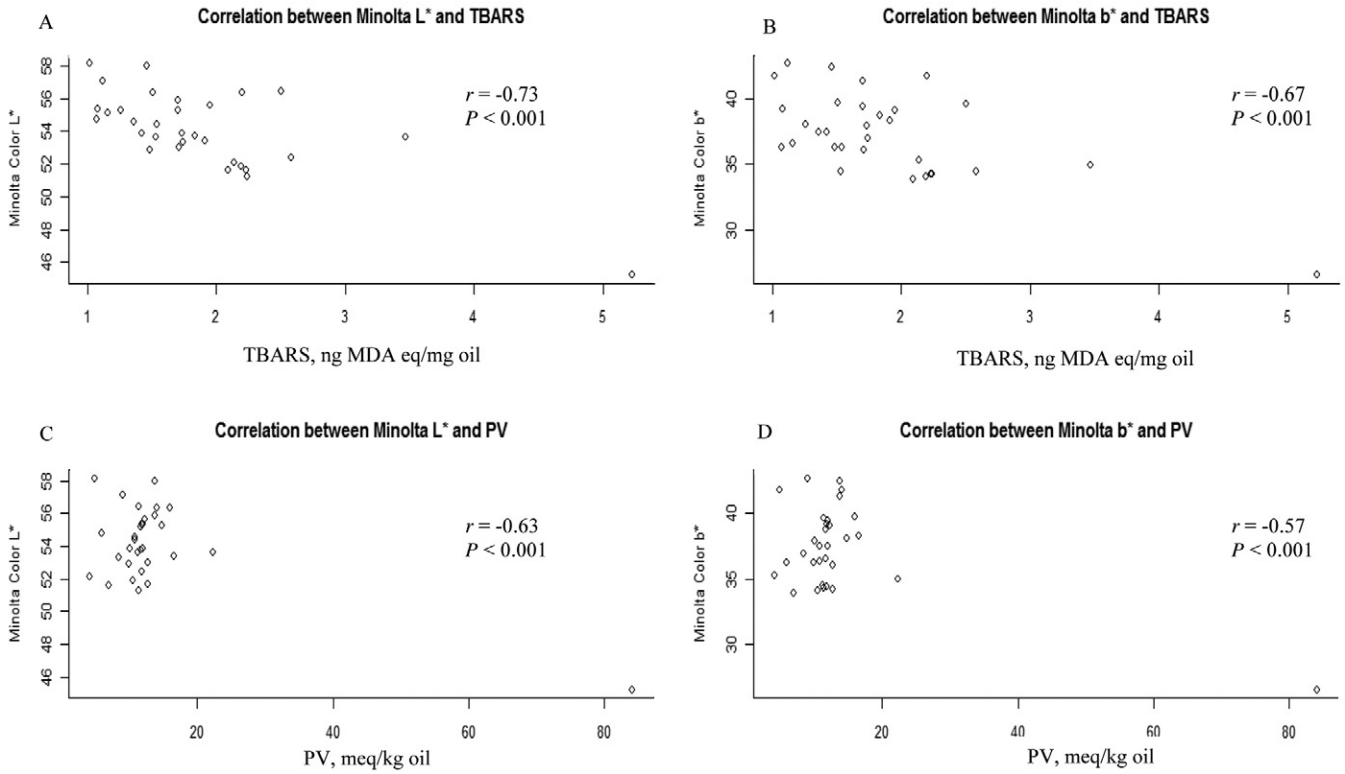

Figure 2. Correlation between thiobarbituric acid reactive substances (TBARS) and Minolta color (A and B), and peroxide value (PV) and Minolta color (C and D) in corn dried distillers grains with solubles; MDA = malondialdehyde.

be considered in addition to PV when evaluating lipid peroxidation level in fat or oil.

The TBARS measures saturated aldehydes, 2-enals, and 2-dienals produced in the termination phase of lipid peroxidation (Palmquist and Jenkins, 2003). Measurement of TBARS is 1 of the most frequently used methods for determining lipid peroxidation because of its simplicity and relatively short assay time. However, limitations of this method also exist. For example, similar to PV, a low TBARS value could be the result of aldehydes that have not yet been produced or volatile aldehydes have been already been lost during processing and storage of the lipid. Furthermore, the TBARS value obtained from the color reaction should not be interpreted to represent absolute levels of peroxidation. In addition to MDA and other aldehydes, several non-peroxidation substances, such as soluble proteins, peptides, AA, and pigments in food samples, can interfere with the TBA reagent and give high false readings (Pegg, 2001). Compared with the TBARS assay used in the current study, a direct quantification of specific aldehydes (i.e., 4-hydroxynonenal; Liu et al., 2012), using HPLC or liquid chromatography-mass spectrometry, can be more accurate, but these methods are relatively complex and expensive, and may not be economically feasible for commercial use. As a result, the combination of PV and TBARS, which measure some of the secondary lipid peroxidation products produced in both initiation and termination phase of lipid peroxidation, were chosen as indicators to evaluate lipid peroxidation level in DDGS in the current study. The strong positive relationship between these 2 peroxidation indicators suggests that PV and TBARS can be used simultaneously when determining lipid peroxidation level in DDGS.

In addition to PV and TBARS of DDGS, color measurement with Minolta spectrophotometers may also be a useful general indicator of lipid peroxidation level in DDGS. Previously, color has been used to predict the digestible AA content in DDGS, based on the strong correlation between dark-colored DDGS and a reduced concentration of digestible AA compared with light-colored DDGS (Cromwell et al., 1993; Fastinger and Mahan, 2006). This association is presumed to be due to heat damage during the DDGS drying process, resulting in increased Maillard products and a darker color. Heating food products at high temperatures increases the production of Amadori compounds, which can be converted to melanoidins and have a dark color (Ferrer et al., 2005; Finot, 2005). Brown-colored oxypolymers are also produced during polymerization reactions of lipid peroxidation products (Buttkus, 1975; Khayat and Schwall, 1983). Furthermore, lipid peroxidation products react with amines, AA, and protein to cause browning observed in fatty foods during processing and storage (El-Zeany and Fattah, 1982; Gillatt and Rossell, 1992; Pokorny, 1998). In the current study, Minolta L*

and b* were negatively correlated with PV and TBARS. Additionally, the DDGS source that had the lowest L* and b* also had the greatest PV and TBARS value among other DDGS sources. These results suggest that darker and less yellow-colored DDGS are more likely to have a greater lipid peroxidation level, as measured by TBARS and PV, compared with lighter-colored DDGS samples. Furthermore, our results are in agreement with results from a preliminary analysis that showed that a dark-colored DDGS sample contained 40% more total polar aldehydes and 12.5% more total nonpolar aldehydes than a light-colored DDGS sample (Shurson and Csallany, University of Minnesota, St. Paul, MN, unpublished). However, although color measurement is relatively fast and simple, it may not be a reliable indicator of the extent of lipid peroxidation because it does not quantify lipid peroxidation products and is also associated with AA digestibility (Cromwell et al., 1993; Fastinger and Mahan, 2006) and phosphorus bioavailability (Amezcua and Parsons, 2007).

Traditional corn DDGS contains ~10% corn oil (Stein and Shurson, 2009). Compared with other vegetable oils, corn oil contains relatively high levels of PUFA (particularly linoleic acid; NRC, 1998) that are vulnerable to lipid peroxidation (Frankel et al., 1984; Linfield et al., 1985; Seppanen, 2005). Large amounts of secondary lipid peroxidation products, such as aldehydes, carbonyls, and ketones, are produced when lipids are heated at relatively high temperatures (Esterbauer et al., 1991). Therefore, corn DDGS may be prone to significant lipid peroxidation. In our survey of 31 ethanol plants, the drying temperature for producing DDGS varied substantially (371 to 593°C inside temperature of the dryer), suggesting that the degree of lipid peroxidation may be different during processing and drying among different DDGS sources. Except for 1 DDGS source, all other DDGS samples contained 2 to 5 times the PV and TBARS values of those found in the corn sample. The DDGS source that had the greatest PV and TBARS values was also subjected to the greatest inside drying temperature (593°C; data not shown) during production. The PV and TBARS of this DDGS source were 27 and 25 times greater than the PV (3.1 meq/kg oil) and TBARS (0.2 ng MDA equivalents/mg oil) of the corn sample, respectively. Furthermore, the wide range in PV and TBARS values obtained for DDGS sources in the current study confirm that lipid peroxidation levels vary greatly among DDGS sources.

Growth suppression from feeding peroxidized lipids has been well documented in several animal species (Dibner et al., 1996; DeRouchey et al., 2004; Harrell et al., 2010). The presence of high amounts of peroxidized lipids in the diet increases the concentration 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, may impair animal health and growth performance (Logani and Davies, 1980; Comporti, 1993). Therefore, one may speculate that peroxidized lipids in DDGS may be 1 of the contributing factors for reduced growth performance of growing-finishing pigs consuming high levels of DDGS (Whitney et al., 2006; Linneen et al., 2008). DeRouchey et al. (2004) suggested that feeding peroxidized lipids (6% dietary inclusion rate) with PV of 40 meq/kg, which is approximately equal to 2.4 meq/kg of PV in the diet (2.4 meq/kg = 40 meq/kg × 6%), resulted in decreased growth performance in nursery pigs. The DDGS source with the greatest PV (84.1 meq/kg oil) in the current study contained 9.66% crude fat (data not shown). Therefore, by including 30% of this DDGS source in the diet, the PV of the diet could be calculated using this equation: PV of the diet, meq/kg = 84.1 meq/kg oil × 9.66% crude fat × 30% inclusion rate = 2.4 meq/kg. As a result, growth performance could be impaired if pigs are fed this highly peroxidized DDGS source. Initial evidence to support the negative growth performance effects from feeding this highly peroxidized DDGS were reported by Song et al. (2012a). These researchers fed diets containing 30% of the most peroxidized DDGS source identified in the current study to wean-finish pigs and observed a reduction in ADG and G:F. Feeding DDGS containing highly peroxidized lipids to pigs may also require supplementation of greater levels of antioxidants than currently recommended for swine. Harrell et al. (2010) reported that dietary supplementation of a blend of synthetic antioxidants improved growth performance of pigs consuming 20% DDGS or peroxidized corn oil. In contrast, no beneficial effects were observed when supplementing vitamin E at 10 times the NRC (1998) recommended requirement in diets containing 30% highly peroxidized DDGS fed to nursery (Song et al., 2012b) or wean-finish pigs (Song et al., 2012a). The lack of consistency of these responses may be due to differences in the degree of lipid peroxidation in the experimental diets fed or the presence of endogenous antioxidants that reduced the need for exogenous antioxidants. The highly peroxidized DDGS source used in these studies also contained 0.95% sulfur, which resulted in an increase in sulfur-containing antioxidant compounds in serum and liver, and may have provided adequate antioxidant protection without the need for supplemental vitamin E (Song et al., 2013). Further studies comparing the effects of feeding a highly peroxidized DDGS source with a low peroxidized DDGS source on pig health and performance may provide a more definitive assessment of whether feeding highly peroxidized DDGS is a contributing factor to reduced growth performance and whether supplemental dietary antioxidants are warranted.

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