RESEARCH REPORT
ANIMAL SCIENCE

Title: Practical evaluation of linoleic acid and antioxidant supplementation for lactating sows housed under high ambient temperatures – **NPB #15-071**

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Industry Summary

High producing sows are under catabolic (resulting in tissue loss) and oxidative stress during lactation and this will impair sow reproductive performance. Moreover, reduced feed intake during high ambient temperatures aggravates these conditions. Providing proper nutrition to sows during periods of stress is extremely important in order to maintain productivity. In particular linoleic acid, which is a precursor of eicosanoids that are important in reproduction, has been shown to be required for optimal subsequent reproductive performance of sows. Linoleic acid is an unsaturated fatty acid prone to peroxidation, which may further exacerbate the oxidative stress status of high producing lactating sows. The purpose of the current study was to determine and verify the impact of linoleic acid supplementation on reproductive performance of sows under practical field conditions and evaluate the impact of commercial antioxidants on oxidative stress and sow performance. A total of 605 sows entered the farrowing room and finished lactation in groups of 22 to 24 sows per group. Sows were fed one of 4 treatments, consisting of diets with 2 levels of linoleic acid (LA; 1.4 or 3.3%) and each of these diets were either not supplemented or supplemented with a commercial antioxidant blend (0 or 0.1%). Sow body weight change and feed intake during lactation were not affected by dietary treatments. Sow body weight at the end of lactation tended (P=0.09) to be higher for mature sows consuming 1.4% LA than other treatments. Feed efficiency was improved (P=0.03) in sows consuming 1.4% versus 3.3% LA in their diets. Litter performance, number of pigs weaned and pre-weaning mortality were not affected by dietary treatment or parity group. The impact of diets on oxidative stress markers varied, were some markers (protein carbonyls, indicating protein damage) were higher and others (malondialdehyde, indicating damage to lipids) were lower in sows fed linoleic acid. Total antioxidant capacity in serum and vitamin E concentrations in serum and milk were lower (P=0.02) in sows fed linoleic acid. Antioxidant supplementation tended (P=0.07) to increase serum vitamin E by 13%. For subsequent reproductive performance, no improvements in wean-to-estrus interval, percentage of sows bred, returns, wean-to-farrow interval, farrowing rate, culling rate, or number of pigs born alive were observed. Collectively, these data indicate that supplementation of antioxidants during lactation did not improve oxidative stress status of sows, nor did it affect performance of sows and litters during lactation or subsequent reproductive performance of sows. Furthermore, inclusion of corn oil to achieve a concentration of 3.3% linoleic acid in lactation diets did not improve performance during lactation or subsequent reproductive performance of sows.

Key Findings:

- Supplementation of lactating sows with linoleic acid (from corn oil) decreased total antioxidant capacity in serum and vitamin E concentrations in serum and milk
- Supplementation of lactating sows with a commercial antioxidant blend increased serum vitamin E concentrations
- Supplementation of linoleic acid (at 3.3%) to lactating sows did not improve lactation performance or subsequent reproductive performance
- Supplementation of a commercial antioxidant blend to sows during lactation did not improve lactation performance or subsequent reproductive performance.

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Scientific Abstract:
Heat stress imposes significant challenges to lactating sows, including decreased feed intake and impaired reproductive performance. Linoleic acid, as a precursor of eicosanoids, has been shown to improve subsequent reproduction of sows. High producing lactating sows are under oxidative stress, and the use of antioxidants could ameliorate adverse effects associated with it. The purpose of the current study was to determine and verify the impact of linoleic acid supplementation on reproductive performance of sows under practical field conditions and evaluate the impact of commercial antioxidants on oxidative stress markers and sow performance. A total of 605 sows entered the farrowing room and finished lactation in groups of 22 to 24 sows per group. Sows were allotted to a RCBD balanced by parity and assigned within groups to 4 dietary treatments, in a 2 x 2 factorial design. Factors consisted of levels of linoleic acid (LA; 1.4 or 3.3%) and antioxidant supplementation (0 or 0.1%). The first 14 groups (n = 313 sows) of sows were used to assess sow and litter performance during lactation. Milk and blood samples were collected from 16 sows per treatment prior to weaning for oxidative stress status assessment. Sow BW change and ADFI was not affected by dietary treatments. Sow BW at 21 d of lactation tended (P=0.09) to be higher for mature sows consuming 1.4% LA than other treatments. Sow G:F was improved (P=0.03) by 6% in sows consuming 1.4% rather than 3.3% LA in their diets. Mature (parity 3 and over) sows had higher BW and lower BW loss (change) at d 21 of lactation (P<0.001). Mature sows consumed (P<0.001) more feed and had lower feed efficiency than young (parity 1 and 2) sows at d 21 of lactation. Litter performance, number of pigs weaned and pre-wean mortality were not affected by dietary treatment or parity group. Total antioxidant capacity in sow serum was 9% higher (P=0.02) and protein carbonyls tended (P=0.07) to be 5% lower in sows fed 1.4% LA compared to sows fed 3.3% LA. Concentrations of MDA increased 20% in sows fed 1.4% LA compared to 3.3% and tended (P=0.05) to be higher in mature sows compared to young sows. Serum 8OHdG increased (P<0.01) 28% in mature sows compared to young sows. Vitamin E concentrations in serum were highest in mature sows fed 1.4% LA compared to other treatments (LA x parity group interaction, P<0.01). Antioxidant supplementation tended (P=0.07) to increase serum vitamin E by 13% compared to dietary treatments without antioxidant. Vitamin E was 17% higher (P<0.01) in milk from sows fed 1.4% LA. All 27 groups (n=603) of sows were used to measure subsequent reproductive performance. Wean-to-estrus interval was not affected by dietary treatment, but young sows came into estrus almost one day earlier than mature sows (P<0.001). Percentage of sows bred, returns and wean-to-farrow interval were not affected by dietary treatment, parity group or their interactions. Farrowing rate tended (P=0.07) to be higher in mature sows fed 3.3% LA with antioxidant than in young sows fed the same

dietary treatment (93.4 vs. 82.0%, respectively). Culling rate was 11 percentage points higher in young sows fed 3.3% LA with antioxidant than in mature sows fed the same diet and young sows fed tallow with antioxidant (antioxidant x LA x parity group interaction, $P<0.02$). Number of pigs born alive was not affected by diet or parity group, but total number of pigs born tended ($P=0.08$) to increase in mature sows fed 3.3% LA with antioxidant compared to young sows fed the same diet. Supplementation of antioxidant during lactation did not improve oxidative stress status of sows, nor did it affect performance of sows and litters during lactation or sow subsequent reproductive cycle. Furthermore, inclusion of 3.3% linoleic acid in lactation diets did not improve performance during lactation or subsequent reproductive performance of sows.

Introduction

High ambient temperatures during the summer months have negative impacts on animal performance in production systems. During this period, swine producers face significant economic losses due to animals experiencing heat stress. Heat stress results from exposure to high ambient temperatures and humidity. The thermo-neutral zone for lactating sows ranges from 12 to 22°C (Black et al., 1993). When ambient temperatures exceed the evaporative critical temperature (25°C), heat stressed sows redistribute blood flow to the periphery in an effort to maximize heat dissipation. Blood pressure is maintained by vasoconstriction of blood vessels elsewhere in the body (Lambert, 2009). The gut is particularly affected during heat stress because blood flow to the intestine is reduced by 40 to 50% (Hales, 1983), which causes hypoxia and increase oxidative stress in intestinal epithelial cells (Lambert, 2009). These conditions compromise gut integrity and impair intestinal epithelial barrier function (Pearce et al., 2011). Moreover, increased intestinal permeability results in endotoxemia and inflammation (Pearce et al., 2012). Furthermore, heat stress alters the metabolic status of the animal (Collier et al., 2005). Observations in birds suggest that heat stress promotes increased mobilization and oxidation of fatty acids in an attempt to meet energy requirements (Mckee et al., 1997). Also, heat stress seems to down-regulate uncoupling proteins in the inner membrane of the mitochondria (Mujahid et al., 2006). Uncoupling proteins are important to prevent the accumulation of protons in the inner membrane of the mitochondria, which reduces the formation of reactive oxygen species. Increased fatty acid load in the mitochondria and the down-regulation of uncoupling proteins results in oxidative stress. Thus, heat stress imposes immunologic and metabolic challenges to lactating sows.

Excessive production of reactive oxygen species can overwhelm antioxidant defense systems resulting in oxidative damage of proteins, lipids, and DNA. Decreased antioxidant capacity during late gestation and lactation can increase oxidative damage by increased production of free radicals, especially when animals are housed under high ambient temperatures (Black et al., 1993, Zhao, 2011). Increased peroxidation of cellular proteins, membrane lipids and DNA may have a direct negative impact on fetal and mammary gland development, and milk production (Zhao et al., 2011). Based on these data, supplementation of antioxidants appears to be warranted in highly prolific sows, especially during heat stress conditions. Indeed, Chauhan et al. (2014) suggested that supra-nutritional levels of antioxidants (specifically vitamin E and Se) were needed to alleviate the negative effects of heat stress on redox homeostasis in sheep. The improved oxidative status resulted in a reduced impact of heat stress in sheep as evidenced by lower respiration rates and maintenance of feed intake. Supplementation of vitamin E and Se appeared to be a suitable nutritional strategy to ameliorate the impact heat stress in sheep and may apply to other livestock (Chauhan et al., 2014).

The modern lactating sow is under pressure for the production of large amounts of milk and suboptimal intake of nutrients could impose even more challenges to sows. Reduced feed intake when sows are exposed to heat stress will negatively affect consumption of essential fatty acids (and antioxidants), which may result in greater mobilization of fat from the body to compensate for the dietary deficiency. This effect is commonly observed in cows during early stages of lactation, in which mobilization of fatty acids from adipose tissue is elevated (Drackey, 1999). Nutritional deficiencies of essential fatty acids (linoleic acid, C18:2, n-6; and α-linolenic acid, C18:3, n-3) during lactation is of important interest because these fatty acids are vital for tissue development of the neonatal pig and they play a role, through prostanoids, in ovulation, luteal regression, implantation, parturition, and post-partum physiology (see the review by Weems et al., 2006). Our previous work (Rosero et al, 2015a) demonstrated that sows are in negative balance for linoleic acid, especially when

feed intake is low. Supplementation of linoleic acid improved subsequent reproductive performance and we suggested including linoleic acid at a minimum of 100 g/d (Rosero, 2016ab).

Inclusion of highly unsaturated fatty acids, such as linoleic acid, but also feed ingredients containing unsaturated lipids (e.g. DDGS) further increases oxidative and metabolic stress in the sow. Inclusion of antioxidants may be especially critical under these circumstances. Although vitamin E functions as an antioxidant and is included in practical sow diets well above NRC (2012) requirements, recent data in finishing pigs suggest that vitamin E (provided at NRC (2012) recommendations) was not effective in preventing oxidative stress associated with feeding a highly oxidized oil (Lu et al., 2014). However, inclusion of ethoxyquin and propyl gallate as antioxidants normalized serum oxidative stress markers and growth performance to levels similar to the control diet without oxidized oil.

There is strong evidence that the supplementation of linoleic acid during lactation could ameliorate the detrimental effects of heat stress on the reproductive cycle of sows. Further, heat stress has significant negative consequences on oxidative status of lactating sows that are already compromised because of oxidative stress associated with high levels of production and the impact of unsaturated dietary lipids on their redox status. We propose to determine and verify the impact of linoleic acid supplementation on reproductive performance of sows under practical field conditions and evaluate the impact of commercial antioxidants on oxidative stress markers and sow performance.

Objectives

The objective of this study was to determine and verify the impact of formulating for a minimum dietary linoleic acid concentration of 3.3% to supply an estimated 100 g/d of linoleic acid (vs. sows fed control diet with approximately 1.4% linoleic acid) on the subsequent reproductive cycle of lactating sows under challenges imposed by heat stress. We further aimed to determine the impact of commercial antioxidants on reproductive performance and oxidative status of sows fed diets low in unsaturated fatty acids or diets supplemented with 3.3% linoleic acid.

Materials & Methods

Animals and dietary treatments

All animal protocols were approved and conducted under the supervision of licensed veterinarians. This experiment was conducted in a commercial research facility owned and operated by The Hanor Family of Companies, located in Mooreland, OK, during the months of June to October.

A total of 605 sows (Camborough, PIC, Hendersonville, TN) entered the farrowing room and finished lactation in groups of 22 to 24 sows per group. A total of 149, 155 and 301 sows representing parity 1, 2 and 3 to 6 were used in the study. Parity 1 and 2 sows were considered young sows, and parity 3 to 6 were regarded as mature sows. Sows were allotted to a RCBD balanced by parity and assigned within groups to 4 dietary treatments in a 2 x 2 factorial design. Factors consisted of levels of linoleic acid (LA) and antioxidant supplementation. Diets were corn-soybean meal based with 12% wheat middlings. Levels of LA were obtained by adding 3.75% of either tallow (3% LA content) or corn oil (54% LA content) for a final dietary concentration of 1.4 and 3.3% LA, respectively. Each of these diets were either not supplemented or supplemented with 0.1% of a synthetic antioxidant blend containing butylated hydroxytoluene, butylated hydroxyanisole and minimum of 3.0% ethoxyquin (Endox® Dry; Kemin Industries, Inc., Des Moines, IA). Diets were formulated to meet or exceed NRC (2012) nutrient recommendations and were manufactured by a commercial feed mill (Enid, OK) owned and operated by The Hanor Family of Companies (Table 1). Diets were color coded for visual confirmation; feed samples were collected weekly on farm and at the feed mill for every new batch that was manufactured. Representative feed samples were submitted to the Agricultural Experiment Station Chemical Laboratories, University of Missouri (Columbia, MO) and proximate analyses were conducted following AOAC (2007) methods.

For the first 14 groups (n = 313 sows), sows were weighed individually when entering the farrowing room, at approximately 110 d of gestation, and again at exactly 21 d of lactation. Sow BW change during lactation was

calculated as the difference between sow BW at d 21 of lactation and the estimated post-farrowing BW, using the following equation by Rosero et al. (2013):

*Post-farrow BW (kg) = -8.246 + 0.981 * BW at 110 d gestation (kg) - 0.679 * total pigs born*

Sow feed efficiency (G:F) was calculated as the sum of total litter gain (kg) and sow BW change (kg), divided by ADFI (kg). Sows were fed a common lactation diet between placement and farrowing. Experimental diets were provided on the day of farrowing until weaning (23 ± 1 d) and feed offered and refusals were recorded daily for ADFI calculations. Sows were fed to satiety twice daily in the morning and afternoon. Feed refusals were removed once daily in the morning. At farrowing, the number of pigs born alive, stillborn pigs and mummies were recorded. Cross-fostering was performed 18 to 24 h after birth to allow for colostrum intake from their dam before moving pigs. All litters were standardized to 12 piglets and initial litter weights and weaning weights were recorded for litter gain calculations. Handling and processing of litters were performed according to standard farm practices under the recommendation of licensed veterinarians. Mortality of pigs throughout lactation was recorded and all litters were weighed at exactly 21 d of lactation. Pigs weighing less than 3.62 kg at weaning were considered no-value pigs. For the remaining 13 groups (n = 292 sows), the same practices were performed, except for sow BW, litter weights and feed intake data recording.

After weaning (23.1 ± 0.5 days), all sows were moved to breeding rooms and housed in individual stalls. Sows had fence-line contact daily with a boar to aid in estrus detection. Once sows came into heat, artificial insemination was performed according to standard farm practices. Sows were checked routinely for vaginal discharge and had daily fence-line contact with a boar to detect any sows returning to estrus. Sows were fed a common gestation diet twice daily. Breeding information was recorded and included days to estrus, return to estrus and sows removed by culling. Subsequent reproductive performance data were also collected and included gestation length, abortions, number of pigs born alive, stillborn pigs and mummies.

During the first two months of the study, ambient temperatures of the farrowing room were recorded hourly using data recorders (LogTag Recorders Ltd., Auckland, New Zealand).

O*xidative status*

Blood samples were collected from 16 sows per treatment (n = 64; equally balanced by parity) at weaning and at approximately 23 d of lactation. Blood samples were used to assess oxidative status (total antioxidant capacity, malondialdehyde, 8-hydroxy-2'-deoxyguanosine, and protein carbonyl) and vitamin E concentrations in serum. Immediately after collection, serum was obtained by centrifugation at 1,000 × g for 12 min at room temperature and subsequently frozen at -20°C for further analyses.

Milk samples were collected from the same 64 sows one day prior to weaning, at approximately d 22 of lactation, from at least 4 functional glands (anterior, middle and posterior). Oxytocin (1 mL) was injected intramuscularly to facilitate milk let down. Samples were immediately frozen at -20°C for analyses of vitamin E concentrations.

Serum and milk samples were sent to the Veterinary Diagnostic Laboratory at Iowa State University (Ames, IA) for analysis of vitamin E concentrations. Serum and milk vitamin E concentrations were determined by HPLC. Serum concentrations of MDA were measured using a commercially available kit, following manufacturer's instructions (OxiSelect TBARS Assay Kit, Cell Biolabs Inc., San Diego, USA). Total antioxidant capacity in serum was determined according to a colorimetric method previously described by Erel (2004). Concentrations of 8-hydroxy-2'-deoxyguanosine (8-OHdG) were measured in serum using a commercially available kit, according to manufacturer's instructions (DNA/RNA Oxidative Damage EIA Kit; Cayman Chemical Company, Ann Arbor, MI). Protein carbonyls were quantified in serum using a commercial kit (Protein Carbonyl Colorimetric Assay Kit, Cayman Chemical Company, Ann Arbor, MI). To normalize the carbonyl content to the amount of protein present, protein concentrations were determined using a bicinchoninic acid assay kit (Pierce BCA Protein Assay Kit, Thermo Fisher Scientific Inc., Rockford, IL). Final carbonyl content was expressed as nmol carbonyl/mg protein. All analyses were conducted in duplicate.

Statistical analyses

Sow, litter performance, markers of oxidative stress and vitamin E concentrations in serum and milk data were analyzed using PROC Mixed of SAS (v. 9.4; SAS Inst. Inc., Cary, NC). The model included fixed effects of linoleic acid level, antioxidant supplementation, parity group (young vs. mature) and relevant interactions. Sow groups entering the farrowing room together were used as random effect. Sow initial BW (at approx. 110 d) and litter weights after cross-fostering were used as covariates for performance analyses. Number of pigs weaned, mortality and no-value pigs were analyzed by fitting the data to a Poisson regression using PROC Glimmix of SAS (v. 9.4; SAS Inst. Inc., Cary, NC). Observations with response means greater than three and a half standard deviations from the mean were excluded from the analysis.

Subsequent reproductive performance data were analyzed using mixed linear models according to the data distribution. Wean-to-estrus and wean-to-farrow interval data were analyzed by fitting the data to a Poisson regression using PROC Glimmix of SAS with the Laplace method of estimation. For dichotomous variables (sows bred, farrowed, returns, culls) data were analyzed using PROC Glimmix of SAS with a Bernoulli distribution. Breeding, farrow and return rates were computed using data from sows that were bred within 18 days after weaning. Total number of pigs born, pigs born alive and stillborn pigs were analyzed using PROC Mixed of SAS, and these variables also excluded data from sows that were bred later than 18 days post-weaning. All models included fixed effects of linoleic acid level, antioxidant supplementation, parity group and relevant interactions. Group of sows was used as the random effect, and group by linoleic acid by antioxidant interaction was also included in the random statement in all models, except for models with Poisson regression and for the variable of sows bred. Mean values were compared pairwise by simple effects least squares mean differences. Differences between treatments were considered significant at $P < 0.05$ and tendencies at $0.05 \leq P < 0.10$.

Results

Sow and litter performance during lactation.

Average temperature in the farrowing room during the months of June-August was 25.4°C; the minimum average temperature recorded was 18.1°C and maximum was 31.0°C during this period. Sow BW change and ADFI were not affected by dietary treatments (Table 2). Sow BW at 21 d of lactation tended (LA x parity group interaction, P=0.09;) to be higher for mature sows consuming 1.4% LA compared to those fed 3.3% LA, but this was not the case for young sows. Sow G:F was improved (P=0.03) by 6% in sows consuming 1.4% LA compared to 3.3% LA in their diets. Sow parity group significantly (P<0.001) affected sow performance, in which mature (parity 3 and over) sows had higher BW and lower BW loss (change) at d 21 of lactation. Mature sows consumed (P<0.001) more feed and had lower feed efficiency than young (parity 1 and 2) sows at d 21 of lactation. Litter performance, number of pigs weaned and pre-weaning mortality were not affected by dietary treatment or parity group (Table 2). Number of no-value pigs was not affected by LA, antioxidant supplementation, or parity group.

Moreover, when analyzing data from all sows put on test (n=605) number of pigs weaned, number of no-value pigs at weaning and mortality displayed similar results to the ones reported above, in which main effects of linoleic acid level, antioxidant supplementation, parity group and their interactions did not affect these variables (Table 3).

Serology and milk.

Total antioxidant capacity was 9% higher (P=0.02) and protein carbonyls tended (P=0.07) to be 5% lower in sows fed 1.4 compared to sows fed 3.3% LA, while antioxidant supplementation, parity group and their interactions did not affect serum TAC or protein carbonyl concentrations (Table 4). Concentrations of MDA increased 20% in sows fed 1.4% LA compared to 3.3% LA and tended (P=0.05) to be higher in mature sows compared to young sows (Table 4). Antioxidant supplementation did not impact serum MDA. Serum 8OHdG increased (P<0.01) by 28% in mature sows compared to young sows, but dietary treatment and interactions did not impact this marker.

Vitamin E concentrations in serum were highest in mature sows fed 1.4% LA compared to other treatments (LA x parity group interaction, P<0.01). Antioxidant supplementation tended (P=0.07) to increase serum vitamin

E by 13% compared to dietary treatments without antioxidant. Vitamin E was 17% higher (P<0.01) in milk from sows fed 1.4% LA, but was not impacted by antioxidant supplementation, parity group and their interactions (Table 4).

Subsequent reproductive performance.

Wean-to-estrus interval was not affected by dietary treatment (Table 5), but young sows came into estrus almost one day earlier than mature sows (4.9 vs. 5.8 days for young and mature, respectively; P<0.001). Percentage of sows bred within the first 18 d post-weaning and percentage of sows that returned to estrus after being bred within 18 d post-weaning were not affected by dietary treatment, parity group or their interactions (Table 5).

Wean-to farrow interval and percentage of sows that farrowed from sows bred within 18 days of weaning were not affected by dietary treatment, parity group or their interactions (Table 5). However, farrowing rate considering all weaned sows displayed a tendency (antioxidant x LA x parity group interaction, P=0.07) in which mature sows fed 3.3% LA with antioxidant had a higher farrowing rate than young sows fed the same dietary treatment (93.4 vs. 82.0%, respectively). Culling rate was 11 percentage points higher in young sows fed 3.3% LA with antioxidant than in mature sows fed the same diet and young sows fed 1.4% LA with antioxidant (antioxidant x LA x parity group interaction, P<0.02).

Number of pigs born alive and stillborn pigs were not affected by diet or parity group, but total number of pigs born tended (antioxidant x LA x parity group interaction, P=0.08) to increase in mature sows fed 3.3% LA with antioxidant compared to young sows fed the same diet (Table 5).

Discussion

High producing sows are under catabolic and oxidative stress status during lactation and these can potentially impair litter performance (Kim et al., 2013). Moreover, reduced feed intake during high ambient temperatures can aggravate these conditions. Proper nutrition of the sow during this period is extremely important, and this study explored several concepts to address these issues.

The supplementation of synthetic antioxidants to sows in the current study did not influence sow or litter performance during lactation or in the subsequent reproductive cycle. Correspondingly, oxidative stress markers in serum of sows taken during lactation were not impacted by antioxidant supplementation. With the exception of a tendency to increase serum vitamin E concentrations, the expected decrease in other markers of oxidative stress due to antioxidant supplementation was not supported by our results. Indeed, we have previously shown that supplementation of antioxidant increased vitamin E concentrations in serum of nursery pigs, but had no impact in serum MDA (Chang and van Heugten, 2016).

Sows fed diets containing 3.3% LA (corn oil based diets) had decreased TAC, vitamin E, and higher protein carbonyls, which could be related to the increased susceptibility of corn oil to peroxidation. Lipids with a higher degree of unsaturation are more prone to peroxidation than saturated lipids (Kerr et al., 2015). Consumption of peroxidized lipids has been shown to induce oxidative stress in pigs (Lu et al., 2014; Rosero et al., 2015b; Shurson et al., 2015). Nonetheless, the increase in serum MDA in sows fed 1.4% LA (tallow based diet) is surprising and does not agree with the expected increase in MDA as an oxidative stress marker reported in other studies when peroxidized lipids were fed. Moreover, supplementation of antioxidant to lactation diets would be expected to protect against lipid peroxidation.

Mature sows seemed to be in increased oxidative stress when compared to young sows as indicated by higher MDA and 8OHdG in serum. 8-hydroxy-2'-deoxyguanosine (8-OHdG) is the product of reactive oxygen species (ROS) attack on purines and is an indicator of nucleic acid damage caused by oxidative imbalance (Sen and Chakraborty, 2011). On the other hand, serum vitamin E was greater in mature vs. young sows suggesting greater antioxidant capacity in mature sows.

Milk vitamin E concentrations increased in sows fed 1.4% LA (tallow), which agrees with results observed in serum. However, this increase did not impact litter performance. Similarly, Pinelli-Saavedra and Scaife (2005) did not report improvements in litter performance due to increased vitamin E content of sow milk.

Sow performance during lactation was improved when sows were fed 1.4% LA (tallow based diets), as indicated by improved feed efficiency and heavier sow body weight at weaning. Moreover, mature sows consuming tallow tended to lose less weight at the end of lactation than sows fed the other dietary treatments. These results were not expected, because ME, protein, lysine, and fat content of diets were formulated to be similar among all treatments. However, feed analysis indicated a lower crude fat content in diets containing corn oil (average 4.8% vs. 7.1% in corn and tallow diets, respectively), and this difference could explain, in part, the improved performance of sows consuming tallow diets. Furthermore, the increased degree of saturation in tallow was expected to have a lower digestibility than more unsaturated corn oil, as reviewed by Rosero et al. (2016b).

Supplementation with increased concentrations of LA did not impact litter performance or piglet mortality during lactation. These results agree with Rosero et al. (2016a), in which litter performance of lactating sows fed increasing levels of linoleic acid were minimally impacted. However, based on results found by these same authors, we expected an improvement in subsequent reproductive performance of sows. The suggested level of 3.3% linoleic acid in lactation diets was reported by Rosero et al. (2016a) to be required to maximize subsequent reproduction of sows. Linoleic acid is a precursor of prostaglandins that have important roles in reproduction, such as $PGF_{2\alpha}$ and PGE_2 (Weems et al., 2006). Indeed, linoleic has shown to be beneficial post-partum in dairy cows (Thatcher et al., 2010).

Nonetheless, in the current study we failed to detect improvements in subsequent reproductive performance of sows with increased linoleic acid concentration in the diet. Similar to Rosero et al. (2016a), we did not find differences in wean-to-estrus interval, proportion of sows farrowing relative to sows bred, and proportion of sows bred within 18 days after weaning due to linoleic acid supplementation. The improvements in farrowing rate and culling rate in young sows fed linoleic acid reported by Rosero et al. (2016a) conflict with our findings, in which young sows fed 3.3% LA with antioxidant tended to have the lowest farrowing rate and highest culling rate. However, the observed tendency towards an increase in total pigs born in mature sows fed 3.3% LA with antioxidant is consistent with findings by Rosero et al. (2016a).

Collectively, these data indicate that supplementation of antioxidant during lactation did not improve oxidative stress status of sows, nor did it affect performance of sows and litters during lactation or sow subsequent reproductive performance. Furthermore, inclusion of corn oil to achieve 3.3% linoleic acid level in lactation diets did not improve performance during lactation or subsequent reproductive performance of sows.

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Table 1. Composition of experimental diets, as-fed basis[1]

Item	1.4% LA	1.4% LA + AO	3.3% LA	3.3% LA + AO
Ingredient, %				
Corn, 8.5% CP	49.59	49.49	49.58	49.48
Soybean meal	31.00	31.00	31.00	31.00
Wheat middlings	12.00	12.00	12.00	12.00
Corn oil	0.00	0.00	3.75	3.75
Tallow	3.75	3.75	0.00	0.00
L-Lysine	0.10	0.10	0.10	0.10
L-Threonine	0.06	0.06	0.07	0.07
Limestone	1.11	1.11	1.11	1.11
Monocalcium phosphate, 21% P	0.77	0.77	0.77	0.77
Salt	0.40	0.40	0.40	0.40
Vitamin-mineral premix[2]	0.20	0.20	0.20	0.20
Choline chloride, 60%	0.13	0.13	0.13	0.13
Antioxidant[3]	0.00	0.10	0.00	0.10
Other	0.92	0.92	0.92	0.92
Calculated composition, %				
ME (kcal/kg)	3429	3426	3436	3433
Crude protein	21.2	21.2	21.1	21.1
Total lysine	1.21	1.21	1.20	1.20
Ca	0.82	0.82	0.82	0.82
Total P	0.57	0.57	0.57	0.57
Linoleic acid, 18:2n-6	1.41	1.41	3.30	3.30
Analyzed composition, %				
Moisture	11.15	10.79	11.23	11.07
Crude protein	20.8	21.3	21.9	21.3
Crude fat	7.10	7.06	4.67	4.92
Ca	0.79	0.67	0.83	0.79
P	0.62	0.66	0.75	0.64
Ethoxyquin (ppm)	59.7	67.6	41.3	57.1

[1] Diets were formulated to meet or exceed NRC (2012) nutrient recommendations. LA, linoleic acid; AO, antioxidant.

[2] Supplied per kg of complete diet: Zn, 125 mg; Fe, 100 mg; Mn, 50 mg; Cu, 25 mg; I, 0.7 mg; Se, 0.3 mg; vitamin A, 11,023 IU; vitamin D3, 1,764 IU; vitamin E, 77 IU; vitamin K, 4.4 mg; vitamin B12, 0.044 mg; riboflavin, 8.8 mg; d-pantothenate, 26.5 mg; niacin 55.1 mg; thiamine, 3.3 mg; pyridoxine, 3.3 mg; folicacid, 1.21 mg; biotin, 0.28 mg; phytase, 476 FTU (Quantum Blue, AB Vista, Marlborough, UK), and chromium, 0.4 mg/kg.

Table 2. Effects of supplemental levels of linoleic acid and antioxidant on performance of lactating sows[1]

Item	Parity 1 and 2				Parity 3+				SEM	*P*-value			
	1.4% LA	1.4% LA + AO	3.3% LA	3.3% LA + AO	1.4% LA	1.4% LA + AO	3.3% LA	3.3% LA + AO		LA	AO	LA*AO	Parity Group
Sows, n[2]	38	40	41	38	39	39	37	41					
BW, kg													<0.001
Gestation, d 110	226.2	228.5	233.3	226.7	286.1	294.7	293.6	282.4	4.2	0.97	0.56	0.02	<0.001
Farrowing[3]	236.9	236.7	236.9	236.5	236.1	236.5	236.5	236.1	0.4	0.76	0.56	0.26	0.21
d 21 of lactation[4]	223.3	221.4	223.6	220.6	234.5	234.9	227.9	229.5	2.8	0.08	0.69	0.99	<0.001
BW change, kg	-13.7	-13.3	-11.6	-13.8	-3.3	-3.9	-8.5	-6.8	2.3	0.25	0.89	0.96	<0.001
ADFI, kg/d	4.12	4.01	4.23	4.27	4.81	4.81	4.79	4.88	0.17	0.31	0.95	0.56	<0.001
Sow G:F[5]	0.51	0.50	0.47	0.46	0.45	0.44	0.42	0.43	0.02	0.03	0.83	0.65	<0.001
Litter weight, kg													
After cross-fostering,[6]	17.80	17.33	18.06	17.48	19.14	18.84	20.47	19.51	0.43	0.05	0.06	0.52	<0.001
d 21 of lactation	72.93	71.69	69.62	71.43	71.28	71.80	70.72	71.29	1.64	0.26	0.69	0.45	0.89
Litter gain, kg	54.36	53.13	51.09	52.86	52.73	54.17	52.10	54.17	1.60	0.16	0.52	0.59	0.95
Pigs weaned, n	11.16	11.30	11.17	11.03	10.82	10.63	10.83	10.68	0.53	0.92	0.86	0.90	0.46
Mortality, n	0.84	0.68	0.83	1.03	1.18	1.37	1.19	1.32	0.16	0.56	0.70	0.55	0.17
No-value pigs, n[7]	0.02	0.08	0.08	0.02	0.09	0.10	0.02	0.08	0.04	0.44	0.56	0.51	0.37

[1]Diets were supplemented with 3.75% of either tallow (1.4% LA) or corn oil (3.3% LA), with or without supplementation of antioxidant (0.1% inclusion; Endox® Dry, Kemin Industries). LA, linoleic acid; AO, antioxidant.
[2]Data collected from the first 14 groups of sows placed on test.
[3]Sow BW after farrowing was calculated using the equation by Rosero et al. (2013).
[4]Linoleic acid by parity group interaction tendency (*P*=0.09).
[5]Sow feed efficiency was calculated as sow BW change plus litter gain at weaning, divided by ADFI.
[6]Litters were standardized to 12 pigs per litter.
[7]Three-way interaction (LA*AO*PARITY) tendency (*P*=0.06).

Table 3. Effects of supplemental levels of linoleic acid and antioxidant on performance of lactating sows[1]

	Parity 1 and 2				Parity 3+					*P*-value			
Item	**1.4% LA**	**1.4% LA + AO**	**3.3% LA**	**3.3% LA + AO**	**1.4% LA**	**1.4% LA + AO**	**3.3% LA**	**3.3% LA + AO**	**SEM**	**LA**	**AO**	**LA*AO**	**Parity Group**
Sows, n[2]	78	77	76	73	75	75	75	76					
Pigs weaned, n	11.06	11.25	11.12	10.94	10.62	10.65	10.64	10.61	0.38	0.84	1.00	0.77	0.33
Piglet mortality, n	0.94	0.75	0.88	1.06	1.38	1.35	1.36	1.39	0.12	0.52	0.93	0.39	0.12
No-value pigs, n[3]	0.01	0.03	0.04	0.01	0.04	0.04	0.02	0.04	0.02	0.82	0.77	0.28	0.18

[1]Diets were supplemented with 3.75% of either tallow (1.4% LA) or corn oil (3.3% LA), with or without supplementation of antioxidant (0.1% inclusion; Endox® Dry, Kemin Industries). LA, linoleic acid; AO, antioxidant.
[2]Data collected from all sows placed on test (27 groups).
[3]Three-way interaction (LA*AO*PARITY) tendency (*P*=0.05).

Table 4. Effects of supplemental levels of linoleic acid and antioxidant on serum oxidative status and milk vitamin E concentrations[1]

	Parity 1 and 2				Parity 3+					*P*-value			
Item	**1.4% LA**	**1.4% LA + AO**	**3.3% LA**	**3.3% LA + AO**	**1.4% LA**	**1.4% LA + AO**	**3.3% LA**	**3.3% LA + AO**	**SEM**	**LA**	**AO**	**LA*AO**	**Parity Group**
TAC, µM Trolox eq/mL	100.1	100.2	90.8	94.4	101.0	100.7	85.4	96.5	5.2	0.023	0.332	0.318	0.899
MDA, µmol/L	12.9	9.9	10.5	10.3	13.5	14.9	10.9	10.7	1.2	<0.01	0.534	0.688	0.051
8OHdG, pg/mL	1344	1264	1280	1431	1382	1670	1877	1903	176	0.102	0.443	0.951	<0.01
Protein Carbonyls, pmol/mg	939	902	948	940	928	860	961	977	40	0.070	0.364	0.292	0.973
Serum Vitamin E, ppm	2.56	2.45	2.40	3.03	4.05	4.38	2.65	3.39	0.30	0.024	0.067	0.185	<0.001
Milk Vitamin E, ppm	3.56	3.52	3.10	3.25	3.86	4.19	3.01	3.53	0.33	<0.01	0.219	0.632	0.158

[1]Diets were supplemented with 3.75% of either tallow (1.4% LA) or corn oil (3.3% LA), with or without supplementation of antioxidant (0.1% inclusion; Endox® Dry, Kemin Industries). LA, linoleic acid; AO, antioxidant.

Table 5. Effects of supplemental levels of linoleic acid and antioxidant on the subsequent reproductive cycle of sows[1]

	Parity 1 and 2				Parity 3+					*P*-value			
Item	1.4% LA	1.4% LA + AO	3.3% LA	3.3% LA + AO	1.4% LA	1.4% LA + AO	3.3% LA	3.3% LA + AO	SEM	LA	AO	LA*AO	Parity Group
Sows, n	78	77	76	72	75	75	74	76					
Wean-to-Estrus interval, d†	5.5	6.3	5.6	5.7	5.2	4.7	4.9	4.6	0.3	0.252	0.940	0.454	<0.001
Wean-to-Farrow interval, d†	124.3	125.9	124.1	124.5	122.8	123.6	124.2	123.6	1.4	0.972	0.575	0.497	0.230
Bred within 18 d:Weaned, %†[2]	93.3	97.2	98.6	95.7	97.2	90.5	95.8	96.0	2.3	0.373	0.417	0.699	0.455
Returns:Bred within 18 d, %‡	10.5	12.0	9.1	18.1	10.9	8.4	14.2	8.7	4.2	0.591	0.922	0.757	0.528
Sows farrowed:Bred within 18 d, %‡	94.3	92.7	91.4	84.9	91.4	94.0	91.3	97.2	3.1	0.739	0.626	0.763	0.253
Sows farrowed:Weaned, %†,[3]	87.2^{ab}	89.6^{ab}	89.5^{ab}	82.0^{b}	89.3^{ab}	85.3^{ab}	87.9^{ab}	93.4^{a}	3.7	0.755	0.938	0.868	0.441
Culling rate, %†,[4]	11.6^{ab}	5.2^{b}	6.6^{ab}	16.7^{a}	9.3^{ab}	12.0^{ab}	9.4^{ab}	5.3^{b}	3.3	0.863	0.885	0.406	0.855
Total pigs born, n‡	13.0^{ab}	13.5^{ab}	13.3^{ab}	12.6^{b}	13.8^{ab}	13.2^{ab}	13.6^{ab}	14.0^{a}	0.5	0.939	0.801	0.896	0.092
Pigs born alive, n‡	12.5	12.7	12.6	12.0	12.4	11.9	12.4	12.6	0.4	0.931	0.492	0.892	0.665
Stillborn pigs, n‡,[5]	0.3^{d}	0.8^{bc}	0.7^{c}	0.4^{dc}	1.2^{a}	1.2^{a}	1.1^{ab}	1.1^{ab}	0.1	0.823	0.678	0.073	<0.001

[1]Diets were supplemented with 3.75% of either tallow (1.4% LA) or corn oil (3.3% LA), with or without supplementation of antioxidant (0.1% inclusion; Endox® Dry, Kemin Industries). LA, linoleic acid; AO, antioxidant.
[2]Three-way interaction (LA*AO*PARITY) tendency, *P*=0.068.
[3]Three-way interaction (LA*AO*PARITY) tendency, *P*=0.069.
[4]Three-way interaction (LA*AO*PARITY), *P*=0.018.
[5]Three-way interaction (LA*AO*PARITY), *P*=0.043.
†Relative to all weaned sows (n=603).
‡Relative to sows that were bred within 18 d post-weaning (n=553).