Effects of fat source and level in finishing pigs and increasing omega-3 fatty acids in nursery pigs

by

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Abstract

This thesis includes two chapters evaluating practical questions facing the swine industry including the effects of fat source and level in finishing pig diets and the evaluation of increasing levels of omega-3 fatty acids in nursery pig diets. Two experiments using a total of 3,4171 finishing pigs were used to evaluate the effects of different fat sources and levels on growth performance, carcass characteristics, and economical return. In Experiment 1, three of the four dietary treatments contained increasing levels of choice white grease (0, 1, and 3% of the diet). The final dietary treatment contained no added fat until pigs were approximately 100 kg, then pigs were fed a diet containing 3% added fat. Results from this experiment determined that increasing fat from 0 to 3% decreased ADFI and improved G:F. Pigs fed 3% added fat only during the late finishing phase had intermediate G:F. Increasing fat increased feed cost and reduced income over feed cost. With 3% added fat fed only in late finishing, feed costs and IOFC were intermediate between diets containing 0% added fat and 3% fat added throughout the entire study. Dietary treatments for Experiment 2 included a control diet containing no added fat. The other 4 dietary treatments included two different fat sources, choice white grease or corn oil, added at 1 or 3% of the diet. Results from this experiment concluded that increasing fat from 0 to 3% increased ADG, reduced ADFI, and improved G:F, regardless of fat source used. Increasing fat also increased HCW, carcass yield, and backfat, while pigs fed diets containing corn oil, had higher carcass fat iodine values. Increasing fat increased feed cost, but also increased revenue. However, increasing fat only increased income over feed cost when feed costs were low and revenue was high. Three experiments were conducted using a total of 92,546 nursery pigs to assess the effects of increasing omega-3 fatty acids (alpha-linolenic acid from O3 Trial Feed) on

nursery pig growth performance, response to an LPS immune challenge, and morbidity and mortality in PRRSV positive pigs in a commercial setting. In Experiment 3, increasing omega-3 fatty acids did not improve growth performance or immune response induced by LPS challenge. In Experiment 4, increasing omega-3 fatty acids improved growth performance and reduced total removals and mortality in PRRSV positive pigs. However, in experiment 5 increasing omega-3 fatty acids in the diet did not improve growth performance and actually increased total removals and mortality in PRRSV positive pigs. In summary, these experiments provided data on different fat sources and levels in fishing pig diets and increasing omega-3 fatty acids in nursery pig diets.

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Chapter 1 - Effects of fat source and level on growth performance and carcass characteristics of commercial finishing pigs

Abstract

Two experiments evaluated different fat sources and levels on growth performance, carcass characteristics, and economic impact in commercial finishing pigs. In Exp. 1, 2,160 pigs (337 × 1050, PIC; initially 37.3 ± 0.93 kg) were used. Pens of pigs were blocked by initial BW and randomly assigned to 1 of 4 dietary treatments. Three of the 4 dietary treatments included: 0, 1, and 3% choice white grease. The final treatment contained no added fat until pigs were approximately 100 kg, and then a diet containing 3% fat was fed until marketing. Experimental diets were fed over 4 phases and were corn-soybean meal-based with 40% dried distillers grains with solubles. Overall, increasing choice white grease decreased (linear, $P = 0.006$) ADFI and increased (linear, $P = 0.006$) G:F. Pigs fed 3% fat only during the late finishing phase (approximately 100 to 129 kg) had similar G:F compared to pigs fed 3% for the entire study during the late finishing phase, and intermediate G:F overall. Increasing fat tended to increase (linear, $P = 0.068$) HCW. Feed cost increased (linear, $P \leq 0.005$) and income over feed cost (IOFC) decreased (linear, $P \leq 0.041$) as choice white grease increased. In Exp. 2, 2,011 pigs (PIC 1050 × DNA 600; initially 28.3 ± 0.53 kg) were used. Pens of pigs were blocked by location in the barn and randomly assigned to 1 of 5 dietary treatments arranged in a $2 \times 2 + 1$ factorial with main effects of fat source (choice white grease or corn oil) and level (1 or 3% of the diet) and a control diet with no added fat. Overall, increasing fat, regardless of source, increased (linear, $P < 0.001$) ADG, decreased (linear, $P = 0.013$) ADFI, and increased (linear, $P < 0.001$) G:F. Increasing fat increased (linear, $P \leq 0.016$) HCW, carcass yield, and backfat depth. There was a fat source × level interaction ($P < 0.001$) in carcass fat iodine value (IV), where IV increased to a greater

extent in pigs fed corn oil with only a small increase in IV in pigs fed diets with choice white grease. In conclusion, these experiments suggest that increasing fat from 0 to 3%, regardless of source, produced variable responses in ADG but consistently improved G:F. Increasing fat increased HCW, carcass yield, and backfat depth, but feeding diets containing corn oil increased carcass IV. With the ingredient prices used, the improvement in growth performance did not justify the extra diet cost from increasing fat from 0 to 3% in most situations.

Keywords: fat level, fat source, finishing pigs, iodine value

Introduction

Fat additions to finishing pig diets has been shown to decrease ADFI and increase ADG and G:F (De la Llata et al., 2001; Liu et al., 2018). However, added fat can also impact carcass characteristics such as increasing fat depth and decreasing carcass lean (De la Llata et al., 2001). In a meta-regression analysis, Nitikanchana et al. (2015) summarized results of 41 studies and observed that increasing dietary NE improved growth rate and feed efficiency. However, these responses were only observed when diets maintained a Lys:calorie ratio. Marçal et al. (2019) also concluded that increasing dietary NE improves feed efficiency, however, if the SID Lys:NE ratio is not balanced, growth rate will not improve and can result in increased backfat depth. Therefore, when evaluating the responses to added fat, it is important that the pig's environment and amino acid intake be considered.

There are many fat sources available for use in swine diets. Each source has a different fatty acid composition, resulting in varying digestibility and energy values (Jørgensen and Fernandez, 2000). It is plausible that different inclusion rates of fat and different dietary fat

sources may impact how adipose and lean tissue are deposited (Apple et al., 2009). This ultimately can play a role in growth performance, carcass characteristics, and carcass quality (Miller et al., 1990). Animal fats, such as choice white grease, are associated with lower energy value compared to vegetable oils (NRC, 2012). This is because animal fats contain more saturated fatty acids, while vegetable oils contain more unsaturated fatty acids (Liu et al., 2018). While choice white grease has been a common source of added fat for finishing pig diets, corn oil availability and use has increased with more widespread ethanol production. However, the unsaturated fatty acid composition of corn oil results in softer carcass fat deposited and overall reduced belly firmness compared with more saturated fat sources (Kim et al., 2013).

Because supplementing fat increases diet cost, the ultimate decision to add fat into finishing diets is that the increase in revenue must be greater than the increase in feed cost (De la Llata et al., 2001a). Although there are many studies that show the positive benefits adding fat to finishing diets on growth performance and carcass characteristics, it is less clear if those benefits vary between different fat sources and if growth and economic benefits can be realized by only supplementing fat during late finishing. Therefore, the objective of these experiments was to evaluate the effects of different fat sources and levels on growth performance, carcass characteristics, and economical return of finishing pigs in a commercial setting.

Materials And Methods

General

The Kansas State University Institutional Animal Care and Use Committee approved the protocols used in these experiments. Experiment 1 was conducted at a commercial research facility in southwestern Minnesota (New Horizon Farms, Pipestone, MN). The barns were naturally ventilated and double-curtain-sided with totally slatted floors. Each pen was equipped

with a 5-hole stainless steel dry self-feeder and a bowl waterer for *ad libitum* access to feed and water.

Experiment 2 was also conducted at a commercial research facility located in southwest Minnesota (Pipestone Applied Research; Edgerton, MN). Pigs were housed in a temperature-controlled wean-to-finish facility. Each pen contained 1 nipple waterer and a 4-hole dry self-feeder to allow for *ad libitum* access to feed and water. In both locations, daily feed additions to each pen were accomplished using a robotic feeding system (FeedPro; Feedlogic Corp., Wilmar, MN) that was able to record feed deliveries for individual pens.

Experiment 1

A total of 2,160 pigs (337 × 1050, PIC; initially 37.3 ± 0.93 kg) were used in two consecutive groups for a 99-d study. There were 27 pigs per pen and 20 pens per treatment (10 pens per group). Pens of pigs were blocked by initial BW and randomly assigned to 1 of 4 dietary treatments in a randomized complete block design. There was a control diet with no added fat, two dietary treatments with increasing choice white grease (1 and 3% of the diet) and the final dietary treatment contained no added fat until pigs were approximately 100 kg, then pigs were fed a diet containing 3% added fat. Experimental diets were fed over 4 phases and were corn-soybean meal-based with 40% dried distillers grains with solubles (DDGS). Phase 1 was fed from approximately 36 to 50 kg, phase 2 from 50 to 75 kg, phase 3 from 75 to 100 kg, and phase 4 from 100 kg to market. Diets were formulated to maintain constant SID Lys:NE ratios of 4.50, 3.85, 3.30, and 3.04 g Lys/Mcal for phase 1, 2, 3, and 4, respectively. The ME and NE values for DDGS were derived from internal nutrient values from the commercial facility with all other ingredient NE and ME values derived from NRC (2012). All nutrients were formulated to meet or exceed NRC (2012) requirement estimates. Pens of pigs were weighed and feed

disappearance was recorded approximately every 2 weeks to determine ADG, ADFI, and G:F. Caloric efficiency was calculated by dividing the total ME or NE calories consumed by total gain. On d 83 and 82 for groups one and two, respectively, the three heaviest pigs within each pen were marketed. Their data was included in the growth performance analysis, but not carcass characteristics. The remaining pigs were then marketed approximately 2 wk later at the conclusion of the experiment for each group. At study completion for each group, final pen weights were recorded, and each pig was tattooed with a pen identification number and transported to a commercial abattoir (JBS Swift, Worthington, MN) for processing and carcass data collection. Carcass measurements included HCW, backfat depth, loin depth, and percentage lean (as per JBS Swift's proprietary calculation). Carcass yields were then calculated by the pen average HCW divided by the pen average final BW.

Experiment 2

A total of 2,011 pigs (PIC 1050 × DNA 600; initially 28.3 ± 0.53 kg) were used in a 113-d trial. There were 21 to 27 pigs per pen and 16 pens per treatment. On d 0, pens were blocked by location in the barn and randomly allotted to 1 of 5 dietary treatments. A similar number of barrows and gilts were placed in each pen. Dietary treatments were arranged in a $2 \times 2 + 1$ factorial with main effects of fat source and fat level. Dietary treatments included a control diet containing no added fat. The other 4 dietary treatments included two different fat sources, choice white grease or corn oil, added at 1 or 3% of the diet. Diets were formulated on an ME basis with these values derived from internal data from the production system. The dietary NE concentrations for each treatment were derived from NRC (2012) ingredient values. All nutrients were formulated to meet or exceed NRC (2012) requirement estimates. Experimental diets were corn-soybean meal-based, fed in meal form, and were fed in 6 different phases. Pigs were fed on a

feed budget with phase 1, 2, 3, 4, and 5 provided at 17, 41, 46, 49 and 41 kg per pig, respectively. Phase 6 was provided for the remainder of the study. Pens of pigs were weighed and feed disappearance was measured approximately every 2 wk to determine ADG, ADFI, and G:F. Caloric efficiency was calculated by taking the total ME or NE calories consumed divided by total gain. On d 92 and 104, eight of the heaviest pigs per pen were weighed individually and transported to a commercial packing plant (WholeStone Farms, Fremont, NE) for processing and determination of carcass characteristics. The remaining pigs were marketed at the conclusion of the trial on d 113 and transported to WholeStone Farms for collection of carcass characteristics. A 5 × 5 cm sample of fat, from all three layers, was collected posterior of the sternum on the midline of the belly from one barrow per pen per marketing event. Iodine value analysis was conducted on the fat sample using NIR at WholeStone Farms.

Economic Analysis

In Exp. 1 and 2, feed cost, cost per kilogram of gain, revenue, and income over feed cost (IOFC) were calculated on a per pig placed basis. Economics were calculated using a low and high feed cost scenario. Feed cost was calculated by multiplying feed cost per kg by feed consumed in each phase. Revenue was calculated by total pen gain multiplied by pen carcass yield multiplied by carcass price ($2.65/kg or $1.21/kg for the high and low revenue scenarios, respectively). Income over feed cost was calculated by subtracting the low or high feed cost from the low or high revenue.

In both experiments, choice white grease and corn oil was assumed to cost $1.32/kg for the high feed cost scenario and $0.73/kg for the low feed cost scenario. The following ingredient costs were used for the high-cost scenario: corn = $0.27/kg, SBM = $0.46/kg, DDGS = $0.29/kg, L-Trp = $9.48/kg, DL-Met = $4.63/kg, L-Lys = $1.59/kg. For the low-cost scenario, the

following ingredient costs were used: corn = $0.13/kg, SBM = $0.31/kg, DDGS = $0.18/kg, L-Trp = $9.48/kg, DL-Met = $4.63/kg, L-Lys = $1.59/kg.

Chemical Analysis

In both experiments, diet samples were collected and sent to a commercial laboratory (Midwest Laboratories; Omaha, NE). Standard procedures (AOAC International, 2006) were followed for dry matter (method 930.15), crude protein (method 990.03), and acid hydrolyzed ether extract [method 954.02 (mod.)] analyses.

Statistical Analysis

Growth performance data in both experiments were analyzed using the lmer function of R (Version 4.0.0, R Foundation for Statistical Computing, Vienna, Austria) as a randomized complete block design with pen serving as the experimental unit. In Exp. 1, the statistical model considered fixed effects of dietary treatment, linear and quadratic contrasts of increasing fat dose, and random effects of group and block. Pigs fed diets with 0% added fat to 100 kg and fed 3% until market were excluded from the linear and quadratic analysis and were analyzed as a pairwise comparison relative to the other treatments. In Exp. 2, treatments were considered as a fixed effect and block as a random effect. Linear and quadratic effects for increasing fat level and main effects of fat source were tested, as well as any interactions. For both experiments, the control diet served as 0% inclusion of fat for linear and quadratic analysis. The model for backfat, loin depth, and lean percentage considered HCW as a covariate in Exp. 1 and the model for backfat considered HCW as a covariate in Exp. 2. For both experiments, the model for mortality and removal data specified a binomial distribution. Differences between treatments were considered significant at $P \leq 0.05$ and marginally significant at $0.05 < P \leq 0.10$.

Results

Chemical Analysis

In Exp. 1, analyzed acid hydrolysis fat in the diets for all phases were similar to formulated values and followed the trend of increasing choice white grease (Table 1). In Exp. 2, analyzed acid hydrolysis fat matched closely with formulated values for treatment diets for all phases (Table 2).

Experiment 1

Data were summarized into an early, mid, and late-finishing periods. In the early period, from approximately 36 to 73 kg, increasing fat decreased (linear, $P = 0.013$; Table 7) ADFI. There were no differences between treatments for ADG and G:F ($P > 0.10$) during this period. During the mid-finishing period, from approximately 73 to 100 kg, increasing dietary fat decreased (linear, $P = 0.043$) ADFI with no effect on ADG ($P > 0.10$), leading to an increase (linear, $P = 0.006$) in G:F. In the late finisher from approximately 100 to 129 kg, increasing fat decreased (linear, $P = 0.028$) ADFI and increased (linear, $P = 0.003$) G:F, with no differences observed in ADG ($P > 0.10$). Within the late finishing phase, G:F did not differ ($P > 0.05$) between the two treatment groups fed 3% added fat, and both had greater ($P < 0.05$) G:F compared to pigs fed 0% fat with those fed 1% fat intermediate.

For overall growth performance, increasing dietary fat decreased ADFI and improved G:F (linear, $P = 0.006$). The ADFI and G:F did not differ ($P > 0.05$) between pigs fed diets with 0% added fat to 100 kg and fed 3% thereafter compared to pigs fed all other treatments. There were no significant differences ($P > 0.10$) in overall ADG between the dietary treatments. There were also no observed differences ($P > 0.10$) in total removals and mortalities over the duration of the study. Also, there were no significant differences ($P > 0.10$) in ME or NE caloric efficiency

between treatments. When looking at Lys intake, there were no significant differences ($P > 0.10$) in Lys intake, g/d and Lys intake, g/kg of gain.

For carcass characteristics, there was a tendency for an increase (linear, $P = 0.068$; Table 7) in HCW, where pigs fed the 3% added fat diet tended to have the heaviest HCW. Backfat depth increased (quadratic, $P = 0.057$) when added fat was increased from 0 to 1% but decreased when added fat further increased from 1 to 3%. Similarly, increasing added fat from 0 to 3% tended to decrease (quadratic, $P = 0.052$) lean percentage when added fat increased from 0 to 1% and then increased when added fat was increased from 1 to 3%. There were no significant differences ($P > 0.10$) in carcass yield or loin depth between the dietary treatments.

For economic analysis, increasing fat increased (linear, $P \leq 0.005$) feed cost per pig placed and feed cost per kg of gain for the high and low feed cost scenarios. There were no significant differences ($P > 0.10$) in revenue for the high and low feed cost scenarios. Though BW at the end of the trial and HCW were numerically greater as added fat increased in the diet, total removals and mortality was also numerically greater in diets containing increased levels of added fat. This resulted in revenue to numerically decrease when added fat increased in the diet. Due to this, increasing fat led to a decrease (linear, $P \leq 0.041$) in IOFC for all high and low feed cost/revenue scenarios. Pigs fed diets containing no fat had the highest IOFC compared to pigs fed diets containing 3% added fat with the other treatments intermediate in the high feed cost, high revenue scenario and the low feed cost, low revenue scenario. Pigs fed 0 and 1% fat for the entire trial and pigs fed 3% fat from 100 kg to market had the highest IOFC compared to pigs fed 3% fat for the entire trial in the high feed cost, low revenue scenario.

Experiment 2

There were no interactions between fat source and level for any growth performance response criteria. From d 0 to 65, increasing added fat from 0 to 3% of the diet increased (linear, $P < 0.001$; Table 8) ADG and G:F. There was no difference in ADFI observed during this period nor a main effect of fat source. From d 65 to 113, increasing added fat in the diet from 0 to 3% decreased (linear, $P < 0.001$) ADFI and improved (linear, $P < 0.001$) G:F. There was also a main effect of fat source ($P = 0.046$) where pigs fed diets containing choice white grease had decreased ADFI compared to pigs fed diets containing corn oil. There were no significant differences ($P > 0.10$) in ADG with increasing levels of dietary fat or main effects of fat source observed.

For overall growth performance (d 0 to 113), increasing dietary fat increased (linear, $P < 0.001$) ADG, decreased (linear, $P = 0.013$) ADFI, and improved (linear, $P < 0.001$) G:F. Like the individual phase data, no differences ($P > 0.10$) between fat sources were observed. There were also no observed differences ($P > 0.10$) in removals, mortality, or total removals and mortalities over the duration of the study. For caloric efficiency, increasing fat in the diet led to improved (linear, $P = 0.005$) ME and tended to improve (linear, $P = 0.066$) NE caloric efficiency. For Lys intake, there was a quadratic response (quadratic, $P < 0.001$) in Lys intake, g/kg of gain where Lys intake, g/kg of gain decreased when fat was increased from 0 to 1% and then leveled out when fat was increased from 1 to 3%. There were no significant differences ($P > 0.10$) in Lys intake, g/d.

Increasing added fat from 0 to 3% led to an increase (linear, $P \leq 0.028$) in BW for the first and second marketing events and to an increase (linear, $P = 0.007$) in overall market weight. For carcass characteristics, increasing fat in the diet increased (linear, $P < 0.001$) HCW and carcass yield. Increasing dietary fat from 0 to 3% also increased backfat depth (linear, $P =$

0.016). For carcass fat iodine value, there was a fat source × level interaction ($P < 0.001$) where the magnitude of iodine value increase was greater in pigs fed diets containing corn oil with only a small increase in iodine value when diets with choice white grease were fed. The increase in iodine value with increasing dietary fat was similar across all 3 marketing events, however, iodine values were collectively higher in the final marketing event ($P < 0.001$).

For economics, increasing fat increased (linear, $P < 0.001$) feed cost for both the high and low feed cost scenarios, but also led to greater (linear, $P = 0.003$) revenue for the high and low pig price scenario. Increasing fat in the diet led to decreasing (linear, $P < 0.001$) IOFC in the high feed cost, low revenue scenario, and a tendency to increase (linear, $P = 0.060$) IOFC in the low feed cost, high revenue scenario. There were no significant differences ($P > 0.10$) in IOFC for the high feed cost, high revenue and low feed cost, low revenue scenarios.

Discussion

Adding fat to swine diets is a common method used to improve growth performance in finishing pigs. However, past reviews have suggested that the addition of fat in the diet does not consistently improve ADG (Pettigrew and Moser, 1991). Engel et al. (2001) and Liu et al. (2018) observed no significant differences in ADG with increasing fat in the diet. These results are similar to Exp. 1, where increasing fat in the diet did not influence ADG throughout the duration of the study. On the other hand, De la Llata et al. (2007) observed that increasing fat in the diet led to an increase in ADG in phase 1 (36 to 59 kg) and overall, but no differences were observed in ADG in phase 2 (59 to 93 kg) or phase 3 (93 to 120 kg). These results are similar to Exp. 2, where ADG increased with increasing fat from approximately 28 to 90 kg BW but not from approximately 90 to 122 kg BW. Data from De la Lata (2007) would suggest that the response in ADG early in Exp. 2 was due to pigs being in an energy dependent stage of growth, creating an

ADG response early in the trial, also affecting overall ADG. It is unknown why increasing fat in the diet had no influence on ADG in the early stages of Exp. 1. However, the higher fiber levels from the inclusion of 40% DDGS in these diets might have reduced the digestibility of energy provided by the added fat. Le Goff and Noblet (2001) state that for each percentage of NDF included in the diet, the digestibility of energy decreases ~ 0.8%. Results from Paternostre et al. (2021) conclude that the addition of fiber may decrease the digestibility of fat, which may then decrease the digestibility of other nutrients.

It is important to express SID Lys in relationship to dietary energy. Balancing the SID Lys to dietary energy ratio allows lysine requirements to be applied to a vast range of energy levels (De la Llata et al., 2007). Nitikanchana et al. (2015) observed that ADG in response to increasing energy intake is dependent on SID Lys intake. This might be another reason for the differing responses seen in ADG between the two experiments. In Exp. 1, all diets were formulated to maintaining a similar ratio between SID Lys and NE. However, in Exp. 2, a constant SID Lys: energy (ME or NE) was not maintained with increasing dietary energy. Therefore, the increase in ADG with increasing fat levels may partially be due to the increased SID Lys in the diet and less due to the increase in dietary fat. However, it is important to note that Lys was not limiting in any treatments of any phase. Overall Lys intake was similar across all treatments and Lys per kg of gain was above 22 g/d in both experiments. This indicates that Lys was adequate across all treatments in all phases.

For nutritionists and producers, different fat sources are available for use and understanding relative differences between them is essential for proper decision making. Stephenson et al. (2016) and Apple et al. (2009) observed no significant ADG differences between beef tallow and soybean oil, and beef tallow, poultry fat, and soybean oil, respectfully.

However, Benz et al. (2011) observed that pigs fed soybean oil, an unsaturated fat source, tended to have greater ADG compared to pigs fed diets containing choice white grease, a saturated fatty acid source. However, in the present Exp. 2 study, ADG was not impacted differently based on the fat source that was fed.

The addition of fat in finishing diets has been shown to reduce ADFI and improve G:F (De la Llata et al., 2001b; Liu et al., 2018). As expected, increasing fat in the diet reduced ADFI and improved feed efficiency in both experiments. These results agree with many previous studies (Smith et al., 1999; Engel et al., 2001; Benz et al., 2011; Liu et al., 2018). Pettigrew and Moser (1991) explain that when fat is added to the diet, energy density of the diet increases because fat contains about 2.25 times the energy compared to carbohydrates. When pigs are fed *ad libitum*, feed consumption in order to meet the pig's energy requirement is lessened, causing a reduction in total feed intake. Even though feed intake decreases, the amount of energy consumed is greater. If the concentration of other nutrients are adjusted to compensate for this reduction in feed intake, the increase in energy density, though added fat, can potentially increase gain and improve feed efficiency.

Past results comparing different saturated and unsaturated fat sources have found no significant differences in ADFI and feed efficiency (Benz et al., 2011; Stephenson et al., 2016). Similarly, Liu et al. (2018) found no differences between fat sources in overall G:F when comparing choice white grease and soybean oil. In Exp. 2, there were no significant differences observed in overall G:F between fat sources. These results are further confirmed by no differences in ME and NE caloric efficiency between fat sources in Exp. 2. Similar to lack of differences between fat sources for the ADG response, these results would suggest that the

energy concentration of the fat sources used in Exp. 2 are comparable or not different enough to demonstrate differences in growth performance.

For carcass characteristics, the effect of adding dietary fat has shown mixed findings. De la Llata et al. (2001b; 2007) observed that increased levels of choice white grease increased carcass weight, but had no effect on yield, backfat, loin depth, or lean percentage. These results are comparable to those of Exp. 1, where increasing fat in the diet tended to increase HCW but had no effect on carcass yield, backfat, loin depth, or lean percentage. However, results from Smith et al. (1999) suggest that increasing fat in the diet from 0 to 3% increased backfat and carcass yield. These findings are comparable to those in Exp. 2, where increasing fat in the diet led to greater HCW, carcass yield, and backfat. Also in Exp. 2, no differences were observed in carcass traits between pigs fed different fat sources, similar to the findings of Stephenson et al. (2016) who observed no significant differences in carcass characteristics between pigs fed diets containing soybean oil or choice white grease. Similar to the ADG response in Exp. 1, the higher fiber levels from the inclusion of 40% DDGS in these experimental diets might have reduced the digestibility of energy provided from increasing the level of fat in the diet. Ultimately, this could have played a role in the limited differences observed in carcass characteristics in Exp. 1.

There has been other research evaluating the effect of added fat level and source on carcass fat fatty acid composition. Weber et al. (2006) observed an increase in carcass fat IV values of the inner and outer layers of fat with the in addition of choice white grease or beef tallow. Similar results were seen in Exp. 2, where increasing dietary fat, regardless of source, increased carcass fat IV values. This might reflect the increased backfat with increased added fat in Exp. 2, in which increased fat deposition can lead to greater carcass IV values (Averette Gatlin et al., 2003 and Benz et al., 2011). Benz et al. (2011) observed an increase in fat IV with pigs fed

soybean oil compared to pigs fed diets containing choice white grease. Kellner et al. (2016) also observed greater fat IV values in pigs fed diets containing corn oil compared to pigs fed diets containing tallow or choice white grease. These results are comparable to those observed in Exp. 2, where pigs fed corn oil had greater fat IV values compared to pigs fed choice white grease. This data indicates that pigs consuming diets containing more unsaturated fatty acids have greater IV values compared to pigs fed diets containing a saturated fat source. Vegetable fats, such as corn oil, are higher in polyunsaturated fatty acids compared to animal fats, such as choice white grease (Clarke et al., 1990). These polyunsaturated fatty acids play a major role in inhibiting de novo fat synthesis and can lead to the alteration of the composition of deposited fat (Chilliard et al., 1993). Therefore, increasing the amount of fat in the diet, especially unsaturated fatty acids, increases unsaturated fat deposited. The deposition of more unsaturated fatty acids leads to softer fat, as indicated by the increase in carcass fat IV values with pigs fed diets containing vegetable fats, such as corn oil.

When formulating diets, caloric efficiency can be calculated to determine if the energy values used in diet formulation for ingredients are accurate. Caloric efficiency can be calculated using multiple energy systems, such as ME and NE. If the given energy value of an ingredient is accurate, a similar caloric efficiency will be calculated, regardless of the inclusion rate for the ingredient. If there is a significant difference in caloric efficiency of treatment diets containing increasing levels of an ingredient, the energy value for that ingredient is either overestimated or underestimated in formulation (DeJong et al., 2014). Past research by Kellner et al. (2014) observed no significant differences in caloric efficiency in pigs fed diets containing no dietary fat compared to pigs fed diets containing corn oil or tallow, thus demonstrating that if ingredient values are estimated correctly, no differences in caloric efficiency will occur. These results are

comparable Exp. 1, where increasing dietary choice white grease did not have an impact on ME or NE caloric efficiency. This would indicate that the ME and NE energy values assigned to choice white grease of 8,124 and 7,149 kcal/kg, respectively, were estimated correctly. However, in Exp. 2, increasing fat in the diet led to an improvement in ME caloric efficiency and tended to improve NE caloric efficiency. These results indicate that ME and NE energy content of choice white grease of 7,954 kcal/kg and 7,149 kcal/kg, respectively, and corn oil 7,954 kcal/kg and 7,549 kcal/kg, respectively, may have been underestimated in formulation.

The ME value assigned to choice white grease was higher in Exp. 1 compared to Exp. 2. The caloric efficiency measurements in both trials supports that the ME energy content assigned to choice white grease in Exp. 1 was more accurate, and the ME energy content assigned in Exp. 2 were underestimated. There was no significant difference in ME or NE caloric efficiency between fat sources. The ME energy content to corn oil is higher compared to choice white grease (NRC, 2012). However, in Exp. 2, ME energy contents were valued the same for both fat sources, confirming the no differences observed in caloric efficiency.

The evaluation of economics is crucial when developing any nutritional program. Past research has indicated that measuring IOFC is a beneficial economic evaluator, as it accounts for the two major economical influences of the diet in a partial budget: income and expenses (De la Llata et al., 2001a). In some cases, adding a nutritional unit to an existing diet can increase the cost of that diet (Boland et al., 1999). However, if the addition of that nutritional unit brings a benefit to performance, it may increase revenue enough to increase IOFC (De la Llata et al., 2001a). In both experiments, increasing fat in the diet led to an increase in feed cost. Collins et al. (2009) observed an increase in diet cost with increasing dietary fat from 0 to 6%. However, these results were offset by the reduction in ADFI and improvement in feed efficiency.

Therefore, the cost of production was lessened, and net income was increased with the increase of fat in the diet. Increasing fat did not increase revenue in Exp. 1 because ADG was not increased by added fat and there was a numerical increase in total removals and mortality in pigs fed diets containing increased fat. This caused total gain, used to calculate revenue, to be less compared to other treatments. Furthermore, increasing fat in the diet decreased ADFI, but the increase in cost of the diet with added fat was of greater magnitude than the value of reducing intake. Even though added fat led to an improvement in feed efficiency, the benefit of adding fat in the diet did not increase revenue. Therefore, the cost to add fat into the diet resulted in reduced IOFC in all feed cost scenarios and did not bring an economic benefit in this study. However, in Exp. 2, increased dietary fat increased total gain and revenue. This resulted in an increase in both feed cost and revenue for both high and low feed cost and pig cost scenarios. Therefore, when feed costs were low and revenue were high, adding fat to the diet was justifiable. However, when feed costs were high and revenue was low, adding fat to the diet was not justifiable.

In conclusion, the results from these experiments suggest that increasing fat from 0 to 3%, regardless of fat source, produced variable responses in ADG and a more consistent improvement in G:F. Increasing fat can also result in improved HCW and carcass yield, but increased backfat, and feeding pigs diets containing corn oil as the fat source results in higher carcass fat IV than choice white grease. With the ingredient prices used, the improvement in growth performance did not justify the extra diet cost from increasing fat from 0 to 3% in most situations.

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Table 1.1 Chemical analysis of diets in Exp. 1[1]

	Added fat, %		
Analyzed composition, %	0	1	3
Phase 1			
CP	19.8	20.5	19.9
Acid hydrolyzed fat	5.7	6.6	8.6
Phase 2			
CP	20.2	16.8	19.0
Acid hydrolyzed fat	4.9	6.0	8.0
Phase 3			
CP	17.0	17.5	17.0
Acid hydrolyzed fat	5.4	6.3	7.8
Phase 4			
CP	15.3	17.3	17.7
Acid hydrolyzed fat	4.8	5.8	7.6

[1]A composite sample of each treatment diet was collected and submitted to Midwest Laboratories (Omaha, NE) for crude protein and fat (acid hydrolysis) analysis.

Table 1.2 Chemical analysis of diets in Exp. 2[1]

		Choice white grease		Corn oil	
Analyzed composition, %	0	1	3	1	3
Phase 1					
CP	19.7	18.5	19.7	19.1	20.8
Acid hydrolyzed fat	3.6	4.4	5.7	4.3	5.4
Phase 2					
CP	15.8	17.1	19.5	17.1	19.1
Acid hydrolyzed fat	3.7	4.2	5.9	4.2	6.5
Phase 3					
CP	15.7	15.6	14.4	15.2	16.6
Acid hydrolyzed fat	3.4	4.1	6.0	4.3	5.7
Phase 4					
CP	14.9	14.0	13.4	13.6	14.1
Acid hydrolyzed fat	3.8	4.7	6.6	4.6	6.5
Phase 5					
CP	13.2	14.5	14.4	13.0	14.8
Acid hydrolyzed fat	3.7	4.4	6.0	4.3	5.8
Phase 6					
CP	11.8	10.5	10.8	10.7	11.6
Acid hydrolyzed fat	3.6	5.1	5.5	4.3	6.1

[1]A composite sample of each treatment diet was collected and submitted to Midwest Laboratories (Omaha, NE) for crude protein and fat (acid hydrolysis) analysis.

Table 1.3 Composition of phase 1 and 2 diets in Exp. 1 (as-fed basis)[1]

	Phase 1			Phase 2		
	Added fat, %			Added fat, %		
Ingredient, %	0	1	3	0	1	3
Corn	47.47	45.65	42.03	52.86	51.18	47.70
Soybean meal (46.5% CP)	9.45	10.29	11.91	4.43	5.11	6.57
Corn DDGS[2]	40.00	40.00	40.00	40.00	40.00	40.00
Choice white grease	---	1.00	3.00	---	1.00	3.00
Limestone, ground	1.50	1.50	1.50	1.40	1.40	1.40
Monocalcium P (21% P)	0.20	0.17	0.15	---	---	---
Salt	0.40	0.40	0.40	0.40	0.40	0.40
L-Lys-HCl	0.62	0.62	0.62	0.60	0.60	0.60
DL-Met	0.04	0.04	0.05	---	---	0.02
L-Trp	0.06	0.06	0.06	0.06	0.06	0.06
Thr[3]	0.13	0.13	0.14	0.13	0.13	0.13
Vitamin trace mineral premix	0.10	0.10	0.10	0.09	0.09	0.09
Tribasic copper chloride	0.03	0.03	0.03	0.03	0.03	0.03
Phytase[4]	0.02	0.02	0.02	0.01	0.01	0.01
Calculated analysis						
Standardized ileal digestible (SID) amino acids, %						
Lys	1.06	1.08	1.12	0.92	0.94	0.97
Ile:Lys	56	56	57	56	56	56
Leu:Lys	166	164	160	178	176	172
Met: Lys	33	33	33	32	31	32
Met and Cys:Lys	60	60	60	61	60	61
Thr:Lys	62	62	62	63	63	63
Trp:Lys	19	19	19	19	19	19
Val:Lys	70	70	70	72	72	71
Total Lys, %	1.27	1.29	1.33	1.12	1.14	1.17
ME, kcal/kg	3,195	3,243	3,336	3,210	3,257	3,350
NE, kcal/kg	2,359	2,400	2,481	2,396	2,437	2,518
SID Lys:ME, g/Mcal	3.32	3.33	3.34	2.87	2.88	2.89
SID Lys:NE, g/Mcal	4.50	4.50	4.50	3.85	3.85	3.85
CP, %	20.4	20.7	21.2	18.4	18.6	19.0
Ca, %	0.65	0.64	0.65	0.56	0.56	0.57
P, %	0.59	0.58	0.58	0.53	0.53	0.53
Standardized total tract digestible (STTD) P, %	0.34	0.34	0.34	0.29	0.30	0.30
Ca:P	1.10	1.10	1.11	1.07	1.07	1.07

[1]Phase 1 was fed from approximately 37.2 to 49.9 kg, and phase 2 was fed from approximately 49.9 to 74.8 kg.

[2]Dried distillers grains with solubles.

[3]Thr Pro; CJ America-Bio, Downers Grove, IL.

[4]Optiphos (Huveoharma, Sofia, Bulgaria) was included at 500 and 250 FTU/kg in phase 1 and 2 providing an estimated release of 0.10 and 0.07% STTD P, respectively.

Table 1.4 Composition of phase 3 and 4 diets in Exp. 1 (as-fed basis)[1]

	Phase 3			Phase 4		
	Added fat, %			Added fat, %		
Ingredient, %	0	1	3	0	1	3
Corn	56.46	54.81	51.60	57.47	55.82	52.64
Soybean meal (46.5% CP)	0.94	1.58	2.79	0.02	0.67	1.84
Corn DDGS[2]	40.00	40.00	40.00	40.00	40.00	40.00
Choice white grease	---	1.00	3.00	---	1.00	3.00
Limestone, ground	1.40	1.40	1.40	1.40	1.40	1.40
Salt	0.40	0.40	0.40	0.40	0.40	0.40
L-Lys-HCl	0.55	0.55	0.55	0.50	0.50	0.50
L-Trp	0.06	0.06	0.06	0.05	0.05	0.05
Thr[3]	0.10	0.10	0.11	0.08	0.08	0.08
Vitamin trace mineral premix	0.08	0.08	0.08	0.07	0.07	0.07
Tribasic copper chloride	0.03	0.03	0.03	0.03	0.03	0.03
Calculated analysis						
Standardized ileal digestible (SID) amino acids, %						
Lys	0.80	0.81	0.84	0.74	0.75	0.78
Ile:Lys	57	57	57	60	60	60
Leu:Lys	195	193	188	209	206	201
Met: Lys	35	34	34	37	37	36
Met and Cys:Lys	67	66	64	71	70	69
Thr:Lys	65	65	64	66	66	65
Trp:Lys	19	19	19	19	19	19
Val:Lys	76	76	75	81	80	79
Total Lys, %	0.99	1.00	1.03	0.92	0.94	0.97
ME, kcal/kg	3,214	3,260	3,354	3,214	3,261	3,355
NE, kcal/kg	2,417	2,458	2,540	2,422	2,463	2,546
SID Lys:ME, g/Mcal	2.48	2.49	2.50	2.29	2.30	2.31
SID Lys:NE, g/Mcal	3.30	3.30	3.30	3.04	3.04	3.04
CP, %	17.00	17.20	17.50	16.60	16.70	17.00
Ca, %	0.55	0.55	0.55	0.54	0.54	0.55
P, %	0.51	0.51	0.51	0.51	0.51	0.51
Standardized total tract digestible (STTD) P, %	0.29	0.29	0.29	0.28	0.28	0.28
Ca:P	1.07	1.07	1.08	1.07	1.07	1.08

[1]Phase 3 was fed from approximately 74.8 to 99.8 kg, and phase 4 was fed from 99.8 kg to market.
[2]Dried distillers grains with solubles.
[3]Thr Pro; CJ America-Bio, Downers Grove, IL.

Table 1.5 Composition of phase 1, 2, and 3 diets in Exp. 2 (as-fed basis)[1]

		Phase 1			Phase 2			Phase 3		
Ingredient, %	Fat level, %:	0	1	3	0	1	3	0	1	3
Corn		65.00	64.00	60.68	70.21	68.53	65.26	75.17	73.62	70.50
Soybean meal (46% CP)		22.40	22.20	23.50	17.25	17.80	19.05	12.40	12.90	14.00
Corn DDGS[2]		10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Fat source[3]		---	1.00	3.00	----	1.00	3.00	---	1.00	3.00
Limestone		0.95	0.95	0.95	0.95	0.95	0.95	0.93	0.93	0.93
Monocalcium phosphate (21% P)		0.20	0.21	0.20	0.19	0.19	0.18	0.14	0.14	0.14
Salt		0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Lys-HCl		0.50	0.57	0.58	0.50	0.54	0.54	0.49	0.50	0.51
DL-Met		0.11	0.15	0.16	0.08	0.11	0.12	0.05	0.06	0.07
L-Trp		0.04	0.05	0.05	0.04	0.05	0.05	0.04	0.04	0.04
L-Thr		0.15	0.19	0.19	0.14	0.16	0.17	0.13	0.14	0.15
L-Val		---	0.04	0.05	---	0.03	0.03	---	0.01	0.01
Vitamin and trace mineral premix		0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Copper chloride		0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Phytase[4]		0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04
Calculated analysis										
Standardized ileal digestible (SID) amino acids, %										
Lys		1.17	1.22	1.25	1.04	1.09	1.12	0.92	0.94	0.97
Ile:Lys		58	55	55	57	55	55	56	55	55
Leu:Lys		132	125	125	136	131	129	143	140	137
Met and Cys:Lys		58	58	58	58	58	58	58	58	58
Thr:Lys		63	63	63	63	63	63	64	64	64
Trp:Lys		19	19	19	19	19	19	19	19	19
Val:Lys		65	65	65	65	65	65	65	65	65
Total Lys, %		1.31	1.36	1.39	1.17	1.21	1.24	1.03	1.05	1.08
ME, kcal/kg		3,199	3,249	3,334	3,226	3,270	3,355	3,252	3,295	3,382
NE, kcal/kg[5]		2,468	2,516	2,598	2,498	2,541	2,623	2,528	2,570	2,653
SID Lys:ME, g/Mcal		3.65	3.76	3.76	3.23	3.33	3.33	2.82	2.85	2.86
SID Lys:NE, g/Mcal[5]		4.73	4.85	4.82	4.18	4.29	4.25	3.62	3.65	3.64

CP, %	18.91	18.77	19.12	16.88	17.03	17.36	14.97	15.09	15.37
Ca, %	0.60	0.60	0.60	0.58	0.58	0.59	0.55	0.55	0.55
P, %	0.43	0.43	0.42	0.40	0.40	0.40	0.37	0.37	0.37
Standardized total tract digestible (STTD) P, %	0.38	0.38	0.38	0.36	0.36	0.36	0.34	0.34	0.34
Ca:P	1.40	1.41	1.42	1.44	1.45	1.46	1.48	1.48	1.49

[1]Pigs were fed on a feed budget with phase 1, 2, and 3, provided at 17, 41, and 46 kg per pig, respectively.
[2]Dried distillers grains with solubles.
[3]Increasing levels of choice white grease or corn oil was added to the diets.
[4]Quantum Blue 5P (AB Vista, Marlborough, UK) was included at 2,000 FTU/kg and provided an estimated release of 0.11% for STTD P.
[5]Calculations derived from NRC (2012) ingredient nutrient values.

Table 1.6 Composition of phase 4, 5, and 6 diets in Exp. 2 (as-fed basis).[1]

	Phase 4			Phase 5			Phase 6		
Ingredient, % Fat level, %:	0	1	3	0	1	3	0	1	3
Corn	77.16	77.03	74.01	79.62	77.29	74.28	88.20	87.03	84.60
Soybean meal (46% CP)	10.65	9.65	10.65	8.35	9.70	10.70	9.80	9.95	10.35
Corn DDGS[2]	10.00	10.00	10.00	10.00	10.00	10.00	---	---	---
Fat source[3]	---	1.00	3.00	---	1.00	3.00	---	1.00	3.00
Limestone	0.90	0.90	0.90	0.85	0.83	0.83	0.75	0.75	0.75
Monocalcium phosphate (21% P)	0.11	0.09	0.09	---	---	---	0.20	0.20	0.20
Salt	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Lys-HCl	0.41	0.48	0.48	0.40	0.40	0.40	0.31	0.32	0.33
DL-Met	---	0.03	0.04	---	---	0.01	---	---	---
L-Trp	0.03	0.03	0.04	0.03	0.03	0.03	0.02	0.02	0.02
L-Thr	0.09	0.13	0.13	0.09	0.09	0.10	0.08	0.08	0.09
Vitamin and trace mineral premix	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Copper chloride	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Phytase[4]	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04
Calculated analysis									
Standardized ileal digestible (SID) amino acids, %									
Lys	0.81	0.84	0.86	0.75	0.78	0.80	0.70	0.69	0.70
Ile:Lys	59	55	55	59	59	59	61	60	59
Leu:Lys	157	148	144	162	159	155	178	154	149
Met and Cys:Lys	58	58	58	60	59	58	61	60	58
Thr:Lys	64	64	64	65	65	65	66	66	66
Trp:Lys	19	19	19	19	19	19	19	19	19
Val:Lys	70	66	65	71	71	70	72	71	69
Total Lys, %	0.92	0.94	0.97	0.85	0.89	0.91	0.81	0.76	0.78
ME, kcal/kg	3,261	3,314	3,400	3,278	3,317	3,403	3,270	3,351	3,441
NE, kcal/kg[5]	2,538	2,590	2,674	2,547	2,618	2,706	2,547	2,615	2,702
SID Lys:ME, g/Mcal	2.49	2.53	2.54	2.29	2.36	2.36	2.04	2.04	2.04
SID Lys:NE, g/Mcal[5]	3.20	3.24	3.23	2.94	3.02	3.00	2.76	2.62	2.60
CP, %	14.26	13.81	14.04	13.37	13.82	14.06	12.01	11.99	11.99

Ca, %	0.53	0.52	0.52	0.48	0.48	0.48	0.48	0.48	0.48
P, %	0.36	0.35	0.34	0.33	0.33	0.33	0.33	0.34	0.33
Standardized total tract digestible (STTD) P, %	0.33	0.32	0.32	0.31	0.31	0.31	0.31	0.32	0.32
Ca:P	1.48	1.50	1.52	1.48	1.45	1.46	1.47	1.42	1.44

[1]Pigs were fed on a feed budget with phase 4 and 5 provided at 49 and 41 kg per pig, respectively. Phase 6 was provided for the remainder of the study.
[2]Dried distillers grains with solubles.
[3]Increasing levels of choice white grease or corn oil was added to the diets.
[4]Quantum Blue 5P (AB Vista, Marlborough, UK) was included at 2,000 FTU/kg and provided an estimated release of 0.11% for STTD P.
[5]Calculations derived from NRC (2012) ingredient nutrient values.

Table 1.7 Effects of increasing added fat on growth performance, carcass characteristics, and economical return of finishing pigs in Exp. 1[1]

	Added fat, %					*P* =[2]	
Item	0	1	3	0 to 3[3]	SEM	Linear	Quadratic
BW, kg							
Initial	37.3	37.3	37.3	37.3	0.93	0.857	0.631
Early-finishing	73.9	73.3	73.4	73.8	2.13	0.471	0.436
Mid-finishing	102.2	102.9	103.1	102.8	3.38	0.291	0.540
Final	128.2	129.3	129.6	130.3	1.36	0.166	0.473
Early-finishing[4]							
ADG, kg	0.88	0.87	0.86	0.87	0.013	0.257	0.674
ADFI, kg	2.13	2.09	2.04	2.12	0.059	0.013	0.622
G:F, g/kg	414	416	423	411	11.0	0.176	0.910
Mid-finishing[5]							
ADG, kg	0.99	1.01	1.01	1.01	0.052	0.384	0.593
ADFI, kg	3.03	2.96	2.90	2.98	0.206	0.043	0.547
G:F, g/kg	331	342	351	340	6.6	0.006	0.439
Late-finishing[6]							
ADG, kg	0.95	0.96	0.96	0.98	0.032	0.429	0.499
ADFI, kg	3.34	3.29	3.20	3.27	0.142	0.028	0.853
G:F, g/kg	285[b]	294[ab]	302[a]	301[a]	4.1	0.003	0.599
Overall (d 0 to 99)							
ADG, kg	0.93	0.93	0.93	0.94	0.022	0.904	0.777
ADFI, kg	2.73[a]	2.67[ab]	2.61[b]	2.69[ab]	0.079	0.006	0.622
G:F, g/kg	342[b]	350[ab]	359[a]	350[ab]	4.2	0.006	0.664
Total removals and mortalities, %	4.44	6.48	8.51	8.15	0.624	0.607	0.888
Caloric efficiency, kcal ME/kg gain	9,405	9,313	9,370	9,336	109.2	0.903	0.544
Caloric efficiency, kcal NE/kg gain	7,051	6,998	7,073	7,016	82.1	0.747	0.547
Lys intake, g/d	22.95	22.83	23.15	23.06	0.690	0.525	0.574
Lys intake g/kg of gain	24.61	24.44	24.84	24.57	0.294	0.476	0.483
Carcass characteristics							
HCW, kg	94.70	96.02	96.55	95.81	0.984	0.068	0.394
Carcass yield, %	71.9	71.9	72.2	72.0	0.511	0.392	0.722
Backfat, mm[7]	15.78	16.44	15.86	16.28	0.427	0.881	0.057
Loin depth, mm[7]	63.05	62.98	63.17	63.53	2.072	0.874	0.894
Lean, %[8]	56.8	56.4	56.8	56.6	0.17	0.861	0.052
Economics, $/pig							
Feed cost/ kg gain (Hi)[8]	0.39[b]	0.39[b]	0.41[a]	0.39[b]	0.005	< 0.001	0.578

Feed cost/ kg gain (Lo)[8]	0.23[b]	0.23[b]	0.25[a]	0.23[b]	0.003	< 0.001	0.569
Feed cost (Hi)[9]	74.72[b]	75.67[ab]	78.94[a]	75.34[ab]	1.263	0.004	0.724
Feed cost (Lo)[10]	44.53[b]	45.07[ab]	46.99[a]	44.88[ab]	0.752	0.005	0.714
Revenue (Hi)[11]	167.42	166.67	165.19	165.84	3.786	0.408	0.996
Revenue (Lo)[11]	76.73	76.39	75.71	76.01	1.735	0.408	0.996
IOFC (HiF-HiR)[12]	92.70[a]	91.00[ab]	86.25[b]	90.50[ab]	2.909	0.003	0.811
IOFC (HiF-LoR)[12]	2.01[a]	0.72[a]	-3.23[b]	0.67[a]	1.090	< 0.001	0.687
IOFC (LoF-LoR)[12]	32.20[a]	31.32[ab]	28.73[b]	31.13[ab]	1.239	< 0.001	0.749
IOFC (LoF-HiR)[12]	122.89	121.60	118.21	120.96	3.250	0.041	0.894

[1]A total of 2,160 pigs (L337 × 1050, PIC; initially 37.3 kg ± 0.93 kg) were used in two groups with 27 pigs per pen and 20 replicates per treatment.

[2]Linear and quadratic contrasts were evaluated based on increasing fat in the diet.

[3]Pigs were fed a diet containing 0% fat until 100 kg and were then fed a diet containing 3% added fat.

[4]The early period was from d 0 to 40 in group 1 and d 0 to 42 in group 2 of the study.

[5]The mid-period was from d 40 to 68 in group 1 and d 42 to 70 in group 2 of the study.

[6]The late period was from d 68 to 102 in group 1 and d 70 to 95 in group 2 of the study.

[7]Adjusted using HCW as a covariate.

[8]Feed cost per kg of gain = total feed cost (high or low) per pen divided by total gain per pen.

[9]Feed cost (high): corn was valued at $0.27/kg, SBM = $0.46/kg, DDGS = $0.29/kg, L-Trp = $9.48/kg, DL-Met = $4.63/kg, L-Lys = $1.59/kg, and choice white grease at $1.32/kg.

[10]Feed cost (low): corn was valued at $0.13/kg, SBM = $0.31/kg, DDGS = $0.18/kg, L-Trp = $9.48/kg, DL-Met = $4.63/kg, L-Lys = $1.59/kg, and choice white grease at $0.73/kg.

[11]Revenue = total gain per pen × carcass yield × $2.65/kg (high) or $1.21/kg (low).

[12]Income over feed cost = revenue – feed cost.

[abc]Means within a row with different superscripts differ ($P < 0.05$).

Table 1.8 Effects of fat source and level on growth performance, carcass characteristics, carcass iodine value, and economical value of finishing pigs in Exp. 2[1]

								$P =$[2]	
Fat source:		Choice white grease		Corn oil				Fat level	
Item	Fat level, %: 0	1	3	1	3	SEM	Source	Linear	Quadratic
BW, kg									
d 0	28.3	28.3	28.3	28.3	28.3	0.531	0.975	0.974	0.966
d 65	87.8	88.5	90.5	89.1	91.2	0.948	0.339	< 0.001	0.997
d 113[3]	120.6	122.7	120.7	122.9	124.3	1.579	0.206	0.492	0.289
Market weights									
Cut 1[4] (d 92)	126.5	127.1	131.4	129.0	130.5	1.107	0.545	< 0.001	0.941
Cut 2[4] (d 104)	129.6	130.0	132.3	130.6	132.2	1.479	0.848	0.028	0.847
Overall market weight[5]	125.9	126.8	128.3	127.7	129.1	1.139	0.331	0.007	0.623
d 0 to 65									
ADG, kg	0.91	0.92	0.95	0.93	0.96	0.010	0.219	< 0.001	0.897
ADFI, kg	2.09	2.05	2.03	2.03	2.05	0.030	0.915	0.182	0.195
G:F, g/kg	436	449	468	457	469	4.0	0.143	< 0.001	0.050
d 65 to 113									
ADG, kg	0.96	0.97	0.98	0.98	0.99	0.012	0.426	0.301	0.546
ADFI, kg	3.15	3.07	2.98	3.12	3.05	0.033	0.046	< 0.001	0.821
G:F, g/kg	307	317	327	314	324	2.8	0.263	< 0.001	0.343
Overall (d 0 to 113)									
ADG, kg	0.93	0.94	0.96	0.95	0.97	0.009	0.183	< 0.001	0.795
ADFI, kg	2.47	2.42	2.37	2.43	2.41	0.029	0.321	0.013	0.353
G:F, g/kg	377	388	404	391	402	2.8	0.884	< 0.001	0.098
Removals, %	4.8	4.9	4.0	5.4	3.0	1.10	0.725	0.201	0.395
Mortality, %	2.0	2.9	1.7	1.9	1.7	0.71	0.557	0.655	0.490
Total removals and mortalities, %	6.8	7.9	5.7	7.4	4.8	1.33	0.545	0.185	0.255
Caloric efficiency, kcal ME/kg gain	8,656	8,531	8,418	8,484	8,450	65.0	0.893	0.005	0.522
Caloric efficiency, kcal NE/kg gain	6,730	6,655	6,608	6,629	6,663	50.7	0.743	0.066	0.531
Lys intake, g/d	21.33	20.98	21.10	21.00	21.44	0.253	0.379	0.661	0.122
Lys intake, g/ kg of gain	22.95	22.33	22.00	22.16	22.08	0.178	0.746	< 0.001	0.013
Carcass characteristics									
HCW, kg	89.93	90.63	92.48	91.56	93.21	0.879	0.224	< 0.001	0.780
Yield, %	71.5	71.5	71.7	72.0	72.2	0.001	0.125	< 0.001	0.217
Fat, mm[6]	23.55	23.44	23.90	23.33	23.83	0.161	0.580	0.016	0.104

Iodine value[7]	69.21	69.87	71.64	72.81	79.91	0.260	< 0.001	< 0.001	<0.001
Economics, $/pig placed									
Feed cost/ kg gain (Hi)[8]	0.79	0.80	0.83	0.80	0.83	0.006	0.853	< 0.001	0.268
Feed cost/ kg gain (Lo)[8]	0.48	0.48	0.49	0.48	0.50	0.004	0.831	< 0.001	0.453
Feed cost (Hi)[9]	74.44	75.76	80.45	76.25	81.72	1.051	0.328	< 0.001	0.469
Feed cost (Lo)[10]	44.50	45.28	47.72	45.56	48.47	0.627	0.336	< 0.001	0.604
Revenue (Hi)[11]	186.01	187.13	192.09	189.49	194.42	2.168	0.262	0.003	0.956
Revenue (Lo)[11]	85.25	85.77	88.04	86.85	89.11	0.994	0.262	0.003	0.956
IOFC (HiF-HiR)[12]	111.57	111.37	111.64	113.24	112.70	1.429	0.307	0.835	0.709
IOFC (HiF-LoR)[12]	10.81	10.01	7.59	10.60	7.39	0.606	0.719	< 0.001	0.275
IOFC (LoF-LoR)[12]	40.76	40.49	40.32	41.29	40.64	0.597	0.348	0.565	0.707
IOFC (LoF-HiR)[12]	141.51	141.85	144.37	143.93	145.96	1.691	0.275	0.060	0.923

[1]A total of 2,011 (PIC 1050 × DNA 600; initially 28.3 ± 0.53 kg) with 21 to 27 pigs per pen and 16 replications per treatment were used in a 113- d finishing trial.

[2]Linear and quadratic contrasts were evaluated based on increasing fat in the diet. No fat source × level interactions observed ($P > 0.10$) in any growth performance criteria.

[3]Values represent weights at final marketing.

[4]Eight of the heaviest pigs were marketed from each pen.

[5]Weighted average of all marketing events.

[6]Adjsuted using HCW as a covariate.

[7]Fat source × level interaction, $P < 0.001$.

[8]Feed cost per kg of gain = total feed cost (high or low) per pen divided by total gain per pen.

[9]Feed cost (high): corn was valued at $0.27/kg, SBM = $0.46/kg, DDGS = $0.29/kg, L-Trp = $9.48/kg, DL-Met = $4.63/kg, L-Lys = $1.59/kg, and choice white grease at $1.32/kg.

[10]Feed cost (low): corn was valued at $0.13/kg, SBM = $0.31/kg, DDGS = $0.18/kg, L-Trp = $9.48/kg, DL-Met = $4.63/kg, L-Lys = $1.59/kg, and choice white grease at $0.73/kg.

[11]Revenue = total gain per pen × carcass yield × $2.65/kg (high) or $1.21/kg (low).

[12]Income over feed cost = revenue – feed cost.

Chapter 2 - Effects of increasing omega-3 fatty acids on growth performance, immune response, and mortality in nursery pigs

Abstract

Three experiments evaluated omega-3 fatty acids, provided by O3 Trial Feed, on nursery pig growth performance, mortality, and response to an LPS immune challenge or natural PRRSV outbreak. In Exp. 1, 350 pigs (241 × 600, DNA; initially 5.8 kg) were used. Pens of pigs were randomly assigned to 1 of 5 dietary treatments containing increasing omega-3 fatty acids (0, 1, 2, 3, and 4% O3 Trial Feed) with 14 replications per treatment. On d 25, 2 pigs per pen were injected intramuscularly with 20 μg *Escherichia coli* LPS per kg BW and 1 pig per pen was injected with saline as a control. Body temperature was taken from all 3 pigs prior to and 2, 4, 6, and 12 h post LPS challenge. IL-1β and TNF-α concentrations were determined in LPS challenged pigs 24 h prior and 4 h post LPS challenge. There was no interaction between treatment and time for change in body temperature ($P > 0.10$). Overall, increasing O3 Trial Feed did not influence ($P > 0.10$) ADG, ADFI, G:F, IL-1β, or TNF-α. In Exp. 2, 1,056 pigs [PIC TR4 × (Fast LW × PIC L02) initially 7.3 kg] were used. Pens of pigs were randomly assigned to 1 of 4 dietary treatments containing increasing omega-3 fatty acids (0, 0.75, 1.5, and 3% O3 Trial Feed) with 12 replications per treatment. Oral fluids tested negative on d 7 and 14, but positive for North American PRRSV virus via PCR on d 21, 28, 35, and 42. Overall, increasing O3 Trial Feed increased (linear, $P < 0.001$) ADG, ADFI, and G:F and decreased (linear, $P = 0.027$) total removals and mortality. In Exp. 3, 91,140 pigs (DNA 600 × PIC 1050; initially 5.1 kg), originating from PRRSV-positive sow farms, were used across 8 nursery sites. Each site contained 5 barns with 2 rooms per barn and ~1,100 pigs per room. Rooms of pigs were blocked

by nursery site and allocated within sow source to 1 of 2 dietary treatments (Control or 3% O3 Trial Feed) with 40 replications per treatment. Oral fluids from 61 of the 80 rooms tested positive for North American PRRSV virus 1 wk post-weaning and 78 of the 80 rooms tested positive 3 wk after weaning. Overall, O3 Trial Feed did not influence ADG, ADFI, or G:F but increased ($P < 0.001$) total removals and mortalities. In summary, increasing omega-3 fatty acids, sourced by O3 Trial Feed, did not improve growth performance or immune response in healthy pigs given an LPS-challenge. However, it appears that if omega-3 fatty acids are fed prior to a natural PRRSV break (as in Exp. 2), growth performance and mortality may be improved.

Keywords: immune response, LPS, mortality, nursery pigs, omega-3, PRRSV

Introduction

The need to better understand the interaction between nutrition and the immune system is critical in order to reach peak production efficiency under disease situations. During a health challenge, an animal will re-direct nutrients away from growth towards the immune response (Klasing and Leshchinsky, 2000; Caroll et al., 2003). Including omega-3 fatty acids in the diet during a health challenge may alter allocation of nutrients to improve pig performance (Liu et al., 2003; Duan et al., 2014).

Inclusion of omega-3 fatty acids in the diet has been used as a nutritional strategy to improve the immune response by reducing the omega-6:3 fatty acid ratio. Research has demonstrated that lowering the ratio of omega-6:3 from the 10:1 or 20:1 observed in typical swine diets, to a range of 3:1 or 5:1 improves immune response (Duan et al., 2014; Huber et al.,

2018). Reducing the omega-6:3 fatty acid ratio increases the incorporation of omega-3 fatty acids into cell membranes to make it available for improved immune function during an immune challenge (Huber et al., 2018).

Porcine Reproductive and Respiratory virus (PRRSV) is a pathogen that results in significant impacts on sow reproduction as well as respiratory disease in weaned and growing pigs. The economic losses due to PRRSV are estimated at $664 million each year for U.S. swine producers (Montaner-Tarbes et al., 2019). PRRSV is in the Arteriviridae family, order Nidovirales, and is an enveloped, positive-stranded RNA virus. The detection of North American and European PRRSV strains can be accomplished by using real-time PCR testing (Kleiboeker, 2005).

Lipopolysaccharide (LPS) is an acceptable challenge model to evaluate the immune system to study the response to Gram-negative bacterial infections (Wyn, 2015). When an LPS challenge is administered, macrophages and neutrophils produce and release cytokines. Pro-inflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α), interleukin-1-beta (IL-1β), and interleukin-6 (IL-6), stimulate the acute phase immune response by activating hepatocytes to produce acute phase proteins. Pro-inflammatory cytokines also stimulate the hypothalamus-pituitary-adrenal axis, which produces prostaglandins, initiating the body to induce increased body temperatures. (Son et al., 2002; Llamas Mota et al., 2006).

O3 Trial Feed is a flax seed and algae-derived source of omega-3 fatty acids (alpha-linolenic acid) that has been used to increase omega-3 content of pork. The fatty acid profile makes it a viable option to reduce the omega-6:3 fatty acid ratio in nursery pig diets and potentially improve immune function. However, there is no published research available with O3 Trial Feed as a source of omega-3 fatty acids in nursery pigs. Our hypothesis was that lowering

the ratio of dietary omega-6:3 fatty acids would enhance the pig's immune system and reduce morbidity and mortality in the light of an LPS or PRRSV challenge. Therefore, the objective of these studies was to determine the influence of omega-3 fatty acids (alpha-linolenic acid), sourced by O3 Trial Feed, on nursery pig growth performance, response to an LPS immune challenge, and morbidity and mortality in PRRSV positive pigs in a commercial setting.

Materials And Methods

General

The Kansas State University Institutional Animal Care and Use Committee approved the protocol used in these experiments. In all three experiments, the omega 6:3 ratio was manipulated by adding a flaxseed-algae-based ingredient, rich in alpha-linolenic acid (O3 Trial Feed, NBO3 Technologies LLC, Manhattan, KS; Table 1).

Experiment 1

Experiment 1 was conducted at the Kansas State University Swine Teaching and Research Center in Manhattan, KS. The facility is completely enclosed, environmentally controlled, and mechanically ventilated. Each pen contains a 4-hole, dry self-feeder and a nipple waterer to provide *ad libitum* access to feed and water. Pens (1.2 × 1.2 m) had metal tri-bar floors and allowed approximately 0.288 m^2/pig. A total of 350 weanling pigs (241 × 600, DNA, Columbus NE; initially 5.8 ± 0.03 kg) were used in a 41-d trial. There were 5 pigs per pen and 14 replications per treatment. Pens of pigs were randomly assigned to 1 of 5 dietary treatments in a completely randomized design. The dietary treatments included increasing levels of omega-3 fatty acids (0, 1, 2, 3, and 4% O3 Trial Feed; Table 3). Experimental diets were fed across 3 phases and were corn-soybean meal-based. Phase 1 diets were fed from d 0 to 13 (approximately 5.8 to 7.3 kg BW). Phase 2 diets were fed from d 13 to 22 (approximately 7.3 to 11.5 kg BW).

Phase 3 was fed from d 22 to 41 (approximately 11.5 to 22.8 kg BW). Diets were formulated to 1.40% SID Lys for phase 1, 1.35% SID Lys for phase 2, and 1.30% SID Lys for phase 3 (Table 2). All other nutrients were formulated to meet or exceed NRC (2012) requirement estimates. For phase 1 and 2, a single base diet was manufactured (Hubbard Feeds, Beloit, KS), then O3 Trial Feed, corn, and soybean meal additions were mixed at the Kansas State University O.H. Kruse Feed Technology Innovation Center (Manhattan, KS) to make the final diets. Complete phase 3 diets were manufactured at Hubbard Feeds (Beloit, KS). Phase 1 was fed in pellet form and phases 2 and 3 were fed in meal form. Individual pigs were weighed, and feed disappearance was recorded on d 0, 7, 13, 20, 22, 32, and 41 to determine ADG, ADFI, and G:F.

On d 25, two pigs per pen (those closest to the average weight of the pen) were injected intramuscularly in the neck with 20 µg *Escherichia coli* (*E. coli*) LPS per kg BW. An additional pig in each pen was injected with 2 mL of saline to serve as a control. The LPS (*Escherichia coli* serotype O55:B5, Sigma Aldrich, Saint Louis, MO) was dissolved in sterile 0.9% NaCl solution. Body temperature was taken from all 3 pigs prior to the injection (0 h) and at 2, 4, 6, and 12 h after injection. A blood sample was taken from pigs injected with the *E. coli* LPS challenge one day prior to the challenge (d 24) and 4 h after the *E. coli* LPS injection to determine immune response criteria. Blood samples were centrifuged at 4°C at 1800 × g for 30 min and, then serum was frozen in separate aliquots for later cytokine analyses.

For IL-1β analysis, samples were analyzed in triplicate within a single assay. Serum concentrations of IL-1β were determined utilizing a porcine IL-1β ELISA kit per the instructions of the manufacturer (R & D Systems, Minneapolis, MN). The dynamic range of the assay was 39.1 to 2,500 pg/mL with a sensitivity of 13.6 pg/mL. For TNF-α analysis, samples were analyzed in triplicate within a single assay. Serum concentrations of TNF-α were determined

using a porcine TNF-α ELISA kit per the instructions of the manufacturer (R & D Systems, Minneapolis, MN). The dynamic range of the assay was 23.4 to 1,500 pg/mL with a sensitivity of 5.0 pg/mL. Any sample with values outside the dynamic range of the assay was diluted and re-analyzed in triplicate.

Experiment 2

This study was conducted at the New Fashion Pork Research Nursery in Jackson, MN. At weaning, pigs were moved to and housed in a temperature-controlled nursery facility. Each pen (1.89 × 3.05 m) consisted of plastic-grated flooring, one cup waterer, and one 3-hole stainless steel self-feeder. Access to feed and water was provided *ad libitum.* Pigs were allowed approximately 0.262 m^2/pig. A total of 1,056 weaned pigs [PIC TR4 × (Fast LW × PIC L02) initially 7.3 ± 0.09 kg] were used in a 46-d nursery trial. There were 22 pigs per pen (equal mixed sex) and 12 replications per treatment. Pens of pigs were randomly assigned to 1 of 4 dietary treatments in a completely randomized design. The dietary treatments included increasing levels of omega-3 fatty acids (0, 0.75, 1.5, and 3% O3 Trial Feed; Table 5). Experimental diets were fed across 4 phases and were corn-soybean meal based. Pigs were fed on a feed budget with Phase 1, 2, 3, and 4 provided at 2.7, 3.8 7.3, and 14.5 kg per pig, respectively. Phase 1, 2, and 3 diets were formulated to 1.40% SID Lys and the phase 4 diet was formulated to 1.34% SID Lys. All other nutrients were formulated to meet or exceed NRC (2012) requirement estimates. All diets were manufactured at the New Fashion Pork Feed Mill (Estherville, IA) and fed in meal form. Pens of pigs were weighed and feed disappearance was recorded weekly during the course of this study to determine ADG, ADFI, and G:F. Cotton ropes were placed in each pen on d 7, 14, 21, 28, 35, and 42 to determine presence of the North American and European PRRSV strains in oral fluid samples. A new rope was placed in each pen for 15 min on each sample day

and then oral fluids collected from 6 random pens were pooled for qRT-PCR testing. Samples were processed at the University of Minnesota Veterinary Diagnostic Laboratory using a commercially available qRT-PCR kit (Thermo-Fisher NA/EU PRRSV PCR; Waltham, MA).

Experiment 3

This study was conducted at Seaboard Foods in northwest Oklahoma and southwest Kansas. At weaning, pigs were moved and housed in temperature-controlled, hotel style, nursery facilities. Each barn contained 2 rooms and each room contained 40 pens with 27 to 28 pigs per pen (approximately 1,100 pigs per room). Each pen (2.97 × 1.97 m) contained one nipple waterer and one 6-hole stainless steel self-feeder. Access to feed and water was provided *ad libitum.* Pigs were allowed approximately 0.201 m^2/pig. A total of 91,140 weaned pigs (DNA 600 × PIC 1050; initially 5.1 ± 0.05 kg), originating from a PRRSV-positive sow farms, were used across 8 nursery sites. Each site contained 5 barns with 2 rooms in each barn. Rooms of pigs were blocked by nursery site and allocated within sow source, to 1 of 2 dietary treatments with 40 groups (rooms) per treatment. The first treatment was a standard nursery diet program specific to the production system and did not contain O3 Trial Feed. The second treatment was the same standard nursery diets with 3% O3 Trial Feed. At placement, all pigs received 0.45 kg/pig of a common pre-starter diet containing no O3 Trial Feed. Pigs were then fed experimental diets across 3 phases (Table 6). Pigs were fed on a feed budget, receiving 2.7 kg/pig of phase 1 and 6.8 kg of phase 2 before being fed phase 3 for the remainder of the study. The SID Lys concentration was formulated to 1.35% for phase 1, 1.30% for phase 2, and 1.28% for phase 3. All other nutrients were formulated to meet or exceed NRC (2012) requirement estimates. All diets were corn-soybean meal-based and fed in pelleted form. O3 Trial Feed was added at the expense of corn while adjusting feed grade amino acids and enzymatically treated soybean meal to

maintain similar soybean meal levels and amino acid profiles. Diets for phase 1 and phase 2 were manufactured at Seaboard Feed Mill (Leoti, KS) and phase 3 was manufactured at Seaboard Feed Mill (Hugoton, KS). One truck load of weaned pigs was weighed each week. The truck weight was divided by the count of weaned pigs to create a weekly initial BW for pigs placed into the nursery. At the end of each nursery turn, pigs were weighed by truck loads to determine close-out weights for each room. Feed intake was determined by the difference between the amount of feed delivered and the feed remaining upon completion of the nursery group. This data was used to determine ADG, ADFI, and G:F. Adjusted ADG was calculated by adding total removal and mortality weight to the total gain to calculate adjusted total gain, which then was divided by pig days. After the shipping of each nursery room, all water and injectable treatment records were collected. Cotton ropes were placed in each room every other week to evaluate for the presence of the North American and European PRRSV strains in oral fluid samples. New ropes was placed in each room for 30 min on each sample day and then oral fluids collected from each rope were pooled to create 2 duplicate samples for each room. The oral fluid samples were then frozen and sent to Kansas State University Swine Lab and stored at -20°C. Samples were processed at Kansas State University Veterinary Diagnostic Laboratory using the Tetracore PRRS Multiplex real time PCR procedure.

Chemical analysis

In Exp. 1, phase 1 and 2 diet samples were collected at manufacturing, and phase 3 diet samples were collected from every fifth 22.7-kg bag using a feed probe to obtain a representative sample for each respective diet and phase. In Exp. 2, diet samples for each treatment were collected with a probe from feeders. Complete diet samples were stored at -20°C until they were homogenized, subsampled, and submitted for analysis. In Exp. 3, diet samples for each treatment

were collected weekly from feeders in each room throughout the study. Complete diet samples were sent to Kansas State University Swine Lab and stored at -20°C Samples were then subsampled to create a composite sample for each treatment and submitted for analysis. Samples of each dietary treatment were analyzed (NBO3 Technologies LLC; Manhattan, KS) for fatty acid profiles (Tables 3, 5, and 7). Also, a representative sample of O3 Trial Feed was collected within each experiment and submitted to NBO3 Technologies LLC (Manhattan, KS) for fatty acid analysis (Table 1).

Statistical Analysis

Growth performance (Exp. 1 and 2) and mortality (Exp. 2 only) data were analyzed as a completely randomized design with pen serving as the experimental unit. Linear and quadratic contrasts in response to increasing omega-3 fatty acids (increasing O3 Trial Feed), were measured among treatments. In Exp. 3, growth performance data were analyzed as randomized complete block design with room serving as the experimental unit. Models were fit with the nlme package of R (Version 4.0.0, R Foundation for Statistical Computing, Vienna, Austria). Mortality and medication data were analyzed using the GLIMMIX procedure of SAS (version 9.4, Cary, NC). Total removals and mortality data were analyzed assuming a binomial distribution with a logit link function. Medication data were analyzed using a Poisson distribution with an offset function using the log transformed number of days at risk for each experimental unit or count of pigs placed and data reported as count of injections per 1,000 pig days and count of injections per pig placed, respectively. In Exp. 1, cytokine and temperature data were analyzed using the lmer package of R with random effects of pen and plate. Values were calculated by subtracting the measured variable at each time point from the baseline level (h 0). Pearson correlation coefficients were determined using PerformanceAnalytics package of

R. Differences between treatments were considered significant at $P \leq 0.05$ and marginally significant at $0.05 < P \leq 0.10$.

Results

Chemical Analysis

For all 3 experiments, fatty acid analysis in treatment diets were similar to formulated values. As O3 Trial Feed increased in the diet, the level of omega-3 fatty acids increased and the omega-6:3 fatty acid ratio decreased (Tables 3, 5, and 7).

Experiment 1

In phase 1 (d 0 to 13), there was a tendency (linear, $P = 0.065$; Table 8) for increased ADG with increasing O3 Trial Feed. For ADFI, there was a quadratic ($P = 0.046$) effect with ADFI decreasing as O3 Trial Feed increased from 0 to 1% and then increasing as O3 Trial Feed increased up to 4%. There were no significant differences observed for G:F. On d 13, due to the numerical increase in ADG, increasing O3 Trial Feed increased BW (linear, $P = 0.042$). In phase 2 (d 13 to 22), there were no differences observed in ADG or G:F. However, increasing O3 Trial Feed increased ADFI as O3 Trial Feed increased up to 2% (quadratic, $P = 0.013$), with ADFI decreasing as O3 Trial Feed inclusion further increased. In Phase 3 (d 22 to 41), no differences were observed in ADG or ADFI. However, during this period, feeding increasing O3 Trial Feed improved (linear, $P = 0.046$) G:F. For overall growth performance (d 0 to 41), there were no significant differences observed in ADG, ADFI, or G:F.

Prior to the LPS challenge (h 0), average body temperature was 39.9 ± 0.28 °C. There was no interaction between treatment and time in change in body temperature with increasing O3 Trial Feed, nor main effect of O3 Trial Feed on temperature ($P > 0.10$; Figure 1). However, there was a main effect of time ($P < 0.001$; Figure 2). Pigs responded as expected with an increase in

body temperature at 2 h post LPS challenge with average body temperature 2 h post LPS challenge being 40.4 ± 0.65 °C. Then, body temperature decreased as time post-challenge increased. After 12 h post LPS challenge, body temperatures were close to baseline levels with an average body temperature of 39.9 ± 0.45 °C. Increased O3 Trial Feed did not influence IL-1β or TNF-α concentrations from baseline to 4 h post LPS challenge ($P > 0.10$). The average IL-1β baseline was 4.1 ± 1.68 pg/mL across all treatments with all values below 21.5 pg/mL. The average TNF-α baseline was 114.5 ± 0.24 pg/mL across all treatments with all values below 527.7 pg/mL.

Experiment 2

The North American strain of PRRSV was not detected in oral fluids taken from ropes that were placed in pens on d 7 and 14, but was detected on d 21, 28, 35, and 42. The European strain of PRRSV was undetectable on each collection day.

From d 0 to 14 and d 14 to 21, there were no differences observed for ADG or ADFI ($P > 0.10$; Table 9). However, from d 0 to 14, increasing O3 Trial Feed resulted in a tendency for an increase then decrease (quadratic, $P = 0.065$) in G:F with G:F improving through 0.75% and decreasing thereafter. From d 21 to 28 corresponding with the detection of PRRSV in the population, increasing O3 Trial Feed increased (linear, $P \leq 0.035$) ADG and ADFI and improved (linear, $P = 0.010$) G:F. From d 28 to 35, ADG (quadratic, $P = 0.009$) and G:F (quadratic, $P = 0.004$) decreased with increasing O3 Trial Feed, but then returned to control values at 3% O3 Trial Feed. During this period, increasing O3 Trial Feed increased (linear, $P = 0.016$) ADFI. Finally, from d 35 to 46, increasing O3 Trial Feed increased (linear, $P \leq 0.001$) ADG and ADFI, while also improving (linear, $P = 0.010$) G:F.

For overall growth performance (d 0 to 46), increasing O3 Trial Feed increased (linear, $P < 0.001$) ADG, resulting in pigs fed 3% O3 Trial Feed having the greatest growth rate. Increasing O3 Trial Feed also increased (linear, $P < 0.001$) overall ADFI and G:F. Percentages of total removals and mortality for the overall study decreased (linear, $P = 0.027$) with increasing O3 Trial Feed.

Experiment 3

Of the oral fluids taken from the ropes placed in each room, the North American strain of PRRSV was detected in 61 of the 80 rooms (76%) at the first sampling period one-week post-placement in the nursery. The North American strain of PRRSV was detected in 78 of the 80 rooms (98%) tested at the second sampling point, 3 weeks post-placement in the nursery, and all oral fluid samples from each room had detectable North American PRRSV for the remainder of the nursery turn. The European strain of PRRSV was not detected in any sample.

Overall, there were no differences observed in ADG, ADFI, or G:F between pigs fed the control diet or those fed 3% O3 Trial Feed ($P > 0.10$; Table 10). Similarly, there were no differences observed for adjusted ADG or G:F ($P > 0.10$). There were also no main effects of sow flow between pigs fed the control diets or those fed diets containing 3% O3 Trial Feed ($P > 0.10$). Pigs fed control diets had reduced ($P < 0.001$) total removals and mortalities compared to pigs fed diets containing 3% O3 Trial Feed.

Pigs fed diets containing 3% O3 Trial Feed had fewer ($P < 0.001$; Table 10) total injections per pigs placed compared to pigs fed diets without O3 Trial Feed. However, there were no significant differences observed in the total number of injections given per 1,000 pig days ($P > 0.10$). 95-97% of the medication administered was enrofloxacin with the remainder of the medication given being dexamethasone, and ceftiofur hydrochloride.

Data were analyzed to understand the potential correlation in PRRSV status and growth performance. A higher cycle threshold (Ct) value means that there is less detectable viral genomic material present. In contrast, a lower Ct value means that there is more viral genomic material present in the sample. For oral fluids collected 1-week post-placement, ADG was significantly and positively correlated with Ct values ($P < 0.01$; r = 0.44; Table 11). This shows that 19% (R^2) of the variability in ADG is explained by the Ct value 1-week post-placement. Similarly, there was a significant and positive correlation between ADFI and Ct values for oral fluids collected 1-week post-placement ($P < 0.05$; r = 0.33). This explains that 11% of the variability in ADFI is described by Ct values 1-week post-placement. However, there was no correlation between G:F and Ct values ($P > 0.10$). These results show that decreased Ct values at placement (greater amount of viral RNA) are associated with reductions in ADG and ADFI. There was a significant and negative correlation between total removals and mortality and Ct values for oral fluids collected 1-week post-placement ($P < 0.01$; r = -0.67), and a significant negative correlation between total removals and mortality and Ct values for oral fluids collected 3-weeks post-placement ($P < 0.05$; r = -0.30). This shows that 45% and 9% of the variability in total removals and mortality is explained by Ct values 1- and 3-weeks post placement, respectfully. There was no evidence of correlation between oral fluid samples collected late in the nursery phase with total removals and mortality ($P > 0.10$).

Discussion

As productivity in the swine industry increases, so has prevalence of bacterial and viral diseases (Davies, 2011). Thus, it is important to continue to improve the interaction between health and nutrition to maximize production efficiency and immune function (Carroll et al.,

2003). One nutritional strategy to potentially influence the immune response is to incorporate omega-3 fatty acids (alpha-linolenic acid) into nursery diets.

Polyunsaturated fatty acids (PUFA) are categorized into two classes: omega-6 and omega-3 fatty acids. Omega-6 fatty acids are derived from linoleic acid and omega-3 fatty acids are derived from alpha-linolenic acid. Both fatty acids cannot be synthesized in the body; therefore, they must be added to the diet. Omega-3 fatty acids are converted into eicosatetraenoic acid (EPA) and later converted into docosahexaenoic acid (DHA; Teitelbaum and Walker, 2001). Omega-6 fatty acids are converted into arachidonic acid. Typical western swine diets contain high levels of arachidonic acid and low levels of EPA and DHA (Liu, 2015). Furthermore, the addition of omega-3 fatty to the diet at the expense of omega-6 fatty acids increase the incorporation of EPA and DHA into the phospholipid layer of cells which can play a role in the regulation of inflammation and immune response through the management of eicosanoid production (Calder, 2009).

Eicosanoids, such a prostaglandin, thromboxanes, and leukotrienes, are products of arachidonic acid. These eicosanoids are the prime mediators in regulating the intensity of inflammation in the body (Calder, 2009). Therefore, omega-6 fatty acids produce pro-inflammatory responses. Conversely, omega-3 fatty acids have anti-inflammatory properties. They decrease the production of eicosanoids, increase anti-inflammatory resolvins through EPA and DHA, and decrease the production of pro-inflammatory cytokines, such as IL-1β and TNF-α (Calder, 2010). When more omega-3 fatty acids are incorporated into the diet, greater amounts of EPA and DHA are incorporated into the phospholipid layer of the cell (Chapkins et al., 1991), and when more EPA and DHA are present, less arachidonic acid is present in the cell, suppressing eicosanoid synthesis. Therefore, lowering the omega-6:3 fatty acid ratio has shown

to lessen the intensity of the inflammatory response allowing the body to allocate energy and nutrients away from stimulating the immune system and more towards growth performance, especially during a health challenge (Liu et al., 2003; Duan et al., 2014).

The benefits of incorporating omega-3 fatty acids on growth performance can be variable. It is thought that the benefit omega-3 fatty acids bring is only present during a heath challenge. Li et al. (2014) observed no differences in growth performance with the incorporation of omega-3 fatty acids, sourced from a marine omega-3 product, when there was no bacterial or environmental challenge present. These results are similar to those of Exp. 1, where no differences in overall growth performance were observed with increasing levels of omega-3 fatty acids in the diet. Liu et al. (2003) observed no differences in growth performance with the incorporation of omega-3 fatty acids, sourced via fish oil, prior to an LPS challenge. However, pigs fed diets containing fish oil had improved ADG and ADFI after pigs were administered an LPS challenge. These results reflect those of Exp. 2, where pigs fed increasing levels of omega-3 fatty acids had increased ADG, ADFI, and G:F once the prevalence of viral shedding of PRRSV increased, as evidenced by the PRRSV positive oral fluid results on d 21. After PRRSV was detected, a linear benefit in growth performance was found with increasing levels of omega-3 fatty acids. The improvement found after the viral challenge resulted in increased overall growth performance and reduced total removals and mortality. Huber et al. (2018) explained that when the omega-6:3 fatty acid ratio is reduced, through the addition of omega-3 fatty acids, energy and other nutrients can be allocated more towards growth performance and less towards maintenance because less energy is needed to mediate inflammation. The benefits observed in Exp. 2 are thought to be because pigs were fed omega-3 fatty acids long enough to allow omega-3 fatty acids to enrich cells with EPA and DHA, which improved immune function. However, in Exp. 3,

there was no improvement in overall growth performance in pigs fed diets containing an increase in omega-3 fatty acids. Because a large portion of pigs in this experiment were positive for PRRSV at arrival to the nursery, there was not enough time for omega-3 fatty acids to enrich the cell and influence immune response.

The activation of the immune system during a health challenge includes many interactions between inflammatory responses, different cell types, and antigens. The complexity of the immune system makes it almost impossible to study all at once. Therefore, researchers analyze different components separately (Teitelbaum and Walker, 2001). LPS is a common and practical model to use to evaluate the overall acute phase immune response (Llamas Moya et al., 2006). LPS is an endotoxin found in the outer membrane of Gram-negative bacteria. During an LPS challenge, macrophages and monocytes produce pro-inflammatory cytokines, which can lead to an induced fever and a reduction in feed intake (Carroll et al, 2003).

IL-1β is a pro-inflammatory cytokine responsible for several mechanisms as part of the immune response. Elevated IL-1β concentrations lead to the activation of the hypothalamic-pituitary-adrenal axis, which produces prostaglandin E2, resulting in a fever in the body (Song and Horrobin, 2004). Liu et al. (2003), observed a decrease in plasma IL-1β concentrations in pigs fed diets containing omega-3 fatty acids, sourced by fish oil, compared to pigs fed diets containing corn oil. The increase in dietary omega-3 fatty acids is thought to have led to a decrease in eicosanoid production, therefore, less production of pro-inflammatory cytokines. In Exp. 1 of our studies, there were no differences observed in IL-1β concentrations in pigs fed diets containing increasing levels of omega-3 fatty acids in the diet.

TNF-α is another pro-inflammatory cytokine that induces a fever during a health challenge by activating the hypothalamic-pituitary-adrenal axis (Wright et al., 2000). Carroll et

al. (2003) observed a decrease in TNF-α concentrations in the serum with increasing levels of omega-3 fatty acids, sourced via menhaden fish oil, compared to pigs fed diets containing corn oil. Similarly, Gaines et al. (2003) observed a reduction in TNF-α serum concentrations with the addition of omega-3 fatty acids, sourced by menhaden fish oil, in the diet compared to pigs fed diets containing corn oil. However, the results from Exp. 1 did not find a similar response, as increasing omega-3 fatty acids in the diet did not influence TNF-α in the serum compared to the baseline concentration.

The stimulation of a febrile response, due to an LPS challenge, can occur as soon as 15 min post LPS-challenge (Wyns et al., 2015). When an LPS challenge is administered, the macrophages produce pro-inflammatory cytokines that stimulate a fever (Johnson and von Borell, 1994). Huber et al. (2018) observed a reduced body temperature 2 h post-LPS challenge compared to controls in pigs fed diets containing omega-3 fatty acids, via fish oil. However, in Exp. 1, increasing dietary omega-3 fatty acids had no effect on body temperature post-LPS challenge. Body temperatures did increase 2 h post-LPS challenge and then gradually began to decrease back to normal levels until pigs were back to baseline body temperatures ~12 h post-LPS challenge. These results indicate that the LPS challenge was executed properly; however, no differences in body temperature or cytokine production in the serum were observed.

Though there are many studies confirming the benefits of omega-3 fatty acids on immune function during a health challenge, there were no benefits observed during the LPS challenge in Exp. 1. One hypothesis that could explain this is that the source of omega-3 fatty acids used for all the current experiments was less efficient at being converted to EPA and DHA. Menhaden fish oil, an alternative source of omega-3 fatty acids used in other trials, is highly concentrated with EPA (Ratnayake et al., 1988). Past research observed a reduction in pro-inflammatory

cytokines and body temperature with increasing levels of dietary omega-3 fatty acids during an LPS challenge when the source of omega-3 fatty acid was fish oil based (Carroll et al., 2003; Gaines et al., 2003; Liu et al., 2003; Huber et al., 2018). In Exp. 1., O3 Trial Feed may not have provided enough EPA and DHA to pigs to impact inflammation and the febrile response during the short-lived LPS challenge. Therefore, increasing omega-3fatty acids in the diet did not impact pro-inflammatory cytokines and body temperature during the LPS.

PRRSV is one of the most impactful pathogens affecting swine production globally (Montaner-Tarbes et al., 2019). The viral envelope glycoproteins are the first to come in contact with host cell receptors to initiate infection and stimulate the immune system (Shi et al., 2015). Once infected, typical symptoms of PRRSV virus include severe respiratory disease in newborns and growing pigs and reproductive failure in sows. However, different PRRSV strains and immune status of host cells can play a role in the severity of the infection (Lunney et al., 2016). The innate immune system is the first response to prevent viral replication of PRRSV. The goal is to stimulate a strong adaptive immune response to fight against the infectious agents PRRSV virus enriches within the host cell (Montaner-Tarbes et al., 2019). Additionally, PRRSV virus has mechanisms that suppress the production of cytokines that help strengthen the innate immune response (Van Reeth et al., 1999).

PUFAs have shown altering responses depending on the type of disease being evaluated and the omega-6:3 ratio of the diet. Studies by Walter et al. (2019) concluded that the competency of PUFA, either omega-6, omega-3, or the ratio of the two, depends on the dosage, the amount of time given, and pathogen present. However, little research has been done on the effects of supplementing PUFAs on respiratory health in swine. As previously stated, results from Exp. 2 indicated that once pigs tested positive for PRRSV virus on d 21, an improvement in

growth performance was observed in pigs fed increasing levels of dietary omega-3 fatty acids. This ultimately led to an improvement in overall growth performance and a reduction in total removals and mortality. However, in Exp. 3, no differences in growth performance were observed with the inclusion of omega-3 fatty acids in the diet in pigs that tested positive for PRRSV virus for the duration of the nursery turn. This supports a component of the conclusion stated by Walters et al. (2019), that omega-3 fatty acids might need to be fed for a certain period of time before eliciting a benefit. This would allow more EPA and DHA to enter the phospholipid layer of cells, which would improve the immune system and develop cells that are better prepared for a health challenge, like PRRSV. However, further research is needed to determine the amount of time needed to enrich cells with EPA and DHA in order to improve immune responses and prepare the host cells for invading diseases and infections. Potential research could be conducted to supplement omega-3 fatty acids into gestation diets of PRRSV positive sow flows, to pass on the benefits of EPA and DHA to subsequent offspring and better prepare host cells and the immune system before pigs are exposed to circulating infections and diseases.

Diagnostic testing using real-time PCR is a common method to detect PRRSV (Trevisan et al., 2019). When interpreting the results of a PCR assay, a higher cycle threshold (Ct) value means that there is less detectable viral genomic material present. In contrast, a lower Ct value means that there is more viral genomic material present in the sample. In Exp. 3, there was a highly significant, positive correlation between ADG and Ct values for oral fluids collected 1-week post-placement into the nursery and, also a significant, positive correlation between ADFI and Ct values for oral fluids collected 1-week post-placement. These results suggest that as Ct values for oral fluid samples collected early in the nursery turn decrease (more viral RNA

present), so does ADG and ADFI. There was a highly significant, negative correlation between total removals and mortality and Ct values for oral fluids collected 1-week post-placement and a significant negative correlation between total removals and mortality and Ct values for oral fluids collected 3-weeks post-placement. These results indicate that as Ct values decrease (greater viral RNA present), total removals and mortality increase. There was no evidence of a correlation between growth and removals and mortality for oral fluid samples collected later in the nursery stage. This could be partially explained by the fact that all oral fluid samples collected at 3- and 4-weeks post-placement were PRRSV-positive with Ct values ranging from 27 to 36, whereas oral fluid samples collected 1 and 2 weeks post-placement included PCR-negative samples which assumed a Ct value of 40, which was the PCR upper detection limit. These results illustrate that high levels of PRRSV genetic material in oral fluid samples early in the nursery phase results in reduced gain and feed intake as well as greater removals and mortality. It is also important to consider that all pigs used in Exp. 3 were sourced from PRRSV-positive sow farms. Magalhaes et al. (2022) explains that there is a strong association between the health of the sow farm and down stream pig performance and mortality and the presence of PRRSV in the sow farm can be a major risk factor on wean-to-finish mortality. The correlation of PRRSV CT and growth performance we observed supports this research. Further research is needed to better explain the relationship between Ct values, growth performance, and mortality rate to understand whether interventions could influence viral shedding and how that could make an impact on growth performance during a heath challenge.

In summary, increasing levels of omega-3 fatty acids in the diet, through the inclusion of O3 Trial Feed, did not improve growth performance or immune response in healthy pigs given an LPS-challenge. This is thought to be due to the high health status of pigs used and O3 Trial

Feed not providing enough EPA and DHA to influence the immune response during a short LPS challenge. If omega-3 fatty acids are fed before a natural PRRSV break, growth performance, immune response, and mortality can be improved during the health challenge. However, it appears that the inclusion of omega-3 fatty acids in the diet will not be beneficial to growth, immune response, or mortality if fed after a health challenge outbreak. Research on the timing of omega-3 fatty acid supplementation and onset of a disease challenge needs to be evaluated.

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Table 2.1 Analyzed fatty acid composition of O3 Trial Feed[1]

Fatty acid, %	Exp. 1	Exp. 2	Exp. 3
Total fatty acids	23.29	23.77	20.13
Total fat	25.87	26.41	22.37
Omega-6:3	0.36	0.37	0.46
C16:0	1.43	1.43	1.38
C18:1n9c	4.17	4.33	4.67
C18:2n6c[3]	4.28	4.41	4.03
C18:3n3[4]	12.09	12.20	8.73

[1]A representative sample of O3 Trial Feed was collected within each experiment and submitted to NBO3 Technologies LLC (Manhattan, KS) for fatty acid analysis.

[3]Major omega-6 fatty acid.

[4]Major omega-3 fatty acid.

Table 2.2 Composition of experimental diets in Exp. 1 (as-fed basis)[1]

Ingredient, %	Phase 1	Phase 2	Phase 3
Corn	40.32	56.37	66.12
Soybean meal (46.5% CP)	18.98	24.31	29.00
Dried whey	25.00	---	---
Whey permeate, 80% lactose	---	9.00	---
Corn DDGS[2], 7.5% oil	5.00	---	---
Enzymatically treated soybean meal[3]	5.00	5.00	---
Corn oil	2.00	1.00	1.00
Calcium carbonate	0.50	0.75	0.75
Monocalcium P (21% P)	0.80	1.10	0.95
Sodium chloride	0.30	0.55	0.60
L-Lys-HCl	0.55	0.55	0.53
DL-Met	0.25	0.25	0.22
L-Thr	0.22	0.25	0.23
L-Trp	0.05	0.04	0.04
L-Val	0.17	0.17	0.16
Vitamin premix with phytase	0.25	0.25	0.25
Trace mineral premix	0.15	0.15	0.15
Choline chloride	0.04	---	---
Zinc oxide	0.41	0.25	---
Phytase[4]	0.02	0.02	0.02
O3 Trial Feed[5]	+/-	+/-	+/-
Calculated analysis			
Standardized ileal digestible (SID) AA, %			
Lys	1.40	1.35	1.30
Ile:Lys	55	54	54
Leu:Lys	110	108	114
Met and Cys:Lys	58	58	58
Thr:Lys	64	63	64
Trp:Lys	19.5	19.1	18.9
Val:Lys	70	70	71
His:Lys	32	34	36
Total Lys, %	1.53	1.48	1.44
NE, kcal/kg	2,588	2,522	2,489
SID Lys:NE, g/Mcal	5.41	5.35	5.22
CP, %	20.5	20.3	20.2
Ca, %	0.65	0.69	0.64
P, %	0.66	0.63	0.58
Standardized total tract digestible (STTD) P, %	0.58	0.51	0.46
Ca:P	0.99	1.10	1.10
EFA, %	2.34	1.91	2.06
Alpha linolenic acid %	0.08	0.08	0.08
Linoleic acid %	2.26	1.83	1.98

Omega-6:3	27.3	23.0	24.4

[1]Phase 1 diets were fed from d 0 to 13 (approximately 5.8 to 7.3 kg BW). Phase 2 diets were fed from d 13 to 22 (approximately 7.3 to 11.5 kg BW). Phase 3 was fed from d 22 to 41 (approximately 11.5 to 22.8 kg BW).

[2]Dried distillers grains with solubles.

[3]Hamlet Protein, Findlay, OH.

[4]Quantum Blue 5G (AB Vista, Marlborough, UK) provided a release of 0.13% STTD P with 411 FTU/kg.

[5]O3 Trial Feed was added at 0, 1, 2, 3, and 4% at the expense of corn from the experimental diets (NBO3 Technologies LLC, Manhattan, KS).

Table 2.3 Analyzed fatty acid composition of experimental diets in Exp. 1[1]

	O3 Trial Feed, %				
Fatty acid, %	0	1	2	3	4
Phase 1 (d 0 to 13)					
Total fatty acids	4.21	4.34	4.72	4.96	4.89
Total fat	4.68	4.82	5.25	5.51	5.44
Omega-6:3	18.57	9.56	6.44	4.94	4.07
C16:0	0.61	0.61	0.64	0.66	0.64
C18:1n9c	1.01	1.01	1.10	1.15	1.12
C18:2n6c[2]	2.26	2.25	2.36	2.11	2.32
C18:3n3[3]	0.12	0.24	0.37	0.49	0.57
Phase 2 (d 13 to 22)					
Total fatty acids	3.95	3.97	4.64	4.53	4.56
Total fat	4.39	4.41	5.15	5.04	5.06
Omega-6:3	15.03	9.60	5.38	4.29	3.78
C16:0	0.57	0.56	0.61	0.59	0.58
C18:1n9c	0.89	0.88	1.03	0.98	0.98
C18:2n6c	2.14	2.11	2.31	2.21	2.17
C18:3n3	0.14	0.22	0.43	0.52	0.58
Phase 3 (d 22 to 41)					
Total fatty acids	4.47	4.77	5.01	4.49	4.71
Total fat	4.96	5.30	5.61	4.99	5.23
Omega-6:3	20.69	10.11	6.71	4.86	3.80
C16:0	0.66	0.67	0.68	0.61	0.61
C18:1n9c	1.06	1.11	1.18	0.99	1.03
C18:2n6c	2.36	2.46	2.53	2.17	2.20
C18:3n3	0.11	0.24	0.38	0.45	0.58

[1]Complete diets contained trace levels of C12:0, C14:0, C15:0, C16:1n7, C17:0, C18:0, C18:1n9t, C18:1n7c, C18:3n6, CLA 9c, 11t (n7), C20:0, C20:2n6, C22:0, C23:0, and C24:0 of < 0.10%. Other fatty acids levels were too low to be detected in the analysis.

[2]Major omega-6 fatty acid.

[3]Major omega-3 fatty acid.

Table 2.4 Composition of experimental diets in Exp. 2 (as-fed basis)[1]

Ingredient, %	Phase 1	Phase 2	Phase 3	Phase 4
Corn	42.46	55.59	52.97	43.46
Soybean meal (46.5% CP)	18.99	19.99	26.75	26.52
AV-E digest[2]	17.45	16.05	3.90	---
Dried whey	2.25	---	---	---
Oat groats	5.00	---	---	---
Cereal blend[3]	7.95	3.00	1.40	---
Corn DDGS[4], 7.5% Oil	---	---	10.00	25.00
Beef tallow	2.50	2.00	1.00	1.00
Monocalcium P (21% P)	---	---	0.40	0.38
Limestone	0.40	0.40	1.15	1.48
Salt	---	0.16	0.55	0.63
L-Lys-HCl	0.45	0.49	0.58	0.56
DL-Met	0.19	0.22	0.32	0.22
L-Trp	0.05	0.06	0.05	0.04
L-Val	0.11	0.14	0.15	0.06
L-Ile	0.03	0.05	0.06	---
L- Thr	0.22	0.26	0.29	0.20
Tribasic copper chloride	0.04	0.04	---	---
Vitamin premix with phytase	0.25	0.25	0.25	0.25
Trace mineral premix	0.15	0.15	0.15	0.15
Choline chloride 60%	0.03	0.03	---	---
Zinc oxide	0.35	0.35	0.02	---
Vitamin E (20,000 IU)	0.05	0.01	0.01	---
Zinc[5]	---	---	---	0.06
LipoVital GL 90[6]	0.10	0.05	---	---
Blue dye	0.01	---	---	---
FXP[7]	0.20	0.10	---	---
AcidoMatrix GH[8]	0.50	0.50	---	---
N-Hance[7]	0.25	0.10	---	---
AlphaGal 280P[9]	0.03	0.01	---	---
Manganese[10]	0.02	0.02	0.02	0.01
O3 Trial Feed[11]	---	---	---	---
Calculated analysis				
Standardized ileal digestible (SID) amino acids, %				
Lys	1.40	1.40	1.40	1.34
Ile:Lys	59	59	60	60
Leu:Lys	111	113	120	141
Met and Cys:Lys	57	59	66	65
Thr:Lys	63	64	64	63
Trp:Lys	19.3	19.1	19.1	19.2
Val:Lys	71	72	72	72
His:Lys	36	36	36	40
Total Lys, %	1.62	1.62	1.58	1.53
NE NRC, kcal/kg	2,255	2,321	2,396	2,412

SID Lys:NE, g/Mcal	6.21	6.04	5.85	5.56
CP, %	23.9	23.5	23.1	24.1
Ca, %	0.87	0.80	0.79	0.80
P, %	0.61	0.58	0.56	0.57
Standardized total tract digestible (STTD) P, %	0.49	0.47	0.44	0.44
Ca:P	1.42	1.38	1.41	1.41
EFA, %	1.67	1.86	1.84	2.17
Alpha linolenic acid %	0.11	0.11	0.08	0.08
Linoleic acid %	1.6	1.75	1.75	2.08
Omega-6:3	15.1	16.5	20.8	25.3

[1]Pigs were fed on a feed budget (kg/pig): Phase 1, 2.7; Phase 2, 3.6; Phase 3, 7.3; and Phase 4, 14.5 lb per pig.

[2]XFE Products, Des Moines, IA.

[3]Quincy Farm Products, Quincy, IL.

[4]Dried distillers grains with solubles.

[5]New Fashion Pork Custom Zinc: 210,000 ppm.

[6]Berg & Schmidt Functional Lipids, Hamburg, Germany.

[7]Ani-Tek, Social Circle, GA.

[8]Novus International, Saint Charles, MO.

[9]Kindstrom-Schmoll Inc., Eden Prairie, MN.

[10]New Fashion Pork Custom Manganese - 200,000 ppm.

[11]O3 Trial Feed was added at 0.75, 1.5, and 3% at the expense of corn and soybean meal to form the experimental diets.

Table 2.5 Analyzed fatty acid composition of experimental diets in Exp. 2[1]

	O3 Trial Feed, %			
Fatty acid, %	0.00	0.75	1.50	3.00
Phase 1				
Total fatty acid	7.21	8.36	8.17	8.86
Total fat	8.01	9.28	9.07	9.84
Omega-6:3	14.34	10.23	7.65	4.05
C16:0	1.38	1.60	1.51	1.62
C18:1n9c	2.16	2.56	2.36	2.63
C18:2n6c[2]	2.02	2.17	2.22	2.17
C18:3n3[3]	0.14	0.22	0.29	0.54
Phase 2				
Total fatty acid	6.54	6.96	7.92	8.25
Total fat	7.27	7.74	8.80	9.16
Omega-6:3	14.36	9.94	6.44	4.18
C16:0	1.23	1.29	1.45	1.47
C18:1n9c	1.93	2.06	2.31	2.39
C18:2n6c	1.95	2.03	2.24	2.27
C18:3n3	0.14	0.21	0.36	0.55
Phase 3				
Total fatty acid	5.48	5.48	5.60	6.39
Total fat	6.08	6.09	6.23	7.10
Omega-6:3	14.45	8.85	6.46	4.10
C16:0	0.89	0.87	0.88	0.96
C18:1n9c	1.34	1.32	1.31	1.52
C18:2n6c	2.32	2.27	2.31	2.49
C18:3n3	0.16	0.26	0.36	0.61
Phase 4				
Total fatty acid	5.65	5.91	6.15	6.48
Total fat	6.28	6.57	6.83	7.20
Omega-6:3	15.34	10.16	7.53	5.53
C16:0	0.90	0.90	0.93	0.97
C18:1n9c	1.35	1.40	1.46	1.53
C18:2n6c	2.56	2.64	2.68	2.73
C18:3n3	0.17	0.26	0.36	0.50

[1]Complete diets contained trace levels of C12:0, C14:0, C15:0, C16:1n7, C17:0, C18:0, C18:1n9t, C18:1n7c, C18:3n6, CLA 9c, 11t (n7), C20:0, C20:2n6, C22:0, C23:0, and C24:0 of < 0.10%. Other fatty acids levels were too low to be detected in the analysis.

[2]Major omega-6 fatty acid.

[3]Major omega-3 fatty acid.

Table 2.6 Composition of experimental diets in Exp. 3 (as-fed basis)[1]

	Phase 1		Phase 2		Phase 3	
	O3 Trial Feed, %		O3 Trial Feed, %		O3 Trial Feed, %	
Ingredient, %	0	3	0	3	0	3
Corn	43.97	40.58	59.30	56.13	56.72	53.30
Soybean meal (47%)	20.00	20.00	32.50	32.50	37.25	37.83
Base mix[2]	21.13	21.13	---	---	---	---
Enzymatically treated soybean meal[3]	6.09	6.42	0.78	1.09	---	---
Lucrafit TM 50[4]	2.15	2.15	1.25	1.25	---	---
Monocalcium phosphate[5]	1.52	1.58	1.15	1.17	0.84	0.86
Beef tallow	0.86	0.86	1.64	1.54	2.48	2.39
Salt	0.73	0.73	0.60	0.60	0.40	0.40
Limestone, ground	0.41	0.40	0.54	0.54	0.66	0.66
L-Lys	0.68	0.64	0.65	0.61	0.46	0.41
L-Trp	0.10	0.10	0.10	0.09	0.08	0.07
L-Val	0.06	0.10	0.09	0.10	---	---
L-Thr	0.20	0.20	0.22	0.20	0.17	0.15
Vitamin premix-nursery[6]	0.05	0.05	0.05	0.05	---	---
Vitamin premix-grow-finish[7]	---	---	---	---	0.08	0.08
Trace mineral premix[8]	0.10	0.10	0.08	0.08	0.08	0.08
Phytase[9]	0.06	0.06	0.06	0.06	0.06	0.06
Copper chloride	0.03	0.03	0.03	0.03	0.03	0.03
Choline chloride	0.03	0.03	---	---	---	---
Zinc oxide 72%	0.32	0.32	0.32	0.32	---	---
FXP[10]	0.40	0.40	0.20	0.20	---	---
Liquid Methionine[11]	0.30	0.31	0.21	0.21	0.17	0.15
N-hance[10]	0.30	0.30	---	---	---	---
CTC 100g	0.25	0.25	---	---	0.25	0.25
Oxytetracycline 200g	---	---	0.13	0.13	---	---
Denagard 10g	0.18	0.18	---	---	0.18	0.18
Synthetic red dye	---	0.01	---	0.01	---	0.01
Synthetic blue dye	0.01	---	0.01	---	0.01	---
O3 Trial Feed[12]	---	3.00	---	3.00	---	3.00
Calculated analysis						
Standardized ileal digestible (SID) amino acids, %						
Lys	1.35	1.35	1.30	1.30	1.28	1.28
Ile:Lys	58	58	58	58	64	64
Met and Cys:Lys	58	58	58	58	58	58
Thr:Lys	64	64	64	64	64	64
Trp:Lys	24	24	24	24	24	24
Val:Lys	72	72	72	72	70	70
Total Lys, %	1.46	1.46	1.43	1.43	1.42	1.43
NE NRC, kcal/kg	2,630	2,620	2,680	2,680	2,770	2,770
SID Lys:NE, g/Mcal	5.15	5.19	4.83	4.83	4.61	4.61
CP, %	21.79	22.68	21.51	22.00	22.58	23.14
Crude fat, %	4.10	4.42	4.10	4.52	4.80	5.22

Ca, %	0.68	0.68	0.67	0.67	0.70	0.70
P, %	0.67	0.68	0.59	0.59	0.54	0.54
Standardized total tract digestible (STTD) P, %	0.50	0.50	0.47	0.47	0.43	0.43

[1]Pigs were fed experimental diets on a feed budget with Phase 1 and 2 provided at 2.7 and 6.8 kg per pig. Phase 3 was provided for the remainder of the study.

[2]Quincy Farms, Quincy IL.

[3]HP300; Hamlet Protein, Findley, OH.

[4]Purina Animal Nutrition, Arden Hills, MN.

[5]NexFos; The Mosaic Company, Plymouth, MN.

[6]Provided per kg of premix: 24,250,869 IU vitamin A; 3,747,862 IU vitamin D_3; 220,462 IU vitamin E; 6614 mg menadione; 19,842 mg riboflavin; 97,003 mg niacin; 79,366 mg pantothenic acid; 93 mg vitamin B_{12}; 220 mg biotin; 3,527 mg folic acid; 6,614 mg pyridoxine; 600 mg selenium; and 32,099,551 BXU xylanase.

[7]Provided per kg of premix: 8,818,498 IU vitamin A; 1,543,237 IU vitamin D_3; 44,092 IU vitamin E; 3,307 mg menadione; 6,614 mg riboflavin; 27,558 mg niacin; 26,455 mg pantothenic acid; 26 mg vitamin B_{12}; 375 mg selenium; and 25,623,400 BXU xylanase.

[8]Provided per kg of premix: 187,500 mg Zn from zinc oxide and zinc sulfate, 95,000 mg Fe from ferrous sulfate, 31,250 mg Mn from manganese sulfate and manganese oxide, 18,750 mg Cu from copper sulfate, and 750 mg I from calcium iodate.

[9]Axtra PHY Gold (Danisco Animal Nutrition, Cedar Rapids, IA) was included to provide approximately 1,730 FTU/kg in phase1, 1,875 FTU/kg in phase 2, and 1,920 FTU/kg in phase 3 providing an estimated release of 0.08, 0.11, and 0.12% STTD P, for phase 1, 2, and 3, respectively.

[10]Ani-Tek, Social Circle, GA.

[11]Alimet; Novus International Inc., Saint Charles, MS.

[12]O3 Trial Feed (NBO3 Technologies LLC, Manhattan, KS) was added at 3% at the expense of corn while adjusting feed grade amino acids and enzymatically treated soybean meal to maintain similar soybean meal levels and amino acid profiles.

Table 2.7 Analyzed fatty acid composition of experimental diets in Exp. 3[1]

	O3 Trial Feed, %	
Fatty acid, %	0	3
Total fatty acid	5.14	5.44
Total fat	5.72	6.05
Omega-6:3	14.74	4.57
C16:0	0.99	0.95
C18:1n9c	1.41	1.44
C18:2n6c[3]	1.67	1.79
C18:3n3[4]	0.11	0.39

[1]Composites of complete diets contained trace levels of C6:0, C8:0, C10:0, C12:0, C14:0, C14:1n5, C15:0, C16:1n7, C17:0, C18:0, C18:1n9t, C18:1n7t, C18:1n7c, C18:3n6, CLA 9c, 11t (n7), C20:0, C201n9, C20:2n6, C22:0, C23:0, C24:0, and C24:1n9 of < 0.10%. Other fatty acids levels were too low to be detected in the analysis.

[3]Major omega-6 fatty acid.

[3]Major omega-3 fatty acid.

Table 2.8 Effects of omega-3 fatty acids, sourced by O3 Trial Feed, on nursery pig performance and cytokine production in Exp. 1[1]

	O3 Trial Feed,[2] %						*P* =	
Item	0	1	2	3	4	SEM	Linear	Quadratic
BW, kg								
d 0	5.8	5.7	5.8	5.8	8.8	0.03	0.366	0.927
d 13	7.2	7.0	7.4	7.3	7.4	0.12	0.042	0.831
d 22	11.6	11.1	11.8	11.8	11.4	0.18	0.361	0.441
d 41	23.0	21.8	23.3	22.9	22.7	0.32	0.534	0.975
Phase 1 (d 0 to 13)								
ADG, g	110	97	126	112	126	8.08	0.065	0.732
ADFI, g	196	174	199	205	237	12.01	0.003	0.046
G:F, g/kg	556	551	630	556	554	34.3	0.993	0.245
Phase 2 (d 13 to 22)								
ADG, g	480	447	482	489	446	12.0	0.502	0.281
ADFI, g	600	608	633	621	576	15.6	0.487	0.013
G:F, g/kg	802	739	763	791	774	16.1	0.947	0.116
Phase 3 (d 22 to 41)[3]								
ADG, g	602	567	606	585	595	12.5	0.914	0.544
ADFI, g	954	950	940	916	926	21.8	0.188	0.869
G:F, g/kg	631	602	645	639	643	9.9	0.046	0.646
Overall (d 0 to 41)								
ADG, g	415	384	425	408	411	7.8	0.497	0.695
ADFI, g	630	615	634	616	627	11.3	0.889	0.726
G:F, g/kg	659	625	670	662	657	9.5	0.264	0.906
IL-1β change, pg/mL[4]	506.1	615.5	777.8	430.6	543.2	147.8	0.796	0.308
TNF-α change, pg/mL[5]	5,002	6,093	5691	5,628	4,463	1,001.8	0.623	0.272

[1]A total of 350 pigs (Line 241 × 600, DNA, Columbus NE; initially 5.8 ± 0.03 kg)) were used with 5 pigs per pen and 14 replications per treatment and were fed trial diets for a 41-day period.

[2]Omega-6:3 ratios for the five treatments within each phase were: Phase 1 (27.3:1, 11.6:1, 7.4:1, 5.4:1, 4.3:1); Phase 2 (23.0:1, 9.6:1, 6.1:1, 4.5:1, 3.6:1); and Phase 3 (24.4:1, 10.1:1, 6.5:1, 4.8:1, 3.8:1), respectively (NBO3 Technologies LLC, Manhattan, KS).

[3]Two pigs per pen were injected intramuscularly with 20 micrograms *Escherichia coli* (*E. coli*) LPS per kg BW on d 25 to measure immune responses.

[4] Change in IL-1β from baseline (0 h) to 4 h after intramuscular injection with 20 µg *Escherichia coli* LPS per kg BW on d 25. The average IL-1β baseline was 4.1 ± 1.68 pg/mL across all treatments and with all baseline values below 21.5 pg/mL.

[5]Change in TNF-α from baseline (0 h) to 4 h after intramuscular injection with 20 µg *Escherichia coli* LPS per kg BW on d 25. The average TNF-α baseline was 114.5 ± 0.24 pg/mL across all treatments and with all baseline values below 527.7 pg/mL.

Table 2.9 Effects of omega-3 fatty acids, sourced by O3 Trial Feed on growth performance and total removals and mortality in PRRSV-challenged nursery pigs in Exp. 2[1]

	O3 Trial Feed,[2] %					*P* =	
Item	0.00	0.75	1.50	3.00	SEM	Linear	Quadratic
BW, kg							
d 0	7.3	7.3	7.3	7.3	0.09	0.315	0.601
d 14	8.9	8.9	9.1	8.9	0.09	0.735	0.138
d 21	11.4	11.3	11.4	11.3	0.12	0.916	0.980
d 28	13.5	13.6	13.6	14.0	0.17	0.023	0.528
d 35	17.8	17.6	17.9	18.5	0.21	0.006	0.169
d 46	22.4	22.9	23.2	24.0	0.24	< 0.001	0.987
d 0 to 14							
ADG, g	99	100	117	108	6.2	0.139	0.142
ADFI, g	175	174	178	178	5.3	0.508	0.870
G:F, g/kg	562	565	653	604	22.8	0.090	0.065
d 14 to 21							
ADG, g	343	337	323	345	9.3	0.926	0.108
ADFI, g	202	494	493	492	10.2	0.184	0.268
G:F, g/kg	668	683	656	702	17.5	0.233	0.339
d 21 to 28							
ADG, g	303	324	319	380	12.9	< 0.001	0.285
ADFI, g	569	604	601	617	13.9	0.035	0.379
G:F, g/kg	539	534	530	615	21.2	0.010	0.079
d 28 to 35							
ADG, g	615	578	594	635	12.1	0.062	0.009
ADFI, g	797	788	903	837	13.1	0.016	0.251
G:F, g/kg	771	734	740	758	8.6	0.732	0.004
d 35 to 46							
ADG, g	411	458	464	493	12.8	< 0.001	0.218
ADFI, g	801	826	842	883	17.1	0.001	0.936
G:F, g/kg	513	554	551	560	10.3	0.010	0.077
d 0 to 46 (Overall)							
ADG, g	315	322	331	355	5.3	< 0.001	0.568
ADFI, g	522	528	239	557	7.5	< 0.001	0.844
G:F, g/kg	603	611	635	637	5.3	< 0.001	0.510
Total removals and mortalities, %	11.5	11.9	8.5	6.7	2.14	0.027	0.856

[1]A total of 1,056 pigs (PIC TR4 × (Fast LW × PIC L02) initially 7.3 ± 0.09 kg) were used with 22 pigs per pen and 12 replications per treatment and were fed trial diets for a 46-day period.

[2] Omega-6:3 ratios for the four treatments within each phase were: Phase 1 (15.1:1, 8.4:1, 5.9:1, 3.7:1); Phase 2 (16.5:1, 9.2:1, 6.4:1, 4.0:1); Phase 3 (20.8:1, 10.4:1, 7.0:1, 4.2:1); and Phase 4 (25.3:1, 12.5:1, 8:3:1, 5.0:1), respectively (NBO3 Technologies LLC, Manhattan, KS).

Table 2.10 Effects of omega-3 fatty acids, sourced by O3 Trial Feed on growth performance, total removals and mortality, and medication usage in PRRSV-challenged nursery pigs in Exp. 3[1]

	O3 Trial Feed, %			
Item	0	3	SEM	*P* =
BW, kg				
d 0	5.2	5.1	0.05	0.316
d 43	17.7	17.6	0.31	0.707
Overall (d 0 to 43)				
ADG, g	285	281	7.0	0.555
ADFI, g	434	426	12.5	0.313
G:F, g/kg	662	663	22.8	0.924
Total removals and mortality, %	7.7	8.9	1.13	< 0.001
Total injections				
Injections per 1,000 pig days, n	18.26	18.03	1.170	0.226
Injections per pig placed, n	0.70	0.68	0.042	< 0.001

[1]A total of 91,140 (Initially 5.1 ± 0.05 kg) were used with 40 rooms per treatment and approximately 1,100 pigs per room.

Table 2.11 Correlations between Ct values, growth performance, and removals and mortality in Exp. 3[1]

	Cycle threshold (Ct) value			
	Week 1	Week 3	Week 5	Week 7
ADG, g	r = 0.44 *P* < 0.01	r = 0.26 *P* < 0.10	r = 0.04 *P* > 0.10	r = -0.07 *P* > 0.10
ADFI, g	r = 0.33 *P* < 0.05	r = 0.18 *P* > 0.10	r = 0.10 *P* > 0.10	r = 0.17 *P* > 0.10
G:F, g/kg	r =0.18 *P* > 0.10	r = 0.11 *P* > 0.10	r = -0.04 *P* > 0.10	r = -0.18 *P* > 0.10
Removals and mortality, %	r = -0.67 *P* < 0.01	r = -0.30 *P* < 0.05	r = 0.07 *P* > 0.10	r = 0.09 *P* > 0.10

[1]A total of 91,140 (Initially 5.1 ± 0.05 kg) were used with 40 rooms per treatment and approximately 1,100 pigs per room.

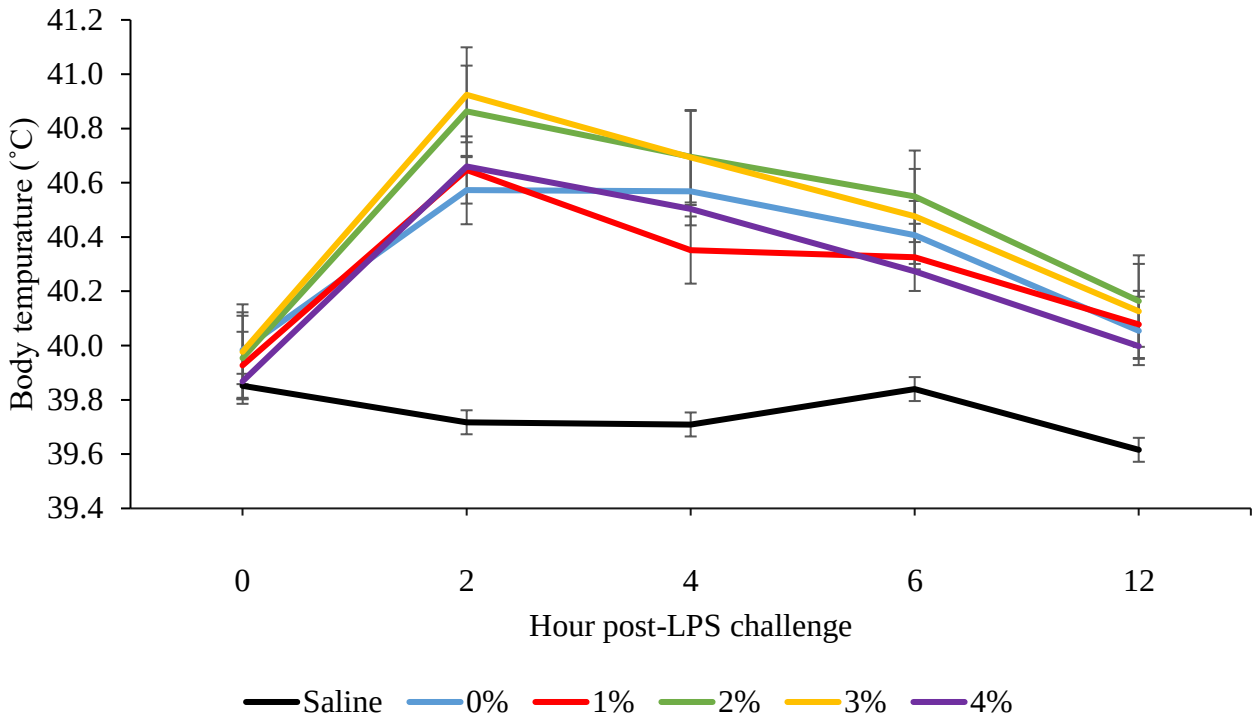

Figure 2.1.Body temperature (˚C) over time between treatments.