RESEARCHREPORT

ANIMAL SCIENCE — Nutritional Efficiency Consortium

Title: The influence of increasing dietary intake of omega-3 fatty acid concentration on postpartum hypophagia and energy output in the milk via alterations in lipolytic activity and insulin sensitivity of the adipose tissue. **– NPB #09-148** **revised**

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Industry Summary:

Introduction:

In the hog industry it is imperative that we maximize the reproductive performance of sows in order to optimize pig flow and thus improve the economics of pork production. Modern sows are hyper-prolific, and due to a continual increase in litter sizes, the productivity of the pork industry is continually improving. This increase in piglet numbers, however, has several consequences on the survival and performance of piglets as well as on sow longevity.

Our overall objective was to improve the reproductive and productive functions of high producing sows. Specifically, we aimed to determine how altering the dietary fatty acid profile of sow diets would affect her whole body metabolism and her ability to provide nutrients and energy to her offspring by looking at milk energy output, piglet growth rate, sow feed intake and the role of leptin on feed intake and the responsiveness of sow adipose tissue to be mobilized when required.

Materials and Methods:

This experiment used five dietary treatments, each divided into a gestation and lactation ration. The diets were formulated to have a constant total fat concentration (5% crude fat), but varied in the ratio of n-6 to n-3 FA's. The treatment groups consisted of a control (tallow), 3 diets with plant oil based n-6:n-3 ratios (10:1, 5:1, and 1:1) as well as a 5:1 fish oil diet.

These research results were submitted in fulfillment of the Nutritional Efficiency Consortium research projects.
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Sows (n=150) were assigned to one of five test diets on d 80 of gestation and remained on these diets for three reproductive cycles. In order to determine the effects of dietary polyunsaturated fatty acids on high producing sows, any sow farrowing less than 11 piglets during cycle 3 was removed from trial, as were any sows that dropped below 10 nursing piglets throughout lactation. A set of sows for the 10:1(n=8) and 1:1 (n=7) diets had jugular catheters inserted on d 5 of lactation and underwent an epinephrine challenge in order to determine their ability to mobilize body fat, and a glucose challenge to determine the dietary effects on insulin sensitivity. Milk samples were collected on d 4 and d 16 of lactation and blood was collected for leptin analysis on d 5 and d 15.

Results and Conclusions:

Reducing the n-6:n-3 fatty acid ratio in sow diets had a wide range of effects on the reproductive performances of sows. A ratio of 5:1 increased piglet performance and sow feed intake. A plant based ratio of 1:1, and a fish based ratio of 5:1 lead to reductions in feed intake. Metabolic adaptations of the sows were measured in the 10:1 and 1:1 fed groups and results of this study show that sows fed at 1:1 ratio diet appeared to be in a state of negative energy balance relative to the 10:1 pigs throughout early lactation. There were no differences between diets on piglet performance, and thus on estimated milk energy and nutrient outputs. With the exception of the fish based diet, there were no major effects on piglet growth rates, indicating that sows will compensate for changes in feed intake through body fat mobilization, ensuring that their offspring are provided with an adequate supply of energy and nutrients for growth. This in turn would potentially have negative long term effects on the sow, in that if she is having to draw on her body reserves for each lactation, she will have a shortened lifespan in the herd and be more costly to a producer relative to a sow that does not have to draw on her own body to provide enough energy to her litter (or does so to a lesser extent). Combining the production data for all 5 dietary groups with the metabolic data of the 10:1 and 1:1 diet sows, it would appear that if the n-6:n-3 ratio becomes too low, negative effects on sow performance are seen, and a ratio of 5:1 appears optimal in terms of sow and piglet performance.

Implications

We observed optimal sow performance when diets with a n-6:n-3 fatty acids ratio of 5:1 were fed throughout gestation. This can be accomplished in a typical corn, soybean meal diet, or a wheat, barley based diet with added corn oil by adding about 0.5 % flaxseed oil.

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Scientific Abstract:

Modern sows are hyper-prolific, and due to a continual increase in litter sizes, the productivity of the pork industry is continually improving. This increase in piglet numbers, however, has several consequences on the survival and performance of piglets as well as on sow longevity. An experiment was conducted to determine the metabolic adaptations of the sow throughout lactation, in response to altering the dietary omega-6 (n-6) to omega-3 (n-3) fatty acid ratios, and to determine the effects on overall sow and piglet performance. Sows (n=150) were assigned to one of five test diets on d 80 of gestation and remained on these diets for three reproductive cycles. Diets (5% crude fat), divided into gestation and lactation rations, consisted of a control (tallow based) and 4 diets with n-6:n-3 ratios of 10:1, 5:1, 1:1 or 5:1 fish based. In order to determine the effects of dietary polyunsaturated fatty acids on high producing sows, any sow farrowing less than 11 piglets during cycle 3 was removed from trial, as were any sows that dropped below 10 nursing piglets throughout lactation. A set of 8 sows for the 10:1 and 1:1 diets had jugular catheters inserted on d 5 of lactation and underwent an epinephrine challenge in order to determine the effect of diet on body fat mobilization, and a glucose challenge to determine the effects on insulin sensitivity. Milk samples were collected on d 4 and d 16 of lactation and blood was collected for leptin analysis on d 5 and d 15.

Piglets raised by sows consuming the 5:1 plant diet had higher birth and weaning weights, while those nursing from sows on the fish based diet had lower weights ($P < 0.05$). During cycle 2 and 3 a significant affect of dietary treatment on feed intake was observed. In cycle 2 there was no difference between the control pigs, and the three plant based treatment groups; however sows consuming the fish diet ate half a kg less than the others ($P = 0.04$). In cycle 3 the control and 5:1 plant diet sows ate the most feed, while the 1:1 and fish diet sows consumed the smallest amount ($P = 0.05$). Altering the n-6:n-3 fatty acid ratio of sow diets did not affect the overall milk composition or output, and with the exception of the fish based diet, there were no major effects on piglet growth rates, indicating that sows will compensate for changes in feed intake through body fat mobilization, ensuring that their offspring are provided with an adequate supply of energy and nutrients for growth. Prior to any form of challenge, sows consuming the 1:1 ratio diet appeared to be in a state of body fat mobilization when compared to those consuming the 10:1 ratio, as they tended to have higher circulating levels of NEFA, glycerol and leptin. When sows underwent a metabolic challenge with exogenous epinephrine we found that the sows consuming a ratio of 10:1 had a greater response, indicated by a lower net incremental area under the curve(niAUC) for glucose ($P < 0.05$) and tendencies for higher niAUC NEFA and glycerol concentrations. It appears that since the 1:1 ratio sows were mobilizing more body fat prior to the challenge, they were less sensitive to a dose of exogenous epinephrine than the 10:1 ratio sows. There were no dietary effects during the glucose challenge, indicating that dietary fat did not alter the insulin sensitivity of sow tissues.

Overall, it appears that if the dietary n-6:n-3 ratio becomes too low, negative effects on sow performance are seen, and a ratio of 5:1 appears optimal in terms of sow and piglet performance. Regardless of diet the sow is able to undergo metabolic adaptations to ensure that the energy and nutrients required by her offspring are met. This means that if she is consuming less feed, she will draw on her own body reserves to meet the energy output demands, which will in turn negatively affect her long term performance and increase producer costs.

Introduction

Modern sows are hyper-prolific, and due to continual improvements in genetics and management, litter sizes have increased dramatically. Industry profitability however, has not necessarily improved. For example, the increase in piglet numbers has had several consequences on the survival and performance of piglets as well as on sow longevity. Currently, a large portion of the increase in litter size is offset by a reduction in the number of piglets born alive, or by an increase in the number of small, weak born piglets (Boulot et al., 2008).

As piglet numbers increase there is an increasing energy demand on the sow to provide enough milk, and if this energy is not provided in the diet, she will draw on her body reserves, potentially having negative impacts on subsequent reproductive cycles.

Lactational homeorhesis is the physiological drive to produce milk at the expense of other body functions. This phenomenon has been extensively researched in the dairy cow, but it also describes the metabolic state of the lactating sow that experiences a period of negative energy balance and adipose tissue mobilization to support milk production (Pettigrew et al. 1993). Hypophagia immediately following farrowing contributes to the inability of sows to meet nutrient demands for maximal milk production. Although milk production increases in response to litter size (Auldist et al. 1998) it may still be a limitation to optimal growth in the larger litters found in modern genotypes despite extensive mobilization of body tissue reserves. Hormonal regulation of appetite and milk production may affect the degree of negative energy balance encountered by modern sows.

Ad libidum consumption of feed during gestation is accompanied by reduced feed intake during the subsequent lactation (Weldon et al. 1994). Several metabolic explanations for this have been suggested including roles for circulating hormones and adipose tissue adaptation. When sows were fed either a standard restricted diet, or ad lib from day 60 of gestation until farrowing, the sows fed ad lib during gestation gained more weight during gestation but lost more weight during lactation (Weldon et al. 1994). During lactation, insulin secretion was increased and plasma NEFA concentration was reduced in the restricted fed sows. The authors (Weldon et al. 1994) concluded that higher insulin concentration in sows fed the restricted diet during gestation may have stimulated appetite by reducing lipid mobilization and increasing peripheral glucose use.

Other hormones which may regulate feed intake around the time of parturition include ghrelin and leptin and while recent evidence doesn't support a role for ghrelin (Govoni et al. 2007) there is evidence to suggest that leptin may be important. Leptin, a hormone secreted by adipose tissue, is an important regulator of appetite, energy metabolism, and body composition (Hossner 1998). When adipose tissue reserves are high, leptin activates satiety centers in the hypothalamus and food intake is reduced. Studies have shown a positive correlation between leptin levels and backfat thickness in growing pigs (Robert et al. 1998) and sows (Estienne et al. 2000). Negative energy balance in sows resulted in a rapid decrease in leptin concentrations in plasma and adipose tissue (Estienne et al. 2000). Insulin, glucose and IGF-I, all reduced in the fasted state, are potent regulators of leptin and a positive correlation was observed between plasma insulin, leptin and luteinizing hormone concentrations in lactating sows fed ad libidum compared to feed restricted sows (reviewed by Barb et al. 2005).

The importance of adipose tissue in the maintenance of lactation, especially during early lactation, has been extensively researched in the high-producing dairy cow. It is important to determine if the lactating sow responds similarly to the cow and thus facilitate extrapolation of the large amount of data available. In the cow, a dramatic increase in catabolism and decrease in anabolism occurs in adipose tissue shortly after parturition (McNamara 1991). This is partly a whole-body response to the negative energy balance resulting from the energy output in milk and decreased feed intake around parturition, but experiments conducted on adipose tissue *in vitro* demonstrate that the adipose tissue responsiveness is also altered. Lipogenesis was decreased by 54 %, esterification by 16 % and epinephrine-stimulated free fatty acid release (maximal) was increased by 77% in bovine adipose tissue biopsies taken at 0.5 months pre-partum when compared to samples taken 1 month pre-partum (McNamara and Hillers 1986). It has been suggested that both selection for milk production and decreased energy intake in early lactation result in decreased adipose tissue anabolism during early lactation (McNamara 1991). *In vitro* rates of epinephrine stimulated lipolysis were strongly correlated with milk energy secretion in dairy cows (McNamara 1989). There is evidence that anabolic and catabolic rates measured in porcine adipose tissue using *in vitro* procedures underestimates the *in vivo* rates of lipolysis and lipogenesis.

Mersmann (1986) suggests that plasma NEFA and glycerol concentrations are more accurate predictors for swine.

It has been shown in several studies that the fatty acid profile of adipocytes reflects the composition of dietary lipids (reviewed by Fickova et al. 1998; Eastwood et al. 2009) and furthermore that replacement of omega-6 (n-6) fatty acids (FA) with fatty acids of the omega-3 (n-3) series has profound effects on reducing plasma triglycerides (reviewed by Fickova et al. 1998). The effect of n-3 fatty acids on lipogenesis and lipolysis is less clear. Lipolysis was decreased when rats were fed diets enriched with n-3 fatty acids for 8 weeks (Gaiva et al. 2001). Conversely rats fed n-3 enriched diets for just 1 week exhibited increased lipolytic response and decreased insulin-stimulated glucose transport and lipogenesis (Fickova et al. 1998). Awad et al. (1990) was unable to show any effect of dietary fatty acid composition on lipid metabolism in the adipose tissue of mature rats which is similar to the early work of Allee et al. (1972) who was unable to demonstrate an effect of dietary fatty acid composition on lipogenesis. All fatty acids in this study were equally effective in inhibiting lipogenesis of adipose tissue of 45 kg pigs (Allee et al. 1972) and 10 % tallow added to the diet of lactating sows, did not alter the rates of lipolysis measured using an exogenous epinephrine challenge (Tilton et al. 1999). A lactation feed supplemented with 2 % oil with a high n-6:n-3 ratio administered 8 days pre-parturition was associated with high leptin levels pre- and post-partum, insulin resistance on the first day of lactation, and decreased feed intake on the first and second days following parturition. Moreover, across all treatment groups there was a significant negative correlation between leptin and litter weight and litter growth (Papadopoulos et al. 2008).

Summary: Extensive research has demonstrated that adipose tissue is actively involved in the support of lactation through the supply of nutrients following increased lipolysis. The role of whole body adaptations in the sow such as alterations in insulin sensitivity or concentrations of hormones (leptin) which may regulate feed intake are less well defined. Moreover, while it is well known that dietary n-3 fatty acids perturb lipid metabolism, their potential role in adipose tissue metabolism during early lactation in the sow has not been examined. We hypothesize that reducing the n-6:n-3 fatty acid ratio in the diets of sows will improve feed intakes of the animals via reducing leptin concentrations and improving insulin sensitivity of adipose tissue, thus improving milk production (energy output) which in turn will improve litter growth throughout lactation.

Objectives:

Our overall program objective was to improve the reproductive and productive functions of high producing sows. This program was divided into several experiments, looking at animal performance as well as biological parameters. The program spanned three reproductive cycles with the aim of improving sow reproduction through altering the fatty acid ratios in the diet, which in turn could improve the economic status of the industry. Experiment 1 examined general parameters including sow and piglet performance, as well as fatty acid and immunoglobulin profiles in milk and serum (Appendix 1). Experiment 2 was designed to look at how the fatty acid ratio affects the conversion of α-linolenic acid into EPA and DHA. Experiment 3 looked at how the dietary fatty acid ratio of sows affects the immune responses of their weaned offspring (Appendix 2), and Experiment 4 was to determine how altering this fatty acid ratio will affect adipose tissue metabolism during lactation. Data from all of the experiments will be combined to draw final conclusions. Experiment 4 is the specific project funded by the National Pork Board and the primary focus of this report.

The aim of this specific project (experiment 4) was to determine if piglet growth rate is improved by altering the adipose tissue metabolism of the sow through the feeding of diets varying in n-6:n-3 ratio. We examined the role of several mechanisms which may explain the observed responses including alterations in sow adipose tissue lipolytic activity and the potential role of leptin in feed intake. This is important for a more

thorough understanding of the metabolic adaptations of the modern, prolific sow during the transition from gestation to lactation.

The specific objectives for experiment 4 were to determine the effect of chronic administration of diets varying in n-6:n-3 ratio to sows on: 1. Milk energy output; 2. Piglet growth rate; 3. Feed intake of the sow during lactation; 4. Lipolytic activity of sow adipose tissue based on plasma NEFA and glycerol concentrations and the response to an epinephrine challenge to determine maximal lipolytic rate; 5. Insulin sensitivity of sow tissue; 6. Role of leptin in feed intake of the sow during early lactation.

Materials and Methods:

The NPB funded experiment was part of a larger series of experiments investigating the role of dietary PUFA's on the reproductive functions of sows. This work was approved by the University of Saskatchewan's Animal Research Ethics Board, (UCACS # 19970020 and 20090129) and adhered to the Canadian Council on Animal Care guidelines for humane animal use.

Treatments: A total of 5 dietary treatments were used for the experiment, each divided into a gestation and a lactation ration. Fatty acids were supplemented at different levels to adjust the ratio of n-6:n-3 fatty acids in the diets. The two fatty acids of interest were the n-6 fatty acid linoleic acid (LA), the source of which was corn oil, and the n-3 fatty acid α-linolenic acid (ALA), the source of which was flax oil (and/or flaxseed meal). Fish oil was also used as a source of long chain n-3 fatty acids such as EPA and DHA. The treatments were: *(1)* Control: devoid of both n-3 and n-6 fatty acids (tallow); (*2)* 10:1 ratio of n-6:n-3 (plant based); (*3)* 5:1 ratio of n-6:n-3 (plant based); (*4)* 1:1 ratio of n-6:n-3 (plant based) and (*5)* 5:1 ratio of n-6:n-3 (fish based).

Diets were balanced for net energy and digestible essential amino acids and were formulated based on the digestible oil content. The control diet contained tallow to balance diets for net energy without providing any additional polyunsaturated fatty acids. Diet formulations are shown in Tables 1 & 2. The 5:1 fish based diet was formulated as a 1:1 ratio, but fatty acid analysis determined that the n-6 content was higher than expected.

Animal Care: A total of 50 sows per treatment (starting as gilts through parity 3) were utilized for the overall experiment (n= 150 sows). Sows were managed according to normal production practices during breeding, gestation and farrowing, and piglets were cared for under normal practices. Sows in gestation were limit fed according to the normal production schedule for the farm (2.5 – 3.0 kg/d, fed once daily).

Data Collection/ Experimental Design: Sows were randomly allocated to a dietary treatment one month pre-farrowing and remained on treatment diets through the next breeding to weaning cycle, followed by a subsequent breeding to weaning cycle allowing for the determination of chronic effects of the diet n-3:n-6 ratio on reproduction performance (Figure 1).

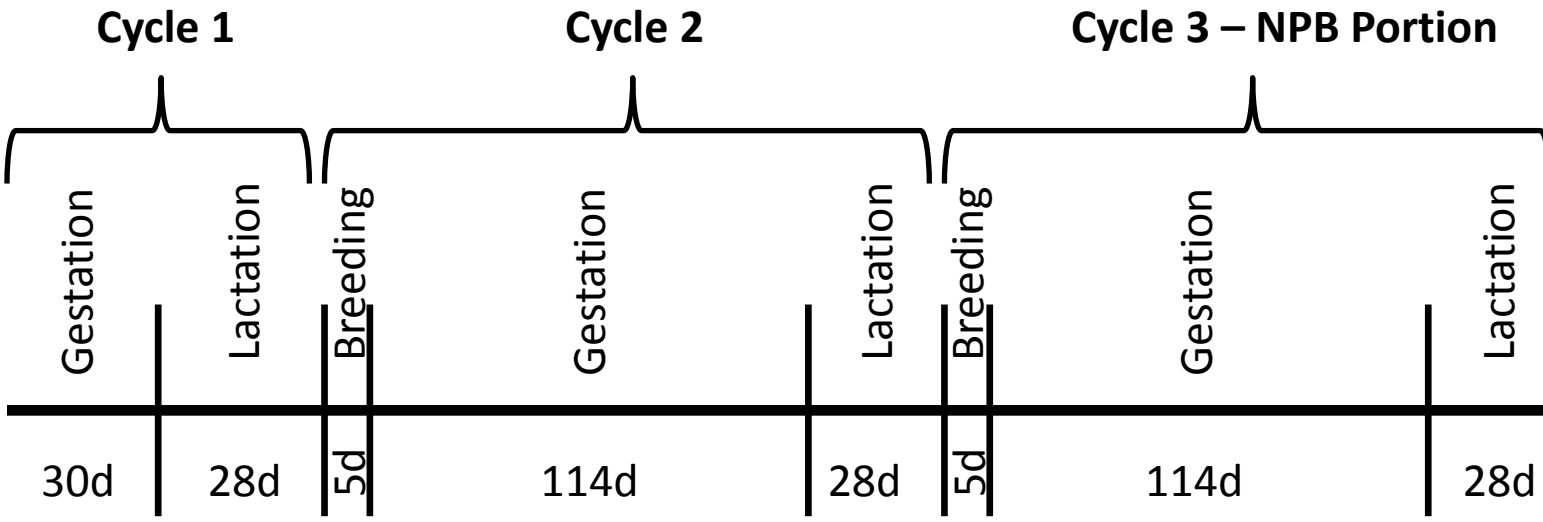

Figure 1: Experimental timeline beginning with first day of treatment assignment

Sows were weighed at the time of dietary assignment and weaning for cycle 1, then at d 110 of gestation, within 24 hours post farrowing, d 7 post farrowing and weaning for cycle 2 and cycle 3. Sow backfat thickness was determined using a Real Time (B Mode) Ultrasound Scanner (Pie Scanner 200 SLC, Pie Medical, The Netherlands) during cycle 2 and 3 when sows were weighed. The right side of each animal was scanned longitudinally between the 10^{th} and last ribs, 5 cm lateral to the dorsal midline. Scan location was marked on each sow, so subsequent scans were determined in the exact same location.

The total number of piglets born, as well as mummies and stillbirths were recorded for each cycle. Pre-weaning mortalities, and treatments administered to sows and/or piglets were tracked. Piglet weights at birth and weaning, as well as sow feed intakes were recorded. Sow blood was collected on d 110 of gestation during cycle 1 for 12 sows/diet in order to determine the circulating plasma fatty acid profile of the animals. During cycle 2, 12 sows/diet were randomly selected and used for colostrum collection at the time of farrowing. Two piglets from each of those litters were used for blood collection (one pre and one post suckle) to determine IgG, IgA and plasma fatty acid concentrations. Colostrum was also analyzed for IgG, IgA and fatty acids.

NPB sponsored experiment. Effect of n-6:n-3 ratio on feed intake of the sow and milk energy output (Cycle 3):
Following parturition of the second breeding to weaning cycle (sows had received diets for approximately 7 months) 10 sows per treatment were selected for an intensive sampling protocol to determine the effect of long term administration of different dietary n-6:n-3 ratios on milk energy output. The sows selected had to farrow a minimum of 11 piglets in order to be included in this component of the trial, as our focus was the high producing sow. Litters were balanced to 12 piglets within 24 hours post farrowing. Any sow which dropped below 10 nursing piglets was removed from trial.

Following farrowing, feed intake was increased by approximately 1 kg per day until ad libitum intake was achieved. The step-up was faster for sows with no feed refusals. Weighed feed was supplied to the sow three times daily.

Piglets were weighed immediately following farrowing, and on days 3, 7, 10, 14, 21 and 28 of lactation. Milk production and energy output in the milk was calculated from piglet growth rate according to Noblet and Etienne (1989). They report a R^2 of 0.88 (n=20) between sow milk DM, and energy and nitrogen in milk from litter BW gain (Noblet and Etienne, 1989). This method was chosen over the weigh-suckle-weigh method of

measuring milk output or isotope dilution because it does not disrupt the normal social interaction of the sow and litter required by this technique, and does not require fasting (Pettigrew et al. 1985).

A milk sample was taken from all functional teats following an injection of oxytocin on day 4 and 16 of lactation. Samples were analyzed for their fatty acid profiles using a method adapted from O'Fallon et al. (2008). Samples were also analyzed for their total solids (dry matter) using AOAC method 990.20 (AOAC 1990).

Determination of effect of n-6:n-3 ratio on adipose tissue metabolism: A subset of sows from 2 of the 5 treatments (10:1 n-6:n-3 ratio and the 1:1 n-6:n-3 ratio) were used to evaluate the effect of the n-6:n-3 ratio on energy mobilization from adipose tissue (n=10 for 10:1 diet; n=8 for 1:1 diet). On d 5 of lactation, sows had a jugular catheter inserted into the lateral auricular vein according to the method of Niiyama et al. (1985). Catheter patency was maintained with a solution of sterile heparinized saline (0.1 % heparin).

An epinephrine challenge was conducted on d 5 of lactation according to the methods of Tilton et al. (1999) and Mersmann (1986). An epinephrine dosage of 1.6 ug/kg BW was used (Tilton et al. 1999). Blood samples were collected 15 min pre-infusion and at time 0, 2, 4, 6, 10, 15, 20, 30, 45, 60 and 120 minutes post-infusion. Plasma was analyzed for glucose, NEFA and glycerol using colorimetric test kits purchased from BioAssay Systems (Hayward, CA; catalogue numbers DIGL-200, EFFA-100 and EGLY-200 respectively).

Similarly, a glucose challenge to evaluate effect of n-6/n-3 ratio on tissue sensitivity to insulin occurred on d 6 of lactation. Sows were infused with glucose (1 mg/kg BW) following a 12 hour fast (Tilton et al. 1999). Blood samples were collected in the same manner as those from the epinephrine challenge except additional samples were taken at 150 and 180 minutes post-infusion. Plasma was analyzed for glucose as described above and for C-peptide (pro-insulin) using a porcine specific C-peptide RIA kit (Millipore Corp., Billerica, MA).

Concentrations of leptin in serum (Multispecies RIA Kit, Millipore Corp., Billerica, MA) was determined on day 6 using a fraction of the 15 minute pre-infusion blood sample, and on d 15 of lactation using a sample collected by jugular venipuncture.

Statistical Analysis: Data was analyzed using the Mixed Model of SAS for a completely randomized design. Data from the glucose and epinephrine challenges were analyzed as repeated measures within Proc Glimmix, accounting for diet, time and diet by time interactions. Area under the curves were calculated using the pre infusion samples as the baseline (Tilton et al. 1999) and were analyzed using the completely randomized design in Proc Mixed. Relationship of serum parameters in the sow were correlated with piglet growth rate using the Proc Corr procedure within SAS. Significance was declared when $P < 0.05$. Tendencies were declared when $P < 0.1$ but > 0.05.

Results:

Objective 1: Effects on milk energy output:

Milk samples were collected from sows on d 4 and d 16 of lactation (early and late lactation sample) and analyzed for fatty acid profile and dry matter (total solids). Milk production, dry matter, energy and nitrogen outputs were estimated based on the equations of Noblet and Etienne (1989). The measured dry matter value and the estimated dry matter values for each diet were similar, indicating that our estimations for other parameters should also be accurate. Table 3 shows the estimated production of milk, as well as the dry matter, nitrogen and energy contents on a pig per day basis. The estimations are for d 1-5 of lactation, d 5-21 of

lactation and d 1-21 of lactation. The measured milk dry matter (total solids) content for d 4 and d 16 of lactation are shown in Table 4. Altering the n-6:n-3 fatty acid ratio of sow diets did not affect the overall milk composition as shown in Tables 3 and 4; however, the fatty acid profile of the early and late lactation samples did differ across dietary treatment groups, as shown in Table 5. In general, milk fatty acid composition mimicked that of the dietary fatty acid composition, with higher amounts of n-3 fatty acids in the lower ratio diets.

Objective 2: Effects on piglet growth rate:

The effects of altering the fatty acid ratio in sow diets on piglet performance and growth varied between reproductive cycles (Table 6). During experimental cycle 1, piglet birth weights were unaffected by sow diet ($P > 0.05$); however, weaning weights were different between groups ($P < 0.05$). Piglets born to sows consuming the 1:1 and 5:1 fish based diets had reduced weaning weights when compared to those raised by sows consuming the 5:1 plant based diets ($P = 0.02$). In experimental cycle 2, both average piglet birth and weaning weights were affected by sow diet ($P < 0.05$). Similar to cycle 1, piglets raised by sows consuming the 5:1 plant diet had higher birth and weaning weights, while those nursing from sows on the fish based diet had lower weights. The average daily gain of the piglets during lactation followed the same pattern for cycle 1 ($P = 0.01$) and cycle 2 ($P = 0.04$).

During cycle 3 any effects on piglet birth and weaning weights were not present, and there was no effect on average daily gain of the piglets throughout lactation ($P > 0.05$). It is possible that this is due to a reduction in the number of sows on trial during cycle 3 ($n = 20$/diet) when compared to cycles 1 and 2 ($n=30$/diet), as numeric differences can be seen however they did not reach statistical significance (Table 6). The numeric pattern was similar to cycle 1 and 2, with piglets raised by the fish diet sows having lower birth and weaning weights, as well as lower average daily gains.

Objective 3: Effects on feed intake of the sow during lactation:

Sow feed intake was measured during cycle 2 and cycle 3 (Table 7). For cycle 2 the intake was determined as a total feed disappearance from farrowing to weaning. During cycle 3, feed intake was measured as a daily disappearance from farrowing to day 3 of lactation, and then total disappearance until weaning. During both cycles a significant affect of dietary treatment on feed intake was observed. In cycle 2 there was no difference between the control pigs, and the three plant based treatment groups; however sows consuming the fish diet ate half a kg less than the others ($P = 0.04$). In cycle 3 the control and 5:1 plant diet sows ate the most feed, while the 1:1 and fish diet sows consumed the smallest amount ($P = 0.05$). There was no significant affect of diet on early lactation feed intake; however it is possible that this is due to such high variability between animals and a reduced number of sows during this experimental cycle. As the time period over which feed intake was measured increased, variability between sows decreased, and sows consuming the 1:1 diet ate significantly less feed when compared to the control and 5:1 diet sows.

Objective 4: Effects on the lipolytic activity of sow adipose tissue based on plasma NEFA and glycerol concentrations and the response to an epinephrine challenge to determine maximal lipolytic rate:

Sows underwent an epinephrine challenge to determine the effects of fatty acid ratios in the diet on the maximal lipolytic activity of sow adipose tissue. The effects of diet on the baseline (pre-challenge) concentrations of plasma NEFA, glycerol and glucose are shown in Table 8. Circulating glucose tended to be

higher in sows fed the 10:1 n-6:n-3 fatty acid ratio when compared to those fed the 1:1 ratio (P = 0.11). Both NEFA and glycerol concentrations were numerically opposite to glucose, with the 1:1 pigs having greater circulating baseline values compared to the 10:1 pigs; however, this did not reach statistical significance potentially due to a high level of variability and a low number of pigs. These baseline results would indicate that, relative to the other treatments, sows consuming the 1:1 fatty acid ratio were in a greater state of body fat mobilization prior to being challenged with epinephrine or glucose.

The net incremental area under the curve (niAUC; AUC minus baseline) and peak concentrations for NEFA and glycerol were not different between the two dietary treatments during the challenge period. The niAUC was numerically lower for the 1:1 pigs in both cases, and it is possible that significance was not achieved again due to high variability between animals. Sows consuming the 10:1 diet had lower glucose peaks when adjusted for baseline and lower niAUC responses during the epinephrine challenge. Results of the glucose, NEFA and glycerol responses to the challenge are shown in Table 9.

Objective 5: Effects on insulin sensitivity of sow tissue:

After a 12 hour fast sows were challenged with glucose to determine the effect of diet on insulin sensitivity of sow tissues. Table 8 outlines the baseline concentrations of fasted glucose and C-peptide (pre-insulin). There was no effect of dietary fatty acid ratio on the circulating concentration of either glucose or c-peptide prior to challenge (P > 0.05).

We found no effect of diet on peak glucose concentration, glucose response, peak c-peptide concentration or c-peptide response during the glucose challenge (P > 0.05; Table 10). Peak glucose during the challenge was similar to the baseline values for fed sows, indicating that a rapid increase in plasma glucose occurred during the challenge. Peak c-peptide values rose above baseline values during the challenge regardless of diet. Our results clearly show that sows responded to the glucose challenge; however this response was unaffected by dietary treatment.

Objective 6: Effects on the role of leptin in feed intake of the sow during early lactation:

Plasma leptin concentration was determined on day 5 and day 15 of lactation. As shown in Table 8, there was no effect of dietary treatment on the leptin concentration on day 5 (P > 0.1); however, sows consuming the 1:1 n-6:n-3 ratio diet had elevated leptin concentrations on day 15 (P = 0.07).

As discussed previously, there was no difference between the 10:1 and 1:1 ratio sows in terms of total feed intake throughout lactation for cycle 2 or cycle 3 (Table 7). When we look at the early lactation feed intake of sows (d 0-3), we can see that sows on the 10:1 diet ate 3 kg more feed during that time than the 1:1 diet sows; however again between animal variability is high and this did not approach significance (P = 0.46). Correlations between leptin (day 5 or 15) and feed intake (d 0-3, total and ADFI) were not significant (P > 0.1).

Additional Findings:

Animal performance data for cycle 3 is shown in Table 11 (performance data for cycles 1 and 2 can be found in Appendix 1). There were no effects of diet on lactation length, wean to estrus intervals or sow weight changes during lactation (P > 0.05). Sows consuming the 5:1 and 1:1 plant based diets had higher backfat thickness prior to farrowing (P = 0.01) and at weaning (P < 0.01) when compared to the other groups. Sows

consuming the 5:1 fish based diet lost the most backfat throughout lactation ($P = 0.06$). Diet had no effect on the number of piglets born alive or born total ($P > 0.05$). There was also no effect of sow diet on litter birth weights (live, dead or total), or on average piglet birth weight, or average piglet weaning weight ($P > 0.05$).

Overall correlations between blood parameters and performance parameters were determined regardless of dietary treatment group. Leptin was correlated with NEFA ($P < 0.1$) and glycerol ($P = 0.1$), indicating that increased leptin concentrations are related to increased NEFA and glycerol in the blood. Glycerol and NEFA were highly correlated with each other ($P < 0.0001$), which was expected since both are products of fat mobilization in the body and were negatively correlated with feed intake ($P < 0.1$). Surprisingly, leptin was not correlated with sow feed intake, but it was negatively correlated to piglet gain ($P < 0.05$), indicating that sows with high leptin concentrations had piglets with reduced growth rates. Glycerol and NEFA concentrations were also positively correlated to backfat thickness ($P < 0.05$ for farrowing backfat; $P < 0.1$ for weaning backfat).

Results from an additional study looking at the immune responses of piglets weaned from sows consuming varied n-6:n-3 fatty acid ratios can be found in Appendix 2.

Discussion:

Milk energy output was estimated using the equations provided by Noblet and Etienne (1989), which are based on the growth rates of piglets throughout lactation. In cycle 3 of our experiment, when litters were standardized, we saw no effect of sow diet on piglet average daily gains, and thus we saw no effects on estimated nutrient and energy output in the milk. We specifically aimed to study the effects on high-producing sows, and thus any sow nursing less than 10 piglets was removed from the trial. Total solids in milk samples were determined, and were similar to the values which were estimated by the equations, thus we are confident that the estimations of energy, nitrogen and total production would be accurate also. It appears that high-producing sows, regardless of the fatty acid ratio they are fed, have similar energy and nutrient outputs in their milk, and thus would have metabolic adaptations in place to ensure that they are able to provide enough to meet the demands of the litters.

During cycle 2 and 3 trends existed between sow feed intake and piglet growth rates. Sows that consumed the least amount of feed had piglets with the smallest growth rates throughout lactation and vice versa. For example, sows fed the fish based diet ate less feed during cycle 2 and 3, lost more backfat during lactation and had piglets with reduced daily gains, whereas those fed a 5:1 ratio consumed more feed, had the fastest growing piglets and did not experience major backfat loss throughout lactation.

Alterations to feed intake may be due to metabolic signals triggering satiety receptors (such as increases in the hormone leptin), or due to palatability. When long chain polyunsaturated fatty acids are included in animal feed there is always the concern for oxidation of the lipid, causing the diets to become rancid and thus lead to palatability issues. It is unlikely that this was a factor in our experiment. The diets contained 0.025% ethoxyquin as an antioxidant, a level greater than commonly found in the literature, and diets were made at frequent intervals to prevent long term storage. Also, when diets were analyzed for their fatty acid profiles, the long chain polyunsaturated fatty acids were present in expected amounts. It is unlikely that diet oxidation was a cause of reductions in feed intake; however this does not rule out that palatability could be a factor. Specifically, the fish diet that it may have tasted too ‘fishy’ and the sows simply did not enjoy the taste of the diet. That being said, sows consuming the 1:1 ratio diet had elevated plasma leptin, and higher backfat values, indicating that it is more likely that the n-6:n-3 fatty acid ratio of sow diets can alter the metabolic signals occurring within the body (as discussed below).

When body fat is used as a source of energy, which typically occurs when an animal is in a state of negative energy balance, the triacylglycerides stored in adipose tissue are broken down into free fatty acids (non-esterified fatty acids) and glycerol. The fatty acids are transported to the liver where they are oxidized to produce energy. In the case of lactating sows, a negative energy balance could be caused by either a reduction in appetite where she does not consume enough metabolizable energy to meet her output demands, or by having such a high level of energy output through her milk that she cannot physically consume enough feed to meet her requirements even at maximal feed intake. In either situation, her energy output exceeds her energy intake, thus putting her into a state of negative energy balance where she must draw on her own body reserves in order to meet her output demands.

Prior to either challenge, on day 5 of lactation, the sows consuming the 1:1 ratio diet were apparently in a state of body fat mobilization when compared to those consuming the 10:1 ratio diet. The 10:1 group had higher circulating glucose and lower NEFA and glycerol levels, as well as having lower plasma leptin on day 15 of lactation. The 1:1 sows were the opposite, with decreased circulating glucose and increased NEFA, glycerol and leptin. Total feed intake for the 1:1 diet sows was 3 kg less than the 10:1 sows over the first 3 days of lactation. This reduction in feed intake combined with higher circulating levels of NEFA and glycerol indicates that these sows had to draw on their own body reserves to meet their energy output demands. The estimated milk energy output based on piglet growth rates was unaffected by dietary treatment group, which means that despite differences in energy intake, energy outputs were the same, and thus the sows consuming the 1:1 diet had no option but to rely more on their own energy reserves. These findings are similar to those of the dairy cow in early lactation, in that she produces milk at the expense of her own body reserves, and in some cases even to the point of illness. The problem therefore may not be observed in piglet performance, but in sow longevity, return to estrus intervals and her ability to rebreed.

As discussed previously, it is unlikely that palatability was the cause of decreased feed consumption in early lactation for the 1:1 diet sows. Sows in this diet group had a higher backfat thickness prior to farrowing, during lactation and at weaning, despite consuming equal amounts of feed relative to the control, 10:1 and 5:1 groups during cycle 2. Leptin is a protein hormone produced primarily from adipose tissue. It plays a key role in regulating energy intake by acting on hypothalamic receptors to inhibit appetite. Levels of circulating leptin are proportional to the total amount of adipose tissue in the body, namely backfat depth (De Rensis et al., 2005). The sows consuming the 1:1 ratio diet had increased circulating leptin levels relative to the 10:1 diet sows, which makes sense given that they also had increased backfat thickness. Most likely, these animals had reduced appetite immediately post farrowing due to this metabolic pathway. These findings agree with those of Weldon et al. (1994), who found that sows with greater fat content pre-farrowing had lower feed intakes post-farrowing.

When sows underwent the epinephrine challenge, those sows consuming a diet with a fatty acid ratio of 10:1 had a greater response, indicated by a lower niAUC glucose and tendencies for higher niAUC NEFA and glycerol concentrations. It appears that since the 1:1 ratio sows were mobilizing more body fat prior to the challenge, they were less sensitive to a dose of exogenous epinephrine than the 10:1 ratio sows. The exogenous epinephrine triggered the release of fatty acids from adipose tissue in the 10:1 fed sows. Similar results were observed by Tilton et al. (1999). They found that the pigs which had a reduced response to the epinephrine challenge (10% added tallow) had higher peak levels of circulating glucose. They proposed that the peripheral tissues of these sows may be sparing glucose since they are relying more on circulating fatty acids for energy. In our study, the 1:1 diet pigs that were in a state of fat mobilization prior to challenge and had a reduced response to challenge, also had increased peak levels of circulating glucose.

In the experiment conducted by Tilton et al. (1999), they found that, although plasma NEFA concentrations increased in response to the epinephrine challenge, they did not see an increase in plasma glycerol. This leads to the conclusion that changes seen were due to alterations in the clearance rates of NEFA from circulation, and were not due to a change in adipose tissue responsiveness. In our experiment, tendencies

for increases in both NEFA and glycerol occurred, indicating that the adipose tissue of sows fed the 10:1 n-6:n-3 fatty acid ratio diet was more responsive to the epinephrine challenge, whereas those fed the 1:1 ratio perhaps had desensitized tissue due to the pre-challenge state of body fat mobilization. Sows with more responsive adipose tissue would be more readily able to mobilize their body fat stores during a period of stress or energy balance challenge, and thus should be able to better cope with the negative energy balance which occurs in early lactation. Overall, it appears that sows consuming diets with reduced n-6:n-3 fatty acid ratios were sent into a state of metabolic energy usage, which reduced the sensitivity of their adipose tissue. The higher ratio sows were able to reach a higher level of lipolysis based on the niAUC of NEFA, glycerol and glucose when challenged.

When sows were presented with an exogenous glucose challenge, no dietary differences were seen. Both Tilton et al. (1999) and Coffey et al. (1987) reported no dietary effects on plasma insulin concentration due to challenge. In our experiment, the glucose challenge caused the expected increase in plasma glucose and C-peptide (pre-insulin), but there were no differences among diets. Glucose concentration rose to a level similar to the baseline values obtained when the sows were in the fed state, indicating that our challenge model did not "push" the sows beyond the capacity of an insulin response and that the insulin response was unaffected by the fatty acid ratio in the diet. Thus our experimental diets did not have an effect on insulin sensitivity of sow tissues, but did affect the responsiveness of adipose tissue when a negative energy balance model was used.

In conclusion, reducing the n-6:n-3 fatty acid ratio in sow diets did affect the reproductive performances of sows. A ratio of 5:1 increased piglet performance and sow feed intake. A plant based ratio of 1:1, and a fish based ratio of 5:1 lead to reductions in feed intake. Metabolic adaptations of the sows were measured in the 10:1 and 1:1 fed groups and results of this study show that sows fed the 1:1 ratio diet appeared to be in a state of negative energy balance relative to the 10:1 pigs throughout early lactation. There were no differences between diets on piglet performance, and thus on estimated milk energy and nutrient outputs. With the exception of the fish based diet, there were no major effects on piglet growth rates, indicating that sows will compensate for changes in feed intake through body fat mobilization, ensuring that their offspring are provided with an adequate supply of energy and nutrients for growth. This could have negative long term effects on the sow, in that if she is having to draw on her body reserves for each lactation, she will have a shortened lifespan in the herd and be more costly to a producer relative to a sow that does not have to draw on her own body to provide enough energy to her litter. Combining the production data for all 5 dietary groups with the metabolic data of the 10:1 and 1:1 diet sows, it would appear that if the n-6:n-3 ratio becomes too low, negative effects on sow performance are seen, and a ratio of 5:1 appears optimal in terms of sow and piglet performance. The fatty acid ratio of a typical gestation or lactation corn/soybean meal diet (containing approximately 70% corn and 20% soybean meal) would have an n-6:n-3 fatty acid ratio between 25:1 and 20:1.

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Table 1: Gestation Sow Diets

	Formulated n-6:n-3 Fatty Acid Ratio				
Ingredient (%)	**Control**	**10:1**	**5:1**	**1:1**	**1:1 Fish**
Barley	69.98	72.17	66.61	57.58	70.08
Wheat	9.60	7.00	12.00	19.00	8.90
Corn	-	-	1.50	4.40	-
Flaxseed	-	-	-	5.00	-
Soybean Meal	12.60	11.40	8.30	5.00	12.70
Canola Meal	1.55	1.40	-	-	1.60
Flaxseed Meal	-	1.80	5.98	5.40	-
Tallow	3.48	-	-	-	-
Canola Oil	-	0.72	-	-	-
Corn Oil	-	2.58	2.29	0.38	0.06
Flax Oil	-	-	0.58	0.46	-
Fish Oil (Herring)	-	-	-	-	3.87
Mineral Mix[1]	0.500	0.500	0.500	0.500	0.500
Vitamin Mix[2]	0.600	0.600	0.600	0.600	0.600
Salt	0.500	0.500	0.500	0.500	0.500
Limestone	0.470	0.468	0.477	0.483	0.467
Mono/dical	0.638	0.601	0.484	0.437	0.645
L-Thr	-	-	0.025	0.041	-
Lys-HCl	-	0.178	0.075	0.140	-
Choline	0.060	0.060	0.060	0.060	0.060
Ethoxyquin (antioxidant)	0.025	0.025	0.025	0.025	0.025
Calculated Analysis					
DM (%)	87.58	87.60	87.64	87.64	87.64
CP (%)	14.12	14.10	13.95	13.30	14.12
DE (Mcal/kg)	3.23	3.22	3.23	3.23	3.23
NE (Mcal/kg)	2.35	2.35	2.35	2.34	2.37
SID Lys (g/kg)	5.44	5.44	5.44	5.44	5.45
SID Thr (g/kg)	4.08	4.08	4.08	4.05	4.08
SID Met (g/kg)	1.93	1.93	1.93	1.93	1.93
SID SAA (g/kg)	4.21	4.21	4.20	4.21	4.21
SID Trp (g/kg)	1.48	1.48	1.48	1.48	1.48
Calcium (g/kg)	4.70	4.70	4.70	4.69	4.70
Available P (g/kg)	2.78	2.78	2.78	2.78	2.78
Lipid (g/kg)	49.80	49.88	49.97	49.80	53.98
C18:2 (g/kg)	7.75	23.56	21.00	11.98	7.69
C18:3 (g/kg)	1.00	2.36	4.20	11.98	7.69
n3/n6	0.13	0.10	0.20	1.00	1.00
PUFA (% of total lipid)	17.57	51.96	50.43	48.11	28.50

***SID** is the amount of standardized ileal digestible amino acids

[1]Mineral premix provided (per kg of diet): Zn, 100 mg as zinc sulphate; Fe, 80 mg as ferrous sulphate; Cu, 50 mg as copper sulphate; Mn, 25 mg as manganous sulphate; I, 0.50 mg as calcium iodate; and Se, 0.10 mg as sodium selenite.

[2]Vitamin premix provided (per kg of diet): vitamin A, 8250 IU; vitamin D, 825 IU; vitamin E, 40 IU; niacin, 35 mg; D-pantothenic acid, 15 mg; menadione, 4 mg; folacin, 2 mg; thiamine, 1 mg; D-biotin, 0.2 mg; and vitamin B_{12}, 25 ug.

Table 2: Lactation Sow Diets

	Formulated n-6:n-3 Fatty Acid Ratio				
Ingredient (%)	**Control**	**10:1 Ratio**	**5:1 Ratio**	**1:1 Ratio**	**1:1 Fish**
Barley	35.00	33.00	27.10	20.70	42.00
Wheat	37.00	39.00	37.50	45.00	29.00
Corn	-	-	7.00	6.40	-
Flaxseed	-	-	-	2.90	-
Soybean Meal	19.48	16.00	12.90	9.13	18.41
Canola Meal	-	-	-	-	1.70
Flaxseed Meal	-	3.85	8.00	9.70	-
Tallow	3.65	-	-	-	-
Canola Oil	-	-	-	-	-
Corn Oil	-	3.16	2.44	0.34	0.14
Flax Oil	-	0.15	0.236	1.029	-
Fish Oil (Herring)					3.90
Mineral Mix	0.500	0.500	0.500	0.500	0.500
Vitamin Mix	0.600	0.600	0.600	0.600	0.600
Salt	0.500	0.500	0.500	0.500	0.500
Limestone	0.885	0.888	0.872	0.881	0.862
Mono/dical	1.803	1.710	1.646	1.526	1.829
L-Thr	0.118	0.133	0.145	0.168	0.113
Lys-HCl	0.379	0.431	0.477	0.545	0.371
Choline	0.060	0.060	0.060	0.060	0.060
Ethoxyquin (antioxidant)	0.025	0.025	0.025	0.025	0.025
Calculated Analysis					
DM (%)	87.91	87.95	87.99	88.03	87.98
CP (%)	16.30	16.15	16.12	15.66	16.37
DE (Mcal/kg)	3.28	3.28	3.29	3.29	3.28
NE (Mcal/kg)	2.37	2.37	2.36	2.36	2.36
SID Lys (g/kg)	9.45	9.45	9.45	9.44	9.44
SID Thr (g/kg)	5.84	5.84	5.84	5.84	5.84
SID Met (g/kg)	2.20	2.20	2.19	2.18	2.16
SID SAA (g/kg)	4.59	4.59	4.59	4.59	4.58
SID Trp (g/kg)	1.70	1.70	1.69	1.70	1.70
Calcium (g/kg)	8.00	8.00	8.00	7.97	8.00
Available P (g/kg)	5.03	5.04	5.03	5.03	5.03
Lipid (g/kg)	49.97	50.00	49.93	49.99	53.97
C18:2 (g/kg)	7.26	25.16	21.93	11.00	7.71
C18:3 (g/kg)	0.94	2.52	4.38	11.00	7.71
n3/n6	0.13	0.10	0.20	1.00	1.00
PUFA (% of total lipid)	16.40	55.35	52.69	44.00	28.57

***SID** is the amount of standardized ileal digestible amino acids

[1]Mineral premix provided (per kg of diet): Zn, 100 mg as zinc sulphate; Fe, 80 mg as ferrous sulphate; Cu, 50 mg as copper sulphate; Mn, 25 mg as manganous sulphate; I, 0.50 mg as calcium iodate; and Se, 0.10 mg as sodium selenite.

[2]Vitamin premix provided (per kg of diet): vitamin A, 8250 IU; vitamin D, 825 IU; vitamin E, 40 IU; niacin, 35 mg; D-pantothenic acid, 15 mg; menadione, 4 mg; folacin, 2 mg; thiamine, 1 mg; D-biotin, 0.2 mg; and vitamin B_{12}, 25 ug.

Table 3: Estimated* Milk Production, Dry Matter, Nitrogen and Energy

	Diets (n-6:n-3 fatty acid ratio)					Statistics	
	Control	**10:1**	**5:1**	**1:1**	**5:1 Fish**	**SEM**	**P-Value**
Production (g/piglet/d)							
d 1-21	789.27	760.33	736.13	726.99	736.21	24.838	0.38
d 1-5	534.57	500.00	533.75	487.99	479.77	25.257	0.35
d 5-21	821.12	808.77	770.99	770.52	781.18	20.238	0.25
Dry Matter (g/piglet/d)							
d 1-21	144.53	139.89	136.01	134.54	136.01	3.985	0.38
d 1-5	108.83	101.52	108.65	98.98	97.25	5.339	0.35
d 5-21	147.97	154.94	139.86	139.72	141.46	3.284	0.25
Nitrogen (g/piglet/d)							
d 1-21	5.86	5.71	5.58	5.54	5.59	0.128	0.38
d 1-5	4.98	4.67	4.97	4.56	4.48	0.230	0.35
d 5-21	5.94	5.87	5.67	5.66	5.72	0.111	0.25
Energy (kcal/piglet/d)							
d 1-21	943.59	914.38	889.60	880.70	889.96	25.170	0.38
d 1-5	728.65	678.10	727.45	660.54	648.52	36.929	0.35
d 5-21	959.64	947.17	910.33	909.21	919.86	20.028	0.25

*Estimations based on equations provided by Noblet and Etienne (1989)

Table 4: Percentage dry matter (total solids) in milk samples on day 4 and 16 of lactation

	Diets (n6:n3 fatty acid ratio)					Statistics	
	Control	**10:1**	**5:1**	**1:1**	**5:1 Fish**	**SEM**	**P-Value**
d 4	18.57	19.12	18.67	19.43	20.29	0.553	0.23
d 16	19.42	18.45	19.50	19.30	19.01	0.353	0.23

Table 5: Fatty acid profile of early (d 4) and late (d 16) lactation milk samples (mg FA/ml Milk)

	Diets (n6:n3 fatty acid ratio)					Statistics	
	Control	**10:1**	**5:1**	**1:1**	**5:1 Fish**	**SEM**	***P* Value**
Early Lactation (d4)							
Caprylic Acid (8:0)	1.10	0.87	0.91	1.13	1.15	0.199	0.775
Capric Acid (10:0)	0.79	0.65	0.74	0.57	0.53	0.099	0.338
Lauric Acid (12:0)	0.85	0.65	0.76	0.77	0.73	0.102	0.730
Myristic Acid (14:0)	12.32	8.64	10.16	11.85	15.07	1.542	0.078
Myristoleic Acid (14:1)	0.98^{a}	0.58^{c}	0.70^{bc}	0.73^{abc}	0.97^{ab}	0.093	0.022
Palmitic Acid (16:0)	115.49	98.93	109.18	117.60	133.77	11.104	0.297
Palmitoleic Acid (16:1)	38.65	29.45	34.33	32.84	38.06	3.587	0.371
Stearic Acid (18:0)	19.44	14.92	16.11	20.76	27.58	3.796	0.185
Oleic Acid (18:1 cis)	116.77	75.42	90.22	111.90	166.18	23.668	0.115
Vacinic Acid (18:1 trans)	8.05^{a}	4.45^{a}	5.37^{a}	7.28^{a}	14.42^{b}	1.768	0.006
Linoleic Acid (18:2 n6)	39.64	69.14	73.30	75.89	64.50	10.703	0.159
y-Linolenic Acid (18:3)	0.56	0.54	0.60	0.60	0.58	0.140	0.998
α-Linolenic Acid (18:3 n3)	3.86^{a}	8.50^{a}	14.22^{a}	49.95^{b}	8.32^{a}	4.292	<0.001
Arachidic Acid (20:0)	1.03^{a}	1.04^{a}	0.89^{a}	0.92^{a}	5.27^{b}	0.402	<0.001
Eicosanoic Acid (20:1)	0.19^{a}	0.23^{a}	0.42^{a}	0.36^{a}	10.61^{b}	0.732	<0.001
Eicosadienoic Acid (20:2)	1.04	0.92	1.02	1.48	1.02	0.338	0.795
Eicosatrienoic Acid (20:3)	0.22^{a}	0.32^{a}	0.51^{a}	2.28^{b}	0.67^{a}	0.225	<0.001
Arachidonic Acid (20:4 n6)	2.10	1.45	1.52	1.61	1.72	0.386	0.774
Eicosapentaenoic Acid (20:5 n3)	0.68^{a}	0.29^{a}	0.52^{a}	2.39^{ab}	3.99^{b}	0.721	0.007
Behenic Acid (22:0)	0.23^{a}	0.13^{a}	0.10^{a}	0.35^{a}	5.72^{b}	0.398	<0.001
Erucic Acid (22:1)	0.26^{a}	0.20^{a}	0.16^{a}	0.30^{a}	2.33^{b}	0.407	0.004
Docosahexaenoic Acid (22:6 n3)	0.38^{a}	0.23^{a}	0.30^{a}	0.97^{a}	7.23^{b}	0.622	<0.001
Lignoceric Acid (24:0)	1.13^{a}	1.06^{a}	1.45^{a}	3.21^{b}	5.34^{c}	0.533	<0.001
Nervonic Acid (24:1)	0.17^{a}	0.12^{a}	0.11^{a}	0.27^{a}	0.70^{b}	0.082	0.001
Total n3	5.15^{a}	9.33^{ac}	15.55^{ac}	55.59^{b}	20.21^{c}	5.103	<0.001
Total n6	43.34	72.06	76.44	79.58	67.82	11.332	0.209
Late Lactation (d16)							
Caprylic Acid (8:0)	1.00	1.32	0.83	1.07	1.06	0.242	0.718
Capric Acid (10:0)	1.41	1.27	1.48	1.37	1.20	0.138	0.632
Lauric Acid (12:0)	1.53	1.39	1.62	1.57	1.47	0.115	0.643
Myristic Acid (14:0)	19.08	16.30	19.40	18.68	20.90	1.276	0.189
Myristoleic Acid (14:1)	1.70	1.20	1.36	1.55	1.19	0.165	0.162
Palmitic Acid (16:0)	160.05	149.21	177.37	113.82	142.05	15.859	0.106
Palmitoleic Acid (16:1)	53.85	43.72	53.12	72.44	47.05	11.043	0.424
Stearic Acid (18:0)	19.84	16.85	20.97	22.76	16.23	3.826	0.721
Oleic Acid (18:1 cis)	125.48	107.83	130.40	77.22	118.01	16.725	0.215
Vacinic Acid (18:1 trans)	7.94^{ab}	6.39^{b}	7.81^{ab}	4.87^{b}	9.89^{a}	1.060	0.037
Linoleic Acid (18:2 n6)	42.36^{a}	82.14^{b}	83.08^{b}	44.69^{a}	45.87^{a}	6.528	0.001
y-Linolenic Acid (18:3)	0.30	0.24	0.30	19.12	0.30	8.474	0.437
α-Linolenic Acid (18:3 n3)	4.52^{a}	10.34^{ab}	17.21^{b}	40.72^{c}	6.46^{a}	2.379	<0.001
Arachidic Acid (20:0)	1.23	0.92	1.19	7.40	4.68	3.080	0.501
Eicosanoic Acid (20:1)	0.21^{a}	0.42^{a}	0.19^{a}	0.45^{a}	9.51^{b}	0.408	<0.001
Eicosadienoic Acid (20:2)	0.88^{bc}	1.18^{ab}	1.52^{a}	0.64^{bc}	0.56^{c}	0.195	0.014
Eicosatrienoic Acid (20:3)	0.35^{a}	0.26^{a}	0.90^{b}	1.08^{b}	0.62^{ab}	0.177	0.016
Arachidonic Acid (20:4 n6)	1.33	1.23	1.46	1.17	0.91	0.238	0.569
Eicosapentaenoic Acid (20:5 n3)	0.78^{a}	0.54^{a}	0.51^{a}	1.34^{a}	4.09^{b}	0.451	<0.001
Behenic Acid (22:0)	0.23^{a}	0.16^{a}	0.16^{a}	0.63^{a}	4.18^{b}	0.385	<0.001
Erucic Acid (22:1)	0.24^{a}	0.27^{a}	0.22^{a}	0.72^{ab}	1.55^{b}	0.284	0.015
Docosahexaenoic Acid (22:6 n3)	0.31^{a}	0.22^{a}	0.19^{a}	0.66^{b}	4.76^{c}	0.118	<0.001
Lignoceric Acid (24:0)	0.75^{a}	0.92^{ac}	1.26^{bc}	1.45^{b}	2.88^{d}	0.154	<0.001
Nervonic Acid (24:1)	0.16^{a}	0.19^{a}	0.17^{a}	0.18^{a}	0.49^{b}	0.047	0.001
Total n3	5.95^{a}	11.36^{ab}	18.81^{b}	43.80^{c}	15.94^{b}	2.615	<0.001
Total n6	44.87^{a}	84.79^{b}	86.35^{b}	65.62^{ab}	47.65^{a}	7.992	0.002

Table 6: Effects of sow dietary fatty acid ratio on piglet weight and growth throughout lactation

	Diets (n6:n3 fatty acid ratio)					Statistics	
	Control	**10:1**	**5:1**	**1:1**	**5:1 Fish**	**SEM**	**P-Value**
Cycle 1							
Number of Sows	31	31	32	32	30	-	-
Avg Number liveborn	12.8	12.6	12.4	13.0	13.0	0.50	0.92
Avg Birth Wt (kg)	1.46	1.46	1.48	1.37	1.33	0.049	0.10
Avg Wean Wt (kg)	8.18^{ab}	8.55^{a}	8.62^{a}	8.01^{b}	7.85^{b}	0.192	0.02
ADG (kg/d)	0.26^{ab}	0.26^{ab}	0.27^{a}	0.25^{bc}	0.25^{c}	0.006	0.01
Cycle 2							
Number of Sows	30	28	31	30	30	-	-
Avg Number liveborn	12.5	12.5	11.5	12.3	13.0	0.60	0.54
Avg Birth Wt (kg)	1.49^{a}	1.43^{ab}	1.52^{a}	1.43^{ab}	1.34^{b}	0.045	0.05
Avg Wean Wt (kg)	8.83^{a}	8.68^{ab}	9.17^{a}	8.65^{ab}	8.22^{b}	0.215	0.04
ADG (kg/d)	0.31^{a}	0.30^{ab}	0.32^{a}	0.30^{ab}	0.29^{b}	0.007	0.05
Cycle 3							
Number of Sows	19	19	20	18	19	-	-
Avg Number liveborn	12.9	12.6	12.3	12.5	13.9	0.64	0.38
Avg Birth Wt (kg)	1.41	1.35	1.40	1.31	1.26	0.057	0.30
Avg Wean Wt (kg)	8.63	8.42	8.30	7.73	8.16	0.273	0.16
ADG (kg/d)	0.27	0.27	0.26	0.26	0.25	0.009	0.38

Table 7: Effects of dietary fatty acid ratio on lactation feed intake

	Diets (n6:n3 fatty acid ratio)					Statistics	
	Control	**10:1**	**5:1**	**1:1**	**5:1 Fish**	**SEM**	**P-Value**
Cycle 2							
Number of Sows	30	28	31	29	29	-	-
ADFI d0-26 (kg/d)	7.5^{a}	7.4^{a}	7.6^{a}	7.5^{a}	6.8^{b}	0.20	0.04
Cycle 3							
Number of Sows	19	19	20	18	19	-	-
Feed consumed d0-3 (kg)	16.3	17.8	15.3	14.6	14.1	1.65	0.46
ADFI d0-3 (kg/d)	4.1	4.4	3.8	3.7	3.5	0.41	0.46
ADFI d0-26 (kg/d)	8.4^{a}	7.7^{ab}	8.2^{a}	7.4^{b}	7.7^{ab}	0.27	0.05

Table 8: Baseline plasma concentrations of glucose, NEFA, glycerol, c-peptide and leptin in lactating sows fed diets containing n-6:n-3 fatty acid ratios of 10:1 or 1:1

	Diets (n6:n3 fatty acid ratio)		Statistics	
	10:1	**1:1**	**SEM**	**P-Value**
Number of Sows	10	8/7*	-	-
Fasted [Glucose], mg/dL	64.67	63.54	5.701	0.88
Fed [Glucose], mg/dL	78.93	56.48	9.818	0.11
[NEFA], uM	93.27	240.02	74.152	0.16
[Glycerol], mg/dL	0.40	0.81	0.214	0.20
[C-Peptide], ng/mL	0.30	0.25	0.070	0.58
Number of Sows	8	8	-	-
Day 5 [Leptin], ng/mL HE[1]	3.24	3.27	0.279	0.92
Day 15 [Leptin], ng/mL HE[1]	3.24[a]	3.82[b]	0.210	0.07

*The 1:1 diet had 8 sows for the epinephrine challenge (fed glucose, NEFA, glycerol) and 7 for the glucose challenge (fasted glucose, c-peptide).

[1]HE = human equivalent

Table 9: Plasma concentrations of glucose, NEFA and glycerol during an epinephrine challenge for lactating sows fed diets containing n-6:n-3 FA ratios of 10:1 and 1:1

	Diets (n6:n3 fatty acid ratio)		Statistics	
	10:1	**1:1**	**SEM**	**P-Value**
Glucose, mg/dL				
Maximum Peak	104.50	104.27	7.725	0.98
Adjusted[1] Maximum Peak	25.58[a]	47.78[b]	9.092	0.09
Total Area Under Curve	10790.0	9971.9	831.85	0.47
Net Incremental Area Under Curve[2]	276.3[a]	2456.0[b]	891.76	0.09
NEFA, uM				
Maximum Peak	281.57	353.12	109.320	0.63
Adjusted* Maximum Peak	50.51	56.47	3.730	0.34
Total Area Under Curve	22195.0	30446.0	11811.00	0.61
Net Incremental Area Under Curve	9802.2	-1560.8	5996.13	0.18
Glycerol, mg/dL				
Maximum Peak	1.12	1.26	0.529	0.85
Adjusted* Maximum Peak	0.71	0.44	0.379	0.63
Total Area Under Curve	106.4	89.0	47.06	0.80
Net Incremental Area Under Curve	51.9	-18.5	32.74	0.15

[1]Adjusted = adjusted to account for baseline concentration

[2]Net incremental area under curve = total area under curve adjusted for baseline

Table 10: Plasma concentrations of glucose and c-peptide during a glucose challenge for fasted lactating sows fed diets containing n-6:n-3 FA ratios of 10:1 and 1:1

	Diets (n6:n3 fatty acid ratio)	Statistics

	10:1	1:1	SEM	P-Value
Glucose, mg/dL				
Maximum Peak	85.89	78.46	5.620	0.33
Adjusted[1] Maximum Peak	21.22	14.92	5.472	0.41
Total Area Under Curve	12371.0	11628.0	1065.41	0.60
Net Incremental Area Under Curve[2]	100.6	-703.3	902.88	0.51
C-Peptide, ng/mL				
Maximum Peak	0.44	0.36	0.094	0.52
Adjusted* Maximum Peak	0.14	0.11	0.045	0.63
Total Area Under Curve	50.4	40.4	12.62	0.55
Net Incremental Area Under Curve	-7.8	-20.9	7.88	0.22

[1]Adjusted = adjusted to account for baseline concentration

[2]Net incremental area under curve = total area under curve adjusted for baseline

Table 11: Sow and piglet performance data for the 3rd reproductive cycle consuming experimental diets

	Diets (n6:n3 fatty acid ratio)					Statistics	
	Control	**10:1**	**5:1**	**1:1**	**5:1 Fish**	**SEM**	**P Value**
Lactation Length (d)	26.1	26.7	26.6	26.1	26.9	0.51	0.66
Wean to Estrus (d)	5.0	6.5	6.0	6.3	8.4	1.95	0.80
d110 Sow Weight (kg)	277.82	292.96	297.28	301.27	295.89	8.458	0.29
Farrowing Sow Weight (kg)	274.83	290.66	288.41	292.55	285.10	8.655	0.57
d7 Sow Weight (kg)	272.98	279.68	289.07	284.48	279.56	9.115	0.69
Weaning Sow Weight (kg)	270.23	274.62	282.03	279.73	274.19	8.733	0.85
Total Wt Change (kg/lactation)	-4.59	-16.05	-6.38	-12.82	-10.91	4.496	0.30
Avg. Daily Wt Change (kg/d/lactation)	-0.18	-0.60	-0.26	-0.49	-0.41	0.170	0.34
d110 Backfat (mm)	13.36^{a}	14.31^{ab}	15.31^{bc}	15.89^{c}	14.66^{abc}	0.556	0.01
Farrowing Backfat (mm)	13.65^{a}	14.40^{ab}	14.98^{ab}	15.78^{b}	14.47^{ab}	0.539	0.06
d7 Backfat (mm)	13.65^{a}	14.02^{a}	14.38^{ab}	15.55^{b}	14.28^{ab}	0.536	0.10
Weaning Backfat (mm)	12.47^{a}	13.47^{ab}	14.06^{bc}	15.17^{c}	13.01^{ab}	0.457	< 0.01
Total Backfat Change (mm/lactation)	-1.18	-0.93	-0.92	-0.62	-1.46	0.308	0.34
Avg Daily Backfat Change (mm/d/lactation)	-0.04^{ab}	-0.02^{a}	-0.02^{a}	-0.02^{a}	-0.07^{b}	0.016	0.06
# Born Alive	12.9	12.6	12.3	12.5	13.9	0.64	0.37
# Stillborn	0.8	1.3	0.8	1.1	2.0	0.37	0.12
# Mummies	0.6	0.3	0.7	0.5	0.4	0.24	0.74
# Born Total	14.3	14.2	13.8	14.0	16.4	0.76	0.11
Live Litter Birth Weight (kg)	18.10	16.90	16.93	16.04	17.49	0.895	0.53
Dead Litter Birth Weight (kg)	1.17	1.44	1.49	1.79	2.55	0.526	0.39
Total Litter Birth Weight (kg)	18.66	18.35	18.43	17.83	20.04	1.000	0.59
Avg Live Piglet Birth Wt (kg)	1.41	1.35	1.40	1.31	1.26	0.057	0.30
Avg Total Piglet Birth Wt (kg)	1.32	1.30	1.35	1.30	1.24	0.063	0.76
# Weaned*	11.1	11.2	11.2	11.3	11.6	0.35	0.85
Total Litter Weaning Wt (kg)	95.61	94.13	93.03	86.93	94.22	3.813	0.43
Avg Piglet Weaning Wt (kg)	8.63	8.42	8.30	7.73	8.16	0.273	0.16

*Litters were standardized and sows were removed from trial if they had less than 10 piglets nursing

Appendix 1: The effects of increasing dietary intake of omega-3 fatty acids on the reproductive functions of sows

Note – the sections presented in this appendix are not funded by NPB but are part of the overall series of experiments. Details are provided to show the overall status of the project and allow interpretation of the NPB funded experiments in the context of the overall project.

EXECUTIVE SUMMARY

Polyunsaturated fatty acids, specifically the omega-3 fatty acids, have many benefits. It has been shown in various animal models that these fatty acids can act on reproductive pathways through altering hormone production. In the pork production industry, maximizing the reproductive performance of sows is a universal goal. With improvements in genetics and nutrition the number of piglets produced per sow has increased, however, this increase is partially offset by increases in the numbers of small and weak-born piglets, as well as in pre-weaning mortality. It is also important to understand that as we increase the number of viable offspring per litter, we are increasing the demands on the sow which may affect subsequent litters and/or her reproductive lifespan. Currently, there is little information on how the ratio of omega-6 to omega-3 fatty acids in the diets of sows can affect her reproductive performance and the performance of her offspring.

This project is divided into several sections, looking at animal performance as well as biological parameters. The project spans several reproductive cycles with the aim of improving sow reproduction through altering the fatty acid ratio in the diet, which in turn could improve the economic status of the industry. Section 1 was designed to examine general parameters including fatty acid and immunoglobulin profiles in milk and serum, and how this affects the sows and piglets. Section 2 was designed to examine how the fatty acid ratio could affect the conversion of α-linolenic acid into EPA and DHA.

Currently, the overall project is not complete but data for several parameters are available. The overall design used 150 sows assigned to one of five test diets on d 80 of gestation and remained on these diets for two reproductive cycles (3 if they were included in an additional NPB funded component of the trial). Diets (5% crude fat), divided into gestation and lactation rations, consisted of a control (tallow based) and 4 diets with omega-6 to omega-3 ratios of 10:1, 5:1, 1:1 or 5:1 fish based. Based on the results of animal performance, a dietary fatty acid ratio of 5:1 omega-6 to omega-3 improved litter weaning weights and maximized sow feed intakes throughout lactation. Serum, colostrum and milk fatty acid profiles shifted as dietary fatty acid intake was altered to reflect dietary intake, but also indicating an effect of the ratio on the conversion of ALA into EPA. Final conclusions will not be drawn until all data and analyses are available.

INTRODUCTION

In the swine production industry, the most critical stages of production are the breeding and farrowing to weaning periods. Modern sows are hyper-prolific, and due to a steady increase in litter sizes, the productivity of the pork industry is continually improving. It is important to realize however, that this increase in litter size has had some negative consequences on both piglet survival and/or performance, as well as on sow longevity and

reproductive performances. As piglet numbers increase there is an increasing energy demand on the sow to provide enough milk, and if this energy is not provided in the diet, she will draw on her body reserves, potentially negatively impacting subsequent reproductive cycles.

Over the years there have been many nutritional strategies implemented with the aim of improving reproductive performance of sows. Recently, there has been a growing interest in the use of dietary long chain polyunsaturated fatty acids (PUFA's) within the pork production industry, due to the fact that PUFA's have been implicated in having many potential health benefits.

Polyunsaturated fatty acids act on many components of the biological system, primarily because they act as precursors for many different hormones and molecules throughout the body. Linoleic acid (LA) and α-linolenic acid (ALA) are precursors for the eicosanoids, which includes prostaglandins (some of the most abundant molecular forms found in the body), leukotrienes and thromboxanes (Lands, 1992). Omega-6 (n-6) fatty acids such as LA are precursors for the F-2 series prostaglandins while the omega-3 (n-3) fatty acids such as ALA are precursors for the F-3 series (Figure A1-1). There is a direct competition for the desaturation and elongation enzymes between the n-6 and n-3 fatty acids, thus increasing the overall concentration of one type of fatty acid in the diet will shift the production of eicosanoids. For example, increasing dietary n-3 will shift the pathway towards the production of $PGF_{3\alpha}$ at the expense of $PGF_{2\alpha}$.

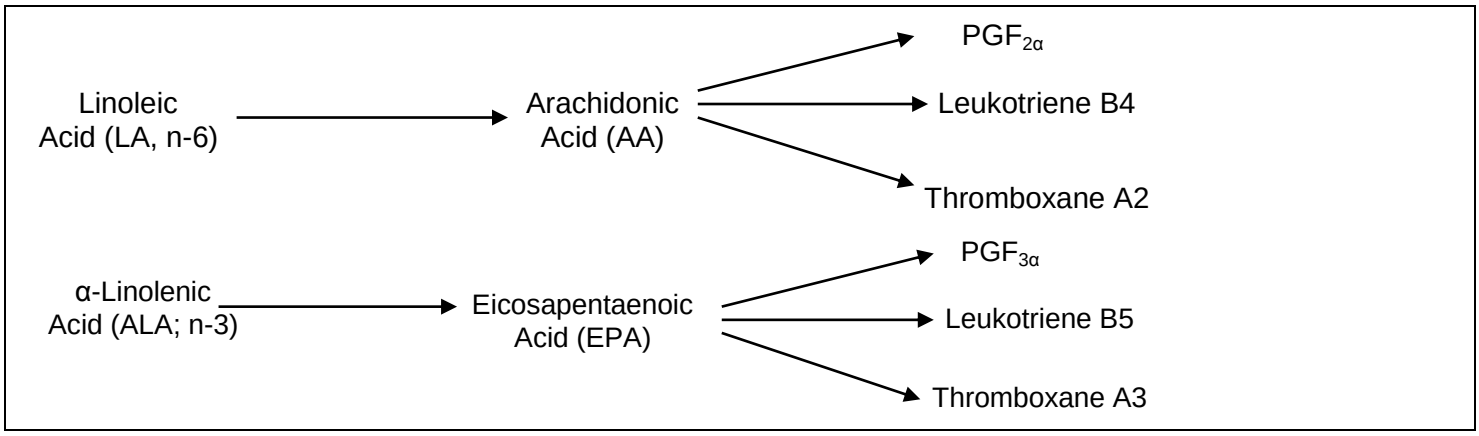

Figure A1-1: Omega-6 and omega-3 fatty acids as precursors for eicosanoids

As discussed by Holub (2002), there is evidence that in humans, n-3 fatty acids reduce the risk of heart disease by reducing ventricular arrhythmias, platelet reactivity and thrombotic effects as well as by reducing plasma viscosity. Omega-3's also increase levels of HDL leading to a reduction in LDL and VLDL, reducing the risk of atherosclerosis. Omega-3 fatty acids have also been shown to have anti-cancer properties (Dolecek, 1992; Johnston, 1995; Baro et al., 1998; Klein et al., 2000).

There are several review papers which discuss the effects of n-3 fatty acids on reproduction in animals and humans (Abayasekara and Wathes, 1999; Allen and Harris, 2001; Wathes et al., 2007). Petit and colleagues have shown in several studies that alteration of dietary PUFA content in cattle can alter the number and size of pre-ovulatory follicles, ovulation rate, conception rate, progesterone production by the corpus luteum and PGF2α production which in turn affects luteolysis and the length of gestation (Petit et al., 2001; Petit et al., 2002; Petit and Twagiramungu, 2006). In pigs, inclusion of dietary n-3 fatty acids into sow rations increases the PUFA content in sow and piglet plasma as well as in milk (Fritsche et al., 1993; Rooke et al., 2001); which has been attributed to improving pre-weaning survival albeit, inconsistently. Webel et al. (2003) were also able to

show an increase in the number of piglets born alive when sows were fed a source of fish oil (high in n-3). A study by Dunstan et al. (2004) found that women fed fish oil had increased IgA concentrations in their milk. Additionally, Jackson et al. (1995) showed that corn fed sows (high n-6 fatty acids) had depressed IgG concentrations in their milk. A recent article by Mateo et al. (2009) presented results of increased IgG concentrations in the colostrum and milk of sows fed a fish product. PUFA's thus have great potential for improving the reproductive performances of sows.

Much of the scientific literature discusses omega-3 fatty acids in two groups, the less biologically active ALA, or DHA and EPA which are believed to be the omega-3's responsible for many of the health and reproductive benefits described above. DHA and EPA within the body are from either direct consumption (ex. fish oil), or through biosynthesis pathways utilizing dietary ALA as the precursor. As mentioned above, synthesis of the long chain n-6 and n-3 fatty acids from their shorter precursors is a competitive process as they both utilize the same enzymes. Many papers provide estimates as to the efficiency of the conversion of ALA into its longer chain counterparts in humans; however, although the estimates are variable, it is apparent that this conversion is not a very efficient process (<10-15%). Within the swine literature, there appear to be no true estimates of this conversion process; however, some groups believe that the conversion is more efficient than in humans (Martinez, personal communication). Importantly, studies looking at the conversion of ALA into EPA and DHA have shown that it is dependent on the ratio of the fatty acids in the diet, not the absolute amount of them (Harnack et al., 2009).

There are several methods which can be utilized to determine the conversion rates of ALA into EPA and DHA. The most accurate method of measuring conversion is to use [13^{C}]-labeled linoleic or α-linolenic acids and measure the recovery of this label in the longer-chain homologues (Harnack et al., 2009); this method, however, is expensive and not practical in most situations. Another method to measure conversion is to measure the activity of the delta-5 and delta-6 desaturase enzymes (which are the rate limiting steps in *de novo* long chain PUFA synthesis) to determine if the ratio of the fatty acids in the diets cause a differential regulation of the enzymes involved in the conversion process, This data can be combined with that of the actual fatty acid profiles of the animals (McNeil et al., 2005). Since the fatty acid profiles can be easily measured, and those animals receiving ALA in their diets at differing ratios can be compared to both the dietary profile and the fatty acid profile of the control animals, we can estimate the conversion of ALA into EPA and DHA using a much more practical and cost effective method.

HYPOTHESIS

Reducing the dietary ratio of n-6 to n-3 polyunsaturated fatty acids in sows will improve reproductive efficiency, as well as improve the immune status of piglets by:

- Altering the fatty acid profile of the sows and piglets, thus shifting production of the eicosanoid and immune cell precursors from the n-6 to n-3 fatty acids
- Increasing the n-3 fatty acid, IgG and IgA content of colostrum, thus improving the immune cell status of piglets after colostrum ingestion
- Altering the enzyme activity levels of Delta-5 and Delta-6 desaturases, thus improving the conversion of ALA into EPA and DHA.

OBJECTIVES

The overall objective of this experiment is to improve the reproductive performances of sows through alteration of dietary n-6 to n-3 polyunsaturated fatty acid ratios, thus improving the economic status of sow reproduction.

This study can be divided into 2 major objective sections as follows:

- The effects of n-3/n-6 on generalized parameters including fatty acid and immunoglobulin profiles, and on sow production parameters.
    - Effects on the sow
    - Effects on the piglet
- Effect of n-3/n-6 on the conversion of ALA to EPA and DHA

MATERIALS AND METHODS

Detailed description of animals and diets can be found in the main report. Data collected for cycles 1 and 2 are:

Cycle 1:

- Sow weights (when first assigned to their diet and at weaning)
- Number of piglets born (total, live, stillborn, mummified)
- Litter weights (at birth and weaning)
- Weaning to estrus interval
- Any other available production data on the animals such as required treatments and cross-foster data.

Cycle 2, all sows:

- Average daily feed intake (ADFI) during lactation
- Sow weights and backfat thickness at d110 of gestation, within 24-hr post farrowing, 1 week post farrowing and again at weaning.
- Number of piglets born (total, live, mummies and stillborn)
- Individual piglet weights (at birth and weaning) to determine the variability in litter weights as well as individual piglet growth performance during lactation.
- Sex ratio of the piglets
- Weaning to estrus interval

Cycle 2, 12 sows/diet:

- Sow blood collection for fatty acid profile analysis (d 110 of gestation)
- Colostrum collection for fatty acid analysis and IgG and IgA analysis
- 2 piglets/litter will be bled for fatty acid and immunoglobulin analysis at birth (pre- and post- suckle samples)
- The pre-suckle blood sample piglet will be euthanized and the liver collected in order to measure the enzyme activity levels of delta-5 and delta-6 desaturases

During Cycle 2 liver samples from 1 pig per litter, at birth, were collected. The pigs were euthanized according to standard procedures for their age/size, and their livers were extracted for enzyme analysis. Livers were stored at -80^{o}C until analysis. The enzymes of interest are the delta 5 and delta 6 desaturase enzymes.

We will be able to correlate the enzyme activity levels with the FA profiles found in the blood, thus allowing us to determine the conversion of ALA into EPA and DHA depending on the ratio of n-3 to n-6 in the diets of the sows.

RESULTS

Nearly all analysis for this section of the overall project is complete; however, laboratory analysis of piglet livers for delta-5 and delta-6 desaturase enzyme activity is pending.

Performance results for cycle 1 and cycle 2 are shown in Table A1-1. There was no affect of diet on the total number of piglets born, born alive, or litter birth weights for either cycle ($P > 0.05$). Average piglet weaning weight was higher for the 10:1 and 5:1 plant based groups when compared to the 1:1 and fish based groups during cycle 1 ($P = 0.02$). During cycle 2, fish oil sows consumed 10% less feed ($P = 0.04$), had reduced piglet birth weights ($P = 0.05$), and average piglet weaning weight was reduced by 0.8 kg/piglet ($P = 0.04$) when compared to control and 5:1 plant oil based sows.

Immunoglobulin (IgA and IgG) concentrations found in colostrum and piglet serum (pre and post suckle) are shown in Table A1-2. There were no effects of sow diet consumption on immunoglobulin production ($P > 0.05$).

The fatty acid profile of colostrum collected at the time of farrowing is shown in Table A1-3, and the profile of sow and piglet (pre and post suckle) plasma can be found in Table A1-4. Total plasma n-3 FA's were greater in sows ($P < 0.0001$) and post-suckle piglets ($P = 0.004$) consuming the 1:1 and fish diets. The ALA content was highest in the 1:1 group whereas EPA and DHA were highest in the fish group. In pre-suckle piglet plasma, ALA and DHA did not differ among treatment groups ($P > 0.05$). Relative to the control piglets, EPA was 2.5 times greater in the 1:1 group and 4 times greater in the fish group ($P < 0.0001$) prior to suckling. In post-suckle samples, ALA was highest in piglets from the 1:1 diet group ($P < 0.005$), and EPA and DHA were highest in piglets from the fish based sows ($P < 0.0001$) which was expected when compared to the colostrum FA profile.

The FA profile of pre-suckle piglet plasma indicates that the conversion of ALA into EPA can be increased by reducing the dietary n-6:n-3 FA ratio of their mothers. It is possible that reduced competition for the enzymes responsible for the desaturation and elongation occurs, allowing for increased selection of the n-3 FA's by these enzymes thus improving conversion efficiency.

CONCLUSIONS

The results from this study indicate that the long term feeding of decreased n-6:n-3 ratio diets to sows can affect reproductive performances. A plant oil based ratio of 5:1 (n-6:n-3) maximized piglet growth and sow feed intakes, and did not affect her return to estrus interval. Sows consuming the fish oil diet consumed less feed and had reduced piglet birth and weaning

weights when compared to the other treatment groups. Increasing the intake of plant based n-3 FA's in a 1:1 (n-6:n-3) ratio increased circulating levels of EPA in addition to ALA in both sows and piglets. This indicates that flaxseed may be a viable option for reducing the n-6:n-3 ratio and increasing the n-3 content in sow diets, and thus the benefits of including n-3 FA's into swine rations can be achieved using a locally grown crop.

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Table A1-1: Reproductive performances of sows consuming differing dietary omega-6 to omega-3 ratios during Cycles 1 and 2

	Control	10:1	5:1	1:1	5:1 Fish	SEM	*P* Value
Cycle 1							
Avg. No. Born Alive	12.8	12.6	12.4	13.0	13.0	0.50	0.919
Avg. No. Born Total	13.7	13.6	13.7	14.3	14.4	0.54	0.729
Live Litter Birth Weight (kg)	18.7	18.3	18.5	17.9	17.7	0.78	0.894
Avg. Birth Weight (kg)	1.5	1.5	1.5	1.4	1.3	0.05	0.101
Avg. Weaning Weight (kg)	8.2^{ab}	8.6^{a}	8.6^{a}	8.0^{b}	7.8^{b}	0.19	0.019
Cycle 2							
Sow ADFI (kg)	7.5^{a}	7.4^{a}	7.6^{a}	7.5^{a}	6.8^{b}	0.20	0.036
Sow Weight Change (kg/lact)[1]	-5.6	-8.0	-5.6	-3.3	-11.7	2.63	0.291
Sow Backfat Change (mm/lact)[1]	-0.8	-1.1	-0.7	-0.9	-0.7	0.22	0.712
Wean to Estrus Interval	4.1	4.9	4.2	3.9	5.1	0.42	0.171
Avg. No. Born Alive	12.5	12.5	11.5	12.3	13.0	0.60	0.538
Avg. No. Born Total	13.3	14.0	12.9	14.0	14.4	0.63	0.464
Live Litter Birth Weight (kg)	18.1	17.5	16.8	17.7	16.9	0.77	0.725
Avg. Birth Weight (kg)	1.5^{a}	1.4^{ab}	1.5^{a}	1.4^{ab}	1.3^{b}	0.05	0.050
Avg. Weaning Weight (kg)	8.8^{a}	8.7^{ab}	9.2^{a}	8.7^{ab}	8.2^{b}	0.21	0.040

[1]Avgerage lactation length of 26 days

Table A1-2: IgA and IgG concentrations (mg/ml) of colostrum and piglet pre- and post-suckle serum samples

	Control	10:1	5:1	1:1	5:1 Fish	SEM	*P* Value
Colostrum							
IgA	16.4	18.3	15.8	17.6	15.6	1.95	0.837
IgG	82.8	90.7	81.4	87.3	82.9	7.55	0.903
Pre-Suckle Plasma							
IgA	ND	ND	ND	ND	ND	-	-
IgG	ND	ND	ND	ND	ND	-	-
Post-suckle							
IgA	7.4	6.5	7.7	7.1	6.8	1.26	0.960
IgG	27.9	23.3	25.9	26.9	24.1	4.17	0.931

ND = not detected, assay detection limits were 0.1 mg/ml for IgA and 0.9 mg/ml for IgG

Table A1-3: Colostrum fatty acid profiles (mg FA/ml colostrum)

Fatty Acid	Control	10:1	5:1	1:1	5:1 Fish	SEM	*P* Value
Caprylic Acid (8:0)	0.47	0.44	0.95	1.19	1.08	0.263	0.153
Capric Acid (10:0)	0.03	0.03	0.05	0.04	0.03	0.006	0.136
Lauric Acid (12:0)	0.14	0.11	0.13	0.13	0.13	0.019	0.922
Myristic Acid (14:0)	6.39^{ab}	4.41^{b}	4.47^{b}	5.03^{b}	7.74^{a}	0.732	0.014
Myristoleic Acid (14:1)	0.14	0.11	0.09	0.15	0.12	0.020	0.298
Palmitic Acid (16:0)	61.77	57.24	49.18	57.71	43.03	6.299	0.278
Palmitoleic Acid (16:1)	9.04^{ab}	5.42^{c}	5.40^{c}	6.98^{bc}	10.25^{a}	1.075	0.009
Stearic Acid (18:0)	13.43	12.28	18.99	15.55	15.53	3.039	0.589
Oleic Acid (18:1 cis)	85.65^{a}	78.67^{a}	47.46^{bc}	76.68^{ab}	39.87^{c}	10.400	0.011
Vacinic Acid (18:1 trans)	6.57^{a}	5.11^{a}	3.01^{bc}	8.78^{ab}	7.01^{c}	1.668	0.171
Linoleic Acid (18:2 n6)	44.90^{a}	96.47^{c}	81.88^{bc}	70.04^{b}	28.40^{a}	8.474	<0.001
y-Linolenic Acid (18:3)	0.39	0.66	0.57	0.47	0.42	0.081	0.131
α-Linolenic Acid (18:3 n3)	4.54^{a}	16.43^{a}	17.31^{a}	50.38^{b}	4.85^{a}	4.683	<0.001
Arachidic Acid (20:0)	0.54	0.49	0.41	0.42	1.14	0.199	0.096
Eicosanoic Acid (20:1)	0.49^{a}	0.70^{a}	0.39^{a}	0.56^{a}	1.55^{b}	0.201	0.002
Eicosadienoic Acid (20:2)	0.78^{a}	1.39^{a}	1.22^{a}	1.26^{a}	3.08^{b}	0.390	0.003
Eicosatrienoic Acid (20:3)	1.50	1.85	1.45	2.01	0.79	0.336	0.155
Arachidonic Acid (20:4 n6)	1.18^{ab}	1.57^{a}	0.88^{bc}	1.07^{bc}	0.59^{c}	0.163	0.003
Eicosapentaenoic Acid (20:5 n3)	0.71^{a}	0.80^{a}	0.75^{a}	1.93^{b}	1.86^{b}	0.304	0.006
Behenic Acid (22:0)	0.20^{a}	0.22^{a}	0.23^{a}	0.78^{a}	2.39^{b}	0.227	<0.001
Erucic Acid (22:1)	0.28^{a}	0.32^{a}	0.37^{a}	1.54^{b}	2.06^{b}	0.346	0.001
Docosahexaenoic Acid (22:6 n3)	0.53^{a}	0.45^{a}	0.38^{a}	0.68^{a}	5.33^{b}	0.346	<0.001
Lignoceric Acid (24:0)	1.55^{a}	1.71^{a}	1.73^{a}	3.05^{b}	3.16^{b}	0.271	<0.001
Nervonic Acid (24:1)	0.24^{a}	0.23^{a}	0.22^{a}	0.24^{a}	0.65^{b}	0.039	<0.001
Total n3	7.29^{a}	19.53^{a}	19.89^{a}	55.01^{b}	12.83^{a}	4.959	<0.001
Total n6	47.24^{a}	100.08^{b}	84.54^{bc}	72.85^{c}	32.49^{a}	8.805	<0.001

Table A1-4: Sow and piglet plasma omega-3 and omega-6 fatty acid profiles (mg FA/ml Plasma)

Fatty Acid	Control	10:1	5:1	1:1	5:1 Fish	SEM	*P* Value
Sow Plasma Fatty Acids							
Linoleic Acid (18:2 n6)	1.20	1.56	1.39	1.12	1.18	0.211	0.599
α-Linolenic Acid (18:3 n3)	0.06^{a}	0.09^{a}	0.17^{a}	0.37^{a}	0.10^{a}	0.042	<0.001
Arachidonic Acid (20:4 n6)	0.20^{a}	0.19^{a}	0.14^{ab}	0.09^{b}	0.11^{b}	0.023	0.006
Eicosapentaenoic Acid (20:5 n3)	0.02^{ab}	0.02^{a}	0.03^{ab}	0.06^{b}	0.26^{c}	0.015	<0.001
Docosahexaenoic Acid (22:6 n3)	0.02^{a}	0.02^{a}	0.02^{a}	0.02^{a}	0.10^{b}	0.009	<0.001
Total n3	0.12^{a}	0.15^{a}	0.25^{a}	0.51^{b}	0.55^{b}	0.050	<0.001
Total n6	1.44	1.82	1.63	1.41	1.33	0.202	0.462
Pre-suckle Piglet Plasma Fatty Acids							
Linoleic Acid (18:2 n6)	0.11	0.10	0.20	0.09	0.10	0.039	0.229
α-Linolenic Acid (18:3 n3)	0.01	0.01	0.02	0.01	0.01	0.006	0.507
Arachidonic Acid (20:4 n6)	0.24^{a}	0.23^{a}	0.21^{a}	0.13^{b}	0.12^{b}	0.021	<0.001
Eicosapentaenoic Acid (20:5 n3)	0.01^{a}	0.01^{a}	0.02^{a}	0.04^{b}	0.06^{c}	0.003	<0.001
Docosahexaenoic Acid (22:6 n3)	0.15	0.08	0.11	0.12	0.16	0.022	0.078
Total n3	0.21^{ab}	0.14^{a}	0.21^{ab}	0.23^{bc}	0.31^{c}	0.029	0.004
Total n6	0.26	0.18	0.32	0.21	0.26	0.049	0.331
Post-suckle Piglet Plasma Fatty Acids							
Linoleic Acid (18:2 n6)	0.57^{a}	1.36^{b}	1.24^{b}	0.61^{a}	0.53^{a}	0.204	0.007
α-Linolenic Acid (18:3 n3)	0.03^{a}	0.11^{ab}	0.16^{bc}	0.22^{c}	0.04^{a}	0.037	0.004
Arachidonic Acid (20:4 n6)	0.20^{a}	0.30^{b}	0.27^{b}	0.14^{a}	0.15^{a}	0.023	<0.001
Eicosapentaenoic Acid (20:5 n3)	0.01^{a}	0.02^{a}	0.03^{a}	0.05^{a}	0.16^{b}	0.011	<0.001
Docosahexaenoic Acid (22:6 n3)	0.09^{a}	0.07^{a}	0.10^{a}	0.09^{a}	0.18^{b}	0.014	<0.001
Total n3	0.18^{a}	0.36^{ab}	0.51^{bc}	0.64^{c}	0.59^{bc}	0.088	0.003
Total n6	0.60^{a}	1.43^{b}	1.34^{b}	0.70^{a}	0.70^{a}	0.207	0.01

Appendix 2: Pre-natal programming: the effects of sow diets on piglet immune responses when challenged with *E.coli* lipopolysaccharide (LPS)

Note – the sections presented in this appendix are not funded by NPB but are part of the overall series of experiments. Details are provided to show the overall status of the project and will allow interpretation of the NPB funded experiments in the context of the overall project.

EXECUTIVE SUMMARY

Omega-3 fatty acids are known to have many different health benefits, including anti-inflammatory properties. In the hog industry, weaning can be considered the most stressful time in a piglet's life, as they are not only removed from the sow, but are exposed to new housing, feeds and pen-mates. All of these parameters combine to trigger an immune response in the animal, which may have negative effects such as reducing feed intake and performance.

This project was designed to determine if feeding a diet high in omega-3 fatty acids to sows could alter the immune responses of piglets when challenged post-weaning with *E. Coli* lipopolysaccharide (LPS). The project is a component of the project entitled "the effects of increasing dietary intake of omega-3 fatty acids on the reproductive functions of sows and on postpartum hypophagia and milk energy output via alterations in the lipolytic activity of adipose tissue". The piglets in this trial were produced from sows being fed one of five diets containing differing ratios of long chain fatty acids provided through a variety of fat sources including flax, fish and corn.

One week post-weaning, piglets were assigned to either a control group (saline injected) or an LPS injected group and a series of blood samples were collected to monitor cytokine responses. Febrile data was also collected for a 24 hr period.

Diet affected body temperature over the 24 hour period, with piglets from sows consuming the 1:1 diet having a greater body temperature than those from the control, 10:1 and 5:1 groups ($P = 0.0004$). Body temperatures of piglets produced from sows consuming the 5:1 fish based diet were intermediate ($39.74^{o}C$). A diet by immune challenge interaction tended to be different for both body temperature ($P=0.1163$) and IL-8 ($P=0.1819$). Piglets from the 1:1 and 5:1 fish diet groups had a greater IL-8 response to the immune challenge when compared to piglets from the other diets. A greater febrile response to the LPS challenge also occurred in piglets originating from sows consuming the 1:1 ratio diet.

Piglets raised by sows consuming diets with altered omega-3 to omega-6 fatty acid ratios responded differently when challenged with LPS as an inflammatory model. It is clear that altering sow rations can have effects on their offspring even after they are weaned.

INTRODUCTION

Over the years there have been many nutritional strategies implemented with the aim of improving animal and human health. Recently, there has been a growing interest in the use of dietary long chain polyunsaturated fatty acids (PUFA's), as PUFA's have been implicated in having many health benefits. Similar to others livestock producers, the pork industry is looking to omega-3 fatty acids to improve the health and productiveness of their herds.

Polyunsaturated fatty acids act on many components of the biological system, which is primarily due to the fact that they act as precursors for many different hormones and molecules. Linoleic acid (LA) and α-linolenic acid (ALA) are precursors for the eicosanoids, which includes prostaglandins (some of the most abundant molecular forms found in the body), leukotrienes and thromboxanes (Lands, 1992). Omega-6 (n-6) fatty acids such as LA, are precursors for the F-2 series prostaglandins while the omega-3 (n-3) fatty acids such as ALA, are precursors for the F-3 series. There is a direct competition for the desaturation and elongation enzymes between the n-6 and n-3 fatty acids, thus increasing the overall concentration of one type of fatty acid in the diet will shift the production of eicosanoids.

As well as influencing the eicosanoids, there is significant evidence in the literature that intake of n-3 fatty acids alters the profile of cytokine molecules. Cytokines are proteins which are secreted by immune cells in response to stimuli, and assist in regulating the development of immune effector cells or by acting as an effector themselves. It is believed that n-3 fatty acids modulate cytokine function by acting upon intracellular signaling pathways, transcription factor activity as well as gene expression (Simopoulos, 2002). Essentially, interactions between immune and inflammatory cells are mediated by cytokines, of which tumor necrosis factor (TNF-α), interleukin (IL)-1 and IL-6 are the most important. They are produced by both monocytes and macrophages, and production of appropriate amounts in response to infection is important and beneficial; however, over or inappropriate production of these cells can be detrimental. Generation of an appropriate immune response is essential when an animal is immunologically challenged but it has been well documented that the pro-inflammatory cytokines divert nutrients to the synthesis of other immune molecules resulting in increased muscle degradation, and reduced protein synthesis (Zhan et al., 2009).

In the swine production industry, weaning is certainly the most stressful time in a piglet's life and new immune challenges are presented to the animal. It is during this time that feed intakes are reduced, and an immune response will be generated. Although a certain degree of immune response would be beneficial during this time, it would be worth exploring if the effects of the sow's diet could influence the immune status of the piglets, and if providing n-3 fatty acids could reduce the production of immune cells and improve the performance of the piglets post-weaning. *E. Coli* lipopolysaccharide (LPS) is commonly used to study the immune response of animals including humans and pigs. LPS triggers the release of pro-inflammatory cytokines, which in turn triggers the hypothalamic-pituitary-axis (HPA) to release cortisol, and also induces a febrile response (Williams et al., 2009). Despite generating an immune response, LPS is only one component of the bacteria and does not contain the ability to replicate thus making it safe for use in healthy herds.

This experiment is one component of a project entitled "the effects of increasing dietary intake of omega-3 fatty acids on the reproductive functions of sows". The piglets studied in this trial were produced from sows being fed one of five diets containing differing ratios of long chain fatty acids provided through a variety of fat sources including flax, fish and corn. The treatment groups included a control group (tallow based, thus primarily saturated), 10:1 ratio of n-6 to n-3 fatty acids, a 5:1 ratio, a 1:1 ratio which was plant based, and a 5:1 fish based ratio. The total fat content of the diets remained constant and only the ratio of n-6 to n-3's changed. The project was designed to look at the immune responses of piglets produced by these sows post-weaning, and to determine if the fatty acid profile of the sow diet significantly impacted piglet health.

HYPOTHESIS

Reducing the dietary ratio of n-6 to n-3 polyunsaturated fatty acids in sows will alter the immune cell profile in response to LPS challenges in newly weaned pigs by reducing the severity of a febrile response and by reducing cytokine production.

OBJECTIVES

The overall objective is to alter the production of pro-inflammatory cytokines in newly weaned piglets through pre-natal programming influenced by the fatty acid profile of the sow's diet.

Using piglets obtained from litters in which sows were fed one of 5 dietary n-6 to n-3 fatty acid ratios, the specific objectives were to:

- Characterize the febrile response of piglets challenged with LPS
- Characterize the production of pro-inflammatory cytokines such as IL-1, IL-6 and TNF-α
- Characterize the profile of blood urea nitrogen (BUN) production as an indicator of muscle breakdown

MATERIALS AND METHODS

This experiment was conducted at Prairie Swine Centre Inc. (PSCI) in the new sow housing facility and nursery.

Animals and Treatments

A total of 100 piglets were used over a period of approximately 6 weeks, dependent upon availability. Barrows, of approximately equal starting body weights were selected. The piglets originated from sows fed one of 5 dietary treatments, divided into a gestation and a lactation ration. Fatty acids in these diets were supplemented at differing levels to adjust the ratio of n-3 to n-6 fatty acids in the diets. The sow diets were 1) control (devoid of both n-3 and n-6 fatty acids), 2) 10:1 ratio of n-6 to n-3, 3) 5:1 ratio of n-6 to n-3, 4) 1:1 ratio, 5) 5:1 ratio (fish based).

Post-weaning, all of the piglets were fed a highly palatable common starter diet. A total of 100 piglets were used; 10 control and 10 LPS challenged piglets for each diet (5 diets x 20 piglets = 100).

Data Collection/Experimental Procedure

Preliminary experiment:

A preliminary experiment was conducted to determine the optimal dose of LPS requied to generate an immune response without harming the animals. Based on the literature and personal communication with Nicolas Gabler (Iowa State), this would be between 10 and 15 µg/kg BW of E. Coli 055B5 based LPS (Sigma Chemicals). Our pre-trial indicated a dose of 15 µg/kg was suitable.

Main experiment:

In order to reduce the effects of handling stress on the immune response of the piglets, all piglets within each of the pre-selected litters were handled a minimum of 4x per week for 2 weeks prior to the trial (prior to weaning), and were placed into a recumbent position to mimic blood collection as well as having a rectal thermometer inserted. This allowed the piglet time to adapt to the handling procedures required for this trial.

Piglets were weaned on Tuesday mornings and moved to an environmentally controlled nursery room, and housed in groups of 2 per pen. All of the piglets were housed with an unfamiliar pen-mate of similar size, thus all piglets was subjected to the stress of mixing. Sow availability depended on week and thus each week had different numbers of piglets put on trial. The piglets were allowed to adapt to their new environment for 6 days followed by the 24 hour immune challenge. Piglets were able to return to the normal production herd following the immune challenge.

Each pen was randomly assigned to either the control treatment (saline injection) or the LPS treatment (injection of 15µg LPS/kg BW), thus the experiment used a 5 x 2 factorial arrangement of treatments (5 sow diets x 2 immune treatment groups). During the 24 hour challenge, feed disappearance for each pen was recorded and all pigs were weighed at time 0 and time 24 hours. At time 0, each piglet's rectal temperature was recorded and a pre-challenge blood sample drawn. This was immediately followed by an injection of either saline or LPS. Rectal temperatures were recorded every hour for the first 6 hours post injection and then at 12 and 24 hours. Blood was collected into evacuated blood tubes at time 0, 2, 6 and 12 hours for analysis of pro-inflammatory cytokines and blood urea nitrogen. To facilitate collections, the pens were started over a 1 hour period.

Analysis

Plasma samples were analyzed in duplicate for TNF-α, IL-1β, IL-6, IL-8 and BUN. Cytokine samples were sent to Aushon Biosystems (Billerica, MA) for analysis. Blood urea nitrogen was analyzed using a commercially available kit (BioAssay Systems, CA).

Statistics

All data (rectal temperatures, feed intakes, body weight changes and blood parameters) was analyzed using the PROC GLIMMIX function of SAS using a factorial design with repeated measures. The factorial design allowed for the comparison both the effects of diet and the effects of the LPS challenge on all parameters. Significance was declared if $P < 0.05$. Time course data was also analyzed using area under the curve and net incremental area under the curve (to account for baseline).

Animal Care

All animals used were cared for and monitored according to PSCI's Standard Operating Procedures.

Animal care protocols (# 19970020 and #20090094) approved by the University of Saskatchewan Committee of Animal Care and Supply for adherence to the standards outlined by the Canadian Council for Animal Care were received for this experiment.

Additional Experiments:

Two additional experiments are currently (August 2011) in progress and data is not available. The additional experiments are as follows:

Experiment 2: This experiment will be run as described above (experiment 1), the only difference is that the piglets used will be obtained from sows consuming normal production diets, and the piglets will be fed starter diets with differing fatty acid ratios (the ratios will be the same as those used in the sow diets). Again, 100 pigs and a 5 x 2 factorial arrangement of treatments (5 diets and 2 immune challenge treatments) will be used. The piglets will begin these diets immediately post-weaning and will be subjected to the immune challenge 6 days later as in experiment 1.

Experiment 3: This component will be designed to look at nitrogen balance and turnover within the piglet bodies during an LPS challenge. As in experiment 2, the piglets will be fed diets containing altered FA ratios (mimicking that of the sow diets). Based on the results obtained from both Experiment 1 and 2, we will select 2 of the dietary treatment groups to pursue in further detail, to determine exactly how the FA profile of the diet during an immune challenge will affect protein deposition, breakdown and excretion from the body. This experiment will be a 3 x 2 factorial design, with 3 challenge/feed intake groups and 2 diets. The 3 challenge groups are a control (saline injected, ad libidum feed intake), the LPS challenge group (LPS injected, ad libidum feed intake) and a saline injected group consuming the same amount of feed as the LPS injected group (ad libidum feed intakes for the LPS group will be less than the ad libidum control group). A total of 42 newly weaned piglets of equal body weights will be used (n=7/trt). Pigs will be housed individually in raised metabolism crates allowing for total urine and fecal collections. Pigs will be given 6 days to adapt to their new diets and surroundings, which will be followed by a nitrogen balance period of 4 days during which total urine and feces will be collected to determine the nitrogen output of each animal. On day 1 and 3 of the N balance

period, the pigs will be injected with either LPS or Saline as described above. Following their respective injections on day 3, the pigs will be gastrically infused with ^{15}N-Glycine at a dose of 5 mg/kg BW. Urine and feces will be collected for 2 days post infusion. Biological samples obtained from the pigs prior to infusion will allow for the determination of the background enrichment of ^{15}N in the pig. Whole body protein turnover, and thus protein synthesis and degradation rates will be calculated using the model of Picou and Taylor-Robert (1969). The effects of an LPS immune challenge on protein turnover have been conducted previously by Daiwen et al. (2007). Our experiment will add the component of feeding altered omega-6:omega-3 FA ratios to this model.

RESULTS

The animal and laboratory work for experiment 1 has been completed. Animal work for experiment 2 has been completed and laboratory work is underway. Experiment 3 will begin once results for experiment 1 and 2 are complete.

Figure A2-1 shows the febrile response of piglets from each diet and challenge group. All pigs injected with LPS had an elevated body temperature, indicating that the challenge model worked ($P < 0.001$). Statistical analysis of the challenge, time and challenge*time interaction for body temperature, IL-1β, IL-8 and TNFα were all significant ($P < 0.05$) and tended to affect IL-6 also ($P < 0.15$). P values for each parameter and their interactions for each of the measurements are shown in Table A2-1.

Diet had a significant effect on body temperature, with piglets produced by sows consuming the 1:1 diet having a greater body temperature (39.90°C) than those from the control (39.62^{o}C), 10:1 (39.56^{o}C) and 5:1(39.54^{o}C) groups ($P = 0.0004$). Body temperatures of piglets produced from sows consuming the 5:1 fish based diet were intermediate (39.74^{o}C).

The diet by immune challenge interaction tended to be different for body temperature ($P=0.1163$). Piglets from the 1:1 and 5:1 fish diet groups had a greater IL-8 response to the immune challenge when compared to piglets from the other diets (Figure A2-2). A greater febrile response to the LPS challenge also occurred in piglets originating from sows consuming the 1:1 ratio diet (Figure A2-3).

Fatty acid analysis of late lactation milk samples revealed that the omega-3 to omega-6 profiles were similar to those of the sow diets with ratios of 7.5:1, 4.5:1, 1.5:1 and 3:1 for the 10:1, 5:1, 1:1 and 5:1 fish diets respectively. These samples were analyzed as part of the project entitled "The influence of increasing dietary intake of omega-3 fatty acid concentration on postpartum hypophagia and energy output in the milk via alterations in lipolytic activity and insulin sensitivity of the adipose tissue".

CONCLUSIONS

Feeding programs for sows can affect how their offspring respond to the immune challenges which are presented at weaning. Altering the omega-3 to omega-6 fatty acid ratio in

sow diets had effects on febrile responses and cytokine responses of their offspring when challenged with LPS post-weaning. Piglets produced by sows consuming a 1:1 ratio diet had elevated rectal temperatures, and had a greater response to the immune challenge when compared to the other diets. It is possible that this could be beneficial, in that the animals have a greater capacity to fight off an immune challenge, or it may cause a sub-clinical, more energetically expensive affect throughout the weaning period. Further experiments will help determine the energetic costs of these immune responses on the animals.

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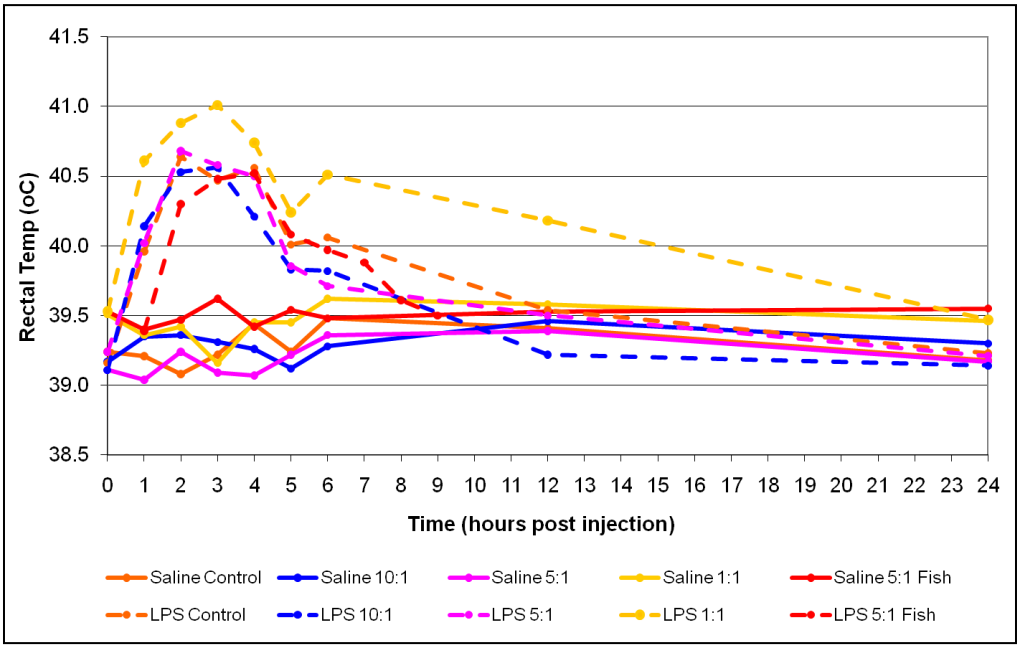

Figure A2-1: Average rectal temperatures of pigs treated with LPS or saline after being raised by sows consuming varying n-6 to n-3 fatty acid ratios

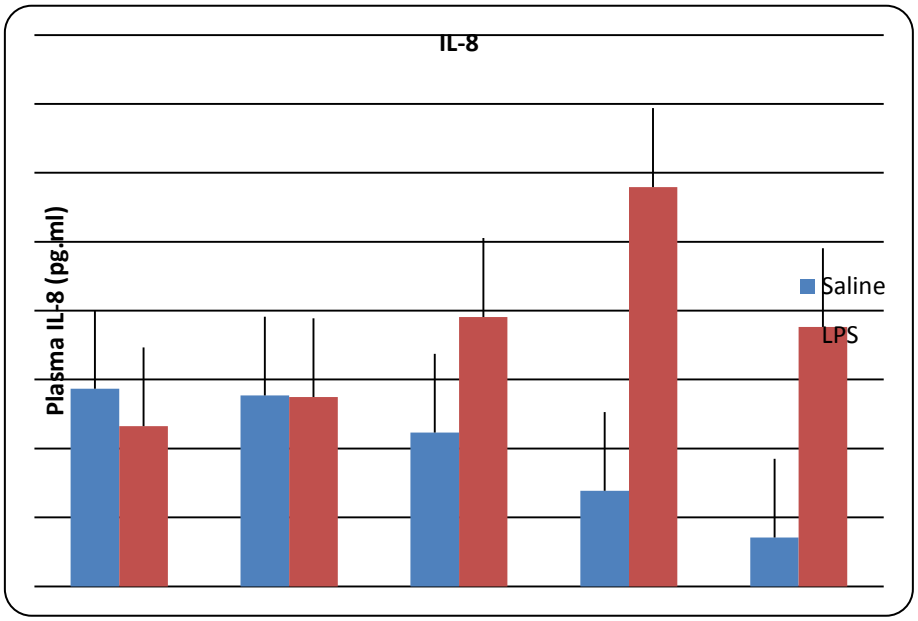

Figure A2-3: Diet x Challenge interaction of plasma IL-8 concentration (bars show the mean ± SEM)

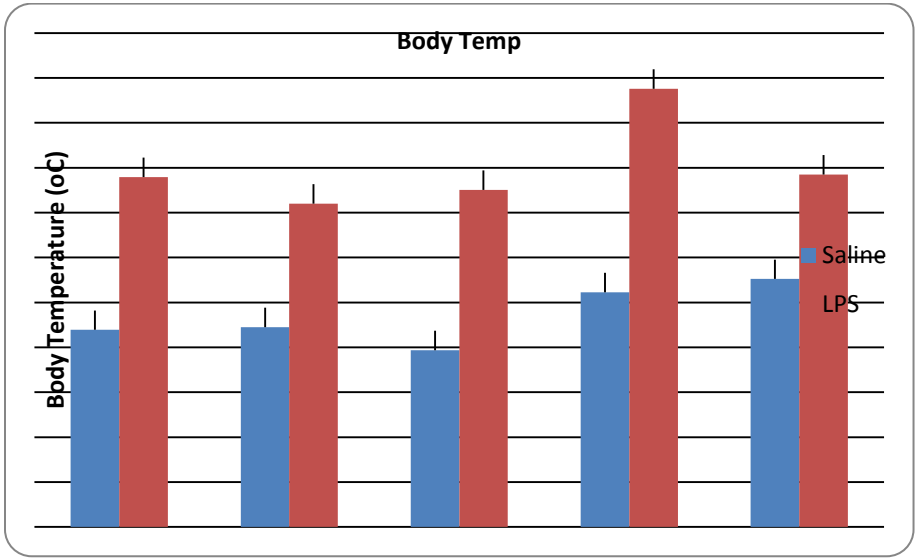

Figure A2-4: Diet x Challenge interaction of body temperature in piglets (bars show the mean ± SEM)

Table A2-1: P-values for each parameter and their interactions for piglet body temperature and plasma cytokines

	P-Values						
	Diet	**Challenge**	**Time**	**Diet x Challenge**	**Diet x Time**	**Challenge x Time**	**Diet x Challenge x Time**
Temp (^{o}C)	**0.0004**	**<0.0001**	**<0.0001**	*0.1163*	0.2938	**<0.0001**	0.6258
IL-1β (pg/ml)	0.7808	**0.0489**	**0.0024**	0.5074	0.9774	**0.0011**	0.8760
IL-6 (pg/ml)	0.5840	*0.0551*	*0.1721*	0.6980	0.9577	0.1407	0.9559
IL-8 (pg/ml)	0.8069	**0.0226**	**<0.0001**	*0.1816*	*0.1157*	**<0.0001**	*0.1430*
TNFα (pg/ml)	0.5435	**<0.0001**	**<0.0001**	0.4743	0.7437	**<0.0001**	0.7224