RESEARCH REPORT
ANIMAL SCIENCE

Title: Effect of GnRH-II on reproductive efficiency and productivity of first parity sows – **NPB #12-184**

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Industry Summary:

Although never investigated, gonadotropin-releasing hormone II (GnRH-II) may be associated with the interaction between nutritional status and reproduction in swine. Therefore, we examined the role of GnRH-II in return to estrus following lactation of first parity sows. The objectives of our study were: 1) Determine GnRH-II levels between first parity sows fed a restricted or ad libitum lactation diet and 2) Do reproductive characteristics improve following GnRH-II treatment of first parity sows with a negative energy balance at weaning? In Objective 1, first parity sows (n = 17) were farrowed, weighed, and backfat thickness was determined by ultrasound. Standard farrowing traits were recorded including weights of corresponding piglets. After farrowing, litter sizes were standardized within treatment and sows were fed either an ad libitum or restricted (80% of ad libitum) standard lactation diet for 25 days. At weaning, sows were weighed, tenth rib backfat thickness was determined by ultrasound, and a blood sample was taken. Three sows from each treatment were sacrificed and tissue samples from the hypothalamus, anterior pituitary gland, ovaries, oviduct, uterus, fat and mammary glands were collected from each sow. The remaining sows in each treatment were exposed twice daily to boars for 15 days to determine wean-to-estrus interval and days in estrus. Blood samples were analyzed for GnRH-II concentrations and gene expression assays for GnRH-II were performed on each tissue sample. As expected, average daily feed intake was reduced in restricted compared to ad libitum fed sows. However, sow weight loss, sow backfat loss, litter weight gain, average piglet weight gain, wean-to-estrus interval and days in estrus did not differ between treatments. Although there were no differences between treatments for GnRH-II gene expression in any of the tissues collected, GnRH-II levels in the blood were significantly higher in restricted vs. ad libitum fed sows. In Objective 2, first parity sows (n = 30) were weighed and farrowed and standard farrowing traits as well as feed intake were recorded. At weaning, a blood sample was collected and sows were weighed and treated with either saline or a synthetic version of GnRH-II. Once daily boar exposure was provided for 15 days to determine wean-to-estrus interval. Next, blood samples were analyzed for GnRH-II concentrations. Unexpectedly, GnRH-II treatment had no effect on wean-to-estrus interval, exhibiting similar effects as saline. Therefore, we examined correlations between farrowing/weaning traits with GnRH-II levels in the blood at weaning. Interestingly, GnRH-II concentrations were negatively correlated with average daily feed intake and total feed consumed. Based on results from both studieswe were unable to link GnRH-II levels at weaning with subsequent return to estrus. However, we did find an exciting result, sows with reduced blood levels of GnRH-II at weaning ate more feed during lactation. Although further studies are warranted, GnRH-II concentrations

measured in the blood may represent an early indicator (i.e., prior to lactation) of appetite issues in lactating sows. In addition, synthetic agents designed to reduce GnRH-II concentrations could be given to stimulate appetite in non-eating sows, enhancing sow productivity and therefore, profitability of pork producers. Dr. Brett R. White - phone: 402-472-6438; email: bwhite2@unl.edu.

Keywords: GnRH-II, GnRH-II Receptor, Wean-to-Estrus Interval, Reproductive Efficiency, Reproductive Axis

Scientific Abstract:

Unlike the native form of gonadotropin-releasing hormone (GnRH-I), the recently identified, second isoform (GnRH-II) is produced in nearly every tissue of the body, including non-reproductive tissues, and has been linked to the interaction between nutrition and reproductive function in mammals. Since this relationship has never been investigated in pigs, we investigated the role of GnRH-II in the transition from first to second parity in sows. First parity sows were farrowed, weighed, backfat thickness was determined by ultrasound and standard farrowing traits were recorded. After farrowing, litter sizes were standardized within treatment and sows were fed either an ad libitum (n = 8) or restricted (80% of ad libitum; n = 9) standard lactation diet for 25 d. At weaning, sows were weighed, tenth rib backfat thickness was determined by ultrasound, and a blood sample was taken. Three sows from each treatment were sacrificed and tissue samples from the hypothalamus, anterior pituitary gland, ovaries, oviduct, uterus, fat and mammary glands were collected from each sow. The remaining sows in each treatment were exposed twice daily to boars for 15 d to determine wean-to-estrus interval and days in estrus. Blood samples were analyzed via ELISA for GnRH-II concentrations, RNA was isolated from each tissue sample and converted into cDNA, and GnRH-II gene expression assays were performed. As expected, average daily feed intake was reduced in restricted compared to ad libitum fed sows ($P < .05$). However, sow weight loss, sow backfat loss, litter weight gain, average piglet weight gain, wean-to-estrus interval and days in estrus did not differ between treatments ($P > .05$). Although there were no differences between treatments for GnRH-II gene expression in any of the tissues collected ($P > .05$), GnRH-II levels in the blood were significantly higher in restricted vs. ad libitum fed sows ($P < .05$). In our second study, first parity sows (n = 30) were weighed and farrowed and standard farrowing traits as well as feed intake were recorded. At weaning, a blood sample was collected and sows were weighed and treated with either an agonist specific to GnRH-II (d-ala^6 GnRH-II; 150 ng/kg body weight; n = 15) or an equivalent volume of 0.9% saline (n = 15). Once daily boar exposure was provided for 15 d to determine wean-to-estrus interval and blood samples were analyzed for GnRH-II concentrations. GnRH-II treatment had no effect on wean-to-estrus interval (5.7 d), exhibiting similar effects as saline (5.8 d; $P > .05$). Therefore, we determined Pearson correlation coefficients between serum GnRH-II levels at weaning and all other traits. Interestingly, GnRH-II concentrations were negatively correlated with average daily feed intake (-0.40; $P < .05$) and there was a tendency for a negative correlation with total feed consumed (-0.34; $P < .07$). GnRH-II concentrations were not correlated with any other trait measured, including wean-to-estrus interval ($P > .05$). Next, we characterized females as having either HIGH (> 100 pg/ml; n = 15) or LOW (< 100 pg/ml; n = 15) GnRH-II concentrations. Sows with HIGH vs. LOW GnRH-II levels at weaning did not differ for any of the traits measured ($P > .05$) except average daily feed intake ($P < .05$) as well as a tendency for total feed intake ($P < .07$). Although we were unable to confirm a role for GnRH-II in return to estrus following lactation of first parity sows, we are intrigued by results suggesting a role of GnRH-II in appetite. Thus, GnRH-II levels in the blood may represent an early marker for potential appetite issues during lactation and could lead to development of novel therapeutic agents to control appetite in swine.

Introduction:

The inability of females to successfully transition from the first to second parity is extremely costly to the swine industry, representing a major reason for culling sows. Successful activation of the reproductive axis (Figure 1) is critical to the ability of lactating, first parity females to return to estrus, become pregnant and produce a litter. Gonadotropin-releasing hormone (GnRH) released from the hypothalamus travels to the anterior pituitary gland, stimulating the release follicle stimulating hormone (FSH) and luteinizing hormone (LH). Both FSH and LH then act on the gonads. FSH promotes follicle development, whereas LH triggers ovulation and maintains progesterone production by the corpus luteum (CL) throughout pregnancy. Steroids produced by the gonads can feedback at the level of the anterior pituitary or hypothalamus.

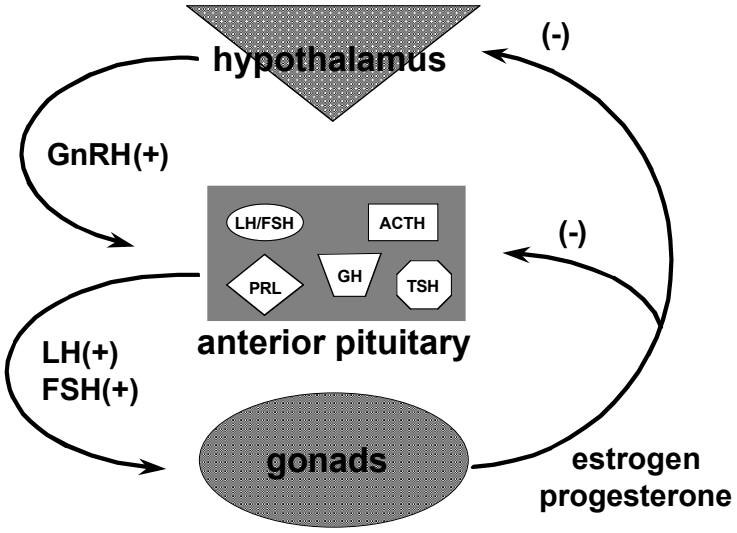

Figure 1. Diagram of the reproductive axis.

Recently, a new form of GnRH has been isolated (GnRH-II) that is considerably different from the well-characterized, original form. Most notably, GnRH-II is produced in many tissues of the body, including non-reproductive tissues. Studies in a number of mammalian species indicate that GnRH-II can rescue reproductive function in nutrient restricted females, suggesting that GnRH-II levels are indicative of the nutritional plane or metabolic status of females. Likely, threshold GnRH-II levels must be reached to activate the reproductive axis (Figure 1) as a sow recovers from the negative energy balance associated with the metabolic stresses of lactation. The potential of using a GnRH-II treatment to offset nutritional deficiencies experienced during lactation could be highly beneficial to swine producers.

Nutrient restriction delays wean-to-estrus interval when body condition is poor at farrowing or when feed intake is below 5.5 pounds per day during lactation. This effect is attributed to inactivation of the reproductive axis, leading to secretion of insufficient LH levels. Similarly, primiparous sows with excessive weight loss require a longer recovery period from their negative energy balance during lactation, have extended wean-to-estrus intervals and exhibit increased incidences of anestrus compared to sows with little or no weight loss during lactation. Short-term starvation during lactation significantly reduces insulin, glucose and IGF-1 levels. Scientists have restricted feed intake during the last week of lactation inducing sows to lose approximately 13% of protein and 17% of fat mass during lactation. Although wean-to-estrus interval and ovulation rate were not different from ad libitum fed sows, embryonic survival was reduced and growth of embryos/fetuses was blunted. However, restricting the diet of primiparous sows to 50% of ad libitum dramatically reduced levels of LH and extended the wean-to-estrus interval. In contrast to short-term starvation, no differences in ovulation rate or embryonic survival were detected between restricted and ad libitum fed sows.

Therefore, we chose two objectives to determine the role of GnRH-II during a period of substantial nutritional drain in sows, the transition from first to second parity. Our hypothesis for these studies was that GnRH-II levels would be significantly lower at weaning in restricted fed first parity sows compared

to those fed ad libitum levels, extending wean-to-estrus intervals. We also hypothesized that GnRH-II treatment of first parity sows in negative energy balance during lactation would improve reproductive characteristics such as wean-to-estrus interval.

Objectives:

Objective I. Determine GnRH-II levels between first parity sows fed a restricted or ad libitum lactation diet.

Objective 2. Do reproductive characteristics improve following GnRH-II treatment of first parity sows with a negative energy balance at weaning?

Material & Methods:

In Objective 1, gestating first parity sows (n = 17) were transported from the UNL Swine Unit at the Agricultural Research and Development Center (ARDC) in Mead, NE to the UNL Animal Science Building. Tenth rib backfat thickness was determined via ultrasound prior to farrowing. After farrowing, sows were weighed and corresponding piglets processed and weighed. Litter sizes were standardized between females in each treatment, making a concerted effort to reduce piglet mortality during the lactation period. All male piglets were castrated at 7 d of age. Each sow was placed on a standard lactation diet prepared by the UNL Feed Mill at ARDC and allowed 4 d to determine ad libitum feed intake. Half the sows were placed on an ad libitum diet and the other half fed a restricted diet (80% of ad libitum) for 21 additional days (total lactation period = 25 d). As required, the feed intake of ad libitum fed sows was increased throughout the lactation period, whereas restricted sows remained at the feed intake level determined previously. At weaning, sows and piglets were weighed and 10^{th} rib backfat thickness determined by ultrasound for each sow. Three sows from each treatment (n = 6) were transported to the UNL Meats Laboratory and sacrificed immediately following weaning. We collected blood as well as tissue samples from the hypothalamus, anterior pituitary gland, ovaries, oviduct, uterus, fat and mammary gland. The remaining 11 sows were bled at weaning, moved to gestation crates, placed on a breeding/gestation diet (4 pounds per day), and detected twice daily with boar exposure for return to estrus. After 15 d post-weaning, sows were removed from the study.

Approximately 100 mg of tissue from each sample was isolated (in duplicate) and immediately placed in 1 ml of RNAlater for storage at -20^{o}C until RNA isolation. Later, tissue was homogenized and RNA was isolated and reverse transcribed into cDNA for quantitative PCR analysis. Quantitative PCR was performed with an ABI 7900 HT instrument (Applied Biosystems, Carlsbad, CA) and the $\Delta\Delta C_T$ method. The cyclophilin rRNA gene was used as a housekeeping gene to normalize data. Data analysis was performed using the SDS RQ program (Applied Biosystems). For detection of GnRH-II gene expression, Taqman MGB probe (Applied Biosystems) and primer pairs (Integrated DNA Technologies, Coralville, IA) were designed for GnRH-II. Power SYBR Green (Applied Biosystems) was used for cyclophilin to normalize data. Blood samples collected at weaning were stored at 4^{o}C overnight, centrifuged (12,000 x *g*) for 20 min and serum isolated for storage at -20^{o}C until ELISA analysis. GnRH-II concentrations were determined according to manufacturer's instructions using an ELISA specific for porcine GnRH-II (Cat. #CSE-EL009636PI; Cusabio, Hubei, China). Briefly, a standard curve (0, 20, 40, 160, 640, 2,000 and 8,000 pg/ml) was included. Optical densities were determined using a Wallac 1420 Victor^2 microplate reader (Perkin Elmer, Waltham, MA) at 450 nm. The data were interpolated based on the standard curve using a 4-parameter logistic (4-PL) curve fit. The minimum sensitivity of the assay was 20 pg/ml and the intra-assay coefficient of variation was 10%.

In Objective 2, gestating first parity sows (n = 30) were weighed upon entry into the farrowing facility following standard operating procedures of the UNL Swine Unit at ARDC. After farrowing, sows were

placed on a standard farrowing diet and daily feed intake was recorded. At weaning, sows were weighed, moved to breeding pens and placed on a breeding/gestation diet (4 pounds per day). In addition, a blood sample was taken and females were randomly allocated to one of two treatments. Sows were given an intra-muscular (I.M.) injection of either an agonist specific for GnRH-II (d-ala^6 GnRH-II) diluted in 0.9% saline (150 ng/kg of body weight) or an equivalent volume of 0.9% saline (Control). Estrous detection was performed with once daily boar exposure to determine return to estrus. After 15 d post-weaning, sows were removed from the study. Based on our results from GnRH-II and saline treated sows, we chose to examine the data from this experiment using an alternative method. We characterized first parity sows based on serum GnRH-II concentrations at weaning. They were placed into two categories: HIGH (> 100 pg/ml; n = 15) or LOW (< 100 pg/ml; n = 15) GnRH-II concentrations. All farrowing/weaning traits measured were then compared between HIGH and LOW groups.

All data were analyzed using the General Linear Models (GLM) procedure of the Statistical Analysis System (SAS) and least squares means for all traits were compared between treatment groups using least significant differences. In Objective 2, we wanted to examine the correlation between serum GnRH-II concentrations and all other measured sow traits. Pearson correlation coefficients between GnRH-II levels and other sow traits were determined using the Correlation (CORR) procedure of SAS.

Results:

Objective 1

For all first parity sows in the experiment, the averages for total number of piglets born and total number of piglets born alive was 15 and 14, respectively. We have summarized the animal data for each treatment (Ad Libitum vs. Restricted Intake) from Objective 1 in Table 1.

TABLE 1. SOW AND LITTER TRAITS FOR FIRST PARITY SOWS FED EITHER AN AD LIBITUM OR RESTRICTED LACTATION DIET

Trait	Ad Libitum Intake	Restricted Intake
Number of First Parity Sows	8	9
Balanced Litter Size	11	11
Sow Farrowing Weight (kg)	215.6	210.8
Sow Weaning Weight (kg)	204.8	189.5
Sow Weight Loss (kg)	11.3	21.3
Sow 10th Rib Farrowing Backfat (cm)	2.5	2.4
Sow 10th Rib Weaning Backfat (cm)	1.8	1.8
Sow 10th Rib Backfat Loss (cm)	0.7	0.6
Total Litter Birth Weight (kg)	14.9	13.6
Total Litter Weaning Weight (kg)	81.7	78.5
Total Litter Weight Gain (kg)	66.8	64.9
Average Piglet Birth Weight (kg)	1.3	1.2
Average Piglet Weaning Weight (kg)	7.3	7.0
Average Piglet Weight Gain (kg)	6.0	5.8
Total Feed Intake (kg)	284.7	248.6
Average Daily Feed Intake (kg)	11.4	10.0*
GnRH-II Serum Concentrations (pg/ml)	38.5	51.6*
Wean-to-Estrus Interval (days)	3.9	6.3^{a}
Period of Standing Estrus (days)	2.5	2.1

* $P < 0.05$ vs. ad libitum intake.

[a] One sow included in this group had not returned to estrus within 15 days after weaning.

Although not significant, sows fed a restricted lactation diet lost approximately 10 kg more body weight than sows provided an ad libitum diet. In fact, one sow fed the ad libitum diet gained 13 kg during the 25-d lactation period. We were unable, however, to detect a difference between treatments for 10th rib backfat thickness loss from farrowing to weaning (0.7 and 0.6 cm for ad libitum and restricted fed sows, respectively; $P > .05$). Although litters gained 2 kg more to weaning in ad libitum fed compared to restricted fed females the difference was not significant ($P > .05$). However, the means for average piglet weight gain from birth to weaning between treatments were similar (6.0 kg for ad libitum fed vs. 5.8 kg for restricted fed; $P > .05$). As expected average daily feed intake was lower in restricted compared to ad libitum fed first parity sows ($P < .05$). Although ad libitum fed sows consumed approximately 36 kg more feed than restricted females during the entire 25-d lactation period, this differences was not significant. With a larger sample size, this difference may have reached significance.

In regard to post-weaning reproductive characteristics, 10 of the 11 sows remaining on trial returned to estrus within 5.5 d after weaning. Regardless of treatment, this wean-to-estrus interval is acceptable within swine industry standards. Although the wean-to-estrus interval for ad libitum fed females was approximately 2.5 d shorter than restricted fed females, the difference was not significant ($P > .05$). Of importance, one female from the restricted fed treatment group did not return to estrus within 15 d post-weaning when all females were removed from the study. Thus, the differences between treatments for wean-to-estrus interval may have actually been larger. We did not detect a difference between sows provided an ad libitum or restricted diet for length of standing estrus either ($P > .05$).

Quantitative PCR was performed on tissue samples from the hypothalamus, anterior pituitary gland, ovaries, oviduct, uterus, fat and mammary gland. Despite the presence of GnRH-II transcripts in all cell types, we were unable to detect any differences between ad libitum and restricted fed sows for GnRH-II gene expression ($P > .05$). However, examination of serum GnRH-II concentrations via ELISA revealed that sows fed a restricted lactation diet had higher levels of GnRH-II at weaning than their ad libitum fed counterparts ($P < .05$).

Since the sows in this study were farrowed within the confines of the Department of Animal Science on the UNL campus, the opportunity also arose to include an educational component to this research project. Consistent with this, 16 students in Animal Science completed a 1 credit hour independent study course, coordinated by Dr. Bryan Reiling and myself, in which they gained valuable experience in: 1) preparing sows for entry into the farrowing house; 2) observing sows for signs indicating the timing of parturition; 3) assisting with farrowing; 4) piglet processing and castration; 5) observing health status of sow/piglets and treating sick animals appropriately; and 5) procedures associated with weaning of both the sow and piglets. Based on my conversations with students, they really enjoyed the course, especially the opportunity to gain hands-on experience in the handling of livestock animals.

Objective 2

We have summarized the sow data for each treatment (d-ala^6 GnRH-II vs. saline) from Objective 2 in Table 2. It is important to remember that only wean-to-estrus interval was examined following injection of treatments. All other sow traits were determined prior to treatment.

TABLE 2. SOW TRAITS FOR LACTATING FIRST PARITY SOWS TREATED WITH EITHER d-ala^6 GnRH-II OR SALINE AT WEANING

Trait	d-ala^6 GnRH-II	Saline
Number of First Parity Sows	15	15
Lactation Length (d)	21.1	20.6

Sow Farrowing Weight (kg)	225.6	218.8
Sow Weaning Weight (kg)	194.6	192.6
Sow Weight Loss (kg)	31.1	26.2
Total Feed Intake (kg)	200.4	219.4
Average Daily Feed Intake (kg)	9.5	10.6
GnRH-II Serum Concentrations (pg/ml)	123.5	121.4
Wean to Estrus Interval (days)	5.7	5.8

As indicated in Table 2, we were unable to detect differences between treatments for any of the sow traits measured. Interestingly, the mean GnRH-II serum concentrations prior to injection of either d-ala^6 GnRH-II (121.4 pg/ml) or saline (123.5 pg/ml) for these randomly selected sows were nearly identical. Therefore, neither treatment group was deficient in GnRH-II levels to begin the trial. Next, we determined the correlations between serum GnRH-II concentrations at weaning and all sow traits measured. The Pearson correlation coefficient for GnRH-II levels and average daily feed intake (-0.40) was significant (P = .03) whereas the relationship between GnRH-II levels and total feed consumed (-0.34) approached significance (P = .06). Correlation coefficients between GnRH-II levels and all other sow traits were not significant (P > .05).

Since there was no difference in return to estrus between d-ala^6 GnRH-II and saline treated first parity sows, we decided to evaluate the data in a different manner. We grouped sows according to GnRH-II levels at weaning. Sows with less than 100 pg/ml GnRH-II were categorized as LOW whereas females with greater than 100 pg/ml were considered to have HIGH GnRH-II concentrations. We have summarized the sow data for each treatment (HIGH vs. LOW) in Table 3.

TABLE 3. SOW TRAITS FOR LACTATING FIRST PARITY SOWS WITH HIGH OR LOW GnRH-II LEVELS AT WEANING[a]

Trait	HIGH	LOW
Number of First Parity Sows	15	15
Lactation Length (d)	21.1	20.7
Sow Farrowing Weight (kg)	223.3	221.6
Sow Weaning Weight (kg)	192.6	194.6
Sow Weight Loss (kg)	30.6	27.0
Total Feed Intake (kg)	191.4	227.1*
Average Daily Feed Intake (kg)	9.1	10.9*
GnRH-II Serum Concentrations (pg/ml)	173.5	71.4*
Wean to Estrus Interval (days)	5.8	5.6

[a] HIGH = GnRH-II levels above 100 pg/ml; LOW = GnRH-II levels below 100 pg/ml.

* P < 0.05 vs. HIGH.

As described in Table 3, grouping first parity sows into HIGH or LOW GnRH-II levels revealed some interesting results. Sow weight loss and wean-to-estrus interval did not differ between sows with HIGH vs. LOW GnRH-II concentrations (P > .05). As expected given the design of this analysis, average GnRH-II serum concentrations were 173.5 pg/ml for the HIGH group compared to 71.4 pg/ml for LOW females (P < .05). Of interest, however, total feed intake was considerably higher for sows with LOW GnRH-II levels than those in the HIGH groups (P < .05) and total feed intake between groups tended to be different (P < .07).

Discussion:

Based on results from both objectives, we have established a negative relationship between serum GnRH-II concentrations at weaning and appetite in lactating first parity sows. This finding was surprising as we would have hypothesized that elevated GnRH-II levels in the blood would be positively correlated with feed intake. However, this result has opened new avenues of research regarding the biological/physiological function of GnRH-II in the pig. There is precedence for a role of GnRH-II in appetite as evidenced by one study in the literature. These investigators determined that GnRH-II reduced feed intake in female musk shrews by 28%. Interestingly, treatment of females with GnRH-I also decreased feed intake (14%). We are extremely excited by this result and interested in further investigation. Although further studies are warranted, GnRH-II concentrations measured in the blood may represent an early indicator (i.e., prior to lactation) of appetite issues in lactating sows. In addition, synthetic agents designed to reduce GnRH-II concentrations could be given to stimulate appetite in non-eating sows, enhancing sow productivity and therefore, profitability of pork producers. Another area of interest may lie in feed efficiency of growing and finishing swine. The possibility of predicting or enhancing feed efficiency could be an invaluable tool in the swine industry. In addition, more efficient food production has become a priority area of investigation given the changes in our global climate. However, a number of questions remain to be answered. Is there a link between GnRH-II levels and feed efficiency? At which stages of the growing or finishing phases would be most appropriate to examine the relationship between GnRH-II and feed efficiency? Could custom nutritional programs be designed for groups of growing/finishing swine with different levels of GnRH-II in the blood? This area of investigation is also intriguing to us and we are currently designing experiments to pursue this avenue of research.

We originally proposed feeding first parity sows at 50% of ad libitum based on published literature. However, the Institutional Animal Care and Use Committee (IACUC) at UNL was not comfortable with a 50% feed restriction and required us to alter our protocol to 80% of ad libitum. This unforeseen alteration to our procedures for Objective 1 may have resulted in no difference between restricted and ad libitum fed first parity sows for weaning-to-estrus interval and days in estrus. In our study, the level of feed restriction (80% of ad libitum) was determined within 4 d after farrowing. We found this extremely challenging to determine since sows fluctuate considerably as they adjust to the lactation diet. This could have contributed to the absence of a significant difference between treatments for total feed consumed during lactation. Although, the intake of ad libitum fed sows was continually increased throughout the lactation period whereas the intake of females fed the restricted diet remained the same. Nonetheless, average daily feed intake of restricted fed sows over the entire 25-d lactation period was significantly lower than that of ad libitum fed sows.

The ability to detect GnRH-II levels in the blood of first parity, lactating sows was exciting as well. Both GnRH-I and GnRH-II consist of 10 amino acids, but due to the short half-life of GnRH-I, it can't be detected reliably in circulating blood. Therefore, GnRH-II may exhibit different properties than GnRH-I allowing it to travel through the vascular system without degradation. Blood sampling is a much more efficient method to track GnRH-II levels in animals at different physiological states compared to gene expression assays. Although both methodologies were utilized in these studies, detection of GnRH-II levels in the blood was the preferred method. To determine GnRH-II gene expression levels, multiple tissues must be collected and analyzed. These assays are more time consuming than the ELISAs used to determine serum GnRH-II concentrations in the blood. In addition, considerably more reagents must be purchased to complete the gene expression assays compared to pre-prepared ELISA kits. In Objective 2, we had hoped to characterize serum GnRH-II concentrations in first parity gilts prior to injection of either d-ala^6 GnRH-II or saline. However, we were unable to run ELISAs on blood samples in that short of a timeframe and therefore, had to inject treatments prior to attaining the data for GnRH-II levels. When we

analyzed the data, however, GnRH-II levels were essentially identical between treatments. Therefore, baseline GnRH-II levels were similar between treatments.