**Title:** Effect of GnRH-II on feed efficiency and immune function of growing/finishing pigs – **NPB #14-240**

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**Industry Summary:**

Feed efficiency and immune response of market hogs can have a profound effect on the profitability of pork producers. Therefore, we examined the impact of the newly isolated hormone, GnRH-II, on feed efficiency and immune function of growing/finishing pigs. The objectives of our study were: 1) Determine GnRH-II levels in growing/finishing pigs provided either a feed-restricted or *ad libitum* diet and 2) Examine the influence of GnRH-II on immune function in growing/finishing pigs. Terminal cross barrows were weaned, moved to nursery facilities at 21 days and fed standard Nursery I and II diets for 6 weeks. Next, pigs were provided *ad libitum* access to Phase I of a 3-phase grower/finisher diet, commonly used at the University of Nebraska-Lincoln swine unit, for 4 weeks. During the last week of the Phase I diet, barrows were randomly allocated to receive either an *ad libitum* or restricted (50% of *ad libitum*) intake diet, placed in individual pens with *ad libitum* access to water and allowed a 7-day acclimation period. Daily feed intake was steadily increased in an attempt to determine the average *ad libitum* feed intake. Next, pigs were provided the Phase II diet and feed intake treatments were initiated, with half of the daily feed provided in the morning and evening. However, after Week 1, barrows fed the restricted intake diet exhibited significant weight losses. To avoid any potential health issues related to weight loss, we adjusted the restricted intake diet to 68% of *ad libitum* for the remainder of the study. Pigs received the Phase II and III diets for 4 weeks each. At the start of Week 2 for each feeding phase, animals received diets mixed with 0.4% titanium dioxide, to estimate the ability of pigs to digest feed, for 10 days. On Day 8 - 10, 3 fecal samples were taken from each barrow (8 am and 5 pm), pooled within pig, and examined for nitrogen, phosphorus, titanium dioxide and energies. Barrows were weighed weekly and blood samples taken every 2 weeks to measure blood levels of GnRH-II and a marker of the immune response. Overall, *ad libitum* fed growing/finishing pigs were heavier, had greater average daily gain and average daily feed intake, and exhibited decreased gain:feed ratios compared to restricted fed pigs. Despite no differences during Phase II, dry matter and nutrient digestibility were greater in restricted pigs compared to *ad libitum* pigs in Phase III. Serum GnRH-II concentrations were relatively low and varied significantly among animals prior to treatment. Although GnRH-II levels were lower for barrows fed a restricted vs. *ad libitum* diet on Day 7 and 14, we did not detect an overall effect of dietary treatment. However, GnRH-II concentrations did decrease with age regardless of treatment. No differences in immune response between treatments were observed through Day 42 of the trial, although the immune response tended to be decreased on Day 7 in barrows fed the restricted diet, likely associated

with severe weight loss from a 50% feed restriction during the first week of the trial. Based on results from this study, we were unable to link GnRH-II levels with feed efficiency or immune response in growing/finishing barrows. However, we did determine that GnRH-II levels vary among animals and decrease with age during the grower/finisher stage, suggesting that it may be a potential marker for growth. Dr. Brett R. White - phone: 402-472-6438; email: bwhite2@unl.edu.

**Keywords:** GnRH-II, Feed Restriction, Feed Efficiency, Immune Response, Digestibility

**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. Due to the ubiquitous production of GnRH-II, it has been linked to both feed intake as well as the immune response. Since these relationships have never been explored in pigs, we investigated the role of GnRH-II in feed efficiency and immune function of growing/finishing pigs. Barrows were weaned, moved to nursery facilities at 21 days and maintained on standard Nursery I and II diets for 6 weeks. Next, pigs were provided *ad libitum* access to Phase I of a 3-phase grower/finisher diet, commonly used at the University of Nebraska-Lincoln swine unit, for 4 weeks. During the last week of the Phase I diet, barrows were randomly allocated to receive either an *ad libitum* or restricted (50% of *ad libitum*) intake diet, placed in individual housing with *ad libitum* access to water and allowed a 7-day acclimation period. Daily feed intake was steadily increased in an attempt to determine the average *ad libitum* feed intake. Following the acclimation period, pigs were provided the Phase II grower/finisher diet and feed intake treatments were initiated, with half of the daily feed provided between 8 and 9 am and the other half delivered between 5 and 6 pm. However, after Week 1, barrows fed the restricted intake diet exhibited a significant loss in body weight. In order to avoid any potential health issues related to weight loss, we adjusted the restricted intake diet to 68% of *ad libitum* for the remainder of the study. Pigs received the Phase II and III diets for 4 weeks each. At the start of Week 2 for each feeding phase, animals received diets mixed with 0.4% titanium dioxide, as an exogenous digestibility marker, for 10 days. On Day 8, 9 and 10, 3 fecal grab samples were taken from each barrow (8 am and 5 pm), pooled within pig, homogenized, and examined for nitrogen, phosphorus, titanium dioxide and energies. Barrows were weighed weekly and blood samples taken every 2 weeks to measure serum levels of GnRH-II and a marker of immune response, c-reactive protein (CRP), via enzyme-linked immunoassays (ELISA). As expected, *ad libitum* feeding resulted in greater body weights during every week of the trial ($P < 0.0001$) and overall, *ad libitum* fed growing/finishing pigs had greater average daily gain and average daily feed intake, as well as decreased gain:feed ratios compared to restricted fed pigs ($P < 0.0001$). Despite observing no differences in Phase II, dry matter and energy digestibility were greater in restricted pigs compared to *ad libitum* pigs during Phase III ($P < 0.05$). Serum GnRH-II concentrations were relatively low and significantly different among animals prior to treatment ($P < 0.05$). Although GnRH-II levels were lower for barrows fed a restricted vs. *ad libitum* diet on Day 7 and 14, we did not detect any overall effects of treatment ($P = 0.16$) or treatment x age interaction ($P = 0.22$). However, GnRH-II concentrations did decrease with age regardless of treatment ($P < 0.01$). No differences in serum CRP levels between treatments were observed through Day 42 of the trial ($P > 0.05$). In contrast, CRP concentrations tended ($P = 0.06$) to be decreased on Day 7 in barrows fed a restricted compared to *ad libitum* diet, likely associated with severe weight loss from a 50% feed restriction during the first week of the trial. Thus, we were unable to establish a relationship between serum GnRH-II concentrations and feed efficiency or immune response in growing/finishing barrows. However, GnRH-II levels varied among animals and decreased significantly with age. Although more research is warranted, circulating GnRH-II levels could represent a potential marker for growth, leading to the development of novel screening methods or growth promoting agents to enhance the productivity and profitability of pork producers.

**Introduction:**

The original form of gonadotropin-releasing hormone (GnRH-I) is considered the master regulator of reproduction. GnRH-I is released from the hypothalamus and 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 in females and spermatogenesis in males, whereas LH triggers ovulation in females and testosterone production in males. 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.

Through funds acquired from the Nebraska Pork Producers Association in 2012, we were able to establish a negative correlation between GnRH-II levels in blood with lactation feed intake in first parity sows. In other words, females with high GnRH-II levels (> 100 pg/ml) had significantly reduced lactation feed intake (approximately 4 lbs/day) compared to sows with low levels of GnRH-II (< 100 pg/ml). Given this exciting result, we wanted to investigate whether GnRH-II has an impact on appetite and feed efficiency in growing/finishing swine as well. Although most research has focused on reproductive function, GnRH-II has been correlated with feed intake/appetite. Direct injection of GnRH-II into the brain of goldfish and zebrafish significantly reduced feeding behavior. However, only a few studies have been performed in mammals. Feed restriction of musk shrews decreased GnRH-II mRNA levels in the brain. Similar to studies reported in fish, direct injection of GnRH-II into the brain of *ad libitum* or restricted fed musk shrews lowered feed intake by 28 and 33%, respectively. This altered feed intake began 90 min after treatment and persisted for 3 h.

Consistent with its ubiquitous production, GnRH-II has also been associated with cells involved in the immune response. GnRH-II gene expression has been detected in bone marrow and peripheral mononuclear blood cells, whereas expression of the receptor specific to GnRH-II has been found in lymph nodes, thymus and peripheral lymphocytes. In genetically engineered zebrafish, microbial infection increased GnRH-II gene expression. In the human, exogenous GnRH-II treatment augments invasion of immune cells (T cells) into bone marrow and spleen. Further, interleukin 2 receptor gamma production was significantly decreased with GnRH-II treatment in peripheral mononuclear blood cells, further suggesting a role for GnRH-II in immune function.

**Objectives:**

Objective 1. Determine GnRH-II levels in growing/finishing pigs provided either a feed-restricted or *ad libitum* diet.

Objective 2. Examine the influence of GnRH-II on immune function in growing/finishing pigs.

**Material & Methods:**

In Objective 1, barrows (n = 36) derived from white crossbred sows inseminated with terminal line boars (DNA Genetics, Columbus, NE) were born in the Animal Science Building at the University of Nebraska-Lincoln as part of the ASCI 150 course entitled, “Animal Production Skills” under the supervision of Dr. Bryan Reiling. As part of this course, students assist with: 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 6) procedures associated with weaning of both the sow and piglets.

Male piglets were castrated at 7 days of age, weaned at 21 days of age, moved to nursery facilities and maintained on a standard Nursery 1 and Nursery 2 diet for 6 weeks. Next, barrows were provided *ad libitum* access to Phase I of a 3-phase grower/finisher diet, commonly used at the University of Nebraska-Lincoln (UNL) Agricultural Research and Development Center (ARDC) swine unit located at Ithaca, NE, for 4 weeks. During the last week of the Phase I diet, barrows were randomly allocated to receive either an *ad libitum* or restricted (50% of *ad libitum*) intake diet, placed in individual housing with *ad libitum* access to water and allowed a 7-day acclimation period. At this time, daily feed intake was steadily increased in an attempt to determine the average *ad libitum* feed intake for these animals. Following the 7-day acclimation period, pigs were provided the Phase

II grower/finisher diet and feed intake treatments were initiated. Barrows receiving the *ad libitum* or restricted intake diet weighed 51.2 and 51.5 kg, respectively. Half of the daily feed provided was given between 8 and 9 am and the other half delivered between 5 and 6 pm. Specific weekly feed intake levels for each treatment are reported in Table 1. Pigs received the Phase II diet for 4 weeks and the Phase III diet for 4 weeks. At the start of Week 2 for each feeding phase, animals received diets mixed with 0.4% titanium dioxide, as an exogenous digestibility marker to estimate the ability of pigs to digest feed, for 10 days. On Day 8, 9 and 10, 3 fecal grab samples were taken from each barrow (at 8 am and 5 pm) and pooled within pig. Samples were stored at -20°C to subsequently determine chemical composition of the pooled homogenized feces for nitrogen, phosphorus, titanium dioxide and energies. Barrows were weighed weekly and blood samples taken every 2 weeks to measure serum GnRH-II levels via an enzyme-linked immunoassay (ELISA) previously validated in our laboratory.

For Objective 2, the same blood samples collected for the GnRH-II ELISAs in Objective 1 were used for analysis of immune function. Serum from each barrow was analyzed with an ELISA specific for a marker of acute immune response, c-reactive protein (CRP), previously validated in the laboratory.

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.

**Results:**

Objective 1

The raw means for beginning and ending weight, average daily gain, feed intake and average daily feed intake of barrows fed either an *ad libitum* or restricted intake diet during each week of the trial are summarized in Table 1.

TABLE 1. GROWTH TRAITS FOR BARROWS FED AN AD LIBITUM OR RESTRICTED INTAKE DIET

| **Trait** | **Ad Libitum Intake** | **Restricted Intake** |
|---|---|---|
| ***Phase II – Week 1*** | | |
| No. barrows | 18 | 18 |
| Beginning weight (kg) | 52.1 | 52.5 |
| Ending Weight (kg) | 59.0 | 53.0 |
| Average daily gain (kg/d) | 0.98 | 0.07 |
| Feed intake (kg) | 17.88 | 9.10 |
| Average daily feed intake (kg/d) | 2.56 | 1.30 |
| ***Phase II – Week 2*** | | |
| No. barrows | 18 | 18 |
| Beginning weight (kg) | 59.0 | 53.0 |
| Ending Weight (kg) | 65.4 | 58.7 |
| Average daily gain (kg/d) | 0.91 | 0.82 |
| Feed intake (kg) | 19.43 | 13.37 |
| Average daily feed intake (kg/d) | 2.78 | 1.91 |
| ***Phase II – Week 3*** | | |
| No. barrows | 18 | 17 [a] |
| Beginning weight (kg) | 65.4 | 58.7 |
| Ending Weight (kg) | 73.6 | 64.3 |
| Average daily gain (kg/d) | 1.17 | 0.83 |
| Feed intake (kg) | 19.48 | 13.37 |

| | | |
|---|---|---|
| Average daily feed intake (kg/d) | 2.78 | 1.91 |
| ***Phase II – Week 4*** | | |
| No. barrows | 18 | 17 |
| Beginning weight (kg) | 73.6 | 64.3 |
| Ending Weight (kg) | 82.5 | 71.5 |
| Average daily gain (kg/d) | 1.28 | 1.04 |
| Feed intake (kg) | 22.74 | 15.71 |
| Average daily feed intake (kg/d) | 3.25 | 2.24 |
| ***Phase III – Week 1*** | | |
| No. barrows | 18 | 17 |
| Beginning weight (kg) | 82.5 | 71.5 |
| Ending Weight (kg) | 90.5 | 78.4 |
| Average daily gain (kg/d) | 1.13 | 0.98 |
| Feed intake (kg) | 23.73 | 17.14 |
| Average daily feed intake (kg/d) | 3.39 | 2.45 |
| ***Phase III – Week 2*** | | |
| No. barrows | 18 | 17 |
| Beginning weight (kg) | 90.5 | 78.4 |
| Ending Weight (kg) | 97.7 | 87.4 |
| Average daily gain (kg/d) | 1.04 | 1.28 |
| Feed intake (kg) | 24.52 | 20.47 |
| Average daily feed intake (kg/d) | 3.50 | 2.92 |
| ***Phase III – Week 3*** | | |
| No. barrows | 18 | 17 |
| Beginning weight (kg) | 97.7 | 87.4 |
| Ending Weight (kg) | 105.1 | 94.7 |
| Average daily gain (kg/d) | 1.05 | 1.04 |
| Feed intake (kg) | 23.69 | 20.39 |
| Average daily feed intake (kg/d) | 3.39 | 2.91 |
| ***Phase III – Week 4*** | | |
| No. barrows | 18 | 17 |
| Beginning weight (kg) | 105.1 | 94.7 |
| Ending Weight (kg) | 112.0 | 100.5 |
| Average daily gain (kg/d) | 0.99 | 0.84 |
| Feed intake (kg) | 24.27 | 20.47 |
| Average daily feed intake (kg/d) | 3.47 | 2.92 |

[a] One barrow was removed from the study due to sickness.

Following statistical analysis, the least squares means for body weight (kg) from each week of the trial is depicted in Table 2. As expected, *ad libitum* feeding resulted in greater body weights during every week of the trial ($P < 0.0001$) compared to restricted fed growing/finishing pigs.

TABLE 2. EFFECT OF EITHER A FEED-RESTRICTED OR AD LIBITUM DIET ON BODY WEIGHT (kg) OF GROWING/FINISHING PIGS

| Day of Trial | Ad Libitum Intake[a] | Restricted Intake[a] | SEM | *P*-value |
|---|---|---|---|---|
| 7 | 59.32 | 53.03 | 0.85 | <0.0001 |
| 14 | 65.80 | 58.77 | 0.88 | <0.0001 |
| 21 | 73.95 | 64.54 | 0.95 | <0.0001 |
| 28 | 82.95 | 71.86 | 1.07 | <0.0001 |

| | | | | |
|---|---|---|---|---|
| 35 | 90.84 | 78.81 | 1.24 | <0.0001 |
| 42 | 98.15 | 87.63 | 1.46 | <0.0001 |
| 49 | 105.64 | 95.02 | 1.67 | <0.0001 |
| 56 | 112.52 | 100.68 | 1.65 | <0.0001 |

[a] Least squares means for body weight (kg).

Table 3 depicts the least squares means for average daily gain, average daily feed intake and gain:feed ratio for growing/finishing barrows fed an *ad libitum* or restricted intake during each week of the trial. Overall, *ad libitum* fed growing/finishing pigs had greater average daily gain and average daily feed intake, as well as decreased gain:feed ratio compared to restricted fed pigs ($P < 0.0001$). Uniquely, average daily gain during Week 2 ($P = 0.11$) and gain:feed ratio during Week 2 ($P = 0.60$) of Phase II were not different between dietary treatments. However, this is likely attributable to compensatory gain as a result of switching from 50% to 68% feed restriction during the first and second weeks of the trial. In addition, treatment effects were not detected ($P > 0.17$) for average daily gain or gain:feed ratio on Week 3, Week 4 and overall during the Phase III feeding period. Due to increased growth rates during the trial, the ad libitum fed pigs were starting to deposit fat rather than build muscle at this point in the growth curve. Consistent with this, restricted fed pigs took between 1 and 2 weeks following the end of the trial to reach market weights attained by *ad libitum* fed barrows on the last week of the trial.

TABLE 3. EFFECT OF EITHER RESTRICTED OR AD LIBITUM INTAKE ON GROWTH PERFORMANCE OF GROWING/FINISHING PIGS

| **Item[a]** | **Ad Libitum Intake** | **Restricted Intake** | **SEM** | ***P*-value** |
|---|---|---|---|---|
| ***Phase II* – Week 1** | | | | |
| ADG (kg/d) | 0.98 | 0.07 | 0.03 | <0.0001 |
| ADFI (g/d) | 2.55 | 1.3 | 0.02 | <0.0001 |
| Gain:Feed | 0.38 | 0.06 | 0.02 | <0.0001 |
| ***Phase II* – Week 2** | | | | |
| ADG (kg/d) | 0.91 | 0.82 | 0.04 | 0.1121 |
| ADFI (g/d) | 2.78 | 1.91 | 0.01 | <0.0001 |
| Gain:Feed | 0.33 | 0.43 | 0.02 | <0.0001 |
| ***Phase II* – Week 3** | | | | |
| ADG (kg/d) | 1.17 | 0.83 | 0.05 | <0.0001 |
| ADFI (g/d) | 2.78 | 1.91 | 0.01 | <0.0001 |
| Gain:Feed | 0.42 | 0.43 | 0.02 | 0.5983 |
| ***Phase II* – Week 4** | | | | |
| ADG (kg/d) | 1.28 | 1.04 | 0.03 | <0.0001 |
| ADFI (g/d) | 3.25 | 2.24 | 0.02 | <0.0001 |
| Gain:Feed | 0.39 | 0.46 | 0.01 | <0.0001 |
| ***Phase II* – Overall** | | | | |
| ADG (kg/d) | 1.09 | 0.69 | 0.02 | <0.0001 |
| ADFI (g/d) | 2.84 | 1.84 | 0.01 | <0.0001 |
| Gain:Feed | 0.38 | 0.35 | 0.01 | <0.0001 |
| ***Phase III* – Week 1** | | | | |
| ADG (kg/d) | 1.13 | 0.98 | 0.05 | 0.0301 |
| ADFI (g/d) | 3.39 | 2.45 | 0.06 | <0.0001 |
| Gain:Feed | 0.33 | 0.4 | 0.01 | <0.0001 |
| ***Phase III* – Week 2** | | | | |
| ADG (kg/d) | 1.04 | 1.28 | 0.06 | 0.0032 |
| ADFI (g/d) | 3.50 | 2.92 | 0.10 | <0.0001 |

| | | | | |
|---|---|---|---|---|
| Gain:Feed | 0.29 | 0.44 | 0.01 | <0.0001 |
| ***Phase III* – Week 3** | | | | |
| ADG (kg/d) | 1.05 | 1.04 | 0.08 | 0.8995 |
| ADFI (g/d) | 3.38 | 2.91 | 0.08 | <0.0001 |
| Gain:Feed | 0.31 | 0.36 | 0.02 | 0.1876 |
| ***Phase III* – Week 4** | | | | |
| ADG (kg/d) | 0.99 | 0.84 | 0.08 | 0.1738 |
| ADFI (g/d) | 3.47 | 2.92 | 0.07 | <0.0001 |
| Gain:Feed | 0.28 | 0.29 | 0.02 | 0.9578 |
| ***Phase III* – Overall** | | | | |
| ADG (kg/d) | 1.05 | 1.04 | 0.03 | 0.7205 |
| ADFI (g/d) | 3.44 | 2.80 | 0.07 | <0.0001 |
| Gain:Feed | 0.31 | 0.37 | 0.01 | <0.0001 |
| ***Phase II and III* – Combined** | | | | |
| ADG (kg/d) | 1.07 | 0.86 | 0.02 | <0.0001 |
| ADFI (g/d) | 3.14 | 2.32 | 0.04 | <0.0001 |
| Gain:Feed | 0.34 | 0.37 | 0.01 | <0.0001 |

[a] ADG = average daily gain; ADFI = average daily feed intake.

Analysis of serum GnRH-II concentrations as determined by ELISA revealed significant differences among animals prior to treatment ($P < 0.05$). To account for this variation, baseline GnRH-II levels for individual barrows were included in the statistical model as a covariate. Despite decreased GnRH-II levels for barrows fed a restricted vs. ad libitum diet on Day 7 and 14, we did not detect a statistical effect of treatment (*ad libitum* vs. restricted; $P = 0.16$) or treatment x age interaction ($P = 0.22$). Least squares means for the entire trial were 70.40 ± 4.14 and 61.92 ± 4.14 for *ad libitum* and restricted fed growing/finishing pigs, respectively ($P > 0.05$). Least squares means for GnRH-II levels were elevated ($P < 0.05$) on Day 7 and tended to be elevated ($P = 0.052$) on Day 14 in *ad libitum* vs. restricted fed barrows, but were not different between dietary treatments on Day 28, 42 or 56 (Table 4). However, we did detect an effect of age ($P < 0.01$). Serum GnRH-II levels decreased with age regardless of treatment. Least squares means were 79.05 ± 4.39 (Week 1), 75.65 ± 6.50 (Week 2), 64.27 ± 4.42 (Week 4), 52.77 ± 5.05 (Week 6) and 59.07 ± 5.09 (Week 8) pg/ml.

TABLE 4. EFFECT OF EITHER RESTRICTED OR AD LIBITUM INTAKE ON SERUM GnRH-II LEVELS (pg/ml) OF GROWING/FINISHING PIGS

| **Day of Trial** | **Ad Libitum Intake** | **Restricted Intake** | **SEM** | ***P*-value** |
|---|---|---|---|---|
| 7 | 89.07 | 69.02 | 6.26 | 0.029 |
| 14 | 88.73 | 62.57 | 9.23 | 0.052 |
| 28 | 67.34 | 61.19 | 6.30 | 0.497 |
| 42 | 50.93 | 54.61 | 7.18 | 0.720 |
| 56 | 55.92 | 62.21 | 7.25 | 0.545 |

There were no differences in energy digestibility observed in Phase II (Table 5). However, during Phase III, dry matter and energy digestibility were greater in restricted pigs compared to *ad libitum* pigs ($P < 0.05$; Table 5).

TABLE 5. EFFECT OF EITHER RESTRICTED OR AD LIBITUM INTAKE ON ENERGY DIGESTIBILITY OF GROWING/FINISHING PIGS

| **Item[a]** | **Ad Libitum Intake** | **Restricted Intake** | **SEM** | ***P*-value** |
|---|---|---|---|---|
| **Overall Phase II** | | | | |

| | | | | |
|---|---|---|---|---|
| DM (%) | 95.82 | 96.37 | 0.46 | 0.394 |
| DM Digestibility (%) | 85.07 | 85.85 | 0.34 | 0.107 |
| GE (cal/g) | 4336.30 | 4401.70 | 22.17 | 0.042 |
| GE Digestibility (cal/g) | 84.64 | 85.31 | 0.36 | 0.190 |
| **Overall Phase II** | | | | |
| DM (%) | 96.24 | 96.18 | 0.37 | 0.904 |
| DM Digestibility (%) | 81.62 | 83.45 | 0.46 | 0.007 |
| GE (cal/g) | 4418.63 | 4398.47 | 14.07 | 0.312 |
| GE Digestibility (cal/g) | 80.87 | 82.88 | 0.51 | 0.008 |

[a] DM = dry matter; GE = gross energy.

Objective 2

Since we did not detect a treatment effect for circulating GnRH-II levels, we were unable to establish a GnRH-II influence on the immune response. However, we have summarized the immune response data for each treatment (restricted vs. *ad libitum* intake) from Objective 2 in Table 6. No differences in serum C-reactive protein (CRP) levels between treatments were observed through Day 42 of the trial ($P > 0.05$). In contrast, CRP concentrations tended ($P = 0.06$) to be decreased on Day 7 in barrows fed a restricted compared to *ad libitum* diet. As indicated in the Materials & Methods section, we began this study (Week 1) using a 50% reduction in feed intake for the restricted-fed diet. Based on growth data from the first week of the experiment, this level of restriction was too severe for the pigs. As a result, we adjusted the restricted intake diet from 50 to 68% of *ad libitum*. Thus, barrows fed the restricted diet were likely stressed significantly during the first week of the trial.

TABLE 6. EFFECT OF EITHER RESTRICTED OR AD LIBITUM INTAKE ON SERUM C-REACTIVE PROTEIN LEVELS (pg/ml) OF GROWING/FINISHING PIGS

| Day of Trial | Ad Libitum Intake | Restricted Intake | SEM | *P*-value |
|---|---|---|---|---|
| 0 | 322.96 | 293.16 | 42.02 | 0.609 |
| 7 | 256.43 | 172.21 | 31.16 | 0.061 |
| 14 | 163.51 | 151.34 | 19.83 | 0.667 |
| 28 | 299.88 | 262.84 | 43.93 | 0.353 |
| 42 | 121.51 | 122.95 | 9.19 | 0.911 |

Discussion:

Although least squares means for GnRH-II levels were elevated ($P < 0.05$) on Day 7 and tended to be elevated ($P = 0.052$) on Day 14 in *ad libitum* vs. restricted fed barrows, we were unable to establish an overall difference in GnRH-II levels between restricted- and *ad libitum* fed barrows. This was in stark contrast to the negative correlation between serum GnRH-II concentrations and feed intake in lactating, first parity sows established in our previous studies. Interestingly, GnRH-II levels in circulating blood were lower for barrows in this trial compared to lactating, first parity sows in our previous trial. For barrows, no GnRH-II levels exceeded 100 pg/ml, whereas half of the first parity sows in our previous study had values greater than 100 pg/ml. In addition, our laboratory has recently reported elevated levels of GnRH-II in boar testes compared to the hypothalamus and anterior pituitary gland. Although studies in musk shrews and old world monkeys indicated that GnRH-II is produced by every tissue in the body, we hypothesize that the majority of circulating GnRH-II is produced by the testis. Studies in these species also indicated that GnRH-II is made in the ovary, however, we have not specifically quantitated GnRH-II levels in ovarian tissue. Therefore, the lack of differences between dietary treatments could be due to the fact that we utilized castrated animals in our current study, essentially removing the organ with the highest production of GnRH-II. Additional studies are underway to determine GnRH-II levels between age-matched, intact boars and barrows.

Further, results from this study indicated that serum GnRH-II levels decreased with age in growing/finishing barrows. The ability to predict or enhance growth rates in growing/finishing pigs is appealing and could be an invaluable tool to pork producers. In addition, more efficient food production has become increasingly important given future world population estimates as well as global climate change. However, a number of questions remain to be answered. Is there a link between GnRH-II levels and growth characteristics? In the current study, there were not enough animals to correlate GnRH-II concentrations in the blood with specific growth traits. Additional studies with more animals are warranted. At which stages of the development (nursery, grower or finisher phases) would be most appropriate to examine the relationship between GnRH-II levels and growth? Circulating levels of GnRH-II were significantly different among animals prior to the start of dietary treatments, suggesting that some factor may have influenced GnRH-II secretion earlier in development. What contributed to this difference or do baseline GnRH-II levels simply vary among individual pigs regardless of age? Customized nutritional programs could potentially be designed for groups of growing/finishing swine with divergent levels of serum GnRH-II. This area of investigation is intriguing to us and we are currently pursuing aspects of this research utilizing a genetically-engineered swine line with reduced endogenous levels of the receptor for GnRH-II.

We began the study restricting feed intake by 50% of *ad libitum* in an attempt to create divergent growth rates and hopefully, circulating GnRH-II levels between animals in each dietary treatment. We successfully reduced average daily gain in restricted fed (0.07 kg/day) compared to *ad libitum* fed (0.98 kg/day) barrows. Furthermore, serum GnRH-II concentrations were significantly reduced in pigs fed the restricted intake diet during the first week and tended to be reduced during the second week. However, too many animals in the restricted intake dietary treatment lost significant amounts of weight during the first week. This was a condition in our animal protocol approved by the Institutional Animal Care and Use Committee (IACUC) at UNL. We were required to alter the restricted intake diet from 50 to 68% of *ad libitum*. We are confident that this was the appropriate course of action as it was apparent that a 50% reduction in feed intake would affect animal health. However, this unforeseen alteration to our procedures may have contributed to the fact that we were unable to establish differences in serum GnRH levels between restricted and *ad libitum* fed growing/finishing pigs.