LATE GESTATION LYSINE AND ENERGY EFFECTS IN SOWS AND DOSE-RESPONSES TO TRYPTOPHAN AND VALINE IN FINISHING PIGS

by

MÁRCIO ANTÔNIO DORNELLES GONÇALVES

D.V.M., Federal University of Rio Grande do Sul, 2011

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Diagnostic Medicine/Pathobiology
College of Veterinary Medicine

KANSAS STATE UNIVERSITY
Manhattan, Kansas

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Abstract

The overall goal of this dissertation was to evaluate the effects of different amino acid (AA) levels on performance of pigs under commercial conditions. To reach this objective, a total of 12 experiments were conducted. For the sow research, 1,102 highly prolific sows were used to determine the effects of AA and energy intake during late gestation on piglet birth weight and reproductive performance of sows. Weight gain depended on the energy and AA intake levels while sows fed increased amount of energy had increased stillborn rate; however, there was no statistical differences due to energy intake in stillborn rate of gilts. The modest increase in individual piglet birth weight is due to energy rather than AA intake during late gestation. Pre-weaning mortality was reduced in piglets suckling from sows fed high AA diets during late gestation while subsequent reproductive performance was not affected. With recent advances in statistical computing capability, linear and non-linear mixed models were refined to estimate the AA ratio dose-response relationships. Then, 4 experiments using 2,420 wean-to-finish pigs were conducted to validate the methods for estimating the standardized ileal digestible (SID) AA to lysine (AA:Lys) ratio requirement. Subsequently, 7 experiments using 7,562 pigs were conducted to estimate the SID tryptophan (Trp) to Lys and Valine (Val) to Lys ratio requirements of wean-to-finish pigs. In 11- to 20-kg pigs, optimum SID Trp:Lys ranged from 16.6% for maximum mean G:F to 21.2% for ADG. In 30- to 125-kg pigs, optimum SID Trp:Lys ratio ranged from 16.9% for maximum mean G:F to 23.5% for ADG. However, 18% SID Trp:Lys captured 96 and 100% of the maximum mean ADG and G:F for finishing pigs, respectively. In 25- to 45-kg pigs, optimum SID Val:Lys ratio ranged from 72.3% for maximum mean G:F to 74.4% for ADG with 99% of the maximum mean ADG and G:F at approximately 69% and 65% SID Val:Lys ratio, respectively. In conclusion, optimum SID Trp:Lys and Val:Lys

were consistently higher for ADG than G:F. This finding is critical for conducting economic evaluations and reference tables such as NRC (2012) should consider presenting requirement values for different response criteria.

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Dedication

Dedicated to the memories of my father César Gonçalves (1954 – 1993) and stepfather Glenn Maciel (1953 – 1998).

Chapter 1 - Effects of amino acids and energy intake during late gestation of high-performing gilts and sows on litter and reproductive performance under commercial conditions[1,2]

M. A. D. Gonçalves*, K. M. Gourley†, S. S. Dritz*,[3], M. D. Tokach†, N. M. Bello‡, J. M. DeRouchey†, J. C. Woodworth†, and R. D. Goodband†

*Department of Diagnostic Medicine/Pathobiology, College of Veterinary Medicine, †Department of Animal Sciences and Industry, College of Agriculture, and ‡Department of Statistics, College of Arts and Sciences, Kansas State University, Manhattan, KS 66506-0201

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[3] Corresponding author: dritz@vet.k-state.edu

ABSTRACT: The objective of this study was to determine the effects of AA and energy intake during late gestation on piglet birth weight and reproductive performance of high-performing gilts and sows housed under commercial conditions. At d 90 of gestation, a total of 1,102 females (PIC 1050) were housed in pens by parity group (P1 or P2+) with approximately 63 P1 and 80 P2+ in each pen, blocked by BW within each pen, and each female was randomly assigned to dietary treatments within BW block. Dietary treatments consisted of combinations of 2 standardized ileal digestible (**SID**) AA intakes (10.7 or 20.0 g/d SID Lys and other AA met or exceeded the NRC [2012] recommendations) and 2 energy intakes (4.50 or 6.75 Mcal/d intake of NE) in a 2 × 2 factorial arrangement. Data were analyzed using generalized linear mixed models specified to recognize pen as the experimental unit for parity and the individual female as the experimental unit for dietary treatments. Results indicate an overall positive effect of high energy intake on BW gain during late gestation, though this effect was more manifest under conditions of high, as opposed to low, AA intake (interaction, $P < 0.001$). Further, the magnitude of BW gain response to increased energy intake was greater ($P < 0.001$) for sows compared to gilts. Sows fed high energy intake had reduced probability of piglets born alive ($P < 0.004$) compared to those fed low energy, but no evidence for differences was found in gilts. This can be explained by an increased probability ($P = 0.002$) of stillborns in sows fed high vs. low energy intake. There were no evidences for differences between dietary treatments in litter birth weight and individual piglet birth weight of total piglets born. However, individual born alive birth weight was approximately 30 ± 8.2 g heavier ($P = 0.011$) for females fed high, as opposed to low, energy intake. Further, born alive piglets were approximately 97 ± 9.5 g heavier ($P < 0.001$) for sows than for gilts. Pre-weaning mortality was decreased ($P = 0.034$) for females fed high, compared to low, AA intake regardless of energy level. In conclusion, 1) BW gain of gilts and sows depended not only on energy but also AA intake levels, 2) sows fed increased amount of

energy had increased stillborn rate, and 3) increased energy intake during late gestation had a positive effect on individual piglet birth weight with no evidence for such effect for AA intake.

Key words: amino acids, birth weight, energy, gestation, gilts, sows

INTRODUCTION

Increased litter size over the last decades reduced the uterine space available for fetal growth and development, thus, reducing individual piglet birth weight (Town et al., 2005). Lower birth weight has been associated with reduced piglet survivability, wean weight, and market weight (Bergstrom et al., 2011; Douglas et al., 2013). However, few nutritional options have been identified to help mitigate the reduction in birth weight associated with large litter sizes (Goodband et al., 2013).

Evidence from recent studies does not support any impact of increased feed intake in early or mid-gestation on piglet birth weight (Heyer et al., 2004; Lawlor et al., 2007). However, increasing feed intake in late gestation has been shown to improve piglet birth weight (Cromwell et al., 1989, Shelton et al., 2009, Soto et al., 2011). Cromwell et al. (1989) observed a 40 g increase in piglet birth weight when gilts and sows were fed an extra 1.4 kg of feed daily during late gestation. Shelton et al. (2009) and Soto et al. (2011) observed an increase in piglet birth weight in litters from gilts fed increased amount of feed during late gestation, though this was not apparent in sow litters. Yet, the effects of increased feed allowance during late gestation on piglet birth weight remains unclear for commercial conditions, particularly in high-performing herds (> 14.5 total piglets born/sow). Further, because all studies reported increased feed intake, which increased both energy and AA, it is unclear if the influences on piglet birth weight are due to dietary AA or energy content.

The objective of this study was to determine the effects of AA and energy intake during late gestation on piglet birth weight and reproductive performance of high-performing gilts and sows housed under commercial conditions. The hypothesis was that both maternal dietary AA and energy in the late gestation period would positively affect piglet birth weight in an additive manner.

MATERIALS AND METHODS

General

The Kansas State University Institutional Animal Care and Use Committee approved the protocol used in this experiment. The experiment was conducted at a commercial sow farm in northern Ohio during the summer season. Females were individually housed from d 0 to 40 of gestation, then were group-housed (1.86 and 1.67 m^2/animal for gilts and sows, respectively) from d 40 to 111 of gestation. Each group pen was equipped with an electronic sow feeding station (Schauer, Prambachkirchen, Austria) and two cup waterers. All females had ad libitum access to water.

Animals and diets

From d 0 to 89 of gestation, females were fed a common diet with 0.59% standardized ileal digestible (**SID**) Lys according to body condition (thin, ideal, and fat females were fed 3.2, 2.0, and 1.8 kg/d, respectively), following standard practice at this commercial farm. To be eligible for enrollment in this experiment at d 90 of gestation, females must not have: 1) recorded a return to estrus event during the previous cycle; 2) had an abortion during the previous cycle; 3) lameness of moderate or greater severity; or 4) body condition score less than 2 on a 1 to 5 scale. At d 90 of gestation, a total of 1,102 females (PIC 1050, Hendersonville, TN; 741 gilts and 361 sows) were housed in pens by parity group (P1 or P2+) with approximately 63 P1 or 80 P2+

in each pen, blocked by BW within each pen, and each female was randomly assigned to dietary treatments within BW block in a pen. The parity for P2+ (sows) after farrowing was 4.0 ± 1.9 (median ± SD). Dietary treatments consisted of combinations of 2 SID AA intakes (10.7 or 20.0 g/d SID Lys and other AA met or exceeded the NRC [2012] recommendations as a ratio to Lys) and 2 energy intakes (4.50 or 6.75 Mcal/d intake of NE) in a 2 × 2 factorial arrangement. All other nutrients met or exceeded the NRC (2012) recommendations. The NRC (2012) estimates the Lys requirement from d 90 of gestation until farrowing at 16.7 g SID Lys/d for gilts and 11.9 g SID Lys/d for sows. The NE requirement estimate is 6.37 Mcal NE/d for gilts and 6.24 Mcal NE/d for sows (NRC 2012). The low AA (10.7 g/d SID Lys) and low energy (4.50 Mcal NE/d) intake dietary treatment in this experiment was structured to be representative of practices used in commercial farms that do not increase the amount of feed in late gestation. The NE intake on the low energy treatment was calculated to meet the maintenance requirement for a 230 kg BW female. Thus, the low energy intake was expected to provide near or just above maintenance for the majority of the females in the study. The high energy treatment was defined as 6.75 Mcal NE/d because it is above the requirement of gilts and sows estimated by NRC (2012) and also to represent the levels of intake used in those farms that increase the amount of feed in late gestation. The NRC (2012) SID Lys requirement estimate for gilts at d 113 of gestation is 19.3 g/d. The high AA treatment was formulated to provide 20 g/d SID Lys to be above NRC (2012) estimated requirement for gilts and sows during the last third of gestation and to be in accordance with findings from Srichana et al. (2006).

Two diets were formulated (Table 1.1) and delivered at 4 different ratios and intake levels in order to achieve the desired dietary treatments for the 2 × 2 factorial treatment structure (Table 1.2). Diets were balanced on a Ca to standardized total tract digestible (STTD) P ratio basis. Phytase was included in both diets at the same level, with release considered to be 0.12% for STTD P. No AA or energy release was considered for phytase.

At d 111 of gestation, females were moved to the farrowing house and fed 3.6 kg/d of a common lactation diet with 1.25% SID Lys provided until farrowing and then provided the same diet ad libitum thereafter. Both gestation and lactation diets were corn-soybean meal-based and presented in meal form.

The response variables measured were: female ADFI from d 90 to 111 of gestation, individual female BW at d 90 and 111 of gestation, total number of piglets born, number of piglets born alive, number of stillborns, number of mummified fetuses, number of dead piglets, and number of removed piglets, individual piglet BW at birth was collected at 0530 h for the litters farrowed overnight and at 1200 h for the litters farrowed between 0530 and 1200 h. Litter birth weight and individual piglet birth weight were then calculated with and without the inclusion of stillborns and mummified fetuses. The coefficient of variation of birth weight within litter was calculated by dividing the individual piglet birth weight standard deviation by the average piglet birth weight of that specific litter.

Following farrowing and data collection, litter size was equalized after weighing individual piglets in a blinded manner regardless of dietary treatment; no pigs were added to litters thereafter. Dead and removed pigs were recorded. Removed pigs were considered pigs removed from the female due to loss of body condition and were put on an off-test nursing female. Lactation length, wean-to-estrus interval, and percentage of females bred until 7 d after weaning were also recorded.

On the subsequent cycle, no dietary treatments were applied and females were fed a common diet with 0.59% SID Lys accordingly to body condition (thin, ideal, and fat females were fed 3.2, 2.0, and 1.8 kg/d, respectively) until d 90 of gestation and then feed allowance was increased by 0.9 kg/d for thin and ideal condition females. To evaluate subsequent female performance, farrowing rate, total number of piglets born, number of born alive, number of stillborns, and number of mummified fetuses from the next reproduction cycle were also recorded.

Diet Sampling and Analysis

Prior to diet formulation, 5 samples of corn and soybean-meal were submitted for proximate and total AA analysis. The analyzed values were used in formulation in conjunction with NRC (2012) digestibility coefficients. Samples of the diets were submitted to Ward Laboratories, Inc. (Kearney, NE) for analysis of DM (method 935.29; AOAC Int., 2012), CF (method 978.10; AOAC Int., 2012 for preparation and Ankom 2000 Fiber Analyzer [Ankom Technology, Fairport, NY]), ash (method 942.05; AOAC Int., 2012), crude fat (method 920.39 a; AOAC Int., 2012 for preparation and ANKOM XT20 Fat Analyzer [Ankom Technology, Fairport, NY]), Ca, and P (method 968.08 b; AOAC Int., 2012 for preparation using ICAP 6500 [ThermoElectron Corp., Waltham, MA]). Diet samples were taken from each electronic feeding station twice a week, then CP and total AA analyses were conducted in duplicate on composite samples by Ajinomoto Heartland Inc. (Chicago, IL). Feeding station calibration was monitored twice a week by weighing 10 samples from each dispenser in each station.

Statistical Analysis

Data were analyzed using generalized linear mixed models whereby the linear predictor included parity group, dietary treatments and all interactions as fixed effects, as well as the random effects of pen nested within parity and BW block crossed with pen nested within parity.

So specified, models recognized pen as the experimental unit for parity and the individual female as the experimental unit for dietary treatments, after accounting for BW blocking.

Female ADFI from d 90 to 111 of gestation, individual female BW at d 90 of gestation, female BW gain from d 90 to 111 of gestation, individual piglet BW at birth, total litter birth weight, lactation length, and WEI were fitted assuming a normal distribution of the response variable. In these cases, residual assumptions were checked using standard diagnostics on studentized residuals and were found to be reasonably met.

In turn, total number of piglets born and litter size after equalization were fitted assuming a negative binomial distribution on the response, whereas born alive, stillborns, mummified, dead, removed, and weaned piglets, as well as females bred until 7 d after weaning and subsequent farrowing were fitted using a binomial distribution. Further, the coefficient of variation of birth weight within the litter considering total piglets born and piglets born alive were approximated with a beta distribution, as all observed values laid between 0 and 1. Overdispersion was assessed using a maximum-likelihood-based Pearson chi-square/DF statistic and accounted for as needed (Stroup 2012). The final models used for inference were fitted using restricted maximum likelihood estimation. Degrees of freedom were estimated using the Kenward-Rogers approach (Kenward and Roger, 1997).

Estimated means and corresponding standard errors (SEM) are reported for all interactive means and also for treatment combinations of interest consistent with significance of interaction or main effects, following hierarchical principle of inference (Milliken and Johnson, 2009). Pairwise comparisons were conducted on such means using a Bonferroni adjustment to prevent inflation of Type I error due to multiple comparisons. Statistical models were fitted using the GLIMMIX procedure of SAS (Version 9.3, SAS Institute Inc., Cary, NC). Results were considered significant at $P \leq 0.05$ and marginally significant at $0.05 < P \leq 0.10$.

RESULTS

General

Chemical analysis of DM, CP, crude fiber, crude fat, Ca, P, ash, and total AA reasonably met the formulated values (Table 1.3). Average daily feed intake from each treatment was reasonably close to the feed allowance (Tables 1.2 and 1.4).

Female BW gain during late gestation

Within each parity group, we observed no evidence for any differences between treatments in initial BW at 90 d of gestation, thus validating our randomization process (Table 1.5). Regarding BW gain during late gestation, significant interactions were apparent, specifically between AA × Energy ($P < 0.001$) and Parity × Energy ($P < 0.001$). An overall positive effect of high energy intake was identified on the magnitude of BW gain during late gestation, though this effect was more manifest (Energy × AA, $P < 0.001$) under conditions of high AA intake compared to low AA intake (8.8 ± 0.36 kg and 6.5 ± 0.37 kg, respectively; Fig. 1.1). Further, the magnitude of BW gain response to increased energy intake was greater for sows compared to gilts (8.8 ± 0.42 kg and 6.5 ± 0.29 kg, respectively; $P < 0.001$; Fig. 1.2).

Piglet birth weight

Considering the total number of piglets born, there was no evidence for differences between the dietary treatments on litter birth weight or on individual piglet birth weight (Tables 1.4 and 1.5). However, litter birth weight and individual piglet birth weight were heavier in sows ($P < 0.001$) than gilts, whereas within-litter birth weight CV was greater ($P < 0.001$) in sows than gilts. Further, a marginally greater (P = 0.091) within-litter birth weight CV was observed in females fed high energy compared to low energy independent of parity level.

When litter birth weight and individual piglet birth weight for piglets born alive were considered, weights were heavier in sow litters (P < 0.001) compared to gilt litters. More specifically, born alive piglets from sows were approximately 97 ± 9.5 g heavier (P < 0.001) than from gilts. Further, individual born alive birth weight (Fig. 1.3) was approximately 30 ± 8.2 g heavier (P = 0.011) for females fed high energy intake compared to low energy intake females, regardless of AA intake or parity level. There was no evidence for differences in the within-litter birth weight CV of born alive piglets between the dietary treatments, though this CV was greater (P < 0.001) in sows than gilts.

Reproductive performance

Litter size. There was no evidence for any differences in the number of total piglets born between dietary treatments. However, across diets, sows had more (P < 0.001) total piglets born than gilts. In turn, energy intake showed a differential effect on the probability of born alive for sows and gilts (Parity × Energy, P < 0.001). Specifically, sows fed high energy intake had a reduced probability of piglets born alive (Parity × Energy, P < 0.004), compared to those fed low energy, but no evidence for differences was found in gilts, regardless of level of AA intake in their diet. This may be partially explained by an increased probability of stillborns (Parity × Energy, P = 0.002) in sows fed high, as opposed to low, energy intake (Fig. 1.4). Additionally, after accounting for the effect of energy intake, the probability of stillborns was reduced (P = 0.049) in females fed high AA intake. Further, an AA × Energy × Parity (P = 0.047) interaction was identified on probability of mummified fetuses, whereby sows fed low energy and high AA intake had increased probability compared to sows fed low energy and low AA intake (P =

0.048); no evidence for dietary effects was apparent in gilts. As expected, there were no statistical differences between litter size after equalization as a function of dietary treatment or parity.

Pre-weaning mortality, removal rate, and piglets weaned. Pre-weaning mortality (**PWM**) was decreased (P = 0.034) in piglets suckling from females fed high, as compared to low, AA intake during late gestation regardless of energy level. After adjusting for dietary treatments, sows showed greater PWM than gilts (P < 0.001). There were no statistically significant differences between dietary treatments on removal rate; however, there was a marginal increase in the probability of piglets weaned (P = 0.087) when females were fed high, as opposed to low, energy.

Lactation length, percentage bred by 7 d, and wean-to-estrus interval. There was no evidence for differences in lactation length between dietary treatment or parity level. For all dietary treatments, the percentage of females bred by 7 d after weaning was greater (P = 0.001) for sows than for gilts. This was explained by a lower (P = 0.001) wean-to-estrus interval (WEI) in sows compared to gilts. However, there was no evidence for any differences between dietary treatments in percentage of females bred by 7 d after weaning or WEI.

Subsequent female performance

For the subsequent reproductive cycle, there was no evidence for any effects of dietary treatments on farrowing rate, number of total piglets born, probability of born alive piglets, and probability of mummified fetuses. However, females previously fed high energy had lower (P = 0.040) probability of stillborn piglets in the subsequent cycle compared to those fed low energy regardless of AA level.

On the subsequent cycle, sows had greater ($P < 0.001$) number of total piglets born compared to gilts regardless of dietary treatments. In turn, gilts had increased ($P < 0.004$) probability of piglets born alive compared to sows, and this was at least partially explained by a decreased ($P < 0.012$) probability of stillborns on the subsequent cycle.

DISCUSSION

The main objective of this study was to evaluate the impact of AA and energy intake levels during late gestation on piglet birth weight and subsequent maternal reproductive performance.

Piglet birth weight

Several experiments in the literature reported piglet birth weight without specifying if it included total born or was limited to only those piglets born alive. In this study, birth weights from total born and from piglets born alive are both reported. This distinction is important because there was no evidence for any differences in litter birth weight or individual total born piglet birth weight between the dietary treatments; however, individual born alive piglet birth weight was heavier in piglets from females fed high, compared to low, energy intake.

Interestingly, the observed dietary energy effect in the current study had similar estimated magnitude to another large sample size study conducted in multiple farms and multiple seasons by Cromwell et al. (1989) where the authors observed a 40 g improvement in birth weight of born alive piglets by feeding increased amount of feed from d 90 of gestation. Additionally, in our study, it is worth noting that parity had more than 3-fold greater effect (approximately 97 vs 30 g) on individual born alive piglet birth weight than energy intake.

Srichana (2006) suggested a SID Lys requirement for gilts in late gestation of 20 g/d estimated through nitrogen balance. Similarly, our findings showed that increasing SID Lys intake from 10.7 to 20 g/d indeed increased female BW gain; however, AA did not significantly affect piglet birth weight. This finding is interesting from the perspective that the SID AA level to maximize growth of the gestating female is probably different from the level to maximize piglet birth weight because fetal growth is a priority during late gestation (Theil et al., 2014). Thus, the gestating female will likely catabolize protein to supply AA to the growing fetuses. Genetic selection has focused on maximized leanness as it improves feed efficiency (Chen et al., 2003). Consequently, gilt and sow body composition have shifted towards increased lean rather than fat (Lewis and Southern, 2000). Therefore, given that individual piglet birth weight was affected by increasing levels of energy, it could be speculated that females during late gestation are limited in energy, rather than limited in AA.

Reproductive performance

Born alive piglets were reduced in sows fed high energy intake due to an increased probability of stillborns but not in gilts. Fat sows have been reported to have longer farrowing duration (Madec and Leon, 1992), which can cause a higher probability of stillborn piglets (Zaleski and Hacker, 1993); however, Borges et al. (2005) did not observe any association between sow body condition and probability of stillborn and Cozler et al. (2002) observed a reduced probability of stillborns in heavier weight sows. Thus, the literature is unclear on the effects of sow body condition or body weight on probability of stillborns. These results from the literature are probably further confounded by others factors such as lean to fat ratio and diet composition. However, existing evidence about a relationship between higher parities and increased probability of stillborns may be related to poorer uterine muscle tone (Zaleski and

Hacker, 1993; Leenhouwers et al., 1999; Borges et al., 2005). Our data is consistent with this line of thought as we observed higher stillborn rate in sows compared to gilts. In addition, it has been reported that stillborn rate is greater in heavier piglets (Arthur et al., 1989). In our study, piglets were heavier at birth in 1) sows compared to gilts, and 2) females fed higher energy compared to low energy. This might explain our result on a greater stillborn rate in sows fed high energy compared to sows fed low energy.

Stillborn rate was reduced in females fed high AA intake, which is in agreement with Magnabosco et al. (2013), who observed a marginally significant reduction of 1.1 percentage points in the probability of stillborns for gestating sows fed higher Lys. Another study fed low or high AA during lactation and also observed a marginal reduction in stillborn in the subsequent farrowing for females fed high AA (Musser et al., 1998). This is an interesting finding as changes would be expected in the body composition (lean to fat ratio) of females fed high AA intake, which could, in turn, impact uterine muscle tone and reduce dystocia (Almond et al., 2006). The only AA × energy × parity interaction was that sows fed low energy and high AA intake had increased probability of mummified fetuses compared to sows fed low energy and low AA intake, though no evidence for any dietary effects was apparent in gilts. This finding has not been previously reported in the literature and the biological reasons for it could not be explained in this experiment.

Pre-weaning mortality improved for litters suckling from females fed high AA intake compared to low AA intake regardless of energy level. This result is consistent with the findings of a proof-of-concept study (DeGeeter et al., 1972) which showed that low CP during gestation negatively influenced pre-weaning mortality. Yet, given that increased AA have not been reported to increase milk fat (Dourmad et al., 1998; Kusina et al., 1999), it remains unclear how dietary AA influence pre-weaning mortality. Sows fed higher AA had marginally higher milk protein content (Yang et al., 2009), which could potentially be related to a change in lean:fat ratio of piglets.

Female BW gain during late gestation

The interactive effects of dietary AA and energy levels on BW have been well documented in nursery pigs (Schneider et al., 2010), finishing pigs (Main et al., 2008; Nitikanchana et al., 2015), and lactating sows (Tokach et al., 1992). To the author's knowledge, this is the first report of an interaction between AA and energy intake on BW gain of reproductive females during late gestation. Our results are in agreement with the body of literature in nursery and finishing pigs in which a simultaneous increase in AA and energy is needed to maximize growth until the genetic ceiling for protein deposition is reached (Campbell and Taverner, 1988). This is an important finding that deserves further quantification given that the current NRC (2012) spreadsheet model only predicts gestating female BW gain based on energy intake, but not based on AA intake or based on a AA:calorie ratio. This study provides evidence that AA intake should be considered when estimating BW gain of gilts and sows during late gestation.

Increasing energy intake increased BW gain during late gestation in both gilts and sows. However, sows fed low energy intake had reduced BW gain compared to gilts. This could be partly explained because gilts have higher growth rate than sows (NRC, 2012) and maintenance in late gestation represents approximately 60% of the energy requirement for gilts and 80% for sows based on NRC (2012). Therefore, partitioning of energy towards growth is greater in gilts compared to sows whereas partitioning of energy towards maintenance is greater in sows compared to gilts.

Parity effects

Even after dietary effects were accounted for, sows had greater number of total piglets born, litter birth weight, piglet birth weight, and birth weight CV compared to gilts. These results are in agreement with the current body of literature (Pettigrew et al., 1986; Gama and Johnson, 1993; Milligan et al., 2002b). Further, we found greater PWM in litters from sows compared to litters from gilts. From an immune status perspective, greater PWM would be expected in litters from gilts due to smaller amount of antibodies transferred through colostrum (Roth and Thacker, 2006). On the other hand, greater PWM in litters from sows than gilts could occur due to the more variable access to functional teats (Cutler et al., 2006), greater variation within the litter, and greater number of total piglets born (Roehe and Kalm, 2000). The current body of literature has mixed results regarding the impact of parity on survivability until weaning (Knol et al., 2002; Milligan et al., 2002a; Milligan et al., 2002b). As shown in past studies (Mabry et al., 1996; Guedes and Nogueira, 2001), sows had shorter WEI than gilts and, consequently, greater percentage of females bred by 7 d after weaning.

Subsequent female performance

Even though there were no statistical differences between dietary treatments in the subsequent parity for total piglets born and piglets born alive, females fed high energy in the cycle on which the dietary treatments were fed had lower probability of stillborn in the subsequent cycle. This suggests no evidence for a long-term impact or carry-over effect of dietary treatments on reproductive performance of gilts and sows, as dietary treatments were only applied in the first cycle, whereas on the subsequent cycle females were under standard farm procedures common to all.

A comment on modern statistical modeling

The statistical analysis in the current study entailed state-of-the-art generalized linear mixed models (**GLMM**), which evaluated each response variable according to the nature of its distribution (Stroup, 2012). By contrast to the general linear model (**GLM**) that assumes normality on the response variable, GLMM are particularly useful for non-normal responses for which a normal approximation may not be the best approach; for example, in the presence of count (i.e., total piglets born), binomial (i.e., piglets born alive), and binary (i.e., farrowing rate) data (Stroup, 2012). In fact, GLMM allows the researcher to recognize the proper nature of a response variable and the corresponding statistical distribution to be used for its modeling. For example, an observation on a given sow farrowing a litter of size 13 with all piglets born alive can be argued to carry different information (and probably health implications) compared to an observation from another sow which may have also farrowed 13 piglets born alive but from a larger litter (20 total born for example). Recording such observations using just a count of 13 born alive in either case, as is often the case with swine farm database management systems, fails to recognize the difference in information contained by both observation and can easily lead to misleading conclusions. Instead, a more insightful understanding of the situation may be feasible if one recognized the nature of the variable born alive as binomial with number of trials given by the litter size and with probability of born alive estimated from the data. Indeed, properly recognizing the nature of the response variable has important implications for sound inference and subsequent decisions making (Stroup, 2012). In turn, inappropriate use of statistical distributions can create misleading interpretations of the data (Limpert and Stahel, 2011). In fact, it is possible that inconsistent finding amongst sow experiments may be explained, at least partially, by inappropriate use of statistical distributions to model non-normal responses that are common in swine production systems.

IMPLICATIONS

In conclusion, 1) body weight gain of swine females depends not only on energy but also on AA intake levels, and it does so differently for gilts and sows, 2) high energy intake caused increased stillborn rate in sows, 3) pre-weaning mortality was reduced in piglets suckling from females with high AA intake, and 4) increased energy intake during late gestation had a positive, though modest, effect on individual piglet birth weight; no evidence for such effect was apparent for levels of AA intake.

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TABLES

Table 1.1. Diet composition (as-fed basis)[1]

	SID Lys, %	
Ingredient	0.40	1.06
Corn	87.97	62.47
Soybean meal (46% CP)	8.06	33.59
Monocalcium phosphate	1.60	1.25
Limestone	1.50	1.45
Salt	0.50	0.50
L-Lys HCl	0.045	0.045
DL-Met	---	0.200
L-Thr	0.035	0.210
Choline chloride 60%	0.100	0.100
Vitamin/mineral premix[2]	0.150	0.150
Phytase[3]	0.035	0.035
TOTAL	100	100
Calculated analysis		
Standardized ileal digestible (SID) AA, %		
Lys	0.40	1.06
Ile:Lys	80	72
Leu:Lys	219	144
Met:Lys	41	47
Met & Cys:Lys	79	71
Thr:Lys	81	80
Trp:Lys	21	22
Val:Lys	94	76
NE, kcal/kg	2521	2386
CP, %	11.20	21.50
Ca, %	0.85	0.86
P, %	0.62	0.66
Available P, %	0.52	0.48
Stand. total tract dig. (STTD) P, %	0.52	0.52
Ca:Total P	1.37	1.29
Ca:STTD P	1.64	1.64

[1] Diets were fed from d 90 to 111 of gestation. Corn and soybean meal were analyzed for total AA content prior to diet formulation and NRC (2012) SID digestibility values were used in the diet formulation.

[2] Provided per kg of diet: 40 mg Mn from manganese oxide, 99 mg Fe from iron sulfate, 132 mg Zn from zinc sulfate, 16.5 mg Cu from copper sulfate, 0.33 mg I from ethylenediamin dihydroiodide, 0.30 mg Se from sodium selenite, 0.23 mg biotin, 1.65 mg folic acid, 3.31 mg pyridoxine, 9,921 IU vitamin A, 2,202 IU vitamin D3, 66 IU vitamin E, 4.3 mg vitamin K, 33 mg

pantothenic acid, 43 mg niacin, 10 mg riboflavin, and 33 µg vitamin B12.
[3] Quantum Blue 2G (AB Vista Feed Ingredients, Marlborough, UK) provided 701 FTU per kg of diet with a release of 0.12% STTD P.

Table 1.2. Experimental dietary treatment structure[1]

	AA intake:	Low		High	
	Energy intake:	Low	High	Low	High
Item	Delivered ratio[2], %:	71/29	100/0	0/100	50.5/49.5
Feed allowance, kg/d		1.81	2.68	1.89	2.75
Energy, Mcal NE/d		4.50	6.75	4.50	6.75
SID Lys, g/d		10.7	10.7	20.0	20.0

[1] Dietary treatment structure based on the two diets presented in Table 1.

[2] Delivered ratio between 0.40% and 1.06% SID Lys diets in order to achieve the desired dietary treatments on an intake basis. Other AA met or exceeded the NRC (2012) recommendations as a ratio to Lys.

Table 1.3. Chemical analysis of the diets (as-fed-basis)[1]

Item	AA intake	Low		High	
	Energy intake	Low	High	Low	High
Proximate analysis, %					
DM		89.3 (87.4)	89.3 (87.2)	90.1 (88.0)	89.8 (87.6)
CP		13.6 (14.2)	10.4 (11.2)	20.9 (21.5)	15.9 (16.3)
Crude fiber		1.6 (2.2)	1.6 (2.1)	2.1 (2.5)	1.9 (2.3)
Ca		0.84 (0.85)	0.74 (0.85)	0.79 (0.85)	0.78 (0.85)
P		0.59 (0.63)	0.59 (0.62)	0.64 (0.66)	0.64 (0.64)
Fat		2.6 (3.0)	2.5 (3.2)	2.8 (2.7)	2.5 (2.9)
Ash		4.5 (5.1)	4.0 (4.9)	5.1 (5.8)	4.5 (5.3)
Total AA, %					
Lys		0.66 (0.69)	0.48 (0.48)	1.14 (1.21)	0.81 (0.84)
Ile		0.50 (0.52)	0.38 (0.38)	0.84 (0.87)	0.61 (0.62)
Leu		1.16 (1.22)	0.96 (1.00)	1.67 (1.74)	1.32 (1.37)
Met		0.26 (0.29)	0.18 (0.19)	0.45 (0.54)	0.31 (0.36)
Met & Cys		0.48 (0.52)	0.36 (0.38)	0.76 (0.85)	0.56 (0.61)
Thr		0.54 (0.56)	0.40 (0.39)	0.92 (0.98)	0.65 (0.68)
Trp		0.13 (0.14)	0.12 (0.10)	0.24 (0.26)	0.17 (0.18)
Val		0.59 (0.59)	0.47 (0.45)	0.90 (0.94)	0.69 (0.69)
His		0.34 (0.39)	0.26 (0.31)	0.52 (0.58)	0.38 (0.45)
Phe		0.63 (0.69)	0.49 (0.54)	1.00 (1.05)	0.75 (0.79)

[1] Diet samples were taken from each electronic feeding station twice a week, then CP and total AA analyses were conducted in duplicate on composite samples by Ajinomoto Heartland Inc.
[2] Values in parentheses indicate those calculated from diet formulation and are based on values from NRC, 2012 (Nutrient Requirements of Swine, 11th ed. Natl. Acad. Press, Washington DC) with the exception of total AA content from corn and soybean-meal, which were analyzed prior to diet formulation by Ajinomoto Heartland Inc. (Chicago, IL).

Table 1.4. Least square mean estimates (and corresponding SEM) of the effects of AA and energy intake during late gestation of high-performing gilts and sows on piglet birth weight and reproductive performance under commercial conditions[1]

	Gilts				Sows			
AA intake[2]	Low	High	Low	High	Low	High	Low	High
Item / Energy intake[2]	Low	Low	High	High	Low	Low	High	High
BW d 90, kg	175.5 ± 1.51	174.7 ± 1.51	175.3 ± 1.52	175.3 ± 1.51	227.3 ± 2.21	227.4 ± 2.19	225.8 ± 2.20	228.7 ± 2.22
BW gain d 90 to d 111, kg	13.4 ± 0.44	16.4 ± 0.44	18.4 ± 0.44	24.5 ± 0.44	10.5 ± 0.67	13.4 ± 0.67	18.5 ± 0.68	23.0 ± 0.67
ADFI d 90 to 111, kg	1.8 ± 0.01	1.8 ± 0.01	2.6 ± 0.01	2.6 ± 0.01	1.8 ± 0.01	1.9 ± 0.01	2.7 ± 0.01	2.7 ± 0.01
Total piglets born, n	14.2 ± 0.22	14.1 ± 0.21	14.1 ± 0.22	14.2 ± 0.21	15.3 ± 0.34	14.8 ± 0.33	15.1 ± 0.32	15.5 ± 0.35
Born alive, %	94.6 ± 0.5	95.0 ± 0.5	93.6 ± 0.5	94.2 ± 0.5	93.3 ± 0.8	93.1 ± 0.8	89.6 ± 1.0	90.8 ± 1.0
Mummified fetuses, %	1.8 ± 0.28	1.7 ± 0.27	2.6 ± 0.36	2.5 ± 0.34	1.6 ± 0.36	3.0 ± 0.54	3.4 ± 0.57	2.8 ± 0.54
Stillborn, %	3.5 ± 0.40	3.2 ± 0.38	3.6 ± 0.40	3.2 ± 0.37	5.1 ± 0.69	3.7 ± 0.58	6.9 ± 0.83	6.1 ± 0.79
Total born								
Litter birth weight, kg	18.0 ± 0.24	17.9 ± 0.24	17.8 ± 0.23	17.9 ± 0.23	20.7 ± 0.34	20.2 ± 0.34	20.6 ± 0.34	21.0 ± 0.35
Piglet birth weight, kg	1.25 ± 0.01	1.27 ± 0.01	1.28 ± 0.01	1.28 ± 0.01	1.36 ± 0.02	1.36 ± 0.02	1.38 ± 0.02	1.36 ± 0.02
Birth weight CV, %	20.2 ± 0.66	20.4 ± 0.65	21.7 ± 0.68	21.3 ± 0.67	25.6 ± 1.04	26.2 ± 1.05	27.3 ± 1.06	25.9 ± 1.07
Born alive								
Litter birth weight, kg	17.2 ± 0.23	17.2 ± 0.23	17.1 ± 0.23	17.3 ± 0.23	19.6 ± 0.34	19.5 ± 0.34	19.1 ± 0.34	20.0 ± 0.35
Piglet birth weight, kg	1.28 ± 0.01	1.28 ± 0.01	1.30 ± 0.01	1.31 ± 0.01	1.36 ± 0.02	1.39 ± 0.02	1.40 ± 0.02	1.41 ± 0.02
Birth weight CV, %	18.0 ± 0.47	18.2 ± 0.47	18.5 ± 0.48	18.3 ± 0.47	23.9 ± 0.77	23.2 ± 0.75	23.2 ± 0.76	21.9 ± 0.77
Litter size after equalization, n	14.5 ± 0.30	14.4 ± 0.30	14.6 ± 0.30	14.3 ± 0.30	14.2 ± 0.42	13.7 ± 0.40	13.9 ± 0.44	14.0 ± 0.43
Piglets weaned, %	84.3 ± 0.82	86.5 ± 0.77	86.2 ± 0.78	86.4 ± 0.78	80.7 ± 1.31	81.5 ± 1.26	82.0 ± 1.35	83.2 ± 1.26
Pre-weaning mortality, %	10.3 ± 0.69	8.0 ± 0.61	8.9 ± 0.64	8.4 ± 0.63	13.7 ± 1.15	13.1 ± 1.11	13.3 ± 1.21	12.1 ± 1.11
Piglet Removal rate, %	5.0 ± 0.55	5.2 ± 0.57	4.5 ± 0.52	4.9 ± 0.55	5.1 ± 0.82	5.00 ± 0.79	4.3 ± 0.77	4.3 ± 0.75
Lactation length, d	24.9 ± 0.27	24.9 ± 0.27	24.7 ± 0.46	24.0 ± 0.46	24.4 ± 0.41	24.1 ± 0.40	24.2 ± 0.72	24.2 ± 0.68
Wean-to-estrus interval, d	6.8 ± 0.43	5.9 ± 0.44	6.6 ± 0.45	6.2 ± 0.44	4.4 ± 0.71	4.2 ± 0.68	4.8 ± 0.77	4.9 ± 0.71
Females bred by 7 d after weaning, %	87.8 ± 2.88	88.9 ± 2.81	85.1 ± 3.26	89.1 ± 2.77	98.3 ± 1.72	98.3 ± 1.68	94.0 ± 3.37	96.2 ± 2.68
Subsequent performance								
Farrowing rate, %	88.3 ± 2.88	88.4 ± 2.91	84.2 ± 3.36	88.6 ± 2.86	93.7 ± 3.20	93.9 ± 3.13	91.3 ± 3.96	87.1 ± 4.67
Total piglets born, n	13.2 ± 0.35	13.2 ± 0.35	13.0 ± 0.36	13.4 ± 0.35	14.7 ± 0.56	15.5 ± 0.56	15.5 ± 0.62	15.0 ± 0.59
Born alive, %	93.9 ± 0.64	93.4 ± 0.68	93.9 ± 0.67	94.3 ± 0.61	91.1 ± 1.12	91.6 ± 1.07	92.2 ± 1.1	92.0 ± 1.11
Mummified fetuses, %	1.8 ± 0.33	1.8 ± 0.34	2.4 ± 0.40	2.0 ± 0.35	1.7 ± 0.46	2.9 ± 0.60	3.1 ± 0.67	2.3 ± 0.57
Stillborn, %	4.3 ± 0.54	4.7 ± 0.58	3.8 ± 0.52	3.7 ± 0.48	7.0 ± 1.01	5.5 ± 0.86	4.7 ± 0.84	5.5 ± 0.92

[1]A total of 1,102 females (PIC 1050) were used with 274 to 278 females per dietary treatment combination.
[2] Refer to Tables 1 and 2 for dietary composition and treatment structure, respectively.

Table 1.5. P-values corresponding to main effects of, and interactions between, AA intake, energy intake, and parity during late gestation of high-performing gilts and sows on piglet birth weight and reproductive performance under commercial conditions[1]

	AA × Energy × Parity	AA × Energy	Parity × AA	Parity × Energy	Parity	AA	Energy
BW d 90, kg	0.463	0.230	0.187	0.856	0.001	0.438	0.926
BW gain d 90 to d 111, kg	0.128	0.001	0.131	0.001	0.028	0.001	0.001
ADFI d 90 to 111, kg	0.608	0.834	0.050	0.707	0.001	0.001	0.001
Total piglets born, n	0.249	0.154	0.938	0.492	0.001	0.901	0.552
Born alive, %	0.569	0.483	0.718	0.092	0.002	0.261	0.001
Mummified fetuses, %	0.047	0.068	0.134	0.910	0.199	0.461	0.001
Stillborn, %	0.456	0.628	0.471	0.014	0.001	0.049	0.013
Mummified fetuses + Stillborn, %	0.569	0.483	0.718	0.092	0.002	0.261	0.001
Total born (TB)							
Litter birth weight, kg	0.453	0.189	0.795	0.241	0.001	0.904	0.489
Piglet birth weight, kg	0.885	0.546	0.446	0.643	0.001	0.993	0.365
Birth weight CV, %	0.610	0.266	0.792	0.533	0.001	0.678	0.091
Born alive							
Litter birth weight, kg	0.405	0.145	0.459	0.954	0.001	0.184	0.945
Piglet birth weight, kg	0.489	0.602	0.641	0.743	0.001	0.292	0.011
Birth weight CV, %	0.955	0.674	0.466	0.204	0.001	0.522	0.564
Litter size after equalization, n	0.462	0.761	0.987	0.986	0.103	0.516	0.904
Piglets weaned, %	0.365	0.516	0.789	0.781	0.001	0.120	0.087
Pre-weaning mortality, %	0.254	0.494	0.443	0.882	0.001	0.034	0.356
Piglet Removal rate, %	0.963	0.804	0.653	0.670	0.724	0.830	0.155
Lactation length, d	0.363	0.578	0.735	0.338	0.448	0.341	0.310
Wean-to-estrus interval, d	0.873	0.581	0.467	0.529	0.001	0.395	0.455
Females bred by 7 d after weaning, %	0.913	0.700	0.990	0.284	0.001	0.595	0.192
Subsequent performance							
Farrowing rate, %	0.436	0.927	0.456	0.428	0.167	0.981	0.163
Total piglets born, n	0.208	0.578	0.859	0.819	0.001	0.710	0.830
Born alive, %	0.459	0.808	0.893	0.875	0.004	0.904	0.284
Mummified fetuses, %	0.250	0.080	0.501	0.975	0.220	0.976	0.212
Stillborn, %	0.172	0.450	0.682	0.921	0.012	0.974	0.040

[1] A total of 1,102 females (PIC 1050) were used with 274 to 278 females per dietary treatment combination.

[2] Other AA met or exceeded the NRC (2012) recommendations as a ratio to Lys.

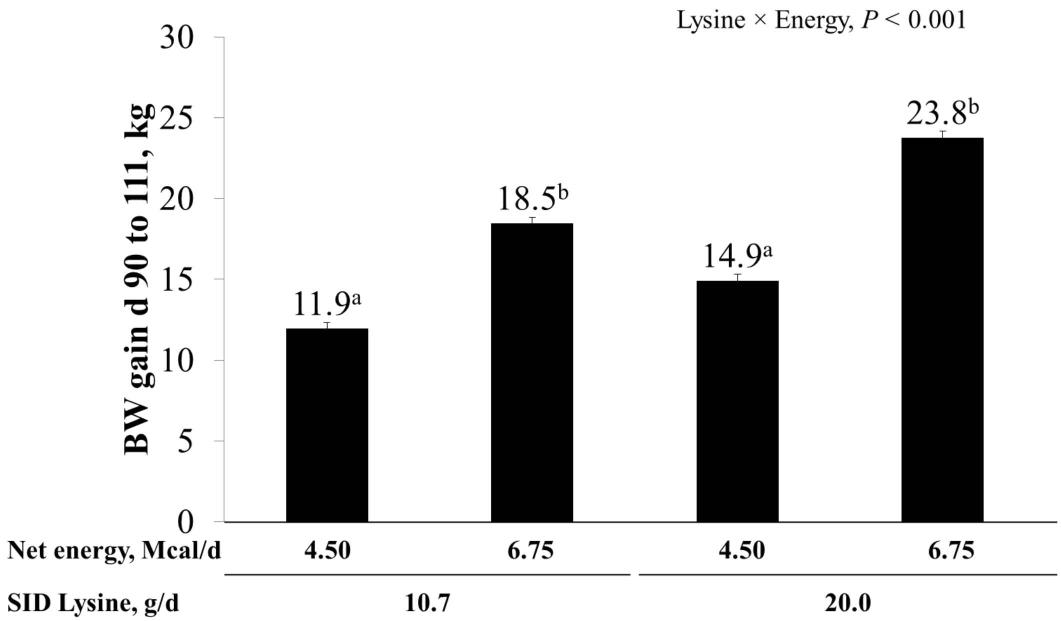

Table 1.6. Estimated mean (± SEM) BW gain of gilts and sows fed different AA and energy intake levels from d 90 to d 111 of gestation. Other AA met or exceeded the NRC (2012) recommendations as a ratio to Lys. [a,b] Within SID Lys level, means with different superscript differ ($P < 0.05$).

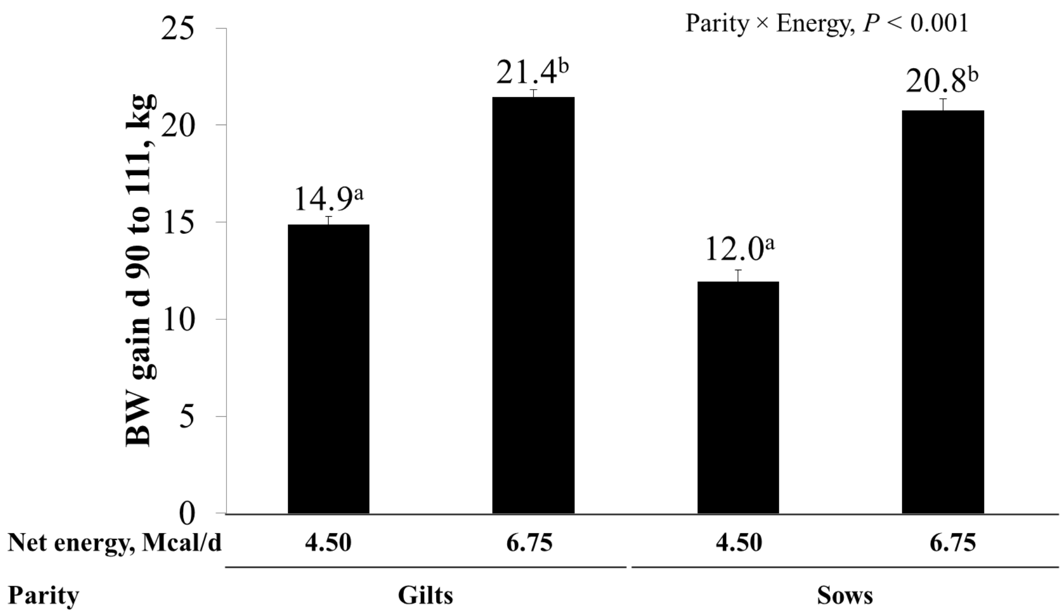

Table 1.7. Estimated mean (± SEM) BW gain of gilts and sows fed different energy intake levels from d 90 to d 111 of gestation. [a,b] Within parity level, means with different superscript differ (P < 0.05).

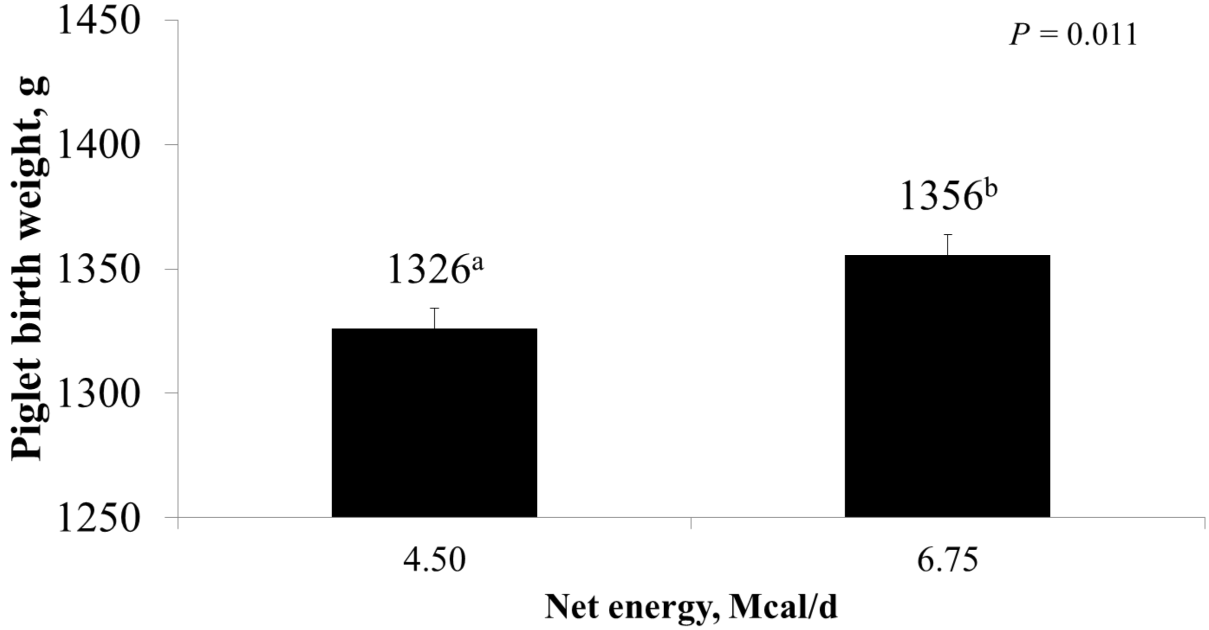

Table 1.8. Estimated mean (± SEM) individual born alive piglet birth weight for different energy intake levels fed from d 90 to d 111 of gestation. [a,b] Means with different superscript differ (P < 0.05).

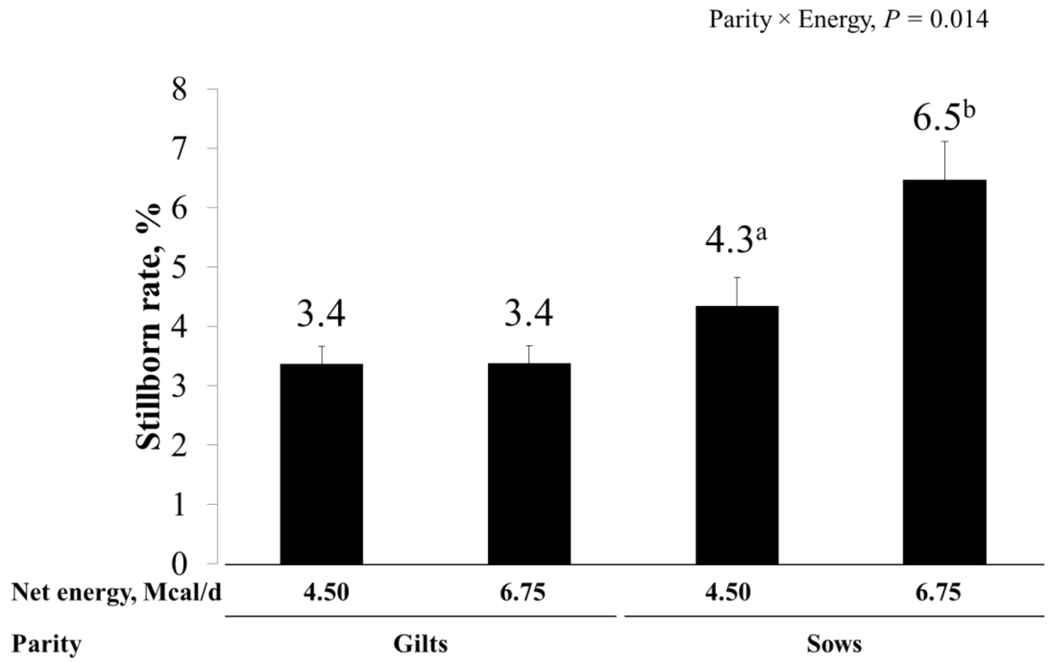

Table 1.9. Estimated stillborn rate (± SEM) for gilts and sows fed different energy intake levels fed from d 90 to d 111 of gestation. [a,b] Within parity level, means with different superscript differ ($P < 0.05$).

Chapter 2 - An update on modeling dose-response relationships: accounting for correlated data structures and heterogeneous variance in linear and non-linear mixed models[4]

M. A. D. Gonçalves*, N. M. Bello[5]‡, S. S. Dritz*, M. D. Tokach†,

J. M. DeRouchey†, J. C. Woodworth†, R. D. Goodband†

*Department of Diagnostic Medicine/Pathobiology, College of Veterinary Medicine,

‡Department of Statistics, College of Arts and Sciences,

†Department of Animal Sciences and Industry, College of Agriculture,

Kansas State University, Manhattan, KS 66506-0201

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[4] Contribution no. 16-152-J from the Kansas Agric. Exp. Stn., Manhattan, KS 66506-0210.

[5] Corresponding author: nbello@ksu.edu

ABSTRACT: Advanced methods for dose-response assessments are used to estimate concentrations of a nutrient that optimize a given outcome of interest, thereby determining nutritional requirements for optimal performance. Traditionally, many dose-response methods use a fixed-effects framework that assumes mutually independent observations with homogeneous variances. Yet, experimental data often present a design structure that includes correlations between observations (i.e. blocking, nesting, etc.), as well as heterogeneity of variances that can mislead inference if disregarded. Our objective in this article is to demonstrate practical implementation of computationally-intensive linear and non-linear mixed models methodology to describe dose-response relationships accounting for correlated data structure and heterogeneous variances. To illustrate, we modeled data from a randomized complete block design study to evaluate the Standardized Ileal Digestible (SID) Trp:Lys ratio dose-response on G:F of nursery pigs. A base linear mixed model was fit to explore the functional form of G:F relative to Trp:Lys ratios and assess model assumptions, in particular residual homoscedasticity. Next, we fitted 3 competing dose-response mixed models to G:F, namely a quadratic polynomial (QP), a broken-line linear (BLL) ascending model, and a broken-line quadratic (BLQ) ascending model, all of which included heteroskedastic specifications, as dictated by the base model, and used parameter estimates from the base model as initial values. The GLIMMIX procedure of SAS (Version 9.4) was used to fit the base and quadratic polynomial models and the NLMIXED procedure was used to fit the non-linear models. We further illustrated the use of a grid-search approach to facilitate convergence and parameter estimation in non-linear mixed models, as this seemed to be the most common implementation problem. Model fit between competing dose-response models was compared using maximum-likelihood-based Bayesian Information Criterion (BIC). The QP, BLL, and BLQ models fitted on G:F of nursery pigs yielded BIC values of 353.7, 343.4, and 345.2, respectively, thus indicating a better fit of BLL followed closely by BLQ. The BLL breakpoint estimate of the SID Trp:Lys ratio was 16.5% (95% CI: [16.1, 17.0%]), whereas the BLQ estimate was 16.0% (95% CI: [15.5, 16.6%]). Importantly, accounting for heterogeneous variance enhanced inferential precision as the breadth of the CI for mean breakpoint decreased by approximately 44%, from [15.8, 17.4%] to [16.1, 17.0%] SID Trp:Lys. In summary, the article illustrates the use of linear and non-linear mixed models for dose-response relationships accounting for heterogeneous residual variances, discusses important diagnostics, and their implications for inference, and provides practical recommendations for computational troubleshooting.

Key words: computational troubleshooting, dose-response, heterogeneous variances, linear and non-linear mixed models

INTRODUCTION

Dose-response models are used to estimate concentrations of a nutrient that optimize a given outcome, thereby determining nutritional requirements for optimal performance. Polynomials and broken-lines are functional forms commonly used in regression models to estimate nutrient dose-response relationships (Robbins et al., 1979; Vendenov and Pesti, 2007; Pesti et al., 2009). These models are often used in a fixed-effects modeling framework that assumes mutually independent observations with homogeneously dispersed errors. Yet, experimental data often present a design structure that includes correlations between observations (i.e. blocking, nesting, etc.) and heteroskedastic errors (Wiggans and Vanraden, 1991; Wolfinger, 1996). In fact, heterogeneity of residual variances, also known as heteroskedasticity, seems to be a relatively common phenomenon in animal production systems

(Cernicchiaro et al., 2013; Gonçalves et al., 2015) that can mislead inference if disregarded (Wiggans and Vanraden, 1991; Wolfinger, 1996).

Mixed models are particularly well-suited to handle correlated data (Littell et al., 2006). Yet, implementation of mixed models is not without challenges including convergence of the iterative estimation process, particularly when fitting non-linear mixed models. A common problem is that models either fail to converge or converge to sub-optimal solutions (i.e. local vs. global maxima). A grid search approach can assist the estimation process by providing initial parameter values over the likelihood surface and guiding the iterative process away from sub-optimal solutions and facilitating a more efficient search for optimal solutions (Kiernan et al., 2012).

The main objective of this paper is to demonstrate practical implementation of linear and non-linear mixed models methodology for dose-response relationships, accounting for correlated data structures and heterogeneous variances. Second, we illustrate techniques to facilitate computational implementation of these models.

MATERIALS AND METHODS

Data

We used the dataset previously presented by Gonçalves et al. (2015) on G:F of nursery pigs fed experimental diets consisting of increasing levels of Standardized Ileal Digestible (**SID**) Trp:Lys ratio. Briefly, data were collected under a randomized complete block design whereby 1,088 pigs arranged in pens of 24 to 27 pigs were blocked by average initial BW and randomly assigned to experimental diets (6 pens/diet) consisting of SID Trp:Lys ratios of 14.5, 16.5, 18.0, 19.5, 21.0, 22.5, and 24.5%. The response variable G:F in its observed scale ranged from 0.520

to 0.610 kg BW gain/kg feed intake. The small magnitude of the scale prompted us to use a multiplier of 1000 and re-express G:F as (kg BW gain/kg feed intake) × 1000 to ensure numerical stability in the estimation process, particularly for variance components. It is well described that when estimates are very small and close to internal tolerances of computational algorithms, convergence can be impaired (Kiernan et al., 2012). The raw data is presented in Fig. 2.1 as a SAS (Version 9.4, SAS Institute Inc., Cary NC) data step and includes BW blocks (i.e. "Block"), SID Trp:Lys treatment (i.e. "Trt"), pen identification (i.e. "PenID"), the response G:F expressed in kg BW gain/kg feed intake (i.e. "GF") multiplied by 1000 (i.e. "y"), which we used for analysis.

Base Mixed Model: specification and implementation

We started by fitting a "base" linear mixed model to 1) explore possible functional forms of the relationship between G:F and SID Trp:Lys ratios, 2) evaluate model assumptions, in particular homogeneity of residual variances, and 3) obtain preliminary estimates of variance components (i.e, block and residual variances) that could later be used as starting values in dose-response linear and non-linear mixed models. The base mixed model was specified as follows:

$$y_{ij} = \eta + \alpha_i + b_j + e_{ij} \tag{1}$$

where y_{ij} is the G:F expressed in kg BW/kg feed intake multiplied by 1000 associated with the experimental unit in block j assigned to SID Trp:Lys ratio i; η corresponds to an intercept whereas α_i represents the differential effect of Trp:Lys ratio i (treated as a categorical variable); in turn, b_j is the random effect of the j^{th} block with $b_j \sim N(0, \sigma_b^2)$, and e_{ij} is a random residual associated with the experimental unit in the j^{th} block that received the i^{th} SID Trp:Lys ratio whereby $e_{ij} \sim N(0, \sigma_e^2)$, and b_j and e_{ij} are assumed to be independent of each other.

The base mixed model was fitted with the GLIMMIX procedure of SAS software (Fig. 2.2) using its default estimation method restricted maximum likelihood (**REML**). The Kenward-Roger's procedure was used to estimate degrees of freedom and adjust estimated standard errors for bias correction (Littell et al., 2006). To assess model assumptions, we plotted studentized residual as a function of levels of the treatment factor (Fig. 2.4). All observations had values of studentized residuals comprised between [-3, 3], thereby indicating no evidence for any extreme observations. However, it was apparent from Fig. 2.4 that the amount of dispersion of studentized residuals around zero was quite uneven across treatments. More specifically, at 18% SID Trp:Lys ratio, residuals were tightly clustered around zero whereas for diets consisting of 14.5, or 24.5 SID Trp:Lys ratio, residuals seemed to have the greatest dispersion around zero, thereby questioning the assumption of a homogeneous residual variance in the base mixed model.

Specification of heterogeneous residual variances. To further evaluate potential heteroskedasticity, we expanded our base mixed model in Eq. (1) to accommodate heterogeneous residual variances such that $e_{ij} \sim N\left(0, \sigma^2_{e_k}\right)$ with subscripts indicating the k^{th} level of a variance group to which the ij^{th} observation corresponded to. We defined alternative variance groups consisting of 2, 3, 4, or 7 levels defined empirically from Fig. 2.4 as Treatment combinations having seemingly comparable residual dispersion. These variance groups are listed in Table 2.1 and are presented for illustration, realizing that this is not an exhaustive list. A commented SAS code is available as a supplementary file to illustrate how the variance groups were defined (Appendix A). The choice of model with the best fitting heterogeneous variance specification was based on Bayesian Information Criterion (BIC; Schwarz, 1978). Please refer to model selection section below for more details.

Dose-response estimation models: specification and implementation

Next, we considered 3 competing linear and non-linear dose-response mixed models, namely a quadratic polynomial model, a broken-line linear ascending model, and a broken-line quadratic ascending model. These competing models represent three commonly used functional forms of the relationship between nutrient requirement and the response G:F, based on the amino acid nutrition literature (Robbins et al., 2006; Pesti et al., 2009). The competing models were specified as follows:

Quadratic polynomial (**QP**):

$$y_{ij} = \beta_0 + \beta_{1,QP}X_i + \beta_{2,QP}X_i^2 + b_j + e_{ij} \qquad (2)$$

Broken-line linear ascending model (**BLL**):

$$y_{ij} = \varphi_{BLL} + \beta_{BLL} \times (\omega_{BLL} - X_i) + b_j + e_{ij} \qquad \text{for } X_i < \omega_{BLL} \text{ and,} \qquad (3)$$

$$y_{ij} = \varphi_{BLL} + b_j + e_{ij} \qquad \text{for } X_i \geq \omega_{BLL}\,.$$

Broken-line quadratic ascending model (**BLQ**):

$$y_{ij} = \varphi_{BLQ} + \beta_{1,BLQ} \times \left(\omega_{BLQ} - X_i\right) + \beta_{2,BLQ} \times \left(\omega_{BLQ} - X_i\right)^2 + b_j + e_{ij} \text{ for } X_i < \omega_{BLQ} \text{ and,}$$

(4)

$$y_{ij} = \varphi_{BLQ} + b_j + e_{ij} \qquad \text{for } X_i \geq \omega_{BLQ}.$$

where y_{ij} is the observed G:F expressed as kg BW gain/kg feed intake multiplied by 1000 associated with the pen randomly assigned to SID Trp:Lys ratio i within block j; X_i indicates the i^{th} known SID Trp:Lys ratio. For all models, b_j is the random effect of the j^{th} block with

$b_j \sim N(0, \sigma_b^2)$, and e_{ij} is a random error associated with the experimental unit in the j^{th} block that received the i^{th} SID Trp:Lys ratio whereby $e_{ij} \sim N\left(0, \sigma_{e_k}^2\right)$, and the k^{th} levels were defined by the best-fitting heteroskedastic base model. Also, b_j and e_{ij} are assumed to be independent of each other. For the QP model, β_0 is the intercept, $\beta_{1,QP}$ and $\beta_{2,QP}$ are the corresponding unknown linear and quadratic regression coefficients as a function of X_i. For the non-linear models, φ_{BLL} and φ_{BLQ} indicate the unknown maximum response (i.e. plateau) under BLL and BLQ models, respectively; in turn, β_{BLL}, $\beta_{1,BLQ}$ and $\beta_{2,BLQ,}$ are the corresponding unknown regression coefficients as a function of X_i for values of X_i smaller than the plateau. Finally, ω_{BLL} and ω_{BLQ} are the unknown minimum levels of SID Trp:Lys to reach the plateau under the BLL and BLQ models, respectively. We note that our implementation of non-linear mixed models takes into consideration the standard recommendation of a hierarchy principle of model building (Kutner et al., 2005).

The SAS software was used to implement all dose-response models. The GLIMMIX procedure was used to fit the QP model whereas NLMIXED procedure was used for non-linear mixed models (i.e. BLL and BLQ). Under both procedures, the method of estimation was specified to be maximum likelihood (**ML**) to enable comparison of competing models. Figures 2.6 to 2.8 show code used to implement the dose-response models using SAS software, in particular QP model (Fig. 2.6), BLL model (Fig. 2.7), and BLQ model (Fig. 2.8).

We note that, for the dataset used in this article, the final dose-response models used for inference needed to account for heterogeneous residual variances, as dictated by preliminary data exploration using the base mixed model (see previous section). We further illustrate the impact that disregarding heterogeneity of variances when modeling can have on the point estimates of the dose-response breakpoint as well as on its inference. Specifically, we compare estimates of nutrient requirements and respective inference based on the best fitting dose-response model with and without specification of heterogeneous residual variances.

Improving computational performance of non-linear mixed models. To facilitate the iterative estimation process of fitting non-linear mixed models, we provided initial values for model key parameters. Initial values for parameters φ_{BLL}, φ_{BLQ}, β_{BLL}, $\beta_{1,BLQ}$, $\beta_{2,BLQ,}$ ω_{BLL}, and ω_{BLQ} were approximated from empirical scatterplots of the data and also from fitted values from the base mixed model. In turn, initial values for σ_b^2, and $\sigma_{e_k}^2$ were elicited using estimates from the base mixed model. For each parameter, at least 3 initial values were used to conduct a grid search so that for the BLL model, a total of 3^5 sets of starting values were evaluated, whereas 3^6 set of starting values were considered for the BLQ model.

Estimating confidence intervals. For the non-linear mixed models, the estimated mean breakpoint and its asymptotic confidence interval (i.e. 95%) follow from ML estimation of model parameters; these values can be obtained directly from the SAS NLMIXED output. For the QP model, the estimated mean dose level at which the maximum response occurred was computed as follows. We first obtained the first derivative of the regression equation with respect to the predictor variable X (Pesti et al., 2009), then equate the derivative to zero and solve for X, thus obtaining the value of X that maximizes the average response; this value was derived to be $-\frac{\beta_1}{2\beta_2}$. A (1-$\alpha$)% confidence interval (CI) for the estimated mean dose level using the QP model can be approximated using a graphical approach (Lavagnini and Magno, 2007). Briefly, the fitted QP model is plotted over the dose levels with the desired estimated CI (i.e. 95% CI). Then, the maximum estimated response is projected on the y-axis using a horizontal line. The points of intersection of this horizontal line with the CI boundaries on the predicted line are then projected onto x-axis as confidence intervals estimators of the optimum dose level.

Model comparison. Competing mixed models used to evaluate the functional form of the dose-response relationship, namely QP, BLL, and BLQ were compared based on model fit using BIC (Schwarz, 1978). When comparing fit between models fitted using of GLIMMIX and NLMIXED, it is important to consider default software specifications to ensure that the underlying methods of estimation are aligned so as to enable meaningful comparisons of information criteria. The default specification for method of estimation in GLIMMIX is REML whereas in NLMIXED, it is ML; therefore, one should explicitly specify ML-based inference in GLIMMIX by indicating method=MSPL (Fig. 2.6) in SAS code.

RESULTS AND DISCUSSION

Base Mixed Model: Implementation and Inference

After fitting a base mixed model to the response G:F assuming a homogeneous residual variance (as in Eq. 1), we assessed model assumptions using a plot of studentized residual over levels of SID Trp:Lys ratios (Fig. 2.4). The residual plot indicated no evidence for outliers, as all studentized residuals were within ± 2.5. However, studentized residuals seemed to be more dispersed around zero for some SID Trp:Lys ratio treatments (i.e. 14.5 and 24.5%) than for others (i.e. 16.5 and 18.0%) thereby questioning the standard assumption of a homogeneous residual variance across all treatments. Instead, the plot of studentized residuals (Fig. 2.4) suggested that the residual variance might differ amongst treatments. To address this departure from model assumptions, we expanded our base mixed model to explicitly accommodate heterogeneous residual variances. Table 2.1 shows BIC statistics for model fit assessment for alternative base mixed models fitted either with a homogeneous residual variance (i.e. a common variance across SID Trp:Lys ratios) or with heterogeneous residual variances defined either on each SID Trp:Lys ratio (i.e. 7 variance groups) or on 2, 3 or 4 so-called "variance groups" consisting of combination of SID Trp:Lys ratio treatments. Base mixed models with heterogeneous residual variances for 2, 3 or 4 groups of treatments fitted the data better than the base mixed model with a common residual variance, as indicated by smaller values of the BIC statistic (Table 2.1). Thus, we selected for further modelling steps the most parsimonious model, that is the base mixed model with fewest variance components that best fits the data, in this case the model with heterogeneous residual variances for 2 groups of SID Trp:Lys ratios consisting of (16.5, 18.0%) vs. (14.5, 19.5, 21.0, 22.5, 24.5%). Indeed, a plot of studentized residuals obtained from fitting the selected base model with heterogeneous residual variances suggested a more even spread of residuals for all SID Trp:Lys ratio treatments. Figure 2.9 shows the estimated least square means of G:F for experimental diets consisting of increasing levels of SID Trp:Lys ratio allowing for heterogeneous residual variance across 2 groups, as specified based on the best fitting base mixed model.

Dose-response models

As previously indicated, our implementation of non-linear dose-response mixed models included elicitation of initial parameter values in order to facilitate the estimation process. Using parameter estimates from the base mixed model (Fig. 2.9), we specified initial values for the scaled plateau level (i.e. φ_{BLL} *or* φ_{BLQ}) at approximately 582 kg BW gain/kg feed intake whereas the slope β_{BLL} for the linear segment of the BLL model was approximated at 1950 [calculated using values from Fig. 2.9 as (0.582 – 0.543) × 1,000 ÷ (0.165 –0.145)]. It is noted that, the sign of the initial value provided for the slope β_{BLL} must be multiplied by (–1) to yield (–1950) given the model parameterization process implemented in SAS. For breakpoint parameters, namely ω_{BLL} or ω_{BLQ}, initial values were specified at 15.0, 16.0, and 17.0% SID Trp:Lys based on descriptive assessments of the data. Initial values for variance components were obtained from corresponding estimates from variance components from the fitted base mixed model and specified at 11 for BW block (σ_b^2), 56 for variance group 1 ($\sigma_{e_1}^2$) and 268 for variance group 2 ($\sigma_{e_2}^2$).

We also set up a grid search approach to facilitate model convergence. For a grid search, each model parameter was assigned an array of initial values selected with the domain of each parameter; this grid search approach can be particularly important for variance components. For the BLL model, we set an array of parameter values consisting of all combinations of φ_{BLL} = [578, 582, 586]; β_{BLL}= [–975, –1950, –3900]; ω_{BLL} = [0.15, 0.16, 0.17]; σ_b^2 = [6, 11, 22]; $\sigma_{e_1}^2$ = [28, 56, 112]; $\sigma_{e_2}^2$ = [134, 268, 536]. Eliciting an array of initial values for each parameter can be challenging. One possible approach may be to "half" or "double" the individual values specified to initiate the iterative estimation process, provided that all values in the grid fall within the bounds of the parameter space. Following a similar rationale, we specified initial values for parameters of the BLQ model. The initial values for $\beta_{2,BLQ}$ were informed based on the β_2 coefficient from the QP model, whereby $\beta_{2,BLQ}$ = [–4685, –9369, –18738]. After fitting the BLL and BLQ models, convergence was reached and all parameter estimates were found to be within the plausible range specified by the grid search and away from the extreme values of each grid search. It is noted that the array of initial values that initiates the grid search can be lengthened and/or tuned using a trial-and-error approach to ensure that, ultimately, the point estimate of the parameter of interest falls within the specified array grid. Such tuning is important to minimize the chances of convergence to a local maxima. As such, more than 3 initial values may be needed for some parameters to enhance the search through the likelihood surface although this increases use of computational resources.

After model fitting, the heteroskedastic versions of QP, BLL, and BLQ models had BIC of 353.7, 343.4, and 345.2, respectively, indicating that the BLL model was the better fitting one, followed closely by the BLQ model. Instead, the QP model showed the poorest fit of all. To characterize this assessment of relative fit, we present fitted equations and conclusions based on all competing dose-response mixed models. However, we note that for the purpose of reporting results in scientific publications it is often recommended that only the best fitting model, or alternatively, models with comparably better fit (i.e. lowest BIC values with differences smaller than 2 points) be used to draw conclusions, in order to avoid misleading readers using inference from models of poor data fit.

Figure 2.10 shows fitted regression lines based on all three competing dose-response mixed models overlaying a scatterplot of the data. Tthe estimated regression equations for these models are presented below:

QP predictive equation:

$$\text{G:F} = 0.1927 + 3.86 \times (\text{Trp:Lys}) - 9.37 \times (\text{Trp:Lys})^2$$

BLL predictive equation:

$$\text{G:F} = 0.5827 - 1.95 \times (0.165 - \text{Trp:Lys}), \text{ if SID Trp:Lys} < 16.5\%,$$

$$\text{G:F} = 0.5827 \qquad\qquad \text{if SID Trp:Lys} \geq 16.5\%.$$

BLQ predictive equation:

$$\text{G:F} = 0.5824 - 1.95 \times (0.16 - \text{Trp:Lys}) - 40 \times (0.16 - \text{Trp:Lys})^2,$$

$$\text{if SID Trp:Lys} < 16.0\%,$$

G:F = 0.5824, if SID Trp:Lys ≥ 16.0%.

As previously mentioned, the QP model showed the poorest data fit. This may be explained by looking at Fig. 2.10, in which it is apparent that the estimated regression line under the QP model was likely to underestimate G:F at 16.5% Trp:Lys ratio while seemingly overestimating it at 14.5% and 19.5% Trp:Lys ratios. Further, the functional form imposed by the QP model on these data forced a maximum predicted G:F at approximately 21% Trp:Lys ratio, followed by a decrease in predicted G:F above this nutrient level, which does not seem consistent with the data at the highest levels of Trp:Lys ratio considered in this study.

The best fitting model for this dataset, that is the BLL model, estimated the SID Trp:Lys ratio breakpoint at 16.5% (95% CI: [16.1, 17.0%]), whereas the closely-ranked second best-fitting model, that is the BLQ model, estimated the breakpoint at 16.0% (95% CI: [15.5, 16.6%]). These BLL- and BLQ-based point estimates seemed to be very close in magnitude; however, a broader 95% CI indicated larger inferential uncertainty under the BLQ model. Figure 2.11 illustrates the fitted BLL model, including the breakpoint estimate and its corresponding 95% confidence interval. The apparently close relative data fit of the BLL and BLQ models (i.e. BIC difference < 2 points) may be explained by the very limited data available along the range of X prior to plateauing of the relationship between G:F and SID Trp:Lys ratio. That is, data on G:F along the non-plateaued section of either piece-wise regression were available only at two values of the explanatory variable, namely 14.5% and 16.5% SID Trp:Lys ratio. The design of future studies may take this limitation into consideration to ensure that more dose levels of the X variable are available on both sides of the expected breakpoint value.

It is important to note that dose-response models should be fit and plotted at the level of the experimental unit so that the underlying variability in the data is properly accounted for.

Instead, dose-response models have been fit to the average values of each treatment for a given response variable (Robbins et al., 2006). This practice may appear to enhance model fit, though at the price of disregarding the underlying experimental error. As a result, inferential precision would be expected to artificially deflate with subsequent undesirable consequences on Type I error and repeatability of results.

Mixed model selection

Commonly used fit statistics in the context of mixed models include BIC, amongst others such as AIC (Milliken and Johnson, 2009). For these information criteria, smaller values are indicative of models with better fit to the data. In this study, we used a ML-based BIC as our fit statistic of choice to select between competing dose-response mixed models. The calculation of BIC yields fit criteria that are slightly more conservative than that of AIC, as BIC tends to put a greater penalty on number of model parameters, thus favoring more parsimonious models (Schwarz, 1978). The BIC value is only meaningful in relative terms, that is, when comparing two or more models applied to the same data set (Milliken and Johnson, 2009). The BIC can take any number on the real line from minus infinite to positive infinite, whereby a smaller BIC value indicates a better fitting model; more specifically, models that differ in their BIC values by at least 2 points are considered to have meaningful differences in their data fit (Raftery, 1996). The best fitting model, that is the model with lowest BIC, would normally be selected for estimation and inference.

Another model selection criterion commonly used to assess goodness of fit in the context of fixed-effects regression models is the coefficient of determination R^2. As intuitive an interpretation of goodness of fit as it might have (i.e. proportion of variability explained by an effect), R^2 is often misused in the context of mixed models (Littell et al., 2006). More specifically, for models that have more than one variance component (i.e. mixed models), R^2 is not uniquely defined (Kvalseth, 1985) and the variety of alternative specifications of $R^2$are not equivalent (Kvalseth, 1985), thus leading to non-trivial pitfalls during interpretation of data analysis (Kvalseth, 1985; Willet and Singer, 1988).

Homogeneous vs. heterogeneous residual variances

In the context of linear models, it is well described that the violation of the homogeneous residual variance assumption poses a considerable risk to inference, more so than violations of the normality assumption on residuals (Milliken and Johnson, 2009). Indeed, incorrectly assuming homogeneous residual variances due to unchecked residual assumptions can impair inferential efficiency when treatment means (or functions thereof) are of interest (Wiggans and VanRaden 1991; Wolfinger, 1996). Indeed, when homogeneous residual variances were incorrectly assumed in this study, the breadth of the BLL-based 95% confidence interval on the estimated SID Trp:Lys ratio breakpoint for maximum G:F was increased by approximately 44% relative to that of the BLL model with heterogeneous residual variances (95% CI: [15.8, 17.4%]) vs. [16.1, 17.0%]). This example of inappropriately calibrated inferential uncertainty illustrates typical inferential inefficiencies associated with erroneous assumptions in the context of dose-response relationship mixed models.

Hierarchy principle in polynomial models

On building of polynomial regression models, abiding by the hierarchy principle is generally recommended to ensure proper model formulation (Peixoto, 1987). The hierarchy principle states that if a higher order polynomial term is retained in the model, then the related lower order terms should also be kept in the model whether or not the coefficients for these lower-order terms are significant (Kutner et al., 2005). This recommendation has not always been heeded in the animal sciences, in particular in the context of estimation of nutritional requirements. For example, broken-line quadratic models lacking a first-order linear term have been used (Robbins et al., 2006; Pesti et al., 2009) counter to the hierarchy model building principle. Only hierarchically well-formulated models are invariant under linear transformation; otherwise, significance tests on regression coefficients can yield artefactual results (Peixoto, 1987). Artefactual results may not be obvious for any given data applications, thus the importance of taking this principle into careful consideration during model building.

Troubleshooting non-linear mixed models

Several useful tips on troubleshooting implementation of non-linear models can be found in the literature (Kiernan et al., 2012). Specific to non-linear mixed models such as BLL and BLQ, some of the most common issues include convergence of the iterative estimation process and estimation failures.

Use of a grid search to facilitate estimation. One of the biggest challenges in non-linear mixed models is to reach convergence of the iterative estimation process. Even when convergence is attained, there is no guarantee that it be on a global maxima (optimal solution) as opposed to a local maxima (sub-optimal solution). The use of a grid search approach can assist in this process by providing plausible initial parameter values over the likelihood surface, thus efficiently guiding the iterative parameter estimation process towards optimal solutions and away from sub-optimal ones (Kiernan et al., 2012). Specification of initial values is particularly important for variance components as well as for non-linear parameters, such as the breakpoint parameter in the piece-wise regression. Initial values can be specified in NLMIXED by using the PARMS statement (Fig. 2.7 and 2.8). The actual initial values inputted into NLMIXED can be adjusted based on preliminary analysis and also to assess sensitivity of the final inference to starting values. For the datasets that we have used, we find that specification of at least three initial values for each parameter facilitates a grid search over the likelihood surface. The START option in the NLMIXED procedure will call for an output which shows exactly which initial value the procedure chose for each of the parameters. A greater number of initial values in the grid search will enhance the search through the likelihood surface. However, one should also consider that grid searches can increase use of computational resources substantially.

Other troubleshooting techniques. Besides using a grid search, failures to converge can be minimized by: 1) specifying reasonable models that reflect the functional form of the dose-response appropriate for a given dataset, whereby “reasonable models” can be informed from preliminary analysis or fitting a base model that does not assume any functional form in the relationship between response and treatment, as in our case; 2) increasing the number of maximum iterations before which the estimation process will abort if convergence has not been reached (i.e. in the NLMIXED procedure, this can be specified with the option MAXITER); 3) careful tuning of the convergence criterion (i.e. in NLMIXED, this can be done by modifying the relative gradient criterion using the option GCONV; in particular, GCONV=0 will force the procedure to continue to a greater number of iterations until it meets the relative change in the next convergence criterion is met, which is the function value criterion (FCONV)), as suggested by Kiernan et al. (2012); 4) using the BOUND option to define reasonable boundaries for selected parameters, in particular the breakpoint parameter, which should not lie outside of the range of dose levels considered in a given study; and 5) last but not least, evaluating the programing code for bugs and coding errors, as with implementation of any statistical software.

SUMMARY

This paper presents concepts underlying the implementation of linear and non-linear mixed models for dose-response relationships accounting for correlated data structure and heterogeneous residual variances. We illustrated the inferential implications of properly checking and addressing model assumptions, particularly pertaining to the assumption of a common residual variance, as well as the utilization of the hierarchy principle for model building. Additionally, we explained the importance of using proper fit statistics for model selection in the context of mixed models and the pitfalls of using the conventional fixed-effect based coefficient of determination (R^2). We further demonstrated practical approaches to facilitate some of the computational challenges associated with fitting of non-linear mixed models, including use of a grid search to facilitate convergence of the iterative estimation process.

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TABLES AND FIGURES

```
data GF;
     input Block Trt PenID$ y  varGrp$;

     datalines;
1    0.145     184  562  Var2
1    0.165     187  593  Var1
1    0.180     182  579  Var1
1    0.195     189  610  Var2
1    0.210     186  583  Var2
1    0.225     188  604  Var2
1    0.245     176  572  Var2
2    0.145     145  549  Var2
2    0.165     185  574  Var1
2    0.180     162  592  Var1
2    0.195     164  586  Var2
2    0.210     183  602  Var2
2    0.225     181  593  Var2
2    0.245     180  594  Var2
3    0.145     168  520  Var2
3    0.165     165  587  Var1
3    0.180     166  590  Var1
3    0.195     147  582  Var2
3    0.210     167  606  Var2
3    0.225     174  588  Var2
3    0.245     173  578  Var2
4    0.145     154  563  Var2
4    0.165     170  588  Var1
4    0.180     169  577  Var1
4    0.195     175  555  Var2
4    0.210     150  566  Var2
4    0.225     146  563  Var2
4    0.245     148  551  Var2
5    0.145     172  529  Var2
5    0.165     149  571  Var1
5    0.180     152  575  Var1
5    0.195     161  568  Var2
5    0.210     159  593  Var2
5    0.225     171  573  Var2
5    0.245     153  604  Var2
6    0.145     156  535  Var2
6    0.165     151  579  Var1
6    0.180     163  581  Var1
6    0.195     155  568  Var2
6    0.210     157  591  Var2
6    0.225     160  585  Var2
```

```
6    0.245     158  580  Var2
;
```

Figure 2.1. Example data set from Gonçalves et al. (2015), which evaluated the effects of SID Trp:Lys ratio on nursery pig performance with two residual variance groups (Var1 for [16.5, 18.0% SID Trp:Lys] and [14.5, 19.5, 21.0, 22.5, 24.5% SID Trp:Lys] for Var2).

```
proc glimmix data=GF plots=studentpanel;
     class block trt;
     model y = trt / ddfm=kr;
     random intercept / subject = block;
     output out=igausout pred=p student=std;
     nloptions tech=nrridg;
     lsmeans trt / cl plot=meanplot(cl join);
run;
```

Figure 2.2. Base model assuming homogeneous residual variance.

```
proc glimmix data=GF plots=studentpanel;
     class block trt varGrp;
     model y = trt / ddfm=kr;
     random intercept / subject = block;
     random _residual_ / group = varGrp;
     output out=igausout pred=p student=std;
     nloptions tech=nrridg;
     lsmeans trt / cl plot=meanplot(cl join);
run;
```

Figure 2.3. Base model allowing for heterogeneous residual variances.

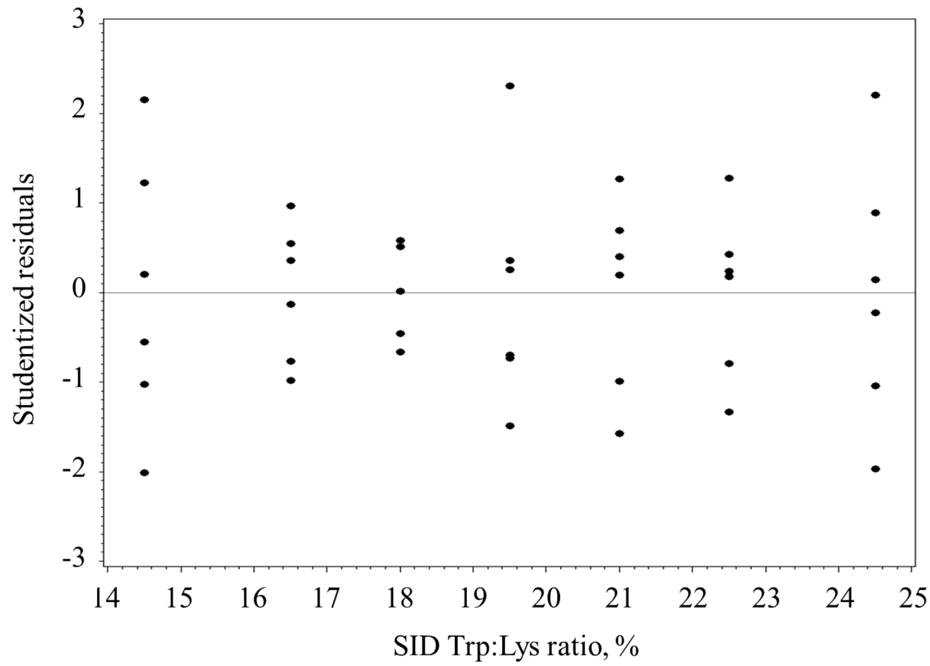

Figure 2.4. Studentized residuals of G:F by treatment levels obtained from a base model fitted with a common residual variance.

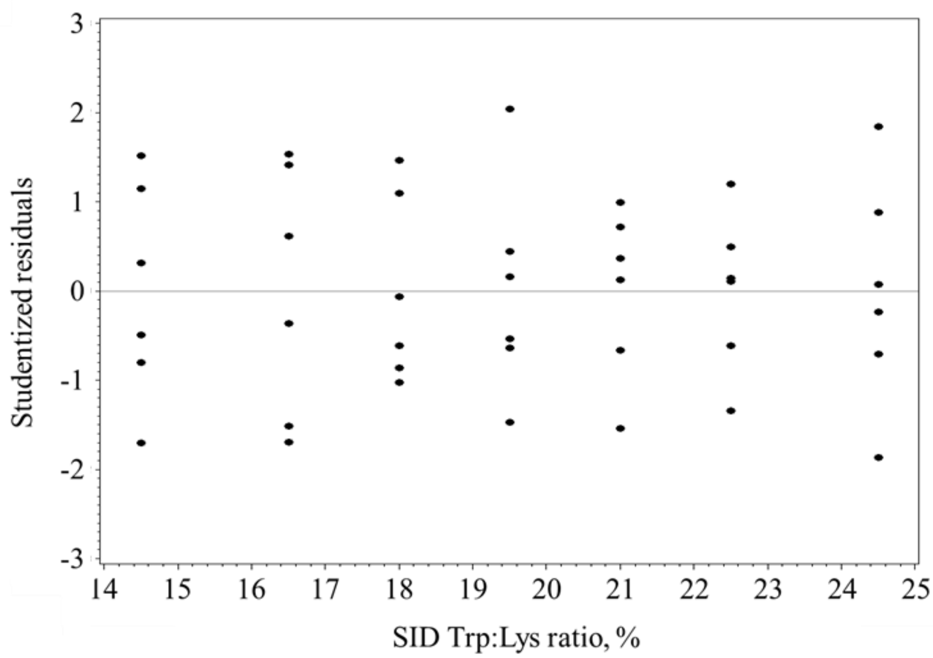

Figure 2.5. Studentized residuals of G:F by treatment levels obtained from a base model allowing for heterogeneous of residual variances with two group variances ([16.5, 18.0% SID Trp:Lys] vs. [14.5, 19.5, 21.0, 22.5, 24.5% SID Trp:Lys]).

Table 2.1. Bayesian Information Criterion (BIC) fit statistics for base models fitted assuming a homogeneous (i.e. common) variance or heterogeneous variances for 2, 3, 4 or 7 groups

Variance component	BIC	Group constituency
Common variance	303.6	No groups
2 variance groups – combination I	299.6	(16.5, 18.0) vs. (14.5, 19.5, 21.0, 22.5, 24.5)
2 variance groups – combination II	300.6	(14.5, 19.5, 24.5) vs. (16.5, 18.0, 21.0, 22.5)
3 variance groups	300.3	(14.5, 19.5, 24.5) vs. (21.0, 22.5) vs. (16.5, 18.0)
4 variance groups	301.1	(14.5, 19.5, 24.5) vs. (21.0, 22.5) vs. (16.5) vs. (18.0)
7 variance groups	306.4	One group per treatment

```
proc glimmix data=GF method=MSPL ;
     class  block varGrp;
     model y = trt trt*trt / solution ;
     random intercept / subject=block;
     random _residual_ / group=varGrp;
     output out=igausout pred=p resid=r;
     nloptions tech=nrridg;
run;
```

Figure 2.6. Quadratic polynomial mixed model with heterogeneous variance.

```
proc nlmixed data=GF maxiter=1000 gconv=0  start;
     bounds .145<R_BLL<.245;
     parms L_BLL= 578 582 586 U_L= -975 -1950 -3900 R_BLL= 0.15 0.16 0.17
     Block_Var= 6 11 22 vareVar1= 28 56 112 vareVar2= 134 268 536;
     z=(trt<R_BLL)*(R_BLL-trt);  * Characterize the model as non-linear;
     s2e = (varGrp="Var1") * vareVar1 + (varGrp="Var2") * vareVar2;
     model y ~ normal(L_BLL + U_L *(z) + beff, s2e);
     random beff ~ normal(0,Block_Var) subject=block out=blups;
     predict L_BLL + U_L*(z) out=ppp;
run;
```

Figure 2.7. Broken-line linear mixed model with heterogeneous variance.

```
proc nlmixed data=GF maxiter=1000 gconv=0  start;
     bounds .145<R_BLQ<.245;
     parms L_BLQ= 578 582 586  U_Q1= -975 -1950 -3900
     U_Q2= -4685 -9369 -18738 -40000 -80000 R_BLQ=0.15 0.16 0.17
     Block_Var= 6 11 22 vareVar1= 28 56 112 vareVar2= 134 268 536;
     z=(trt<R_BLQ)*(R_BLQ-trt);
     s2e = (varGrp="Var1") * vareVar1 + (varGrp="Var2") * vareVar2;
     model y ~ normal(L_BLQ + U_Q1*z + U_Q2*(z*z) + beff, s2e);
     random beff ~ normal(0,Block_Var) subject=block out=blups;
     predict L_BLQ + U_Q1*z + U_Q2*(z*z) out=ppp;
run;
```

Figure 2.8. Broken-line quadratic mixed model with heterogeneous variance.

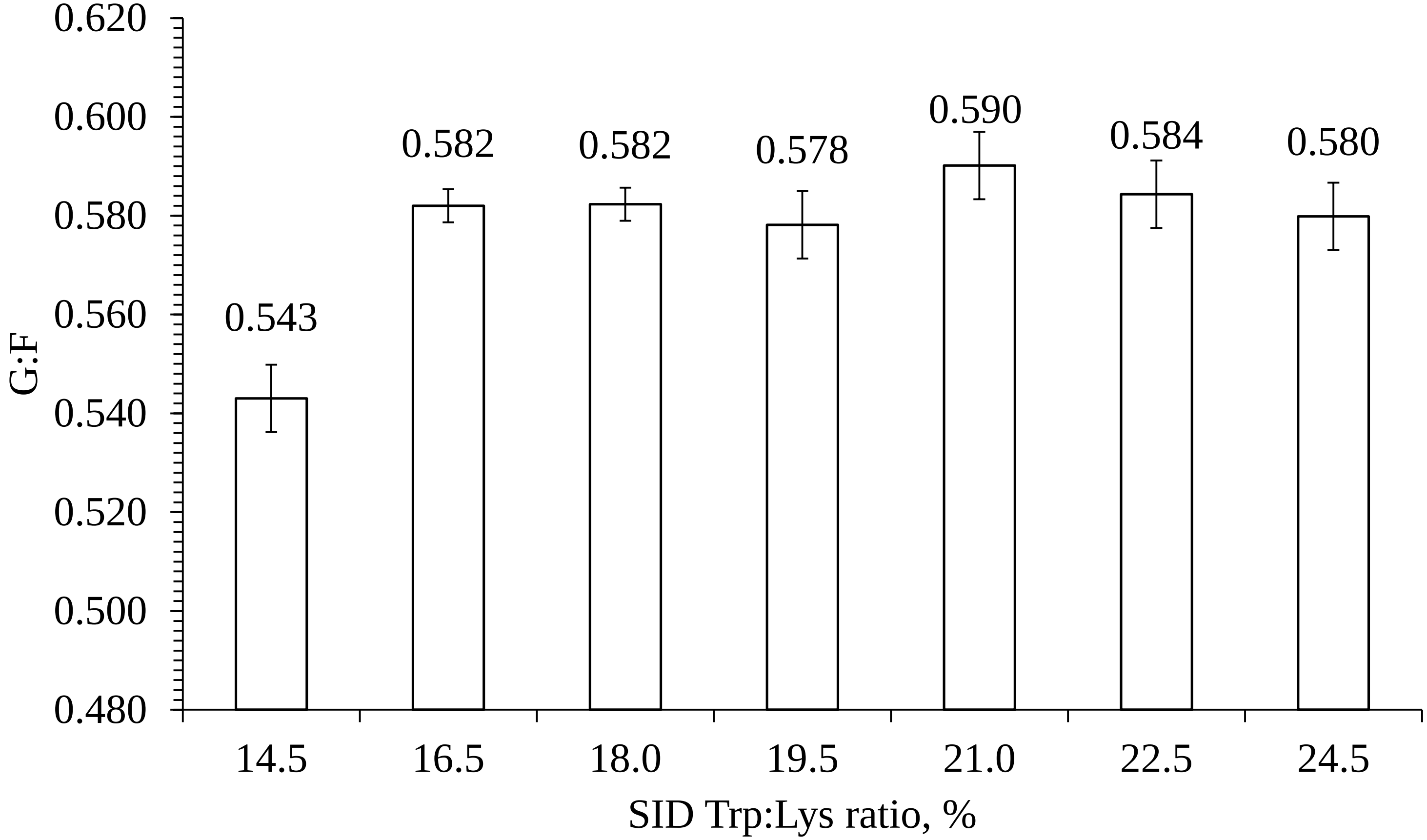

Figure 2.9. Estimated least square means of G:F ± SEM for experimental diets consisting of increasing levels of SID Trp:Lys ratio using the selected base mixed model with heterogeneous residual variances.

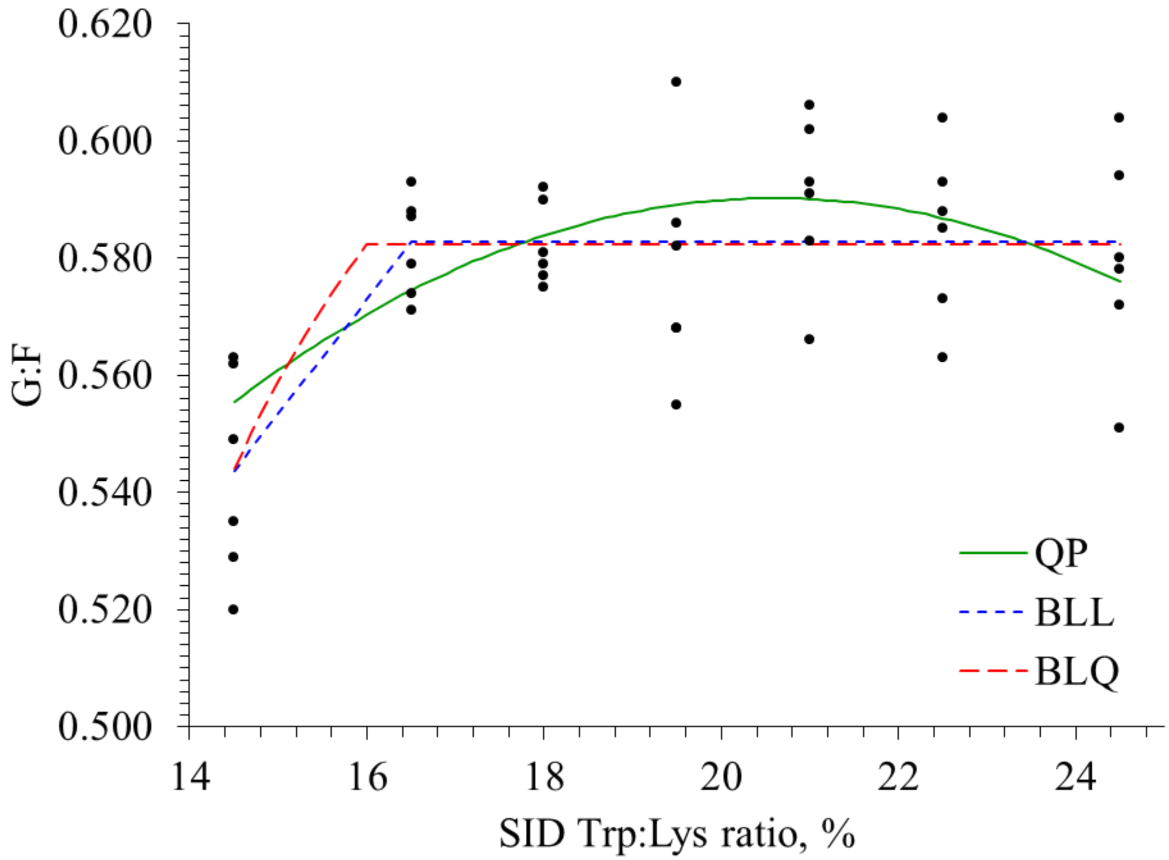

Figure 2.10. Fitted regression lines for competing dose-response linear and non-linear mixed models accounting for heterogeneous residual variances, including quadratic polynomial (QP) model, broken-line linear (BLL) ascending model and broken-line quadratic (BLQ).

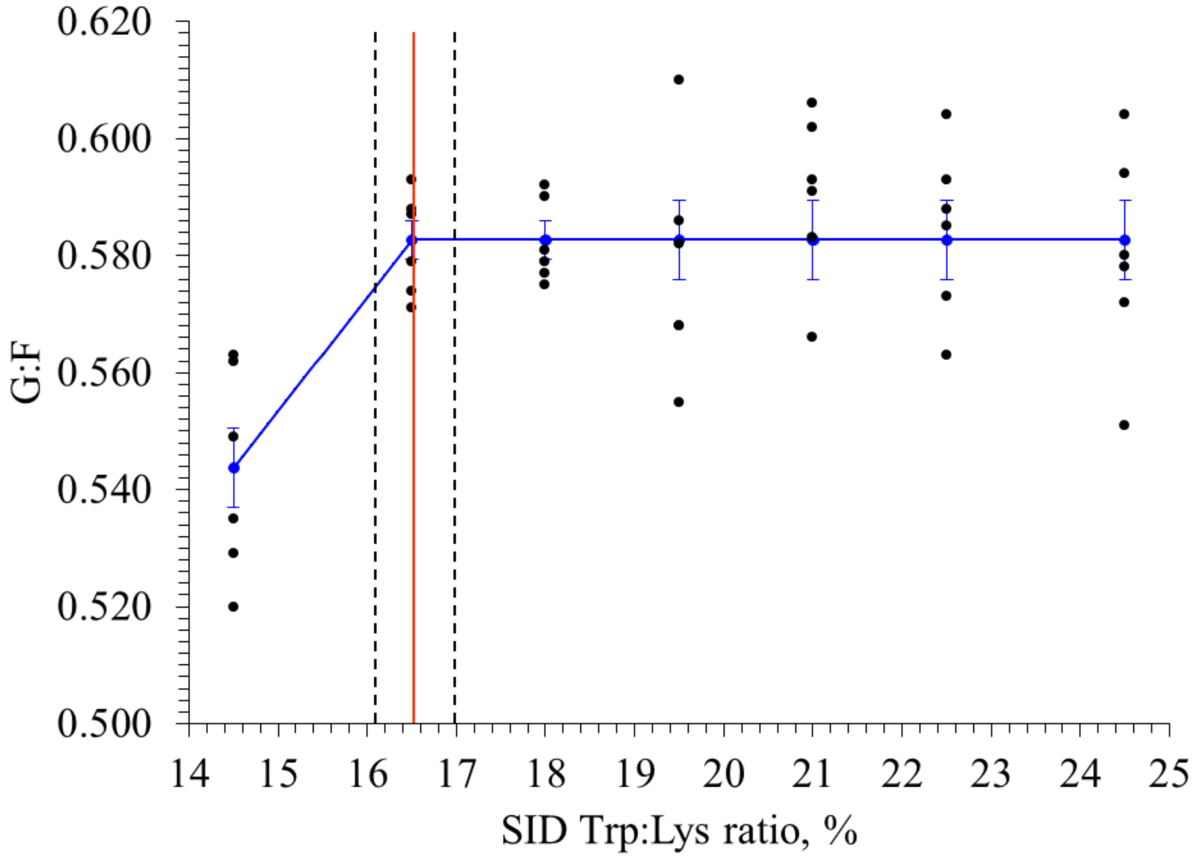

Figure 2.11. Non-linear broken-line linear ascending mixed model for G:F accounting for heterogeneous residual variances, including mean predictions (ascending and horizontal lines), 95% confidence interval on the mean (whiskers), and estimated SID Trp:Lys breakpoint (vertical line at 16.5% SID Trp:Lys) with corresponding 95% confidence interval (vertical dashed lines; 16.1, 17.0%).

Chapter 3 - Validating a dietary approach to determine the AA:Lys ratio for pigs [6,7]

M. A. D. Gonçalves*, M. D. Tokach†[8], S. S. Dritz*, K.J. Touchette‡, J. M. DeRouchey†, J. C. Woodworth†, and R. D. Goodband†

*Department of Diagnostic Medicine/Pathobiology, College of Veterinary Medicine,
†Department of Animal Sciences and Industry, College of Agriculture, Kansas State University, Manhattan 66506-0201, KS and ‡ Ajinomoto Heartland Inc., Chicago, IL

[6] Contribution no. 14-414-J from the Kansas Agric. Exp. Stn., Manhattan, KS 66506-0210.
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[8] Corresponding author: mtokach@k-state.edu

ABSTRACT: The objective of the studies was to validate a dietary approach to determine the optimal SID AA:Lys ratio for pigs using Trp as a model. Four 21-d experiments were conducted in which pigs (PIC 337 × 1050) were fed corn-soybean meal-based diets with 30% corn dried distillers grains with solubles (DDGS). A total of 1,188, 1,232, 1,204, and 1,183 pigs with initial BW of 13.0 ± 0.2, 22.8 ± 0.6, 57.7 ± 1.1, and 87.4 ± 1.2 kg were used in Exp. 1, 2, 3, and 4, respectively. Each experiment had 11 pens per treatment with 24 to 28 pigs per pen. In Exp. 1, each pen housed the same number of barrows and gilts whereas in Exp. 2 to 4 only gilts were used. Dietary treatments were: (1) High CP, High Lys, and High Trp:Lys ratio (**HHH**); (2) Low CP, High Lys, and High Trp:Lys ratio (**LHH**); (3) Low CP, Low Lys, and High Trp:Lys ratio (**LLH**); and (4) Low CP, Low Lys, and Low Trp:Lys ratio (**LLL**). The SID Trp concentrations used were 14.5 vs. 20% of Lys, CP was at least 3 percentage units different, and SID Lys levels were 0.01 percentage unit above the estimated requirement at the expected initial BW and 0.10 or 0.05 percentage units below requirement at the expected final BW of the Exp. 1 (nursery) and Exp. 2, 3, and 4 (finishing), respectively. In Exp. 1, decreasing CP (HHH vs. LHH) did not influence ADG but reduced ($P < 0.05$) G:F of pigs. Decreasing Lys (LHH vs. LLH) and decreasing the SID Trp:Lys ratio (LLH vs. LLL) reduced ($P < 0.05$) ADG and G:F. In Exp. 2, decreasing CP did not affect ADG but decreased ($P < 0.05$) G:F of gilts. Decreasing Lys and the SID Trp:Lys ratio decreased ($P < 0.05$) both ADG and G:F. In Exp. 3, decreasing CP or Lys did not influence ADG or G:F of gilts. Decreasing the SID Trp:Lys ratio reduced ($P < 0.05$) ADG and G:F. In Exp. 4, decreasing CP did not influence ADG but decreased G:F ($P < 0.05$) of gilts. Decreasing Lys had no effect on performance, but decreasing the SID Trp:Lys ratio reduced ($P < 0.05$) ADG and G:F. In conclusion, low-CP diets formulated 0.10 and 0.05 percentage units below the SID Lys requirement at the end of the experiment's weight range appear to ensure pigs are below their Lys requirement when determining the optimal SID Trp:Lys ratio for 13- to 24-

kg pigs and 23- to 37-kg gilts, respectively. For gilts heavier than 37 kg, formulating diets at 0.05 percentage units below the SID Lys requirement at the end of the experiment's weight range may limit the ability to provide statistical evidence that gilts are under their Lys requirement.

Key words: amino acid ratio, growth, lysine, pigs, tryptophan

INTRODUCTION

Low-CP, AA-fortified diets are commonly fed in the swine industry due to the increased availability and decreased cost of feed-grade AA and to reduce the environmental impact of excess N excretion. The literature suggests that pigs fed low-CP diets have similar performance to pigs fed high-CP diets as long as essential AA are supplemented to meet pigs' requirements (Kerr et al., 1995, Bellego et al., 2001). Lysine is the first limiting AA in most of the cereal grains used in swine diets (Lewis, 2000). The pig's Lys requirement depends on the rate of lean accretion, environment, feed intake, and health status (Williams et. al, 1997; NRC, 2012). Because the Lys requirement when reported as a percentage of the diet decreases as BW increases, if the experimental diet is not limiting in Lys at the end of the experiment's BW range, the ratio of other AA to Lys will be underestimated (Susenbeth and Lucanus, 2005). Therefore, Lys must be the second limiting AA throughout the experiment. Thus, the objective of these studies was to validate a dietary approach to establishing basal diets for determining the optimal standardized ileal digestible (**SID**) AA:Lys ratio for pigs using Trp as a model. The hypotheses are: 1) pigs fed low crude protein diets with adequate Lys and Trp do not reduce growth performance, 2) low Lys diets formulated at a level that is deficient for the pigs will reduce growth performance, and 3) pigs fed 14.5% Trp:Lys will have poorer growth performance compared to pigs fed 20.0% SID Trp:Lys.

MATERIALS AND METHODS

General

The Kansas State University Institutional Animal Care and Use Committee approved the protocol used in this experiment. The studies were conducted at two commercial research barns in southwestern Minnesota.

The nursery barn in which Exp. 1 was conducted was totally enclosed, environmentally controlled, and mechanically ventilated. Each pen (3.7 × 2.3 m) was equipped with a 6-hole stainless steel dry self-feeder (SDI Industries, Alexandria, SD) and a pan waterer.

The finishing barn used for Exp. 2, 3, and 4 was naturally ventilated and double-curtain-sided. Each pen (5.5 × 3.0 m) was equipped with a 4-hole stainless steel dry self-feeder (Thorp Equipment, Thorp, WI) and a cup waterer.

Both barns had completely slatted flooring and deep pits for manure storage. Each facility was equipped with a computerized feeding system (FeedPro; Feedlogic Corp., Willmar, MN) that delivered and recorded daily feed additions and diets as specified. Pigs had ad libitum access to feed and water.

Animals and diets

Four 21-d growth experiments were conducted with two groups of pigs. Exp. 1 was conducted with a group of nursery pigs and Exp. 2, 3, and 4 were conducted with a single group of finishing pigs. A total of 1,188, 1,232, 1,204, and 1,183 pigs (PIC 337 × 1050; Hendersonville, TN) with initial BW of 13.0 ± 0.2, 22.8 ± 0.6, 57.7 ± 1.1, and 87.4 ± 1.2 kg and final BW of 24.0 ± 0.3, 36.4 ± 0.8, 73.6 ± 1.2, and 107.8 ± 1.2 were used in Exp. 1, 2, 3, and 4, respectively. The reduction in the total number of pigs for Exp. 2 to 4 was due to mortality or removal of unhealthy pigs. Each experiment had 11 pens per treatment with 24 to 28 pigs per pen. In Exp. 1, each pen housed 14 gilts and 13 barrows. Only gilts were used in Exp. 2, 3, and 4. Dietary treatments (Tables 3.1, 3.2, 3.3, and 3.4) were: (1) High CP, High Lys, and High Trp:Lys ratio (**HHH**); (2) Low CP, High Lys, and High Trp:Lys ratio (**LHH**); (3) Low CP, Low Lys, and High Trp:Lys ratio (**LLH**); and (4) Low CP, Low Lys, and Low Trp:Lys ratio (**LLL**). Corn-soybean meal-based diets with 30% DDGS were used with different SID Trp:Lys ratios (14.5% vs. 20%), CP (at least 3 percentage units difference), and SID Lys levels (0.01 percentage unit above requirement at the expected initial BW and 0.10 or 0.05 percentage units below requirement at the expected final BW of the nursery and finishing, respectively). Lysine requirements were estimated using the NRC (2012) model for mixed gender pens of pigs for the nursery phase and for gilts only for the finishing phase. Diets were balanced to have the same NE and Ca to standardized total tract digestible (**STTD**) P ratio. Phytase was included in all diets at the same level, with release considered to be 0.12% for P (STTD basis) and 0.10% for Ca. No amino acids releases were considered for phytase.

Five representative samples of corn, soybean meal, and DDGS were collected each week for 5 wk and analyzed in duplicate for total Trp (method 13904:2005; ISO, 2005), other AA (method 994.12; AOAC Int., 2012), and CP (method 990.03; AOAC Int., 2012) by Ajinomoto Heartland Inc. (Chicago, IL), and values were used in diet formulation. Other nutrients and SID AA digestibility coefficient values used for diet formulation were obtained from NRC (2012). Diets were formulated using the following minimum ratios relative to Lys: Thr:Lys (65, 65 and 68% in nursery, early and late finishing, respectively), Val:Lys (70%), Ile:Lys (55%), Met & Cys:Lys (60%), Leu:Lys (100%), and His:Lys (32%).

Pens of pigs were weighed and feed disappearance was measured at the beginning and at d 21 of each experiment to determine ADG, ADFI, and G:F. There was a 29-d interval between

Exp. 2 and 3 and 16-d interval between Exp. 3 and 4, in which pigs were fed a common diet that met or exceeded NRC (2012) nutrient requirements and contained 20% SID Trp:Lys.

Diet samples were taken from 6 feeders per dietary treatment 3 d after the beginning and 3 d before the end of each experiment and stored at -20°C, then total Trp, other AA, and CP analysis (conducted with the same methods described above) were conducted on composite samples from each dietary treatment by Ajinomoto Heartland Inc. Diet samples were also submitted to Ward Laboratories Inc. (Kearney, NE) for analysis of DM (method 935.29; AOAC Int., 2012), crude fiber [method 978.10; AOAC Int., 2012 for preparation and Ankom 2000 Fiber Analyzer (Ankom Technology, Fairport, NY)], ash (method 942.05; AOAC Int., 2012), crude fat [method 920.39 a; AOAC Int., 2012 for preparation and ANKOM XT20 Fat Analyzer (Ankom Technology, Fairport, NY)], Ca, and P [method 968.08 b; AOAC Int., 2012 for preparation using ICAP 6500 (ThermoElectron Corp., Waltham, MA)].

Statistical analysis

Data were analyzed using the MIXED procedure of SAS (SAS Version 9.3; SAS Institute, Inc., Cary, NC) as a randomized complete block design. Pen was the experimental unit for all data analysis. At d 0 of each experiment, pens of pigs were ranked from lightest to heaviest by initial average pen BW. Thus, initial average pen BW was the blocking factor, with a total of 11 blocks. Treatments were randomly assigned within each block. The model included terms for the fixed effects of dietary treatment with the block (initial average pen BW) as a random effect. In addition, for Exp. 3 and 4, dietary treatment from the previous experiment (2 and 3, respectively) was also considered a random effect. Treatment means were separated using pairwise comparisons of means performed using the DIFFS option from the LSMEANS statement of SAS. Results were considered significant at $P \leq 0.05$ and were considered marginally significant at $P > 0.05$ and $P \leq 0.10$.

RESULTS

Based on the chemical analysis before diet formulation, the soybean meal used in these studies had numerically lower CP (46.0 vs. 47.7%) and Lys (2.81 vs. 2.96%) than NRC values (2012; Table 3.5). Similarly, compared with NRC values (2012), corn had numerically lower CP (7.5 vs. 8.2%), and DDGS had lower Lys (0.81 vs. 0.90%) but higher Trp (0.23 vs. 0.20%). These differences in major ingredient compositions emphasize the importance of chemically analyzing major ingredients prior to diet formulation when conducting AA requirement experiments. The CV of ingredient analysis is in agreement with the variability shown by Cromwell et al., (1999). The nutrient and total AA analysis of the diets (Tables 3.6, 3.7, 3.8 and 3.9) were within the expected variation. The Ca levels in a few instances analyzed at higher than expected levels, but we do not believe the levels influenced the results of these experiments.

In Exp. 1, decreasing CP (Table 3.10; HHH vs. LHH) did not influence ($P > 0.05$) ADG and final BW of pigs but increased ($P < 0.05$) ADFI and, consequently, reduced G:F. Decreasing Lys (LHH vs. LLH) and decreasing the SID Trp:Lys ratio (LLH vs. LLL) reduced ($P < 0.05$) ADG, ADFI, G:F, and final BW.

In Exp. 2, decreasing CP did not influence ($P > 0.05$) ADG and final BW of gilts but increased ($P < 0.05$) ADFI and, consequently, reduced G:F. Decreasing Lys (LHH vs. LLH) reduced ($P < 0.05$) ADG, G:F, and final BW, with no change in ADFI. Decreasing the SID Trp:Lys ratio (LLH vs. LLL) reduced ($P < 0.05$) ADG, ADFI, G:F, and final BW.

In Exp. 3, decreasing CP (HHH vs. LHH) did not influence ($P > 0.05$) ADG, G:F, or final BW of gilts but increased ($P < 0.05$) ADFI. Decreasing Lys (LHH vs. LLH) had no effect ($P >$

0.05) on pig performance. Decreasing the SID Trp:Lys ratio (LLH vs. LLL) decreased (P < 0.05) ADG, ADFI, G:F, and final BW.

In Exp. 4, decreasing CP (HHH vs. LHH) did not influence (P > 0.05) ADG, ADFI or final BW of gilts but reduced (P < 0.05) G:F. Decreasing Lys (LHH vs. LLH) had no effect (P > 0.05) on pig performance. Decreasing the SID Trp:Lys ratio (LLH vs. LLL) reduced (P < 0.05) ADG, G:F, and final BW, but ADFI was not affected (P > 0.05).

DISCUSSION

Low-CP, AA-fortified diets did not influence ADG or final BW in any experiment compared with pigs fed the high-CP diets with increased soybean meal. These results are in agreement with previous research (Bellego et al., 2001, Bellego et al., 2002, Tous et al., 2014). Bellego et al. (2001) observed that pigs fed low-CP, AA-fortified diets had decreased urinary energy losses and reduced heat increment compared with pigs fed high-CP diets, which emphasizes the need to compare diets with different CP concentrations on a NE basis. Pigs fed low-CP, AA-fortified diets in Exp. 1, 2 and 3 had increased ADFI and, consequently, had increased NE intake compared with those fed high-CP diets. However, Bellego et al. (2002) observed decreased ADFI in pigs fed low-CP diets but no differences in NE intake under thermoneutral conditions. In addition, G:F was reduced in Exp. 1, 2 and 4 in pigs fed low-CP diets (HHH vs. LHH), which was contrary to Bellego et al. (2002) and Tous et al. (2014). However, Bellego et al. (2002) and Tous et al. (2014) reduced CP from 17.5 to 13.3% (4.8 and 5.9 Lys:CP ratio, respectively) and from 13 to 12% (5.7 and 6.2 Lys:CP ratio, respectively). Tuitoek et al. (1997) did not observe any changes in growth performance when CP level was reduced from 16.6 to 13% in diets for growing pigs and from 14.2 to 12.8% for finishing pigs. Kerr and Easter (1995) conducted two experiments where CP was reduced (from 16 to 12%; 5.0 and 6.7 Lys:CP ratio, respectively) and fortified with crystalline AA. In the first experiment, no differences were observed in ADG; however, in the second experiment, pigs fed low-CP, AA-fortified diets (Lys, Trp, and Thr) had reduced ADG compared with pigs fed high-CP diets. Because G:F was reduced in pigs fed low-CP diets in Exp. 1, 2, and 4, these findings may suggest that the NE used for corn was overestimated or that NE values used for soybean meal and added fat sources were underestimated which is supported by a recent study conducted by Sotak and Stein (2014) who observed a higher NE for soybean meal compared with that reported by NRC (2012).

The SID Lys concentrations used in diet formulation were 92, 95, 94, and 93% of SID Lys requirement estimates suggested by NRC (2012) at the end of the BW range for Exp. 1, 2, 3, and 4, respectively. Using diets with 92 and 95% of the estimated SID Lys requirement at the end of the BW range for 13- to 24-kg pigs and for 23- to 37-kg gilts was sufficient to statistically reduce growth performance (LHH vs. LLH); however, for 58- to 74- and 87- to 108-kg gilts, using diets with 94 and 93% of SID Lys requirement at the end of the BW range resulted in only a numerical reduction in ADG, G:F, and final BW between the LHH and LLH diets. Conversely, another approach would be to formulate SID Lys to be less than 93% of the NRC (2012) requirement estimate of the final BW of the experiment when determining AA:Lys in pigs heavier than 37 kg. These differences in results for heavier weight ranges might be partially explained by the slightly higher than expected Lys level in the chemical diet analysis. In Exp. 3, Lys had higher chemically analyzed values than expected. Grams of SID Lys per kilogram of BW gain in the LLH treatment was below the estimated requirement of finishing pigs in four experiments with gilts conducted in the same facilities (Main et al., 2008) for our experiments 2 and 4 but was above the requirement for experiment 3. However, in all experiments the amount in g of SID Lys/d was below the requirement suggested by NRC (2012).

In all experiments, pigs fed diets with 14.5% SID Trp:Lys had decreased performance compared with pigs fed diets with 20% SID Trp:Lys. This result indicates that Trp was limiting in the LLL diet. Furthermore, this indicates that in Trp:Lys ratio studies, a Trp ratio of 14.5% of Lys may be a good starting point for observing a response to increasing Trp.

In conclusion, low-CP diets formulated 0.10 and 0.05 percentage units below the SID Lys requirement at the end of the experiment's weight range appear to ensure pigs are below their Lys requirement when determining the optimal SID Trp:Lys ratio for 13- to -24-kg pigs and 23- to 37-kg gilts, respectively. For gilts heavier than 37 kg, formulating diets at 0.05 percentage units below the SID Lys requirement at the end of the experiment's weight range may limit the ability to provide statistical evidence that gilts are under their lysine requirement.

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TABLES

Table 3.1.Diet composition, Exp. 1 (as-fed basis)[1]

Item	HHH[2]	LHH	LLH	LLL
Ingredient, %				
Corn	31.48	41.59	55.10	55.16
Soybean meal (46% CP)	32.79	23.09	10.91	10.91
DDGS[1]	30.00	30.00	30.00	30.00
Corn oil	3.00	1.80	0.50	0.50
Calcium phosphate (dicalcium)	0.15	0.30	0.50	0.50
Limestone	1.50	1.49	1.48	1.48
Salt	0.35	0.35	0.35	0.35
Trace mineral premix[3]	0.100	0.100	0.100	0.100
Vitamin premix[4]	0.125	0.125	0.125	0.125
L-Lys-HCl	0.340	0.625	0.575	0.575
DL-Met	0.075	0.160	0.070	0.070
L-Thr	0.065	0.190	0.140	0.140
L-Trp	---	0.053	0.054	---
L-Val	---	0.105	0.060	0.060
L-Ile	---	---	0.010	0.010
Phytase[5]	0.025	0.025	0.025	0.025
TOTAL	100	100	100	100
Calculated analysis				
Standardized ileal digestible (SID) AA, %				
Lys	1.29	1.29	0.97	0.97
Ile:Lys	67	55	55	55
Leu:Lys	152	135	153	153
Met:Lys	34	37	35	35
Met & Cys:Lys	60	60	60	60
Thr:Lys	65	65	65	65
Trp:Lys	20.0	20.0	20.0	14.5
Val:Lys	73	70	70	70
His:Lys	43	37	38	38
Total Lys, %	1.51	1.48	1.13	1.13
ME, kcal/kg	3,443	3,395	3,330	3,329
NE, kcal/kg	2,469	2,470	2,471	2,469
SID Lys:ME, g/Mcal	3.74	3.80	2.91	2.91
SID Lys:NE, g/Mcal	5.22	5.22	3.92	3.92
CP, %	26.1	22.9	18.2	18.1
Ca, %	0.71	0.71	0.71	0.71
P, %	0.52	0.51	0.49	0.49
Available P, %	0.37	0.38	0.40	0.40
Stand. Dig. P with phytase, %	0.40	0.40	0.40	0.40
Ca:P	1.36	1.39	1.44	1.44

Ca:P (STTD P with phytase)	1.79	1.79	1.79	1.79

[1] Diets were fed from 13.0 to 24.0 kg BW. Corn, dried distillers grains with solubles (DDGS) and soybean meal were analyzed for total amino acid content and NRC (2012) SID digestibility values were used in the diet formulation.
[2] HHH: high CP, high SID Lys, and high SID Trp:Lys; LHH: low CP, high SID Lys, and high SID Trp:Lys; LLH: low CP, low SID Lys, and high Trp:Lys; LLL: low CP, low SID Lys, and low SID Trp:Lys.
[3] Provided per kg of diet: 33 mg Mn from manganese oxide, 110 mg Fe from iron sulfate, 110 mg Zn from zinc oxide, 16.5 mg Cu from copper sulfate, 0.33 mg I from ethylenediamine dihydriodide, and 0.30 mg Se from sodium selenite.
[4] Provided per kg of diet: 8,816 IU vitamin A; 1,378 IU vitamin D_3; 44.1 IU vitamin E; 4.41 mg vitamin K; 27.6 mg pantothenic acid; 50 mg niacin; 7.7 mg riboflavin and 33 μg vitamin B_{12}.
[5] OptiPhos 2000 (Huvepharma, Peachtree City, GA) provided 500 phytase units (FTU) per kg of diet.

Table 3.2. Diet composition, Exp. 2 (as-fed basis)[1]

Item	HHH[2]	LHH	LLH	LLL
Ingredient, %				
Corn	39.59	49.60	57.14	57.20
Soybean meal (46% CP)	25.32	16.10	9.56	9.55
DDGS[1]	30.00	30.00	30.00	30.00
Choice white grease	2.70	1.35	0.50	0.50
Limestone	1.49	1.45	1.43	1.43
Salt	0.35	0.35	0.35	0.35
Trace mineral premix[3]	0.100	0.100	0.100	0.100
Vitamin premix[4]	0.075	0.075	0.075	0.075
L-Lys-HCl	0.305	0.575	0.550	0.550
DL-Met	0.020	0.100	0.050	0.050
L-Thr	0.035	0.150	0.125	0.125
L-Trp	---	0.050	0.051	---
L-Val	---	0.070	0.050	0.050
Phytase[5]	0.025	0.025	0.025	0.025
TOTAL	100	100	100	100
Calculated analysis				
Standardized ileal digestible (SID) AA, %				
Lys	1.09	1.09	0.92	0.92
Ile:Lys	68	55	55	55
Leu:Lys	165	147	159	159
Met:Lys	32	36	34	34
Met & Cys:Lys	60	60	60	60
Thr:Lys	65	65	65	65
Trp:Lys	20.0	20.0	20.0	14.5
Val:Lys	76	70	70	70
His:Lys	45	38	39	39
Total Lys, %	1.29	1.26	1.08	1.08
ME, kcal/kg	3,425	3,379	3,344	3,343
NE, kcal/kg	2,489	2,489	2,489	2,488
SID Lys:ME, g/Mcal	3.18	3.22	2.75	2.75
SID Lys:NE, g/Mcal	4.37	4.37	3.69	3.69
CP, %	23.2	20.2	17.6	17.6
Ca, %	0.65	0.61	0.58	0.58
P, %	0.46	0.42	0.40	0.40
Available P, %	0.33	0.32	0.31	0.31
Stand. Dig. P with phytase, %	0.36	0.33	0.32	0.32
Ca:P	1.40	1.44	1.46	1.46
Ca:P (STTD P with phytase)	1.82	1.82	1.82	1.82

[1] Diets were fed from 22.8 to 36.4 kg BW. Corn, dried distillers grains with solubles (DDGS) and soybean meal were analyzed for total amino acid content and NRC (2012) SID digestibility values were used in the diet formulation.

[2] HHH: high CP, high SID Lys, and high SID Trp:Lys; LHH: low CP, high SID Lys, and

high SID Trp:Lys; LLH: low CP, low SID Lys, and high Trp:Lys; LLL: low CP, low SID Lys, and low SID Trp:Lys.

[3] Provided per kg of diet: 33 mg Mn from manganese oxide, 110 mg Fe from iron sulfate, 110 mg Zn from zinc oxide, 16.5 mg Cu from copper sulfate, 0.33 mg I from ethylenediamine dihydriodide, and 0.30 mg Se from sodium selenite.

[4] Provided per kg of diet: 5,290 IU vitamin A; 825 IU vitamin D_3; 26.4 IU vitamin E; 2.64 mg vitamin K; 16.5 mg pantothenic acid; 30 mg niacin; 4.6 mg riboflavin and 20 μg vitamin B_{12}.

[5] OptiPhos 2000 (Huvepharma, Peachtree City, GA) provided 500 phytase units (FTU) per kg of diet.

Table 3.3. Diet composition, Exp. 3 (as-fed basis)[1]

Item	HHH[2]	LHH	LLH	LLL
Ingredient, %				
Corn	46.84	57.00	60.82	60.87
Soybean meal (46% CP)	18.95	9.52	6.23	6.23
DDGS[1]	30.00	30.00	30.00	30.00
Corn oil	2.10	0.85	0.50	0.50
Limestone	1.28	1.26	1.25	1.25
Salt	0.35	0.35	0.35	0.35
Trace mineral premix[3]	0.100	0.100	0.100	0.100
Vitamin premix[4]	0.075	0.075	0.075	0.075
L-Lys-HCl	0.275	0.552	0.495	0.495
DL-Met	---	0.050	0.005	0.005
L-Thr	0.005	0.125	0.090	0.090
L-Trp	---	0.052	0.045	---
L-Val	---	0.045	0.010	0.010
Phytase[5]	0.025	0.025	0.025	0.025
TOTAL	100	100	100	100
Calculated analysis				
Standardized ileal digestible (SID) amino acids, %				
Lys	0.92	0.92	0.80	0.80
Ile:Lys	70	55	56	56
Leu:Lys	181	159	174	174
Met:Lys	33	34	32	32
Met & Cys:Lys	63	60	60	60
Thr:Lys	65	65	65	65
Trp:Lys	20.0	20.0	20.0	14.5
Val:Lys	80	70	70	70
His:Lys	48	39	41	41
Total Lys, %	1.10	1.08	0.95	0.95
ME, kcal/kg	3,419	3,371	3,353	3,352
NE, kcal/kg	2,513	2,512	2,513	2,512
SID Lys:ME, g/Mcal	2.69	2.72	2.38	2.38
SID Lys:NE, g/Mcal	3.65	3.65	3.18	3.18
CP, %	20.7	17.6	16.3	16.2
Ca, %	0.55	0.52	0.51	0.51
P, %	0.44	0.40	0.38	0.38
Available P, %	0.32	0.31	0.30	0.30
Stand. Dig. P with phytase, %	0.34	0.32	0.31	0.31
Ca:P	1.27	1.31	1.32	1.32
Ca:P (STTD P with phytase)	1.63	1.63	1.63	1.63

[1] Diets were fed from 57.7 to 73.6 kg BW. Corn, dried distillers grains with solubles (DDGS), and soybean meal were analyzed for total amino acid content, and NRC (2012) SID digestibility values were used in the diet formulation.

[2] HHH: high CP, high SID Lys, and high SID Trp:Lys; LHH: low CP, high SID Lys, and high SID Trp:Lys; LLH: low CP, low SID Lys, and high Trp:Lys; LLL: low CP, low SID

Lys, and low SID Trp:Lys.

[3] Provided per kg of diet: 33 mg Mn from manganese oxide, 110 mg Fe from iron sulfate, 110 mg Zn from zinc oxide, 16.5 mg Cu from copper sulfate, 0.33 mg I from ethylenediamine dihydriodide, and 0.30 mg Se from sodium selenite.

[4] Provided per kg of diet: 5,290 IU vitamin A; 825 IU vitamin D_3; 26.4 IU vitamin E; 2.64 mg vitamin K; 16.5 mg pantothenic acid; 30 mg niacin; 4.6 mg riboflavin and 20 μg vitamin B_{12}.

[5] OptiPhos 2000 (Huvepharma, Peachtree City, GA) provided 500 phytase units (FTU) per kg of diet.

Table 3.4. Diet composition, Exp. 4 (as-fed basis)[1]

Item	HHH[2]	LHH	LLH	LLL
Ingredient, %				
Corn	51.86	61.19	65.19	65.23
Soybean meal (46% CP)	14.19	5.65	2.21	2.21
DDGS[1]	30.00	30.00	30.00	30.00
Choice white grease	2.10	0.90	0.50	0.50
Limestone	1.12	1.11	1.10	1.10
Salt	0.35	0.35	0.35	0.35
Trace mineral premix[3]	0.050	0.050	0.050	0.050
Vitamin premix[4]	0.050	0.050	0.050	0.050
L-Lys-HCl	0.250	0.500	0.423	0.423
DL-Met	---	0.005	---	---
L-Thr	0.005	0.115	0.065	0.065
L-Trp	---	0.046	0.036	---
L-Val	---	0.015	---	---
Phytase[5]	0.025	0.025	0.025	0.025
TOTAL	100	100	100	100
Calculated analysis				
Standardized ileal digestible (SID) amino acids, %				
Lys	0.79	0.79	0.65	0.65
Ile:Lys	73	56	60	60
Leu:Lys	198	174	201	201
Met:Lys	36	32	36	36
Met & Cys:Lys	68	60	67	67
Thr:Lys	68	68	68	68
Trp:Lys	20.0	20.0	20.0	14.5
Val:Lys	84	70	75	75
His:Lys	50	41	45	45
Total Lys, %	0.96	0.94	0.79	0.79
ME, kcal/kg	3,421	3,378	3,361	3,361
NE, kcal/kg	2,538	2,538	2,539	2,539
SID Lys:ME, g/Mcal	2.30	2.33	1.93	1.93
SID Lys:NE, g/Mcal	3.11	3.11	2.55	2.56
CP, %	18.9	16.0	14.6	14.6
Ca, %	0.48	0.45	0.44	0.44
P, %	0.42	0.38	0.37	0.37
Available P, %	0.31	0.30	0.30	0.30
Stand. Dig. P with phytase, %	0.33	0.31	0.30	0.30
Ca:P	1.16	1.20	1.20	1.20
Ca:P (STTD P with phytase)	1.47	1.47	1.47	1.47

[1] Diets were fed from 87.4 to 107.8 kg BW. Corn, dried distillers grains with solubles (DDGS) and soybean meal were analyzed for total amino acid content, and NRC (2012) SID digestibility values were used in the diet formulation.

[2] HHH: high CP, high SID Lys, and high SID Trp:Lys; LHH: low CP, high SID Lys, and high SID Trp:Lys; LLH: low CP, low SID Lys, and high Trp:Lys; LLL: low CP, low SID

Lys, and low SID Trp:Lys.

[3] Provided per kg of diet: 16.5 mg Mn from manganese oxide, 55 mg Fe from iron sulfate, 55 mg Zn from zinc oxide, 8.3 mg Cu from copper sulfate, 0.17 mg I from ethylenediamine dihydriodide, and 0.15 mg Se from sodium selenite.

[4] Provided per kg of diet: 3,526 IU vitamin A; 551 IU vitamin D_3; 17.6 IU vitamin E; 1.76 mg vitamin K; 11 mg pantothenic acid; 20 mg niacin; 3.1 mg riboflavin and 13 μg vitamin B_{12}.

[5] OptiPhos 2000 (Huvepharma, Peachtree City, GA) provided 500 phytase units (FTU) per kg of diet.

Table 3.5. Amino acid analysis of soybean meal, corn, and dried distillers grains with solubles (DDGS)[1]

Item	Soybean meal		Corn		DDGS	
	Mean	CV	Mean	CV	Mean	CV
CP, %	46.0	0.71	7.5	3.51	27.5	2.77
Total amino acid, %						
Lys	2.81	1.60	0.24	4.24	0.81	3.09
Ile	1.98	2.47	0.25	3.64	0.96	2.61
Leu	3.33	1.71	0.84	3.21	3.04	3.03
Met	0.63	3.32	0.16	3.16	0.56	2.70
Met + Cys	1.30	1.77	0.32	2.79	1.03	3.10
Thr	1.79	2.52	0.26	3.45	0.99	2.62
Trp	0.65	2.01	0.06	1.61	0.23	3.04
Val	2.04	3.34	0.33	3.07	1.22	2.79
His	1.17	2.90	0.20	2.48	0.68	2.34
Phe	2.22	2.75	0.34	3.86	1.28	2.65

[1] Soybean meal, corn, and dried distillers grains with solubles were analyzed for total amino acid content by Ajinomoto Heartland Inc., Chicago, IL. These values are means of 5 samples analyzed in duplicate each week for 5 weeks before the start of the study. These values, along with standardized digestibility coefficients from NRC (2012) for soybean meal, corn, and DDGS, were used in diet formulation.

Table 3.6. Chemical analysis of the diets, Exp. 1 (as-fed basis)[1,2]

Item	HHH	LHH	LLH	LLL
Proximate analysis, %				
DM	91.28 (82.06)	91.11 (82.26)	90.79 (83.23)	90.8 (83.28)
CP	27.6 (26.1)	23.9 (22.9)	20.2 (18.2)	20.1 (18.1)
Crude fiber	4.3 (4.6)	3.9 (4.4)	3.6 (4.2)	3.4 (4.2)
Ca	0.87 (0.71)	1.05 (0.71)	0.74 (0.71)	0.88 (0.71)
P	0.52 (0.52)	0.51 (0.51)	0.51 (0.49)	0.50 (0.49)
Fat	7.1 (7.2)	6.0 (6.2)	4.7 (5.2)	4.6 (5.2)
Ash	5.57 (3.68)	5.32 (3.20)	4.28 (2.61)	4.71 (2.61)
Total amino acids, %				
Lys	1.48 (1.51)	1.52 (1.48)	1.21 (1.13)	1.14 (1.13)
Ile	1.07 (1.01)	0.96 (0.85)	0.76 (0.65)	0.76 (0.65)
Leu	2.38 (2.27)	2.21 (2.03)	1.94 (1.74)	1.95 (1.74)
Met	0.51 (0.50)	0.57 (0.54)	0.41 (0.39)	0.42 (0.39)
Met + Cys	0.95 (0.91)	0.97 (0.90)	0.76 (0.70)	0.76 (0.70)
Thr	1.03 (1.03)	1.08 (1.01)	0.86 (0.78)	0.87 (0.78)
Trp	0.29 (0.30)	0.29 (0.30)	0.22 (0.23)	0.18 (0.17)
Val	1.23 (1.14)	1.18 (1.08)	0.97 (0.83)	0.98 (0.83)
His	0.67 (0.65)	0.60 (0.56)	0.49 (0.44)	0.49 (0.44)
Phe	1.29 (1.22)	1.18 (1.04)	0.93 (0.81)	0.94 (0.81)
Free Lys	0.26 (0.34)	0.45 (0.63)	0.49 (0.58)	0.44 (0.58)
Free Thr	0.09 (0.07)	0.22 (0.19)	0.18 (0.14)	0.20 (0.14)
Free Trp	0.02 (---)	0.04 (0.05)	0.04 (0.05)	0.01 (---)

[1] Values in parentheses indicate those used in diet formulation and are from NRC, 2012 (Nutrient Requirements of Swine, 11th ed. Natl. Acad. Press, Washington DC), with the exception of CP and total AA content from corn, soybean meal, and DDGS, which were analyzed prior to diet formulation by Ajinomoto Heartland Inc. (Chicago, IL).

[2] Diet samples were collected from feeders, stored at -20ºC, and submitted to Ward Laboratories Inc. (Kearney, NE) for proximate analysis, with the exception of CP and total AA, which were analyzed by Ajinomoto Heartland Inc. (Chicago, IL).

Table 3.7. Chemical analysis of the diets, Exp. 2 (as-fed basis)[1,2]

Item	HHH	LHH	LLH	LLL
Proximate analysis, %				
DM	91.16 (86.94)	91.10 (88.04)	90.79 (88.67)	90.97 (88.66)
CP	24.0 (23.2)	20.7 (20.2)	20.5 (17.6)	18.9 (17.6)
Crude fiber	4.2 (4.4)	3.6 (4.3)	3.2 (4.2)	3.6 (4.2)
Ca	0.78 (0.65)	0.84 (0.61)	0.74 (0.58)	0.64 (0.58)
P	0.44 (0.46)	0.40 (0.42)	0.39 (0.40)	0.40 (0.40)
Fat	6.0 (7.1)	5.2 (6.0)	4.7 (5.3)	4.9 (5.3)
Ash	4.87 (4.92)	4.71 (4.44)	4.53 (4.10)	4.15 (4.10)
Total amino acids, %				
Lys	1.30 (1.29)	1.23 (1.26)	1.16 (1.08)	1.08 (1.08)
Ile	0.93 (0.89)	0.81 (0.73)	0.77 (0.62)	0.72 (0.62)
Leu	2.19 (2.09)	2.04 (1.86)	1.96 (1.71)	1.91 (1.71)
Met	0.42 (0.41)	0.48 (0.45)	0.39 (0.37)	0.40 (0.37)
Met + Cys	0.82 (0.79)	0.83 (0.78)	0.73 (0.67)	0.75 (0.67)
Thr	0.91 (0.89)	0.89 (0.86)	0.81 (0.74)	0.78 (0.74)
Trp	0.26 (0.26)	0.24 (0.25)	0.23 (0.22)	0.17 (0.17)
Val	1.08 (1.01)	1.02 (0.92)	0.94 (0.80)	0.92 (0.80)
His	0.60 (0.58)	0.53 (0.49)	0.50 (0.43)	0.47 (0.43)
Phe	1.15 (1.08)	1.01 (0.91)	0.96 (0.79)	0.90 (0.79)
Free Lys	0.23 (0.31)	0.37 (0.58)	0.37 (0.55)	0.38 (0.55)
Free Thr	0.06 (0.04)	0.15 (0.15)	0.12 (0.13)	0.13 (0.13)
Free Trp	0.01 (---)	0.03 (0.05)	0.02 (0.05)	0.01 (---)

[1] Values in parentheses indicate those used in diet formulation and are from NRC, 2012 (Nutrient Requirements of Swine, 11th ed. Natl. Acad. Press, Washington DC), with the exception of CP and total AA content from corn, soybean meal, and DDGS, which were analyzed prior to diet formulation by Ajinomoto Heartland Inc. (Chicago, IL).
[2] Diet samples were collected from feeders, stored at -20ºC, and submitted to Ward Laboratories Inc. (Kearney, NE) for proximate analysis, with the exception of CP and total AA, which were analyzed by Ajinomoto Heartland Inc. (Chicago, IL).

Table 3.8. Chemical analysis of the diets, Exp. 3 (as-fed basis)[1,2]

Item	HHH	LHH	LLH	LLL
Proximate analysis, %				
DM	91.44 (87.33)	90.86 (88.34)	90.35 (88.57)	90.77 (88.57)
CP	21.5 (20.7)	19.1 (17.6)	17.2 (16.3)	17.6 (16.2)
Crude fiber	3.9 (4.3)	3.1 (4.2)	3.1 (4.1)	3.2 (4.1)
Ca	0.77 (0.55)	0.64 (0.52)	0.62 (0.51)	0.56 (0.51)
P	0.49 (0.44)	0.42 (0.40)	0.4 (0.38)	0.42 (0.38)
Fat	7.2 (6.6)	5.4 (5.6)	4.9 (5.4)	5.1 (5.4)
Ash	4.54 (4.41)	3.74 (3.93)	3.57 (3.77)	3.40 (3.77)
Total amino acids, %				
Lys	1.23 (1.10)	1.37 (1.08)	1.03 (0.95)	1.11 (0.95)
Ile	0.86 (0.78)	0.71 (0.62)	0.65 (0.56)	0.65 (0.56)
Leu	2.10 (1.94)	1.95 (1.71)	1.85 (1.63)	1.85 (1.63)
Met	0.39 (0.36)	0.40 (0.37)	0.33 (0.31)	0.34 (0.31)
Met + Cys	0.77 (0.71)	0.74 (0.67)	0.65 (0.59)	0.66 (0.59)
Thr	0.84 (0.76)	0.80 (0.74)	0.71 (0.66)	0.74 (0.66)
Trp	0.21 (0.22)	0.20 (0.22)	0.18 (0.19)	0.16 (0.15)
Val	1.02 (0.90)	0.89 (0.79)	0.80 (0.70)	0.81 (0.70)
His	0.55 (0.52)	0.49 (0.43)	0.45 (0.40)	0.46 (0.40)
Phe	1.06 (0.96)	0.91 (0.79)	0.84 (0.73)	0.85 (0.73)
Free Lys	0.31 (0.28)	0.68 (0.55)	0.43 (0.50)	0.47 (0.50)
Free Thr	0.05 (0.01)	0.12 (0.13)	0.10 (0.09)	0.13 (0.09)
Free Trp	0.02 (---)	0.04 (0.05)	0.03 (0.05)	0.01 (---)

[1] Values in parentheses indicate those used in diet formulation and are from NRC, 2012 (Nutrient Requirements of Swine, 11th ed. Natl. Acad. Press, Washington DC) with the exception of CP and total AA content from corn, soybean meal, and DDGS, which were analyzed prior to diet formulation by Ajinomoto Heartland Inc. (Chicago, IL).

[2] Diet samples were collected from feeders, stored at -20ºC, and submitted to Ward Laboratories Inc. (Kearney, NE) for proximate analysis, with the exception of CP and total AA, which were analyzed by Ajinomoto Heartland Inc. (Chicago, IL).

Table 3.9. Chemical analysis of the diets, Exp. 4 (as-fed basis)[1,2]

Item	HHH	LHH	LLH	LLL
Proximate analysis, %				
DM	90.43 (87.31)	89.92 (88.21)	89.79 (88.46)	89.83 (88.46)
CP	20.9 (18.9)	17.3 (16.0)	15.7 (14.6)	15.9 (14.6)
Crude fiber	3.7 (4.3)	3.8 (4.1)	3.3 (4.1)	3.8 (4.1)
Ca	0.86 (0.48)	0.65 (0.45)	0.66 (0.44)	0.52 (0.44)
P	0.42 (0.42)	0.40 (0.38)	0.39 (0.37)	0.38 (0.37)
Crude fat	6.4 (6.6)	5.7 (5.7)	5.0 (5.5)	5.4 (5.5)
Ash	4.70 (3.96)	3.87 (3.54)	3.45 (3.36)	3.48 (3.36)
Amino acids, %				
Lys	1.00 (0.96)	0.93 (0.94)	0.84 (0.79)	0.81 (0.79)
Ile	0.81 (0.70)	0.65 (0.55)	0.58 (0.49)	0.56 (0.49)
Leu	2.05 (1.82)	1.81 (1.61)	1.75 (1.53)	1.75 (1.53)
Met	0.37 (0.34)	0.33 (0.30)	0.31 (0.28)	0.31 (0.28)
Met + Cys	0.74 (0.66)	0.65 (0.59)	0.61 (0.55)	0.60 (0.55)
Thr	0.77 (0.69)	0.72 (0.67)	0.64 (0.57)	0.64 (0.57)
Trp	0.21 (0.19)	0.18 (0.19)	0.16 (0.16)	0.13 (0.12)
Val	0.96 (0.82)	0.81 (0.70)	0.71 (0.62)	0.71 (0.62)
His	0.52 (0.48)	0.43 (0.39)	0.41 (0.36)	0.41 (0.36)
Phe	1.00 (0.87)	0.82 (0.72)	0.76 (0.65)	0.77 (0.65)
Free Lys	0.17 (0.25)	0.32 (0.50)	0.30 (0.42)	0.31 (0.42)
Free Thr	0.04 (0.01)	0.12 (0.12)	0.08 (0.07)	0.08 (0.07)
Free Trp	0.01 (---)	0.02 (0.05)	0.02 (0.04)	0.01 (---)

[1] Values in parentheses indicate those used in diet formulation and are from NRC, 2012 (Nutrient Requirements of Swine, 11th ed. Natl. Acad. Press, Washington DC), with the exception of CP and total AA content from corn, soybean meal, and DDGS, which were analyzed prior to diet formulation by Ajinomoto Heartland Inc. (Chicago, IL).

[2] Diet samples were collected from feeders, stored at -20ºC, and submitted to Ward Laboratories Inc. (Kearney, NE) for proximate analysis, with the exception of CP and total AA, which were analyzed by Ajinomoto Heartland Inc. (Chicago, IL).

Table 3.10. Effects of different SID Trp:Lys ratios, CP, and SID Lys levels on pig performance[1]

Item	HHH[2]	LHH	LLH	LLL	SEM	Probability, *P* <
Exp. 1						
d 0 BW, kg	12.9	13.0	12.9	13.0	0.17	0.976
ADG, g	557[a]	574[a]	471[b]	406[c]	9.6	0.001
ADFI, g	768[b]	827[a]	786[b]	721[c]	14.8	0.001
G:F	0.726[a]	0.696[b]	0.599[c]	0.564[d]	0.012	0.001
d 21 BW, kg	24.7[a]	25.1[a]	22.9[b]	21.5[c]	0.28	0.001
Exp. 2						
d 0 BW, kg	22.8	22.8	22.8	22.8	0.6	0.988
ADG, g	713[a]	727[a]	654[b]	492[c]	13.8	0.001
ADFI, g	1,160[b]	1,249[a]	1,262[a]	1,012[c]	37.3	0.001
G:F	0.617[a]	0.586[b]	0.521[c]	0.487[d]	0.010	0.001
d 21 BW, kg	37.8[a]	38.0[a]	36.7[b]	33.2[c]	0.8	0.001
Exp. 3						
d 0 BW, kg	57.6	57.6	57.7	57.7	1.2	0.967
ADG, g	760[a]	779[a]	754[b]	661[c]	17.1	0.001
ADFI, g	1,828[b]	1,924[a]	1,932[a]	1,826[b]	24.2	0.001
G:F	0.415[a]	0.405[a]	0.391[a]	0.363[b]	0.010	0.005
d 21 BW, kg	74.3[a]	74.5[a]	73.8[a]	71.8[b]	1.2	0.001
Exp. 4						
d 0 BW, kg	87.4	87.4	87.4	87.4	1.2	0.999
ADG, g	1,033[a]	986[ab]	965[b]	868[c]	18.3	0.001
ADFI, g	2677	2667	2680	2558	43.2	0.167
G:F	0.386[a]	0.370[b]	0.360[b]	0.339[c]	0.004	0.001
d 21 BW, kg	109.2[a]	108.3[ab]	107.9[b]	105.8[c]	1.2	0.001

[1]A total of 1,188, 1,232, 1,204, and 1,183 pigs (PIC 337 × 1050) were used for experiments 1, 2, 3 and 4, respectively, in 21-d growth trials. Each treatment had 11 replications with 24 to 28 pigs per pen.
[2] Dietary treatments were HHH (High CP, High Lys, and High Trp), LHH (Low CP, High Lys, and High Trp), LLH (Low CP, Low Lys, and High Trp), LLL (Low CP, Low Lys, and Low Trp).
[a,b,c,d] Means in same row with different superscripts differ ($P < 0.05$).

Chapter 4 - Effects of standardized ileal digestible tryptophan:lysine ratio on growth performance of nursery pigs[9,10]

M. A. D. Gonçalves*, S. Nitikanchana*, M. D. Tokach†[11], S. S. Dritz*, N. M. Bello‡, R. D. Goodband†, K. J. Touchette§, J. Usry§, J. M. DeRouchey†, and J. C. Woodworth†

*Department of Diagnostic Medicine/Pathobiology, College of Veterinary Medicine, †Department of Animal Sciences and Industry, College of Agriculture, ‡Department of Statistics, College of Arts and Sciences, Kansas State University, Manhattan, KS 66506-0201, and **§** Ajinomoto Heartland Inc., Chicago, IL

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[11] Corresponding author: mtokach@k-state.edu

ABSTRACT: Two experiments were conducted to estimate the standardized ileal digestible (**SID**) Trp:Lys ratio requirement for growth performance of nursery pigs. Experimental diets were formulated to ensure that lysine was the second limiting amino acid throughout the experiments. In Exp. 1 (6-10 kg BW), 255 nursery pigs (PIC 327 × 1050, initially 6.3 ± 0.15 kg, mean ± SD) arranged in pens of 6 or 7 pigs were blocked by pen weight and assigned to experimental diets (7 pens/diet) consisting of SID Trp:Lys ratios of 14.7, 16.5, 18.4, 20.3, 22.1, and 24.0% for 14 d with 1.30% SID Lys. In Exp. 2 (11 to 20 kg BW), 1,088 pigs (PIC 337 × 1050, initially 11.2 kg ± 1.35 BW, mean ± SD) arranged in pens of 24 to 27 pigs were blocked by pen weight and assigned to experimental diets (6 pens/diet) consisting of SID Trp:Lys ratios of 14.5, 16.5, 18.0, 19.5, 21.0, 22.5, and 24.5% for 21 d with 30% dried distillers grains with solubles (DDGS) and 0.97% SID Lys. Each experiment was analyzed using general linear mixed models with heterogeneous residual variances. Competing heteroskedastic models included broken-line linear (BLL), broken-line quadratic (BLQ), and quadratic polynomial (QP). For each response, the best-fitting model was selected using Bayesian information criterion. In Exp. 1 (6 to 10 kg BW), increasing SID Trp:Lys ratio linearly increased ($P < 0.05$) ADG and G:F. For ADG, the best-fitting model was a QP in which the maximum mean ADG was estimated at 23.9% (95% CI: [<14.7, >24.0]%) SID Trp:Lys ratio. For G:F, the best-fitting model was a BLL in which the maximum mean G:F was estimated at 20.4% (95% CI: [14.3, 26.5]%) SID Trp:Lys. In Exp. 2 (11 to 20 kg BW), increasing SID Trp:Lys ratio increased ($P < 0.05$) ADG and G:F in a quadratic manner. For ADG, the best-fitting model was a QP in which the maximum mean ADG was estimated at 21.2% (95% CI: [20.5, 21.9]%) SID Trp:Lys. For G:F, BLL and BLQ models had comparable fit and estimated SID Trp:Lys requirements at 16.6 (95% CI: [16.0, 17.3]%) and 17.1% (95% CI: [16.6, 17.7]%), respectively. In conclusion, the estimated mean SID Trp:Lys requirement in Exp. 1 ranged from 20.4% for maximum mean G:F to 23.9% for

maximum mean ADG, whereas in Exp. 2 it ranged from 16.6% for maximum mean G:F to 21.2% for maximum mean ADG. Our results suggest that standard NRC (2012) recommendations may underestimate the SID Trp:Lys requirement for nursery pigs from 11 to 20 kg BW.

Key words: amino acid ratio, growth, lysine, nursery pig, tryptophan

INTRODUCTION

Tryptophan (Trp) is an important limiting amino acid in corn and soybean meal–based diets of nursery and finishing pigs (Lewis, 2000). As the availability of feed-grade amino acids, including Trp, increases, so does their use as replacement for intact protein sources in swine diets. The Trp requirement in swine diets can be expressed in different ways. In particular, the standardized ileal digestible (**SID**) Trp requirement expressed as a ratio to Lys (**Trp:Lys**) is considered a practical approach for diet formulation (Stein et al., 2007); however, the observed SID Trp:Lys ratio requirement varies considerably among studies. For example, the NRC (2012) estimates the SID Trp requirement at 16.3% of Lys for nursery pigs, but Guzik et al. (2005) estimated a SID Trp:Lys requirement of greater than 19.5%, and Simongiovanni et al. (2012) concluded that it was 17 to 22% of Lys. The objective of these studies was to estimate the SID Trp:Lys ratio requirement for growth performance in nursery pigs.

MATERIALS AND METHODS

The Kansas State University Institutional Animal Care and Use Committee approved the protocols used in these experiments. Experiment 1 was conducted at the Kansas State University

Swine Teaching and Research Center in Manhattan, KS, and Exp. 2 was conducted at a commercial research-nursery barn in southwestern Minnesota.

Experiment 1: 6 to 10 kg BW

A total of 255 nursery pigs (PIC 327 × 1050, with initial and final BW of 6.3 ± 0.15 and 9.8 ± 0.46 kg, respectively, mean ± SD) were used in a 14-d growth trial. Pigs were weaned at 21 d of age and placed in the nursery facility, where they were fed a common diet for 3 d. At d 3 after weaning, pigs were weighed in pens, and pens were randomly assigned to dietary treatments in a randomized complete block design blocked by initial average pen BW. Therefore, d 3 after weaning was d 0 of the trial. Each treatment consisted of 7 pens of 6 to 7 pigs/pen, and each pen comprised barrows and gilts. A 4-hole, dry self-feeder and a nipple waterer were used in each pen (1.2 × 1.5 m) to provide ad libitum access to feed and water. Experimental diets consisted of corn and soybean meal and had 6 ratios of SID Trp:Lys, namely 14.7, 16.5, 18.4, 20.3, 22.1, and 24.0%. Feed-grade L-Trp was added at the expense of corn starch in the basal diet to achieve the desired ratios of SID Trp:Lys (Table 4.1). Nutrients and SID AA digestibility values used for diet formulation were obtained from NRC (1998). Large batches of the 14.7% and 24.0% SID Trp:Lys diets were manufactured, then blended to achieve the intermediate SID Trp:Lys ratios. The percentages of low- and high-SID Trp:Lys blends to create the treatment diets were 100 and 0, 80 and 20, 60 and 40, 40 and 60, 20 and 80, and 0 and 100% for 14.7, 16.5, 18.4, 20.3, 22.1, and 24.0% SID Trp:Lys, respectively. Diets were formulated to 1.30% SID Lys based on data of Nemechek et al. (2011). Based on the NRC model (2012), 1.34% SID Lys is the requirement in a diet with 3,341 kcal ME/kg for 10-kg nursery pigs. Thus, experimental diets were 0.04 percentage point below the NRC requirement at the end of the 6- to 10-kg BW nursery phase to ensure that lysine was the second limiting amino acid throughout the experiment. The

14.7% SID Trp:Lys ratio diet was also reported to be deficient in Trp (Nemechek et al., 2011). Diets contained 10% spray-dried whey and no specialty protein sources, such as spray-dried blood meal or select menhaden fishmeal. Experimental diets were fed in meal form and were prepared at the O.H. Kruse Feed Technology Innovation Center.

Experiment 2: 11 to 20 kg BW

A total of 1,088 pigs (PIC 337 × 1050, with initial and final BW of 11.2 kg ± 1.35 and 20.3 ± 2.16 kg, respectively, mean ± SD) were used in a 21-d growth trial. Pigs were weaned at 16 d of age and grouped into pens of 27 animals (14 gilts and 13 barrows). After weaning, pigs were fed a common pelleted diet for 7 days, followed by common meal diets containing 10 and 20% dried distillers grains with solubles (DDGS) from d 7 to 14 and 14 to 28 after weaning, respectively, with 20% SID Trp:Lys ratio. On d 28 after weaning, pigs were weighed in pens, and pens were blocked by initial average pen BW and randomly assigned dietary treatments in a randomized complete block design with 6 pens per treatment. Therefore, d 28 after weaning was d 0 of the trial. The facility was totally enclosed, environmentally controlled, and mechanically ventilated. Pens had completely slatted flooring and deep pits for manure storage. Each pen (3.7 × 2.3 m) was equipped with a 6-hole stainless steel dry self-feeder and a pan waterer for ad libitum access to feed and water. Daily feed additions to each pen were accomplished through a robotic feeding system (FeedPro; Feedlogic Corp., Willmar, MN) capable of providing and measuring feed amounts for individual pens. This system is capable of feeding each individual pen any of the individual diets as well as a blend of two diets. Five representative samples of corn, soybean meal, and DDGS were collected each week for 5 wk and analyzed in duplicate for total AA (method 994.12; AOAC Int., 2012) and CP (method 990.03; AOAC Int., 2012) by Ajinomoto Heartland Inc. (Chicago, IL) before diet formulation. These values along with standardized digestibility coefficients from NRC (2012) for corn, soybean meal, and DDGS were used in diet formulation. Diets were balanced on an NE basis using NRC (2012) values.

Two experimental corn-soybean meal–based diets with 30% DDGS were formulated (Table 4.2) to be limiting in Lys and have 14.5 and 24.5% SID Trp:Lys ratios, then the diets were blended using the robotic feeding system to achieve intermediate SID Trp:Lys ratios, thereby defining dietary treatments. The percentage of low- and high-SID Trp:Lys ratios blended to create the treatment diets were 100 and 0, 80 and 20, 65 and 35, 50 and 50, 35 and 65, 20 and 80, and 0 and 100% for 14.5, 16.5, 18.0, 19.5, 21.0, 22.5, and 24.5% SID Trp:Lys, respectively. The SID Trp:Lys ratio was increased by adding feed-grade L-Trp to the control diet at the expense of corn. The NRC (2012) model was used to estimate the SID Lys requirement of pigs fed diets with 2,466 kcal NE/kg at the expected BW at the end of the experiment (22.7 kg). The SID Lys requirement (i.e., 1.07%) was reduced by 0.10 percentage point for diet formulation to ensure that lysine was the second limiting amino acid throughout the experiment. Diets were fed in meal form and were manufactured at the New Horizon Farms Feed Mill (Pipestone, MN). A preliminary experiment was conducted prior to Exp. 2 in the same facility and with pigs of the same BW to validate that diets were indeed limiting in Lys. For that preliminary experiment, a total of 1,188 pigs (PIC 337 x 1050, initially 12.9 kg ± 0.66 BW, mean ± SD) were used in a 21-d growth trial with 27 pigs per pen and 11 pens per treatment. Pigs were fed either a high-Lys diet [SID Lys levels were 0.01% above the estimated NRC (2012) requirement at the expected initial BW] or a low-Lys diet [0.97%, which is 0.10 percentage point below the estimated NRC (2012) requirement at the expected final BW]. In the preliminary study, pigs fed low-Lys diets had lower ($P < 0.05$) ADG, ADFI, and G:F compared with pigs fed high-Lys diets, thus validating the below-requirement SID Lys level of diets used in Exp. 2.

Diet Sampling and Analysis

In Exp. 1 and 2, samples of the diets were submitted to Ward Laboratories, Inc. (Kearney, NE) for analysis of DM (method 935.29; AOAC Int., 2012), CF [method 978.10; AOAC Int., 2012 for preparation and Ankom 2000 Fiber Analyzer (Ankom Technology, Fairport, NY)], ash (method 942.05; AOAC Int., 2012), crude fat [method 920.39 a; AOAC Int., 2012 for preparation and ANKOM XT20 Fat Analyzer (Ankom Technology, Fairport, NY)], Ca, and P [method 968.08 b; AOAC Int., 2012 for preparation using ICAP 6500 (ThermoElectron Corp., Waltham, MA)]. In Exp. 1, CP was analyzed by Ward Laboratories, Inc. (method 990.03; AOAC Int., 2012); in Exp. 2, CP was analyzed by Ajinomoto Heartland Inc. (method 990.03; AOAC Int., 2012).

In Exp. 1, diet samples were collected from feeders at the beginning of the trial and on d 7 and 14. At the end of the trial, samples of the diets were combined within dietary treatment, and a composite sample from each treatment was analyzed in duplicate for total AA content by Ajinomoto Heartland Inc.

In Exp. 2, diet samples were taken from 6 feeders per dietary treatment 3 days after the beginning of the trial and 3 days prior to the end of the trial and stored at -20° C, then CP and total AA analyses were conducted in duplicate on composite samples by Ajinomoto Heartland Inc.

Data Collection

Pig BW and feed disappearance were measured on d 0 and 14 in Exp. 1 and on d 0 and 21 in Exp. 2 to calculate ADG, ADFI, G:F, grams of SID Trp intake per day, and grams of SID Trp intake per kilogram of gain. Total grams of SID Trp intake per day was calculated based on formulated values by multiplying ADFI by SID Lys level by SID Trp:Lys. The total grams of

SID Trp intake was divided by total BW gain to calculate the grams of SID Trp intake per kilogram of gain.

Statistical Analysis

Responses of interest (ADG, ADFI, G:F, BW, grams of SID Trp intake per day, and grams of SID Trp intake per kilogram of gain) measured at the pen level were analyzed using general linear and non-linear mixed models to accommodate the randomized complete block design of the study. The linear predictor included the fixed effect of dietary treatment presented as a factor and initial average pen BW as a random blocking factor. Pen was the experimental unit. Models were expanded to account for heterogeneous residual variances, as needed. Residual assumptions were checked using standard diagnostics on studentized residuals and were found to be reasonably met. Linear and quadratic polynomial contrasts were built to evaluate the functional form of the dose-response to increasing dietary SID Trp:Lys ratio on ADG, ADFI, G:F, BW, grams of SID Trp intake per day, and grams of SID Trp intake per kilogram of gain. Polynomial contrast coefficients were adjusted for unequally spaced treatments using the IML procedure of SAS. Degrees of freedom were estimated using the Kenward-Rogers approach. Statistical models were fitted using the GLIMMIX procedure of SAS (Version 9.3, SAS Institute Inc., Cary, NC). Results were considered significant at $P \leq 0.05$ and marginally significant at $0.05 < P \leq 0.10$.

Additional models adapted from Robbins et al. (2006) and Pesti et al. (2009) were fitted to ADG and G:F to further estimate SID Trp:Lys requirements using an inverse prediction strategy. Specifically, competing statistical models fitted to the data included a broken-line linear ascending (BLL) model, a broken-line quadratic ascending (BLQ) model, and a quadratic polynomial (QP) model. As in Robbins et al. (2006), competing models described growth performance as a function of SID Trp:Lys levels as follows:

Broken-line linear ascending model:

$$y_{ij} = L_{BLL} + U_l \times (R_{BLL} - X_i) + b_j + e_{ij} \qquad \text{for } X_i < R_{BLL} \text{ and}$$

$$y_{ij} = L_{BLL} + b_j + e_{ij} \qquad \text{for } X_i \geq R_{BLL},$$

Broken-line quadratic ascending model:

$$y_{ij} = L_{BLQ} + U_q \times (R_{BLQ} - X_i)^2 + b_j + e_{ij} \qquad \text{for } X_i < R_{BLQ} \text{ and}$$

$$y_{ij} = L_{BLQ} + b_j + e_{ij} \qquad \text{for } X_i \geq R_{BLQ},$$

Quadratic polynomial model:

$$y_{ij} = \beta_0 + \beta_1 X_i + \beta_2 X_i^2 + b_j + e_{ij}$$

where y_{ij} is the response associated with the pen in block j assigned to dietary treatment i, X_i is the SID Trp:Lys level of the i^{th} dietary treatment, and L_{BLL} and L_{BLQ} indicate the unknown maximum growth response to dietary treatments (i.e., plateau) under BLL and BLQ models, respectively. R_{BLL} and R_{BLQ} are the unknown minimum levels of SID Trp:Lys requirement to reach the plateau under the BLL and BLQ models, respectively. β_0 is the intercept; U_l, U_q, β_1, and β_2 are the corresponding unknown rates of change of the response as a function of X_i; b_j is the random blocking effect of initial average pen BW associated with j^{th} block, where $b_j \sim N\left(0, \sigma_b^2\right)$; e_{ij} is a random error associated with the pen in the j^{th} block that received the i^{th} treatment whereby $e_{ij} \sim N\left(0, \sigma_{e_i}^2\right)$; and b_j and e_{ij} are assumed to be independent of each other.

Broken-line regression models were fitted using the NLMIXED procedures of SAS. The optimization technique used was the Dual Quasi-Newton algorithm, as specified by default in the NLMIXED procedure. Competing statistical models were compared using maximum-likelihood-based fit criteria, specifically the Schwarz's Bayesian Criterion (BIC; Milliken and Johnson, 2009). Results reported here correspond to inference yielded by the best-fitting models.

For the best-fitting models, the estimated requirement of SID Trp:Lys to reach plateau performance (i.e., R_{BLL} and R_{BLQ} in the broken-line models) or to reach maximum performance (i.e., in the QP) of ADG and G:F are reported with a 95% confidence interval. In the quadratic polynomial model, the level of SID Trp:Lys ratio that maximized ADG and G:F was estimated by equating the first derivative of the regression equation to zero, then solving for the SID Trp:Lys ratio (Pesti et al., 2009). The corresponding 95% confidence intervals were computed using the inverse regression approach proposed by Lavagnini and Magno (2006).

RESULTS AND DISCUSSION

The analyzed total AA, DM, CP, CF, Ca, P, fat, and ash contents of diets for Exp. 1 and 2 (Tables 4.3 and 4.4, respectively) were consistent with calculated values based on variation reported by Cromwell et al. (1999).

In Exp. 1 (6 to 10 kg BW), increasing SID Trp:Lys ratio linearly increased (Table 4.5) ADG ($P = 0.022$), G:F ($P = 0.012$), grams of SID Trp intake per day ($P = 0.001$), and grams of SID Trp intake per kilogram of gain ($P = 0.001$). Increasing SID Trp:Lys ratio also induced marginal linear increases in ADFI ($P = 0.057$) and final BW ($P = 0.052$).

For ADG in the 6- to 10-kg BW pigs (Exp. 1), the best-fitting model was a QP (BIC: 215.3) compared with BLL and BLQ models (BIC: 217.4 and 217.8, respectively). The estimated regression equation for the best-fitting QP model (Figure 4.1) was:

$$\text{ADG} = 42.7 + 1819.7 \times (\text{Trp:Lys}) - 3810.1 \times (\text{Trp:Lys})^2$$

whereby the SID Trp:Lys explanatory variable is expressed as a proportion (i.e., 0.180) rather than a percentage (i.e., 18.0%) for numerical stability of computations. Based on the best-fitting QP model, the maximum mean ADG (Fig. 4.1) was estimated at a 23.9% (95% CI: [<14.7,>24.0]%) SID Trp:Lys ratio. We acknowledge the substantial width of this confidence interval, thereby indicating considerable uncertainty for inference on SID Trp:Lys requirements that maximized ADG. This uncertainty is probably related to the large amount of unaccounted variability in ADG relative to an apparently minor effect of SID Trp:Lys ratios on ADG in Exp. 1, which is further supported by the non-significant P-value of the quadratic regression coefficient for ADG ($P = 0.484$) during this phase.

Also for Exp. 1 (6 to 10 kg BW), the best-fitting model for G:F was a BLL (BIC: 253.6) compared with QP and BLQ (BIC: 255.0 and 255.0, respectively). The best-fitting estimated regression equations for the best-fitting BLL model (Fig. 4.2) were:

$$\text{G:F} = 0.733 - 0.6034 \times (0.204 - \text{Trp:Lys}) \quad \text{if SID Trp:Lys} < 20.4\%$$

$$\text{G:F} = 0.733 \quad \text{if SID Trp:Lys} \geq 20.4\%$$

whereby the SID Trp:Lys explanatory variable is expressed as a proportion (i.e., 0.180) rather than a percentage (i.e., 18.0%) for numerical stability. Based on the best-fitting BLL model, the estimated minimum SID Trp:Lys requirement to achieve maximum mean G:F was 20.4% (95% CI: [14.3, 26.5]%).

To the author’s knowledge, this is the first attempt to try to quantify uncertainty around the SID Trp:Lys ratio requirement. Therefore, after this first experiment, a subsequent study was done on a larger scale in a commercial facility with a 7-point titration and slightly wider treatment ranges to reduce the uncertainty around the estimates and evaluate the response to increasing SID Trp:Lys ratios.

In Exp. 2 (11 to 20 kg BW), increasing SID Trp:Lys ratio quadratically increased (Table 4.6) ADG (P = 0.001), ADFI (P = 0.006), G:F (P = 0.002), final BW (P = 0.001), grams of SID Trp intake per day (P = 0.026), and grams of SID Trp intake per kilogram of gain (P = 0.006).

For ADG in the 11- to 20-kg BW pigs (Exp. 2), the best-fitting model was a QP (BIC: 198.1) compared with BLL and BLQ models (BIC: 204.8 and 204.8, respectively). The estimated regression equation for the best-fitting QP model (Figure 4.3) was:

$$\text{ADG} = -317 + 7259 \times (\text{Trp:Lys}) - 17110 \times (\text{Trp:Lys})^2.$$

Based on the best-fitting QP model, the maximum mean ADG was estimated at 21.2% (95% CI: 20.5 to 21.9%) SID Trp:Lys. Note the reduced uncertainty (i.e., narrower confidence interval) around the estimated Trp:Lys ratio requirement for maximum mean ADG compared with that in Exp 1.

For G:F in the 11- to 20-kg BW pigs (Exp. 2), BLL and BLQ models had comparable fit (BIC: 346.1 and 346.1, respectively), whereas QP showed less adequate fit (BIC: 355.2). The comparable fit of these models may be explained by the scarcity of information on G:F for the range of SID Trp:Lys ratios for which the functional relationship is estimated; that is, G:F observations were available for only 2 SID Trp:Lys ratios before the estimated plateau in G:F was detected (Figure 4.4). We also note that the same number of unknown fixed-effects parameters occur in the BLL model (i.e. L_{BLL}, U_l and R_{BLL}) and the BLQ model (i.e. L_{BLQ}, U_q and R_{BLQ}), so the principle of parsimony favoring simpler models would, in this case, not contribute to model selection. Taken together, these issues can help explain, at least partially, the impaired discrimination in differential fit between BLL and BLQ models. Based on the best-fitting BLL and BLQ models, alternative estimated regression equations (Figure 4.4) were:

Based on the BLL model:

$$\text{G:F} = 0.5844 - 1.95 \times (0.166 - \text{Trp:Lys}), \text{ if SID Trp:Lys} < 16.6\%,$$

G:F = 0.5844 if SID Trp:Lys ≥ 16.6%.

Based on the BLQ model:

$$G{:}F = 0.5844 - 59.80 \times (0.171 - Trp{:}Lys)^2, \text{ if SID Trp:Lys} < 17.1\%,$$

$$G{:}F = 0.5844, \quad \text{if SID Trp:Lys} \geq 17.1\%.$$

The estimated SID Trp:Lys requirements for G:F (Fig. 4.4) were 16.6 (95% CI: 16.0 – 17.3) and 17.1% (95% CI: 16.6 to 17.7) based on BLL and BLQ models, respectively. Again similar to the estimated SID Trp:Lys requirements for maximum mean ADG, the confidence interval for SID Trp:Lys requirements for maximum mean G:F was narrower than that in Exp. 1.

Broken-line models and QP models can be used (through inverse prediction) to estimate the minimum level of mean requirement that maximizes average growth performance in the intended swine population. From a conceptual standpoint, both types of models may address the question of interest, but finer differences between the fit of the models become apparent when describing the relationship between growth performance and SID Trp:Lys. This illustrates the importance of objectively selecting a model based on its fit to the data (Littell et al., 2006). When developing requirement curves, the coefficient of determination R^2 traditionally has been used as the primary indicator of model fit to select among competing models (Pesti et al., 2009). However, within a mixed models framework as in our study, the calculation and interpretation of R^2 is fraught with ambiguities. First, R^2 is not uniquely defined when multiple sources of random variability are present in the data (Schabenberger and Pierce, 2002). Such multiple definitions of R^2 in the mixed models framework impair the arguably intuitive interpretation of R^2 as "proportion of variability explained by X" that has been so appealing in the animal sciences. Furthermore, attempts to calculate R^2 in the mixed models framework fail to take into account model complexity in the presence of random effects (Kvalseth, 1985). In turn, other information criteria that take into consideration the design structure of the experiment are available for model selection in the mixed model framework. These include, but are not limited to, maximum likelihood–based Akaike information criterion and Schwarz's Bayesian Criterion (Milliken and Johnson, 2009). The Schwarz's Bayesian Criterion (BIC) is often used in mixed models, and its calculation is slightly more conservative than the AIC and tends to favor more parsimonious models (Schwarz, 1978). In this study, we chose BIC as our primary indicator of model fit when assessing the competing dose-response models. A 2-point difference in BIC is usually considered indicative of improved fit of the model with lower BIC (Raftery, 1996). Our results indicate that for ADG in both early and late nursery pigs, the QP model was the single best-fitting model. In contrast, for G:F the BLL was the best-fitting model in early nursery pigs. In late nursery pigs, BLL and BLQ models had comparable BICs, indicating comparable fit to the data, such that we provided the regression equations for both models. Although the confidence intervals for SID Trp:Lys ratio requirements overlap for BLL and BLQ models, the functional forms assumed by the models induced a point estimate for BLQ that was numerically slightly higher than that of the BLL model.

In addition to considering biologic performance, nutritionists should also take into account economic considerations during diet formulation. It is generally economically unfeasible to formulate diets that meet requirements to achieve 100% of the maximized average performance, so arbitrary target levels of average performance (i.e., 95%) are commonly used in the industry (Pesti et al., 2009). Therefore, the current study also provides fitted prediction equations that can be used to estimate mean ADG and G:F based on different SID Trp:Lys requirements. The SID Trp:Lys ratio needed to achieve different target average ADG and G:F levels for nursery pigs are demonstrated in Table 4.7. Approximately 18% SID Trp:Lys ratio would be needed to achieve 95% of the maximum mean ADG and 98% of the maximum mean G:F in Exp. 1, whereas the same ratio would achieve 96% of the maximum mean ADG and

100% of the maximum mean G:F in Exp. 2. In early and late nursery pigs, 99% of maximum mean ADG was reached at 21.3% and 19.6% SID Trp:Lys, respectively. These relative differences in performance can be translated into economic terms based on localized economic conditions while taking into account the underlying biologic performance.

In general, our findings add to the body of literature in which low levels of SID Trp in the diets of pigs reduce ADFI and, consequently, reduce ADG and G:F (Simongiovanni, et al. 2012). The absorbed Trp is converted into 5-hydroxytryptophan, which is a metabolite that can cross the blood-brain barrier and work as a precursor for serotonin after being decarboxylated (Floc'h et al., 2011). As a result, the inclusion of different levels of Trp in pig diets has been shown to manipulate serotonin-mediated feed intake (Batterham, et al., 1994; Simongiovanni, et al. 2012). Although our results on SID Trp:Lys requirements for the 6- to 10-kg BW phase (Experiment 1) were inconclusive, our study indicates that for the 11- to 20-kg BW phase (Experiment 2), the SID Trp:Lys requirements for maximum mean ADG were greater than those for maximum mean G:F, which is consistent with other studies (Ma et al., 2010; Petersen, 2011). In turn, the NRC (2012) recommended SID Trp:Lys ratio is 16.3% for nursery pigs, whereas the National Swine Nutrition Guide (2010) recommended SID Trp:Lys at 16.8% for nursery pigs. It is worth noticing that neither of these two standards distinguish between requirements for ADG and requirements for G:F; in fact, these standards correspond closely with our estimates of SID Trp:Lys requirements for maximum mean G:F, but they may underestimate requirements for maximum mean ADG.

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TABLES

Table 4.1. Diet composition, Exp. 1 (as-fed basis)[1]

Item	Basal diet
Ingredient, %	
Corn	58.10
Soybean meal (46.5% CP)	25.20
Spray-dried whey	10.00
Soybean oil	1.00
Monocalcium P (21% P)	1.10
Limestone	0.90
Salt	0.35
Zinc oxide	0.25
Trace mineral premix[2]	0.15
Vitamin premix[3]	0.25
L-Lys HCl	0.533
DL-Met	0.220
L-Thr	0.230
L-Ile	0.100
L-Val	0.160
Gln	0.630
Gly	0.630
Phytase[4]	0.085
Corn starch[5]	0.123
L-Trp	---
TOTAL	100
Calculated analysis	
Standardized ileal digestible (SID) AA, %	
Lys	1.30
Ile:Lys	60
Leu:Lys	111
Met:Lys	36
Met & Cys:Lys	58
Thr:Lys	64
Trp:Lys	14.7
Val:Lys	70
Total Lys, %	1.42
ME, kcal/kg	3,341
NE, kcal/kg	2,239
SID Lysine:ME, g/Mcal	3.89
SID Lysine:NE, g/Mcal	5.27
CP, %	20.4
Ca, %	0.72
P, %	0.64

Available P, %	0.47

[1] Diets were fed from 6.8 to 9.8 kg BW.
[2] Provided per kg of diet: 39.6 mg Mn from manganese oxide, 165 mg Fe from iron sulfate, 1,965 mg Zn from zinc sulfate, 16.5 mg Cu from copper sulfate, 0.30 mg I from calcium iodate, and 0.30 mg Se from sodium selenite.
[3] Provided per kg of diet: 11,020 IU vitamin A; 1,378 IU vitamin D3; 44 IU vitamin E; 4 mg vitamin K; 8 mg riboflavin; 28 mg pantothenic acid; 50 mg niacin; and 0.039 mg vitamin B12.
[4] Phyzyme 600 (Danisco Animal Nutrition, St. Louis, MO) provided 509 FTU per kg, with a release of 0.10% available P.
[5] Feed-grade L-Trp was added at the expense of corn starch at 0, 0.024, 0.049, 0.074, 0.098, and 0.123% of the diet to provide Trp:Lys ratios of 14.7, 16.5, 18.4, 20.3, 22.1, and 24.0% to form the experimental treatments.

Table 4.2. Diet composition, Exp. 2 (as-fed basis)[1]

Item	SID Trp:Lys	
	Low (14.5%)	High (24.5%)
Ingredient, %		
Corn	55.16	55.06
Soybean meal (46% CP)	10.91	10.92
DDGS[2]	30.00	30.00
Beef tallow	0.50	0.50
Dicalcium phosphate (18.5% P)	0.50	0.50
Limestone	1.48	1.48
Salt	0.35	0.35
Trace mineral premix[3]	0.100	0.100
Vitamin premix[4]	0.125	0.125
L-Lys HCL	0.575	0.575
DL-Met	0.070	0.070
L-Thr	0.140	0.140
L-Trp	---	0.098
L-Ile	0.010	0.010
L-Val	0.060	0.060
Phytase[5]	0.025	0.025
TOTAL	100	100
Calculated analysis		
Standardized ileal digestible (SID) AA, %		
Lys	0.97	0.97
Ile:Lys	55	55
Leu:Lys	153	153
Met:Lys	35	35
Met & Cys:Lys	60	60
Thr:Lys	65	65
Trp:Lys	14.5	24.5
Val:Lys	70	70
His:Lys	38	38
Phe & Tyr:Lys	106	106
Trp:BCAA[6]	3.9	6.6
Trp:LNAA[7]	2.8	4.8
ME, kcal/kg	3,325	3,328
NE, kcal/kg	2,466	2,468
SID Lysine:ME, g/Mcal	2.91	2.91
SID Lysine:NE, g/Mcal	3.93	3.93
CP, %	18.1	18.2
Ca, %	0.71	0.71
P, %	0.49	0.49
Available P, %	0.40	0.40

[1] Diets were fed from 11.2 to 20.3 kg BW. Corn, dried distillers grains with solubles (DDGS) and soybean meal were analyzed for CP and total amino acid content and NRC (2012) SID digestibility values were used in the diet formulation.
[2] Dried distillers grains with solubles.
[3] Provided per kg of diet: 33 mg Mn from manganese oxide, 110 mg Fe from iron sulfate, 110 mg Zn from zinc oxide, 16.5 mg Cu from copper sulfate, 0.33 mg I from ethylenediamin dihydroiodide, and 0.30 mg Se from sodium selenite.
[4] Provided per kg of diet: 5,290 IU vitamin A; 827 IU vitamin D3; 26.4 IU vitamin E; 2.64 mg vitamin K; 16.5 mg pantothenic acid; 30 mg niacin; 4.6 mg riboflavin and 0.02 mg vitamin B12.
[5] OptiPhos 2000 (Enzyvia LLC, Sheridan, IN) provided 1,251 FTU per kg of diet with a release of 0.13% available P.
[6] Amount of Trp in the diet as a ratio to branched-chain AA (BCAA; Ile, Leu, Val) on SID basis.
[7] Amount of Trp in the diet as a ratio to large neutral AA (LNAA; Ile, Leu, Val, Phe, and Tyr) on SID basis.

Table 4.3. Chemical analysis of the diets, Exp. 1 (as-fed-basis)[1]

	SID Trp:Lys, %					
Item	14.7	16.5	18.4	20.3	22.1	24.0
Proximate analysis, %						
DM	91.07 (89.43)[2]	91.06 (89.43)	91.23 (89.43)	91.12 (89.43)	91.17 (89.43)	91.21 (89.43)
CP	20.2 (20.4)	20.5 (20.4)	20.3 (20.4)	20.4 (20.4)	20.6 (20.5)	21.0 (20.5)
CF	2.2 (2.3)	2.2 (2.3)	2.3 (2.3)	2.3 (2.3)	2.4 (2.3)	2.3 (2.3)
Ca	0.87 (0.72)	0.85 (0.72)	0.76 (0.72)	0.84 (0.72)	0.86 (0.72)	0.87 (0.72)
P	0.69 (0.64)	0.63 (0.64)	0.67 (0.64)	0.70 (0.64)	0.73 (0.64)	0.77 (0.64)
Fat	3.3 (3.7)	3.2 (3.7)	3.4 (3.7)	3.1 (3.7)	3.2 (3.7)	3.2 (3.7)
Ash	6.04 (5.54)	5.93 (5.54)	5.71 (5.54)	5.81 (5.54)	6.10 (5.54)	6.11 (5.54)
Amino acids, %						
Lys	1.43 (1.42)	1.43 (1.42)	1.42 (1.42)	1.38 (1.42)	1.37 (1.42)	1.42 (1.42)
Ile	0.90 (0.87)	0.95 (0.87)	0.93 (0.87)	0.94 (0.87)	0.91 (0.87)	0.93 (0.87)
Leu	1.61 (1.61)	1.63 (1.61)	1.60 (1.61)	1.60 (1.61)	1.53 (1.61)	1.60 (1.61)
Met	0.50 (0.50)	0.46 (0.50)	0.49 (0.50)	0.47 (0.50)	0.49 (0.50)	0.49 (0.50)
Met & Cys	0.82 (0.82)	0.78 (0.82)	0.81 (0.82)	0.78 (0.82)	0.80 (0.82)	0.81 (0.82)
Thr	0.95 (0.93)	0.97 (0.93)	0.95 (0.93)	0.94 (0.93)	0.94 (0.93)	0.95 (0.93)
Trp	0.22 (0.22)	0.23 (0.24)	0.24 (0.26)	0.27 (0.29)	0.30 (0.31)	0.30 (0.34)
Val	1.07 (1.02)	1.05 (1.02)	1.04 (1.02)	1.05 (1.02)	1.03 (1.02)	1.05 (1.02)
His	0.47 (0.48)	0.46 (0.48)	0.46 (0.48)	0.45 (0.48)	0.44 (0.48)	0.46 (0.48)
Phe	0.92 (0.86)	0.92 (0.86)	0.91 (0.86)	0.90 (0.86)	0.88 (0.86)	0.89 (0.86)

[1] Diet samples were collected from feeder at the beginning of the trial and on d 7 and 14. At the end of the trial, samples of each diet were combined and a composite sample was analyzed for total AA analysis by Ajinomoto Heartland Inc. (Chicago, IL). Samples of the diets were also submitted to Ward Laboratories, Inc. (Kearney, NE) for analysis of DM, CP, CF, Ca, P, ash and crude fat.

[2] Values in parentheses indicate those calculated from diet formulation and are based on values from NRC, 1998 (Nutrient Requirements of Swine, 11th ed. Natl. Acad. Press, Washington DC).

Table 4.4. Chemical analysis of the diets, Exp. 2 (as-fed-basis)[1]

	SID Trp:Lys, %						
Item	14.5	16.5	18.0	19.5	21.0	22.5	24.5
Proximate analysis, %							
DM	90.48 (88.26)[2]	90.06 (88.27)	90.21 (88.27)	90.25 (88.27)	90.35 (88.27)	89.91 (88.27)	89.78 (88.28)
CP	19.0 (18.1)	19.4 (18.2)	18.8 (18.2)	18.7 (18.2)	18.9 (18.2)	19.1 (18.2)	18.2 (18.2)
CF	3.8 (4.2)	3.8 (4.2)	4 (4.2)	3.9 (4.2)	3.5 (4.2)	3.8 (4.2)	4.0 (4.2)
Ca	0.88 (0.71)	0.93 (0.71)	0.97 (0.71)	1.11 (0.71)	1.04 (0.71)	1.10 (0.71)	1.25 (0.71)
P	0.52 (0.49)	0.52 (0.49)	0.55 (0.49)	0.54 (0.49)	0.52 (0.49)	0.53 (0.49)	0.54 (0.49)
Fat	4.8 (5.2)	4.7 (5.2)	4.9 (5.2)	4.9 (5.2)	4.7 (5.2)	4.7 (5.2)	4.7 (5.2)
Ash	4.89 (4.73)	4.75 (4.73)	4.82 (4.73)	5.39 (4.72)	5.35 (4.72)	5.18 (4.72)	5.57 (4.72)
Amino acids, %							
Lys	1.19 (1.13)	1.18 (1.13)	1.22 (1.13)	1.22 (1.13)	1.17 (1.13)	1.16 (1.13)	1.19 (1.13)
Ile	0.73 (0.65)	0.75 (0.65)	0.75 (0.65)	0.75 (0.65)	0.74 (0.65)	0.76 (0.65)	0.77 (0.65)
Leu	1.82 (1.74)	1.86 (1.74)	1.86 (1.74)	1.87 (1.74)	1.85 (1.74)	1.90 (1.74)	1.89 (1.74)
Met	0.40 (0.39)	0.39 (0.39)	0.40 (0.39)	0.40 (0.39)	0.40 (0.39)	0.39 (0.39)	0.40 (0.39)
Met & Cys	0.70 (0.70)	0.71 (0.70)	0.72 (0.70)	0.71 (0.70)	0.72 (0.70)	0.72 (0.70)	0.73 (0.70)
Thr	0.82 (0.78)	0.81 (0.78)	0.83 (0.78)	0.81 (0.78)	0.83 (0.78)	0.80 (0.77)	0.81 (0.77)
Trp	0.19 (0.17)	0.19 (0.19)	0.19 (0.21)	0.20 (0.22)	0.23 (0.24)	0.23 (0.25)	0.24 (0.27)
Val	0.93 (0.83)	0.96 (0.83)	0.96 (0.83)	0.96 (0.83)	0.95 (0.83)	0.96 (0.83)	0.96 (0.83)
His	0.47 (0.44)	0.48 (0.44)	0.48 (0.44)	0.48 (0.44)	0.47 (0.44)	0.49 (0.44)	0.49 (0.44)
Phe	0.88 (0.81)	0.91 (0.81)	0.90 (0.81)	0.91 (0.81)	0.90 (0.81)	0.93 (0.81)	0.93 (0.81)

[1] Diet samples were taken from 6 feeders per dietary treatment 3 d after the beginning of the trial and 3 d prior to the end of the trial and stored at -20°C, then CP and amino acid analysis was conducted on composite samples by Ajinomoto Heartland Inc. Samples of the diets were also submitted to Ward Laboratories, Inc. (Kearney, NE) for analysis of DM, CF, Ca, P, ash and crude fat.

[2] Values in parentheses indicate those calculated from diet formulation and are based on values from NRC, 2012 (Nutrient Requirements of Swine, 11th ed. Natl. Acad. Press, Washington DC) with the exception of CP and total AA content from corn, soybean-meal, and DDGS which were analyzed prior to diet formulation by Ajinomoto Heartland Inc. (Chicago, IL).

Table 4.5. Least square mean estimates (± SEM) for growth performance of 6- to 10-kg nursery pigs fed dietary treatments consisting of standardized ileal digestible (SID) Trp:Lys ratios ranging from 14.7 to 24.0% (Exp. 1)[1]

	SID Trp:Lys, %						Probability, *P* <	
	14.7	16.5	18.4	20.3	22.1	24.0	Linear	Quadratic
ADG, g	226 ± 14.1	244 ± 8.5	244 ± 8.5	266 ± 14.1	258 ± 14.1	260 ± 8.5	0.022	0.484
ADFI, g	325 ± 11.6	342 ± 11.6	342 ± 11.6	349 ± 11.6	341 ± 11.6	363 ± 11.6	0.057	0.939
G:F	0.718 ± 0.011	0.697 ± 0.011	0.694 ± 0.011	0.750 ± 0.011	0.751 ± 0.011	0.716 ± 0.011	0.012	0.500
BW, kg								
d 0	6.3 ± 0.06	6.3 ± 0.06	6.2 ± 0.06	6.3 ± 0.06	6.2 ± 0.06	6.3 ± 0.06	0.753	0.870
d 14	9.4 ± 0.19	9.7 ± 0.19	9.7 ± 0.09	10.0 ± 0.19	9.9 ± 0.19	9.9 ± 0.19	0.052	0.294
SID Trp intake, g/d	0.621 ± 0.029	0.736 ± 0.029	0.818 ± 0.029	0.917 ± 0.029	0.978 ± 0.029	1.132 ± 0.029	0.001	0.687
SID Trp g/kg gain	2.8 ± 0.11	3.0 ± 0.06	3.4 ± 0.06	3.5 ± 0.11	3.8 ± 0.11	4.4 ± 0.11	0.001	0.075

[1]A total of 255 nursery pigs (PIC 327 × 1050, initially 6.3 kg and 3 d postweaning) were used in a 14-d trial with 6 to 7 pigs per pen and 7 pens per treatment.
[2]Diets were formulated to 1.30% SID Lys based on data of Nemechek et al. (2011).

Table 4.6. Least square mean estimates (±SEM) for growth performance of 11- to 20-kg nursery pigs fed dietary treatments of standardized ileal digestible (SID) Trp:Lys ratio ranging from 14.5 to 24.5% (Exp. 2)[1,2]

	SID Trp:Lys, %							Probability, $P <$	
Item	14.5	16.5	18.0	19.5	21.0	22.5	24.5	Linear	Quadratic
d 0 to 21									
ADG, g	369 ± 20.2	428 ± 20.2	442 ± 20.2	432 ± 20.2	453 ± 17.6	451 ± 17.6	435 ± 17.6	0.001	0.001
ADFI, g	682 ± 35.2	735 ± 31.1	759 ± 35.2	749 ± 35.2	768 ± 31.1	773 ± 31.1	750 ± 31.1	0.001	0.006
G:F	0.543 ± 0.008	0.582 ± 0.005	0.582 ± 0.005	0.578 ± 0.008	0.590 ± 0.005	0.584 ± 0.005	0.580 ± 0.008	0.002	0.002
BW, kg									
d 0	11.3 ± 0.55	11.3 ± 0.55	11.2 ± 0.55	11.2 ± 0.55	11.2 ± 0.55	11.3 ± 0.55	11.2 ± 0.55	0.844	0.952
d 21	19.0 ± 0.87	20.2 ± 0.87	20.7 ± 0.87	20.3 ± 0.94	20.8 ± 0.87	20.7 ± 0.87	20.4 ±0.87	0.001	0.001
SID Trp intake, g/d	0.959 ± 0.069	1.176 ± 0.063	1.325 ± 0.069	1.417 ± 0.069	1.564 ± 0.063	1.686 ± 0.063	1.783 ± 0.063	0.001	0.026
SID Trp, g/kg gain	2.6 ± 0.04	2.8 ± 0.02	3.0 ± 0.02	3.3 ± 0.04	3.5 ± 0.04	3.7 ± 0.04	4.1 ± 0.04	0.001	0.006

[1] A total of 1,088 pigs (PIC 337 × 1050, initially 11.2 kg BW and 28 d postweaning) were used in a 21-d growth trial with 24 to 27 pigs per pen and 6 pens per treatment.
[2] The NRC (2012) model was used to determine the Lys requirement of mixed gender pens of pigs at the end of the BW range (22.7 kg) and that value was reduced by 0.10 percentage point.

Table 4.7. Standardized ileal digestible (SID) Trp:Lys ratio at different target performance levels of nursery pigs[1]

	Percent of maximum performance, %					
Item	95%	96%	97%	98%	99%	100%
Exp. 1 (6- to 10-kg BW pigs)						
ADG						
QP	18.1	18.7	19.4	20.2	21.3	23.9
G:F						
BLL	14.3	15.5	16.7	18.0	19.2	20.4
Exp. 2 (11- to 20-kg BW pigs)						
ADG						
QP	17.6	18.0	18.4	18.9	19.6	21.2
G:F						
BLL	15.1	15.4	15.7	16.0	16.3	16.6
BLQ	14.9	15.1	15.4	15.7	16.1	17.1

[1]Derived from equations presented in the text.

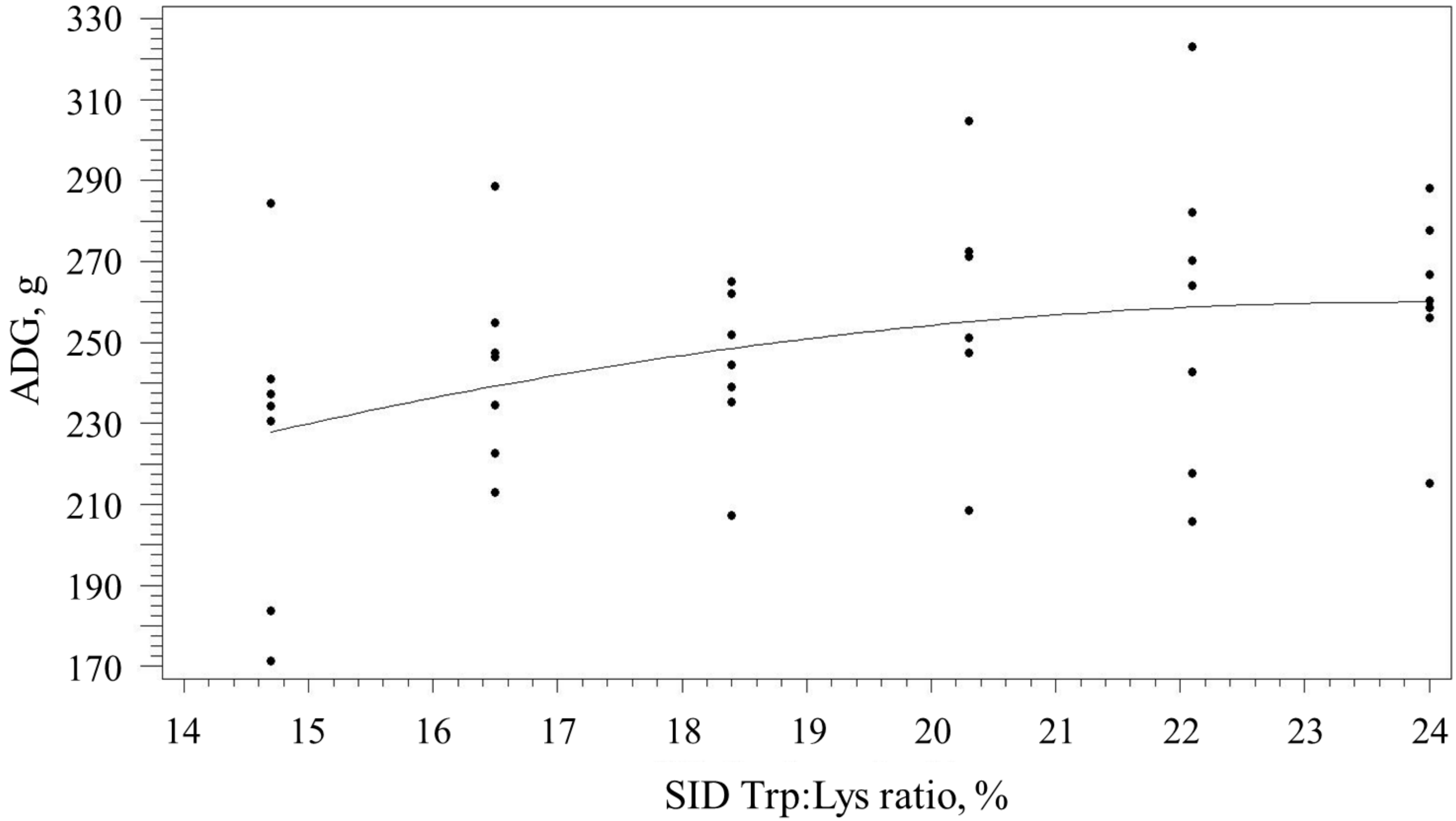

Figure 4.1. Fitted quadratic polynomial (QP) regression model on ADG as a function of increasing standardized ileal digestible (SID) Trp:Lys in 6- to 10-kg pigs (Exp. 1). The maximum mean ADG was estimated at 23.9% (95% CI: [<14.5, >24.5]%) SID Trp:Lys.

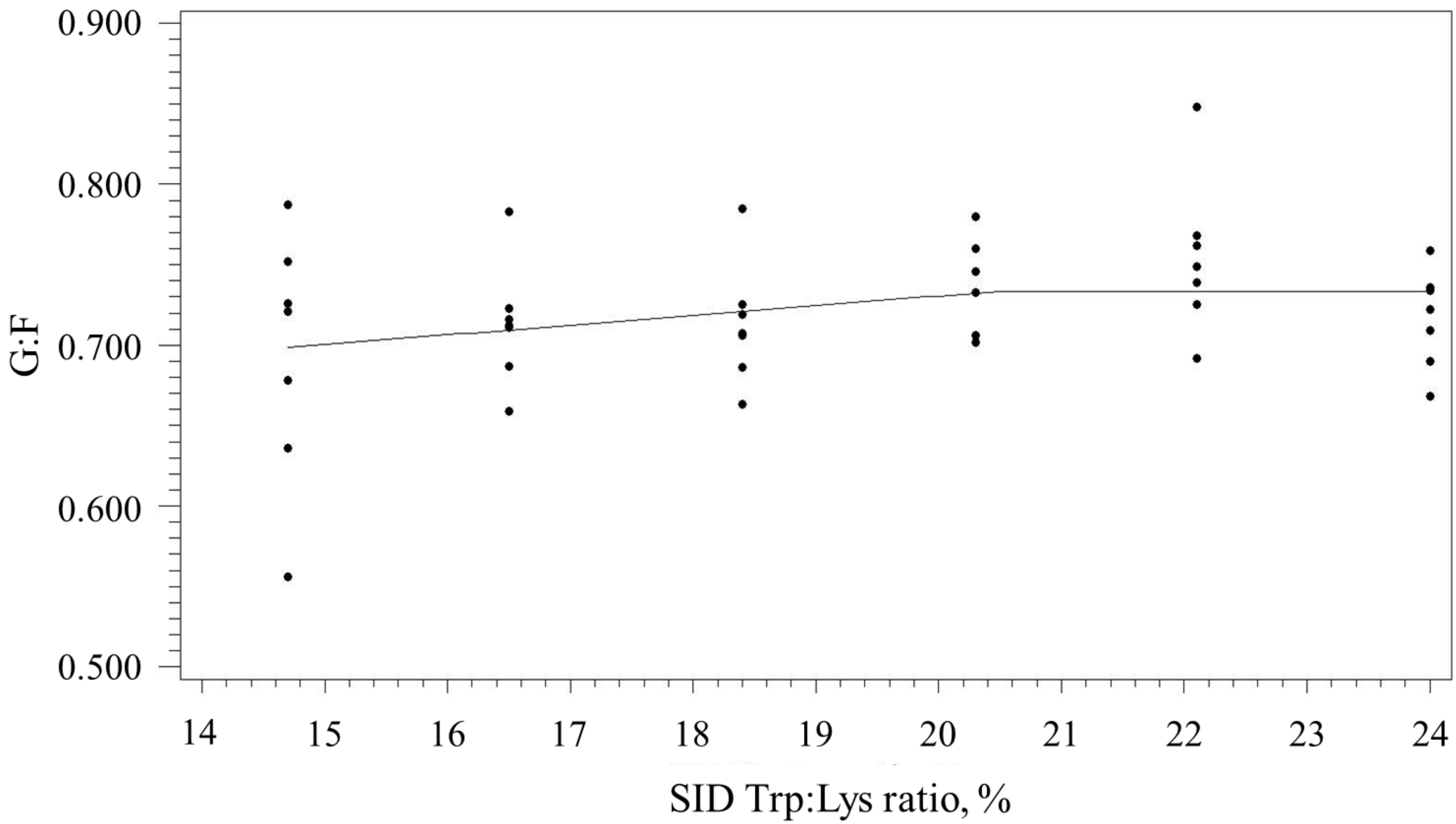

Figure 4.2. Fitted broken-line linear (BLL) regression model on G:F as a function of increasing standardized ileal digestible (SID) Trp:Lys in 6- to 10-kg pigs (Exp. 1). The maximum mean G:F was estimated at 20.4% (95% CI: [14.3, 26.5]%).

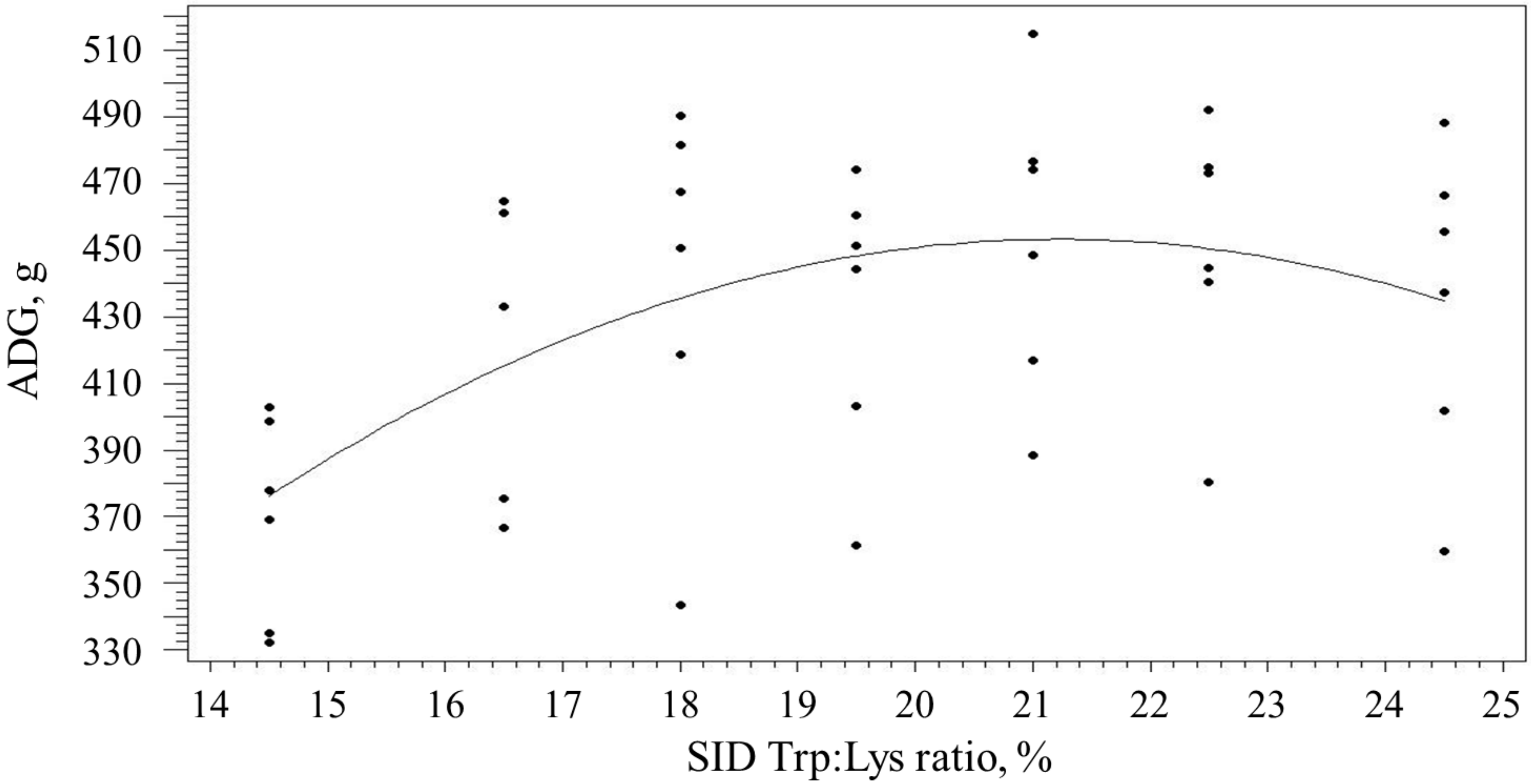

Figure 4.3. Fitted quadratic polynomial (QP) regression model on ADG as a function of increasing standardized ileal digestible (SID) Trp:Lys in 11- to 20-kg pigs (Exp 2.). The maximum mean ADG was estimated at 21.2 (95% CI: [20.5, 21.9]%) SID Trp:Lys.

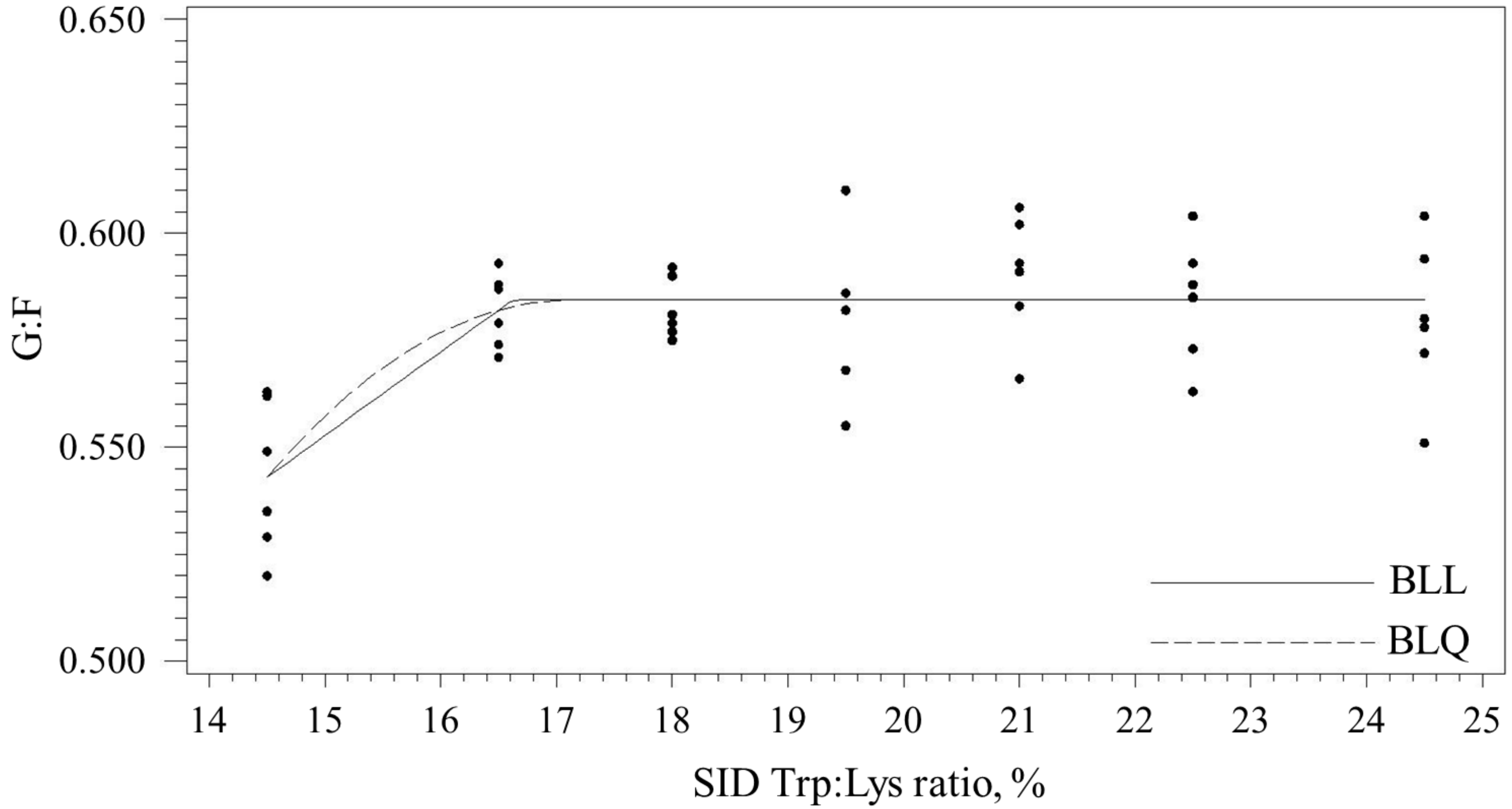

Figure 4.4. Fitted broken-line linear (BLL) and broken-line quadratic (BLQ) regression models on G:F as a function of increasing standardized ileal digestible (SID) Trp:Lys in 11- to 20-kg pigs (Exp 2.). The maximum mean G:F was estimated at 16.6 % (95% CI: [16.0, 17.3]%) and 17.1% (95% CI: [16.6, 17.7]%) SID Trp:Lys in the BLL and BLQ models, respectively.

Chapter 5 - Effect of standardized ileal digestible tryptophan:lysine ratio on growth performance of finishing gilts under commercial conditions[12,13]

M. A. D. Gonçalves*, M. D. Tokach†[14], S. S. Dritz*, N. M. Bello‡, K. J. Touchette§, R. D. Goodband†, J. M. DeRouchey†, and J. C. Woodworth†

*****Department of Diagnostic Medicine/Pathobiology, College of Veterinary Medicine, †Department of Animal Sciences and Industry, College of Agriculture, ‡Department of Statistics, College of Arts and Sciences, Kansas State University, Manhattan, KS 66506-0201, and **§** Ajinomoto Heartland Inc., Chicago, IL.

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[14] Corresponding author: mtokach@k-state.edu

ABSTRACT: A study was conducted to estimate the standardized ileal digestible (**SID**) Trp:Lys ratio requirement for growth performance of finishing gilts under commercial conditions. Dietary treatments consisted of SID Trp:Lys ratios of 14.5, 16.5, 18.0, 19.5, 21.0, 22.5, and 24.5%. The study was conducted in 4 experimental rounds of 21 d of duration each, and used corn-soybean meal-based diets with 30% DDGS formulated to be deficient in Lys at the end of each experiment. A total of 1,166, 1,099, 1,132, and 975 gilts (PIC 337 × 1050, initially 29.9 ± 2.0, 55.5 ± 4.8, 71.2 ± 3.4, and 106.2 ± 3.1 kg BW, mean ± SD) were used in experimental rounds 1 to 4, respectively. Within each round, pens of gilts were blocked by BW and assigned to one of the 7 dietary treatments in a randomized complete block design. Each experimental round consisted of 6 pens/treatment with 20 to 28 gilts per pen. First, a linear mixed model was fitted to data from each experimental round to characterize performance. Next, data of experimental rounds were combined to fit competing dose-response linear and non-linear models and estimate SID Trp:Lys ratio requirements for performance. Competing models included broken-line linear (BLL), broken-line quadratic (BLQ), and quadratic polynomial (QP). For each response, the best fitting model was selected using Bayesian information criterion. Increasing SID Trp:Lys ratio increased ADG in a quadratic manner ($P < 0.022$) in all rounds except 2, for which the increase was linear ($P < 0.001$). Increasing SID Trp:Lys ratio increased ($P < 0.049$) G:F quadratically in experimental rounds 1 and 3, linearly ($P < 0.024$) in round 4, and cubically in round 2 ($P < 0.002$). For maximum mean ADG, QP was the best fitting dose-response model and estimated SID Trp:Lys ratio at 23.5% (95% CI: [22.7, 24.3%]). For maximum mean G:F, BLL and BLQ dose-response models had comparable fit and estimated SID Trp:Lys ratio at 16.9 (95% CI: [16.0, 17.8%]) and 17.0% (95% CI: [15.0, 18.9%]), respectively. Thus, the estimated SID Trp:Lys ratio for 30- to 125-kg gilts ranged from 16.9%

for maximum estimated mean G:F to 23.5% for maximum estimated mean ADG. Furthermore, 95% of the maximum estimated mean ADG was obtained feeding 17.6% SID Trp:Lys ratio and 98% of the maximum estimated mean ADG was obtained feeding 19.8% SID Trp:Lys ratio.

Key words: amino acid ratio, finishing pig, growth, lysine, tryptophan

INTRODUCTION

With the increasing usage of dried distillers grains (**DDGS**) and feed-grade AA in commercial swine diets during the last decade, Trp went from being the fourth limiting AA (Naatjes et al., 2014) in corn-soybean meal based diets to being the second or third limiting AA in diets with DDGS (Johnson et al., 2013). Tryptophan plays a role in a wide range of functions besides protein synthesis, having a large impact on feed intake regulation by manipulation of ghrelin and serotonin pathways (Le Floc'h et al., 2011).

The AA requirements of pigs can be expressed in various ways (Stein et al., 2007), though probably one of the most practical approaches for diet formulation is the expression of the standardized ileal digestible (**SID**) Trp requirement as a ratio to Lys (**Trp:Lys**). The NRC (2012) estimates the SID Trp:Lys ratio requirement for finishing gilts at 17.4%. However, recent studies suggest requirement estimates ranging from 16.5 to 23.6% SID Trp:Lys ratio for finishing pigs (Simongiovanni et al., 2012; Zhang et al., 2012; Salyer et al., 2013). These studies indicate the requirement may be significantly higher than suggested by NRC (2012). Furthermore, to accurately determine the SID Trp:Lys ratio requirement, Lys must also be limiting. Otherwise, the SID Trp:Lys ratio requirement estimate will be underestimated (Susenbeth and Lucanus, 2005; Susenbeth, 2006). Additionally, the AA requirement estimation is likely to depend on the statistical model used and on the response variable selected (Baker et al., 1986; Robbins et al., 2006; Simongiovanni et al., 2012; Naatjes et al., 2014). The current body of literature lacks a study in which results can be applied to finishing gilts across a range of BW and lacks use of best fitting statistical mixed models that take into account the experimental design structure to estimate the requirement for the different response variables. Therefore, the objective of this study was to determine the SID Trp:Lys ratio requirement for ADG and G:F in 30- to 125-kg gilts under commercial conditions.

MATERIALS AND METHODS

General

The Kansas State University Institutional Animal Care and Use Committee approved the protocol used in these experiments. All experiments were conducted at a commercial research finishing complex in southwestern Minnesota. The barns were naturally ventilated and double-curtain sided. Pens had completely slatted flooring and deep pits for manure storage. Each pen (5.5 × 3.0 m) was equipped with a 4-hole stainless steel dry self-feeder (Thorp Equipment, Thorp, WI) and a cup waterer. Each barn was equipped with a computerized feeding system (FeedPro; Feedlogic Corp., Willmar, MN) that delivered and recorded daily feed additions and diets as specified. This system is capable of feeding each individual pen any of the individual diets as well as a blend of two diets. The equipment provided gilts with ad libitum access to feed and water.

Animals and diets

A growth experiment was conducted in four 21-d-long experimental rounds consisting of a total of 1,166, 1,099, 1,132, and 975 gilts (337 × 1050; PIC Hendersonville, TN) with initial

BW of 29.9 ± 2.0, 55.5 ± 4.7, 71.2 ± 3.2, and 106.2 ± 3.1 kg and final BW of 45.6 ± 2.7, 75.0 ± 5.1, 91.2 ± 3.4, and 124.7 ± 4.7 (mean ± SD) in experimental rounds 1, 2, 3 and 4, respectively. Experimental rounds 1 and 3 were conducted with a single group of gilts and fed a common diet with 20% SID Trp:Lys ratio for 32 d between studies. Each experimental round had 6 pens per treatment with 20 to 28 gilts per pen.

Five representative samples of corn, soybean meal, and DDGS were collected each week for 5 wk and analyzed in duplicate for total AA (except Trp; method 994.12; AOAC Int., 2012), Trp (method 13904:2005; ISO, 2005), and CP (method 990.03; AOAC Int., 2012) by Ajinomoto Heartland Inc. (Chicago, IL), and values were used in diet formulation. Other nutrients and SID AA digestibility coefficient values used for diet formulation were obtained from NRC (2012).

Two experimental corn-soybean meal-based diets with 30% DDGS were formulated for each of the experiments (Table 5.1) to be limiting in Lys and have SID Trp:Lys ratios of 14.5 or 24.5%. These diets were blended using the robotic feeding system to achieve dietary treatments of intermediate SID Trp:Lys ratios. The proportion of low and high SID Trp:Lys blended to create the treatment diets were 100 and 0, 80 and 20, 65 and 35, 50 and 50, 35 and 65, 20 and 80, and 0 and 100% for 14.5, 16.5, 18.0, 19.5, 21.0, 22.5, and 24.5% SID Trp:Lys ratios, respectively. The SID Trp:Lys ratio was increased by adding crystalline L-Trp to the control diet at the expense of corn. The NRC (2012) model was used to estimate the Lys requirement of gilts at the expected BW at the end of each experimental round. The SID Lys as a percentage of the diet was reduced by 0.05 to 0.10 percentage points below the requirement at the expected BW at the end of each experimental round to ensure that Lys was the second limiting amino acid throughout the experiment. This reduction was based on results of a preliminary study conducted by Gonçalves et al. (2014) in the same commercial research finishing complex. Diets were fed in meal form and were manufactured at the New Horizon Farms feed mill (Pipestone, MN).

In each experimental round, diet samples were taken from 6 feeders per dietary treatment 3 d after the beginning and 3 d before the end of each experiment and stored at -20°C, then total AA and CP analysis (conducted with the same methods previously described) were conducted in duplicate on composite samples of each treatment by Ajinomoto Heartland Inc. Additionally, diet samples were also submitted for analysis of DM (method 935.29; AOAC Int., 2012), crude fiber (method 978.10; AOAC Int., 2012 for preparation and Ankom 2000 Fiber Analyzer [Ankom Technology, Fairport, NY]), ash (method 942.05; AOAC Int., 2012), ether extract (method 920.39 a; AOAC Int., 2012 for preparation and ANKOM XT20 Fat Analyzer [Ankom Technology, Fairport, NY], Ca, and P (method 968.08 b; AOAC Int., 2012 for preparation using ICAP 6500 [ThermoElectron Corp., Waltham, MA], Ward Laboratories, Inc. Kearney, NE).

Data collection

Pig BW and feed disappearance were measured on d 0 and 21 of each experiment to calculate ADG, ADFI, G:F, g of SID Trp daily intake, and g of SID Trp intake per kg of gain. Total g of SID Trp daily intake was calculated based on formulated values by multiplying ADFI by SID Lys level by SID Trp:Lys ratio. The total g of SID Trp intake were divided by total BW gain to calculate the g of SID Trp intake per kg of gain.

Statistical analysis

As a first step, responses of interest (ADG, ADFI, G:F, BW, g of SID Trp daily intake, and g of SID Trp intake per kg of gain) measured at the pen level were each analyzed separately for each experimental round using a linear mixed model to accommodate the randomized complete block design structure of each round. These initial analyses were used to characterize performance as a function of dietary treatments consisting of increasing SID Trp:Lys ratios. For these analyses, the linear predictor included the fixed effect of dietary treatment presented as a factor and initial average pen BW as a random blocking factor. Pen was the experimental unit. Orthogonal linear, quadratic and cubic polynomial contrasts were built using coefficients adjusted for unequally spaced treatments and were used to evaluate the functional form of the dose-response to increasing dietary SID Trp:Lys ratio on ADG, ADFI, G:F, BW, g of SID Trp daily intake, and g of SID Trp intake per kg of gain. Heterogeneous residual variances as a function of treatment combinations were fitted as needed. Model assumptions were checked using studentized residuals and were considered to be appropriately met. Degrees of freedom were estimated using the Kenward-Roger's method (Kenward and Roger, 1997). Statistical models were fitted using the GLIMMIX procedure of SAS (Version 9.3, SAS Institute Inc., Cary, NC). Results were considered significant at $P \leq 0.05$ and marginally significant at $0.05 < P \leq 0.10$.

Next, data from all 4 experimental rounds were combined and linear and non-linear regression models adapted from Robbins et al. (2006) and Pesti et al. (2009) were fitted to ADG and G:F to further estimate SID Trp:Lys ratio requirements using an inverse prediction strategy. Specifically, competing statistical models fitted to the data were: a broken-line linear ascending (BLL) model, a broken-line quadratic ascending (BLQ) model, and a quadratic polynomial (QP). Models were expanded to consider 1) random effects of experiment, block nested within experiment, and the crossproduct of treatment by experiment, as well as 2) linear and quadratic effects of initial BW as explanatory covariates. Competing models described growth performance in relation to SID Trp:Lys ratio, adapted from Robbins et al. (2006) and Gonçalves et al. (2015), as follows:

Quadratic polynomial (QP) model:

$$y_{ijk} = \beta_0 + \beta_1 X_i + \beta_2 X_i^2 + \beta_3 W_{ijk} + \beta_4 W^2_{ijk} + t_j + b_{k(j)} + (tX)_{ij} + e_{ijk},$$

Broken-line linear (BLL) ascending model:

$$y_{ijk} = L_{BLL} + U_l \times (R_{BLL} - X_i) + \beta_3 W_{ijk} + \beta_4 W^2_{ijk} + t_j + b_{k(j)} + (tX)_{ij} + e_{ijk} \quad \text{for } X_i < R_{BLL} \text{ and}$$

$$y_{ijk} = L_{BLL} + \beta_3 W_{ijk} + \beta_4 W^2_{ijk} + t_j + b_{k(j)} + (tX)_{ij} + e_{ijk} \quad \text{for } X_i \geq R_{BLL},$$

Broken-line quadratic (BLQ) ascending model:

$$y_{ijk} = L_{BLQ} + U_{q1} \times (R_{BLQ} - X_i) + U_{q2} \times (R_{BLQ} - X_i)^2 + \beta_3 W_{ijk} + \beta_4 W^2_{ijk} + t_j + b_{k(j)} + (tX)_{ij} + e_{ijk} \quad \text{for } X_i < R_{BLQ} \text{ and}$$

$$y_{ijk} = L_{BLQ} + \beta_3 W_{ijk} + \beta_4 W^2_{ijk} + t_j + b_{k(j)} + (tX)_{ij} + e_{ijk} \quad \text{for } X_i \geq R_{BLQ},$$

where y_{ijk} is the response associated with a pen assigned to dietary treatment i within BW block k of experiment j; X_i is the SID Trp:Lys ratio of the i^{th} dietary treatment; W_{ijk} is the initial BW associated with ijk^{th} observation; L_{BLL} and L_{BLQ} indicate the unknown maximum response to dietary treatments (i.e. plateau) under BLL and BLQ models, respectively; R_{BLL} and R_{BLQ} are the unknown minimum levels of SID Trp:Lys ratio requirement to reach the plateau under the BLL and BLQ models, respectively; β_0 is the QP model intercept; U_l, U_{q1}, U_{q2}, β_1, and β_2 are the corresponding unknown coefficients characterizing change of the response as a function of X_i; β_3 and β_4 are the corresponding unknown linear and quadratic coefficients on W_{ijk}; t_j is the random effect associated with the j^{th} experiment assumed $NIID(0, \sigma_t^2)$; $b_{k(j)}$ is the random effect of the k^{th}

block within the j^{th} experiment assumed $NIID(0, \sigma_b^2)$; $(tX)_{ij}$ is the random effect of the i^{th} treatment implemented for the j^{th} experiment and assumed $NIID(0, \sigma_{tX}^2)$; and, e_{ijk} is a random error associated with the ijk^{th} observation such that $e_{ijk} \sim NIID(0, \sigma_e^2)$. Finally, we assume all random and residual effects to be mutually independent.

Broken-line regression models were fitted using the NLMIXED procedures of SAS. The optimization technique used was the dual Quasi-Newton algorithm, as specified by default in the NLMIXED procedure. Competing statistical models were compared using maximum-likelihood-based fit criteria, specifically the Bayesian information criteria (BIC; Milliken and Johnson, 2009). Results reported here correspond to inference yielded by the best fitting model for ADG and G:F data combined across experimental rounds.

For the best-fitting models, the estimated requirement of SID Trp:Lys ratio of ADG and G:F to reach plateau performance (i.e., R_{BLL} and R_{BLQ} in the broken-line models) or to reach maximum performance (i.e., in the QP) are reported with a 95% confidence interval. In the QP model, the level of SID Trp:Lys ratio that maximized the response variable was estimated by equating the first derivative of the regression equation to zero, then solving for the SID Trp:Lys ratio (Pesti et al., 2009). The corresponding 95% confidence intervals were computed using the inverse regression approach proposed by Lavagnini and Magno (2006).

RESULTS

The analyzed nutrient and total AA content of experimental diets for experimental rounds 1, 2, 3, and 4 (Tables 5.2, 5.3, 5.4, and 5.5, respectively) were reasonably consistent with calculated values. Gilts consumed a total of 17.7, 19.3, 18.3, and 19.6 g of SID Lys per kg of gain in experimental rounds 1 to 4, respectively. These levels were all lower than the g/kg estimate requirements of Main et al. (2008) confirming that Lys was limiting.

Characterization of growth performance of finishing gilts

Increasing SID Trp:Lys ratios increased ADG in a quadratic manner ($P < 0.022$) in all experimental rounds (Table 5.6) except round 2, for which the increase was linear ($P < 0.001$). Increasing SID Trp:Lys ratios increased ADFI in a quadratic manner ($P < 0.017$) in experimental round 1 and linearly ($P < 0.001$) in round 2. There was no evidence for treatment differences ($P > 0.610$) in ADFI in round 3, and ADFI was marginally increased in a quadratic manner ($P < 0.073$) in round 4 as SID Trp:Lys ratio increased.

Increasing SID Trp:Lys ratios increased ($P < 0.049$) G:F quadratically in experimental rounds 1 and 3, linearly ($P < 0.024$) in rounds 4, and cubically in round 2 ($P < 0.002$). Final BW increased linearly ($P < 0.030$) in response to increasing SID Trp:Lys ratios in all experiments except round 1, where the increase in final BW was in a quadratic manner ($P < 0.017$).

Increasing SID Trp:Lys ratios increased g of SID Trp daily intake linearly ($P < 0.001$) in all experiments. Increasing SID Trp:Lys ratios increased g of SID Trp intake per kg of gain linearly ($P < 0.001$) in all experiments except round 3, where the increase was in a quadratic manner ($P < 0.005$).

Estimation of SID Trp:Lys ratio requirements

When dose-response models were fitted to the response ADG, the QP model had the best fit (BIC: 1655.4) whereas BLL and BLQ models showed poorer fit (BIC: 1668.7 and 1659.8, respectively). The overall estimated SID Trp:Lys ratio requirement for ADG was 23.5% (95% CI: [22.7, 24.3%]) based on the QP dose-response model (Fig. 5.1), fitted as follows:

QP predictive equation for ADG = (– 0.329) + 6.3 × (SID Trp:Lys ratio) – 13.5 × (SID Trp:Lys ratio)^2 + 0.015 × (Initial BW, kg) – 0.000098 × (Initial BW, kg)^2,

where the Trp:Lys ratio is expressed in decimal form (i.e., 0.180) rather than as a percentage (i.e., 18.0%).

For G:F, BLL and BLQ had comparable fit (BIC: 1316.3 and 1316.1, respectively) whereas the QP model showed poorer fit (BIC: 1322.6). The estimated SID Trp:Lys ratio breakpoint for G:F were 16.9 (95% CI: [16.0, 17.8%]) and 17.0% (95% CI: [15.0, 18.9%]) for BLL and BLQ models, respectively (Fig. 5.2):

BLL predictive equation for G:F = 0.599 – 1.0 × (0.169 – SID Trp:Lys ratio) – 0.004 × (Initial BW, kg) + 0.000017 × (Initial BW, kg)^2 if SID Trp:Lys ratio < 16.9%; otherwise G:F was predicted at a maximum plateau value dependent on initial BW.

BLQ predictive equation for G:F = 0.6014 – 0.603 × (0.170 – SID Trp:Lys ratio) – 20.0 × (0.170 – SID Trp:Lys ratio)^2 – 0.004 × (Initial BW, kg) + 0.000017 × (Initial BW, kg)^2 if SID Trp:Lys ratio < 17.0%; otherwise G:F was predicted at a maximum plateau value dependent on initial BW.

DISCUSSION

The goal of this study was to estimate the SID Trp:Lys ratio requirements for ADG and G:F in 30- to 125-kg gilts under commercial conditions. The estimated SID Trp:Lys ratio requirements ranged from 16.9% for maximum mean G:F to 23.5% for ADG of finishing gilts under commercial conditions. Consistent with our findings, a recent study by Zhang et al., (2012) conducted on early finishing pigs observed an ideal SID Trp:Lys ratio ranging from 19.7 to 23.6%, depending on the response variable. In that study, the authors concluded that the SID Trp:Lys ratio requirement was at least 22% for 25- to 50-kg pigs. Our results are also consistent with those of the review by Moehn et al., (2012), in which Trp requirement across growing monogastric animals was determined to range from 17 to 22% of Lys content. Along similar lines, the classical work on ideal dietary protein in pigs estimated a total Trp:Lys ratio of 18% (Wang and Fuller, 1989). As a reference, 18% total Trp:Lys ratio is approximately equivalent to 17.6% SID Trp:Lys ratio in a corn-soybean meal based diet with 30% DDGS. In a review of the literature, Susenbeth (2006) used descriptive statistics to conclude upon a total Trp:Lys ratio requirement of 17%. The NRC (2012) model estimates a similar SID Trp:Lys ratio of 17.3% for gilts fed a diet containing 2,150 kcal NE/kg. Further, a recent study by Young et al. (2013) concluded that the SID Trp:Lys ratio to maximize growth and economic performance for 34 to 125 kg pigs housed under commercial conditions was estimated at 18%. Salyer et al. (2013) studied the SID Trp:Lys ratio requirement in diets with 30% DDGS through two trials in a commercial research facility; the authors concluded that the requirement was 16.5% for finishing pigs up to 72.6 kg and greater than 19.5% SID Trp:Lys ratio for pigs heavier than 72.6 kg.

Contrary to our findings, Kendall et al. (2007) conducted three studies with barrows and concluded that the SID Trp:Lys ratio for 90 to 125 kg BW was at most 17%; however, the g of SID Lys intake per kg of gain was above 20 g in two of the three trials, which suggests diets were above the SID Lys requirement for barrows (Chiba et al., 1991; Main et al., 2008). It is possible that this may have led to an underestimation of the SID Trp:Lys ratio. Additionally, the CP levels in those experiments were low (8.4 to 10.5%), which could potentially limit some of the non-essential AA (Kerr and Easter, 1995). The fact that Kendall et al. (2007) used only barrows also may have played a role in the SID Trp:Lys ratio requirement estimation, because barrows were found to be less susceptible to Trp deficiency than gilts (Henry et al., 1995; Henry et al., 1996; Salyer et al., 2013).

Quant et al. (2012) observed no evidences for difference in SID Trp:Lys ratio requirement comparing corn-based vs. non-corn-based (barley and Canadian field peas) diets. In these studies, the authors observed a SID Trp:Lys ratio requirement of 15.6 and 15.8% for plasma urea N and ADG, respectively. These studies by Quant et al. (2012) were adequately deficient in Lys (14.0 to 14.6 g of SID Lys intake per kg of gain). The range of SID Trp:Lys ratios in these experiments (12.7 to 17.9 and 13.0 to 18.1%) encompasses Trp deficiency, but probably only marginally reaches adequacy and the current body of literature would argue that it does not have much of a surplus of Trp to correctly model the data (Wang and Fuller, 1989; Susenbeth, 2006; Zhang et al., 2012). This is important because, as showed by Susenbeth (2006), for a study to adequately estimate an AA requirement, it must take into consideration not only deficiency but also adequacy, and surplus. Thus, our large scale study conducted under commercial conditions agrees with most of the literature regarding the SID Trp:Lys requirement for G:F, but shows a greater requirement for ADG.

Trp requirement estimation

The estimation of the Trp requirement is influenced by a variety of factors such as content of Lys and other AA in the diet, range of Trp levels used, response variable, models used, target performance level, and sex. To estimate the requirements of AA other than Lys, diets must be formulated to ensure that Lys is deficient at the end of the BW range of the experiment, so that the Trp:Lys ratio is not underestimated (Susenbeth, 2006). Preferably, the dose-response experiment should contain six or more levels of the nutrient being studied and should have similar number of levels below and above the anticipated requirement to correctly model the requirement (Baker, 1986). Also, different response variables will have different Trp requirements, i.e., the SID Trp:Lys ratio to maximize G:F was estimated at a lower value than that to maximize ADG. This was also the case in the meta-analysis by Simongiovanni et al. (2012). Conversely, Zhang et al. (2012) observed that the Trp requirement for G:F was higher than that for weight gain.

Baker (1986) reviewed some of the common pitfalls in establishing dietary requirements and concluded that a quantitative evidence-based assessment through statistical modelling was a more adequate approach than subjective estimation (i.e., defining the requirement as simply the dietary treatment level that maximized the response variable). Many statistical models are available and the estimation of nutrient requirement also depends on the specific model selected. Pesti et al. (2009) demonstrated a range in requirement from 8 to 12.8 g of Lys per kg of broiler diets depending on the model chosen. Robbins et al. (2006) suggested that in cases in which the broken-line linear model was not appropriate to the data set, an alternative might be to evaluate models that include a quadratic component. Otherwise, one might underestimate the requirement.

More specifically, the choice of statistical model should explore and align with the functional form of the response of interest as a function of the nutrient being tested. The broken-line linear model assumes that the animal will respond linearly to the increase in the nutrient being studied until a plateau is reached (Robbins et al., 1979). The broken-line quadratic model assumes diminishing marginal productivity (Pesti et al., 2009) until reaching plateau performance at requirement (i.e. break-point), after which there is no improvement in performance. The quadratic polynomial model will also assume diminishing marginal productivity until maximum performance is reached; however, after such maximum, a reduction in performance can be observed (Pesti et al., 2009). To ensure meaningful inference, it is important to select the model that best fits the behavior of the data and report estimated requirements based on such best-fitting model (Milliken and Johnson, 2009; Pesti et al., 2009). In this study, we used model fit criteria, in particular Bayesian Information Criteria, to select the best fitting dose-response model for each response of interest and reported estimated requirements based on such best-fitting models.

Once the best fit model for a given response is selected, the nutritionist can use it to determine the target level of performance. Because the levels of a nutrient needed to meet the requirement of 100% of the animals can be economically costly, some nutritionists have arbitrarily chosen levels such as 90, 95, or 99% of the requirement (Robbins et al., 1979; Pesti et al., 2009). Table 5.7 shows the SID Trp:Lys ratio to achieve different target performance levels based on the best fitting models for ADG and G:F. Note that at 96% of the optimum performance, the SID Trp:Lys ratio for ADG and G:F is approximately 18% and 15%, respectively, whereas at 99% of the optimum performance, the SID Trp:Lys ratio for ADG and G:F is approximately 21% and 16 %, respectively. The equations of the best fit models presented herein provide an opportunity for nutritionists to determine the economic return at each level of nutrient addition.

IMPLICATIONS

Increasing levels of a nutrient in diet formulation will often produce a linear increase in diet cost; therefore, if the law of diminishing returns applies, formulating diets slightly below the requirement for some nutrients can potentially be more economical. Nutritionists may use the growth and efficiency performance equations available herein to aid in decision making using an economic approach that entails feed costs and pig price to define the most economical SID Trp:Lys ratio. We highlight that the estimated mean requirements for the SID Trp:Lys ratio of 30- to 125-kg gilts ranged from 16.9% for G:F to 23.5% for maximum ADG in this commercial operation. Furthermore, 95% of the maximum estimated ADG was obtained by feeding 17.6% SID Trp:Lys ratio and 98% of the maximum estimated ADG was obtained by feeding 19.8% SID Trp:Lys ratio.

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TABLES AND FIGURES

Table 5.1. Diet composition, Exp. rounds 1, 2, 3, and 4 (as-fed basis)[1]

	Standardized ileal digestible (SID) Trp:Lys ratio							
	Exp. 1		Exp. 2		Exp. 3		Exp. 4	
Item	Low (14.5%)	High (24.5%)	Low (14.5%)	High (24.5%)	Low (14.5%)	High (24.5%)	Low (14.5%)	High (24.5%)
Ingredient, %								
Corn	57.77	57.67	62.69	62.61	63.07	62.99	63.53	63.45
Soybean meal (46% CP)	9.03	9.03	4.51	4.51	4.13	4.14	3.43	3.43
DDGS[2]	30.00	30.00	30.00	30.00	30.00	30.00	30.00	30.00
Corn oil	0.50	0.50	-	-	-	-	0.50	0.50
Beef tallow	-	-	0.50	0.50	-	-	-	-
Choice white grease	-	-	-	-	0.50	0.50	-	-
Limestone	1.40	1.40	1.28	1.28	1.20	1.20	1.40	1.40
Salt	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
Trace Mineral premix[3]	0.100	0.100	0.100	0.100	0.100	0.100	0.050	0.050
Vitamin premix[4]	0.075	0.075	0.075	0.075	0.075	0.075	0.050	0.050
L-Lys HCl	0.540	0.540	0.431	0.431	0.455	0.455	0.415	0.415
DL-Met	0.045	0.045	-	-	-	-	-	-
L-Thr	0.125	0.125	0.045	0.045	0.090	0.090	0.055	0.055
L-Trp	-	0.091	-	0.076	-	0.073	-	0.072
L-Val	0.045	0.045	-	-	-	-	-	-
Phytase[5]	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025
Ractopamine HCl, 5 g/kg	-	-	-	-	-	-	0.200	0.200
TOTAL	100	100	100	100	100	100	100	100
Calculated analysis								
Standardized ileal digestible (SID) AA, %								
Lys	0.90	0.90	0.75	0.75	0.72	0.72	0.71	0.71
Ile:Lys	55	55	63	63	58	58	64	64

Leu:Lys	161	161	196	195	187	187	203	203
Met:Lys	34	34	34	34	34	34	35	35
Met & Cys:Lys	60	60	64	64	63	63	66	66
Thr:Lys	65	65	65	65	68	68	68	68
Trp:Lys	14.5	24.5	14.5	24.5	14.5	24.5	14.5	24.5
Val:Lys	70	70	76	76	72	72	78	78
His:Lys	39	39	46	46	43	43	47	47
Trp:BCAA[6]	5.8	9.8	4.3	7.3	3.0	5.1	4.8	8.1
Trp:LNAA[7]	4.7	7.9	3.1	5.3	2.2	3.7	3.9	6.6
ME, kcal/kg	3,346	3,349	3,348	3,350	3,353	3,355	3,344	3,346
NE, kcal/kg	2,493	2,495	2,517	2,519	2,523	2,524	2,520	2,521
SID Lys:ME, g/Mcal	3.60	3.60	2.23	2.23	2.14	2.14	2.81	2.81
SID Lys:NE, g/Mcal	2.68	2.68	2.97	2.97	2.85	2.85	2.12	2.12
CP, %	17.4	17.5	16.4	16.5	15.4	15.4	16.0	16.0
Ca, %	0.57	0.57	0.51	0.51	0.48	0.48	0.55	0.55
P, %	0.39	0.39	0.38	0.37	0.37	0.37	0.37	0.37
Available P, %	0.31	0.31	0.30	0.30	0.30	0.30	0.27	0.27

[1] Diets were fed from 29.9 to 45.6 kg, 55.5 to 75.0 kg, 71.2 to 91.2 kg, and 106.2 to 124.7 kg BW. Corn, dried distillers grains with solubles (DDGS) and soybean meal were analyzed for CP and total amino acid content and NRC (2012) SID digestibility values were used in the diet formulation.
[2] Dried distillers grains with solubles.
[3] Provided per kg of premix: 33 g Mn from manganese oxide, 110 g Fe from iron sulfate, 110 g Zn from zinc oxide, 16.5 g Cu from copper sulfate, 0.33 g I from ethylenediamin dihydroiodide, and 0.30 g Se from sodium selenite.
[4] Provided per kg of premix: 7,054,720 IU vitamin A; 1,102,300 IU vitamin D3; 35,274 IU vitamin E; 3,527 mg vitamin K; 6.173 mg riboflavin; 22,046 mg pantothenic acid; 39,683 mg niacin; and 26.5 mg vitamin B12.
[5] OptiPhos 2000 (Huvepharma, Peachtree City, GA) provided 500 FTU per kg of diet and a release of 0.10% in Available P was considered.
[6] Amount of Trp in the diet as a ratio to branched-chain AA (BCAA; Ile, Leu, Val) on an SID basis.
[7] Amount of Trp in the diet as a ratio to large neutral AA (LNAA; Ile, Leu, Val, Phe, and Tyr) on an SID basis.

Table 5.2. Chemical analysis of the diets, Exp. round 1 (as-fed-basis)[1]

	Standardized ileal digestible (SID) Trp:Lys ratio, %						
Item	14.5	16.5	18.0	19.5	21.0	22.5	24.5
Proximate analysis, %							
DM	90.77 (88.65)[2]	90.91 (88.65)	90.68 (88.65)	90.81 (88.65)	90.84 (88.65)	90.73 (88.66)	90.66 (88.66)
CP	19.7 (17.4)	19.6 (17.4)	19.4 (17.4)	18.4 (17.4)	19.5 (17.4)	18.7 (17.5)	18.9 (17.5)
Crude fiber	3.8 (4.2)	3.9 (4.2)	3.5 (4.2)	3.6 (4.2)	3.4 (4.2)	3.4 (4.2)	3.3 (4.2)
Ca	0.74 (0.57)	0.87 (0.57)	0.72 (0.57)	0.78 (0.57)	0.85 (0.57)	0.77 (0.57)	0.78 (0.57)
P	0.45 (0.39)	0.46 (0.39)	0.44 (0.39)	0.44 (0.39)	0.45 (0.39)	0.42 (0.39)	0.45 (0.39)
Ether extract	5.6 (5.3)	5.9 (5.3)	6.0 (5.3)	5.9 (5.3)	5.9 (5.3)	5.3 (5.3)	5.4 (5.3)
Ash	4.44 (2.53)	4.79 (2.53)	4.33 (2.53)	4.2 (2.53)	4.44 (2.53)	3.95 (2.53)	4.10 (2.53)
Total AA, %							
Lys	1.13 (1.06)	1.16 (1.06)	1.15 (1.06)	1.11 (1.06)	1.13 (1.06)	1.11 (1.06)	1.10 (1.06)
Ile	0.70 (0.67)	0.69 (0.67)	0.70 (0.67)	0.70 (0.67)	0.72 (0.67)	0.69 (0.67)	0.69 (0.67)
Leu	1.92 (1.92)	1.84 (1.92)	1.89 (1.92)	1.89 (1.92)	1.91 (1.92)	1.90 (1.92)	1.89 (1.92)
Met	0.38 (0.36)	0.38 (0.36)	0.39 (0.36)	0.40 (0.36)	0.39 (0.36)	0.38 (0.36)	0.37 (0.36)
Met + Cys	0.72 (0.67)	0.70 (0.67)	0.72 (0.67)	0.73 (0.67)	0.73 (0.67)	0.70 (0.67)	0.71 (0.67)
Thr	0.79 (0.75)	0.82 (0.75)	0.81 (0.75)	0.78 (0.75)	0.80 (0.75)	0.80 (0.75)	0.78 (0.75)
Trp	0.18 (0.16)	0.21 (0.18)	0.22 (0.19)	0.21 (0.21)	0.22 (0.22)	0.23 (0.23)	0.23 (0.25)
Val	0.89 (0.85)	0.85 (0.85)	0.87 (0.85)	0.86 (0.85)	0.88 (0.85)	0.86 (0.85)	0.86 (0.85)
His	0.48 (0.44)	0.47 (0.44)	0.48 (0.44)	0.48 (0.44)	0.49 (0.44)	0.48 (0.44)	0.48 (0.44)
Phe	0.91 (0.87)	0.88 (0.87)	0.90 (0.87)	0.90 (0.87)	0.92 (0.87)	0.90 (0.87)	0.89 (0.87)

[1] Diet samples were taken from 6 feeders per dietary treatment 3 d after the beginning of the trial and 3 d to the end of the trial and stored at -20°C, then CP and amino acid analysis was conducted on composite samples by Ajinomoto Heartland Inc. Samples of the diets were also submitted to Ward Laboratories, Inc. (Kearney, NE) for analysis of DM, CF, Ca, P, ash and crude fat.

[2] Values in parentheses indicate those calculated from diet formulation and are based on values from NRC, 2012 (Nutrient Requirements of Swine, 11th ed. Natl. Acad. Press, Washington DC) with the exception of CP and total AA content from corn, soybean-meal, and DDGS which were analyzed to diet formulation by Ajinomoto Heartland Inc. (Chicago, IL).

Table 5.3. Chemical analysis of the diets, Exp. round 2 (as-fed-basis)[1]

	Standardized ileal digestible (SID) Trp:Lys ratio, %						
Item	14.5	16.5	18.0	19.5	21.0	22.5	24.5
Proximate analysis, %							
DM	89.97 (88.52)[2]	89.47 (88.53)	89.77 (88.53)	90.17 (88.53)	90.02 (88.53)	89.6 (88.53)	90.08 (88.53)
CP	16.7 (16.4)	16.6 (16.4)	15.5 (16.5)	15.9 (16.5)	16.8 (16.5)	16.6 (16.5)	16.7 (16.5)
Crude fiber	3.5 (4.1)	3.7 (4.1)	3.5 (4.1)	3.3 (4.1)	3.3 (4.1)	3.5 (4.1)	3.7 (4.1)
Ca	0.74 (0.51)	0.61 (0.51)	0.75 (0.51)	0.69 (0.51)	0.72 (0.51)	0.79 (0.51)	0.62 (0.51)
P	0.41 (0.38)	0.40 (0.38)	0.40 (0.38)	0.40 (0.38)	0.41 (0.38)	0.41 (0.38)	0.42 (0.37)
Ether extract	5.2 (5.4)	5.4 (5.4)	5.3 (5.4)	5.2 (5.4)	5.3 (5.4)	5.0 (5.4)	5.5 (5.4)
Ash	4.22 (3.71)	3.98 (3.71)	4.34 (3.71)	4.06 (3.71)	4.25 (3.71)	4.33 (3.71)	3.98 (3.71)
Total AA, %							
Lys	0.94 (0.93)	0.92 (0.93)	0.92 (0.93)	0.91 (0.93)	0.90 (0.93)	0.93 (0.93)	0.95 (0.93)
Ile	0.72 (0.60)	0.66 (0.60)	0.69 (0.60)	0.72 (0.60)	0.67 (0.60)	0.68 (0.60)	0.76 (0.60)
Leu	1.71 (1.73)	1.68 (1.72)	1.67 (1.72)	1.69 (1.72)	1.67 (1.72)	1.68 (1.72)	1.76 (1.72)
Met	0.30 (0.31)	0.31 (0.31)	0.29 (0.31)	0.29 (0.31)	0.30 (0.31)	0.29 (0.31)	0.32 (0.31)
Met + Cys	0.60 (0.61)	0.60 (0.61)	0.57 (0.61)	0.58 (0.61)	0.60 (0.61)	0.58 (0.61)	0.62 (0.61)
Thr	0.62 (0.67)	0.64 (0.67)	0.63 (0.67)	0.64 (0.67)	0.63 (0.67)	0.63 (0.67)	0.67 (0.67)
Trp	0.16 (0.14)	0.16 (0.16)	0.17 (0.17)	0.17 (0.18)	0.19 (0.19)	0.20 (0.20)	0.21 (0.22)
Val	0.78 (0.73)	0.78 (0.73)	0.76 (0.73)	0.77 (0.73)	0.76 (0.73)	0.78 (0.73)	0.82 (0.73)
His	0.41 (0.43)	0.42 (0.43)	0.40 (0.43)	0.41 (0.43)	0.42 (0.43)	0.41 (0.43)	0.44 (0.43)
Phe	0.78 (0.76)	0.80 (0.76)	0.77 (0.76)	0.78 (0.76)	0.80 (0.76)	0.78 (0.76)	0.83 (0.76)

[1] Diet samples were taken from 6 feeders per dietary treatment 3 d after the beginning of the trial and 3 d to the end of the trial and stored at -20°C, then CP and amino acid analysis was conducted on composite samples by Ajinomoto Heartland Inc. Samples of the diets were also submitted to Ward Laboratories, Inc. (Kearney, NE) for analysis of DM, CF, Ca, P, ash and crude fat.

[2] Values in parentheses indicate those calculated from diet formulation and are based on values from NRC, 2012 (Nutrient Requirements of Swine, 11th ed. Natl. Acad. Press, Washington DC) with the exception of CP and total AA content from corn, soybean-meal, and DDGS which were analyzed to diet formulation by Ajinomoto Heartland Inc. (Chicago, IL).

Table 5.4. Chemical analysis of the diets, Exp. round 3 (as-fed-basis)[1]

	Standardized ileal digestible (SID) Trp:Lys ratio, %						
Item	14.5	16.5	18.0	19.5	21.0	22.5	24.5
Proximate analysis, %							
DM	89.74 (88.52)[2]	90.49 (88.52)	90.12 (88.52)	90.54 (88.52)	90.61 (88.52)	90.85 (88.52)	90.45 (88.53)
CP	16.2 (15.4)	16.7 (15.4)	16.3 (15.4)	16.8 (15.4)	17.1 (15.4)	16.4 (15.4)	16.2 (15.4)
Crude fiber	3.8 (4.1)	4.0 (4.1)	4.1 (4.1)	3.9 (4.1)	3.9 (4.1)	3.9 (4.1)	3.9 (4.1)
Ca	1.10 (0.48)	0.62 (0.48)	0.73 (0.48)	0.75 (0.48)	0.73 (0.48)	0.76 (0.48)	0.81 (0.48)
P	0.40 (0.37)	0.37 (0.37)	0.37 (0.37)	0.35 (0.37)	0.38 (0.37)	0.36 (0.37)	0.37 (0.37)
Ether extract	4.9 (5.4)	5.2 (5.4)	5.0 (5.4)	5.0 (5.4)	5.0 (5.4)	5.1 (5.4)	4.9 (5.4)
Ash	4.63 (2.29)	3.62 (2.29)	4.02 (2.29)	4.03 (2.29)	3.90 (2.29)	4.01 (2.29)	4.02 (2.29)
Total AA, %							
Lys	0.87 (0.87)	0.87 (0.87)	0.88 (0.87)	0.93 (0.87)	0.9 (0.87)	0.91 (0.87)	0.90 (0.87)
Ile	0.59 (0.60)	0.61 (0.60)	0.60 (0.60)	0.62 (0.60)	0.61 (0.60)	0.60 (0.60)	0.59 (0.60)
Leu	1.73 (1.82)	1.78 (1.82)	1.79 (1.82)	1.83 (1.82)	1.80 (1.82)	1.76 (1.82)	1.75 (1.82)
Met	0.31 (0.29)	0.32 (0.29)	0.32 (0.29)	0.33 (0.29)	0.33 (0.29)	0.32 (0.29)	0.31 (0.29)
Met + Cys	0.60 (0.58)	0.61 (0.58)	0.63 (0.58)	0.64 (0.58)	0.62 (0.58)	0.62 (0.58)	0.61 (0.58)
Thr	0.66 (0.65)	0.68 (0.65)	0.68 (0.65)	0.68 (0.65)	0.70 (0.65)	0.68 (0.65)	0.67 (0.65)
Trp	0.13 (0.14)	0.16 (0.15)	0.17 (0.16)	0.18 (0.17)	0.18 (0.18)	0.19 (0.19)	0.19 (0.21)
Val	0.71 (0.74)	0.73 (0.74)	0.74 (0.74)	0.76 (0.74)	0.75 (0.74)	0.73 (0.74)	0.72 (0.74)
His	0.41 (0.39)	0.42 (0.39)	0.43 (0.39)	0.44 (0.39)	0.43 (0.39)	0.42 (0.39)	0.42 (0.39)
Phe	0.77 (0.78)	0.80 (0.78)	0.79 (0.78)	0.81 (0.78)	0.8 (0.78)	0.78 (0.78)	0.78 (0.78)

[1] Diet samples were taken from 6 feeders per dietary treatment 3 d after the beginning of the trial and 3 d to the end of the trial and stored at -20°C, then CP and amino acid analysis was conducted on composite samples by Ajinomoto Heartland Inc. Samples of the diets were also submitted to Ward Laboratories, Inc. (Kearney, NE) for analysis of DM, CF, Ca, P, ash and crude fat.

[2] Values in parentheses indicate those calculated from diet formulation and are based on values from NRC, 2012 (Nutrient Requirements of Swine, 11th ed. Natl. Acad. Press, Washington DC) with the exception of CP and total AA content from corn, soybean-meal, and DDGS which were analyzed to diet formulation by Ajinomoto Heartland Inc. (Chicago, IL).

Table 5.5. Chemical analysis of the diets, Exp. round 4 (as-fed-basis)[1]

Item	Standardized ileal digestible (SID) Trp:Lys ratio, %						
	14.5	16.5	18.0	19.5	21.0	22.5	24.5[3]
Proximate analysis, %							
DM	91.89 (88.54)[2]	91.63 (88.54)	92.03 (88.54)	91.74 (88.54)	91.71 (88.54)	91.70 (88.54)	---
CP	14.7 (16.0)	14.1 (16.0)	15.0 (16.0)	15.0 (16.0)	14.7 (16.0)	15.1 (16.0)	---
Crude fiber	3.0 (4.1)	3.2 (4.1)	3.2 (4.1)	3.3 (4.1)	3.2 (4.1)	3.2 (4.1)	---
Ca	0.69 (0.55)	0.76 (0.55)	0.72 (0.55)	0.70 (0.55)	0.78 (0.55)	0.66 (0.55)	---
P	0.43 (0.37)	0.42 (0.37)	0.44 (0.37)	0.44 (0.37)	0.43 (0.37)	0.43 (0.37)	---
Ether extract	5.3 (5.4)	5.3 (5.4)	5.4 (5.4)	5.3 (5.4)	5.1 (5.4)	5.0 (5.4)	---
Ash	3.82 (3.72)	3.91 (3.72)	3.69 (3.72)	3.76 (3.72)	3.81 (3.72)	3.62 (3.72)	---
Total AA, %							
Lys	0.82 (0.87)	0.79 (0.87)	0.80 (0.87)	0.79 (0.87)	0.80 (0.87)	0.84 (0.87)	---
Ile	0.62 (0.57)	0.58 (0.57)	0.60 (0.57)	0.58 (0.57)	0.59 (0.57)	0.58 (0.57)	---
Leu	1.62 (1.69)	1.59 (1.69)	1.66 (1.69)	1.61 (1.69)	1.63 (1.69)	1.61 (1.69)	---
Met	0.29 (0.3)	0.28 (0.30)	0.29 (0.30)	0.29 (0.30)	0.29 (0.30)	0.29 (0.30)	---
Met + Cys	0.60 (0.59)	0.56 (0.59)	0.58 (0.59)	0.55 (0.59)	0.57 (0.59)	0.56 (0.59)	---
Thr	0.60 (0.63)	0.57 (0.63)	0.58 (0.63)	0.58 (0.63)	0.59 (0.63)	0.59 (0.63)	---
Trp	0.14 (0.13)	0.13 (0.15)	0.15 (0.16)	0.15 (0.17)	0.16 (0.18)	0.17 (0.19)	---
Val	0.77 (0.70)	0.72 (0.70)	0.75 (0.70)	0.73 (0.70)	0.73 (0.70)	0.72 (0.70)	---
His	0.40 (0.41)	0.38 (0.41)	0.40 (0.41)	0.38 (0.41)	0.39 (0.41)	0.39 (0.41)	---
Phe	0.74 (0.73)	0.70 (0.73)	0.73 (0.73)	0.71 (0.73)	0.72 (0.73)	0.71 (0.73)	---

[1] Diet samples were taken from 6 feeders per dietary treatment 3 d after the beginning of the trial and 3 d to the end of the trial and stored at -20°C, then CP and amino acid analysis was conducted on composite samples by Ajinomoto Heartland Inc. Samples of the diets were also submitted to Ward Laboratories, Inc. (Kearney, NE) for analysis of DM, CF, Ca, P, ash and crude fat.
[2] Values in parentheses indicate those calculated from diet formulation and are based on values from NRC, 2012 (Nutrient Requirements of Swine, 11th ed. Natl. Acad. Press, Washington DC) with the exception of CP and total AA content from corn, soybean-meal, and DDGS which were analyzed to diet formulation by Ajinomoto Heartland Inc. (Chicago, IL).
[3] Sample for 24.5% SID Trp:Lys ratio was lost.

Table 5.6. Least square mean estimates (and corresponding SEM) for growth performance of finishing gilts fed dietary treatments of standardized ileal digestible (SID) Trp:Lys ratio ranging from 14.5 to 24.5% [1]

	SID Trp:Lys ratio, %							Probability, *P* <	
Item	14.5	16.5	18.0	19.5	21.0	22.5	24.5	Linear	Quadratic
Exp. round 1									
Initial BW, kg	30.0 ± 0.82	29.9 ± 0.82	29.9 ± 0.82	29.9 ± 0.82	30.0 ± 0.82	29.9 ± 0.82	29.9 ± 0.82	0.994	0.922
ADG, g	628 ± 20.4	716 ± 20.4	744 ± 12.9	765 ± 12.9	766 ± 20.4	780 ± 12.9	792 ± 20.4	0.001	0.004
ADFI, g	1342 ± 41.3	1417 ± 41.3	1453 ± 41.3	1500 ± 41.3	1475 ± 41.3	1499 ± 41.3	1499 ± 41.3	0.001	0.017
G:F	0.469 ± 0.007	0.505 ± 0.007	0.512 ± 0.007	0.511 ± 0.003	0.520 ± 0.007	0.521 ± 0.007	0.528 ± 0.007	0.001	0.011
Final BW, kg	43.3 ± 1.13	45.0 ± 1.13	45.6 ± 1.13	46.0 ± 1.13	46.1 ± 1.13	46.8 ± 1.13	46.7 ± 1.13	0.001	0.017
SID Trp intake, g/d	1.75 ± 0.069	2.11 ± 0.069	2.35 ± 0.069	2.63 ± 0.069	2.79 ± 0.069	3.04 ± 0.069	3.31 ± 0.069	0.001	0.086
SID Trp, g/kg gain	2.78 ± 0.053	2.94 ± 0.027	3.16 ± 0.53	3.44 ± 0.027	3.64 ± 0.053	3.89 ± 0.053	4.18 ± 0.053	0.001	0.131
Exp. round 2									
Initial BW, kg	55.5 ± 1.94	55.5 ± 1.94	55.5 ± 1.94	55.5 ± 1.94	55.5 ± 1.94	55.5 ± 1.94	55.5 ± 1.94	0.902	0.976
ADG, g	881 ± 13.2	900 ± 13.2	938 ± 13.2	915 ± 13.2	934 ± 13.2	936 ± 13.2	962 ± 13.2	0.001	0.647
ADFI, g	2310 ± 77.7	2214 ± 51.4	2306 ± 77.7	2400 ± 51.4	2453 ± 77.7	2519 ± 77.7	2441 ± 51.4	0.001	0.825
G:F[2]	0.382 ± 0.010	0.407 ± 0.006	0.409 ± 0.010	0.382 ± 0.010	0.382 ± 0.010	0.373 ± 0.010	0.395 ± 0.006	0.169	0.810
Final BW, kg	74.1 ± 2.07	74.5 ± 2.07	75.2 ± 2.07	75.0 ± 2.07	75.1 ± 2.07	75.2 ± 2.07	75.7 ± 2.07	0.030	0.737
SID Trp intake, g/d	2.51 ± 0.110	2.74 ± 0.079	3.11 ± 0.110	3.51 ± 0.079	3.86 ± 0.110	4.25 ± 0.110	4.49 ± 0.79	0.001	0.975
SID Trp, g/kg gain	2.85 ± 0.070	3.04 ± 0.070	3.31 ± 0.070	3.84 ± 0.102	4.14 ± 0.102	4.54 ± 0.102	4.66 ± 0.070	0.001	0.773
Exp. round 3									
Initial BW, kg	71.3 ± 1.21	71.2 ± 1.40	71.3 ± 1.21	71.2 ± 1.21	71.3 ± 1.21	71.2 ± 1.21	71.3 ± 1.21	0.958	0.916
ADG, g	891 ± 21.7	929 ± 21.7	922 ± 14.5	962 ± 21.5	998 ± 14.5	954 ± 21.6	961 ± 11.5	0.001	0.018
ADFI, g	2404 ± 38.8	2394 ± 38.8	2385 ± 38.8	2401 ± 41.8	2421 ± 38.8	2378 ± 38.8	2428 ± 38.8	0.652	0.610
G:F	0.375 ± 0.010	0.387 ± 0.010	0.388 ± 0.004	0.400 ± 0.010	0.410 ± 0.005	0.402 ± 0.010	0.397 ± 0.005	0.006	0.049
Final BW, kg	90.1 ± 1.45	90.9 ± 1.45	90.6 ± 1.45	91.4 ± 1.45	92.3 ± 1.45	91.2 ± 1.45	91.6 ± 1.45	0.022	0.256
SID Trp intake, g/d	2.51 ± 0.052	2.84 ± 0.052	3.09 ± 0.052	3.37 ± 0.052	3.66 ± 0.052	3.85 ± 0.052	4.28 ± 0.052	0.001	0.525
SID Trp, g/kg gain	2.80 ± 0.046	3.06 ± 0.068	3.35 ± 0.044	3.43 ± 0.072	3.67 ± 0.044	4.04 ± 0.067	4.46 ± 0.067	0.001	0.005
Exp. round 4									
Initial BW, kg	106.3 ± 1.25	106.3 ± 1.23	106.2 ± 1.23	106.3 ± 1.23	106.2 ± 1.23	106.2 ± 1.23	106.2 ± 1.23	0.823	0.999
ADG, g	759 ± 15.8	883 ± 30.1	875 ± 30.1	904 ± 30.1	908 ± 14.4	881 ± 30.1	945 ± 14.4	0.001	0.022
ADFI, g	2261 ± 28.3	2429 ± 44.0	2419 ± 25.8	2447 ± 25.8	2481 ± 44.0	2411 ± 44.0	2515 ± 44.0	0.001	0.073
G:F	0.336 ± 0.007	0.363 ± 0.006	0.361 ± 0.012	0.370 ± 0.011	0.366 ± 0.006	0.365 ± 0.006	0.376 ± 0.006	0.001	0.160

Final BW, kg	122.3 ± 1.22	124.8 ± 1.22	124.8 ± 1.22	125.3 ± 1.22	125.2 ± 1.22	124.6 ± 1.22	126.0 ± 1.22	0.003	0.140
SID Trp intake, g/d	2.33 ± 0.037	2.85 ± 0.065	3.09 ± 0.037	3.39 ± 0.037	3.70 ± 0.065	3.85 ± 0.065	4.38 ± 0.065	0.001	0.415
SID Trp, g/kg gain	3.07 ± 0.055	3.23 ± 0.050	3.55 ± 0.096	3.76 ± 0.096	4.08 ± 0.050	4.39 ± 0.096	4.63 ± 0.096	0.001	0.440

[1] A total of 1,166, 1,099, 1,132, and 975 gilts (PIC 337 x 1050, initially 29.9 ± 0.82, 55.5 ± 1.94, 71.2 ± 1.40, and 106.2 ± 1.25 kg BW) were used in Exp. 1, 2, 3, and 4, respectively, in a series of 21-d growth trials with 20 to 28 gilts per pen and 6 pens per treatment.

[2] Gain:feed in Exp. 2 was significant ($P < 0.002$) using a cubic polynomial contrast.

Table 5.7. Standardized ileal digestible (SID) Trp:Lys ratio at different target performance levels of finishing gilts

	Percent of maximum performance, %					
Item	95%	96%	97%	98%	99%	100%
ADG						
QP[1]	17.6%	18.3%	18.9%	19.8%	20.8%	23.5%
G:F						
BLL[2]	13.9%	14.5%	15.1%	15.7%	16.3%	16.9%
BLQ[3]	14.4%	14.7%	15.2%	15.7%	16.2%	17.0%

[1] QP equation for ADG: $[-328.6 + 6342.5 \times (\text{SID Trp:Lys}) - 13514 \times (\text{SID Trp:Lys})^2 + 15.07 \times (\text{Initial BW, kg}) - 0.098 \times (\text{Initial BW, kg})^2] / 1000 \times 2.2046$.

[2] BLL equation for G:F: if SID Trp:Lys < 16.9%, equation is $0.599 - 1.0 \times (0.169 - \text{SID Trp:Lys}) - 0.004 \times (\text{Initial BW, kg}) + 0.000017 \times (\text{Initial BW, kg})^2$, otherwise G:F is predicted at maximum for doses greater than 16.9%.

[3] BLQ equation for G:F: if SID Trp:Lys < 17.0%, equation is $0.6014 - 0.603 \times (0.170 - \text{SID Trp:Lys}) - 20.0 \times (0.170 - \text{SID Trp:Lys})^2 - 0.004 \times (\text{Initial BW, kg}) + 0.000017 \times (\text{Initial BW, kg})^2$, otherwise G:F is predicted at maximum for doses greater than 17.0%.

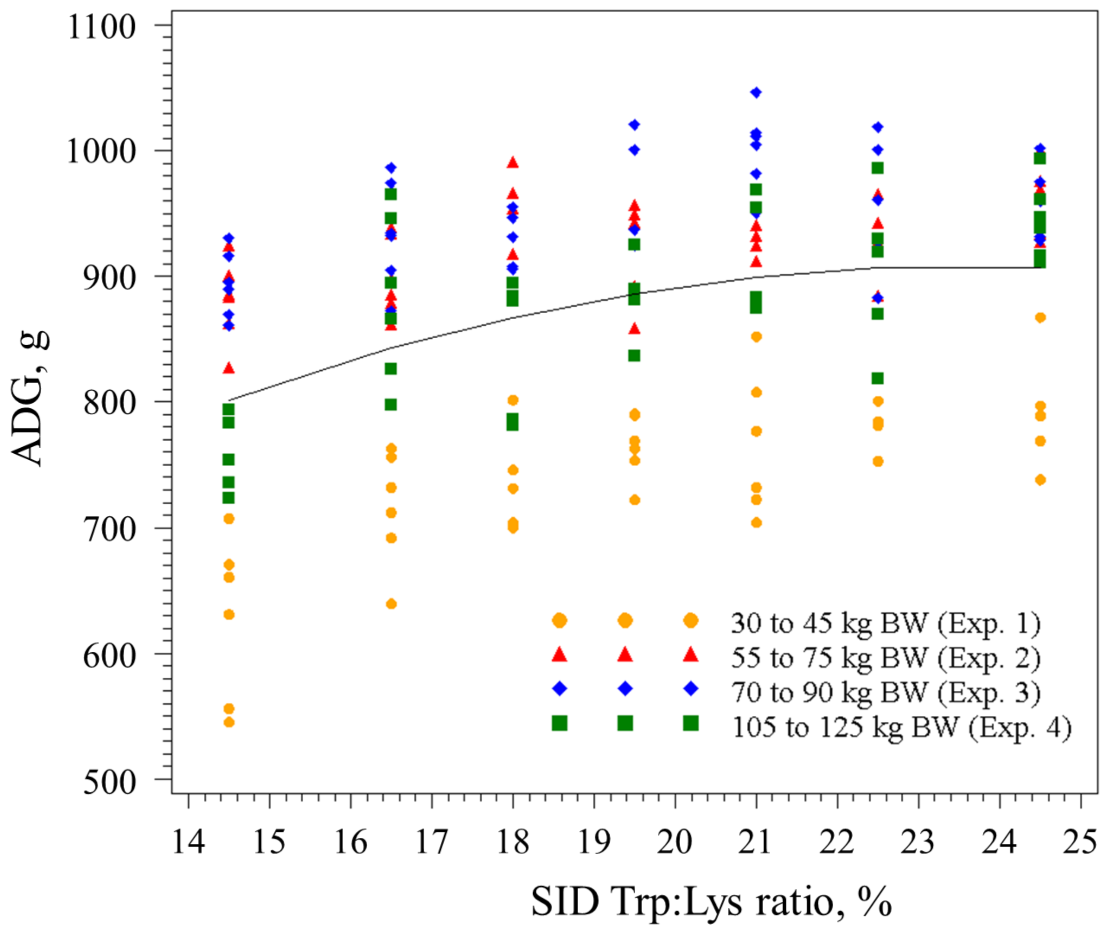

Figure 5.1 Quadratic polynomial (QP) regression of the ADG response to increasing standardized ileal digestible (SID) Trp:Lys ratio in 30- to 125-kg gilts. The maximum ADG was achieved at 23.5% (95% CI: [22.7, 24.3%]) SID Trp:Lys ratio in the QP model.

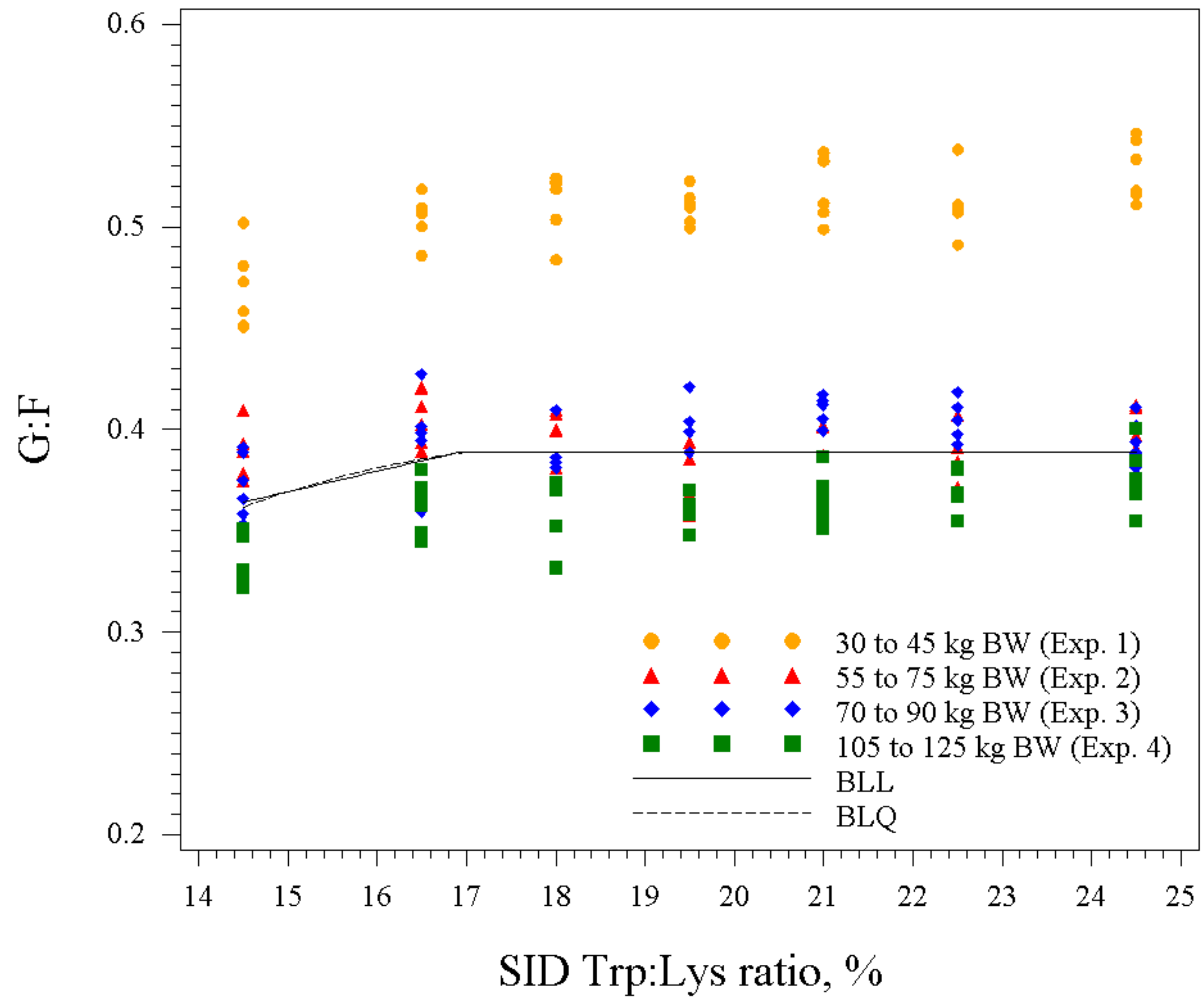

Figure 5.2. Broken-line linear (BLL) and broken-line quadratic (BLQ) regressions of the G:F response to increasing standardized ileal digestible (SID) Trp:Lys ratio in 30- to 125-kg gilts. The maximum G:F was achieved at 16.9 (95% CI: [16.0, 17.8%]) and 17.0% (95% CI: [15.0, 18.9%]) SID Trp:Lys ratio in the BLL and BLQ models, respectively.

Chapter 6 - Effects of standardized ileal digestible valine:lysine ratio on growth performance of 25- to 45-kg pigs under commercial conditions[15,16]

M. A. D. Gonçalves*, M. D. Tokach†[17], S. S. Dritz*, N. M. Bello‡, K. J. Touchette§, R. D. Goodband†, J. M. DeRouchey†, and J. C. Woodworth†

*Department of Diagnostic Medicine/Pathobiology, College of Veterinary Medicine, †Department of Animal Sciences and Industry, College of Agriculture, ‡Department of Statistics, College of Arts and Sciences, Manhattan, KS 66506-0201, and **§** Ajinomoto Heartland Inc., Chicago, IL

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[17] Corresponding author: mtokach@k-state.edu

ABSTRACT: Two experiments were conducted to estimate the standardized ileal digestible (**SID**) Val:Lys ratio requirement for growth performance in 25- to 45-kg pigs under commercial conditions. In Exp. 1, a total of 1,134 gilts (PIC 337 × 1050), initially 31.2 kg ± 2.0 kg BW (mean ± SD) were used in a 19-d growth trial with 27 pigs/pen and 7 pens/treatment. In Exp. 2, a total of 2,100 gilts (PIC 327 × 1050), initially 25.4 ± 1.9 kg BW were used in a 22-d growth trial with 25 pigs/pen and 12 pens/treatment. In both experiments, treatments were blocked by initial BW in a randomized complete block design. In Exp. 1, there were a total of 6 dietary treatments with SID Val at 59.0, 62.5, 65.9, 69.6, 73.0, and 75.5% of Lys and for Exp. 2 there were a total of 7 dietary treatments with SID Val at 57.0, 60.6, 63.9, 67.5, 71.1, 74.4, and 78.0% of Lys. Experimental diets were formulated to ensure that Lys was the second limiting amino acid throughout the experiments. Linear mixed models were fitted to data from each experiment. Then, data from the two experiments were combined to fit competing linear and non-linear models. Competing statistical models were: broken-line linear ascending (BLL) model, broken-line quadratic ascending (BLQ) model, and quadratic polynomial (QP). Competing models were compared using Bayesian information criterion (BIC). In Exp. 1, ADG increased linearly (P = 0.009) with increasing SID Val:Lys ratio whereas ADFI only marginally increased (linear, P = 0.098) with no impact on G:F. In Exp. 2, ADG and ADFI increased in a quadratic manner (P < 0.002) with increasing SID Val:Lys ratio whereas G:F increased linearly (P < 0.001). Overall, the best-fitting model for ADG was a QP. The maximum mean ADG was estimated at a 74.4% (95% CI: [69.5, >78.0%]) SID Val:Lys ratio. The overall best-fitting model for G:F was also a QP. The maximum mean G:F was estimated at 72.3% (95% CI: [64.0, >78.0]) SID Val:Lys ratio. However, 99% of the maximum mean performance for ADG and G:F were achieved at, approximately, 67 and 66% SID Val:Lys ratio, respectively. In conclusion, the SID Val:Lys ratio requirement ranged from 72.3% for maximum G:F to 74.4% for maximum ADG with 99% of

maximum mean ADG and G:F achieved at 67% and 66% SID Val:Lys ratio, respectively, in 25- to 45-kg pigs.

Key words: amino acid ratio, growth, growing pig, lysine, valine

INTRODUCTION

Valine is commonly considered to be the fifth limiting amino acid after Trp in corn-soybean meal–based diets for finishing pigs (Figueroa et al., 2003). Valine can become limiting in diets supplemented with feed-grade amino acids, such as Lys, Met, Thr, and Trp. Stein et al. (2007) suggest that the most practical way to express amino acid requirement is as a ratio to Lys. The NRC (2012) estimated the SID Val:Lys ratio requirement at 65% for 25- to 45-kg pigs. However, even though the NRC provides a single estimate for AA requirements the estimates to optimize ADG and G:F can vary significantly. Additionally, there are recent evidences that the SID Val:Lys ratio requirement may be greater than 65% for pigs ranging from 8- to 120-kg BW (Liu et al. 2015; Soumeh et al., 2015).

The current body of literature tries to define a specific point estimate of the requirement for the Val:Lys ratio (Lewis and Nishimura, 1995; Waguespack et al., 2012); however, there is a need to 1) understand the variability around that point estimate by reporting confidence intervals, and 2) to understand the response surface by evaluating the rate of change of the point estimate as a percentage of the maximum ADG and G:F. Therefore, the objective of these studies was to estimate the SID Val:Lys ratio requirement in low CP and Lys-limiting diets for growth performance in 25- to 45-kg pigs under commercial conditions.

MATERIALS AND METHODS

General

The Kansas State University Institutional Animal Care and Use Committee approved the protocols used in these experiments. Experiments 1 and 2 were conducted at two commercial research-finishing barns in Minnesota. Both barns were naturally ventilated and double-curtain-sided and pens had completely slatted flooring and deep pits for manure storage. In Exp. 1, pens were equipped with a 4-hole stainless steel dry self-feeder (Thorp Equipment, Thorp, WI) and a cup waterer. In Exp. 2, each pen was equipped with a 3-hole stainless steel dry self-feeder (Thorp Equipment, Thorp, WI) and a cup waterer. Both facilities were equipped with a computerized feeding system (FeedPro; Feedlogic Corp., Willmar, MN) that delivered and recorded daily feed additions. During the experiments, pigs had ad libitum access to feed and water.

In Exp. 1, a total of 1,134 gilts (PIC 337 × 1050), initially 31.2 kg ± 2.0 kg BW (mean ± SD) were used in a 19-d growth trial with 27 pigs per pen (0.62 m^2/pig) and 7 pens per treatment. In Exp. 2, a total of 2,100 gilts (PIC 327 × 1050), initially 25.4 ± 1.9 kg BW (mean ± SD) were used in a 22-d growth trial with 25 pigs per pen (0.67 m^2/pig) and 12 pens per treatment. In both experiments, treatments were blocked by initial BW in a randomized complete block design. In Exp. 1, there were 6 dietary treatments with dietary SID Val at 59.0, 62.5, 65.9, 69.6, 73.0, and 75.5% of Lys. For Exp. 2, there were 7 dietary treatments with SID Val at 57.0, 60.6, 63.9, 67.5, 71.1, 74.4, and 78.0% of Lys fed in meal form. In both experiments, the intermediate Val:Lys ratios were obtained by blending different proportions of the low and high Val:Lys ratio diets (Tables 6.1 and 6.2). The NRC (2012) model was used to estimate the Lys requirement of pigs at the expected BW at the end of each experimental round. The SID Lys as a percentage of the diet was reduced by 0.10 percentage points below the requirement at the expected BW at the end of each experimental round to ensure that Lys was the second limiting amino acid throughout the experiment based in a preliminary study conducted by Gonçalves et al. (2014).

Diet Sampling and Analysis

Five representative samples of corn, soybean meal, and DDGS were collected each wk for 5 wk and analyzed in duplicate for total AA (except Trp; method 994.12; AOAC Int., 2012), Trp (method 13904:2005; ISO, 2005), and CP (method 990.03; AOAC Int., 2012) by Ajinomoto Heartland Inc. (Chicago, IL), and values were used in diet formulation. Other nutrients and SID AA digestibility coefficient values used for diet formulation were obtained from NRC (2012).

Diet samples were taken from 6 feeders per dietary treatment 3 d after the beginning of the trial and 3 d prior to the end of the trial and stored at -20° C, then CP (method 990.03; AOAC Int., 2012) and total AA analyses were conducted in duplicate on composite samples (Ajinomoto Heartland Inc., Chicago, IL)

In Exp. 1 and 2, samples of the diets were analyzed for DM (method 935.29; AOAC Int., 2012), crude fiber (method 978.10; AOAC Int., 2012 for preparation and Ankom 2000 Fiber Analyzer [Ankom Technology, Fairport, NY]), ash (method 942.05; AOAC Int., 2012), ether extract (method 920.39 a; AOAC Int., 2012 for preparation and ANKOM XT20 Fat Analyzer [Ankom Technology, Fairport, NY], Ward Laboratories, Inc. Kearney, NE).

Data Collection

Pens of pigs were weighed and feed disappearance measured at the beginning and at the end of each experiment to determine ADG, ADFI, and G:F. The total g of SID Val intake based on formulated values were divided by total BW gain to calculate the g of SID Val intake per kg of gain.

Statistical Analysis

For the statistical analysis, initially, responses measured at the pen level were analyzed within experiment using a general linear mixed model. Within each experiment, linear and quadratic polynomial contrasts with coefficients adjusted for unequally spaced treatments were used to evaluate the dose-response effect of increasing dietary SID Val:Lys ratio on ADG, ADFI, G:F, BW, g of SID Val intake per d, and g of SID Val intake per kg of gain. Heterogeneous residual variances as a function of the response variables were fitted as needed. Model assumptions were checked and considered to be appropriately met. The experimental data was analyzed using the GLIMMIX procedure of SAS (SAS Institute Inc., Cary, NC).

Prior to fitting the dose-response models in the combined data set, a base model was fit to estimate and assess variance components of experiment and weight block nested within experiment, for each of the response variables. Subsequently, the effects of initial BW as a covariate and of the random variance components on model fit were evaluated for each of the response variables. Linear and non-linear regression models adapted from Robbins et al. (2006) and Gonçalves et al. (2015) were expanded to accommodate random effects and were fitted to ADG and G:F to further estimate SID Val:Lys ratio dose-response using an inverse prediction strategy. Specifically, competing statistical models fitted to the data were: a broken-line linear ascending (BLL) model, a broken-line quadratic ascending (BLQ) model, and a quadratic polynomial (QP). Broken-line regression models were fitted using the NLMIXED procedures of SAS. The optimization technique used was the dual Quasi-Newton algorithm, as specified by default in the NLMIXED procedure. Competing statistical models were compared using maximum-likelihood-based fit criteria, specifically the Bayesian information criteria (BIC; Milliken and Johnson, 2009). Results were considered significant at $P \leq 0.05$ and marginally significant at $0.05 < P \leq 0.10$.

RESULTS AND DISCUSSION

The analyzed total AA, DM, CP, crude fiber, ether extract, and ash contents of diets for Exp. 1 and 2 (Tables 6.3 and 6.4, respectively) were reasonably consistent with formulated values (AFFCO, 2009).

In Exp. 1, ADG increased linearly ($P = 0.009$) with increasing SID Val:Lys ratio whereas ADFI only marginally increased (linear, $P = 0.098$) with no impact on G:F (Table 6.5). Final BW marginally increased (linear, $P = 0.064$) with increasing SID Val:Lys ratio. Grams of SID Val intake per d and g of SID Val per kg of gain increased (linear, $P < 0.001$) with increasing SID Val:Lys ratio.

In Exp. 2, ADG and ADFI increased in a quadratic manner ($P < 0.002$) with increasing SID Val:Lys ratio whereas G:F linearly increased ($P < 0.001$; Table 6.6). Final BW also increased (quadratic, $P = 0.010$) with increasing SID Val:Lys ratio. Increasing SID Val:Lys ratio increased g of SID Val intake per d (quadratic, $P = 0.005$) and g of SID Val per kg of gain (linear, $P < 0.001$).

Figures 6.1 and 6.2 show the influence of SID Val:Lys ratio on ADG and G:F, respectively, across 2 experiments in 25- to 45-kg pigs. When combining the data from the two experiments, the best-fitting model for ADG in the 25- to 45-kg BW pigs was a QP (BIC: 1482.9) compared with BLL and BLQ models (BIC: 1491.0 and 1488.6, respectively). The estimated regression equation for the best-fitting QP model (Fig. 6.3) was:

QP equation for ADG = $-1.15 + 4.13 \times$ (SID Val:Lys ratio) $- 2.78 \times$ (SID Val:Lys ratio)^2 $+ 0.012 \times$ (Initial BW, kg) where the SID Val:Lys ratio explanatory variable is expressed as a proportion (i.e., 0.700) rather than a percentage (i.e., 70.0%). Based on the best-fitting QP model, the maximum mean ADG was estimated at a 74.4% (95% CI: [69.5, >78.0%]) SID Val:Lys ratio.

The overall best-fitting model for G:F was also a QP (BIC: 1156.3) compared with BLL and BLQ models (BIC: 1158.7 and 1161.7, respectively). The estimated regression equation for the best-fitting QP model (Fig. 6.4) was:

$$\text{QP equation for G:F} = -0.04 + 1.36 \times (\text{SID Val:Lys ratio}) - 0.94 \times (\text{SID Val:Lys ratio})^2$$

Based on the best-fitting QP model, the maximum mean G:F was estimated at 72.3% (95% CI: [64.0, >78.0]) SID Val:Lys ratio.

The SID Val:Lys ratio observed in the current studies under commercial conditions is in agreement with the current body of literature mostly conducted under university settings. The classical work of Fuller and Wang (1989) estimated the total Val:Lys ratio for 25- to 50-kg pigs was 75%. This would be similar to the 74.4% SID Val:Lys ratio that reached maximum ADG in the current studies; however, higher than the 72.3% SID Val:Lys ratio for maximum G:F. Similarly, Lewis and Nishimura (1995) conducted a study with 70- to 80-kg pigs and estimated the requirement at 11 g SID Val intake/d which would be over 74% SID Val:Lys ratio in the studies presented herein.

Our results are similar to the results from Barea et al. (2009), where the authors observed a greater SID Val:Lys ratio requirement for ADG compared to G:F in 12 to 25 kg pigs. In that study, the SID Val:Lys ratio requirement for ADG was 70% in the BLL model and 75% in the BLQ whereas for G:F the requirements were 68% in the BLL and 72% in the BLQ. Additionally, different statistical models can have different requirement estimates as shown by Gaines et al.

(2011) where approximately 65% SID Val:Lys ratio was the requirement using a BLL model, while approximately 71% was the requirement using a QP model for 13 to 32 kg pigs. It is important to note that dose-response studies should infer on the best fitting model, thus, researchers should report quantitative information about the fit of each model, using criteria such as Bayesian Information Criterion or Akaike Information Criterion (Milliken and Johnson, 2009).

The NRC (2012) estimated the SID Val:Lys ratio requirement of 25- to 45-kg pigs at 65%. Based on our results, the NRC (2012) estimation appears to be adequate to maximize G:F because 65% was within the 95% CI in our study; however, it may be below the pigs' requirement to maximize ADG. Recent studies have shown that the SID Val:Lys requirement of nursery and finishing pigs range from 67 to 70% (Waguespack et al. 2012; Liu et al. 2015; Soumeh et al., 2015). In reality, the optimal ratio will vary given different ingredient and market hog prices. Ultimately, swine nutritionists should use the equations provided herein to determine which SID Val:Lys ratio is most economical for a given situation.

To illustrate how statistical analysis accounts for the variability in the observed data, the observed ADG and G:F for each pen of pigs are shown in Fig. 6.1 and 6.2, respectively; subsequently, we presented the predicted ADG of each pen of pigs by accounting for the pen to pen variability after adjustment for random effects, heterogeneous variance, and initial body weight in Fig. 6.3. Similarly, Fig. 6.4 shows the predicted G:F of each pen of pigs by accounting for the pen to pen variability after adjustment for random effects.

Target performance levels based on the best fitting models for ADG and G:F are listed in Table 6.7. Note that 99% of the maximum mean performance can be achieved with a SID Val:Lys ratio of approximately 67 and 66% for ADG and G:F. A recent meta-analysis conducted by van Milgen et al. (2013) which evaluated 28 dose-response experiments in young pigs concluded that the SID Val:Lys ratio requirement was 69%. The 69% SID Val:Lys ratio in our study was able to capture more than 99% of maximum mean ADG and G:F.

In conclusion, the SID Val:Lys ratio estimates ranged from 72.3% for maximum G:F to 74.4% for maximum ADG with 99% of the maximum mean ADG and G:F achieved at approximately 67% and 66% SID Val:Lys ratio, respectively, in 25- to 45-kg pigs. The growth performance prediction equations from this research can be used along with market prices to determine the optimal SID Val:Lys ratio for a given economic scenario.

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TABLES

Table 6.1. Diet composition (as-fed basis; Exp. 1)[1]

	Standardized ileal digestible Val:Lys ratio	
Item	59.0%	75.5%
Ingredient, %		
Corn	73.31	73.16
Soybean meal (46% CP)	8.20	8.21
Corn dried distillers grains w/ solubles	15.00	15.00
Corn oil	0.50	0.50
Limestone	1.20	1.20
Monocalcium phosphate (21.5% P)	0.30	0.30
Salt	0.35	0.35
Trace mineral premix[2]	0.100	0.100
Vitamin premix[3]	0.075	0.075
L-Lys HCl	0.540	0.540
DL-Met	0.105	0.105
L-Thr	0.175	0.175
L-Trp	0.071	0.071
L-Val	---	0.142
L-Ile	0.043	0.043
Phytase[4]	0.025	0.025
TOTAL	100	100
Calculated analysis		
Standardized ileal digestible (SID) AA, %		
Lys	0.85	0.85
Ile:Lys	55.0	55.0
Leu:Lys	139	139
Met:Lys	36	36
Met & Cys:Lys	60	60
Thr:Lys	65	65
Trp:Lys	20.1	20.1
Val:Lys	59.0	75.5
ME, kcal/kg	3,353	3,358
NE, kcal/kg	2,553	2,555
SID Lys:NE, g/Mcal	3.33	3.33
CP, %	13.9	14.0
Ca, %	0.57	0.57
Available P, %	0.31	0.31
Stand. Total Tract Dig. (STTD) P, %	0.33	0.33
Ca:P	1.41	1.41
Ca:P (STTD P)	1.74	1.74

[1] Diets were fed from 31.3 to 44.9 kg BW. Corn, dried distillers grains with solubles (DDGS), and soybean meal were analyzed for CP and total amino acid concentrations and NRC (2012) SID digestibility values were used in the diet formulation. These diets were blended to make the intermediate dietary treatments: 62.5, 65.9, 69.6, and 73.0, SID Val:Lys ratio.

[2] Provided per kg of premix: 33 g Mn from manganese oxide, 110 g Fe from iron sulfate, 110 g Zn from zinc oxide, 16.5 g Cu from copper sulfate, 0.33 g I from ethylenediamine dihydriodide, and 0.30 g Se from sodium selenite.
[3] Provided per kg of premix: 7,054,720 IU vitamin A; 1,102,300 IU vitamin D3; 35,274 IU vitamin E; 3,527 mg vitamin K; 6.173 mg riboflavin; 22,046 mg pantothenic acid; 39,683 mg niacin; and 26.5 mg vitamin B12.
[4] OptiPhos 2000 (Huvepharma, Peachtree, GA) provided 500 phytase units (FTU) per kg of diet.

Table 6.2. Diet composition (as-fed basis; Exp 2)[1]

	Standardized ileal digestible Val:Lys ratio	
Item	57.0%	78.0%
Ingredient		
Corn	74.56	74.37
Soybean meal (46% CP)	6.77	6.78
Dried distillers grains with solubles	15.00	15.00
Choice white grease	0.50	0.50
Limestone	1.10	1.10
Dicalcium phosphate (18.5% P)	0.45	0.45
Salt	0.35	0.35
Vitamin-mineral premix[2]	0.200	0.200
Phytase[3]	0.050	0.050
L-Lys HCl	0.591	0.591
DL-Met	0.105	0.105
L-Thr	0.195	0.195
L-Trp	0.073	0.073
L-Val	---	0.181
L-Ile	0.062	0.062
TOTAL	100	100
Calculated analysis		
Standardized ileal digestible (SID) AA, %		
Lys	0.85	0.85
Ile:Lys	55.0	55.0
Leu:Lys	139	139
Met:Lys	36	36
Met & Cys:Lys	60	60
Thr:Lys	65	65
Trp:Lys	20.1	20.1
Val:Lys	57.0	78.0
ME, kcal/kg	3,351	3,355
NE NRC, kcal/kg	2,557	2,560
SID Lysine:NE, g/Mcal	3.32	3.32
CP, %	13.6	13.7
Ca, %	0.57	0.57
P, %	0.42	0.41
Available P, %	0.27	0.27
Stand. Total Tract Dig. (STTD) P, %	0.29	0.29
Ca:P	1.38	1.38
Ca:P (STTD P)	2.00	2.00

[1] Diets were fed from 25.4 to 40.7 kg BW. Corn, dried distillers grains with solubles (DDGS), and soybean meal were analyzed for CP and total amino acid concentrations and NRC (2012) SID digestibility values were used in the diet formulation. These diets were blended to make the intermediate dietary treatments: 60.6, 63.9, 67.5, 71.1, and 74.4 SID Val:Lys ratio.

[2] Provided per kg of premix: 3.3 g Mn from manganese oxide, 30.9 g Fe from iron sulfate, 30.9 g Zn from zinc oxide, 3.1 g Cu from copper sulfate, 0.16 g I from ethylenediamine dihydriodide, and 0.12 g Se from sodium selenite, 2,910,072 IU vitamin A; 440,920 IU vitamin D3; 8,047 IU vitamin E; 1,047 mg vitamin K; 1,984 mg riboflavin; 6,854 mg pantothenic acid; 14,991 mg niacin; and 7.94 mg vitamin B12.

[3] Axtra PHY (DuPont, Wilmington, DE) provided 330 phytase units (FTU) per kg of diet.

Table 6.3. Chemical analysis of the diets (as-fed-basis; Exp. 1)[1]

	Standardized ileal digestible Val:Lys ratio, %					
Item	59.0	62.5	66.0	69.5	73.0	75.5
Proximate analysis, %						
DM	86.8 (87.01)[2]	86.87 (87.01)	87.18 (87.02)	86.91 (87.02)	87.02 (87.03)	87.22 (87.03)
CP	14.6 (13.9)	14.2 (13.9)	14.4 (13.9)	14.2 (14.0)	14.3 (14.0)	14.5 (14.0)
Crude fiber	2.2 (3.1)	2.2 (3.1)	2.3 (3.1)	2.2 (3.1)	2.1 (3.1)	2.4 (3.1)
Ether extract	3.2 (4.5)	3.1 (4.5)	3.2 (4.5)	3.1 (4.5)	3.1 (4.5)	2.9 (4.5)
Ash	2.8 (2.5)	3.1 (2.5)	3.4 (2.5)	3.3 (2.5)	3.4 (2.5)	3.1 (2.5)
Amino acids, %						
Lys	0.97 (0.97)	0.98 (0.97)	1.03 (0.97)	0.94 (0.97)	0.94 (0.97)	0.96 (0.97)
Ile	0.55 (0.53)	0.53 (0.53)	0.57 (0.53)	0.52 (0.53)	0.52 (0.53)	0.54 (0.53)
Leu	1.38 (1.34)	1.38 (1.34)	1.44 (1.34)	1.32 (1.34)	1.34 (1.34)	1.40 (1.34)
Met	0.33 (0.36)	0.33 (0.36)	0.34 (0.36)	0.32 (0.36)	0.31 (0.36)	0.32 (0.36)
Met & Cys	0.60 (0.60)	0.59 (0.60)	0.62 (0.60)	0.56 (0.60)	0.56 (0.60)	0.57 (0.60)
Thr	0.65 (0.66)	0.67 (0.66)	0.68 (0.66)	0.63 (0.66)	0.63 (0.66)	0.64 (0.66)
Trp	0.18 (0.20)	0.18 (0.20)	0.18 (0.20)	0.18 (0.20)	0.18 (0.20)	0.19 (0.20)
Val	0.65 (0.59)	0.64 (0.62)	0.69 (0.65)	0.66 (0.68)	0.69 (0.71)	0.73 (0.73)

[1] Diet samples were taken from 6 feeders per dietary treatment 3 d after the beginning of the trial and 3 d prior to the end of the trial and stored at -20°C, then CP and amino acid analysis was conducted on composite samples by Ajinomoto Heartland, Inc. (Chicago, IL). Samples of the diets were also submitted to Ward Laboratories, Inc. (Kearney, NE) for analysis of DM, crude fiber, Ca, P, ash and crude fat.

[2] Values in parentheses indicate those calculated from diet formulation and are based on values from NRC (2012), with the exception of CP and total amino acid content from corn, soybean meal, and dried distillers grains with solubles, which were analyzed prior to diet formulation by Ajinomoto Heartland, Inc.

Table 6.4. Chemical analysis of the diets (as-fed-basis; Exp. 2)[1]

	Standardized ileal digestible Val:Lys ratio, %						
Item	57.0	60.6	63.9	67.5	71.1	74.4	78.0
Proximate analysis, %							
DM	87.91 (86.71)[2]	87.79 (86.71)	86.9 (86.71)	87.01 (86.71)	87.38 (86.71)	87.64 (86.71)	87.50 (86.73)
CP	13.8 (13.6)	13.8 (13.7)	14.5 (13.7)	13.8 (13.7)	13.8 (13.7)	13.9 (13.7)	13.9 (13.7)
Crude fiber	2.7 (3.1)	3.1 (3.1)	3.0 (3.1)	3.0 (3.1)	3.1 (3.1)	3.1 (3.1)	3.1 (3.1)
Ether extract	3.7 (4.5)	3.7 (4.5)	3.5 (4.5)	3.4 (4.5)	3.6 (4.5)	3.9 (4.5)	3.8 (4.5)
Ash	3.6 (2.4)	3.8 (2.4)	3.5 (2.4)	3.9 (2.4)	3.8 (2.4)	3.4 (2.4)	4.0 (2.4)
Amino acids, %							
Lys	0.97 (0.96)	0.95 (0.96)	0.98 (0.96)	1.01 (0.96)	1.05 (0.96)	1.03 (0.96)	0.98 (0.96)
Ile	0.54 (0.52)	0.54 (0.52)	0.57 (0.52)	0.54 (0.52)	0.55 (0.52)	0.55 (0.52)	0.55 (0.52)
Leu	1.34 (1.31)	1.37 (1.31)	1.38 (1.31)	1.34 (1.31)	1.30 (1.31)	1.35 (1.31)	1.33 (1.31)
Met	0.32 (0.35)	0.30 (0.35)	0.33 (0.35)	0.32 (0.35)	0.33 (0.35)	0.32 (0.35)	0.33 (0.35)
Met & Cys	0.57 (0.59)	0.55 (0.59)	0.57 (0.59)	0.56 (0.59)	0.57 (0.59)	0.58 (0.59)	0.57 (0.59)
Thr	0.69 (0.66)	0.64 (0.66)	0.67 (0.66)	0.63 (0.66)	0.66 (0.66)	0.67 (0.66)	0.69 (0.66)
Trp	0.17 (0.19)	0.17 (0.19)	0.17 (0.19)	0.17 (0.19)	0.17 (0.19)	0.17 (0.19)	0.17 (0.19)
Val	0.63 (0.56)	0.64 (0.59)	0.68 (0.62)	0.66 (0.65)	0.72 (0.68)	0.73 (0.71)	0.75 (0.74)

[1] Diet samples were taken from 6 feeders per dietary treatment 3 d after the beginning of the trial and 3 d prior to the end of the trial and stored at -20°C, then CP and amino acid analysis was conducted on composite samples by Ajinomoto Heartland, Inc. (Chicago, IL). Samples of the diets were also submitted to Ward Laboratories, Inc. (Kearney, NE) for analysis of DM, crude fiber, Ca, P, ash and crude fat.

[2] Values in parentheses indicate those calculated from diet formulation and are based on values from NRC (2012), with the exception of CP and total amino acid content from corn, soybean meal, and dried distillers grains with solubles, which were analyzed prior to diet formulation by Ajinomoto Heartland, Inc.

Table 6.5. Effects of standardized ileal digestible (SID) Val:Lys ratio on the growth performance of finishing pigs from 30 to 45 kg, Exp. 1[1]

	SID Val:Lys ratio, %							Probability, $P <$	
Item	59.0	62.5	66.0	69.5	73.0	75.5	SEM[2]	Linear	Quadratic
d 0 to 19									
ADG, g	680	717	717	712	744	726	17.1	0.009	0.305
ADFI, g	1461	1538	1520	1501	1551	1542	48.7	0.098	0.578
G:F	0.467	0.467	0.472	0.474	0.481	0.472	0.0084	0.370	0.648
BW, kg									
d 0	31.3	31.3	31.2	31.3	31.2	31.2	0.77	0.762	0.962
d 21	44.2	45.0	44.8	45.0	45.4	45.0	1.09	0.064	0.349
SID Val intake, g/d	7.33	8.17	8.53	8.87	9.63	9.89	0.28	0.001	0.716
SID Val, g/kg gain	10.7	11.3	11.9	12.3	12.9	13.6	0.23	0.001	0.490

[1] A total of 1,134 gilts (PIC 337 × 1050), initially 31.2 ± 2.0 kg BW (mean ± SD) were used in a 19-d growth trial with 27 pigs per pen and 7 pens per treatment.
[2] Represents the greatest SEM across treatments.

Table 6.6. Effects of standardized ileal digestible (SID) Val:Lys ratio on the growth performance of finishing pigs from 25 to 40 kg, Exp. 2[1]

	SID Val:Lys ratio, %								Probability, *P* <	
Item	57.0	60.6	63.9	67.5	71.1	74.4	78.0	SEM[2]	Linear	Quadratic
d 0 to 22										
ADG, g	621	662	717	708	708	726	717	16.1	0.001	0.002
ADFI, g	1488	1569	1633	1642	1633	1651	1637	28.2	0.001	0.001
G:F	0.415	0.420	0.437	0.429	0.433	0.441	0.439	0.0046	0.001	0.132
BW, kg										
d 0	25.4	25.4	25.5	25.4	25.4	25.4	25.4	0.55	0.989	0.584
d 21	39.1	39.9	41.2	41.1	41.1	41.5	41.2	0.78	0.001	0.010
SID Val intake, g/d	7.6	8.5	9.3	9.9	10.3	11.0	11.4	0.17	0.001	0.005
SID Val, g/kg gain	10.5	11.1	11.2	12.0	12.6	13.0	13.6	0.12	0.001	0.368

[1] A total of 2,100 gilts (PIC 327 × 1050), initially 25.4 ± 1.9 kg BW (mean ± SD) were used in a 22-d growth trial with 25 pigs per pen and 12 pens per treatment.
[2] Represents the greatest SEM across treatments.

Table 6.7. Standardized ileal digestible (SID) Val:Lys ratio at different target performance levels of growing pigs

	Percent of maximum performance, %					
Item	95%	96%	97%	98%	99%	100%
ADG[1]	58.9	60.5	62.3	64.5	67.3	74.4
G:F[2]	<57.0	58.5	60.4	62.6	65.5	72.3

[1] QP equation for ADG =–1.15 + 4.13 × (SID Val:Lys ratio) – 2.78 × (SID Val:Lys ratio)^2 + 0.012 × (Initial BW, kg), estimated to 35 kg pigs.
[2] QP equation for G:F = – 0.04 + 1.36 × (SID Val:Lys ratio) – 0.94 × (SID Val:Lys ratio)^2.

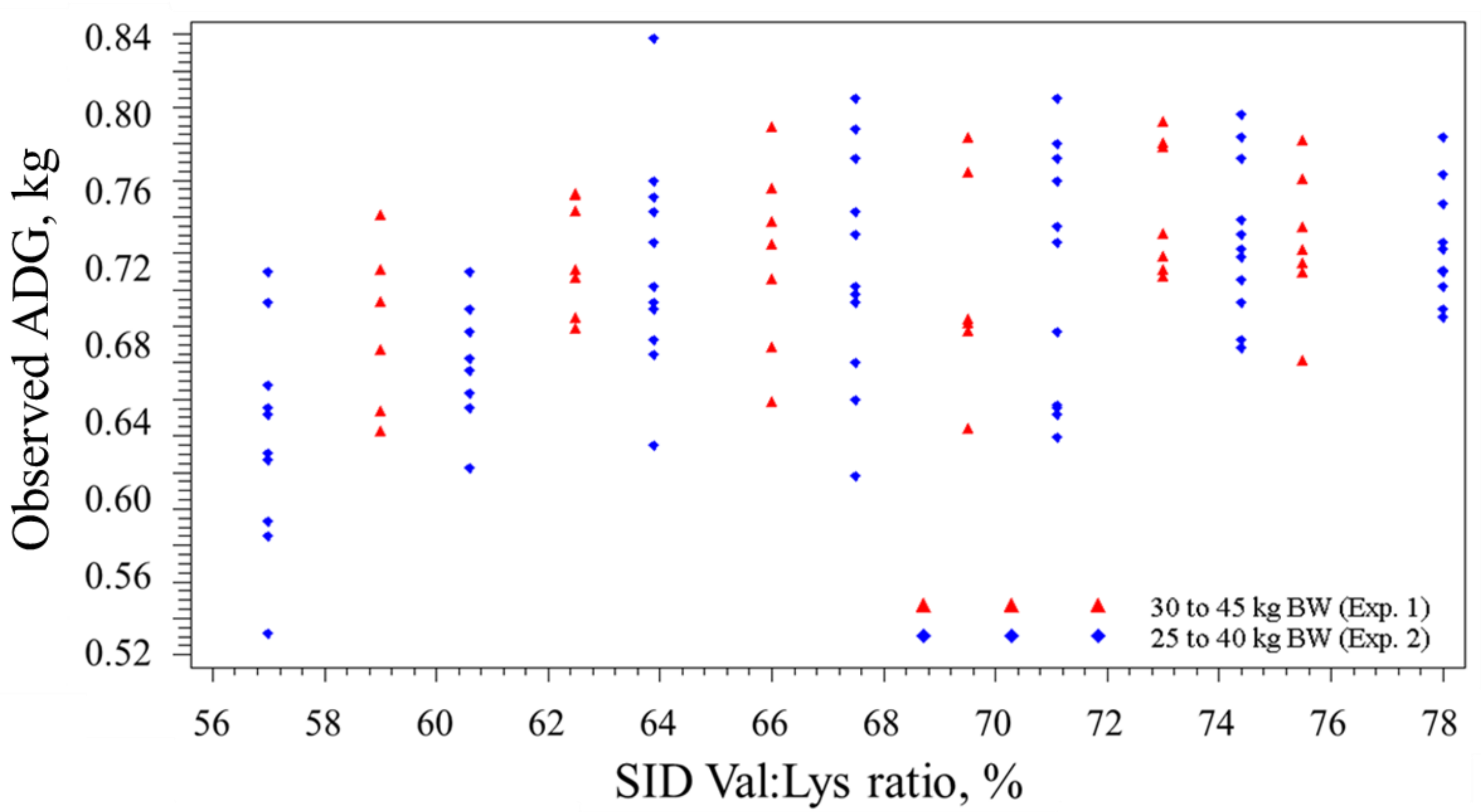

Figure 6.1. Influence of standardized ileal digestible (SID) Val:Lys ratio on ADG values across 2 experiments in 25- to 45-kg pigs. Each data point represents a pen of pigs with 25 or 27 pigs per pen.

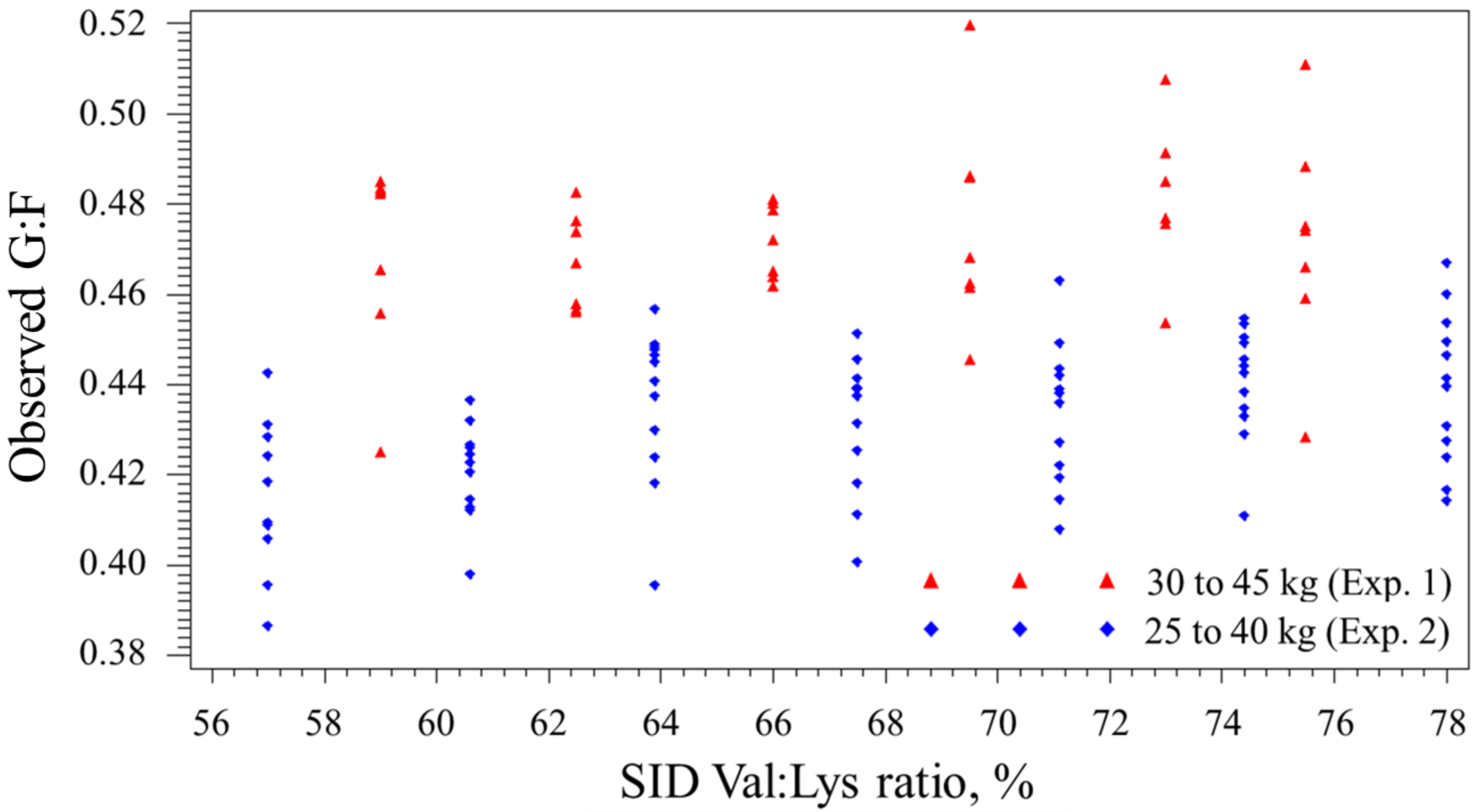

Figure 6.2. Influence of standardized ileal digestible (SID) Val:Lys ratio on G:F values across 2 experiments in 25- to 45-kg pigs. Each data point represents a pen of pigs with 25 or 27 pigs per pen.

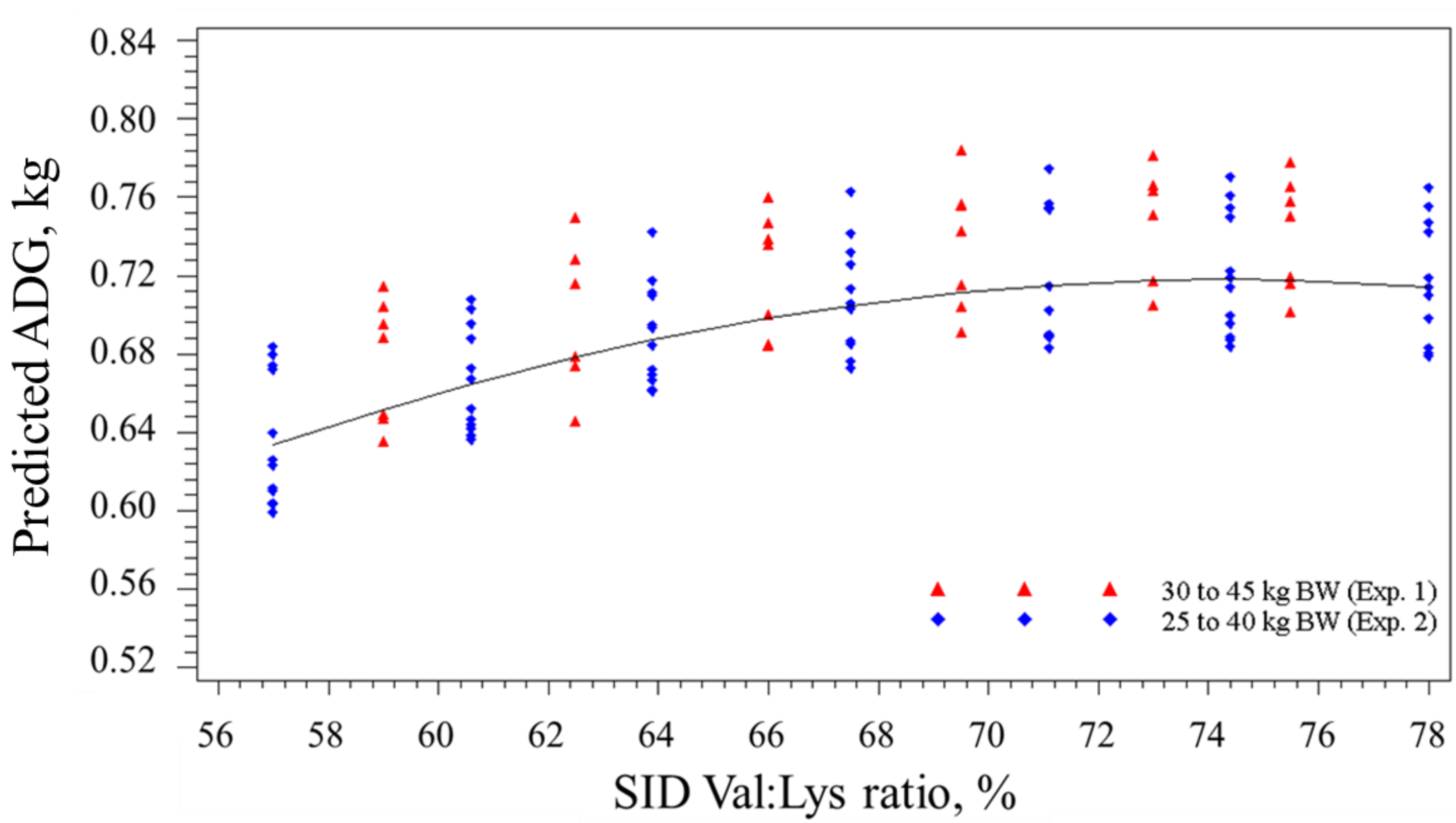

Figure 6.3. Fitted quadratic polynomial (QP) regression model with ADG as a function of increasing standardized ileal digestible (SID) Val:Lys ratio in 25- to 45-kg pigs. The maximum mean ADG was estimated at 74.4% (95% CI: [69.5, >78.0%]) SID Val:Lys ratio. Each point represents the predicted value for the typical pen of pigs after adjustment for random effects, heterogeneous variance, and initial body weight.

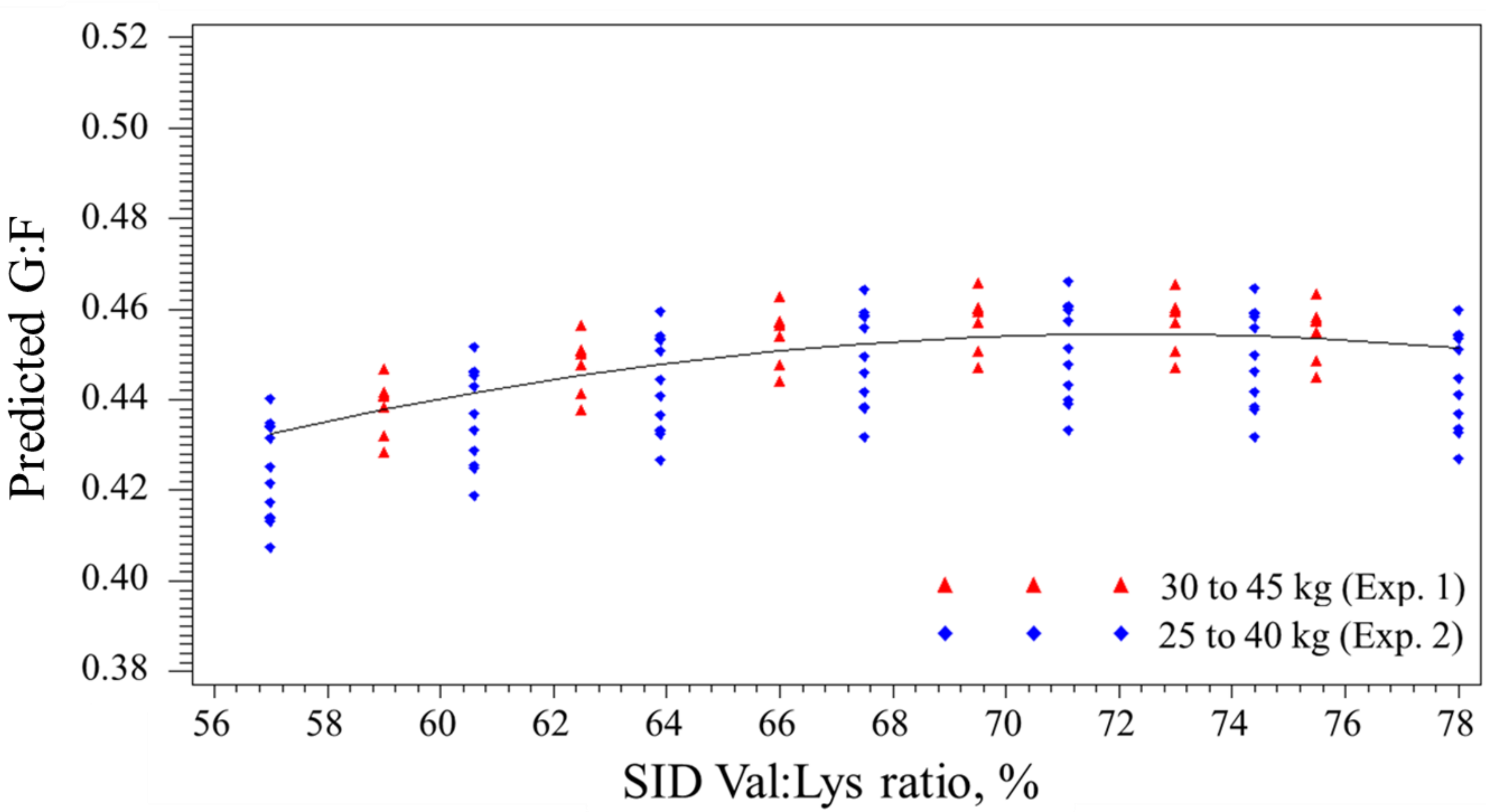

Figure 6.4. Fitted quadratic polynomial (QP) regression model with G:F as a function of predicted values of increasing standardized ileal digestible (SID) Val:Lys ratio in 25- to 45-kg pigs. The maximum mean G:F was estimated at 72.3% (95% CI: [64.0, >78.0]) SID Val:Lys ratio. Each point represents the predicted value for a typical pen of pigs after adjustment for random effects.

Future directions for research

Future research should evaluate the interactive dose-response relationship between energy and AA on sow body weight gain because NRC (2012) model that predicts sow body weight changes does not account for the response to different AA intake levels. Similarly, dose-response relationships should target specifically the effects of energy on individual piglet birth weight and stillborn rate. Finally, the effects of different levels of AA intake during late gestation on pre-weaning mortality have not been reported previously in the scientific literature. While the effects of late gestation dietary composition effects on piglet birthweight are modest we have characterized negative effects mediated through increased energy intake on stillborn rate as well as positive effects of AA intake on pre-weaning mortality.

In the course of these experiments we have refined the characterization of dose-response curve fitting. We demonstrated how to implement linear and non-linear model accounting for heterogeneity of variance and following the hierarchical principle of model fitting. Future efforts should demonstrate how to implement non-linear mixed models with covariates and in factorial treatment structure.

Critical findings from the Trp and Val experiments were: 1) the optimum SID Trp:Lys and Val:Lys were higher for ADG than G:F partially due to the effects of Trp and Val on feed intake regulation; this is particularly important given that NRC (2012) does not give differential requirements between ADG and G:F, and 2) pigs responded in a diminishing return manner to increased AA levels in the diet and, thus, economical models can be built to evaluate the economic optimum AA:Lys ratio. Due to the difficulty categorizing responses to dietary Trp and evidence in the literature that females are more sensitive than males to Trp deficiencies we chose to utilize gilts to categorize the responses. Therefore caution should be applied when inferring these results to barrows. Given that current market weight has reached levels above 130 kg, future research should evaluate the effects of SID Trp:Lys in 130 to 150-kg BW. Future research should evaluate the SID Val:Lys requirement for ADG and G:F in pigs heavier than 45 kg under commercial conditions.

Swine nutrition and production factsheets

Impact of increased feed intake during late gestation on reproductive performance of gilts and sows

Márcio A. D. Gonçalves*[18], DVM, PhD, Steve S. Dritz*, DVM, PhD,

Mike D. Tokach†, MS, PhD, Joel M. DeRouchey†, MS, PhD,

Jason C. Woodworth†, MS, PhD, Robert D. Goodband†, MS, PhD

*Department of Diagnostic Medicine/Pathobiology, College of Veterinary Medicine, and

†Department of Animal Sciences and Industry, College of Agriculture, Kansas State University,

Manhattan, KS 66506-0201

[18] Corresponding author: marcio@k-state.edu

Fast facts

- Each 1 kg/d increase in feed intake increases body weight gain of gilts and sows by 7 kg from d 90 to farrowing.

- Effects of "bump feeding" on individual piglet birth weight are modest and average +28 g/piglet.

- The impact on piglet birth weight is due to increased energy rather than amino acid intake.

Introduction

Bump feeding during late gestation is a widely used practice generally defined as increasing feed intake by about 1 kg from d 90 of gestation to farrowing. The goal is to provide the gestating sow the extra energy and amino acids needed in late gestation to satisfy the exponential conceptus growth[1].

What is the impact of increasing feed intake in late gestation on sow characteristics?

- Body weight gain: Increasing feed allowance by 1 kg/d during late gestation increased sow body weight gain by 6.9 ± 0.8 kg (Table 7.1)[2-4].

- Backfat: Gilts and sows fed 2.3 kg during late gestation lost 1.6 mm of backfat until farrowing whereas those fed 3.9 kg did not alter their backfat[5] and this difference was maintained until weaning. However, other research[3] did not find evidence that increasing feed intake influenced backfat.

- Lactation feed intake: Two studies[2,5] had no evidence for differences when evaluating control vs. increased feed intake during late gestation, whereas one study[3] observed that gilts with increased feed intake during late gestation consumed 17% less feed during lactation. However, increased feed allowance during the whole gestation period has been reported to reduce feed intake during lactation[6].

- Weight loss during lactation: One study[2] observed more weight loss during lactation when increasing feed intake by 1.4 kg/d in late gestation. Whereas, another[3] observed a marginal interaction (P=0.12) between parity and feeding level in which weight loss during lactation was greater in gilts fed increased feed intake but there was no difference for sows.

- Wean-to-estrus interval (WEI): Two studies observed no evidence of altered wean-to-estrus interval[2,4], whereas one[3] observed a marginal reduction of 0.4 d in WEI for gilts fed increased feed intake with no evidence for differences in sows.

What is the impact of increasing feed intake in late gestation on litter characteristics?

- Total born: As expected due to late gestation feeding, several studies have observed no evidence for differences in total born when comparing control fed females vs. females fed increased feed[3-5,7]. One experiment observed a marginal increase in total born when gilts and sows were fed increased feed during late gestation[2].

- Born alive and stillborn rate: The impact on born alive and stillborn rate is not consistent. An earlier study[2] observed a marginal positive effect on number of piglets born alive (9.7 vs 10.0; P=0.06), whereas other studies[3,5] have not observed any difference. A recent study conducted in a large scale commercial research facility[4] observed a small reduction in born alive due to an increase in stillborn rate in sows (6.5 vs. 4.4%) fed high energy intake but no effect in gilts.

- Total litter birth weight: Increased feed intake during late gestation had a positive impact in one study[7] for gilt litters; however, three other studies[3-5] did not observe differences in total litter birth weight.

- Individual piglet birth weight: An earlier study[2] observed an improvement of 40 g/piglet in individual born alive piglet birth weight for females fed increased amounts of feed during late gestation independent of parity. Two additional studies[3,7] observed this positive impact of increasing feed intake during late gestation in gilts, but not in sows. However, individual piglet birth weight could have been confounded with litter size in one of the studies[3], whereas amino acids could have been deficient in the control diet in the other study[7]. However, another study[5] evaluated increasing feed intake at higher levels (7.5 vs. 12.7 ME Mcal/d) and did not find differences. A recent study[4] observed that increased feed intake during late gestation improved individual born alive piglet birth weight by 30 g/piglet. The later study concluded that this improvement was influenced by high energy rather than high amino acid intake.

- Pre-weaning mortality: Several researchers were not able to detect evidence of an influence from increasing feed intake during late gestation on pre-weaning mortality[2,3,5]. A recent study[4] observed an association between lysine levels during late gestation in which increasing lysine reduced pre-weaning mortality (10.4 vs. 11.6%).

- Piglet weaning weight: While one study[2] observed an improvement in piglet wean weight (5.20 vs. 5.37 kg) from females fed increased amounts of feed during late gestation, two others[3,5] did not observe any differences. Other studies measured birth weight, but not weaning weight.

- Estimated economic impact: An economic model accounting for changes in hot carcass weight and survivability from birth-to-market (assuming 0.9 kg extra feed/d for the last 21 d of gestation and a feed cost of $0.24/kg) estimated that the impact of moving the population's average piglet birth weight by 28 g has a modest net impact over feed cost of approximately $0.46 per marketed pig.

In conclusion, each 1 kg increase in daily feed allowance during late gestation is associated with approximately 7 kg of additional body weight gain for gilts and sows. The impact of increased feed intake during late gestation on piglet birth weight is modest and is due to the increase in energy rather than the increase in amino acid intake. A descriptive summary of the literature showed that piglets from females with increased feed intake during late gestation were on average 28 ± 20.4 g heavier.

Table 0.1. Descriptive summary of experiments evaluating increased feed intake during late gestation.

Exp.*	Type	Start, d of gestation	Litters per treatment, n	Total born, n	Control, Mcal ME/d	Control, g SID Lys/d	Increased feed intake, Mcal ME/d	Increased feed intake, g SID Lys/d	Increased by treatment	
									Female BW gain, kg/kg of extra daily feed***	Piglet birth weight, g
2	Both	90	540	10.6	5.8	10.6	10.2	18.4	5.7	40
3	Gilts	90	21	14.3	6.8	11.9	9.8	17.1	5.7	86
3	Sows	90	32	12.4	7.9	11.9	11.4	19.9	5.4	-109
4	Gilts	90	371	14.2	5.9	10.7	8.9	10.7	5.6	24
4	Gilts	90	371	14.2	5.9	20.0	8.9	20.0	9.1	28
4	Sows	90	181	15.1	5.9	10.7	8.9	10.7	9.0	47
4	Sows	90	181	15.3	5.9	20.0	8.9	20.0	10.8	19
5	Both	100	57	11.2	7.5	10.8	12.7	18.3	4.8	10
7	Gilts	100	24	12.5	7.0	9.8	12.9	18.2	---	126
7	Sows	100	51	12.9	7.9	11.2	13.9	19.5	---	-69
Average**	**---**	**90.6**	**---**	**12.6**	**6.0**	**13.5**	**9.6**	**16.6**	**6.9 ± 0.8**	**28 ± 20.4**

[*] Experiment as identified in the references.
[**] Weighted based on the number of sows in each study.
[***] Assuming a corn-soybean meal based diet with 3,252 kcal/kg of ME, is the amount in kg of BW gain per kg of extra daily feed above the basal level. For example, increasing the amount of daily feed from 2 to 3 kg in late gestation, the gilt or sow will be 7 kg heavier at farrowing.

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Ingredient database management: I. Overview and sampling procedures

Márcio A. D. Gonçalves*[19], DVM, PhD, Steve S. Dritz*, DVM, PhD,

Cassandra K. Jones‡, MS, PhD, Mike D. Tokach†, MS, PhD,

Joel M. DeRouchey†, MS, PhD, Jason C. Woodworth†, MS, PhD,

Robert D. Goodband†, MS, PhD

*Department of Diagnostic Medicine/Pathobiology, College of Veterinary Medicine, ‡ Department of Grain Science and Industry, and †Department of Animal Sciences and Industry, College of Agriculture, Kansas State University, Manhattan, KS 66506-0201

[19] Corresponding author: marcio@k-state.edu

Fast facts

- Maintaining an accurate ingredient database is important for predictable growth performance of pigs and economic optimization of the production system.

- A standardized sampling procedure is key to manage a successful ingredient database.

Introduction

Maintaining an accurate ingredient database is important for predictable growth performance of pigs and economic optimization of the production system. Chemical analysis to verify ingredient database values is important for signaling when ingredient values should be updated. To maintain a consistent database, appropriate sampling procedure is needed.

Database management overview

A common best practice for diet formulation ingredient database management is to start by selecting energy and nutrient values for ingredients from one or more sources, such as NRC (2012). Ingredient chemical analysis can be used to confirm or modify these differences in nutrient profiles from reference sources, customizing to specific ingredient sources or local agronomic conditions. Additionally, as new alternative ingredients are available in the market, an accurate estimation of its nutrient profile is necessary. Therefore, a critical factor in obtaining accurate ingredient analysis is appropriate sampling.

Sampling procedures

The sampling procedures are separated in 4 steps[1,2]: 1) define the number of samples to be collected, 2) select the appropriate equipment for sampling, 3) define the sampling location and size, and 4) thoroughly mix sub samples and conduct a sample reduction.

Number of samples

To determine the number of samples needed, one must have previous information from the standard deviation of the chemical analysis (i.e., from NRC or from previous information). For example, if the goal is to collect the correct number of samples to estimate the crude protein of soybean meal within 0.5%, one can determine the number of samples by using the equation:

$$n = \left(\frac{z_{0.975} \times s}{0.5}\right)^2,$$

where $z_{0.975}$ is 1.96 for a 95% confidence limit, s is standard deviation of the sample, and n is the number of samples needed. If crude protein in soybean meal has a standard deviation of 0.99, then

$$n = \left(\frac{1.96 \times 0.99}{0.5}\right)^2$$, thus, n = 15 samples needed.

If one is sampling from bagged or sacked products, the number of bags to sample may vary with the size of the load or shipment. For bagged shipments with multiple pallets, each pallet should be sampled to reach the total amount of samples required (i.e, 15 samples total and 3 pallets, 5 from each pallet should be collected). For shipments involving different lots, samples from each lot should be obtained and retained separately. If one is sampling from bulk products loaded into vehicles with multiple compartments, each compartment should be sampled to reach the total amount of samples required (i.e, 15 samples total and 3 compartments, 5 from each compartment should be collected).

Sampling equipment

The correct selection of sampling equipment is necessary to obtain a representative sample. The most common sampling equipment is the slotted grain probe (Fig. 7.1 and 7.2), which can be manual or automated[1,2]. Probes are available in a variety of sizes to appropriately sample the bag, container, or truck where a representative sample is being obtained. For trucks or railcars, the cylinder slotted or automatic probe should be long enough to reach the bottom of the vehicle to obtain samples. An advantage of the slotted grain probe is that it obtains sample throughout the entire depth of the material.

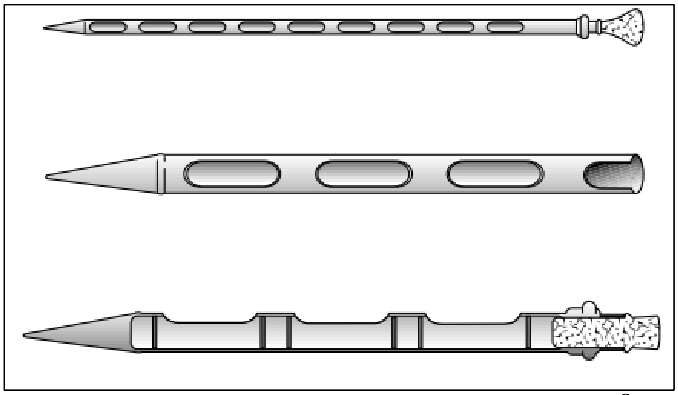

Figure 0.1. Manual slotted grain probe diagram[2].

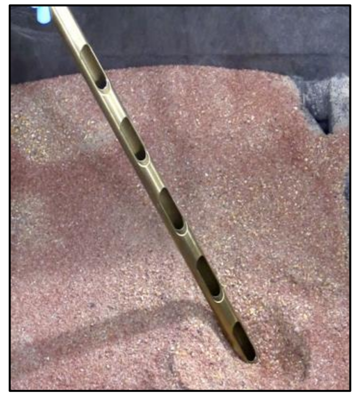

Figure 0.2. Manual slotted grain probe.

Sampling location and size

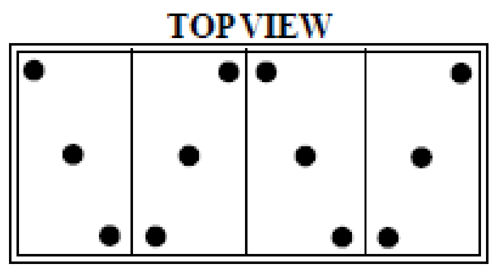

Figure 0.3. Example of location for sampling[1] (top view in a vehicle, i.e., truck or railcar).

Sampling patterns by probe should ensure that a representative sample is collected. For bulk grain, Fig. 7.3 shows an example of locations for collection from a top view in a vehicle (truck or railcar). This pattern may be varied, but demonstrates product in four compartments being sampled from the corners, sides, and middle. Realistically, many mills cannot afford to collect such a large number of probes because it slows down the receiving procedure. Although collecting a single probe sample from a truck is not as representative as collecting the multiple samples shown in Fig. 7.3, it may be the best option for mills that struggle with receiving efficiency. If only one probe is collected, particular care should be taken to vary the compartment and location within the compartment during sampling, Some automatic probes only collect sample from the end, as opposed to the entire length of the probe. If this is the case, care should also be taken to vary the depth of the probe.

While sampling by probe is the most common method, many mills instead sample with either a pelican sampler or catch method during unloading. If this is the case, the sample should be comprised by small collections throughout the entire unloading process. Regardless of the sampling method, the sample size for grain should not be less than 1 kg[1].

Figure 0.4. Bagged ingredient sampling.

When sampling bagged feeds and ingredients (Fig. 7.4), the bag should be stood on end and the probe, or bag trier, inserted diagonally into the bag so that it reaches the opposite corner. The probe should be withdrawn and the sample poured into a container. Approximately 500 g should be collected from each bag. If the lot is 10 bags or fewer, each bag should be sampled, if the lot is 11 bags or more, select 10 bags representative of varying locations in the lot to sample.

Figure 0.5. Riffle divider[2].

- Liquid ingredients and fats: Sampling procedures for liquid ingredients and fats use the same principles as sampling dry ingredients, but with modified liquid probes or collection devices that can be affixed to hoses to collect representative samples during unloading[1].

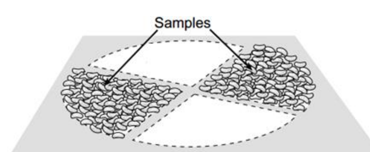

Figure 0.6. Quartering method[2].

- Aseptic feed sampling: When sampling feeds or ingredients for analysis of biological hazards, aseptic sampling should be used[3]. Further information on aseptic sampling can be found at: www.ksuswine.org.

Sample reduction

After samples are collected, they often must be blended and sample size reduced for analysis. If this is necessary, subsamples should be thoroughly mixed together. The samples can be split with a riffle divider[2] (Fig. 7.5) or by the quartering method (Fig. 7.6). Proper division using a riffle divider involves pouring the sample evenly over the divider, then combining the catch pans, and pouring the combined sample through the divider a second time. One of the pans can then be discarded and the process repeated to reduce sample size. The desirable end result will be two samples of approximately 500 g each: one that may be submitted for chemical analysis and a second that may be retained as a backup. Normally, the samples are retained until the livestock is slaughtered.

Take home message

It is critical to have accurate nutrient values for all ingredients by using a standardized sampling procedure to monitor chemical composition of incoming ingredients.

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Ingredient database management: II. Energy

Márcio A. D. Gonçalves*[20], DVM, PhD, Steve S. Dritz*, DVM, PhD,

Cassandra K. Jones‡, MS, PhD, Mike D. Tokach†, MS, PhD,

Joel M. DeRouchey†, MS, PhD, Jason C. Woodworth†, MS, PhD,

Robert D. Goodband†, MS, PhD

*Department of Diagnostic Medicine/Pathobiology, College of Veterinary Medicine, ‡ Department of Grain Science and Industry, and †Department of Animal Sciences and Industry, College of Agriculture, Kansas State University, Manhattan, KS 66506-0201

[20] Corresponding author: marcio@k-state.edu

Fast facts

- There are different methodologies for assigning a net energy value to an ingredient; however, consistently using the same methodology to assign energy values to ingredients is essential for developing a successful database.

Introduction

Dietary energy is an important and expensive component of swine diet. The net energy (NE) system is the most correlated to performance and accounts for the heat increment generated by different ingredients. The most common ingredient values are from NRC[1] and INRA[2]. A well-founded energy system in formulation is especially important with the increasing use of alternative ingredients.

How to assign or update energy values in ingredient databases?

Ingredients with a different chemical profile will generally have a different energy concentration. In order to assign or update an energy value for an ingredient, different approaches are possible:

- **Estimation and validation trials**: Two other approaches are possible by conducting experiments in commercial research barns to generate more information regarding the ingredient.

a. **Estimation trial:** This approach uses energetic efficiency of gain (kcal of NE/kg of gain) to estimate the NE per kg of the ingredient[3,4]. After calculating the energetic efficiency of gain, the researchers calculate what the energy content of the ingredient would be to provide the same energetic efficiency as a standard corn-soybean meal based diet.

b. **Validation trial:** In this approach, the nutritionist assigns the estimated energy value and then conduct a trial with different inclusions of the ingredient compared to a standard corn-soybean meal based diet. In this case, the diets are formulated to have the same level of energy. The expectation in the latter is to have equal performance across the different inclusion levels and is tested by evaluating if the slope of the linear regression between feed efficiency and the ingredient inclusion level is different from zero or not[5].

- **NRC (2012)[1] model equations:** Different equations for predicting NE are presented in the 2012 NRC. However, the equation from Noblet et al. (1994)[6] was used in that publication to calculate NE content of feedstuffs because of the difficulty to acquire some of the nutrient required by other equations (i.e., sugar, digestibility values). The equation by Noblet et al. (1994)[6] requires chemically analyzed values of CP, ADF, ether extract (EE), and starch values. Additionally, this equation requires a ME value. Therefore, if no ME value is available for the ingredient, the ME equation presented in NRC (2012) can be used to estimate a ME value using ash, CP, EE, and NDF.

- **INRA/EvaPig (Saint-Gilles, France) software[2]:** This program integrates equations for several different classes of ingredients to predict a NE and nutrient profile. If the ingredient is biologically similar to any other ingredient family (cereals, cereal by-product, vegetable protein sources, dairy by-products, etc) one can use the closest ingredient as reference. This is the recommended method[2] compared to creating an ingredient profile from scratch because the energy values of the ingredient will be calculated by using specific energy equations related to the reference ingredient. The inclusion of proximate, calcium, phosphorus and total amino acids analysis results for the ingredient in the software is recommended to fine tune the nutrient profile. For example, creating a cereal by-product with 88% dry matter (DM), 9% crude protein (CP), 2% crude fiber, 2% ash, 3% crude fat, 63% starch, and using corn as the reference ingredient in EvaPig, the ingredient is calculated with 2,523 kcal of NE per kg for growing pigs, whereas corn in EvaPig is estimated at 2,651 kcal of NE per kg.

If there is no ingredient available or family of ingredient to use as reference, then an ingredient can be created using equations in the software. To calculate the metabolizable energy (ME) or NE value of the ingredient, the chemical analysis of DM, ash, CP, fiber [crude fiber, neutral detergent fiber (NDF) or acid detergent fiber (ADF)], and either crude fat or gross energy are mandatory. The analysis of starch is required to calculate ME and NE. The analysis of sugars will add precision to the calculations. The EvaPig software manual[2] has step-by-step instructions on this process. For example, the same cereal by-product described above is calculated to 2,550 kcal of NE /kg for growing pigs. Note the slightly different NE estimate (2,550 vs. 2,523 kcal/kg) is because the latter example uses only generic equations whereas the former uses not only generic but also specific equations since corn was used as a reference ingredient. Additionally, this software accounts for differences in energy digestibility between the growing pigs and adult sows[7].

- **Supplier information:** Some nutritionists use energy values provided by the ingredient supplier. It is important to have an understanding of the methodology of how those values were derived and to gauge if they are logical given its nutrient composition. Another approach is to use chemical analysis provided by the supplier and use either approaches 1 or 2 from above to predict the energy value.

Table 0.2. Energy value related to corn for growing pigs

	NRC[8]		EvaPig[2]	
Ingredient	ME	NE	ME	NE
Corn	100	100	100	100
Corn DDGS (6-9% oil)	100	88	101	78
Sorghum (Milo)	104	104	100	99
Soybean meal, dehulled	97	78	99	75
Soybean hulls	57	37	56	38
Wheat middlings	87	79	77	69

Ratio the energy value relative to a reference ingredient across databases

This method generates a relative value between any of the methods cited above, in which the ingredient is assigned an energy value as a ratio to the corn (as an example) which is obtained from the reference database (i.e., EvaPig). From there, the nutritionist would use that ratio to corn from the current database. For example, the new ingredient has 2,000 kcal of NE/kg whereas corn has 2,651 kcal/kg, therefore the ratio is 2,000 / 2,651 = 0.754. On the nutritionist's database, corn is valued at 2,600 kcal/kg, so the new ingredient would be valued at 2,600 × 0.754 = 1,960 kcal NE/kg. The energy values of common alternative ingredients as a ratio to corn are presented in Table 7.2.

It is important to emphasize consistently using the same methodology to assign energy values to ingredients is essential for developing a successful database.

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Ingredient database management: III. Amino acids

Márcio A. D. Gonçalves*[21], DVM, PhD, Steve S. Dritz*, DVM, PhD,

Cassandra K. Jones‡, MS, PhD, Mike D. Tokach†, MS, PhD,

Joel M. DeRouchey†, MS, PhD, Jason C. Woodworth†, MS, PhD,

Robert D. Goodband†, MS, PhD

*Department of Diagnostic Medicine/Pathobiology, College of Veterinary Medicine, ‡ Department of Grain Science and Industry, and †Department of Animal Sciences and Industry, College of Agriculture, Kansas State University, Manhattan, KS 66506-0201

[21] Corresponding author: marcio@k-state.edu

Fast facts

- There are different options to estimate total amino acids (wet chemistry, NIR, or estimating from CP content by regression equations).

- To estimate the standardized ileal digestibility coefficient value, options range from conducting a digestibility experiment, utilizing available literature, to estimating from a similar ingredient.

- It is important to use a single wet chemistry lab for AA analysis to maintain consistency of the database.

Introduction

Assigning digestible amino acid values to ingredients is critical for diet formulation. Steps include: 1) determining the total amino acid levels; 2) assigning digestibility coefficients; and 3) determining how variability in the ingredient will be managed in the database. Typically, base standard ingredient values are obtained from a reference source such as NRC 2012 and then modified if there is evidence of differences in chemical composition relative to the reference source.

How to assign or update amino acids (AA) values in ingredient databases?

To assign or update AA values one needs to:

- **Estimate total AA:** The options below are in order of preference.

a. **Wet chemistry:** This is the best option because it actually measures the total amount of each amino acid in the sample. However, it is important that one utilizes a single laboratory over time to maintain the consistency of the ingredient database. Although measuring total amino acid content is simple, it can be expensive, so particular care should be taken to collect representative samples. Furthermore, the preferred analytical method should be determined, and laboratories selected that utilize that methodology. Approved methods should be recognized by AOAC International, the American Association of Cereal Chemists, or other professional organization.

b. **Near infrared (NIR) spectroscopy from a similar ingredient:** Near infrared spectroscopy predicts nutrient composition from a sample based on its reflectance. Results from the wet chemistry analysis are used to generate and update nutrient prediction equations used in the NIR determination. Thus, it is important that an accurate and the same wet chemistry laboratory is used to update the calibration of the equations. It is less expensive than wet chemistry.

c. **Estimate from crude protein (CP) from a similar ingredient by regression equation:** Estimates the amino acid content from the crude protein (CP) level (wet chemistry or NIR) of the samples[1]. Caution must be taken with this approach because the coefficient of correlation (r) is low for some AA in some ingredients (i.e., lysine in corn; r=0.62). A standardized ileal digestible (SID) amino acid calculator is cited below, which uses either CP or total amino acid in regression equations to estimate the SID AA values[2].

d. **Estimate from CP from similar ingredient by assuming proportionality:** Another approach that can be used, but is the least preferred, is one that assumes that the amino acid content and CP content are directly proportional (i.e., if CP decreases from 48 to 46.5%, which is a 3.1% reduction, thus all AA are reduced by 3.1%).

- Assign or update SID coefficient values for individual AA:

a. **Determine in an 'in vivo' experiment (best option):** To obtain SID coefficient values for individual AA, one needs to collect ileal digesta[3]. This can be accomplished through different surgical procedures; however, the most commonly used is the T-cannula method[4]. This procedure is normally performed in a university research setting. This is the best option if there is economic value for the ingredient and could be used for a long period of time. Because it is not very practical to conduct in swine production companies, other options are listed below.

b. **Utilize available scientific literature:** Search for SID coefficient values in the literature for the specific ingredient.

c. **Estimate from similar ingredients or class of ingredients with known values:** In this case, one would assume similar SID coefficient values between the reference and the ingredient. If a similar ingredient is not available, a similar class of ingredients can be used. Thus, evaluate from which family the ingredient pertains or is closest too (cereals, cereal by-product, vegetable protein sources, dairy by-products, etc). This can be done through understanding of the origin/background of the ingredient as well as through proximate analysis.

d. **Default SID coefficient values from EvaPig (Saint-Gilles, France)[5]:** When no information is available about the ingredient, EvaPig has a default option for digestibility values. This is the least preferred option, since it can be inaccurate. Thus, caution should be used[5].

- Determine how variability in the ingredient will be managed in the database: One challenge regarding some ingredients such as corn DDGS or bakery by-product is accounting for batch-to-batch variation. As far as variability, being conservative will normally be the correct approach, however, this can be a costly mistake. Therefore, understanding the variability in the nutrient values (total AA or coefficient values), as well as the analytical procedure used is important. After having an average as well as a measure of variability (i.e., standard deviation) of the values, one approach is to assign a value that is the mean minus half of the standard deviation. Thus, the actual value would be higher than the assigned value approximately 69% of the time. For an alternative ingredient, such as corn DDGS, for example, most companies will submit samples for total amino acid analysis and use book values for digestibility coefficients. Some nutritionists will segregate DDGS in the database, for example, by source and will also update the amino acid values quarterly or for each new crop. Additionally, some nutritionists monitor crude protein (i.e., via NIR) and submit samples for total amino acid analysis via wet chemistry if it changes above or below a certain limit previously defined.

How should lab-to-lab variability in amino acid analysis be handled to build a database?

To eliminate lab-to-lab variability use a single laboratory. To define which laboratory to use, some nutritionists send several (i.e., five) similar samples of an ingredient (i.e., soybean meal) to several labs. Thus, the nutritionist compares those results with the expected variation from the literature and then selects a laboratory to work with. This is known in the industry as “ring test”. It is important to know their laboratory internal standards and if they conduct any result benchmark. Some companies conduct amino acid analysis mainly via NIR and quarterly cross validate the NIR analysis with wet chemistry. Suggested analytical variation of nutrients is published by American Association of Feed Control Officials. For example, the acceptable analytical range for crude protein is (20/x) + 2, with x representing the target crude protein concentration[6].

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Ingredient database management: IV. Phosphorus

Márcio A. D. Gonçalves*[22], DVM, PhD, Steve S. Dritz*, DVM, PhD,

Cassandra K. Jones‡, MS, PhD, Mike D. Tokach†, MS, PhD,

Joel M. DeRouchey†, MS, PhD, Jason C. Woodworth†, MS, PhD,

Robert D. Goodband†, MS, PhD

*Department of Diagnostic Medicine/Pathobiology, College of Veterinary Medicine, ‡ Department of Grain Science and Industry, and †Department of Animal Sciences and Industry, College of Agriculture, Kansas State University, Manhattan, KS 66506-0201

[22] Corresponding author: marcio@k-state.edu

Fast facts

- Defining available P for ingredients is expensive and requires bone samples. An alternative, Standardized Total Tract Digestible (STTD) P is less expensive and only requires fecal sample collection to be estimated.

- Formulating diets on a STTD P basis is more accurate than Apparent Total Tract Digestible because it accounts for basal endogenous losses, and thus, is additive for diet formulation.

Introduction

Phosphorus (P) is an inorganic element that is important for development and maintenance of the skeletal system[1]. Diets formulated with excess P can have negative impact on the environment due to increased P excretion[2]. This fact sheet will briefly explain the different ways that the P can be expressed, how to assign a P value to an ingredient, and the effects of naturally occurring phytase and diet form on P digestibility.

How dietary P can be expressed?

Phosphorus can be expressed as total or bioavailable.

- Total P: Total P represents all P that the ingredient contains (including the non-available P which is mostly bound in phytic acid, and represents 60 to 75% of the total P in cereal grains and oilseed meals)[1,3].

- **Bioavailable P:** Bioavailable P is the proportion of P that can be absorbed and available for use or storage[4]. The most common methods to estimate P bioavailability are the slope-ratio assay or digestibility experiments. Slope-ratio assay method theoretically estimates the digestible plus post-absorptive utilization of P at the tissue level and is known as available P (AvP) whereas digestibility experiments measure only the digestible utilization, known as Digestible P[5].

a. **Available P:** In the slope-ratio assay method, linear regression is fitted to the response criteria (ex. bone ash) for each set of titrated diets (new vs. inorganic standard ingredient) and the slope of the equation from the ingredient is divided by the slope from the inorganic standard. The drawbacks of slope-ratio assay method are mainly[1,5]: 1) assumption that the inorganic standard is 100% bioavailable thus it is important to use the same standard for all ingredients; 2) dependent on the response criteria used (bone ash vs. P retention); and 3) relatively expensive to complete.

b. Digestible P: Digestible P can be expressed as Apparent Total Tract Digestible (ATTD) P or Standardized Total Tract Digestible (STTD) P. The difference between ATTD P and STTD P is that the later corrects for basal endogenous P losses (Figure 7.7). The concept of STTD P is similar to the concept of standardized ileal digestible amino acids because there is no net P absorption or secretion in the large intestine[1]. The main drawback of the ATTD P method is that it underestimates the true amount of digestible P because does not account for basal endogenous losses. Basal endogenous losses account for approximately 25.6% of the animal's daily P requirement[5]; therefore, expressing P is on a STTD basis is more accurate than on a ATTD basis because is additive for diet formulation.

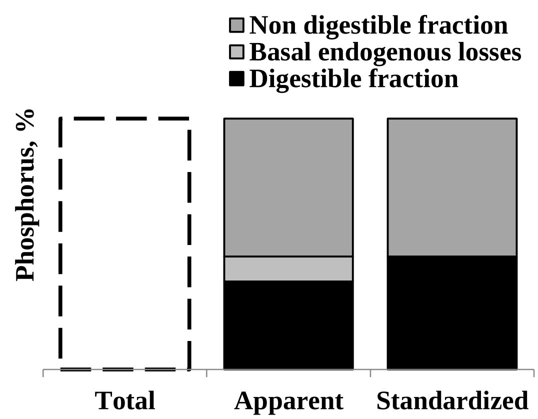

Figure 0.7. Total, standardized and apparent digestible phosphorus and their respective fractions.

How to assign or update P values in ingredient databases?

To assign or update a P value to an ingredient, two steps are needed:

- Analyze the ingredient samples for total P: This step is simple and low cost and just requires a total P analysis of the ingredient.

- Assign a digestibility value: Different databases in the literature express P on different basis (Table 7.3). If the ingredient is from a similar family (cereals, cereal by-product, vegetable protein sources, dairy by-products, etc), then a family ingredient can be used as a reference ingredient for the digestibility.

Table 0.3. Basis of expressing P in different ingredient databases and its availability or digestibility values for corn and soybean meal.

	NRC (1998)	NRC (2012)		EvaPig (2008)
Ingredient	Availability, %	ATTD, %	STTD, %	ATTD, %
Corn	14	26	34	28
Soybean meal, dehulled	23	39	48	32

An alternative approach is to search for information in the literature about the ingredient digestibility. However, if no reference ingredient is available and there is no information in the literature, the default apparent P digestibility value of 20% is used in one software program (EvaPig; Saint-Gilles, France).

What is the impact of naturally occurring phytase and diet form on P digestibility?

Naturally occurring phytase (also known as endogenous dietary phytase) can play a role in P digestibility in some ingredients, such as wheat and wheat by-products[1,6]. However, pelleting can inactivate the naturally occurring phytase in these ingredients[1,6]. For example, apparent P digestibility in wheat middlings is 50% in mash diets whereas only 25% in pelleted diets. Naturally occurring phytase is assumed to have an additive effect with exogenous phytase[6]. In pelleted diets, only exogenous phytase contributes to P release assuming the exogenous phytase is heat stable or applied post-pelleting[6]. EvaPig accounts for naturally occurring phytase and the impact of diet form on P digestibility. Even though NRC (2012) acknowledges the effects of naturally occurring phytase in wheat and its by-products and the negative effects of pelleting on endogenous dietary phytase, no adjustments are made in the ingredient values of NRC to account for these factors.

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Feed efficiency adjustments to compare group close-outs in finishing pigs

Márcio A. D. Gonçalves*[23], DVM, PhD, Steve S. Dritz*, DVM, PhD,

Mike D. Tokach†, MS, PhD, Joel M. DeRouchey†, MS, PhD,

Jason C. Woodworth†, MS, PhD, Robert D. Goodband†, MS, PhD

*Department of Diagnostic Medicine/Pathobiology, College of Veterinary Medicine, and

†Department of Animal Sciences and Industry, College of Agriculture, Kansas State University,

Manhattan, KS 66506-0201

[23] Corresponding author: marcio@k-state.edu

Fast facts

- Feed efficiency of group close-outs can be compared after adjusting for known factors that can influence it.

- Body weight, dietary energy and lysine, gender, ractopamine, mortality, pelleting, grain particle size, and immunocastration are major factors affecting feed efficiency and thus adjusting for them can lead to more meaningful benchmark comparisons.

Introduction

Feed efficiency (F/G) is not always related with profit but is a useful metric in benchmarking group close-outs, especially within a production system. In order to evaluate F/G across group close-outs, adjustment factors can be used to account for known sources of variation.

Feed efficiency adjustment in finishing close-outs

Initial and final weight are major factors affecting F/G because fat is less efficient than protein deposition and the rate of fat deposition increases in relation to protein deposition as body weight increases[1]. If dietary net energy values are accurate, a 1% increase in dietary net energy results in 1% improvement in feed efficiency[2]. This assumes dietary lysine is not limiting. Equations accounting for different factors include:

– **Equation (1), accounts for initial and final weight**:

Adjusted F/G[3] = observed F/G + [standardized initial BW (kg) – actual initial BW(kg)] × slope estimate + [standardized final BW (kg) – actual final BW (kg)] × slope estimate

– **Equation (2), accounts for initial/final weight and energy level of the diet:**

Adjusted F/G[4] = observed F/G + [standardized initial BW (kg) – actual initial BW (kg)] × slope estimate

+ [standardized final BW (kg) – actual final BW (kg)] × slope estimate –

– [(standardized energy level – actual energy level)/standardized energy level) × observed F/G]

The slope estimate varies based on energy level of the diet and genetic line[5,6]. The slope estimates per kg BW range from 0.007 to 0.011[5,6].

– **Equation (3), accounts for NE, average BW, and Standardized ileal digestible (SID) Lysine (Lys):** This equation predicts F/G and, then, is modified to calculate an adjusted F/G based on the observed F/G.

F/G prediction[7] = $1/(0.000004365 \times NE - 0.00162 \times AvgBW - 0.08023 \times SID\ Lys + 0.000094 \times NE \times SID\ Lys + 0.3496)$.

$$\text{Adjusted F/G} = \frac{\text{(F/G from Eq. 3 using standardized values)}}{\text{(F/G from Eq. 3 using actual values)}} \times \text{observed F/G}$$

where NE is the weighted average kcal of NE/kg, AvgBW is the average between initial and final weight (kg), and SID Lys (%) is the weighted average SID Lys. The NE and SID Lys are weighted based on the amount of feed from each phase during the finishing period.

– **Other factors to consider when adjusting for F/G:** The impact of mortality on F/G can be calculated by using the average day in which the mortality occurred in the close-out. If mortality is assumed to occur at the mid-point during the finishing phase, for every 1% increase in mortality, F/G will be poorer by 0.5 to 0.8%[8]. Pelleting improves F/G by about 4 to 6% when feeding pelleted diets with less than 20% fines[4]. Feed efficiency will be poorer by 0.002857 for each 1% fines in the pelleted diet[9]. Grain particle size improves F/G by 1.0 to 1.2%[10] for each 100 micron reduction. Gilts have approximately 1.7% improved F/G compared to mixed gender whereas barrows have 1.7% poorer F/G compared to mixed gender[1]. Ractopamine fed for 21 d prior to market improves finisher F/G by 1.8% for 5 and 3.4% 10 ppm inclusion, in a summary of 12 experiments[11]. Immunocastration improved F/G by 4% over surgically castrated barrows for the whole finishing phase in a meta-analysis with 10 studies[12]. Analyses only included data from studies with animals slaughtered between 4 and 6 weeks after the second immunization (market weight between 107 to 110 kg).

Examples of differences in F/G adjustment based on the change of a single factor compared to the baseline system values are shown in Table 7.4. For example, when comparing two close-outs with similar observed F/G, if one was fed diets with higher energy, the adjusted F/G would be poorer than observed F/G to reflect how that group would perform if the pigs would have received the same amount of dietary energy as the lower energy group.

These adjustments are useful by accounting for the different known factors that affect F/G that are normally present in production systems. A feed efficiency adjustment calculator that accounts for the aforementioned factors can be found at www.ksuswine.org in metric and imperial versions.

Table 0.4. Feed efficiency adjustment simulations for different factors in a barn close-out accounting for mortality and pelleting[7]

Parameters	Baseline	Entry weight	Final weight	Dietary energy	Mortality	Pelleting	Gender
Observed F/G	2.90	2.90	2.90	2.90	2.90	2.90	2.90
Initial Weight, kg	22	25	22	22	22	22	22
Final weight, kg	130	130	135	130	130	130	130
Weighted SID Lys, %	0.78	0.78	0.78	0.78	0.78	0.78	0.78
Weighted Energy, kcal NE/kg	2527	2527	2527	2653	2527	2527	2527
Mortality, %	2.5%	2.5%	2.5%	2.5%	7.5%	2.5%	2.5%
Average mortality, d	60	60	60	60	60	60	60
Pelleting, Y or N	N	N	N	N	N	Y	N
If pelleted, % fines	0%	0%	0%	0%	0%	20%	0%
Gender	Mixed	Mixed	Mixed	Mixed	Mixed	Mixed	Barrows
Adjusted F/G	---	2.88	2.87	2.98	2.77	3.10	2.85

* Developed using equation 3. Assumed impact of mortality over the baseline F/G. † Assumed to reduce F/G by 5% when diets were in pellet form, increase F/G by 0.002857 for each 1% fines in the pelleted diet. § Assumed that barrows have approximately 1.7% improved F/G compared to mixed gender based on NRC (2012) model.

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Comparing different phytase sources for pigs

Márcio A. D. Gonçalves*[24], DVM, PhD, Steve S. Dritz*, DVM, PhD,

Mike D. Tokach†, MS, PhD, Joel M. DeRouchey†, MS, PhD,

Jason C. Woodworth†, MS, PhD, Robert D. Goodband†, MS, PhD

*Department of Diagnostic Medicine/Pathobiology, College of Veterinary Medicine, and

†Department of Animal Sciences and Industry, College of Agriculture, Kansas State University,

Manhattan, KS 66506-0201

[24] Corresponding author: marcio@k-state.edu

Fast facts

- Phytase sources differ in the amount of P released per phytase unit. Similarly, laboratories can analyze phytase activity differently. Thus, caution must be taken when comparing phytase sources and inclusion rates.
- One approach to compare different phytase sources and determine replacement rates between sources is to compare their efficacy at a particular P release value (ex. 0.10% available P release).
- When phytase is included in premixes, using a coated or heat stable product is preferred and to use within 60-days of manufacture date.

Introduction

Phytase is an enzyme that hydrolyzes phytate (or phytic acid) and consequently increases phosphorus availability in feedstuffs[1]. Recently, there has been an increase in the number of phytase sources available in the market. Phytase efficiency can be influenced by factors related to the phytase itself, the animal, or the diet substrate[2].

How to measure phytase activity?

Phytase activity is expressed as the amount of phytase units (FTU or FYT) per unit of feed. The standard AOAC method defines one phytase unit as the quantity of phytase enzyme required to liberate 1 μmol of inorganic phosphorus (P) per min, at pH 5.5, from an excess of 15 μmol/L of sodium phytate at 37°C[3,4]. However, 1 FTU from one source does not necessarily have the same P release as 1 FTU from another source[1]. This is because of the different methods of manufacturing phytase utilized by different suppliers as well as the modified AOAC analytical methods used by the different manufactures to determine FTU[3,4].

- **Analytical methods:** Analytical methods to quantify phytase activity differ across laboratories.

For instance, the reaction time between different methods can range from 15 to 65 min[3]. This is reported to be related to the fact that different phytases have a different biochemical nature[5], thus laboratories have modified the initial standard AOAC method analysis.

Phytase sources and their characteristics

Table 7.5 shows examples of currently commercially available phytase sources and their characteristics.

Table 0.5. Examples of currently commercially available phytase sources and their characteristics[2,6,7]

Trade name	Type*	Protein origin	Expression	Maximal recommended temperature**, ˚C
Natuphos G	3	*Aspergillus niger*	*Aspergillus niger*	85.0
Axtra PHY	6	*Buttiauxella* spp.	*Trichoderma reesei*	95.0
OptiPhos PF	6	*Escherichia coli*	*Pichia pastoris*	85.0
Quantum Blue G	6	*Escherichia coli*	*Trichoderma reesei*	90.5
Ronozyme Hiphos GT	6	*Citrobacter braakii*	*Aspergillys oryzae*	95.0

* Initial carbon site of cleavage. Natuphos (BASF, Florham Park, NJ), Axtra PHY (DuPont, Wilmington, DE); Optiphos (Huvepharma, Peachtree City, GA), Quantum Blue (AB Vista, Marlborough, UK), Ronozyme Hiphos (DSM, Parsippany, NJ).
** Caution must be taken to review maximal recommended feed processing temperatures because most manufacturers have heat stable and non-heat stable products.

Phytase sources can differ in several aspects such as storage time/temperature, product form, coating, and activity after feed processing:

a. **Storage time**: Different phytase sources will have different storage stability. In a published study, one commercially available pure phytase product retained more activity over time compared to two other sources[5]. At room temperature (23 ˚C) or less, pure products had 91, 85, 78, and 71% of their initial activity by 30, 60, 90, and 120-d of storage, respectively[5]. Increased temperature significantly increased the rate of degradation.

b. **Storage temperature**: Storage at 37˚C significantly reduced phytase activity compared to storage at (23 ˚C)[5]. Heat stable products generally retain activity longer during storage under higher temperatures[5].

c. **Product form**: Pure products had 85% recovery, vitamin premixes had 73% recovery, and vitamin-trace mineral premixes had only 60% activity of d 0 values after being stored for for 180 d at room temperature (23 °C)[5]. Thus, rate of phytase degradation is more rapid in vitamin-trace mineral containing premixes compared to premixes containing only vitamins.

d. **Coating**: Coated products were approximately 4, 20, and 39% more stable than uncoated products at 30, 60, and 90-d of storage[5]. Thus, coating mitigated some of the negative effects of long storage times and high temperatures on product stability in premixes[5].

e. **Feed processing**: Most manufacturers have heat stable and non-heat stable products. Pelleting feed with phytase can significantly reduce activity in non-heat stable phytase sources whereas heat-stable sources can withstand higher temperatures[8-14]. Post pellet application is one method to retain phytase activity after thermal processing.

Replacement rates for different phytase sources

Due to their different characteristics, phytase sources have different stability and P release values[3,5]. One approach to compare different phytase sources is to compare the phytase activity needed to reach a particular available P (AvP) release value (ex. 0.10% AvP release). This allows for products to be compared on the same level of activity to determine replacement rates for each phytase source. Table 7.6 illustrates the amount of FTU or FYT needed to achieve specific AvP releases from some commercially available phytase products. The effect of phytase on components of the diet beyond P is a current area of research and at this point results are not consistent[15]. The effects of superdosing phytase on pig growth performance are summarized in a different fact sheet.

Table 0.6. Examples of available P (AvP) release for different commercially available phytase sources*

AvP, % release	STTD P**, % release	Phytase activity (FTU or FYT/kg)				
		Axtra PHY	Natuphos	Optiphos	Quantum Blue	Ronozyme Hiphos
0.100	0.088	270	400	200	250	400
0.120	0.106	360	550	250	315	600
0.140	0.124	500	650	500	430	1000
0.160	0.141	750	900	565	585	1500

* Values provided here are derived or estimated from supplier's recommendation. Axtra PHY (DuPont, Wilmington, DE), Natuphos (BASF, Florham Park, NJ), Optiphos (Huvepharma, Peachtree City, GA), Quantum Blue (AB Vista, Marlborough, UK), Ronozyme Hiphos (DSM, Parsippany, NJ).

** Standardized Total Tract Digestible Phosphorus, assuming that the conversion in P release due to phytase from AvP to STTD P is 88.3%, using monocalcium phosphate as reference.

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Effects of superdosing phytase on growth performance of nursery and finishing pigs

Márcio A. D. Gonçalves*[25], DVM, PhD, Steve S. Dritz*, DVM, PhD,

Mike D. Tokach†, MS, PhD, Joel M. DeRouchey†, MS, PhD,

Jason C. Woodworth†, MS, PhD, Robert D. Goodband†, MS, PhD

*Department of Diagnostic Medicine/Pathobiology, College of Veterinary Medicine, and

†Department of Animal Sciences and Industry, College of Agriculture, Kansas State University,

Manhattan, KS 66506-0201

[25] Corresponding author: marcio@k-state.edu

Fast facts

- The current body of literature suggests that superdosing phytase has the potential for a greater effect on nursery pig performance and with less evidence of its effect on finishing pig performance.

- The relative effect to superdosing phytase appears to be greater if the levels of P, amino acids, or other nutrients are marginal in the diet.

Introduction

Phytase is a highly effective enzyme used to release phosphorus (P) from phytic acid. Recent reports have suggested there are additional mechanisms that can lead to enhanced growth response beyond the P release by feeding high doses of phytase. This has been termed "superdosing".

How does superdosing phytase affect growth performance of pigs?

- Nursery pigs: Increasing phytase concentrations up to 2,500 FTU/kg from *E. coli* derived phytase[1-3] in P adequate diets have resulted in improved growth performance. Another commercial nursery[4] study evaluated the impact of phytase up to 3,000 FTU Ronozyme HiPhos (DSM, Parsippany, NJ)/kg in a low lysine diet compared to an adequate lysine diet with 250 FTU/kg. Average daily gain and feed efficiency were restored to levels similar to the adequate lysine diet when pigs

Table 0.7. Impact of phytase activity (FTU)/kg on average daily gain (ADG) and gain:feed (G:F) of nursery pigs as a percentage of positive control*

	Kies et al. (2006)[5]		Zeng et al. (2014)[2]	
FTU/kg	ADG, %	G:F, %	ADG, %	G:F, %
0	79	94	85	95
100	83	96	---	---
250	93	97	---	---
500	98	98	99	98
750	100	98	---	---
1,000	---	---	100	101
1,500	107	99	---	---
15,000	110	103	---	---
20,000	---	---	109	104

*adapted from Kies et al., 2006 (*Aspergillus niger* phytase) and Zeng et al., 2014 (*Escherichia coli* phytase).

were fed low lysine diets with 1,000 FTU/kg. However, a similar study[4] conducted in university settings did not observe difference on growth performance. Two studies[2,5] feeding nursery pigs as high as 20,000 FTU/kg resulted in increased growth rate and feed efficiency compared to the control treatment suggested to have adequate P release (750 or 1,000 FTU/kg; Table 7.7).

- Finishing pigs: A study feeding up to 2,500 FTU/kg Quantum Blue did not impact energy, crude protein or dry matter digestibility of growing pigs[8]. Another study with growing pigs fed up to 2,000 FTU/kg Quantum Blue (AB Vista, Marlborough, UK) observed linear improvements in ADG and F/G[6]. However, a finishing commercial study evaluating another phytase source observed an improvement in F/G only up to 500 FTU OptiPhos (Huvepharma, Peachtree City, GA)/kg[7]. Additionally, a finishing study in a university setting did not observe an impact of 0 vs. 2,000 FTU/kg from three different sources of phytase on growth performance in diets with adequate P[9].

It is important to note that the relative effect to superdosing phytase will be greater if the levels of P, amino acids, and other nutrients are marginal in the diet. It will also depend on the concentration of added of phytase that is already in the diet. One caution is that a large portion of the superdosing studies have been performed or sponsored by the phytase manufacturers. Little data has been generated by independent third-party entities to evaluate the impact of superdosing different phytase sources.

The actual mechanism of superdosing phytase remains unknown[10], but it is most likely to be a combination of the following:

- Releasing an increased amount of P: In theory, releasing P above the requirement would not bring any benefit; however, if the requirement is underestimated marginal releases in P will improve growth performance.

- **Improving utilization of energy, amino acids, and trace minerals**: Phytate may be an antinutritional factor for other nutrients beside P[11,12]. There is some evidence[13] that superdosing could increase the utilization of energy, amino acids, and minerals digestibility. A review[10] speculated that these effects are likely to be a result of changes in threonine, cysteine, glycine, serine, proline, Ca, Na, Zn, and Fe digestibility.

- **Improving nutrient intake**: It is suggested that superdosing improves digestible nutrient intake due to stimulation of intake, which is speculated to be because phytate could be acting as an appetite suppressant. However, the literature is not clear whether superdosing phytase increases feed intake[4,7].

- **Restoration of proportional Ca:P release**: Superdosing phytase may restore the digestible Ca: P ratio. It is suggested that P and Ca release by phytase is not necessarily on a 1:1 ratio[10]. Thus, this could explain the responses to high levels of phytase because P would continue to be released whereas Ca would approach maximum release.

- **Generating *myo*-inositol***: Myo*-inositol has a vitamin-like effect and its deficiency is difficult to demonstrate in pigs because of endogenous synthesis, variable turnover rates, and interaction with other vitamins or nutrients[14]. As phytate is cleaved with increased levels of phytase, m*yo*-inositol is released[6]; however, the literature is not clear regarding a dietary requirement for *myo*-inositol when pigs are fed typical diets[14].

Interaction between phytase and P-release: There is some evidence that 1,500 ppm of zinc or 20 kg/ton of citric acid reduces the P-releasing efficacy of phytase in young pigs or chickens[15,16]. In a study in sheep, 3,000 ppm of formaldehyde applied to soybean meal and then included as 10% of the diet is reported to affect phytate degradation[17]. Therefore, superdosing may potentially restore available P release from inactivation of phytase when release efficacy has been compromised.

In conclusion, the current body of literature has stronger evidence supporting improvements in growth performance in nursery pigs with less evidence for effects with finishing pigs. However, the exact mechanism by which superdosing phytase impacts performance remains unknown.

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Considerations regarding marketing heavy weight pigs

Márcio A. D. Gonçalves*[26], DVM, PhD, Steve S. Dritz*, DVM, PhD,

Mike D. Tokach†, MS, PhD, Joel M. DeRouchey†, MS, PhD,

Jason C. Woodworth†, MS, PhD, Robert D. Goodband†, MS, PhD

*Department of Diagnostic Medicine/Pathobiology, College of Veterinary Medicine, and

†Department of Animal Sciences and Industry, College of Agriculture, Kansas State University,

Manhattan, KS 66506-0201

[26] Corresponding author: marcio@k-state.edu

Fast facts

- Adequate pen space and marketing strategies are crucial to maximize the value of heavier market weight pigs.
- New facilities and equipment (feeder space, drinker height, gate height, alley width, loading ramp) must account for heavier market weight.
- There is a need for empirical data on nutrient requirements of heavier weight pigs.

Introduction

Market weight has linearly increased by 5.8 kg for every 10 years for the last four decades[1]. This trend is driven by the dilution of fixed costs over more weight per pig.

Genetic and nutrition considerations

- Genetic line: Genetic lines will perform differently when raised to heavier market weights, due to differences in lean and fat deposition[2-4].

- Average daily gain (ADG), feed efficiency (F/G), and carcass yield: Cumulative ADG is expected to be 0.5 to 1.5% lower in pigs fed to 145 kg BW compared to those fed to 125 kg BW[4,5]. Space allowance is one of the main factors that will limit gain when pigs get heavier. Cumulative F/G is expected to worsen by 4 to 9% when average final weight increases from approximately 125 to 145 kg[3-6]. Also, as body weight increases, a slight increase in carcass yield has been reported[7,8].

- Lysine requirements: Factorial approaches estimate the SID Lys requirement for pigs fed from 125 to 140 kg[5] and from 140 to 160 kg[9] to be 0.56 and 0.51%, respectively. However, caution must be taken due to a lack of a body of empirical studies in these weight ranges to increase the confidence in these estimates.

- Ractopamine: Feeding ractopamine increased hot carcass weight in pigs up to 130 kg BW[10]. It appears that ractopamine is still effective at higher market weights. The NRC model estimates the SID Lys requirement from 125 to 140 kg BW is 0.77% when using 10 ppm of ractopamine[5]; however, again, there is a need for empirical studies to confirm this estimate.

Health considerations

- Mortality: Assuming the same rate per day in mortality, a longer feeding duration will incur a slight increase in mortality. In addition, loss of additional heavy weight pigs will increase the overall F/G of a barn due to the amount of feed consumed[11].

- Immunity: Based on the time of the finishing period that diseases are occurring and duration of vaccine immunity, adding 2 to 4 weeks of age may require altered vaccination strategies[12].

Management considerations

- Pen space and marketing strategy: If pen space is limited, feed intake, and thus growth, will decrease. Compared to a market weight of 120 kg, space allowance requirements increase per pig of 5% for 130 kg BW or 11% for 140 kg BW[13]. A 136 kg market weight requires 0.90 m^2/pig for maximum ADG while 0.77 m^2/pig causes a 5% reduction in ADG[13]. Marketing strategies that market pigs at regular intervals before closing out a barn provide more space to remaining pigs and allows them to increase their growth. For example, removing pigs to increase space allowance from 0.65 to 0.84 m^2/pig over the last 3 weeks before 140 kg increased growth rate by 4.8%[14].

- Heat production and ventilation: Pigs produce approximately 8% more heat for each 10 kg increase in BW[15]. It is estimated that from 110 to 132 kg BW, there is approximately 15% increase in heat production per pig[1]. The recommended air flow in the barn for pigs with 115 kg BW is 19.9 m^3/h/pig, 127 kg BW is 22.1 m^3/h/pig, and 138 kg BW is 24.3 m^3/h/pig.

- Pubertal estrus: Adding 4 extra weeks of growth (i.e., 125 to 145 kg) could potentially increase the proportion of gilts that would present pubertal estrus[16]. This could have a modest impact on feed intake and ease of handling market gilts.

- Transportation: Heavier pigs require more space during transport to maintain welfare and reduce transport losses[17]. Thus, the recommended space allowance on trucks for pigs marketed in the summer is 0.46 m^2/pig at 114 kg BW, 0.55 m^2/pig at 136 kg BW, or 0.65 m^2/pig at 182 kg BW[17]. Therefore, fewer pigs will be marketed in each load as pig body weight increases.

Facility and equipment design

Due to continued trends for increased body weight of pigs at marketing, building designs should account for this change. Heavier pigs are wider and taller, thus, feeder space, drinker height, gate height, and alley width must be carefully considered.

- Feeder space: The amount of feeder space needed is normally 1.1 times shoulder width[1]. Because shoulder width increases from 31.5 to 32.7 cm when pigs grow from 125 to 140 kg BW[18], the requirement for width of a feeder space increases from 34.7 to 36.0 cm.

- Drinker height: For a 140 kg BW pig, drinker height should be approximately 77 cm for 90 degree nipple drinker and 92 cm for a downward mounted nipple drinker[19]. However, the drinker height should be adjusted to shoulder height of the smallest pig in the pen[19].

- Gate height: Shoulder height increases by 2.8 cm when pigs grow from 125 to 140 kg BW[19].

- Loading ramp: For pigs heavier than 125 kg, 15° or less is the recommended ramp angle compared to 20° for lighter pigs[17].

Packing plant

- Rail capacity and height: Pigs could be heavier than the facility is designed for, thus, the amount of weight that rails support can be a limiting factor. Increased length of the carcass could pose challenge for food safety if the rail is not high enough.

- Primal cut size: Increased size of the primal cut will require adjustment of cut sizes from the retail market perspective.

- Cooling capacity: Increased weight will require an extra amount of time to cool the carcass.

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Dietary fiber withdrawal strategy before slaughter in finishing pigs

Márcio A. D. Gonçalves*[27], DVM, PhD, Steve S. Dritz*, DVM, PhD,

Mike D. Tokach†, MS, PhD, Joel M. DeRouchey†, MS, PhD,

Jason C. Woodworth†, MS, PhD, Robert D. Goodband†, MS, PhD

*Department of Diagnostic Medicine/Pathobiology, College of Veterinary Medicine, and

†Department of Animal Sciences and Industry, College of Agriculture, Kansas State University,

Manhattan, KS 66506-0201

[27] Corresponding author: marcio@k-state.edu

Fast facts

- High fiber diets fed until market reduces carcass yield and increases carcass fat iodine value if high fiber ingredients contain elevated concentrations of unsaturated fatty acids.

- Fiber withdrawal of approximately 15 to 20 d is able to restore carcass yield and reduce impact on iodine value.

- If high fiber diets are economical, a withdrawal of 15 to 20 d prior to market maximizes income over feed cost across different market scenarios.

Introduction

High-fiber ingredients, such as distillers dried grains with solubles (DDGS) and wheat middlings, are often economically viable to use in finishing pig diets. Because most swine producers are paid on a carcass basis, it is important to understand the impact of high-fiber diets on carcass characteristics and economics. Feeding high-fiber diets up to market reduces carcass yield[1]. It also increases iodine value[1] (IV) due to increased unsaturated fatty acids in most high fiber byproducts.

What is fiber withdrawal?

Fiber withdrawal is the replacement of the high-fiber ingredients in finishing diets by low fiber ingredient(s) (ex. corn-soybean meal based diet) for a specific time before market.

Impact of fiber withdrawal on carcass yield and carcass weight

Pigs fed high-fiber diets until market have a reduction in carcass yield[2,3]. Carcass yield is restored in a 15 to 51 d withdrawal time compared to corn-soybean meal diets[2-6]. The reduction in carcass yield is a result of increased large intestine weight and fecal volume when pigs are fed a diet high in insoluble fiber[7,8]. Because yield is the ratio between carcass and live weight, an increase in live weight without a change in carcass weight leads to a lower yield. A summary of

8 experiments[8] suggests an increase in 0.16% in carcass yield for each 1% reduction in neutral detergent fiber. The negative impact of feeding high fiber diets until market on carcass yield is reported to be greater in immunocastrated compared to physically castrated pigs[5].

Impact of fiber withdrawal on carcass fat quality

Iodine value is a practical means of measuring unsaturated ("soft") fat, by measuring the relative amount of double bonds in the fatty acids. More unsaturated fat is associated with a higher carcass fat IV. From a dietary fat perspective, linoleic (C18:2n-6) and α-linoleic (C18:3n-3) acids are the main drivers of IV[9]. Therefore, withdrawing feeding ingredients, such as DDGS and wheat middlings, which have higher levels of unsaturated fatty acids (i.e. linoleic acid) will reduce the amount of unsaturated fat in the carcass and consequently reduce IV. Iodine value was linearly improved with up to 20 d withdrawal but this was not long enough to fully restore IV[8]. However, IV value was fully restored by using a 9 wk withdrawal[10].

Length of fiber withdrawal to mitigate negative yield effects

Two recent studies evaluated withdrawal of diets with 30% DDGS and 19% wheat middlings for 5, 10, 15, 20 d (Exp. 1) and 9, 14, 19, 24 d (Exp. 2) before market[8]. In Exp. 1, carcass yield was restored in a quadratic manner with increase in withdrawal time, being fully restored at 15 d. In Exp. 2, hot carcass weight linearly increased when withdraw time was increased. The data suggested a withdrawal time of approximately 15 to 20 d is needed to fully restore carcass yield.

Impact of fiber withdrawal on economic performance

Economic calculations have demonstrated[8] that when feeding high fiber diets, a withdrawal period of approximately 15 to 20 d appears to maximize income over feed cost across widely variable ingredient and pork market prices. The economics are driven by pigs fed a withdrawal diet maintaining feed intake while consuming a more caloric dense diet which leads to improved carcass weight relative to live weight.

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Appendix A - Supplement SAS code from Chapter 2

```
** Data set from Chapter 4, which evaluated the effects of SID Trp:Lys ratio
on nursery pig performance
** with two residual variance groups: Var1 for (16.5, 18.0% SID Trp:Lys) and
Var2 for (14.5, 19.5, 21.0, 22.5, 24.5% SID Trp:Lys)
*****************************************************************************
;

data GF;
      input Block Trt PenID$ y  varGrp$;

      datalines;
1     0.145 184   562   Var2
1     0.165 187   593   Var1
1     0.180 182   579   Var1
1     0.195 189   610   Var2
1     0.210 186   583   Var2
1     0.225 188   604   Var2
1     0.245 176   572   Var2
2     0.145 145   549   Var2
2     0.165 185   574   Var1
2     0.180 162   592   Var1
2     0.195 164   586   Var2
2     0.210 183   602   Var2
2     0.225 181   593   Var2
2     0.245 180   594   Var2
3     0.145 168   520   Var2
3     0.165 165   587   Var1
3     0.180 166   590   Var1
3     0.195 147   582   Var2
3     0.210 167   606   Var2
3     0.225 174   588   Var2
3     0.245 173   578   Var2
4     0.145 154   563   Var2
4     0.165 170   588   Var1
4     0.180 169   577   Var1
4     0.195 175   555   Var2
4     0.210 150   566   Var2
4     0.225 146   563   Var2
4     0.245 148   551   Var2
5     0.145 172   529   Var2
5     0.165 149   571   Var1
5     0.180 152   575   Var1
5     0.195 161   568   Var2
5     0.210 159   593   Var2
5     0.225 171   573   Var2
5     0.245 153   604   Var2
6     0.145 156   535   Var2
6     0.165 151   579   Var1
6     0.180 163   581   Var1
6     0.195 155   568   Var2
6     0.210 157   591   Var2
6     0.225 160   585   Var2
6     0.245 158   580   Var2
```

```
;

*Set up the data set to generate estimates through the entire range of the
dose-response to generate the broken-line plots;
*Replace number of blocks and treatment range accordingly;
data fill;
      do block= 1 to 6; *If there are not blocks in a given experiment, make
sure to delete this sentence as well as one of the "end" commands below;
      do trt = .145 to .245 by 0.001;
      y =.;
      output;
      end;
      end;
run;

data GF;
      set GF fill;
run;

** Base model assuming homogeneous residual variance;
      proc glimmix  data=GF plots=studentpanel;
      class block trt;
      model y = trt / ddfm=kr;
      random intercept / subject = block;
      output out=igausout pred=p student=std;
      nloptions tech=nrridg;
      lsmeans trt / cl plot=meanplot(cl join);
run;


proc sort data=igausout;
      by trt;

proc gplot data=igausout;
      plot std*trt / vref=(0);
run;

** Base model allowing for heterogeneous residual variances;

proc glimmix data=GF plots=studentpanel;
      class block trt varGrp;
      model y = trt / ddfm=kr;
      random intercept / subject = block;
      random _residual_ / group = varGrp; *Fit heterogeneous residual
variance for each level of varGrp;
      output out=igausout pred=p student=std;
      nloptions tech=nrridg;
      lsmeans trt / cl plot=meanplot(cl join);
run;


proc sort data=igausout;
      by trt;

proc gplot data=igausout;
      plot std*trt / vref=(0) ;
run;
```

```
** Quadratic polynomial mixed model with heterogeneous variance;

proc glimmix data=GF method=MSPL ; *method=MSPL calls for the same method of
estimation as the one used by NLMIXED (Maximum likelihood);
      class  block varGrp;
      model y = trt trt*trt / solution ;
      random intercept / subject=block;
      random _residual_ / group=varGrp;
      output out=igausout pred=p resid=r;
      nloptions tech=nrridg;
run;


data igausout1000; set igausout;
      p1000=p/1000;
      y1000=y/1000;
run;

proc sort data=igausout1000 ;
       by trt;
      symbol1 v=dot c=black i=rq;
proc gplot data=igausout; plot y*trt; run;


** Broken-line linear mixed model with heterogeneous variance;

proc nlmixed data=GF maxiter=1000 gconv=0  start; * Maxiter, Gconv, and Start
are options that help troubleshooting and model convergence;
      bounds .145<R_BLL<.245; * BOUND option which will define boundaries for
the dose levels;
      parms L_BLL= 578 582 586 U_L= -975 -1950 -3900 R_BLL= 0.15 0.16 0.17
Block_Var= 6 11 22 vareVar1= 28 56 112 vareVar2= 134 268 536; * Parms
statement with at least three initial values allows for a grid search over
the likelihood surface;
      z=(trt<R_BLL)*(R_BLL-trt);  * Characterize the model as non-linear;
      s2e = (varGrp="Var1") * vareVar1 + (varGrp="Var2") * vareVar2; *
Specify for fitting different variance groups;
      model y ~ normal(L_BLL + U_L *(z) + beff, s2e);
      random beff ~ normal(0,Block_Var) subject=block out=blups;
      predict L_BLL + U_L*(z) out=ppp;
run;

proc sort data=ppp; by trt;
      symbol1 v=dot height=1.2  c=black i=none;
      symbol2 v=none c=black i=join;

data ppp1000;set ppp;
      y1000=y/1000;
      pred1000=pred/1000;

proc gplot data=ppp1000;
    plot y1000*trt pred1000*trt /overlay;
run;
```

```
** Broken-line quadratic mixed model with heterogeneous variance; *The only
difference compared to the BLL model is the inclusion of the quadratic term
and U_Q2 coefficient in the model;

proc nlmixed data=GF maxiter=1000 gconv=0  start;
      bounds .145<R_BLQ<.245;
      parms L_BLQ= 578 582 586  U_Q1= -975 -1950 -3900 U_Q2= -4685 -9369 -
18738 -40000 -80000 R_BLQ= 0.15 0.16 0.17 Block_Var= 6 11 22 vareVar1= 28 56
112 vareVar2= 134 268 536;
      z=(trt<R_BLQ)*(R_BLQ-trt);
      s2e = (varGrp="Var1") * vareVar1 + (varGrp="Var2") * vareVar2;
      model y ~ normal(L_BLQ + U_Q1*z + U_Q2*(z*z) + beff, s2e);
      random beff ~ normal(0,Block_Var) subject=block out=blups;
      predict L_BLQ + U_Q1*z + U_Q2*(z*z) out=ppp;
run;

proc sort data=ppp; by trt;
      symbol1 v=dot height=1.2  c=black i=none;
      symbol2 v=none c=black i=join;

data ppp1000;set ppp;
      y1000=y/1000;
      pred1000=pred/1000;

proc gplot data=ppp1000;
    plot y1000*trt pred1000*trt /overlay;
run;
```