PREDICTING MARKET PIG WEIGHTS AND FAT IODINE VALUE AND EFFECT OF ZINC ON GROWTH PERFORMANCE AND IMMUNE FUNCTION OF FINISHING PIGS

by

CHAD BENNETT PAULK

B.S., University of Georgia, 2009
M.S., Kansas State University, 2011

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Animal Science
College of Agriculture

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Abstract

The optimum sampling method swine producers should use to estimate the mean and SD of pig BW within a barn was determined based upon both the time required to conduct and the precision and accuracy of each sampling method. Weighing 5 pigs from 15 pens was determined to be the optimal sampling method. This should require approximately 55 min to complete. Weighing 5 pigs from 15 pens had a CI range of 7.2 to 8.0 kg for estimating the mean BW and 5.6 kg for estimating SD. Next, a meta-analysis was conducted using data from existing literature to generate equations to predict finishing pig back, belly, and jowl fat iodine value. While numerous factors were evaluated, dietary essential fatty acids, dietary net energy content, and backfat thickness had the greatest influence on predicting iodine value of the 3 distinct fat depots. Lastly, 6 experiments were conducted to determine the effects of added Zn on growth performance, pork quality, plasma Zn, and ileal mucosal inflammation mRNA expression of finishing pigs fed diets containing ractopamine-HCl (RAC; Elanco Animal Health, Greenfield, IN). Additional Zn increased plasma Zn and reduced relative expression of *IL-1β*, but did not improve growth performance of pigs fed diets containing RAC in 5 of the experiments. However, in 1 of the experiments, adding Zn to diets containing RAC resulted in a trend for improved growth performance of pigs. Supplementing the RAC diets with dietary Zn decreased the percentage of type IIA fibers and tended to increase the percentage of type IIX fibers compared to pigs fed the RAC diet without added Zn. Ractopamine-HCl produced chops that were lighter and less red, but possessed reduced metmyoglobin reducing ability at the end of the display period. However, adding Zn to RAC diets increased metmyoglobin reducing ability levels at the end of the display period.

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Approved by:

Co-Major Professor
Michael D. Tokach

Approved by:

Co-Major Professor
Jim L. Nelssen

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The optimum sampling method swine producers should use to estimate the mean and SD of pig BW within a barn was determined based upon both the time required to conduct and the precision and accuracy of each sampling method. Weighing 5 pigs from 15 pens was determined to be the optimal sampling method. This should require approximately 55 min to complete. Weighing 5 pigs from 15 pens had a CI range of 7.2 to 8.0 kg for estimating the mean BW and 5.6 kg for estimating SD. Next, a meta-analysis was conducted using data from existing literature to generate equations to predict finishing pig back, belly, and jowl fat iodine value. While numerous factors were evaluated, dietary essential fatty acids, net energy content, and backfat thickness had the greatest influence on predicting iodine value of the 3 distinct fat depots. Lastly, 6 experiments were conducted to determine the effects of added Zn on growth performance, pork quality, plasma Zn, and ileal mucosal inflammation mRNA expression of finishing pigs fed diets containing ractopamine HCl (RAC; Elanco Animal Health, Greenfield, IN). Additional Zn increased plasma Zn and reduced relative expression of *IL-1β*, but did not improve growth performance of pigs fed diets containing RAC in 5 of the experiments. However, in 1 of the experiments, adding Zn to diets containing RAC resulted in a trend for improved growth performance of pigs. Supplementing the RAC diets with dietary Zn decreased the percentage of type IIA fibers and tended to increase the percentage of type IIX fibers compared to pigs fed the RAC diet without added Zn. Ractopamine-HCl produced chops that were lighter and less red, but reduced metmyoglobin reducing ability at the end of the display period. However, adding Zn to RAC diets increased metmyoglobin reducing ability levels at the end of the display period.

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Dedication

I dedicate my dissertation to my family, new and old, for everything they have done for me. This would not be possible without their love and support.

Chapter 1 - Effect of sample size and method of sampling pig weights on the accuracy and precision of estimating the mean weight of the population

ABSTRACT

One contributing factor to economic discounts when marketing pigs is the error associated with estimating the barn population's mean BW. The objective was to determine the best method for selecting pigs to improve the accuracy and precision of estimating the population's mean BW. Using a computer program developed in R (R Foundation for Statistical Computing, Vienna, Austria), we were able to generate 10,000 sample means for different sampling procedures on 3 datasets. Datasets A, B, and C were obtained from 3 different production systems where all of the pigs within a single barn were individually weighed on a single day. Sampling methods evaluated were: (1) random sample of 10 to 200 pigs, (2) varying number of pigs within varying numbers of pens; (3 and 4) selecting the heaviest and lightest pig from 15 pens and calculating the mean or median BW, respectively; and (5) selecting the heaviest and lightest pigs from 15 pens and using the calculated mean and median in the following equation:

Estimated population mean, kg = 0.77 * sample mean, kg + 0.25 * sample median, kg

For Method 1, increasing the sample size of a random sample, regardless of pen arrangement, reduced the CI range for estimating the mean pig BW. For Method 2, as both the number of pigs and the number of pens increased, the CI range decreased. Also, increasing the number of pens sampled while keeping the sample size constant reduced the CI range. For Method 3, selecting the heaviest and lightest pigs in 15 pens and calculating the mean reduced

the CI range compared to taking a random sample of 2 pigs from 15 pens. However, the mean of the 10,000 estimated mean BW was less than the actual population mean. For Method 4, selecting the heaviest and lightest pigs in 15 pens and calculating the median did not reduce the CI range compared to taking a random sample of 2 pigs from 15 pens. For Method 5, selecting the heaviest and lightest pigs in 15 pens and calculating the weighted average of the mean and median resulted in a CI range similar to Method 3. For Method 5, the mean of the 10,000 estimated mean BW was similar to the actual population mean and was validated on 2 additional datasets which showed the estimated means were similar to the actual population means. Method 5 provides the most precise and accurate estimation of population mean BW using 30 pigs from a barn population.

INTRODUCTION

Swine producers must meet processing plant specific pig BW and BW ranges to avoid economic penalties. In attempts to reduce these economic penalties, producers have adopted different marketing strategies to reduce the variation in pig BW. Producers also use growth curves based on performance data from previous groups of pigs to predict the age when pigs will meet optimum market weights (Schinckel et al., 2004). However, factors that influence growth rate, such as environment and health status, may not be consistent between groups of pigs (de Lange et al., 2001). Therefore, the ability to model weight distributions for each group of pigs can enhance the swine producer's ability to market pigs at optimum BW.

Because the BW of a population of pigs typically approximates a normal distribution, subsampling methods to predict the average BW of pigs in the barn can be used to model distributions of BW within the barn. These models can then be used to simulate optimum marketing strategies. One critical assumption is that these models assume there is no error in

estimating the mean pig BW and there is no error associated with correctly selecting the appropriate pigs indicated by the simulation modeling. The error associated with estimating the mean pig BW can be defined by the accuracy and precision of the sampling method used for the estimation. Accuracy is defined as "how well the observed value agrees with the true value," and precision is defined as "how well repeated observations agree with one another" (Petrie and Watson, 2006). Using an accurate and precise sampling method will improve a swine producer's chances to maximize economic return when marketing pigs. Therefore, the objective was to use the developed model to determine the best sampling method for estimating the mean pig BW within a barn.

MATERIALS AND METHODS

Datasets

Three datasets (A, B, and C) were used to evaluate sample size and method of sampling on the precision of estimating the pig SD of BW in the barn. Briefly, these 3 datasets were obtained from 3 different production systems where all of the pigs within a single barn were weighed on a single day. If the barn included a pen in which sick or lame pigs were placed, pigs from this pen were not weighed and not included in the analysis. The actual individual pig weights and distributions from the 3 barns were used to simulate various sampling strategies. Normality of pig BW for each dataset was tested using the Shapiro-Wilk statistical test using the UNIVARIATE procedure of SAS (SAS Institute Inc., Cary, NC).

Dataset A was derived from an experiment conducted at a commercial research facility (Groesbeck et al., 2007). This dataset comprised a total of 1,260 pigs (337 × 1050, PIC,

Hendersonville, TN) in 48 pens with 23 to 28 pigs per pen. The barn was filled with pigs over a 1-wk period. Pigs were randomly distributed to pens as they came off the delivery truck.

Dataset B was obtained from a commercial finishing site in northern IA. This dataset comprised a total of 1,261 pigs (359 × 1050, PIC, Hendersonville, TN) in 19 pens with 56 to 81 pigs per pen. The barn was filled with pigs over a 1-wk period. Pigs were randomly distributed to pens as they came off the delivery truck.

Dataset C was derived from a different commercial site in northern IA. This dataset comprised a total of 1,069 pigs (F25 × G performer, Genetiporc, Alexandria, MN) in 40 pens with 20 to 35 pigs per pen. The barn was filled with pigs over a 1-wk period and pigs were randomly distributed to pens as they came off the delivery truck.

Sample Simulation Model

A simulation model was developed using R (R Foundation for Statistical Computing, Vienna, Austria) to demonstrate the error of different sample sizes. The program also determined the error that methods of selecting pigs have on estimating the pig population mean BW. The model was designed to conduct designated sample sizes within each sample method. The estimated mean BW was calculated for each sample. Each sample size was conducted 10,000 times, generating 10,000 estimated means. These were used to determine the accuracy and precision for each sample method. The accuracy was determined by comparing the mean of the 10,000 sample means to the actual pig population mean BW. The precision was determined by calculating a 95% CI for the 10,000 sample means. The 10,000 sample means for each sample size were sorted from least to greatest, and the 95% CI was generated by selecting the 9,751st observation, the upper limit, and the 250th observation, the lower limit. The distances between the upper and lower confidence limits represent the estimated means CI range.

Sampling Methods

Using the developed sample simulation model, 5 sampling methods were tested. For method 1, the model was designed to take a completely random sample of the designated sample size of pigs (10, 20, 30, up to 200), disregarding pen arrangements, and calculate the mean BW of each sample. Therefore, the model generated 10,000 sample means for each sample size (10, 20, 30, up to 200). Method 2 accounted for the nesting of pens within barn. It evaluated the sampling error by taking a completely random sample from a varying number of pigs within varying numbers of pens, with 1 to 15 pigs sampled from 1 to all of the pens. Therefore, the model generated 10,000 samples means for each combination of the number of pigs from the number of pens. For Methods 3, 4, and 5, the model randomly selected 15 pens from the barn. The heaviest and lightest pig was selected from the 15 pens (30 pigs total) and the mean (Method 3) and median (Method 4) BW was calculated to estimate the population mean pig BW. Because an even number of pigs were selected (30 pigs), calculating the median of the sample resulted in taking the mean of the 2 intermediate pig weights or the heaviest pig in the selected light pigs and the lightest pig from the selected heaviest pigs. Method 5 consisted of taking a weighted average of the mean and median BW calculated using methods 3 and 4. The development of the equation used to calculate the weighted average is discussed later in this section.

Because it is not practical to weigh all the pigs in a pen and precisely know which are the heaviest and lightest, a preliminary study was carried out to determine the accuracy when selecting the heaviest and lightest pigs for Methods 3, 4, and 5. The heaviest and lightest pigs were visually selected by production personnel specialized in selecting pigs for market. The probability for selecting the 1st, 2nd, 3rd, 4th, or 5th heaviest pig was 50, 25, 15, 5, and 5%, respectively, and the probability for selecting the 1st, 2nd, 3rd, 4th, or 5th lightest pig was 70, 15,

5, 5, and 5%, respectively. These probabilities were used in the simulation model when selecting the heaviest and lightest pigs from selected pens.

The coefficients used to calculate the weighted average for Method 5 were developed using the PROC MIXED procedure of SAS (SAS institute, Inc., Cary, NC). Inputs for the model were derived from randomly selecting the heaviest and lightest pig from 15 randomly selected pens (30 pigs total) and calculating the mean and median BW. This was simulated 1,000 times for each Dataset A, B, and C. Therefore, the mean and median (n = 3,000) of each simulated sample were used as predictor variables and the actual population mean was the response variable. The following equation was developed to estimate the mean BW of the population:

Estimated population mean, kg = 0.77 * sample mean, kg + 0.25 * sample median, kg,

where sample mean and median are the mean and median BW of the heavy and lightest pigs selected from 15 randomly selected pens (Method 5; 30 total pigs).

The coefficients for the weighted average were developed using Datasets A, B, and C. Next, the equation used in method 5 to estimate the mean pig weights was validated using 2 additional datasets, D and E. These datasets were not included in data used to develop the original equation. Dataset D contained a total of 1,176 pigs individually weighed (population mean = 74.3 kg, median = 74.8 kg, standard deviation= 10.6 kg, and CV = 14.2%) with 20 to 35 pigs per pen and a total of 38 pens. Dataset E contained a total of 961 pigs weighed (population mean = 93.1 kg, median = 93.9 kg, standard deviation= 9.2 kg, and CV = 9.8%) with 16 to 23 pigs per pen and a total of 48 pens. Sampling methods 3, 4, and 5 were simulated in dataset D and E using the model previously described.

Results and Discussion

Datasets

Frequency histograms and descriptive statistics for dataset A, B, and C are provided in Figure 1. From visualizing each frequency histogram, each dataset seemed to approximate a normal distribution. However, the Shapiro-Wilk test rejected ($P < 0.05$) the normality of each dataset. For Dataset A, the mean, median, standard deviation and coefficient of variation (**CV**) of the population were 114.8 kg, 115.2 kg, 14.9 kg, and 13.0%, respectively. For Dataset B, the mean, median, standard deviation and CV of the population were 96.8 kg, 97.1 kg, 9.8 kg, and 10.1%, respectively. For Dataset C, the mean, median, standard deviation and CV of the population were 100.9 kg, 101.6 kg, 14.5 kg, and 14.4%, respectively.

Sampling Method 1

For Method 1, increasing the sample size of a random sample, regardless of pen arrangement, improved the precision for estimating the mean pig BW. However, this improvement occurred at a decreasing rate (Figure 2). Moreover, a majority of the improvement in the precision of the estimation occurred when the sample size was increased from 10 to 30 pigs (Table 1). Although increasing the sample size of a random sample increases the precision in estimating the mean pig BW of a barn, it also requires additional labor cost with the return over investment decreasing as the sample size increases.

If pork producers implement Method 1 to estimate mean pig BW, there are two concerns that are important to note. First, since the standard deviation of the pigs within the barn is unknown, the sample size to achieve a desired precision cannot be determined. The datasets used herein had a standard deviation ranging from 9.8 to 14.9 kg. Based on the sample size

formula, to achieve a 95% CI of ± 5 kg from the mean BW or a range of 10 kg a producer would need to take a random sample of 14 pigs assuming the population standard deviation was 9.8 kg. However, the producer would need a random sample of 31 pigs if the population standard deviation was 14.9 kg. Second, pigs within a barn are typically not individually numbered. Therefore, it is not possible to use a random number generator to conduct a random sample. This makes it difficult for producers to collect a true random sample, and may lead to bias in the sampling process.

Sampling Method 2

Sampling Method 2 was evaluated to determine the importance of stratifying the random sample across an increasing number of pens. As both the number of pigs and the number of pens were increased or as the total number of pigs sampled increased, the CI range decreased (data not shown). In addition, increasing the number of pens sampled while keeping the total number of pigs sampled constant (30 pigs) led to a reduction in CI range (Table 2). The CI range was reduced from 3.8 to 11.3 kg when 2 pigs from 15 pens were sampled vs 15 pigs from 2 pens. Therefore, increasing the number of pens used when sampling the barn can improve the range between the CI upper and lower limits; however, the magnitude of improvement can vary between barns.

In a finishing pig barn, pigs are typically housed with 25 to 60 pigs per pen depending on the design of the barn. Weighing pigs from multiple pens requires more resources and time, including opening gates and entering pens to select pigs, sorting pigs, and moving the scale throughout the barn. Therefore, situations in the field have occurred where convenient samples are selected for estimating BW. However, the simulation model demonstrated that the sample

needed to be stratified across as many pens as possible to optimize the precision and accuracy of the sampling method.

Although taking a random sample from an increasing number of pens can improve the precision for estimating the mean pig BW, the CI range can still be 10.5 kg or ± 5.25 kg from the mean. Tokach and Henry (2008) observed losses of $1.00 and $1.50 per pig when marketing the first load of 170 pigs 4.5 kg BW lighter and heavier, respectively, than the optimum BW. This suggests taking a random sample of 30 pigs does not meet the accuracy and precision needed to optimize marketing pigs.

Sampling Method 3

Sampling Method 3 consisted of taking the heaviest and lightest pigs in 15 randomly selected pens and calculating the mean. Method 3 reduced the CI range compared to randomly selecting 2 pigs from 15 pens and calculating the mean (Method 2; Table 3); therefore, improving the precision when estimating the mean of the population. This is expected because targeting specific portions of the population reduces possible outcomes compared to taking a random sample of the entire population. However, because specific pigs were selected, bias was introduced into the sampling procedure. This bias resulted in increased systematic error or reduced accuracy, with the mean of the 10,000 simulation means being 3.7, 2.0, and 0.9 kg less than the actual means of Datasets A, B, and C, respectively.

Selecting specific pigs (Method 3) can bias the accuracy of the estimation if the correct pig BW is not selected or if the population pig BW distribution is not symmetrical. When production personnel were tested to visually selected the heaviest and lightest pigs in each pen, it was determined that they selected the actual heaviest pig 50% of the time and the actual lightest pig 70% of the time. This can explain why calculating the mean of the selected pigs can be

biased toward light BW pigs. In addition, the pig weights in the datasets were not determined to be a normal distribution or perfectly symmetrical. Therefore, it is important to consider that the accuracy of the generated sampling model is dependent upon the ability to select the appropriate pigs and the symmetry of the distribution of pig BW.

Sampling Method 4

Sampling Method 4 consisted of selecting the heaviest and lightest pigs in 15 randomly selected pens and calculating the median. Method 4 did not reduce the CI range compared to taking a random sample of 2 pigs from 15 pens (Method 2) and increased the CI range compared to Method 3. Because an even number of pigs were selected (30 pigs) for Method 4, calculating the median of the sample resulted in taking the mean of the 2 intermediate pig BW, i.e. the heaviest of the light pigs and the lightest of the heavy pigs. Thus, using only the BW of 2 pigs from the sample caused a decrease in the precision of the estimation compared to calculating the mean of the 30 selected pigs (Method 3). Although Method 4 did not improve the CI range, it reduced the bias observed in Method 3 for Datasets A and C. This was observed because the median defines the middle observation of the sample, thus it is not influenced by extreme values (Petrie and Watson, 2006).

Sampling Method 5

Sampling method 5 was developed in attempts to obtain the precision from calculating the mean of the heaviest and lightest pigs selected from 15 pens (Method 3) while maintaining the accuracy from calculating the median of the selected pigs (Method 4). Therefore, Method 5 consisted of selecting the heaviest and lightest pigs in 15 pens and calculating a weighted average of the sample mean (Method 3) and median (Method 4) BW. The CI range was similar

to the CI range observed in Method 3. Method 5 resulted in the mean of the 10,000 estimated mean BW being within ± 0.9 kg of the actual population mean BW. Therefore, it reduced the bias observed using Method 3. The equation used in Method 5 accounted for this bias in 2 ways. First, the sum of the coefficients equals 1.02; therefore, if both the mean and median BW of the sample are equal, the estimation will still be increased by 2%. Second, the weighted average uses the median of the sample to influence a portion of the estimation. However, due to the improved precision from calculating the mean of the selected pigs, the mean coefficient is weighted 3x more than the median coefficient.

Method 5 validation

In order to validate Method 5 in comparison to Methods 3 and 4, simulations were conducted on 2 additional datasets (D and E). For Datasets D and E, Method 5 had a CI range similar to Method 3 and reduced the CI range compared to Method 4. As observed in the previous datasets, Method 3 introduced bias or reduced the accuracy in estimating the mean BW of Datasets E and F. This was determined because the mean of the 10,000 estimated mean BW were lighter than the actual population means for Datasets D and E. For Method 5, the mean of the 10,000 estimated mean BW were similar to the actual population means of Dataset D and E. This demonstrates the ability of Method 5 to reduce the bias that was observed when using Method 3 to estimate the mean BW. Therefore, Method 5 was able to accurately and precisely estimate the population mean of the 2 validation datasets.

In conclusion, sample size, method, variation, and distribution of pigs within a barn can substantially affect the precision of estimating the mean weight of all pigs in the barn. Producers should take this into consideration when weighing pigs to make marketing decisions. Using the mean and median of the selected heaviest and lightest pigs in each of 15 pens and

applying it to the developed weighted average equation can improve the precision while maintaining the accuracy to estimate the mean pig weight of the population.

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Table 1.1 The mean and 95% CI of simulated sample means when taking a completely random sample of 10, 30, 60, 90, or 120 pigs from dataset A, B, or C[1]

Sample Size	Mean of simulated sample means, kg	CI upper limit, kg	CI lower limit, kg	CI range, kg
Dataset A[2]				
10 pigs	114.8	123.7	105.1	18.6
30 pigs	114.8	119.8	109.4	10.4
60 pigs	114.7	118.4	111.0	7.4
90 pigs	114.8	117.7	111.9	5.8
120 pigs	114.8	117.3	112.1	5.2
Dataset B[3]				
10 pigs	96.8	102.6	90.6	12.0
30 pigs	96.8	100.2	93.4	6.8
60 pigs	96.8	99.2	94.4	4.8
90 pigs	96.8	98.7	94.8	3.9
120 pigs	96.8	98.5	95.2	3.3
Dataset C[4]				
10 pigs	100.8	109.6	91.7	17.9
30 pigs	100.8	105.8	95.6	10.2
60 pigs	100.9	104.4	97.2	7.2
90 pigs	100.9	103.8	98.0	5.8
120 pigs	100.9	103.3	98.4	4.9

[1] Datasets were analyzed by taking random samples, disregarding pen arrangements, of different sample size (10, 20, 30, etc.) and calculating the mean. This was completed 10,000 times for each sample size. The mean and CI were calculated for the 10,000 sample means of each sample size.

[3]A total of 1,260 pigs (mean = 114.8 kg, median = 115.2 kg, standard deviation = 14.9 kg, and CV = 13.0%) with 23 to 28 pigs per pen and a total of 48 pens.

[4]A total of 1,261 pigs were weighed (population mean = 96.8 kg, median = 97.1 kg, standard deviation = 9.8 kg, and CV = 10.1%) with 19 pens and 56 to 81 pigs per pen.

[5]A total of 1,069 pigs were weighed (population mean = 100.9 kg, median = 101.6 kg, standard deviation = 14.5 kg, and CV = 14.4%) with 40 pens and 20 to 35 pigs per pen.

Table 1.2 The resulting mean and 95% confidence interval (CI) of simulated sample means when sampling a varying number of pig BW and pens to give a total sample size of 30 pigs.[1]

Sampling method	Mean of simulated sample means, kg	CI upper limit, kg	CI lower limit, kg	CI range, kg
Dataset A[2]				
15 pigs from 2 pens	114.9	121.8	107.3	14.5
10 pigs from 3 pens	114.8	121.2	108.1	13.1
6 pigs from 5 pens	114.8	120.7	108.6	12.1
5 pigs from 6 pens	114.8	120.5	108.7	11.8
3 pigs from 10 pens	114.8	120.3	109.2	11.2
2 pigs from 15 pens	114.8	120.1	109.4	10.7
1 pig from 30 pens	114.8	119.9	109.4	10.5
Dataset B[3]				
15 pigs from 2 pens	96.8	101.6	89.6	11.9
10 pigs from 3 pens	96.9	101.3	91.3	10.0
6 pigs from 5 pens	96.9	100.9	92.5	8.4
5 pigs from 6 pens	96.8	100.7	92.8	7.9
3 pigs from 10 pens	96.9	100.4	93.3	7.1
2 pigs from 15 pens	96.8	100.2	93.5	6.7
Dataset C[4]				
15 pigs from 2 pens	101.2	110.9	89.3	21.7
10 pigs from 3 pens	101.2	109.8	91.4	18.3
6 pigs from 5 pens	101.2	108.2	93.4	14.8
5 pigs from 6 pens	101.2	107.7	94.2	13.6
3 pigs from 10 pens	101.2	106.8	95.2	11.7
2 pigs from 15 pens	101.2	106.3	95.8	10.4
1 pig from 30 pens	101.2	105.7	96.5	9.2

[1]Datasets were analyzed by taking random samples of varying number of pigs within varying numbers of pens, with 1 to 15 pigs sampled from 1 to all of the pens and calculating the mean. This was completed 10,000 times for each sampling method. The mean and CI were calculated for the 10,000 sample means of each sampling method.

[2]A total of 1,260 pigs (mean = 114.8 kg, median = 115.2 kg, standard deviation = 14.9 kg, and CV = 13.0%) with 23 to 28 pigs per pen and a total of 48 pens.

[3]A total of 1,261 pigs were weighed (population mean = 96.8 kg, median = 97.1 kg, standard deviation = 9.8 kg, and CV = 10.1%) with 19 pens and 56 to 81 pigs per pen.

[4]A total of 1,069 pigs were weighed (population mean = 100.9 kg, median = 101.6 kg, standard deviation = 14.5 kg, and CV = 14.4%) with 40 pens and 20 to 35 pigs per pen.

Table 1.3 The mean and 95% confidence interval (CI) of simulated sample means for the various sampling methods to give a total sample size of 30 pigs.[1]

Sampling method	Mean of simulated sample means, kg	CI upper limit, kg	CI lower limit, kg	CI range, kg
Dataset A[2]				
Method 3[3]	111.1	114.5	107.8	6.7
Method 4[4]	114.1	119.5	108.9	10.6
Method 5[5]	113.9	117.2	110.5	6.7
Dataset B[6]				
Method 3	94.8	96.3	93.4	2.9
Method 4	94.8	99.1	90.0	9.1
Method 5	96.6	98.6	94.8	3.8
Dataset C[7]				
Method 3	100.0	103.5	96.5	7.0
Method 4	100.6	105.7	95.9	9.8
Method 5	102.0	105.4	98.5	6.9
Dataset D[8]				
Method 3	72.6	75.1	69.9	5.2
Method 4	74.0	78.5	68.7	9.8
Method 5	74.3	77.0	71.5	5.5
Dataset E[9]				
Method 3	91.3	93.4	89.0	4.4
Method 4	93.2	96.6	89.1	7.5
Method 5	93.5	95.6	91.2	4.4

[1] Each sampling method was simulated 10,000 times. The mean and CI were calculated for the 10,000 sample means of each sampling method.

[2]A total of 1,260 pigs (mean = 114.8 kg, median = 115.2 kg, standard deviation = 14.9 kg, and CV = 12.98%) with 23 to 28 pigs per pen and a total of 48 pens.

[3] Selecting the heaviest and lightest pigs from 15 randomly selected pens and calculating the mean from the 30 pigs.

[4]Selecting the heaviest and lightest pigs from 15 randomly selected pens and calculating the median from the 30 pigs.

[5]Selecting the heaviest and lightest pigs from 15 pens and estimated the mean using following equation: Estimated mean, kg = 0.77 * sample mean, kg + 0.25 * sample median, kg.

[6]A total of 1,261 pigs (population mean = 96.8 kg, median = 97.1 kg, standard deviation = 9.8 kg, and CV = 10.1%) with 56 to 81 pigs per pen and a total of 19 pens.

[7]A total of 1,069 pigs weighed (population mean = 100.9 kg, median = 101.6 kg, standard deviation= 14.5 kg, and CV = 14.4%) with 20 to 35 pigs per pen and a total of 40 pens.

[8]A total of 1,176 pigs weighed (population mean = 74.3 kg, median = 74.8 kg, standard deviation= 10.6 kg, and CV = 14.2%) with 20 to 35 pigs per pen and a total of 38 pens.

[9]A total of 961 pigs weighed (population mean = 93.1 kg, median = 93.9 kg, standard deviation= 9.2 kg, and CV = 9.8%) with 16 to 23 pigs per pen and a total of 48 pens.

Figure 1.1 Datasets (A, B, and C) used to evaluate sample size and method of sampling on the precision of estimating the pig mean BW of the barn.

A) Frequency histogram of Dataset A, a total of 1,260 pigs (mean = 114.8 kg, median = 115.2 kg, standard deviation = 14.9 kg, and CV = 13.0%) with 23 to 28 pigs per pen and a total of 48 pens. B) Frequency histogram of Dataset B. A total of 1,261 pigs were weighed (population mean = 96.8 kg, median = 97.1 kg, standard deviation = 9.8 kg, and CV = 10.1%) with 19 pens and 56 to 81 pigs per pen. C) Frequency histogram of Dataset C. A total of 1,069 pigs were weighed (population mean = 100.9 kg, median = 101.6 kg, standard deviation = 14.5 kg, and CV = 14.4%) with 40 pens and 20 to 35 pigs per pen.

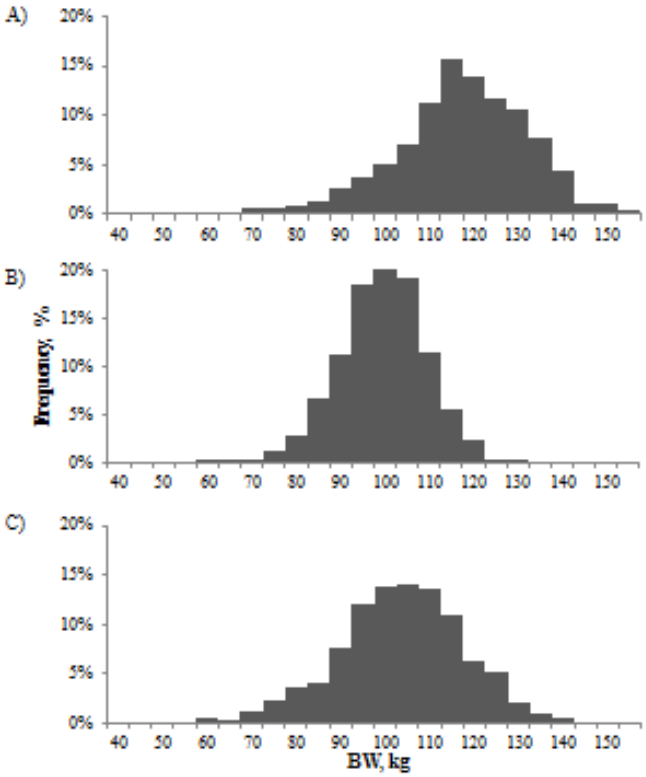
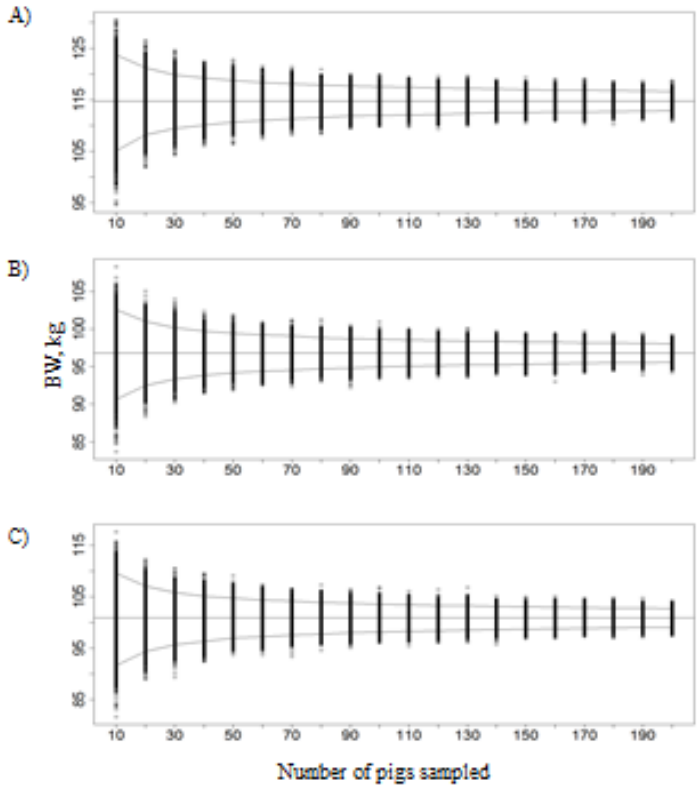


Figure 1.2 Simulated sample means and the mean and 95% CI of simulated sample means when taking a completely random sample of 10 to 200 pigs from dataset A, B, or C.

For dataset A, individual pig weights were collected on a total of 1,260 pigs (actual population mean = 114.8 kg, median = 115.2 kg, standard deviation = 14.9 kg, and CV = 13.0%) with 23 to 28 pigs per pen. For Dataset B, individual pig weights were collected on a total of 1,261 pigs (actual population mean = 96.8 kg, median = 97.1 kg, standard deviation = 9.8 kg, and CV = 10.1%) with 19 pens and 56 to 81 pigs per pen. For dataset C, individual pig weights were collected on a total of 1,069 pigs (actual population mean = 100.9 kg, median = 101.6 kg, standard deviation = 14.5 kg, and CV = 14.4%) with 40 pens and 20 to 35 pigs per pen. The datasets were analyzed by taking random samples, disregarding pen arrangements, of different sample size (10, 20, 30, etc.) and calculating the mean. This was completed 10,000 times for each sample size. Each point represents the mean calculated for the respective sample. Reference lines representing the 95% confidence interval have been drawn, and the center line represents the actual population mean.



Chapter 2 - Effect of sample size and sampling method of pig body weights on the accuracy and precision of estimating the distribution within a population

ABSTRACT

Swine producers must sell pigs weighing within an optimum BW range in order to maximize profitability and not receive economic discounts. In order to do so producers have adopted marking strategies such as marketing the heaviest pigs several weeks before the whole group or "topping" pigs. Knowing how much variation or dispersion exists in individual pig weights from the mean is needed to determine when to sell various loads of pigs in the barn. In statistics and probability theory, the amount of variation in a population is represented by the SD; therefore, our objective was to determine the optimal sample size and method for estimating the SD of BW for a group of pigs in a barn. Using a computer program developed in R (R Foundation for Statistical Computing, Vienna, Austria), we were able to generate 10,000 sample SD for different sampling procedures on 3 different datasets. Using this program, we evaluated weighing: (1) a completely random sample of 10 to 200 pigs from the barn, (2) an increasing number of pigs per pen from 1 to 15 pigs and increasing the number of pens until all pens in the barn had been sampled, and (3) selecting the heaviest and lightest pig (determined visually) in each pen, and subtracting the lightest weight from the heaviest weight and dividing by 6. For all 3 datasets, increasing the sample size of a completely random sample from 10 to 200 pigs decreased the range between the upper and lower CI when estimating the SD; however, this occurred at a diminishing rate. For a sample size of 30 pigs, weighing 2 pigs from 15 pens reduced the CI range by 4.4, 11.9, and 23.6% compared to weighing 15 pigs from 2 pens for Datasets A, B, and C, respectively. Sampling Method 3 resulted in a reduction of the range

between the upper and lower CI from 13 to 72% compared to weighing 2 pigs from 15 pens (Method 2) for the 3 datasets. These data indicated that the distribution of pig weights can be practically estimated by weighing the heaviest and lightest pigs in 15 pens.

INTRODUCTION

Packing plants have implemented a marketing grid encouraging swine producers to sell pigs within a specific BW range. Swine producers must sell pigs weighing within this optimum BW range in order to maximize profitability and not receive economic discounts. In order to do so producers have adopted marking strategies such as marketing the heaviest pigs several weeks before the whole group or "topping" pigs. Therefore, the heaviest pigs are sold at optimum weights and the pigs left in the barn are allowed additional time to achieve optimal market weights. Knowing how much variation or dispersion exists in individual pig weights from the mean is needed to determine when to sell various loads of pigs in the barn. Not accounting for variability in pig BW can result in overestimation of optimal marketing BW and reduce profitability (Huang and Miller, 2004).

Factors, such as birth BW, weaning BW, and health status, may influence differences in variation of pig BW making it difficult to predict (Shull et al., 2013). Because pig BW typically approximates a normal distribution, subsampling methods to predict the average weight and SD of pigs in the barn can be used to model distributions of BW within the barn. Paulk et al. (2014) determine the accuracy and precision of sampling methods producers use to estimate the mean weight of the population. However, knowledge of the distribution of pig BW allows producers to better estimate the ideal timing for removing groups of pigs from a barn. In statistics and probability theory, the amount of variation in a population is represented by the SD. Therefore,

our objective was to determine the optimal sample size and method for estimating the SD of weights for the population of pigs in the barn.

MATERIALS AND METHODS

Datasets

Three datasets (A, B, and C) were used to evaluate sample size and method of sampling on the precision of estimating the pig SD of BW in the barn. Briefly, these 3 datasets were obtained from 3 different production systems where all of the pigs within a single barn were weighed on a single day. If the barn included a pen in which sick or lame pigs were placed, pigs from this pen were not weighed and not included in the analysis. The actual individual pig weights and distributions from the 3 barns were used to simulate various sampling strategies. Normality pig BW for each dataset was tested using the Shapiro-Wilk statistical test using the UNIVARIATE procedure of SAS (SAS Institute Inc., Cary, NC).

Dataset A was derived from an experiment conducted in a commercial research facility (Groesbeck et al., 2007). This dataset comprised a total of 1,260 pigs (337 × 1050, PIC, Hendersonville, TN) in 48 pens with 23 to 28 pigs per pen. The barn was filled with pigs over a 1-wk period. Pigs were randomly distributed to pens as they came off the delivery truck ("gate-cut"). Pigs within the barn were determined to be positive for porcine reproductive and respiratory syndrome, mycoplasma, ileitis, and porcine circovirus Type 2.

Dataset B was obtained from a commercial finishing site in Northern IA. This dataset comprised a total of 1,261 pigs (359 × 1050, PIC, Hendersonville, TN) in 19 pens with 56 to 81 pigs per pen. The barn was filled with pigs over a 1-wk period. Pigs were randomly distributed to pens as they came off the delivery truck. Pigs within the barn were determined to be healthy.

Dataset C was derived from a different commercial site in Northern IA. This dataset comprised a total of 1,069 pigs (F25 × G performer, Genetiporc, Alexandria, MN) in 40 pens with 20 to 35 pigs per pen. The barn was filled with pigs over a 1-wk period. Pigs were randomly distributed to pens as they came off the delivery truck. Pigs within the barn were determined to be positive for porcine reproductive and respiratory syndrome.

Sample Simulation Model

A simulation model was developed using R (R Foundation for Statistical Computing, Vienna, Austria) to demonstrate the error for different sample sizes and for different methods of selecting pigs on estimating the SD of the pig BW in a population. The model was designed to conduct designated sample sizes within each sample method. The estimated SD for BW was calculated for each sample. Each sample size was conducted 10,000 times, generating 10,000 estimated SD of the population. These were used to determine the accuracy and precision for each sample method. The accuracy was determined by comparing the mean of the 10,000 sample SD to the actual population SD pig BW. The precision was determined by calculating a 95% CI for the 10,000 sample SD. The 10,000 sample SD for each sample size were sorted from least to greatest, and the 95% CI was generated by selecting the 9,751st observation, the upper limit, and the 250th observation, the lower limit. The distances between the upper and lower confidence limits represent the estimated SD CI range.

Sampling Methods

Using the developed sample simulation model, 3 sampling methods were tested. For Method 1, the model was designed to take a completely random sample of the designated sample size (10, 20, 30, etc.), disregarding pen arrangements, and calculate the BW SD of each sample.

Therefore, the model generated 10,000 sample SD for each sample size (10, 20, 30, etc.). The sample SD was calculated as:

SD = $\sqrt{\frac{1}{N-1}\sum_{i=1}^{N}(x_i - \bar{x})^2}$, where n is the sample size, {x1, x2, … xn} were the observed values of the sample items, and $\bar{x}$ was the mean value of these observations (Ott and Longnekcer, 2004). Method 2 accounted for the nesting of pens within barn by evaluating the sampling error from taking a completely random sample from a varying number of pigs within varying numbers of pens, with 1 to 15 pigs sampled from 1 to all of the pens. Therefore, the model generated 10,000 sample SD for each number of pigs from number of pens combination. For Method 3, the model randomly selected 15 pens from the barn. The heaviest and lightest pigs were selected from the 15 pens (30 pigs total) and the difference in weight between the lightest and heaviest pigs in the total sample were divided by 6.

Because it is not possible to weigh all the pigs in a pen and precisely know which are the heaviest and lightest, a preliminary study was carried out to determine the accuracy when selecting the heaviest and lightest pigs for Methods 3. The heaviest and lightest pigs were visually selected by production personnel specialized in selecting pigs for market. The probability for selecting the 1st, 2nd, 3rd, 4th, or 5th heaviest pig was 50, 25, 15, 5, and 5%, respectively, and the probability for selecting the 1st, 2nd, 3rd, 4th, or 5th lightest pig was 70, 15, 5, 5, and 5%, respectively. These probabilities were used in the simulation model when selecting the heaviest and lightest pigs from selected pens.

RESULTS AND DISSCUSION

Datasets

Frequency histograms and descriptive statistics for Datasets A, B, and C are provided in Figure 1. From visualizing each frequency histogram, each dataset seemed to approximate a normal distribution. However, the Shapiro-Wilk test rejected ($P < 0.05$) the normality of each dataset. For Dataset A, the mean, median, SD and CV of the population were 114.8 kg, 115.2 kg, 14.9 kg, and 13.0%, respectively. For Dataset B, the mean, median, SD and CV of the population were 96.8 kg, 97.1 kg, 9.8 kg, and 10.1%, respectively. For Dataset C, the mean, median, SD and CV of the population were 100.9 kg, 101.6 kg, 14.5 kg, and 14.4%, respectively. The difference in distribution of pig weights between datasets, as described by the SD and CV, can be attributed to multiple factors. Schinckel et al. (2003) observed increasing pig BW from 93 to 115 kg increased the BW SD from 7.99 to 8.52 kg and reduced the CV from 8.6 to 7.4%. Shull et al. (2013) observed increasing pig BW from 92.6 to 108.2 kg in a commercial facility increased the BW SD from 9.4 to 10.2 and decreased the CV from 10.2 to 9.4, respectively. In addition, other factors, such as birth weight, weaning weight, and health status, may also influence differences in variation of pig BW (Shull et al., 2013).

Sampling Method 1

For all 3 datasets, increasing the sample size of a completely random sample from 10 to 200 pigs decreased the 95% CI range when estimating the SD (Figure 1). A majority of the improvement in the precision of the estimation occurred when the sample size increased from 10 to 30 pigs (Table 1). However, one concern with taking a truly random sample from within a barn is that pigs within a barn are typically not individually numbered. Therefore, it is not

possible to use a random number generator to conduct a random sample. This makes it difficult for producers to collect a true random sample and may lead to bias in the sampling process.

Sampling Method 2

Sampling Method 2 was evaluated to determine the importance of stratifying the random sample across an increasing number of pens. As both the number of pigs and pens were increased when sampling, the CI range decreased (data not shown). For all 3 datasets, increasing the number of pens sampled while keeping the total number of pigs sampled constant led to a reduction in the CI range (Table 2). For a sample size of 30 pigs, weighing 2 pigs from 15 pens reduced the CI range by 4.4, 11.9, and 23.6% compared to weighing 15 pigs from 2 pens for Datasets A, B, and C, respectively.

Stratifying the sample across more pens had the greatest magnitude of improvement in Dataset C. This was caused by the increased amount of variability in pig BW between pens in Dataset C. Individual pen mean BW ranged from 81.6 to 103.4 kg (range equals 21.8 kg) and SD of BW within a pen ranged from 7.3 to 20.0 kg (12.7 kg range) in Dataset C. For Dataset A, the range of individual pen means and SD were 10.4 and 12.7 kg, respectively. Therefore, the range of individual pen means was decreased for Dataset A vs Dataset C, but the range of individual pen SD was similar. For Dataset B, the range of individual pen means and SD were 16.3 kg and 4.5 kg, respectively. Therefore, the range of individual pen means and SD were both decreased for Dataset B vs Dataset C. Having a larger variability between pen mean and SD BW increases the importance of stratifying the sample across multiple pens.

In a finishing pig barn, pigs are typically housed with 25 to 60 pigs per pen depending on the design of the barn. Weighing pigs from multiple pens requires more resources and time, including opening gates and entering pens to select pigs, sorting pigs, and moving the scale

throughout the barn. Therefore, situations in the field can occur where convenient samples are selected for estimating BW. However, the simulation model demonstrated that the sample needed to be stratified across as many pens as possible to optimize the precision and accuracy of the sampling method. This in agreement with research requiring sampling from human populations in which convenience sampling introduces bias (Sousa et al., 2004).

Sampling Method 3

Sampling Method 3 consisted of selecting the heaviest and lightest pigs in 15 pens and dividing the difference between the heaviest and lightest pig of the 30 selected pigs by 6. Method 3 reduced the CI range compared to randomly selecting 2 pigs from 15 pens and calculating the sample SD (Method 2; Table 3); therefore, improving the precision when estimating the SD of the population. Amongst the various datasets, the range was reduced from 9 to 62% compared to randomly selecting 2 pigs from 15 pens. Method 3 is expected to be a good estimator of the SD, because in a population that approximates a normal distribution, 99.9% of observations should be within plus or minus 3 SD of the mean or a total of 6 SD between the heaviest and lightest observation (Ott and Longnecker, 2004). Therefore, selecting the heaviest and lightest BW of the distribution and dividing by 6 should approximate the SD of the population. Although Method 3 improved the precision for estimating the SD, the non-normality, as determined by the Shapiro-Wilk test, of the current datasets possibly reduced this precision.

In conclusion, sample size, method, variation, and distribution of pigs within a barn can substantially affect the precision of estimating the distribution of pig weights. As expected, sample size to obtain similar CI estimates is reduced if the population is less variable. Finally, these data indicate that the distribution of pig weights or SD can be estimated by selecting and weighing the heaviest and lightest pigs in 15 pens.

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Table 2.1 The mean and 95% CI of simulated sample SD when taking a completely random sample of 10, 30, 60, 90, or 120 pigs from dataset A, B, or C[1]

Sample size	Mean of simulated sample SD, kg	CI upper limit, kg	CI lower limit, kg	CI range, kg
Dataset A[2]				
10 pigs	14.4	22.4	7.8	14.6
30 pigs	14.7	19.1	10.7	8.5
60 pigs	14.8	17.8	12.0	5.9
90 pigs	14.8	17.2	12.5	4.7
120 pigs	14.9	16.9	12.8	4.1
Dataset B[3]				
10 pigs	9.5	14.7	5.2	9.4
30 pigs	9.7	12.4	7.1	5.3
60 pigs	9.7	11.6	7.9	3.7
90 pigs	9.7	11.2	8.3	2.9
120 pigs	9.8	11.0	8.5	2.6
Dataset C[4]				
10 pigs	14.1	22.1	7.7	14.5
30 pigs	14.4	18.8	10.5	8.3
60 pigs	14.5	17.5	11.7	5.8
90 pigs	14.5	16.9	12.2	4.7
120 pigs	14.5	16.5	12.6	4.0

[1] Datasets were analyzed by taking random samples, disregarding pen arrangements, of different sample size (10, 20, 30, etc.) and calculating the SD. This was completed 10,000 times for each sample size. The mean and CI were calculated for the 10,000 sample SDof each sample size.

[3]A total of 1,260 pigs (mean = 114.8 kg, median = 115.2 kg, SD = 14.9 kg, and CV = 13.0%) with 23 to 28 pigs per pen and a total of 48 pens.

[4]A total of 1,261 pigs were weighed (population mean = 96.8 kg, median = 97.1 kg, SD = 9.8 kg, and CV = 10.1%) with 19 pens and 56 to 81 pigs per pen.

[5]A total of 1,069 pigs were weighed (population mean = 100.9 kg, median = 101.6 kg, SD = 14.5 kg, and CV = 14.4%) with 40 pens and 20 to 35 pigs per pen.

Table 2.2 The resulting mean and 95% CI of simulated sample SD when sampling a varying number of pig BW and pens to give a total sample size of 30 pigs.[1]

Sampling method	Mean of simulated sample means, kg	CI upper limit, kg	CI lower limit, kg	CI range, kg
Dataset A[2]				
15 pigs from 2 pens	14.5	19.5	10.4	9.0
10 pigs from 3 pens	14.6	19.3	10.4	8.9
6 pigs from 5 pens	14.7	19.3	10.5	8.8
5 pigs from 6 pens	14.7	19.2	10.6	8.6
3 pigs from 10 pens	14.7	19.3	10.7	8.5
2 pigs from 15 pens	14.8	19.3	10.7	8.6
1 pig from 30 pens	14.7	19.2	10.8	8.4
Dataset B[3]				
15 pigs from 2 pens	9.0	12.5	6.6	5.9
10 pigs from 3 pens	9.3	12.7	6.8	5.9
6 pigs from 5 pens	9.5	12.5	6.9	5.6
5 pigs from 6 pens	9.6	12.5	6.9	5.6
3 pigs from 10 pens	9.6	12.5	7.2	5.3
2 pigs from 15 pens	9.7	12.5	7.2	5.2
Dataset C[4]				
15 pigs from 2 pens				
10 pigs from 3 pens	13.2	20.4	9.0	11.4
6 pigs from 5 pens	13.6	19.9	9.2	10.7
5 pigs from 6 pens	13.8	19.3	9.6	9.7
3 pigs from 10 pens	13.9	19.2	9.7	9.5
2 pigs from 15 pens	14.1	18.9	10.2	8.7
1 pig from 30 pens	14.2	18.7	10.3	8.4

[1]Datasets were analyzed by taking random samples of varying number of pigs within varying numbers of pens and the SD was calculated. This was completed 10,000 times for each sampling method. The mean and CI were calculated for the 10,000 sample SD of each sampling method.

[2]A total of 1,260 pigs (mean = 114.8 kg, median = 115.2 kg, SD = 14.9 kg, and CV = 13.0%) with 23 to 28 pigs per pen and a total of 48 pens.

[3]A total of 1,261 pigs were weighed (population mean = 96.8 kg, median = 97.1 kg, SD = 9.8 kg, and CV = 10.1%) with 19 pens and 56 to 81 pigs per pen.

[4]A total of 1,069 pigs were weighed (population mean = 100.9 kg, median = 101.6 kg, SD = 14.5 kg, and CV = 14.4%) with 40 pens and 20 to 35 pigs per pen.

Table 2.3 The mean and 95% CI of simulated sample SD for Sampling Method 3.[1]

Method 3	Mean of simulated sample SD, kg	CI upper limit, kg	CI lower limit, kg	CI range, kg
Dataset A[2]	14.6	17.8	12.4	5.4
Dataset B[3]	10.4	11.0	9.5	1.5
Dataset C[4]	14.7	18.3	10.7	7.6

[1] The heaviest and lightest pigs from 15 pens were selected and used to estimate the SD by calculating the difference in BW between the lightest and heaviest pigs in the total sample and then divided the difference by 6. This was completed 10,000 times and the mean and CI were calculated for the 10,000 sample SD.

[2]A total of 1,260 pigs (mean = 114.8 kg, median = 115.2 kg, SD = 14.9 kg, and CV = 12.98%) with 23 to 28 pigs per pen and a total of 48 pens.

[3]A total of 1,261 pigs (population mean = 96.8 kg, median = 97.1 kg, SD = 9.8 kg, and CV = 10.1%) with 56 to 81 pigs per pen and a total of 19 pens.

[4]A total of 1,069 pigs weighed (population mean = 100.9 kg, median = 101.6 kg, SD= 14.5 kg, and CV = 14.4%) with 20 to 35 pigs per pen and a total of 40 pens.

Figure 2.1 Datasets (A, B, and C) used to evaluate sample size and method of sampling on the precision of estimating the pig mean BW of the barn.

A) Frequency histogram of Dataset A, a total of 1,260 pigs (mean = 114.8 kg, median = 115.2 kg, SD = 14.9 kg, and CV = 13.0%) with 23 to 28 pigs per pen and a total of 48 pens. B) Frequency histogram of Dataset B. A total of 1,261 pigs were weighed (population mean = 96.8 kg, median = 97.1 kg, SD= 9.8 kg, and CV = 10.1%) with 19 pens and 56 to 81 pigs per pen. C) Frequency histogram of Dataset C. A total of 1,069 pigs were weighed (population mean = 100.9 kg, median = 101.6 kg, SD = 14.5 kg, and CV = 14.4%) with 40 pens and 20 to 35 pigs per pen.

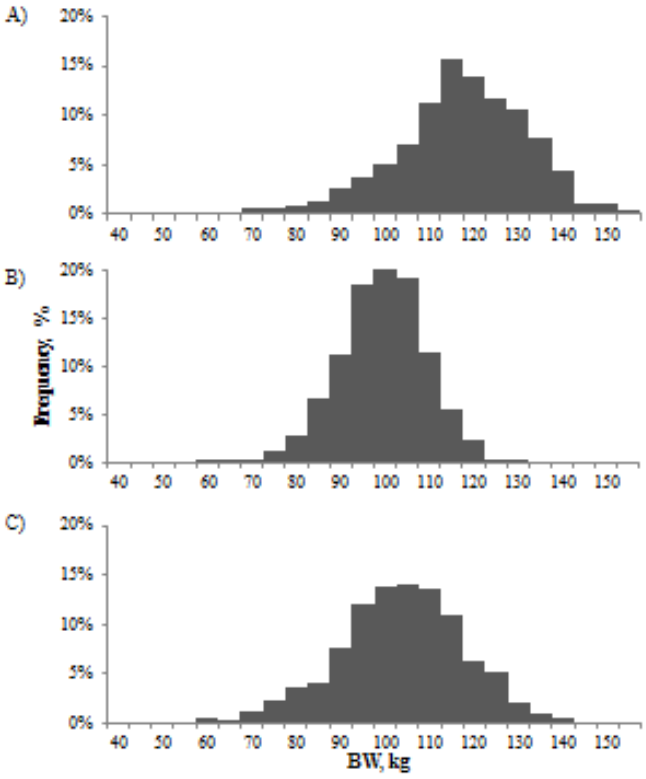
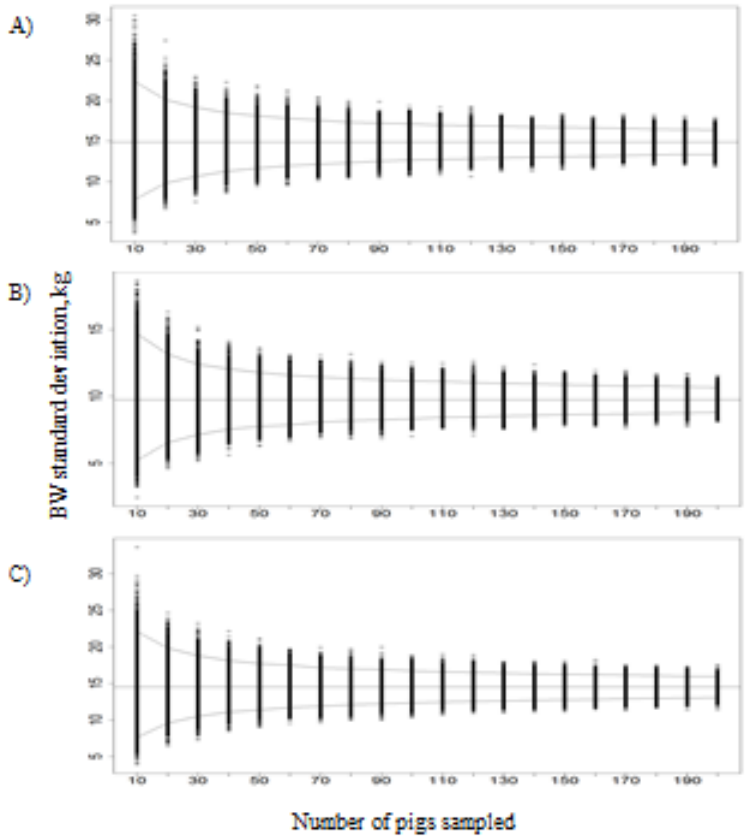


Figure 2.2 Simulated sample means and the mean and 95% CI of simulated sample means when taking a completely random sample of 10 to 200 pigs from dataset A, B, or C.

For dataset A, individual pig weights were collected on a total of 1,260 pigs (actual population mean = 114.8 kg, median = 115.2 kg, SD = 14.9 kg, and CV = 13.0%) with 23 to 28 pigs per pen. For Dataset B, individual pig weights were collected on a total of 1,261 pigs (actual population mean = 96.8 kg, median = 97.1 kg, SD = 9.8 kg, and CV = 10.1%) with 19 pens and 56 to 81 pigs per pen. For dataset C, individual pig weights were collected on a total of 1,069 pigs (actual population mean = 100.9 kg, median = 101.6 kg, SD = 14.5 kg, and CV = 14.4%) with 40 pens and 20 to 35 pigs per pen. The datasets were analyzed by taking random samples, disregarding pen arrangements, of different sample size (10, 20, 30, etc.) and calculating the SD. This was completed 10,000 times for each sample size. Each point represents SD calculated for the respective sample. Reference lines representing the 95% confidence interval have been drawn, and the center line represents the actual population SD.



Chapter 3 - Determining the optimal sampling method to estimate the mean and SD of pig body weights within a population

ABSTRACT

The accuracy and precision of pig subsampling methods can determine the swine producer's ability to sell pigs at optimal market BW in order to reduce economic discounts. The first objective of this experiment was to determine the time required to weigh pigs for different sampling methods used to estimate the mean and SD of a population. The second objective was to determine the optimal sampling method using the time required to weigh pigs and the precision and accuracy of each sampling method. A total of 68 pens of pigs (359 × 1050, PIC, Hendersonville, TN; 77 kg BW) in 2 commercial finishing facilities with 20 to 35 pigs per pen were used. Pens of pigs were blocked by location within barn and randomly allotted to 1 of 4 treatments with 17 pens per treatment. The 4 treatments included: (1) selecting and weighing the heaviest and lightest pig per pen and (2, 3, and 4) weighing the first 5, 10 and 15 pigs out of the pen, respectively. The time required for 2 people to complete each treatment was recorded. To determine the time required to conduct a specific total sample, the time required to weigh the specific number of pigs per pen was multiplied by n pens. The accuracy and precision for estimating the mean BW and SD for each sampling method was determined in Paulk et al. (2012) and Paulk et al. (2013) using their reported Datasets A and C. The precision was determined by calculating a 95% CI for the sample means and SD. The time taken to select and weigh the heaviest and lightest pigs in a pen (Treatment 1) did not differ from weighing 5 pigs per pen (Treatment 2). Increasing the number of pigs weighed per pen (Treatment 3 and 4) increased ($P < 0.05$) the amount of time to weigh a single pen. Therefore, the time taken to select and weigh the heaviest and lightest pigs (Treatment 1) in 15 pens (30 pigs) did not differ

from weighing the first 5 pigs (Treatment 2) from 15 pens (75 pigs), 10 pigs (Treatment 3) from 9 pens (90 pigs) and 15 pigs (Treatment 4) from 6 pens (90 pigs). For Dataset A, these 4 sampling methods had a similar CI range for estimating the mean BW and SD. For Dataset C, Treatment 1 (30 pigs) and 2 (75 pigs) had a reduced CI range for estimating the mean BW compared to Treatment 3 (90 pigs) and 4 (90 pigs). However, Treatment 2 (75 pigs) and 3 (90 pigs) had a reduced CI range for estimating the SD compared to Treatment 1 (30 pigs) and 4 (90 pigs). Therefore, it is concluded that swine producers should weigh 5 pigs from 15 pens to estimate the mean BW and SD within a barn.

INTRODUCTION

Because pig BW typically approximates a normal distribution, subsampling methods to predict the mean and SD can be used to model distributions of BW within the barn. The accuracy and precision of these subsampling methods can determine the swine producer's ability to sell pigs at optimal market BW in order to reduce economic discounts. Paulk et al. (2012) and Paulk et al. (2013) determined the accuracy and precision of varying sampling methods used to estimate the mean and SD of pig BW within a population. Increasing the sample size of a random sample, regardless of pen arrangement, improved the precision for estimating the mean and SD of pig BW; however, a majority of the improvement occurred when the sample size was increased from 10 to 30 pigs. Increasing the sample size of a random sample requires additional labor and cost.

Because the greatest improvement in estimating the mean and SD was at 30 pigs, Paulk et al. (2012, 2013) also evaluated methods to improve the estimates without increasing the sample size of 30 pigs. When the total sample size was held constant, increasing the number of pens sampled improved the precision. However, the precision of estimating the mean and SD

could be further improved by selecting the heaviest and lightest pigs from 15 pens. In order to determine the optimum sampling method swine producers should use both the time required to conduct and the precision and accuracy of each sampling method. Therefore, the first objective of this experiment was to determine the time required to weigh pigs for different sampling methods used to estimate the mean and SD of pig BW of a population. The second objective was to determine the optimal sampling method using the time required to weigh pigs and the precision and accuracy of each sampling method.

Materials and Methods

Time required to weigh pigs for different sampling methods

A total of 68 pens of pigs (359 × 1050, PIC, Hendersonville, TN) in 2 commercial finishing facilities (Barn 1and 2) in northern IA were used in the experiment. Pigs in Barn 1 and 2 were approximately 74.3 and 79.8 kg BW, respectively. Pigs were housed in curtain sided finishing barns with 20 to 35 pigs per pen. Pens of pigs were blocked by location within barn and randomly allotted to 1 of 4 treatments with 9 replicate pens in Barn 1 and 8 replicate pens in Barn 2 for a total 17 pens per treatment.

The 4 treatments included: (1) selecting and weighing the heaviest and lightest pig per pen and (2, 3, and 4) weighing the first 5, 10 and 15 pigs out of the pen, respectively. The time required to complete each treatment was recorded. All treatments were completed by 2 people using an individual pig scale with a digital weight indicator (SW600, Digi-Star, Ft. Atkinson, WI). The scale was made out of aluminum and had 2 wheels attached to the front. Therefore, the scale could easily be moved by 1 person. The scale contained 2 swinging gates at the front and back end. The back gate was opened and closed using a latch on top of the gate. The front gate was attached to an aluminum arm with a handle. The arm extended the length of the scale so that

the handle was located in close proximity to the back gate. The handle was lifted up and pushed forward to open the gate and lifted up and pulled back to close the gate. Therefore, 1 person was able to open and close both gates while standing in the same spot. The same 2 people completed the treatments on all 68 pens in the experiment. Treatments were conducted in Barn 1 on d 1 and Barn 2 on d 2. Person 1's first responsibility was to place the scale a pens length away from the pen to be weighed. Once the scale was set in place, Person 1 and 2 would meet at the gate of the pen to be weighed. Once both persons were ready, Person 1 would record the time and then retrieve the scale, placed it in position next to the current pen to be weighed, and zeroed the scale. For Treatment 1, Person 2 would begin searching for the heaviest and lightest pig in the pen while Person 1 set up the scale. After Person 1 zeroed the scale, he would help Person 2 decide which pigs were the heaviest and lightest by visual evaluation. Once the heaviest and lightest were determined, Person 2 would mark those pigs with marking paint. Person 1 then opened the gate while Person 2 started sorting the heaviest and lightest pig toward the scale. For Treatments 2, 3, and 4, while Person 1 set up the scale, Person 2 opened the gate and was positioned in the pen ready to start assisting pigs onto the scale. For all treatments, while weighing pigs, Person 1's responsibilities were to open and close the scale gates and record pig BW and Person 2's responsibility was to use a 76.2 × 91.4 cm sorting board to assist pigs onto the scale. For Treatment 1, after the 1st pig was weighed, Person 1 would back that pig off of the scale back into the pen while Person 2 sorted the 2nd pig to the scale. Once the 2nd pig was weighed, Person 1 would back that pig off of the scale back into the pen and Person 2 would close the pen gate once the pig was in the pen. After the pen gate was closed, Person 1 would record the time. For Treatment 2, 3, and 4, after Person 1 recorded the BW of each pig, the gate at the front of the scale was opened and the pig was ran into the aisle. After all pigs were

weighed, Person 2 would move the scale to the other side of the open gate to allow Person 1 to move the pigs back into the pen. After all pigs were returned to the pen, the gate was shut and the time was recorded. The same person assumed the same responsibilities for completing treatments on all 68 pens. Treatments were conducted on assigned pens in order of location block. Therefore, each of the 4 treatments was conducted on the designated pen within block before starting on the next block. When Person 1 and 2 took a break, it was taken between blocks.

Treatments were initially analyzed using 2 response criteria: 1) time to complete each treatment per pen; and 2) time to conduct each treatment on a total of 30 pigs. To obtain the time required to conduct a sample size of 30 pigs, the time required to conduct each treatment (select and weigh the heaviest and lightest pig per pen or weigh the first 5, 10 and 15 pigs out of the pen) was multiplied by a factor of 15, 6, 3, and 2, respectively. After preliminary analysis, it was determined that to achieve a total sample size of 30 pigs, selecting and weighing the heaviest and lightest pigs (Treatment 1) from 15 pens required more time than weighing the first 5, 10, or 15 pigs from 6, 3, or 2 pens, respectively. Therefore, the time required to weigh a total of 30 pigs by selecting and weighing the heaviest and lightest pigs (Treatment 1) from 15 pens was compared to the time required to weigh a total of 60, 75, and 90 pigs by weighing the first 5, 10, or 15 pigs (Treatments 2, 3, and 4) from the required number of pens. This was completed to determine the number of total pigs that could be weighed in a similar amount of time as required to selecting the heaviest and lightest pigs (Treatment 1) in 15 pens (30 pigs). This led to 3 additional response criteria: 3) time to conduct Treatments, 2, 3, and 4 so that the total pigs weighed equaled 60; 4) time to conduct Treatments 2 and 4 so that the total pigs weighed equaled 75; and 5) time to conduct Treatments 2, 3, and 4 so that the total pigs weighed equaled 90. Regression analysis was also completed in order to predict the time required to weigh 5 to

15 pigs per pen. The slope of the line from the regression analysis represents the additional time required to weigh each additional pig per pen.

The time analysis did not account for the time required to change clothes for biosecurity measures and set up the barn. This was not included because it was considered to be consistent across all treatments. Changing clothes and setting up the scale and preparing the barn took approximately 27 min in both barns.

Data was analyzed as a randomized complete block design using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC) with pen as the experimental unit. Treatment was included as the fixed effect and location block as a random effect. Differences between treatments were determined using the PDIFF option of SAS. Sampling methods were analyzed using 4 response criteria: 1) time to complete each method per a pen; 2) time to conduct each sample method so that the total pigs weighed equaled 30; 3) time to conduct Treatment 1 so that the total pigs weighed equaled 30 and time to conduct Treatments 2, 3, and 4 so that the total pigs weighed equaled 60; 4) time to conduct Treatment 1 so that the total pigs weighed equaled 30 and time to conduct Treatments 2 and 4 so that the total pigs weighed equaled 75; and 5) time to conduct Treatment 1 so that the total pigs weighed equaled 30 and time to conduct Treatments 2, 3, and 4 so that the total pigs weighed equaled 90. Significant differences were declared at $P < 0.05$ and trend at $P < 0.10$. In addition, the REG procedure of SAS was used to develop a regression equation to predict the time required to weigh 5 to 15 pigs per pen.

Precision for estimating the mean and SD

For a sample size of 30 pigs, the heaviest and lightest pigs in 15 pens can be selected and weighed to achieve a CI range of 6.7 to 6.9 kg when estimating the mean and 5.4 to 7.6 when estimating the SD (Paulk et al., 2012; 2013). However, preliminary analysis determined that

when weighing the first 5, 10, or 15 pigs per pen, a larger sample size can be conducted in the same amount of time as selecting and weighing the heaviest and lightest pigs in 15 pens. Therefore, Datasets A and C from Paulk et al. (2012; 2013) were used herein to determine CI range for a total sample size of 60, 75, and 90 pigs. These sample sizes were achieved by taking random samples of 5 pigs within 12, 15 or 18 pens, 10 pigs within 6 or 9 pens, and 15 pigs within 4, 5 or 6 pens. Datasets A and C were used because they had similar pen arrangements to the 2 barns used in the experiment conducted herein, (i.e., approximately 20 to 35 pigs per pen). These sampling methods were evaluated using a simulation model developed using R (Paulk et al., 2012; 2013; R Foundation for Statistical Computing, Vienna, Austria). Each sample size was conducted 10,000 times, generating 10,000 estimated means and SD. These were used to determine the accuracy and precision for each sample method. The accuracy was determined by comparing the mean of the 10,000 sample means and SD to the actual population mean and SD pig BW, respectively. The precision was determined by calculating a 95% CI for the 10,000 sample means and SD. The distances between the upper and lower confidence limits represent the estimated means and SD CI range. When the heaviest and lightest pigs were selected from 15 pens, the mean was estimated using following equation: Estimated mean, kg = 0.77 * sample mean, kg + 0.25 * sample median, kg, and the SD was estimated by subtracting the sample's lightest pig BW from the heaviest pig BW and divide the difference by 6 (Paulk et al. 2014).

RESULTS

Time required to weigh pigs for different sampling methods

The time taken to select and weigh the heaviest and lightest pigs in a pen (Treatment 1) did not differ from weighing 5 pigs per pen (Treatment 2; Table 1). Increasing the number of pigs weighed per pen (Treatment 2, 3, and 4) increased ($P < 0.05$) the amount of time required to

weigh a single pen. For conducting a sample size of 30 pigs, selecting and weighing the heaviest and lightest pigs in 15 pens (Treatment 1) increased ($P < 0.05$) the time required compared to weighing the first 5, 10, or 15 pigs (Treatment 2, 3, and 4), from 6, 3, or 2 pens, respectively. Weighing 5 pigs (Treatment 2) from 6 pens tended to increase ($P < 0.10$) time required compared to weighing 15 pigs (Treatment 4) from 2 pens with the time needed to weigh 10 pigs (Treatment 3) from 3 pens being intermediate. For conducting a sample size of 60 pigs, selecting and weighing the heaviest and lightest pigs in 15 pens (Treatment 1) increased ($P < 0.05$) the time required compared to weighing the first 5 pigs (Treatment 2) from 12 pens. Both of these treatments increased ($P < 0.05$) time required compared to weigh 10 or 15 pigs (Treatment 3 and 4) from 6 or 4 pens, respectively. For conducting a random sample of 75 pigs, the time required for selecting and weighing the heaviest and lightest pigs in 15 pens (Treatment 1) did not differ from weighing the first 5 pigs (Treatment 2) from 15 pens. However, both of these treatments required more ($P < 0.05$) time than to weigh the first 15 pigs (Treatment 4) from 5 pens. For conducting a random sample of 90 pigs, the time taken to select and weigh the heaviest and lightest pigs in 15 pens (Treatment 1), weigh the first 10 pigs (Treatment 3) from 9 pens and weigh the first 15 pigs (Treatment 4) in 6 pens did not differ, but all took less ($P < 0.05$) time compared to weighing the first 5 pigs (Treatment 2) from 18 pens.

The following regression equation ($R^2 = 0.74$; SE = 2.53) was developed to predict the time needed to weigh 5 to 15 pigs per pen:

$$\mathbf{y = 30.23x + 64.18}$$

Where y = time (s) required to way x number of pigs and x = the number of pigs per pen to be weighed. The predicted time needed to weigh 5 to 15 pigs per pen can then be multiplied by the number of pens to determine time needed to conduct the total sample.

Precision for estimating the mean and SD

For Dataset A, selecting and weighing the heaviest and lightest pigs (Treatment 1) in 15 pens and weighing 5 or 10 pigs per pen (Treatments 2 and 3, respectively) to equal a total of 75 or 90 pigs had a similar (within 0.6 kg) CI range for estimating the mean and SD of BW (Table 2). For Dataset C, selecting and weighing the heaviest and lightest pigs (Treatment 1) in 15 pens and weighing 5 pigs per pen (Treatment 2) to equal a total of 75 or 90 pigs had a similar (within 1.1 kg) CI range for estimating the mean BW. Selecting and weighing the heaviest and lightest pigs (Treatment 1) in 15 pens and weighing 5, 10, or 15 pigs per pen (Treatments 2, 3, and 4, respectively) to equal a total of 75 or 90 pigs had a CI range within 2.5 kg of each method for estimating the SD, with Treatment 1 having the highest CI range and Treatment 2 having the lowest.

DISCUSSION

In a finishing pig barn, pigs are typically housed with 25 to 60 pigs per pen and 19 to 48 pens per a barn depending on the design of the barn. For weighing a set number of pigs, the precision for estimating the mean and SD BW is improved by increasing the number of pens sampled (Paulk et al. 2012; 2013). However, weighing pigs from multiple pens requires more resources and time, including opening gates and entering pens and moving the scale throughout the barn. The intercept and slope of the developed regression equation represent the estimated time required to set up the scale for each pen and the time to weigh each pig, respectively.

Therefore, it took approximately 64 s to move the scale 1 pen's length, zero the scale, and open the gate before weighing any pigs and 30 s for each pig weighed per pen.

For a sample size of 30 pigs, the precision for estimating the mean and SD of pig BW can be further improved by selecting the heaviest and lightest pigs from 15 pens vs weighing n random pigs from n random pens to equal a total sample size of 30 pigs (Paulk et al., 2012; 2013). Although this improved the precision without increasing the number of pigs weighed, selecting and weighing pigs from 15 pens includes additional time to select and sort pigs and weigh multiple pens as previously discussed. Personnel weighing pigs altered the work load and time required by backing each pig off of the scale; however, it took additional time to select pigs and sort them to the scale. It took the same amount of time to select and weigh the heaviest and lightest pig per pen vs. weighing the first 5 pigs per pen. In addition, for a total sample size of 30 pigs, selecting and weighing the heaviest and lightest pigs in 15 pens took 2.5, 3.0, and 3.1x longer to complete compared to weighing 5 pigs from 6 pens, 10 pigs from 3 pens, and 15 pigs from 2 pens, respectively. Therefore, the comparison of sampling methods needed to be reevaluated based upon the time required to conduct the sample instead of the number of pigs weighed.

It took a similar amount of time, approximately 52 to 54 min, to conduct the following sampling methods: selecting and weighing the heaviest and lightest pig in 15 pens (30 pigs), weighing 5 pigs from 15 pens (75 pigs), 10 pigs from 9 pens (90 pigs), and 15 pigs from 6 pens (90 pigs). Based on the CI range, an optimal sampling method was not clearly defined for estimating both the mean and SD. However, for Dataset A and C, weighing 5 pigs from 15 pens had a CI range similar to or reduced compared to the other 3 methods when estimating the mean and SD of BW. Also, weighing 10 pigs from 9 pens (90 pigs), and 15 pigs from 6 pens (90 pigs)

increased the CI range when estimating the mean for Dataset C. Weighing 5 pigs from 15 pens increased the CI range by 0.5 to 1.1 kg for estimating the mean but reduced the CI range by 0.1 and 2.5 kg for estimating the SD compared selecting and weighing the heaviest and lightest pig in 15 random pens.

In addition to improvements in the CI range, weighing 5 vs 10 or 15 pigs per pen may have caused less stress when moving pigs back to the pen after being weighed. Although stress levels were not measured in this experiment, Lewis and McGlone (2006) observed elevated heart rates of pigs moved in groups larger than 5 or 6 pigs. Also, when the heaviest and lightest pigs were selected and weighed, each pig was backed off of the scale into the pen instead of let into the aisle. Therefore, moving pigs back to their pens was not a concern; however, stress related measurements of backing each pig off the scale were not determined.

Determining whether to select and weigh the heaviest and lightest pigs in 15 random pens or weigh the first 5 pigs in 15 random pens may also depend on personnel skill. The time required to select the heaviest and lightest pigs can depend on the person's ability to assess the BW of pigs within a pen and make the decision. The accuracy and precision for estimating the mean and SD can also depend on their ability to accurately select the heaviest and lightest pigs. Personnel not experienced at selecting pigs may prefer to weigh 5 pigs per pen because it can be done by randomly selecting pens and weighing the first 5 pigs in each of those pens.

In conclusion, based on time required to conduct the sample and the precision and accuracy of the sampling method, weighing the first 5 pigs in 15 pens is the recommended sampling method. In addition, weighing the first 5 pigs per pen does not include the assumption that personnel can select the correct pigs and reduces the possibility of bias occurring. It is

expected to take 2 employees approximately 55 min to weigh the first 5 pigs from 15 pens not including time to prepare and clean up.

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Table 3.1 Time required to select and weigh pigs for designated sampling methods[1]

	Treatment[2]					
Total sample	HL	5 pigs	10 pigs	15 pigs	SEM	*P* <
Time per pen,[3] min	3.6[a]	3.6[a]	6.0[b]	8.7[c]	0.3	0.001
Time required for weighing,[4] min						
30 pigs	53.4[a]	21.6[b]	17.9[b]	17.4[b]	2.4	0.001
60 pigs[5]	53.4[a]	43.2[b]	35.9 [c]	34.8[c]	2.1	0.001
75 pigs[5]	53.4[a]	54.0[a]	—	43.5[b]	3.5	0.009
90 pigs[5]	53.4[a]	64.8[b]	53.9[a]	52.3[a]	3.5	0.003

[1]A total of 68 pens in 2 barns with 25 to 30 pigs per pen were used to conduct sampling methods.

[2]Treatments included: (HL) selecting weighing the heaviest and lightest pig per pen and weighing the first 5, 10 and 15 pigs out of the pen.

[3]Time required to conduct sampling method on a single pen.

[4]The time observed for selecting and weighing the heaviest and lightest pig per pen and weighing the first 5, 10 and 15 pigs out of the pen was multiplied a factor of n in order to equal the total sample size.

[5]The time observed for selecting and weighing the heaviest and lightest pig per pen was kept constant at a total sample size of 30 pigs.

[a,b,c]Within a row, means without a common superscript differ ($P < 0.05$).

Table 3.2 The CI range (kg) when a varying number of pigs and pens are sampled to estimate the mean and SD BW of the population.[1]

	Treatment[2]			
Total sample	HL[3]	5 pigs	10 pigs	15 pigs
Dataset A[4]				
Mean				
30 pigs,[5]	6.7	11.8	13.1	14.5
60 pigs,[6]	–	8.0	9.3	10.0
75 pigs,[7]	–	7.2	–	8.9
90 pigs,[8]	–	6.3	7.3	8.2
SD				
30 pigs,[5]	5.4	8.6	8.9	9.0
60 pigs,[6]	–	6.3	6.1	6.3
75 pigs,[7]	–	5.6	–	5.3
90 pigs,[8]	–	5.3	5.1	5.0
Dataset C[9]				
Mean				
30 pigs,[5]	6.9	13.6	18.3	21.7
60 pigs,[6]	–	9.9	12.4	15.1
75 pigs,[7]	–	8	–	13
90 pigs,[8]	–	7.2	9.7	12
SD				
30 pigs,[5]	7.6	9.5	10.7	11.4
60 pigs,[6]	–	6.8	7.4	8.4
75 pigs,[7]	–	5.6	–	7.6
90 pigs,[8]	–	5.1	6.1	6.8

[1] Samples were simulated using datasets from Paulk et al. 2012 and Paulk et al. 2013. Samples were completed 10,000 times for each sampling method. The CI range was calculated for the 10,000 sample means and SD of each sampling method.

[2]Treatments included: (HL) selecting weighing the heaviest and lightest pig per pen and weighing the first 5, 10 and 15 pigs out of the pen.

[3]The mean was estimated using following equation: Estimated mean, kg = 0.77 * sample mean, kg + 0.25 * sample median, kg, and the SD was estimated by subtracting the sample's lightest pig BW from the heaviest pig BW and divide the difference by 6.

[4]A total of 1,260 pigs (mean = 114.8 kg, median = 115.2 kg, SD = 14.9 kg, and CV = 13.0%) with 23 to 28 pigs per pen and a total of 48 pens.

[5]Samples included selecting the heaviest and lightest pig from 15 pens, 5 random pigs from 6 pens, 10 random pigs from 3 pens, and 15 random pigs from 2 pens for Treatments 1, 2, 3, and 4, respectively.

[6]Samples included selecting the heaviest and lightest pig from 15 pens, 5 random pigs from 12 pens, 10 random pigs from 6 pens, and 15 random pigs from 4 pens for Treatments 1, 2, 3, and 4, respectively.

[7]Samples included 5 random pigs from 15 pens and 15 random pigs from 5 pens for Treatments 2 and 4, respectively.

[8]Samples included 5 random pigs from 18 pens, 10 random pigs from 9 pens, and 15 random pigs from 6 pens for Treatments 2, 3, and 4, respectively.

[9]A total of 1,069 pigs were weighed (population mean = 100.9 kg, median = 101.6 kg, SD = 14.5 kg, and CV = 14.4%) with 40 pens and 20 to 35 pigs per pen.

Chapter 4 - Utilizing meta-analyses to generate equations to predict iodine value of pork carcass back, belly, and jowl fat

ABSTRACT

Meta-analyses used data from existing literature to generate equations to predict finishing pig back, belly, and jowl fat IV and an experiment was conducted to validate these equations. The final database included 24, 21, and 29 papers for back, belly, and jowl fat IV, respectively. For Exp. that changed dietary fatty acid composition, initial diets (**INT**) were defined as those fed before the change in diet composition and final diets (**FIN**) were those fed after. The predictor variables tested were divided into 5 groups: 1) diet fat composition (dietary % C16:1, C18:1, C18:2, C18:3, EFA, unsaturated fatty acids, and iodine value product) for both INT and FIN diets; 2) d feeding the INT and FIN diets; 3) ME or NE of the INT and FIN diet; 4) performance criteria (initial BW, final BW, ADG, ADFI, and G:F); 5) carcass criteria (HCW and backfat thickness). The PROC MIXED procedure of SAS (SAS Institute, Inc., Cary, NC) was used to develop regression equations. Evaluation of models with significant terms was then conducted based on the Bayesian Information Criterion (BIC). The optimum equations to predict back, belly, and jowl fat IV were: backfat IV =84.83+(6.87*INT EFA)-(3.90*FIN EFA)-(0.12*INT d)-(1.30*FIN d)-(0.11*INT EFA*FIN d)+(0.048*FIN EFA*INT d)+(0.12*FIN EFA*FIN d)-(0.0060*FIN NE)+(0.0005*FIN NE*FIN d)-(0.26*backfat depth); belly fat IV = 106.16+(6.21*INT EFA)-(1.50*FIN d)-(0.11*INT EFA*FIN d)-(0.012*INT NE)+(0.00069*INT NE*FIN d)-(0.18*HCW)-(0.25*BF); and jowl fat IV =85.50+(1.08*INT EFA)+(0.87*FIN EFA)-(0.014*INT d)-(0.050*FIN d)+(0.038*INT EFA*INT d)+(0.054*FIN EFA*FIN d)-(0.0066*INT NE)+(0.071*INT BW)-(2.19*ADFI)-(0.29*backfat depth). Dietary treatments from the validation experiment consisted of a corn-soybean meal control diet with no added fat

or a 3 × 3 factorial arrangement with main effects of fat source (4% tallow), 4% soybean oil, or a blend of 2% tallow and 2% soybean oil) and feeding duration (d 0 to 42, 42 to 84, or 0 to 84). The back, belly, and jowl fat IV equations tended to overestimate IV when actual IV were less than approximately 65 g/100g and underestimate belly fat IV when actual IV are greater than approximately 74 g/100g or when the fat blend was fed from d 0 to 84 or d 42 to 84. Overall, with the exceptions noted, the regression equations were an accurate tool for predicting carcass fat quality based on dietary and pig performance factors.

INTRODUCTION

Over the last decade, the pork industry has placed considerable importance on pork fat quality. Iodine value (**IV**), a measure of fatty acid unsaturation, is one method used by pork processors for assessing pork fat quality. Increases in fatty acid unsaturation or IV are associated with negative impacts on pork fat quality. This can lead to problems with belly slicing efficiency, fat smearing, and reduced shelf life because of oxidative rancidity (NRC, 2012).

Currently, several swine packers impose penalties on carcasses that possess carcass fat IVs above (more unsaturated) certain thresholds (Benz et al., 2011c). Carcass fat composition of monogastric animals, particularly pigs, is directly related to the fatty acid composition of the diet (Madsen et al., 1992). Thus, feeding ingredients with high amounts of dietary unsaturated fatty acids will increase carcass fat IV. Examples of these ingredients include dried distiller's grains with solubles (DDGS), bakery meal, or added fats such as animal-vegetable blends, choice white grease, or soybean oil (NRC, 2012). The increased use of these ingredients in swine diets has led to concerns by pork processors related to the associated negative impacts on carcass fat quality correlated with high carcass fat IV values.

Carcass fat IV varies between the three important fat depots (back, belly, and jowl) and the IV of these depots show differential responses to the fatty acid composition of dietary feedstuffs (Benz et al., 2010; 2011b; Weigand et al., 2011). While many studies have been conducted to measure carcass fat IV based on different levels of dietary fatty acid composition, accurately predicting final carcass fat IV of the various fat depots is challenging for producers and processors. Therefore, the objective of this study was to utilize a meta-analysis of existing literature to generate predictive equations for back, belly, and jowl fat IV of finishing pigs. In addition, an experiment was conducted to validate the developed equations.

MATERIALS AND METHODS

The term, meta-analysis, is defined as the quantitative summarization of past research (Sauvant et al., 2008). A literature review was conducted to compile studies that examined the effects of dietary fatty acids and dietary energy on variables associated with growth and carcass characteristics and back, belly, and jowl fat IV. The literature search was conducted via the Kansas State University Libraries, utilizing the CABI search engine, and using the keywords "iodine value and pig" or "iodine value and swine." Data was derived from both refereed and non-refereed publications including theses, technical memos, and university publications. The final database resulted in publication dates from 2002 to 2013.

In order to be included in the final database, experiments had to meet the following criteria: 1) pigs used in experiments had ad libitum access to feed and water; 2) gender of the pigs was classified as either barrows, gilts, mix gender or immunocastrate barrows; 3) the percentage of dietary ingredients fed throughout the experiment was adequately defined; 4) the pigs were fed diets without added conjugated linoleic acid; 5) the experiments provided information including duration of the feeding period, initial BW, final BW, ADG, ADFI, G:F,

HCW, and backfat depth. The initial screen yielded 46 publications. Papers were eliminated from the analysis because pigs were not allowed ad libitum access to food and water (1 paper), dietary conjugated linoleic acid was fed (2 papers and 3 treatments from 1 paper), carcass criteria were not included (4 papers), and growth criteria were not reported (5 papers). The final database resulted in 24 papers with 169 observations for backfat IV, 21 papers with 124 observations for belly fat IV, and 29 papers with 197 observations for jowl fat IV (Table 1). In all papers, back, belly, or jowl fat IV was determined by either fatty acid analysis (NRC 2012) or near-infrared analysis (Zamora-Rojas et al., 2013).

The dietary composition of experimental diets was used to calculate percent dietary C16:1, C18:1, C18:2, and C18:3 fatty acids, EFA (sum of C18:2 and C18:3), total unsaturated fatty acids (**USFA**), dietary iodine value product (**IVP**), and dietary ME (kcal/kg) and NE (kcal/kg) concentrations. Reported individual fatty acid percentages from analyzed ingredients or complete diets were calculated as a percent of total fatty acids. When analyzed values were not reported, fatty acids, as a percentage of total fatty acids, were obtained from Sauvant et al. (2004) or from the U.S. Department of Agriculture (2010). The fatty acid profile of corn oil from Sauvant et al. (2004) was used for DDGS. Dietary fatty acid concentrations were calculated by multiplying the percent of each fatty acid by the reported analyzed ether extract of the ingredient or diet. If ether extract was not reported, it was derived from the NRC (2012). Iodine value was calculated using the following equation (NRC, 2012): Total IV = % C16:1 (0.9502) + % C18:1 (0.8598) + % C18:2 (1.7315) + % C18:3 (2.6152) + % C20:4 (3.2008) + % C20:5 (4.0265) + % C22:1 (0.7225) + % C22:5 (3.6974) + % C22:6 (4.4632). In the equation, % is the percentage that each fatty acid methyl ester represents of the sum total of all fatty acid methyl esters in the gas chromatographic analysis. The dietary IVP was calculated for all dietary treatments using the

the following equation (NRC, 2012): IVP = (IV of ingredient fat) × (% fat in the ingredient) × (0.1). The ME and NE content of every diet was determined by using the ingredient ME and NE values provided in the NRC (2012). The ME and NE values for glycerol was obtained from Lammers et al. (2008) and Hinson (2009), respectively.

Some observations (back [n=36], belly [n=37], and jowl [n=45]) changed diet composition during the experiment resulting in changes in dietary fatty acid composition. Therefore, dietary variables were determined for initial (**INT**) and final (**FIN**) diets. Initial diets are defined as diets fed prior to the change in ingredient composition and final diets are defined as diets fed after the change in diet composition. Feeding duration of both the INT and FIN diets were used in the meta-analyses. In the database, observations that did not change dietary fatty acid composition had equal INT and FIN dietary variables and the initial duration was defined as the total duration of the experiment and final duration equaled 0 days. For INT or FIN diets applied over more than one dietary phase, a weighted average of each variable, based on feeding duration within the INT or FIN period, was calculated to describe the treatment applied within that period.

Statistical analysis

Descriptive statistics of candidate variables were evaluated using the PROC UNIVARIATE procedure of SAS. All candidate variables were then evaluated for correlation using the PROC CORR procedure of SAS. This was used to determine relationships between variables and prevent multicolinearity. Based on descriptive statistics and correlations the predictor variables tested were divided into the following groups: 1) diet fat composition (C16:1, C18:1, C18:2, C18:3, EFA, USFA, and IVP); 2) duration of feeding for initial and final diets; 3) energy content of the diet (ME or NE); 4) performance criteria (initial BW, final BW, ADG,

ADFI, and G:F); 5) carcass criteria (HCW and backfat thickness). The PROC MIXED procedure of SAS (SAS institute, Inc., Cary, NC) was then used to develop regression equations to separately predict back, belly, and jowl fat IV. The method of maximum likelihood (ML) was used in the model selection. The treatment applied within each experiment was the experimental unit for modeling of the equations, and experiment within paper was included as a random effect. The statistical significance for inclusion of terms in the models was determined at $P < 0.10$. Further evaluation of models with significant terms was then conducted based on the Bayesian Information Criterion (BIC). A model comparison with a reduction in BIC of more than 2 was considered improved (Kass and Raftery, 1995). Throughout the selection process, studentized residual plots were observed to determine if quadratic terms or interaction terms needed to be tested in the model. The model was determined using a manual forward selection procedure while progressing through the groups of the predictor variables. First, the best single predictor for back, belly, or jowl fat IV was determined. Variables from the dietary fat composition group had the lowest BIC value. Next, the chosen initial and final dietary fat composition variables and the initial and final duration and their interactions were added to the model. Once the best dietary fat composition × duration model was determined, dietary energy content (ME or NE) was added to the model to determine if either were significant and improved the precision of the model. The model was then evaluated for improvement by adding the significant growth performance and carcass criteria parameters.

The method of residual maximum likelihood (REML) was then used to obtain the estimate of the parameters for the candidate models. The adequacies of candidate models were also examined by evaluating a histogram of residuals for evidence of normality and plotting residuals against predicted values of Y (back, belly, or jowl IV; Kuehl, 2000 and St-Pierre,

2003). Actual IV was plotted against predicted IV and was evaluated using the line of equality to determine if there was bias in estimation (Altman and Bland, 1983). Residual plots were also used to investigate outliers. Any residual greater or less than 3 standard deviations from the mean were deemed outliers under review. Outliers were reviewed to determine if they were biologically significant. As a result, one observation for back and belly fat IV was removed.

Validation Experiment

An experiment was conducted in order to validate the regression equations used to estimate back, belly, and jowl fat IV. Data from this experiment was not included in the meta-analysis dataset. The procedures of the validation experiment are described by Stephenson et al. (2015). Dietary treatments consisted of: a corn-soybean meal control diet with no added fat fed from d 0 to 84 (C); 4% tallow from d 0 to 84 (T); 4% tallow from d 0 to 42 and the control from d 42 to 84 (T-C); control from d 0 to 42 and 4% tallow from d 42 to 84 (C-T); blend of 2% tallow and 2% soybean oil from d 0 to 84 (B); blend of 2% tallow and 2% soybean oil from d 0 to 42 and the control from d 42 to 84 (B-C); control from d 0 to 42 and blend of 2% tallow and 2% soybean oil from d 42 to 84 (C-B); 4% soybean oil from d 0 to 84 (SBO); 4% soybean oil from d 0 to 42 and the control from d 42 to 84 (SBO-C); control from d 0 to 42 and 4% soybean oil from d 42 to 84 (C-SBO). Soy oil, tallow, and a blend of the two were added to create treatments of high levels of dietary unsaturated fatty acids, high levels of saturated fatty acids, and a blend of the two, respectively. Back, belly, and jowl fat IV means and the 95% confidence interval determined in the experiment were used to validate the estimated means derived from the equations.

RESULTS

The backfat IV database included INT diets that were fed from 21 to 125 days and were analyzed to contain an IVP range of 21.3 to 107.2 g/100g, an EFA range of 0.80 to 4.88 %, and a NE range of 2,262 to 2,787 kcal/kg (Table 2). The FIN diets were fed up to 66 d prior to market and were analyzed to consist of an IVP range of 21.3 to 107.2 g/100g, an EFA range of 0.80 to 4.90%, and NE range of 2,262 to 2,787 kcal/kg. Before beginning the INT period diet, pigs had an average BW range of 21.9 to 94.3 kg. These pigs' ADFI intake ranged from 1.56 to 3.64 kg/d, and they produced carcasses with HCW ranging from 28.1 to 100.5 kg and backfat thicknesses ranging from 10.5 to 29.5 mm. The backfat IV values ranged from 58.3 to 86.1 g/100g.

The belly fat IV database included INT diets that were fed from 21 to 125 days and were analyzed to contain an IVP range of 33.8 to 96.2 g/100g, an EFA range of 1.51 to 4.09 %, and a NE range of 2,262 to 2,772 kcal/kg. The FIN diets were fed up to 66 d prior to market and were analyzed to consist of an IVP range of 33.8 to 88.1 g/100g, an EFA range of 1.50 to 3.60 %, and a NE range of 2,262 to 2,772 kcal/kg. These pigs' ADFI ranged from 2.04 to 3.31 kg/d, and they produced carcasses with HCW ranging from 79.5 to 100.5 kg and backfat thickness ranging from 14.0 to 29.2 mm. The belly fat IV values ranged from 58.9 to 87.3 g/100g.

The jowl fat IV database included INT diets that were fed from 21 to 125 days and were analyzed to contain an IVP range of 22.1 to 101.1 g/100g, an EFA range of 1.08 to 4.63 %, and a NE range of 2,262 to 2,787 kcal/kg. The FIN diets were fed up to 66 d before market and were analyzed to contain an IVP range of 22.1 to 101.1 g/100g, an EFA range of 1.10 to 4.60 %, and a NE range of 2,262 to 2,787 kcal/kg. These pigs' ADFI ranged from 2.03 to 3.35 kg/d, and they produced carcasses with HCW ranging from 73.5 to 100.5 kg and backfat thickness ranging from 10.4 to 26.0 mm. The jowl fat IV ranged from 61.4 to 86.2 g/100g.

Correlations between predictor variables were determined and as expected some of the variables within each category were highly correlated. For variables determining dietary fat composition in all 3 fat depots, IVP was positively correlated ($R^2 > 0.83$; $P < 0.001$) with C18:2, EFA, and USFA for both INT and FIN diets (Table 3). It was also determined that C18:2 was positively correlated ($R^2 = 1.00$; $P < 0.001$) with EFA for INT and FIN diet in all 3 datasets. The ME content of the diet was positively correlated ($R^2 > 0.86$; $P < 0.001$) with the NE content. For growth and carcass characteristics in all 3 fat depots, FIN BW was positively correlated ($R^2 > 0.64$; $P < 0.001$) with HCW (Table 4).

Significant single variable models used to predict back, belly, and jowl fat IV for the dietary fat composition category included the INT and FIN diet IVP, C18:1, C18:2, C18:3, EFA, and USFA ($P < 0.01$; Table 5). Also, INT C16:1 ($P < 0.07$) was a significant predictor of backfat IV. For the dietary energy content category, the INT and FIN ME were significant predictors for back fat IV ($P < 0.001$). For belly and jowl fat IV, the INT and FIN dietary NE were significant predictors ($P < 0.01$). Common significant single variable models used to predict back, belly, and jowl fat IV for the growth and carcass characteristic category included ADG, ADFI, HCW, and BF ($P < 0.05$; Table 6). In addition, FIN BW and G:F were significant predictors of backfat IV($P < 0.07$), FIN BW for belly fat IV($P < 0.04$), and INT BW for jowl fat IV($P < 0.06$). Predictors C18:2 and EFA had the lowest BIC values within INT (back BIC = 870.6 and 871.6, belly BIC = 624.5 and 622.6, jowl BIC = 853.7 and 962.1, respectively) and FIN (back BIC = 886.6 and 888.1, belly = 629.1 and 627.3, jowl BIC = 961.4 and 962.1, respectively) diets.

For backfat IV, using variables from the dietary fat composition and duration of feeding categories, INT EFA, FIN EFA, INT d, FIN d, INT EFA*FIN d, FIN EFA*INT d, and FIN EFA*FIN d had the lowest BIC (755.2) for all models tested (Table 7). Next variables from the

dietary energy category were tested and the prediction equation developed was improved (BIC = 744.9) by adding FIN NE and FIN NE*FIN d to the model. Lastly, pig growth and carcass characteristics were investigated for inclusion in the model. Adding backfat depth resulted in the best final model (BIC = 734.5).

Utilizing variables from the dietary fat composition and duration of feeding categories for belly fat IV, INT EFA, FIN d, and INT EFA*FIN d resulted in the lowest BIC (586.0) compared to all models tested. Next dietary energy was tested with the addition of INT NE and INT NE*FIN d improving the model (BIC = 566.9). Lastly, pig growth and carcass characteristics were tested, and the model was further improved by adding HCW and backfat thickness (BIC = 557.9).

For jowl fat IV, dietary fat composition and duration of feeding variables including INT EFA, FIN EFA, INT d, FIN d, INT EFA*INT d, and FIN EFA*FIN d were determined to be components of the best model (BIC = 814.6). Next the inclusion of diet energy content was tested, with the model being further improved by adding INT NE (BIC = 792.6). The final step determined the growth and carcass characteristics that should be included. Adding INT BW, ADFI, and backfat thickness improved (BIC =756.2) the final model.

For back, belly, and jowl fat IV, the residual plots showed no evidence of any prediction bias (Figure 1). The residual plots portray the improved precision for the estimation of back and jowl fat IV compared to the precision when predicting belly fat IV. When evaluating bias for all 3 fat depots, the final equations tended to overestimate carcass fat IV when the actual fat IVs were at the lower end of the range (Figure 2). The final equation for belly fat IV tended to underestimate IV when the actual IV values were at the upper end of the range.

Validation Experiment

. Regression equation input variables derived from the validation experiment are presented in Table 8. Back, belly, and jowl fat IV means determined in the experiment and estimated IV are presented in Table 9. For backfat IV, the means estimated using the regression equations fell within 3.77 g/100g of the actual IV for all dietary treatments except C-T, which was 7.47 g/100g greater than the actual value. For belly fat IV, the means estimated using the regression equations fell within 9.22 g/100g of the actual IV for all dietary treatments. However, estimated IV for the C, T, T-C, C-T, B-C, and SBO-C treatments were within 3.77 g/100g of the actual IV. For jowl fat IV, the means estimated using the regression equations fell within 3.43 g/100g of the actual IV for all dietary treatments.

DISCUSSION

Fatty acid composition of pig adipose tissue is highly influenced by amounts and proportions of fatty acids in the diet (Wood et al. 2008). The meta-analysis would support this finding based on the significant variables generated. The equations generated utilizing single predictors demonstrate that the IV of pork fat is primary influenced by dietary unsaturated fatty acid concentration. Similarly, Boyd et al. (1997) and Madsen et al. (1992) developed equations to predict backfat IV using IVP as the predictor variable; however, in contrast to these equations, our regression analyses determined that dietary EFA was a better predictor for back, belly, and jowl fat IV than IVP. In pork fat, EFA (sum of C18:2 and C18:3) are derived directly from the diet, whereas C16 and C18 saturated and monounsaturated fatty acids are mainly the products of de novo synthesis. As a result, the dietary concentrations of EFA have a direct effect on pork fat IV, while dietary C16 and C18 based FA have only minimal direct incorporation into the adipose (Wood et al., 2008). Calculated IVP was shown to be correlated with dietary levels of PUFA and

MUFA. Therefore, it may be less accurate in predicting pork fat IV because of the association with dietary FA that are not directly deposited. The present model overcame this situation by using only the unsaturated fatty acids (EFA) that are directly deposited into the pork fat and as a result, our model was improved compared to using an IVP-based model. Our findings are in agreement with Benz et al. (2011c) who reported that dietary C18:2 is a better predictor of backfat and jowl fat IV than the IVP of the diet.

Some experiments had observations that changed dietary fatty acid composition during the experiment (i.e. switching diets from a high to low or low to high unsaturated or IVP). To account for the changes, both INT and FIN dietary EFA were included in the model to predict back and jowl fat IV. Benz et al. (2011a) demonstrated the influence of initial dietary EFA on back and jowl fat IV. When increasing the time pigs were initially fed a diet with 2.2% EFA from 26 to 82 d, or decreasing the final diet (EFA=1.6%) from 56 to 0 d, the authors reported a 4.0 and 2.7 g/100g increase in back and jowl fat IV, respectively. Furthermore in pigs fed a 4.6% INT EFA diet, there was a 16.7 and 8.7 g/100g increase in back and jowl fat IV, respectively. In addition, Asmus et al. (2014) demonstrated the importance of accounting for FIN EFA and FIN d when estimating jowl fat IV by feeding an initial diet containing 3.4% EFA followed by a FIN diet containing 1 of 2 levels of EFA. For pigs fed FIN diets for 47 d immediately prior to harvest with 2.6 or 1.7% FIN EFA, the authors reported a 2.7 and 7.9 g/100g decrease in jowl fat IV, respectively, when compared to pigs fed a diet containing 3.4% EFA the entire experiment. However, when FIN diets with a 2.6 and 1.7% FIN EFA were only fed for the final 23 d, there was only a 1.9 and 2.7 g/100g decrease in jowl fat IV, respectively. These studies are in agreement with our models used to estimate back and jowl fat IV which included INT EFA, FIN EFA, INT d, and FIN d as well as the interactions of these variables.

The importance of diet EFA and duration of feeding on estimating carcass fat IV can further be explained by the mechanisms of adipose tissue deposition and turnover. Pig adipose tissue maintains a certain level of C18:2 derived from the diet, but when extra C18:2 is provided by the diet, the amount in adipose tissue is increased at the expense of other fatty acids (Koch et al., 1968; Warnants et al., 1999). If dietary levels are reduced, adipose tissue begins eliminating excess levels of C18:2. It appears that the theoretical capacity for changing carcass fat IV is about 60 to 70% within the first 2 wk of dietary change, while the full capacity for change is only reached in 6 to 8 wk (Warnants et al., 1999; Xu et al., 2010b). However, the elimination rates of C18:2 from backfat is variable and is dependent on the initial C18:2 content in backfat (Camoes et al., 1995, and Wiseman and Agunbiade, 1998). This would support our model's improvement for predicting carcass fat IV when the diet × duration interaction is also included. The rate of change in jowl fat IV resulting from either reducing the duration of feeding or the level of unsaturated fatty acids is less than that of back and belly fat IV. For instance, when the FIN d is increased from 0 to 60 d and the INT d is reduced from 120 to 60 d, while all other variables are kept constant, the estimated jowl fat IV is reduced from 81.7 to 71.6 while the backfat is reduced from 80.0 to 66.7g/100g. These differences in fat depot specific IV change can be explained by the fact that finishing pigs would likely deposit fat earlier in the jowl before depositing it in the back and belly (Wiegand et al., 2011). Therefore, the fat that is initially deposited in the jowl is less likely to change.

For predicting belly fat IV, INT EFA, FIN d, and INT EFA*FIN d provided the best model. Previous research has demonstrated considerable intra-belly variation in belly fat IV (Trusell et al., 2011). Therefore, we speculate that variation between sites of collection of the belly fat and fewer total observations is the reason the model is not more complex and robust.

As a result, the belly fat IV prediction equation is less precise compared to the prediction equations for back and jowl fat IV.

The equations generated utilizing single predictors demonstrate the influence of dietary energy content on the IV of pork fat. Bee et al. (2002) previously reported an increase in PUFA and a decrease in SFA and MUFA in carcass backfat inner and outer layers and omental fat of pigs fed low energy diets (2,102 kcal DE/kg) compared to those fed high energy diets (3,343 kcal DE/kg). This was explained by reductions in the activity of lipogenic enzymes resulting from restricted energy intake. Reductions in the activity of these enzymes represent less *de novo* fatty acid synthesis which leads to a greater proportion of unsaturated fatty acids being deposited. Bee et al. (2002) investigated the effects of DE on pork fat IV, while the current analysis tested ME and NE as predictors of carcass fat IV. In addition, including dietary EFA and NE content improved the precision of the model to predict back, belly, and jowl fat IV more than dietary ME. The models demonstrated the negative correlation between NE and carcass fat IV.

Other variables are also known to influence the amount, composition, and quality of pork fat. Wood et al. (2008) described these various factors (such as backfat thickness, gender, age, BW, and maturity) affecting fat composition of pigs. Younger, lighter, and leaner pigs were found to have lower concentrations of C18:0 and C18:1 and greater concentrations of C18:2 in their subcutaneous adipose tissue. This is also the case when intact males and gilts are compared to castrates. Genetic line influence on the fatty acid composition of adipose tissue in swine has been described by several authors (Wood et al., 2003; Kloareg et al., 2007; Monziols et al., 2007), but the differences observed between genotypes are likely attributable to their differences in leanness and subcutaneous fat depth (Hugo & Roodt, 2007). Gender differences in fat composition are also a function of the differences in subcutaneous fat depth and leanness, and

differences found between intact males and females with the same backfat thickness indicate that the adipose tissue of intact males may be less mature than that of castrates and females (Wood et al., 2008). The observations collected for the meta-analyses were from a variety of genetic lines and not distributed evenly across individual genders; therefore, the equations created from our meta-analyses did not include genetic line or gender. The current analyses support the conclusion that backfat depth accounts for much of the differences observed between carcass fat IV, and that backfat depth is negatively correlated with the IV of carcass fat.

Prediction equations are tools that can become an integral part of a pork enterprise; however, it is essential that they are used correctly to prevent the generation of faulty information. It is important to realize that the equations are only valid as long as the input variables consist of values within the ranges used to generate the predictive equation. For example, backfat IV is estimated to be reduced from 73.4 to 68.7 g/100g by lowering the INT EFA from 4 to 2.7% when the INT diet is fed for 90 d followed by a final diet containing 2.7% EFA fed for 30 d (FIN NE of 2,580, backfat depth of 20 mm). However, if FIN d is increased to a value outside of the range used in generating the equations (d 0 to 66), the equation does not behave appropriately and will generate predictions that are not accurate. For example, when INT d equals 30 and FIN d equals 90, while all other variables were kept constant, the estimated backfat IV is increased from 60.0 to 64.0 g/100g. Previous research has documented that reducing the INT EFA will result in decreased carcass backfat IV (Xu et al., 2010b; Benz et al., 2011a). Therefore, in the example, the increase in backfat IV results from using values outside of the range of the predictor variables.

Other factors have been shown to affect the fatty acid content of pork fat, but were not included in these analyses because the data are limited. When 10 ppm of ractopamine-HCl was

fed for 28 d (Carr et al., 2005) and 35 d (Apple et al., 2008) prior to slaughter, the backfat depth was reduced and the backfat IV was increased by approximately 0.07 and 0.08 g/100g per day, respectively. Weber et al. (2006) also reported that when pigs were fed diets with 10 ppm ractopamine HCl for 28 d, the IV of the inner and outer backfat increased about 0.08 g/100g per day, but the IV of belly fat increased only 0.04 g/100g per day. However, Duttlinger et al. (2008) did not observe differences in backfat, belly fat, or jowl fat IV when 7.5 ppm of ractopamine HCl was fed for 28 d. Weber et al. (2006) also reported a reduction in fat IV from feeding 0.6% conjugated linoleic acid (CLA) for 56 d. White et al. (2009) reported a reduction in the IV of the outer and middle backfat layers and belly fat when 0.6% CLA was added to diets containing up to 40% DDGS. They reported that feeding 0.6% CLA during the last 10 d prior to slaughter successfully minimized the effects of feeding 20% DDGS for the last 30 d. Lastly, pigs fed pelleted finishing pig diets compared to meal form has also been shown to increase belly fat IV (Nemechek et al., 2013). The authors reported that pelleting diets with 1.7 and 2.6% EFA increased belly fat IV 1.3 and 3.7 g/100g, respectively, compared to meal form diets fed to pigs for 81 d.

The final prediction equations developed herein were compared to data generated from an additional experiment which was not included in the meta-analysis. For backfat IV, the means estimated using the regression equations fell within 3.77 g/100g of the actual IV for all dietary treatments except C-T. It was determined by the line of equality that when the actual IV values are below approximately 65 g/100g the equation will overestimate backfat IV. Therefore, the overestimation of IV for the C, T, T-C, and C-T diets were expected based on the line of equality. However, the equation tended to overestimate the IV for the C-B and SBO-C treatment by 3.77 and 3.22 g/100g, respectively, and underestimate the IV for the SBO treatment by 2.5

g/100g. For belly fat IV, the means estimated using the regression equations fell within 9.22 g/100g of the actual IV for all dietary treatments. It was determined by the line of equality that when the actual IV values are less than approximately 65 g/100g and greater than approximately 70 g/100g the equation will over and under estimate IV, respectively. Therefore, the overestimation of the IV for the B, C-B, SBO, and C-SBO diets is expected based on the line of equality. The equation also underestimated the IV for the T treatment. For jowl fat IV, the means estimated using the regression equations fell within 3.43 g/100g of the actual IV for all dietary treatments. It was determined by the line of equality that when the actual IV values are less than approximately 65 g/100g the equation will overestimate backfat IV. Therefore, the overestimation of the IV for the C, T, and C-T diets is expected based on the line of equality. However, the equation tended to overestimate the IV for the C-SBO treatment by 2.06 g/100g. Overall, with the exceptions noted, the regression equations were an accurate tool for predicting carcass fat quality based on dietary and pig performance factors.

CONCLUSION

There are many factors, both dietary and biological, that affect the fatty acid composition of adipose tissue in pigs. Iodine value is a measure of fatty acid unsaturation and is commonly used for assessing pork fat quality. Equations incorporating the appropriate factors to estimate carcass fat IV will allow producers to feed their pigs appropriately to avoid monetary discounts associated with IV that are higher than acceptable at harvest. While a number of different factors were evaluated, dietary EFA, NE content, and backfat thickness exhibited the greatest influence on predicting IV of 3 distinct fat depots. Regression equations from this paper can be used to predict back, belly, and jowl fat IV.

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Table 4.1 Summary of papers used in the regression analysis to predict back, belly, and jowl fat iodine value (IV).

										Fat IV, g/100g		
Author	n[1]	EFA,%	NE, kcal/kg	INT d[4]	FIN d[5]	INT BW,[6] kg	ADFI, kg	HCW, kg	Backfat depth,mm	Back	Belly	Jowl
Apple et al., 2008	4	2.1-4.9	2,667-2,699	35	0	77.9	2.03-2.06	80.9-84.0	17.2-18.7	69.0-80.6	–	–
Apple et al., 2009a	4	1.6-4.5	2,480-2,702	23	0	28.1	1.56-1.68	28.1	10.5	70.5-85.2	–	–
	4	1.6-4.5	2,499-2,720	51	0	28.1	1.87-1.91	46.5-48.2	14.7-18	67.9-83.9	–	–
	4	1.7-4.6	2,514-2,738	78	0	28.1	2.15-2.18	64.4	21.7	65.2-85.72	–	–
	4	1.7-4.6	2,526-2,663	102	0	28.1	2.31-2.41	80.6	27.9	65.3-82.6	–	–
Asmus et al., 2011	6	1.7-3.4	2,353-2,512	43-90	0-47	40.9-41.1	2.57-2.7	87.0-89.0	16.8-18.9	–	–	68.4-78.5
Asmus et al., 2012a	7	1.7-3.2	2,366-2,510	73-92	0-19	45.9-46.0	2.46-2.56	88.9-91.2	16.5-18.9	–	–	69.4-81.2
Asmus et al., 2012b	3	1.6-3.1	2,417-2,474	74-107	0-33	24.2-24.3	2.28-2.33	93.1-94.7	20.0-21.5	60.3-74.2	62.2-71.0	64.8-74.2
	3	1.6-3.1	2,417-2,474	74-107	0-33	24.0-24.1	2.16-2.20	92.9-95.0	17.3-20.4	63.5-72.0	64.6-72.1	68.7-74.9
	3	1.6-3.1	2,417-2,484	74-125	0-51	24.2-24.3	2.35-2.39	95.5-97.0	22.8-26.0	58.8-70.7	61.0-72.4	66.9-76.6
	3	1.6-3.1	2,417-2,484	74-125	0-51	24.0-24.1	2.27-2.33	96.3-98.4	23.2-24.3	58.8-69.9	61.7-71.0	68.5-76.9
Asmus et al., 2014	6	1.7-3.6	2,460-2,527	85	0	38-38.1	2.87-3.00	91.1-95.9	23.1-25.1	–	–	68.2-77.0
Averette Gatlin et al., 2002	12	1.6-4.7	2,725-2,757	21	28-56	62.5	2.58-2.96	77.0-96.0	15.8-22.4	72.6-86.1	–	–
	7	1.7-3.3	2,508-2,721	42	0	80.0	3.14-3.64	92.0-95.0	23.2-26.9	70.5-73.3	–	–
	7	1.7-3.3	2,508-2,721	42	0	80.0	2.68-3.18	90.0-92.0	18.3-21.2	70.2-76.0	–	–
Averette Gatlin et al., 2003	4	1.6-3.1	2,753	52	0	73.3-73.7	2.85-3.06	91.6-92.8	22.3-25.5	66.8-73.3	66.6-73.6	–
	4	1.6-3.1	2,753	52	0	79.4-81	3.12-3.30	97.0-99.8	26.0-27.0	66.2-75.0	66.6-72.5	–
Benz et al., 2010	5	2.1-2.9	2,729-2,772	78	0	49.8	2.29-2.39	87.4-89.2	17.9-18.8	67.2-71.1	67.7-72.4	68.6-72.2
Benz et al., 2011a	9	1.6-4.6	2,494-2,722	26-82	0-56	44.0	2.95-3.31	90.6-96.1	17.3-20.1	62.7-83.4	–	66.4-78.8
Benz et al., 2011b	6	1.6-2.0	2,501-2,787	83	0	54.4	2.55-2.73	93.9-100.4	16.5-20.6	63.2-66.6	–	65.7-71.6
Benz et al., 2011c	6	1.6-2.6	2,493-2,653	82	0	47.9	2.52-2.89	88.6-95.3	19.7-21.4	59.4-70.1	–	64.1-71.6
Bergstrom et al., 2009	2	2.5-4.2	2,391-2,478	78	0	35.8	2.50-2.53		19.8-21.3	–	–	68.7-80.2
	2	2.5-4.2	2,391-2,478	78	0	35.5-35.6	2.20-2.21		17.8-18.3	–	–	71.0-81.2
	2	2.5-4.2	2,391-2,478	78	0	34.1-34.3	2.36-2.37	76.3-79.2	15.7-17.5	–	–	70.8-81.3
	2	2.5-4.2	2,391-2,478	78	0	34.7-35.1	2.03-2.06	73.5-74.1	14.5-14.7	–	–	74.1-86.2
	2	2.5-4.2	2,391-2,472	78-99	0-21	35.8	2.69-2.70	96.3-97.5	19.8-21.3	–	–	70.3-79.3
	2	2.5-4.2	2,391-2,472	78-99	0-21	35.5-35.6	2.36-2.39	91.8-93.0	17.8-18.3	–	–	72.0-81.0

	2	2.5-4.2	2,391-2,472	78-99	0-21	34.1-34.3	2.50	94.5-96.1	15.7-17.5	—	—	73.8-81.4
	2	2.5-4.2	2,391-2,472	78-99	0-21	34.7-35.1	2.21-2.22	90.4-90.1	14.5-14.7	—	—	75.0-82.9
Browne et al., 2013	6	1.9-3.0	2,519-2,605	37-103	0-66	26.5	2.41-2.55	90.6-92.7	20.2-22.1	69.9-74.7	68.5-73.3	72.6-76.3
Carr et al., 2005	6	0.8-1.4	2,291-2,523	63	0	46.7-47.0	2.59-2.83	78.3-84.6	15.8-20.7	62.3-67.7	—	—
Coble et al., 2013	6	1.7-2.6	2,364-2,549	68-88	0-20	38.3-38.6	2.79-2.92	89.1-92.4	16.3-20.1	—	—	66.8-74.5
	6	1.7-2.6	2,364-2,549	68-88	0-20	38.3-38.6	2.79-2.95	89.1-92.4	16.3-20.1	61.8-72.1	60.2-68.6	65.0-72.4
Cromwell et al., 2011	4	1.6-3.6	2,448-2,495	92-94	0	32.4-32.7	2.68-2.76	89.0-91.9	21.4-22.7	61.6-82.9	—	—
Duttlinger et al., 2008	4	1.5-1.7	2,483-2,530	28	0	94.3	2.88-3.04	85.9-90.6	17.0-18.0	68.7-70.0	69.5-70.6	69.6-71.5
Duttlinger et al., 2012	6	1.8-2.9	2,616-2,638	97	0	30.8-31.3	2.39-2.51	91.4-93.1	19.0-19.9	62.8-72.5	64.6-73.3	67.7-74.0
Feoli et al., 2007a	4	1.7-2.6	2,494-2,688	65	0	71.7	2.87-3.32	89.1-95.6	16.3-19.3	—	—	67.9-74.2
Feoli et al., 2007b	4	1.6-3.2	2,443-2,480	72	0	64.0	2.92-3.24	94.4-98.8	15.8-16.3	—	—	69.3-80.2
Feoli et al., 2008a	5	1.7-3.3	2,474-2,512	65	0	64.0	2.72-3.05	83.5-90.8	10.4-16.5	—	—	70.3-80.4
Feoli et al., 2008b	4	1.7-3.1	2,493-2,709	69	0	63.6	2.88-3.35	88.8-97.5	18.2-19.3	—	—	67.9-73.2
	4	1.7-2.6	2,492-2,708	67	0	68.1	2.61-2.87	85.4-89.7	16.3-17.5	—	—	66.6-71.7
Goehring et al., 2012a	4	1.1-1.6	2,409-2,536	61	0	72.4-72.5	2.65-2.71	89.7-91.8	19.9-21.2	—	—	67.1-68.9
Goehring et al., 2012b	4	1.7-3.5	2,490-2,522	73	0	58.9-59.6	2.77-2.92	90.0-96.8	20.6-21.0	—	—	69.8-78.0
Graham et al., 2012a	4	1.7-2.9	2,417-2,510	67	0	68.9	2.66-2.74	88.5-93.4	18.7-19.8	—	—	70.2-76.3
Graham et al., 2012b	5	1.6-3.1	2,262-2,498	82	0	46.1-46.2	2.58-2.75	92.9-95.4	15.3-15.7	66.5-78.8	62.1-76.2	67.4-78.7
	5	1.6-3.7	2,410-2,491	75	0	46.0-46.4	22.72-2.86	84.7-89.2	18.3-19.1	—	—	66.8-80.0
Graham et al., 2013	6	1.6-2.9	2,353-2,526	49-73	0-24	55.7-56.0	2.82-2.91	88.5-97.8	17.7-25.5	64.6-78.4	59.1-74.1	65.0-74.1
	6	1.6-2.7	2,353-2,526	49-73	0-24	55.7-56.0	2.82-2.92	88.5-97.8	17.7-25.5	—	62.3-70.7	64.5-71.4
Jacela et al., 2009a	6	1.9-2.0	2,469-2,628	48-89	0-41	38.6-39.0	2.40-2.43	89.8-91.2	17.3-18.8	66.9-74.9	67.8-75.9	68.6-74.7
Jacela et al., 2009b	3	2.4-4.1	2,478-2,480	78	12	46.0-46.1	2.29-2.38	90.0-91.4	17.0-17.3	—	77.2-87.3	—
Jacela et al., 2011	5	1.6-2.3	2,496-2,599	99	0	29.6	2.04-2.17	86.3-91.1	16.4-17.0	68.4-73.5	67.5-73.7	67.5-73.3
Jackson et al., 2009	6	1.6-2.0	2,495-2,659	93.5	0	29.4	2.40-2.58	79.5-84.6	14.0-18.1	65.2-72.2	62.7-68.1	—

Lee et al., 2013	6	1.4-3.6	2,464-2,589	88	0	43.5-44.1	2.54-2.85	95.1-99.4	17.3-19.6	73.5-79.6	75.6-80.4	–
Nitikanchana et al., 2013	5	1.6-2.7	2,294-2,638	74	0	56.8	2.51-2.67	87.5-94.2	15.2-19.4	–	–	69.6-74.3
Paulk et al., 2012	6	1.9-2.9	2,436-2,541	64	20-38	35.4-36.4	2.35-2.46	88.6-95.7	15.2-17.8	–	75.2-81.1	–
Pompeu et al., 2013	4	1.8-2.4	2,524-2,630	27	0	100.3-100.6	2.59-2.81	90.5-96.8	19.1-21.0	–	66.3-72.5	68.2-72.3
Salyer et al., 2012	4	1.7-3.2	2,400-2,536	84	0	46.6	3.09-3.22	93.6-100.1	21.9-24.8	–	–	70.6-77.4
	6	2.4-2.9	2,414-2,625	87	0	42.3-42.4	2.97-3.07	93.4-100.5	20.0-22.7	–	–	71.6-75.1
Sotak et al., 2011	6	1.0-2.2	2,425-2,574	72	0	58.7-58.9	3.04-3.18	91.6-96.9	22.6-26.2	58.8-69.2	–	–
Widmer et al, 2008	7	1.9-2.5	2,479-2,592	114	0	21.9-22.7	2.36-2.78	82.5-93.8	21.1-26.0	–	64.7-75.3	–
Wiegand et al., 2011	5	1.7-2.0	2,521-2,588	21	0	99.8-99.8	3.00-3.31	89.0-93.8	21.6-25.1	–	60.6-61.4	61.4-70.0
Xu et al., 2010a	9	1.7-3.0	2,450-2,551	42-105	0-63	29.7-30.2	2.62-2.74	92.4-95.6	25.0-28.2	–	58.9-71.3	–
Xu et al., 2010b	6	1.7-3.0	2,487-2,516	104	0	21.9-22.3	2.41-2.63	98.8-100.5	27.4-29.5	58.3-72.4	61.5-72.3	–
Ying et al., 2013	6	1.7-2.5	2,448-2,521	82-109	0-27	36.0	2.40-2.55	92.4-95.4	16.5-17.5	–	–	65.8-73.2

[1]Refers to the number of observations from each experiment.
[2]B = Barrows, G = Gilts, M = Mixed (barrows and gilts), IC = Immunocastrates.
[3]Iodine value product (IVP = [iodine value of the dietary lipids] × [percentage dietary lipid] × 0.10); and IV = iodine value (IV = [C16:1] × 0.95 + [C18:1] × 0.86 + [C18:2] × 1.732 + [C18:3] × 2.616 + [C20:1] × 0.785 + [C20:4] × 3.2008 + [C20:5] × 4.0265 + [C22:1] × 0.7225 + [C22:5] × 3.6974 + [C22:6] × 4.4632; NRC, 2012).
[4]Refers to the number of days pigs were fed initial dietary treatments or total days if dietary treatments were not changed during the experiment.
[5] Refers to the number of days pigs were fed final dietary treatments. FIN d = 0 if dietary treatments were not changed during the experiment.
[6]Refers to BW of pigs at the beginning of the experiment.
[7]Refers to BW of pigs at the end of the experiment.
[8]NPD genetic source (Ham-line × Manor hybrid; Roanoke Rapids, NC).
[9]PIC genetic source (Hendersonville, TN).
[10]*ad libitum* fed using a wet-dry feeder.
[11]*ad libitum* fed using a dry feeder.

Table 4.2 Descriptive statistics for data included in the evaluation.

	Initial period[1]				Final period[2]										
Item	IVP,[3] g/100g	EFA,%	NE, kcal/kg	d	IVP,[3] g/100g	EFA,%	NE, kcal/kg	d	INT BW,[4] kg	FIN BW,[5] kg	ADG, kg	ADFI, kg	HCW, kg	Backfat depth, mm	Fat IV, g/100g
Backfat IV[6]															
Mean	60.9	2.48	2,579	69	55.3	2.23	2582	8	48.2	118.7	0.94	2.63	88.0	20.1	70.5
SD	21.0	0.99	127	27	18.7	0.82	115	17	20.2	16.8	0.08	0.38	13.3	3.8	6.0
Minimum	21.3	0.80	2,262	21	21.3	0.80	2,262	0	21.9	45.5	0.73	1.56	28.1	10.5	58.3
Maximum	107.2	4.88	2,787	125	107.2	4.90	2,787	66	94.3	138.6	1.10	3.64	100.5	29.5	86.1
Belly fat IV[7]															
Mean	57.3	2.33	2,525	76	51.9	2.10	2,548	9	46.1	123.9	0.95	2.61	92.1	20.5	69.3
SD	13.7	0.56	111	27	13.5	0.49	97	17	24.0	6.2	0.07	0.28	4.2	3.8	5.4
Minimum	33.8	1.51	2,262	21	33.8	1.50	2,262	0	21.9	106.0	0.83	2.04	79.5	14.0	58.9
Maximum	96.2	4.09	2,772	125	88.1	3.60	2,772	66	100.6	138.6	1.23	3.31	100.5	29.2	87.3
Jowl fat IV[8]															
Mean	59.1	2.49	2,501	75	54.0	2.25	2,519	7	49.7	124.6	0.94	2.70	91.4	18.9	72.1
SD	16.8	0.75	108	21	16.0	0.65	92	14	18.7	6.6	0.08	0.30	4.5	2.6	4.3
Minimum	22.1	1.08	2,262	21	22.1	1.10	2,262	0	24.0	97.4	0.77	2.03	73.5	10.4	61.4
Maximum	101.1	4.63	2,787	125	101.1	4.60	2,787	66	100.6	138.6	1.23	3.35	100.5	26.0	86.2

[1]Characteristics of initial diets fed during the experiment.
[2]Characteristics of final diets fed during the experiment.
[3]Iodine value product (IVP = [iodine value of the dietary lipids] × [percentage dietary lipid] × 0.10); and IV = iodine value (IV = [C16:1] × 0.95 + [C18:1] × 0.86 + [C18:2] × 1.732 + [C18:3] × 2.616 + [C20:1] × 0.785 + [C20:4] × 3.2008 + [C20:5] × 4.0265 + [C22:1] × 0.7225 + [C22:5] × 3.6974 + [C22:6] × 4.4632; NRC, 2012).
[4]Refers to BW of pigs at the beginning of the experiment.
[5]Refers to BW of pigs at the end of the experiment.
[6]The final database resulted in 24 papers with 169 observations for backfat IV.
[7]The final database resulted in 21 papers with 124 observations for belly fat IV.
[8]The final database resulted in 29 papers with 197 observations for jowl fat IV.

Table 4.3 Pearson's correlation coefficients between dependent dietary variables used to predict back, belly, and jowl fat iodine value (IV)[1]

	Initial period[2]								Final Period[3]							
	Fatty acids, %						Energy, kcal/kg		Fatty acids, %						Energy, kcal/kg	
Item	C16:1	C18:1	C18:2	C18:3	EFA	USFA[5]	ME	NE	C16:1	C18:1	C18:2	C18:3	EFA	USFA[5]	ME	NE
IVP, [4] g/100g	0.13	0.57	0.93	0.82	0.94	0.97	0.68	0.58	0.33	0.68	0.91	0.71	0.92	0.97	0.65	0.55
	0.48	0.73	0.83	0.47	0.83	0.97	0.47	0.17	0.59	0.81	0.82	0.28	0.83	0.97	0.54	0.34
	0.30	0.71	0.90	0.59	0.91	0.98	0.40	0.12	0.43	0.79	0.89	0.43	0.90	0.98	0.46	0.14
C16:1, %	1.00	0.71	-0.17	-0.12	-0.17	0.33	0.43	0.36	1.00	0.71	0.01	-0.08	0.01	0.49	0.43	0.38
	1.00	0.83	-0.02	0.33	-0.01	0.64	0.70	0.57	1.00	0.79	0.12	0.09	0.14	0.69	0.57	0.49
	1.00	0.86	-0.12	0.11	-0.11	0.50	0.73	0.70	1.00	0.84	0.01	0.13	0.02	0.58	0.65	0.67
C18:1, %		1.00	0.25	0.17	0.24	0.76	0.79	0.70		1.00	0.34	0.16	0.34	0.84	0.71	0.65
		1.00	0.24	0.26	0.24	0.88	0.84	0.66		1.00	0.34	0.14	0.36	0.92	0.78	0.67
		1.00	0.35	0.31	0.35	0.85	0.75	0.59		1.00	0.44	0.28	0.46	0.90	0.72	0.56
C18:2, %			1.00	0.84	1.00	0.82	0.45	0.36			1.00	0.78	1.00	0.80	0.42	0.32
			1.00	0.38	1.00	0.67	-0.01	-0.29			1.00	0.24	1.00	0.68	0.11	-0.11
			1.00	0.53	1.00	0.79	0.06	-0.23			1.00	0.40	1.00	0.79	0.14	-0.22
C18:3, %				1.00	0.88	0.69	0.53	0.51				1.00	0.81	0.57	0.46	0.44
				1.00	0.41	0.42	0.29	0.10				1.00	0.28	0.22	0.23	0.10
				1.00	0.58	0.53	0.41	0.32				1.00	0.42	0.39	0.27	0.18
EFA, %					1.00	0.82	0.46	0.38					1.00	0.80	0.44	0.35
					1.00	0.67	-0.01	-0.28					1.00	0.69	0.14	-0.09
					1.00	0.80	0.09	-0.20					1.00	0.80	0.16	-0.19
USFA, [5] %						1.00	0.78	0.67						1.00	0.71	0.61
						1.00	0.63	0.36						1.00	0.65	0.47
						1.00	0.53	0.26						1.00	0.56	0.28
ME, kcal/kg							1.00	0.94							1.00	0.94
							1.00	0.91							1.00	0.93
							1.00	0.89							1.00	0.86

[1]The 1st, 2nd, and 3rd row within each variable represents Pearson's correlation coefficients for back, belly, and jowl fat IV datasets, respectively.
[2]Correlations between characteristics of initial diets fed during the experiment.
[3]Correlations between characteristics of final diets fed during the experiment.
[4]Iodine value product (IVP = [iodine value of the dietary lipids] × [percentage dietary lipid] × 0.10); and IV = iodine value (IV = [C16:1] × 0.95 + [C18:1] × 0.86 + [C18:2] × 1.732 + [C18:3] × 2.616 + [C20:1] × 0.785 + [C20:4] × 3.2008 + [C20:5] × 4.0265 + [C22:1] × 0.7225 + [C22:5] × 3.6974 + [C22:6] × 4.4632; NRC, 2012).
[5]Refers to total dietary unsaturated fatty acids.

Table 4.4 Pearson's correlation coefficients between dependent growth performance and carcass characteristic variables used to predict back, belly, and jowl fat iodine value (IV)[1]

	FIN BW, [7] kg	ADG, kg	ADFI, kg	GF	HCW, kg	Backfat depth, mm
INT BW, [2] kg	0.25	0.27	0.57	-0.62	0.21	0.05
	0.14	0.49	0.62	-0.43	0.01	-0.05
	0.03	0.03	0.47	-0.57	-0.03	-0.02
FIN BW, [3] kg	1.00	0.70	0.63	-0.44	0.96	0.41
	1.00	0.53	0.32	0.08	0.64	0.24
	1.00	0.47	0.45	-0.13	0.89	0.36
ADG, kg		1.00	0.72	-0.24	0.64	0.25
		1.00	0.66	0.02	0.45	0.15
		1.00	0.54	0.29	0.50	0.39
ADFI, kg			1.00	-0.79	0.59	0.35
			1.00	-0.70	0.19	0.31
			1.00	-0.59	0.30	0.19
G:F				1.00	-0.46	-0.38
				1.00	0.20	-0.26
				1.00	0.04	0.04
HCW, kg					1.00	0.47
					1.00	0.47
					1.00	0.40

[1]The 1st, 2nd, and 3rd row within each variable represents Pearson's correlation coefficients for back, belly, and jowl fat IV datasets, respectively.
[2]Refers to BW of pigs at the beginning of the experiment.
[3]Refers to BW of pigs at the end of the experiment.

Table 4.5 Dietary characteristic single variable models used to predict back, belly, and jowl fat iodine value (IV)

Item	IVP, [1] g/100g	C16:1, %	C18:1, %	C18:2, %	C18:3, %	EFA, %	USFA, [2] %	ME, kcal/kg	NE, kcal/kg
Initial period[3]									
Backfat IV									
Probability, *P* <	0.001	0.07	0.01	0.001	0.001	0.001	0.001	0.001	0.16
BIC[4]	897.9	1,040.9	1,034.6	870.6	959.6	871.7	942.1	1,032.7	1,042.3
Belly fat IV									
Probability, *P* <	0.001	0.29	0.001	0.001	0.001	0.001	0.001	0.34	0.01
BIC[4]	632.5	716.1	695.5	624.5	695.9	622.6	648.4	716.3	705.2
Jowl fat IV									
Probability, *P* <	0.001	0.92	0.001	0.001	0.001	0.001	0.001	0.83	0.001
BIC[4]	896.8	1,104.5	1,065.4	853.7	1,066.7	858.9	940.7	1,104.4	1,078.8
Final period[5]									
Backfat IV									
Probability, *P* <	0.001	0.17	0.001	0.001	0.001	0.001	0.001	0.001	0.12
BIC[4]	918.2	1,042.3	1031	886.6	986.7	888.1	951.1	1,031.3	1,041.8
Belly fat IV									
Probability, *P* <	0.001	0.67	0.001	0.001	0.46	0.001	0.001	0.42	0.001
BIC[4]	644.2	717	702	629.1	716.7	627.3	659.4	716.6	707
Jowl fat IV									
Probability, *P* <	0.001	0.77	0.001	0.001	0.2	0.001	0.001	0.56	0.01
BIC[4]	992	1,104.4	1,075.1	961.4	1,102.8	962.1	1,013.1	1,104.2	1,090.5

[1]IVP = iodine value product (IVP = [iodine value of the dietary lipids] × [percentage dietary lipid] × 0.10); and IV = iodine value (IV = [C16:1] × 0.95 + [C18:1] × 0.86 + [C18:2] × 1.732 + [C18:3] × 2.616 + [C20:1] × 0.785 + [C20:4] × 3.2008 + [C20:5] × 4.0265 + [C22:1] × 0.7225 + [C22:5] × 3.6974 + [C22:6] × 4.4632; NRC, 2012).

[2]Dietary unsaturated fatty acids.

[3]Characteristics of initial diets fed during the experiment.

[4] Bayesian Information Criterion (BIC) values were used to compare the precision of the model. Models that minimized Bayesian Information Criterion (BIC) Variables within fat depot were used to select variables for initial model building.[5]Characteristics of final diets fed during the experiment.

Table 4.6 Pig growth and carcass characteristic single variable models used to predict back, belly, and jowl fat iodine value (IV)

Item	INT BW,[1] kg	FIN BW,[2] kg	ADG, kg	ADFI, kg	G:F	HCW, kg	Backfat depth, mm
Backfat IV							
Probability, *P* <	0.19	0.02	0.05	0.03	0.07	0.02	0.01
BIC[3]	1,042.5	1,038.3	1,040.4	1,039.5	1,041.1	1,038.3	1,036.5
Belly fat IV							
Probability, *P* <	0.97	0.04	0.01	0.01	0.77	0.001	0.001
BIC[3]	717.2	713.2	710.1	709.8	717.1	704.8	705.5
Jowl fat IV							
Probability, *P* <	0.06	0.15	0.01	0.05	0.76	0.01	0.001
BIC[3]	1,101.1	1,102.4	1,097.8	1,100.5	1,104.4	1,094.7	1,082.0

[1]Refers to BW of pigs at the beginning of the experiment.
[2]Refers to BW of pigs at the end of the experiment.
[3]Bayesian Information Criterion (BIC) values were used to compare the precision of the model. Models that minimized Bayesian Information Criterion (BIC) Variables within fat depot were used to select variables for initial model building.

Table 4.7 Regression equations generated from meta-analyses of existing data for prediction of back, belly, and jowl fat iodine value (IV)[1]

Dependent Variable	Models	BIC[2]
Backfat IV	= 60.30 + (3.70*INT EFA) + (2.37*FIN EFA) - (0.051*INT d) - (0.086*FIN d)	817.0
	= 69.40 + (0.55*INT EFA) + (2.06*FIN EFA) - (0.18*INT d) - (0.088*FIN d) + (0.053*INT EFA*INT d)	782.4
	= 70.66 + (1.22*INT EFA) + (0.86*FIN EFA) - (0.20*INT d) - (0.20*FIN d) + (0.058*INT EFA*INT d) + (0.047*FIN EFA*FIN d)	775.8
	= 69.00 + (6.66*INT EFA) - (4.31*FIN EFA) - (0.18*INT d) - (0.13*FIN d) - (0.095*INT EFA*FIN d) + (0.055*FIN EFA*INT d) + (0.13*FIN EFA*FIN d)	755.2
	= 86.93 + (6.67*INT EFA) - (3.91*FIN EFA) - (0.17*INT d) - (0.14*FIN d) - (0.90*INT EFA*FIN d) + (0.051*FIN EFA*INT d) + (0.13*FIN EFA*FIN d) - (0.0073*INT NE)	746.9
	=87.76 + (7.03*INT EFA) - (3.96*FIN EFA) - (0.17*INT d) - (1.34*FIN d) - (0.11*INT EFA*FIN d) + (0.047*FIN EFA*INT d) + (0.12*FIN EFA*FIN d) - (0.0079*FIN NE) + (0.0005*FIN NE*FIN d)	744.9
	=84.83 + (6.87*INT EFA) - (3.90*FIN EFA) - (0.12*INT d) - (1.30*FIN d) - (0.11*INT EFA*FIN d) + (0.048*FIN EFA*INT d) + (0.12*FIN EFA*FIN d) - (0.0060*FIN NE) + (0.0005*FIN NE*FIN d) - (0.26*BF)	734.5
Belly fat IV	= 54.59 + (6.73*INT EFA) + (0.31*FIN d) - (0.14*INT EFA*FIN d)	586.0
	= 82.77 + (6.37*INT EFA) + (0.28*FIN d) - (0.13*INT EFA*FIN d) - (0.01*INT NE)	580.1
	= 93.05 + (6.45*INT EFA) - (1.43*FIN d) - (0.12*INT EFA*FIN d) - (0.015*INT NE) + (0.00067*INT NE*FIN d)	566.9
	= 111.08 + (6.20*INT EFA) - (1.42*FIN d) - (0.11*INT EFA*FIN d) - (0.014*INT NE) + (0.00066*INT NE*FIN d) - (0.21*HCW)	561.3
	=90.53 + (6.41*INT EFA) - (1.53*FIN d) - (0.12*INT EFA*FIN d) - (0.012*INT NE) + (0.00071*INT NE*FIN d) - (0.30*BF)	560.7
	= 106.16 + (6.21*INT EFA) - (1.50*FIN d) - (0.11*INT EFA*FIN d) - (0.012*INT NE) + (0.00069*INT NE*FIN d) - (0.18*HCW) - (0.25*BF)	557.9
Jowl fat IV	= 58.11 + (3.86*INT EFA) + (1.54*FIN EFA) + (0.013*INT d)	831.1
	= 65.14 + (0.87*INT EFA) + (0.85*FIN EFA) - (0.073*INT d) - (0.078*FIN d) + (0.045*INT EFA*INT d) + (0.051*FIN EFA*FIN d)	814.6
	= 85.28 + (1.18*INT EFA) + (0.95*FIN EFA) - (0.058*INT d) - (0.087*FIN d) + (0.038*INT EFA*INT d) + (0.051*FIN EFA*FIN d) - (0.0083*INT NE)	792.6
	= 86.17 + (0.64*INT EFA) + (0.91*FIN EFA) - (0.065*INT d) - (0.080*FIN d) + (0.043*INT EFA*INT d) + (0.053*FIN EFA*FIN d) - (0.0057*INT NE) - (0.35*BF)	767.7

= 77.88 + (1.04*INT EFA) + (1.01*FIN EFA) - (0.0063*INT d) - (0.041*FIN d) + (0.038*INT EFA*INT d) + (0.053*FIN EFA*FIN d) - (0.0056*INT NE) + (0.066*INT BW) - (0.36*BF)	759.3
= 85.50 + (1.08*INT EFA) + (0.87*FIN EFA) - (0.014*INT d) - (0.050*FIN d) + (0.038*INT EFA*INT d) + (0.054*FIN EFA*FIN d) - (0.0066*INT NE) + (0.071*INT BW) - (2.19*ADFI) - (0.29*BF)	756.2

[1]INT EFA = initial period dietary essential fatty acids, %; FIN EFA = final period dietary essential fatty acids, %; INT d = initial period days; FIN d=final period days; INT NE= initial period dietary net energy, kcal/kg; FIN NE= final period dietary net energy, kcal/kg; BF= backfat depth, mm; INT BW = BW at the beginning of the experiment, kg.

[2]Bayesian Information Criterion (BIC) values were used to compare the precision of the model. Models that minimized BIC were preferred candidate models, with a reduction of more than 2 considered improved (Kass and Raftery, 1995).

Table 4.8 Inputs from validation experiment used in the regression equations to predict back, belly, and jowl fat iodine value (IV)[1]

Treatment[2]:	A	B	C	D	E	F	G	H	I	J
d 0 to 42:	Control	Tallow	Tallow	Control	Blend	Blend	Control	Soy	Soy	Control
d 42 to 84:	Control	Tallow	Control	Tallow	Blend	Control	Blend	Soy	Control	Soy
Initial diet EFA, %	1.50	1.91	1.87	1.47	2.53	2.65	1.47	3.44	3.44	1.47
Initial diet NE, kcal/kg	2,501	2,654	2,654	2,501	2,667	2,667	2,501	2,680	2,680	2,501
Initial diet days	84	84	42	42	84	42	42	84	42	42
Final diet EFA, %	1.50	1.91	1.52	1.94	2.53	1.52	2.41	3.44	1.52	3.45
Final diet NE, kcal/kg	2,536	2,692	2,536	2,692	2,705	2,536	2,705	2,717	2,536	2,717
Final diet days	0	0	42	42	0	42	42	0	42	42
Backfat, mm	17.02	19.30	19.56	18.54	22.35	20.83	19.56	21.84	18.03	19.30
HCW, kg	97.25	99.16	98.52	96.53	96.62	96.57	98.02	98.16	97.75	96.66
ADFI, kg	2.76	2.71	2.79	2.79	2.78	2.70	2.65	2.76	2.71	2.62
Initial BW, kg	45.63	45.68	45.59	45.59	45.86	45.36	45.77	45.59	45.50	45.45

[1]Inputs were obtained from the experiment conducted for validation of regression equations (Stephenson et al., 2014).
[2] Control= no added fat; Tallow= 4% beef tallow; Soy= 4% soybean oil; Blend= 2% tallow and 2% soybean oil

Table 4.9 Validation of regression equations used to predict back, belly, and jowl fat iodine value (IV)

Treatment[1]:	A	B	C	D	E	F	G	H	I	J	
d 0 to 42:	Control	Tallow	Tallow	Control	Blend	Blend	Control	Soy	Soy	Control	
d 42 to 84:	Control	Tallow	Control	Tallow	Blend	Control	Blend	Soy	Control	Soy	SEM
Backfat IV											
Actual[2]	63.29	64.03	63.83	62.72	71.17	66.92	67.83	79.43	67.87	73.86	1.16
Predicted[5]	65.61	66.92	67.16	70.19	70.42	68.59	71.60	76.93	71.09	75.13	
Belly fat IV											
Actual	66.23	67.25	67.50	66.15	72.42	69.91	70.39	79.45	72.44	74.96	0.94
Predicted [6]	63.70	63.48	68.57	65.95	66.89	70.07	65.43	72.29	72.03	65.74	
Jowl fat IV											
Actual	64.68	65.10	65.43	64.66	69.96	67.56	67.84	75.94	71.07	70.90	0.96
Predicted [7]	67.79	68.32	66.54	68.09	70.42	68.36	69.59	75.23	71.18	72.96	

[1] Control= no added fat; Tallow= 4% beef tallow; Soy= 4% soybean oil; Blend= 2% tallow and 2% soybean oil

[2]Means were obtained from the experiment conducted for validation of regression equations (Stephenson et al., 2015).

[3]Lower 95% confidence interval obtained from the experiment conducted for validation of regression equations (Stephenson et al., 2015).

[4]Upper 95% confidence interval obtained from the experiment conducted for validation of regression equations (Stephenson et al., 2015).

[5]Backfat IV=84.83 + (6.87*INT EFA) - (3.90*FIN EFA) - (0.12*INT d) - (1.30*FIN d) - (0.11*INT EFA*FIN d) + (0.048*FIN EFA*INT d) + (0.12*FIN EFA*FIN d) - (0.0060*FIN NE) + (0.0005*FIN NE*FIN d) - (0.26*BF) where INT EFA = initial period dietary essential fatty acids, %; FIN EFA = final period dietary essential fatty acids, %; INT d = initial period days; FIN d=final period days; FIN NE= final period dietary net energy, kcal/kg; BF= backfat depth, mm.

[6]Belly fat IV= 106.16 + (6.21*INT EFA) - (1.50*FIN d) - (0.11*INT EFA*FIN d) - (0.012*INT NE) + (0.00069*INT NE*FIN d) - (0.18*HCW) - (0.25*BF) where INT NE = initial period dietary NE, kcal/kg.

[7]Jowl fat IV= 85.50 + (1.08*INT EFA) + (0.87*FIN EFA) - (0.014*INT d) - (0.050*FIN d) + (0.038*INT EFA*INT d) + (0.054*FIN EFA*FIN d) - (0.0066*INT NE) + (0.071*INT BW) - (2.19*ADFI) - (0.29*BF) where INT BW = BW at the beginning of the experiment, kg.

Figure 4.1 Plot of residuals against predicted A) back, B) belly, and C) jowl fat iodine value (IV) from each mixed model analysis.

The following equations were used: a) backfat IV =81.84 + (7.74*INT EFA) - (4.33*FIN EFA) - (0.12*INT d) - (1.29*FIN d) - (0.12*INT EFA*FIN d) + (0.049*FIN EFA*INT d) + (0.14*FIN EFA*FIN d) - (0.0051*FIN NE) + (0.00049*FIN NE*FIN d) - (0.25*BF); b) belly fat IV = 106.16 + (6.21*INT EFA) - (1.50*FIN d) - (0.11*INT EFA*FIN d) - (0.012*INT NE) + (0.00069*INT NE*FIN d) - (0.18*HCW) - (0.25*BF); c) jowl fat IV = 85.50 + (1.08*INT EFA) + (0.87*FIN EFA) - (0.014*INT d) - (0.050*FIN d) + (0.038*INT EFA*INT d) + (0.054*FIN EFA*FIN d) - (0.0066*INT NE) + (0.071*INT BW) - (2.19*ADFI) - (0.29*BF) where INT EFA = initial period dietary essential fatty acids, %; FIN EFA = final period dietary essential fatty acids, %; INT d = initial period days; FIN d=final period days; INT NE= initial period dietary net energy, kcal/kg; FIN NE= final period dietary net energy, kcal/kg; BF= backfat depth, mm; I-BW = BW at the beginning of the experiment, kg.

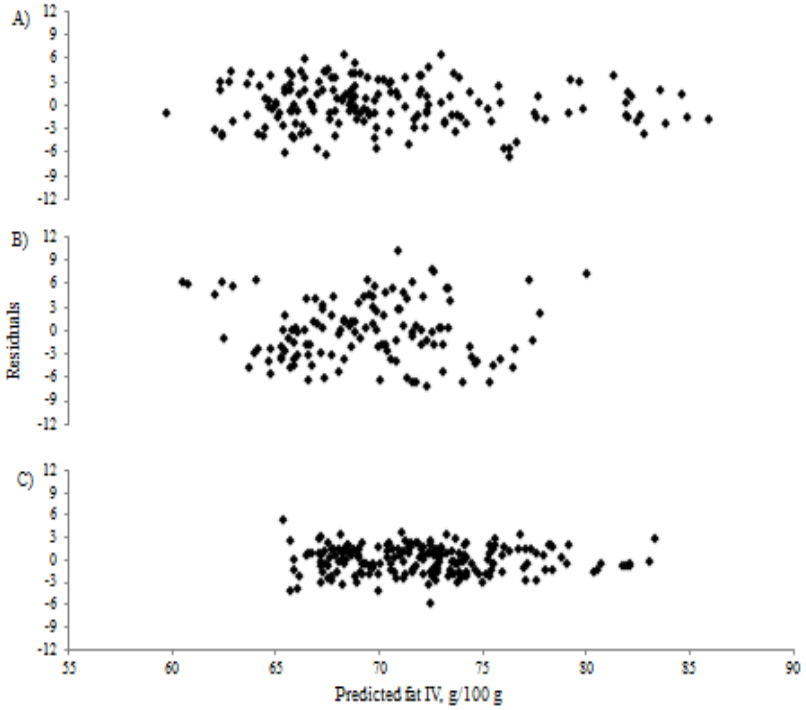
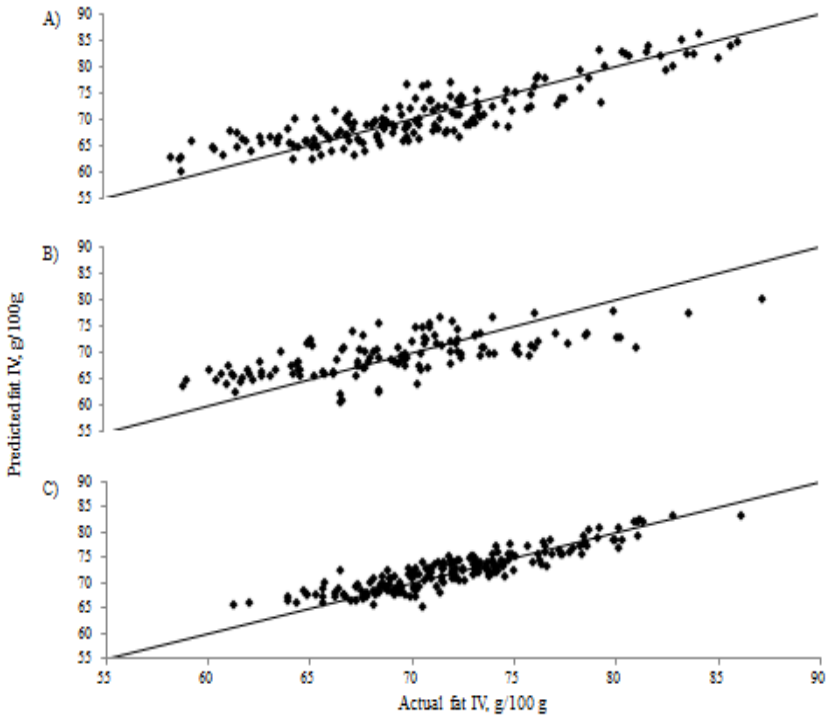


Figure 4.2 Plot of actual iodine value (IV) vs predicted IV relative to the line of equality for A) back, B) belly, and C) jowl fat IV from each mixed model analysis.

The following equations were used: A) backfat IV =84.83 + (6.87*INT EFA) - (3.90*FIN EFA) - (0.12*INT d) - (1.30*FIN d) - (0.11*INT EFA*FIN d) + (0.048*FIN EFA*INT d) + (0.12*FIN EFA*FIN d) - (0.0060*FIN NE) + (0.0005*FIN NE*FIN d) - (0.26*BF); B) belly fat IV = 106.16 + (6.21*INT EFA) - (1.50*FIN d) - (0.11*INT EFA*FIN d) - (0.012*INT NE) + (0.00069*INT NE*FIN d) - (0.18*HCW) - (0.25*BF); C) jowl fat IV = 85.50 + (1.08*INT EFA) + (0.87*FIN EFA) - (0.014*INT d) - (0.050*FIN d) + (0.038*INT EFA*INT d) + (0.054*FIN EFA*FIN d) - (0.0066*INT NE) + (0.071*INT BW) - (2.19*ADFI) - (0.29*BF) where INT EFA = initial period dietary essential fatty acids, %; FIN EFA = final period dietary essential fatty acids, %; INT d = initial period days; FIN d=final period days; INT NE= initial period dietary net energy, kcal/kg; FIN NE= final period dietary net energy, kcal/kg; BF= backfat depth, mm; INT BW = BW at the beginning of the experiment, kg.



Chapter 5 - Effect of increasing zinc content of finishing pig diets containing ractopamine-HCl on growth performance, carcass characteristics, and ileal mucosal inflammation mRNA expression

ABSTRACT

Two experiments were conducted to determine the effects of increasing the dietary Zn content on growth performance, carcass characteristics, plasma Zn, and ileal mucosal inflammation mRNA expression of finishing pigs fed diets containing ractopamine HCl (RAC; Elanco Animal Health, Greenfield, IN). In Exp. 1, 312 pigs (327 × 1050, PIC; 94 kg BW) were used in a 27-d study. There were 2 pigs per pen and 26 pens per treatment. Treatments included a corn-soybean meal diet (control: 0.66% standardized ileal digestible [**SID**] Lys), a diet (0.92% SID Lys) with 10 ppm RAC, the RAC diet plus 50, 100, or 150 ppm added Zn from ZnO, or 50 ppm added Zn from a Zn AA chelate (**ZnAA**; Availa Zn, Zinpro, Eden Prairie, MN). All diets contained 83 ppm Zn from $ZnSO_4$ in the trace mineral premix. Pigs fed RAC diet without added Zn had increased ($P < 0.05$) ADG, G:F, HCW, carcass yield, and loin weight compared with pigs fed the control diet. Increasing Zn from ZnO in diets containing RAC tended to increase (linear, $P = 0.067$) G:F and loin weight (quadratic, $P = 0.064$). Pigs fed diets with 50 ppm added ZnAA tended to have increased ($P = 0.057$) ADG compared with pigs fed the RAC diet. In Exp. 2, 320 pigs (327 × 1050: PIC; 98 kg BW) were used in a 35-d study. There were 2 pigs per pen and 20 pens per treatment. Treatments included a control diet (0.66% SID Lys), a diet (0.92% SID Lys) with 10 ppm RAC, or the RAC diet plus 75, 150, and 225 ppm added Zn from ZnO or ZnAA. All diets contained 55 ppm Zn from $ZnSO_4$ from the trace mineral premix. Pigs fed the RAC diet had increased ($P < 0.05$) ADG, G:F, HCW, loin depth, percentage lean, and liver weight compared with pigs fed the control diet. No Zn level or source effects or level × source

interactions were observed for growth performance. A Zn level × source interaction (quadratic, $P = 0.007$) was observed in liver Zn concentrations. This resulted from liver Zn concentrations plateauing at 150 ppm when ZnO was supplemented, while there was a linear increase when utilizing ZnAA. Increasing Zn in diets containing RAC increased (linear, $P < 0.05$) plasma Zn on d 18 and 32. The expression of *IL-1β* was increased ($P = 0.014$) in mucosa of pigs fed the RAC diet compared to those fed the control diet. Expression of *IL-1β* decreased (linear; $P = 0.026$) in the mucosa of pigs fed increasing added Zn. In conclusion, adding Zn to diets containing RAC resulted in a trend for improved growth performance of pigs in 1 of 2 experiments. Also, additional Zn increased plasma Zn and reduced *IL-1β*.

INTRODUCTION

Ractopamine HCl (RAC; Paylean®; Elanco Animal Health, Greenfield, IN) is a common feed additive used in late finishing pig diets to improve growth performance and carcass leanness (Apple et al., 2007). In addition, recent research demonstrates further improved ADG and G:F of pigs fed diets containing RAC with added Zn from a Zn AA chelate compared to those fed added Zn from an inorganic source (ZnO or $ZnSO_4$; Patience et al. 2011; Rambo et al. 2012). Fry et al. (2013) also observed increased G:F in pigs fed added Zn in diets containing RAC. Although previous research demonstrated improvements in growth performance of pigs fed added Zn in diets containing RAC, the mechanism for these improvements is unknown.

Klasing et al. (1992) suggested that the requirement for Zn may be greater for optimum immune response as Zn plays an important role in multiple aspects of the immune system (Shankar and Prasad, 1998). Increases in the proinflammatory cytokines, *IL-1β* or *TNF-α,* increase intestinal injury leading to a decrease in intestinal barrier function (Ma et al., 2004; Al-Sadi and Ma, 2007). These increases also stimulate macrophages to produce *IL-8*, which is

responsible for attracting neutrophils to the site of inflammation (Baggiolini and Clark-Lewis, 1992). Bao et al. (2003) determined in an *in vitro* Exp. that increasing Zn reduced gene expression of *IL-1β*, *TNF-α*, and *IL-8* in the monocyte-macrophage cell line. However, to the best of our knowledge, the response to RAC on gene expression of proinflammatory cytokines has not been studied. Thus, our objective was to determine the effects of adding various concentrations of Zn from ZnO or a Zn AA chelate (Availa-Zn; Zinpro, Eden Prairie, MN) on growth performance, carcass characteristics, plasma and tissue Zn concentrations, and ileal mucosal inflammation mRNA expression of finishing pigs fed diets containing RAC.

MATERIALS AND METHODS

General

The Kansas State University Institutional Animal Care and Use Committee approved the protocol used in these experiments. Both experiments were conducted at the K-State Swine Teaching and Research Center in Manhattan, KS. Pigs were housed in an environmentally controlled finishing building in 1.5 m^2 pens containing slatted flooring. Each pen was equipped with a single hole, dry self-feeder and a nipple waterer to provide *ad libitum* access to feed and water.

Experiment 1

A total of 312 finishing pigs (327 × 1050, PIC Hendersonville, TN), initially 94 kg BW from 2 consecutive groups were used with treatments replicated equally in both groups. Pens of pigs were allotted to 1 of 6 dietary treatments, with either 2 barrows or 2 gilts per pen and 26 pens per treatment. Dietary treatments consisted of: a corn-soybean meal–based control diet formulated to contain 0.66% standardized ileal digestible (**SID**) Lys; a RAC diet formulated to contain 0.92% SID Lys and 10 ppm RAC; the RAC diet plus 50, 100, or 150 ppm added Zn from

ZnO; or the RAC diet plus 50 ppm added Zn from a Zn AA chelate (**ZnAA**; Availa-Zn; Table 1). All diets contained 83 ppm Zn from $ZnSO_4$ provided by the trace mineral premix. Experimental diets were fed in meal form, and ZnO or ZnAA was added to the RAC diet at the expense of corn. A subsample of experimental diets was collected and analyzed for dietary Zn (Ward Laboratories, Inc., Kearney, NE). Samples were prepared using the method outlined by AOAC int. (2012) and analyzed using an iCAP 6000 series ICP Emission Spectrometer (Thermo Electron Corporation, Marietta, OH). Analyzed total Zn concentrations were 168 and 131 ppm in the control and RAC diets, respectively, 163, 188, and 267 ppm in the diets containing added ZnO, and 185 ppm in the diet containing added ZnAA. Pigs and feeders were weighed on d 0, 14, and 27 to determine ADG, ADFI, and G:F.

On d 27, all pigs were weighed, tattooed, and shipped approximately 2 h to a commercial harvesting plant (Farmland Foods Inc., Crete, NE). Immediately after harvest, HCW was collected and percent carcass yield was calculated by dividing HCW by live weight obtained at the farm before transport to the packing plant. For the second group of pigs, last-rib backfat measurements and boneless loin weights were collected and percentage lean was calculated (NPPC, 2000).

All data were analyzed as a completely randomized design using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC) with pen as the experimental unit. In addition to treatment, the effects of gender and group were included as random effects. Hot carcass weight was used as a covariate for analyses of backfat thickness, percentage lean, and boneless loin weight. Contrast statements consisted of: (1) control vs. RAC diet, (2) increasing ZnO linear and quadratic polynomials, (3) RAC diet vs. ZnAA, and (4) 50 ppm added Zn from ZnO vs ZnAA.

Statistical significance was determined at $P < 0.05$ and P-values falling within $P > 0.05$ and $P < 0.10$ were considered a trend.

Experiment 2

A total of 320 finishing pigs (327 × 1050; PIC, Hendersonville TN) initially 98 kg BW from 4 consecutive groups were used with treatments replicated equally in both groups. Pens of pigs were randomly allotted to 1 of 8 dietary treatments with 20 replicate pens per treatment. Dietary treatments included: a corn-soybean meal–based control diet formulated to 0.66% SID lysine; a RAC diet formulated to 0.92% SID lysine and 10 ppm of RAC; the RAC diet plus 75, 150, or 225 ppm added Zn from either ZnO or ZnAA (Availa-Zn; Table 1). All diets contained 55 ppm Zn from $ZnSO_4$ provided by the trace mineral premix. Experimental diets were fed in meal form, and ZnO or ZnAA was added to the RAC diet at the expense of corn. Diets were fed for the last 41 d before slaughter for group 1 and the last 35 d for group 2, 3, and 4. Analyzed total Zn concentrations were 66 and 77 ppm in the control and RAC diets, respectively, 134, 241, and 308 ppm in the diets containing added ZnO, and 154, 256, and 318 ppm in the diets containing added Availa-Zn.

One pig was randomly selected from 16 pens per treatment (balanced across sex and group for blood collection on d 0, 8, 18, and 32 of the experiment and ileal mucosal swabs at harvest. On the final day of the experiment, pigs were harvested at 1 of 2 locations. The pigs that were selected for bleeding and one randomly selected pig from the remaining pens were weighed, tattooed, and shipped to the Kansas State University Meats Laboratory for harvest. The remaining pigs were weighed, tattooed, and shipped approximately 2.5 h to a commercial harvesting plant (Triumph Foods LLC., St. Joseph, MO).

Pigs harvested at the commercial packing plant were tattooed to allow individual identification for carcass data collection. Hot carcass weight was collected immediately following evisceration, and each carcass was evaluated for percent yield, backfat and loin depth, and percent lean. Fat and loin depth were collected using an optical probe (Fat-O-Meter, SFK Technology A/S, Denmark) inserted between the third and fourth rib (counting from the ham end of the carcass) approximately 7 cm from the dorsal midline. Percent lean was calculated using equations from NPPC (2000).

Pigs harvested at the Kansas State University Meats Laboratory were tattooed to allow for individual carcass identification during data collection. Immediately following evisceration, HCW and liver weights were collected. In addition, liver samples were taken from the top left lobe for Zn analysis and mucosal swabs of the distal ileum were collected for mRNA expression analysis (Jones et al., 2013). Carcasses were chilled (-18° C) for 24 h then the left side of each carcass was ribbed between the 10th and 11th rib interface. At this time, 10^{th} rib backfat and loin depth were measured utilizing similar procedures employed at the commercial packing plant. Percent lean was calculated as previously described. A 30 cm portion of the *Longissimus lumborum* muscle (beginning at the 10th rib) from the left side of each pig was collected for immunohistochemical and fresh pork quality analysis. The results for the fresh pork quality analysis are reported in Paulk et al. (2014).

Zinc analysis

Samples were collected via jugular venipuncture into heparinized (143 USP unite of NA heparin) vacutainer tubes (Tyco Health Care Group LP, Mansfield, MA), inverted, and immediately placed on ice until samples were processed. Whole blood was centrifuged (2,000 × *g*, 15 min, 4°C) and plasma removed and frozen at -20°C. Plasma was deproteinized by diluting

1:4 in 12.5% trichloroacetic acid followed by centrifugation at 2,000 × *g* for 15 min (GS-6KR, Beckman-Coulter, Brea, CA) with the resulting supernatant collected for analysis. Zinc analysis was determined by flame atomic absorption spectrophotometry according to the methods of Shaw et al. (2002) (UNICAM 989 Solar AA Spectrometer, Thermo Elemental Corp., Franklin, MA).

Liver, loin, and feed samples were microwave digested (MARS 5, CEM Corp., Matthews, NC) in 10 mL of HNO_3 followed by addition of 2 mL of H_2O_2 (Shaw et al. 2002). Samples were brought to constant volume and diluted appropriately for Zn analysis described previously.

Ileal Mucosal Gene Expression

Approximately 100 mg of ileal mucosa was homogenized in Trizol (Life Technologies, Grand Island, NY) for the isolation of nucleic acids (Gonzalez et al., 2013). Extracted nucleic acids were purified using the Purelink RNA Mini kit (Life Technologies, Carlsbad, CA). Total RNA was collected with the addition of 90 µl of RNase-Free water on the membrane for 1 min, followed bycentrifuging the column at 12,000 × *g* for 2 min at room temperature. Total RNA concentration and quality [absorbance (A) ratio at 260 and 280 nm] was quantified using a NanoDrop 1000 spectrophotometer (Thermo Scientific, Wilmington, DE). All extractions yielded RNA with A260:280 ratios greater than 1.9 and all samples possessed A260:230 ratios greater than 1.8. Extracted RNA was stored at -80°C until PCR analysis.

Fifty nanograms of total RNA was reverse transcribed (High Capacity cDNA Archive kit; Invitrogen) in a 20 µl reaction volume, according to the manufacturers recommendations. One nanogram equivalent of total RNA was amplified with gene-specific primers (Table 2), DNA polymerase, and SYBRGreen chemistry (SYBR select Master mix; Invitrogen) in a Realplex^2 S

PCR System (Eppendorf North America, Hauppauge, NY). Thermal cycling parameters include an initial heating step of 50°C for 2 min, a initial denaturing step of 95°C for 10 min, followed by 50 cycles of 15s at 95.0ºC, an annealing step for 30 s at the appropriate temperature for each primer, and an extension step of 20 s at 68.0ºC. A final dissociation step was included at 95°C for 15 s, 60°C for 30 s, and 95°C for 15 s. Primer efficiencies were determined from the slope of varying input concentrations, with an acceptable range for amplification being -3.0 to -3.8. All sequences and efficiencies can be found in Table 2. Sequencing of the amplicon products ensured that all primers amplified the gene of interest. Due to sample number and the need to conduct the PCR analysis for each gene over multiple plates, plates were balanced so that an equal number of treatments represented on each plate. Additionally, a pooled sample representing all treatment groups was run on each plate as an internal standard. Relative gene expression levels were calculated as $2^{-Ct\ \text{gene of interest}}/2^{-Ct\ RPL4}$, in which C_t denotes threshold cycle (Gonzalez et al., 2013).

All data was analyzed as a generalized randomized complete block design using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC) with pen as the experimental unit. Dietary treatment served as the fixed effect while gender, group, and barn were included as random blocking factors. For plasma Zn concentration analysis, the statistical structure was the same except day of bleeding served as a fixed effect in addition to dietary treatment. Day of bleeding also served as the repeated measure with animal as the subject. Contrast statements consisted of: (1) negative control vs. positive control RAC diet, (2) the 3-way and all possible 2-way interactions between increasing added Zn and source and d, (3) increasing Zn linear and quadratic polynomials, (4) added Zn from ZnO vs. ZnAA, (5) increasing day linear and quadratic

polynomials. Statistical significance was determined at $P < 0.05$ and P-values falling within $P > 0.05$ and $P < 0.10$ were considered a trend or tendency.

RESULTS

Experiment 1

From d 0 to 14, pigs fed the RAC diet had improved ($P < 0.004$) ADG, G:F, and d-14 BW compared to pigs fed the control diet (Table 3). Increasing dietary Zn from ZnO tended to improve (linear, $P = 0.072$) G:F. Pigs fed diets with RAC plus 50 ppm of added Zn from ZnAA had increased ($P < 0.041$) ADG and G:F compared with pigs solely fed RAC. No differences in growth performance were observed among pigs fed diets containing 50 ppm added Zn from ZnO or ZnAA.

From d 14 to 27, pigs fed the RAC diet had a tendency for increased ($P = 0.060$) ADG and reduced ($P = 0.016$) ADFI, resulting in improved ($P < 0.05$) G:F compared to those fed the control diet. No differences were observed in growth performance when Zn from ZnO or ZnAA was added to the RAC diet. Performance did not differ among pigs fed diets with 50 ppm added Zn from either source.

Overall (d 0 to 27), pigs fed the RAC diet had improved ($P < 0.05$) ADG, G:F, final BW, HCW, and boneless loin weight. Ractoapmine-HCl supplemented pigs also had a tendency for increased ($P = 0.053$) carcass yield, and a tendency for reduced ($P = 0.075$) ADFI compared with those fed the control diet without RAC. Increasing Zn from ZnO tended to increase (linear, $P = 0.067$) G:F, and boneless loin weights (quadratic, $P = 0.064$). Pigs fed the diet with 50 ppm added Zn from ZnAA tended to have increased ($P = 0.057$) ADG compared with pigs fed the RAC diet. No differences were observed in performance between pigs fed diets with 50 ppm added Zn from either source.

Experiment 2

Growth performance and carcass characteristics

From d 0 to 14, pigs fed the RAC diet had improved ($P < 0.05$) ADG and G:F compared to pigs fed the control diet (Table 4). There were no interactions between Zn source and level or a Zn level or source main effect. There was a trend for increased ($P < 0.10$) ADG and ADFI in pigs fed diets with added Zn from ZnO compared to pigs fed diets with added Zn from ZnAA.

From d 14 to 35 and compared to pigs fed the control diet, pigs fed the RAC diet had improved ($P = 0.006$) G:F which resulted from decreased ($P = 0.011$) ADFI. There was no Zn level × source interactions nor a Zn level or source main effects for growth performance.

Overall (d 0 to 35), pigs fed the RAC diet had improved ($P < 0.05$) ADG, G:F, d 35 BW, HCW, loin depth, and percentage lean and reduced ($P < 0.05$) ADFI and backfat depth compared with those fed the control diet. There was no Zn level × source interaction or Zn level or source main effect for growth performance and carcass characteristics.

Plasma and tissue Zn levels and liver weights

Plasma Zn concentrations were not affected by the treatment × day interaction (Table 5). As d increased, there was an increase (quadratic, $P < 0.05$) in plasma Zn. On each of the plasma collection days, Zn concentration was not different between the RAC and control pigs. Also, there was no Zn level × source interaction or Zn source effect. However, pigs fed RAC diets with added Zn had increased (linear, $P < 0.05$) plasma Zn concentrations on d 18 and 32.

There was no difference in liver Zn concentrations between pigs fed either the RAC or control diet (Table 5). A Zn level × source interaction (quadratic, $P = 0.007$) was observed and this resulted from concentrations plateauing at 150 ppm for ZnO supplemented pigs and

concentrations increasing linearly when ZnAA was added. There were no treatment effects on loin Zn concentration. Dietary treatments did not affect loin Zn concentrations.

Pigs fed the RAC diets without added Zn had increased ($P = 0.028$) liver weights compared to those fed the control diet (Table 5). There was no Zn level × source interaction or a Zn level effect for liver weight. Pigs fed the RAC diets with added Zn from ZnO tended to have heavier ($P = 0.091$) liver weights compared to pigs fed the RAC diet with added Zn from ZnAA.

Ileal Mucosal mRNA Expression

There was no Zn level × source interaction or a Zn source effect for *IL-1β* mRNA expression (Table 6). The expression of *IL-1β* was increased ($P = 0.014$) in mucosa of pigs fed the RAC diet compared to those fed the control diet. However, the relative mRNA expression of *IL-1β* decreased (linear; $P = 0.026$) in the mucosa of pigs fed added Zn. There were no treatment differences in *IL-8* or *TNF-α* relative mRNA expression.

DISCUSSION

Both Exp. consisted of a control diet without RAC and a RAC diet without added Zn. This allowed us to confirm the effects of the RAC diet on finishing pig growth performance and carcass characteristics independent of added Zn. Apple et al. (2007) summarized 23 publications determining the effects of RAC on growth performance and carcass characteristics of finishing pigs. They concluded that adding 10 ppm RAC to finishing pig diets resulted in a 11.7%, 13.3%, 2.4 kg, and 3.5 cm^2 average increase in ADG, G:F, HCW, and LM area, respectively, and a 1.4 mm reduction in 10^{th} rib fat depth. In Exp. 1 conducted herein, improvements in ADG, G:F, and HCW were greater than the average improvement determined in the meta-analysis; however, values still fell within the range of differences observed. Similarly, RAC diets increased loin weight in Exp. 1. Although Apple et al. (2007) determined that RAC reduces 10^{th} rib backfat

thickness, it is not as consistent of a response with the change ranging from -16.1 to 6.6%. Data from Exp. 1 did not observe reductions in backfat thickness. In Exp. 2 conducted herein, improvements in ADG and HCW were similar to the average improvement determined in the meta-analysis. Improvements in G:F and reductions in backfat depth were greater than the average; however, values still fell within the range of differences observed. Similarly, RAC diets increased loin depth in Exp. 2.

The NRC (2012) has a Zn requirement estimate of 50 ppm for growing pigs from 100 to 135 kg BW. Although the NRC (2012) estimates increased requirements of AA for 115 to135 kg pigs when RAC is added to the diet, there is not a similar increase in the Zn requirement estimate. However recent data might suggest otherwise, as Fry et al. (2013) observed a tendency for improved G:F in finishing pigs fed diets containing 5 ppm RAC (trace mineral premix provided 79 ppm Zn) with 40 ppm added Zn from either $ZnSO_4$ or ZnAA. Our results from Exp. 1 agree with Fry et al. (2013) in that the addition of up to 150 ppm added Zn from ZnO tended to improve G:F in finishing pigs fed diets containing RAC. However, data from Exp. 2 did not support this observation as growth performance was not influence by increasing level of added Zn Although Fry et al. (2013) determined that added Zn may enhance the response to RAC, they also reported 2 additional experiments that did not observe improved growth performance when Zn was added to RAC diets (79 ppm Zn added from the trace mineral premix). Other studies have also failed to demonstrate improvements in the response to RAC when supplemental Zn was added at levels above that contributed in the trace mineral premix (Rambo et al., 2013; Gowanlock et al., 2013).

Previous research observed improved ADG in pigs fed RAC diets with 50 ppm added Zn from a ZnAA, but ADG was not improved when supplementing a different inorganic Zn source

(Patience et al., 2011; Rambo et al., 2012). However, these studies did not include a RAC treatment without added Zn. Therefore, it is not possible to determine if the supplemental Zn elicited additional benefits over that observed from a diet containing only RAC. Both of the Exp. herein did not observe a difference in growth performance and carcass characteristics among finishing pigs supplemented Zn from ZnO vs ZnAA. This is similar to observations of Fry et al. (2013) and Rambo et al. (2013). In attempt to explain the variability in the response to added Zn from ZnAA vs inorganic Zn, Patience et al. (2013) conducted an experiment to determine if the Lys:calorie ratio of a finishing pig diet with RAC could affect the response to different added Zn sources. They observed no difference in growth performance of pigs fed added Zn independent of the Lys:calorie ratio.

If growth is the primary response criterion used to establish Zn requirements in finishing pigs fed RAC diets, then the Zn provided by the premix (83 ppm Zn from $ZnSO_4$) and endogenous Zn from the ingredients may not have been sufficient for pigs in Exp. 1. However, Zn provided by the premix (55 ppm Zn from $ZnSO_4$) and endogenous Zn from the ingredients was sufficient to support maximum growth performance in Exp. 2. The results from the two experiments were inconsistent and do not provide a clear conclusion for Zn concentrations in RAC containing diets fed to finishing pigs. Published data has also provided inconsistent results and has not explained the variability of this response through the factorial arrangement of treatments. However, research has suggested that the requirement for Zn may be greater for immune response when animals are stressed (Klasing, 1992). In addition, Rambo et al. (2013) attributed the inconsistency of obtained results to health status of the pigs used.

Ractopamine is a member of the phenylethanolamine class of beta-adrenergic agonists which are structurally and functionally similar to the endogenous catecholamines, epinephrine

and norepinephrine (Barnes, 1995; Beermann, 2002). These compounds act as repartitioning agents directing nutrients towards skeletal muscle accretion and away from adipose tissue deposition (Beermann, 2002; Mersmann, 1998). This was demonstrated in Exp. 2 through increased loin depth and reduced backfat thickness. In addition, the data indicated that the RAC diet increased the relative expression of proinflammatory cytokine *IL-1β* in distal ileum mucosa of pigs compared to those fed the control diet. Increasing IL-1β is correlated with elevated intestinal inflammation (Reinecker et al., 1991) and is also associated with increases in intestinal tight junction permeability (Al-Sadie and Ma, 2007). Therefore, our data would suggest that feeding RAC diets results in elevated inflammation of the pig's small intestine based on increased relative expression of *IL-1β*. To the best of our knowledge, there has been no previous data that demonstrates the effects of RAC diets on the relative expression of proinflammatory cytokines in the small intestines of pigs. However, the response to other β-agonist and endogenous catecholamines on proinflammatory cytokine expression in various tissues has been studied. Research in mice also determined that catecholamines released from the sympathetic nerve terminals and adrenal gland resulted in increased *IL-1β* expression in the liver and spleen (Jung et al., 1999).

Although there were increases in *IL-1β* in our study, there were no differences in relative expression of proinflammatory cytokines *TNF-α* or *IL-8*. Verghese et al. (1994) evaluated different isoforms that inhibited degradation of cyclic AMP to determine if these isoforms could regulate cytokine release from LPS-challenged human monocytes. The authors concluded that increasing cellular levels of cAMP reduced the accumulation of *TNF-α* gene expression; however, they increased accumulation of *IL-1β* mRNA. Additional research has also determined increasing cAMP can inhibit LPS-induced production of *TNF-α* and *IL-8* in human

promonocytic THP-1 cells (Farmer and Pugin, 2000). When feeding the β-adrenergic agonist RAC, it binds to a β-adrenergic receptor which activates intracellular adenylyl cyclase leading to increases in intracellular levels of cAMP (Mersmann, 1998). Therefore, increasing intracellular cAMP by feeding RAC may explain the observed differences in *IL-1β* but not *TNF-α* and *IL-8* in the current experiment.

Although pigs fed RAC had increased relative expression of proinflammatory cytokine *IL-1β* in distal ileum mucosa, increasing Zn in RAC diets reduced the relative expression of *IL-1β*. Previous data conducted *in vitro* has determined that Zn deficiency increased gene expression of *IL-1β, TNF-α* and *IL-8* in the monocyte-macrophage cell line (Bao et al., 2003). The authors reported that the reduction in these proinflammatory cytokines due to added Zn is not clearly defined. They speculated that the mechanism revolves around the increase in a Zn finger protein, A20, when increasing levels of Zn are added to the cell media. Jaattela et al. (1996) determined that A20 protein prevents an increase in the expression of *IL-1* and *TNF-α* by inhibiting the activation of nuclear factor-κβ (NF-$_k$B) like transcription factors.

CONCLUSION

Adding Zn to diets containing RAC resulted in a trend for improved growth performance of pigs in 1 of 2 experiments. Thus, adding Zn to diets containing RAC may improve finishing pig performance; however, the results are inconsistent. Due to the decrease in relative expression of *IL-1β*, we speculate that the variability of the response in pigs fed RAC diets with added Zn is mediated at the intestinal level and variability in the response may possibly be due to levels of stressors present in the environment. Therefore, more research is warranted to better define the relationship between RAC, Zn, and intestinal inflammation.

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Table 5.1 Diet composition (as-fed basis)

	Exp. 1[1,2]		Exp. 2[3,4]	
Item	Control	RAC	Control	RAC
Ingredient, %				
Corn	84.29	73.91	83.06	74.24
Soybean meal, (46.5% CP)	13.65	24.00	15.22	23.97
Monocalcium P, (21% P)	0.50	0.45	0.25	0.20
Limestone	0.90	0.90	0.75	0.78
Salt	0.35	0.35	0.35	0.35
Vitamin premix[5]	0.075	0.075	0.075	0.075
Trace mineral premix[6,7]	0.075	0.075	0.075	0.075
L-lysine HCl	0.15	0.15	0.15	0.15
DL-methionine	–	0.015	–	0.015
L-threonine	–	0.025	–	0.025
Phytase[8]	–	–	0.075	0.075
Ractopamine HCl[9]	–	0.05	–	0.05
Calculated analysis, %				
ME, kcal/kg	3,351	3,347	3,362	3,358
NE, kcal/kg	2,511	2,445	2,301	2,269
Standardized ileal digestible Lys, %	0.66	0.92	0.70	0.92
Total lysine, %	0.75	1.03	0.79	1.03
SID lysine: ME/Mcal	1.97	2.75	2.08	2.74
Ca, %	0.51	0.53	0.41	0.44
Total P, %	0.44	0.47	0.39	0.42
Available P, %	0.16	0.16	0.21	0.21

[1]Diets were fed in meal form from d 0 to 27 of the experiment. Basal diets contained 83 ppm Zn from $ZnSO_4$ provided by the trace mineral premix.

[2]Dietary treatments were obtained by replacing corn in the RAC diet to achieve 50, 100, and 150 ppm added Zn from ZnO and 50 ppm added Zn from Zn AA chelate (**ZnAA**; Availa-Zn; Zinpro, Eden Prairie, MN). Analyzed total Zn concentrations were 168 and 131, ppm in the control and RAC diets, respectively, 163, 188, and 267 ppm in the diets containing added ZnO, and 185 ppm in the diet containing added ZnAA.

[3]Diets were fed in meal form from d 0 to 35 of the experiment. Basal diets contained 55 ppm Zn from ZnSO4 provided by the trace mineral premix.

[4]Dietary treatments were obtained by replacing corn in the RAC diet to achieve 75, 150, and 225 ppm added Zn from ZnO or ZnAA. Analyzed total Zn concentrations were 66 and 77 ppm in the control and RAC diets, respectively, 134, 241, and 308 ppm in the diets containing added ZnO, and 154, 256, and 318 ppm in the diets containing added ZnAA .

[5]For Exp. 1 and 2, provided per kilogram of premix: 4,409,200 IU vitamin A; 551,150 IU vitamin D_3; 17,637 IU vitamin E; 1764 mg vitamin K; 3307 mg riboflavin; 11,023 mg pantothenic acid; 19,841 mg niacin; and 15.4 mg vitamin B_{12}.

[6] For Exp. 1, Provided per kilogram of premix: 26.5 g Mn from manganese oxide; 110 g Fe from iron sulfate; 110 g Zn from zinc sulfate; 11 g Cu from copper sulfate, 198 mg I from calcium iodate, and 198 mg Se from sodium selenite.

[7]For Exp. 2, Provided per kilogram of premix: 22 g Mn from manganese oxide; 73 g Fe from iron sulfate; 73 g Zn from zinc sulfate; 11 g Cu from copper sulfate, 198 mg I from calcium iodate,

and 198 mg Se from sodium selenite.

[8]Phyzyme 600 (Danisco Animal Nutrition, St. Louis, MO) provided 408 phytase units/kg of feed, with a release of 0.1% available P.

[9]Provided 10 ppm of ractopamine HCl (Paylean; Elanco Animal Health, Greenfield, IN).

Table 5.2 Sequences, annealing temperatures, amplicon length, and efficiency of primers used for real-time PCR quantification of gene expression

Small intestine genes[1]	Forward Primer (5′ to 3′)	Reverse Primer (5′ to 3′)	T_m, °C	Amplicon Length	Efficiency
IL1B	CCTCCTCCCAGGCCTTCTGT	GGGCCAGCCAGCACTAGAGA	62.0	178	1.01
IL8	TCCTGCTTTCTGCAGCTCTC	GGGTGGAAAGGTGTGGAATG	60.5	100	1.09
TNFA	GCAGGAGCCACCACGCTCTT	CGTGGGCGACGGGCTTATCT	62.0	147	.90
Normalizing gene[2]					
RPL4	AGGAGGCTGTTCTGCTTCTG	TCCAGGGATGTTTCTGAAGG	60.5	184	1.06

[1]Abbreviations: *IL1B* = interleukin-1 beta; *IL8* = interleukin-8; *TNFA* = tumor necrosis factor alpha.
[2]Abbreviation: *RPL4* = ribosomal protein L4.

Table 5.3 Effects of added zinc and ractopamine HCl (RAC) on growth performance and carcass characteristics of finishing pigs, Exp. 1[1]

								Probability, *P* <				
			Zn from ZnO,[2] ppm			Zn from ZnAA,[2,3] ppm			ZnO		ZnAA vs.	
Item	Control	RAC[2]	50	100	150	50	SEM	Control vs. RAC	Linear	Quadratic	RAC	50 ppm Zn (ZnO)
d 0 to 14												
ADG, kg	1.082	1.328	1.351	1.347	1.386	1.423	0.032	0.001	0.242	0.810	0.041	0.122
ADFI, kg	3.351	3.285	3.264	3.230	3.262	3.329	0.061	0.443	0.712	0.670	0.618	0.466
G:F	0.324	0.406	0.415	0.418	0.425	0.430	0.008	0.001	0.072	0.929	0.029	0.153
d 14 to 27												
ADG, kg	0.989	1.07	1.114	1.100	1.074	1.095	0.030	0.060	0.988	0.256	0.572	0.658
ADFI, kg	3.27	3.045	3.119	3.061	2.981	3.101	0.065	0.016	0.394	0.239	0.548	0.847
G:F	0.302	0.354	0.359	0.359	0.362	0.355	0.008	0.001	0.516	0.913	0.997	0.686
d 0 to 27												
ADG, kg	1.036	1.204	1.237	1.228	1.236	1.265	0.022	0.001	0.385	0.568	0.057	0.382
ADFI, kg	3.311	3.169	3.194	3.149	3.127	3.219	0.056	0.075	0.492	0.675	0.535	0.759
G:F	0.314	0.383	0.389	0.391	0.397	0.395	0.005	0.001	0.067	0.930	0.116	0.437
Weight, kg												
d 0	93.5	93.5	93.4	93.0	93.7	93.7	0.68	0.957	0.979	0.536	0.854	0.750
d 14	108.6	112.1	112.3	111.8	113.1	113.6	0.85	0.004	0.524	0.535	0.220	0.285
d 27	121.3	126.0	126.8	126.1	127.0	127.8	0.96	0.001	0.578	0.933	0.190	0.445
Carcass characteristics												
HCW, kg	89.5	93.8	94.9	94.0	94.2	95.3	0.70	0.001	0.876	0.494	0.120	0.686
Carcass yield,[4] %	73.9	74.4	74.8	74.5	74.4	74.65	0.179	0.053	0.758	0.184	0.365	0.501
Backfat depth, mm[5,6]	24.62	23.63	23.70	23.36	22.34	22.81	0.922	0.430	0.264	0.517	0.502	0.465
Loin wt, kg[5,6]	3.86	4.05	3.97	4.03	4.13	3.99	0.054	0.015	0.165	0.064	0.461	0.678

Lean, %[5,6,7]	51.74	52.15	52.12	52.25	52.63	52.48	0.37	0.426	0.300	0.546	0.495	0.460

[1] A total of 312 pigs (PIC 327 × 1050; two consecutive groups of 156 pigs) were used in a 27-d study with 2 pigs per pen and 26 pens per treatment.
[2] Diets contained 10 ppm of ractopamine-HCl (Paylean; Elanco Animal Health, Greenfield, IN).
[3]ZnAA = Zn AA chelate (Availa-Zn; Zinpro, Eden Prairie, MN).
[4] Percentage carcass yield was calculated by dividing HCW by live weight obtained at the farm before transport to the packing plant.
[5] Data was collected on the second group of pigs (13 pens per treatment).
[6] Adjusted using HCW as a covariate.
[7] Percentage lean was calculated using equations from NPPC (2000).

Table 5.4 Effects of level and source of added Zn on growth performance and carcass characteristics of finishing pigs fed ractopamine HCl (RAC), Exp. 2[1]

			Zn from ZnO,[2] ppm			Zn from ZnAA,[2,3] ppm				Probability,[4] $P <$			
Item	Control	RAC[2]	75	150	225	75	150	225	SEM	Control vs RAC	Zn Linear	Zn Quadratic	Source
d 0 to 14													
ADG, kg	1.097	1.333	1.309	1.309	1.320	1.262	1.305	1.225	0.042	0.001	0.242	0.826	0.100
ADFI, kg	3.282	3.158	3.068	3.150	3.107	3.004	3.078	2.954	0.135	0.178	0.223	0.714	0.074
G:F	0.338	0.426	0.427	0.418	0.425	0.422	0.425	0.417	0.013	0.001	0.604	0.998	0.774
d 14 to 35													
ADG, kg	0.998	1.034	1.055	1.071	1.062	1.076	1.027	1.048	0.042	0.419	0.683	0.586	0.619
ADFI, kg	3.464	3.218	3.160	3.181	3.232	3.166	3.187	3.142	0.093	0.011	0.777	0.587	0.636
G:F	0.293	0.324	0.336	0.339	0.332	0.343	0.324	0.335	0.017	0.006	0.497	0.272	0.762
d 0 to 35													
ADG, kg	1.037	1.152	1.158	1.166	1.165	1.151	1.138	1.118	0.029	0.001	0.737	0.740	0.173
ADFI, kg	3.389	3.187	3.120	3.168	3.181	3.101	3.140	3.065	0.103	0.014	0.494	0.612	0.245
G:F	0.311	0.365	0.373	0.371	0.369	0.373	0.365	0.367	0.014	0.001	0.885	0.419	0.599
BW, kg													
d 0	97.9	97.8	98.0	97.9	98.1	98.1	97.9	97.9	1.65	0.869	0.794	0.918	0.997
d 14	113.4	116.5	113.3	116.3	116.7	115.8	116.3	115.2	2.16	0.105	0.990	0.463	0.766
d 35	131.6	137.7	138.4	138.6	138.8	138.3	137.2	137.0	2.29	0.008	0.980	0.803	0.415
Carcass characteristics													
HCW, kg	99.0	101.7	102.5	101.7	102.8	101.9	101.7	101.0	1.34	0.047	0.966	0.814	0.288
Carcass yield,[5] %	72.62	73.17	73.39	72.90	73.56	72.75	73.53	73.03	0.271	0.120	0.575	0.647	0.381
Loin depth,[6] mm	65.03	68.88	69.53	71.62	69.65	70.30	69.76	69.26	1.271	0.033	0.602	0.256	0.629
Backfat depth,[6] mm	22.82	19.28	18.72	19.01	20.09	19.11	19.18	18.81	0.888	0.005	0.837	0.607	0.738
Lean,[6,7] %	51.37	53.65	54.53	54.22	53.75	54.14	54.10	53.95	0.640	0.012	0.858	0.326	0.836

[1] A total of 320 pigs (PIC 327 × 1050) were used with 2 pigs per pen and 20 pens per treatment.

[2] Diets contained 10 ppm of ractopamine-HCl (Paylean; Elanco Animal Health, Greenfield, IN).
[3]ZnAA = Zn AA chelate (Availa-Zn; Zinpro, Eden Prairie, MN).
[4] No interactive effects ($P > 0.12$) of Zn level × source.
[5] Percentage yield was calculated by dividing HCW by live weight obtained at the farm before transport to the packing plant.
[6] Adjusted using HCW as a covariate.
[7] Percentage lean was calculated using equations from NPPC (2000).

Table 5.5 Effects of level and source of added Zn on plasma, liver, and loin Zn concentrations and liver weights of finishing pigs fed ractopamine HCl (RAC), Exp. 2

			Zn from ZnO,[1] ppm			Zn from ZnAA,[1,2] ppm				Probability, $P <$			
Item	Control	RAC[1]	75	150	225	75	150	225	SEM	Control vs RAC	Zn Linear	Zn Quadratic	Source
Plasma,[3,4,5] μg/mL													
d 0	1.06	1.01	1.04	1.05	1.04	1.01	1.06	1.06	0.038	0.328	0.248	0.743	0.845
d 8	1.08	1.07	1.09	1.06	1.15	1.08	1.11	1.16	0.046	0.920	0.114	0.395	0.512
d 18	1.13	1.06	1.12	1.16	1.11	1.10	1.18	1.17	0.039	0.159	0.023	0.157	0.441
d 32	1.08	1.01	1.07	1.07	1.13	1.07	1.09	1.13	0.039	0.187	0.005	0.981	0.769
DM basis,[6] (μg/g)													
Liver[7]	306.24	292.84	314.12	345.38	329.26	289.59	326.44	394.80	17.44	0.551	0.001	0.500	0.570
Loin[8]	61.47	59.15	62.43	58.05	56.41	60.15	55.79	58.68	2.80	0.501	0.326	0.695	0.702
Liver wt,[6,8,9]kg	1.90	2.05	2.06	2.02	2.00	1.96	1.95	1.97	0.058	0.028	0.203	0.555	0.091

[1] Diets contained 10 ppm of ractopamine-HCl (Paylean; Elanco Animal Health, Greenfield, IN).
[2]ZnAA = Zn AA chelate (Availa-Zn; Zinpro, Eden Prairie, MN).
[3]Values represent 128 pigs, 1 pig randomly selected from 16 pens per treatment.
[4] No interactive effects ($P > 0.212$) of Zn level × source or treatment × day.
[5]There was an increase (quadratic, $P < 0.001$) in plasma Zn from day 0 to 18.
[6]Values represent 160 pigs, 1 pig randomly selected from 20 pens per treatment.
[7]There was a Zn level × source interaction (quadratic, $P = 0.007$).
[8]No interactive effects ($P > 0.234$) of Zn level × source.
[9]Liver weights were measured with the gallbladder still intact.

Table 5.6 Effects of level and source of added Zn on mRNA expression of inflammatory cytokine genes in the distal ileum of finishing pigs fed ractopamine HCl (RAC), Exp. 2[1,2]

			Zn from ZnO,[3] ppm			Zn from ZnAA,[3,4] ppm				Probability,[5] $P <$			
Item	Control	RAC[3]	75	150	225	75	150	225	SEM	Control vs RAC	Zn Linear	Zn Quadratic	Source
IL-1β	4.62	10.12	5.63	7.62	5.54	5.74	4.91	5.61	1.776	0.014	0.026	0.122	0.498
IL-8	3.84	3.75	4.82	4.53	3.90	3.94	3.10	3.65	1.867	0.942	0.881	0.621	0.233
TNF-α	7.22	6.65	7.20	5.95	6.00	6.15	5.87	4.82	1.305	0.727	0.302	0.769	0.414

[1] Values represent 128 pigs, 1 pig randomly selected from 16 pens per treatment.

[2] All values indicate relative expression of genes. Relative expression = $2^{-\Delta Ct\ gene\ of\ interest}/2^{-\Delta Ct RPL4}$ (Gonzalez et al., 2013). C_t denotes threshold cycle.

[3] Diets contained 10 ppm of ractopamine-HCl (Paylean; Elanco Animal Health, Greenfield, IN).

[4] ZnAA = Zn AA chelate (Availa-Zn; Zinpro, Eden Prairie, MN).

[5] No interactive effects ($P > 0.333$) of Zn level × source.

Chapter 6 - Effect of added zinc in diets with ractopamine-HCl on growth performance, carcass characteristics, and zinc fecal concentration of finishing pigs reared in a commercial environment

ABSTRACT

Three experiments were conducted to determine the effects of added Zn and Zn source on growth performance and carcass characteristics of finishing pigs fed ractopamine HCL (RAC; Elanco Animal Health, Greenfield, IN). In all experiments, pigs (337×1050, PIC) were housed in a commercial finishing facility with approximately 25 per pen. In Exp. 1, a total of 1,234 pigs (103.7 kg BW) were used in the 28-d study with 4 pens per treatment. Dietary treatments consisted of a corn-soybean meal–based control diet (0.70% standardized ileal digestible [SID] Lys), a RAC diet (0.92% SID Lys) containing 10 ppm RAC, and RAC + 50 ppm added Zn from either ZnO or Zn AA complex (**ZnAA**; Availa-Zn, Zinpro, Eden Prairie, MN). All diets contained 80 ppm Zn from ZnO in the trace mineral premix. In Exp. 2, 1,234 pigs (102 kg BW) were used in a 28-d study with 24 pens per treatment. Treatments were a RAC diet (0.92% SID Lys) with 5 ppm RAC and 80 ppm Zn supplied from the premix or the RAC diet plus 50 ppm added Zn from ZnO. In Exp. 3, a total of 1,197 pigs (58.7 kg BW) were used in a 72-d study with 6 pens per treatment. Pens were randomly assigned to a 2 × 2 × 2 factorial in a split-plot design. The whole plot consisted of diets with or without 75 ppm added Zn from ZnO fed from d 0 to 45 and the subplots were diets with or without 75 ppm added Zn and with or without 10 ppm RAC fed from d 45 to72. All diets contained 50 ppm Zn supplied from the premix. In Exp. 1 and 3, pigs fed the RAC diet had increased ($P < 0.05$) ADG, G:F, and loin depth. In Exp. 1 and 3, pigs fed the RAC diet had increased ($P < 0.05$) final BW, HCW, percentage lean, and decreased ($P =$

0.001) backfat thickness. In all 3 experiments, added Zn did not influence overall performance of pigs fed RAC diets. In Exp. 3, there was an added Zn grower phase × RAC interaction (P = 0.034) from d 45 to 72. Adding Zn from d 0 to 45 increased the response to RAC from d 45 to 72 compared to not feeding Zn from d 0 to 45. However, pigs fed diets with added Zn from d 45 to 72 had decreased ($P < 0.05$) ADG and G:F compared to those fed diets without. In conclusion, added Zn in RAC diets did not improve performance of finishing pigs; however, added Zn during the grower phase may influence the ADG response to RAC in finishing pigs.

INTRODUCTION

The NRC (2012) has a requirement estimate of 50 ppm Zn for growing pigs from 100 to 135 kg BW. Although the NRC (2012) estimates increased requirements of AA for 115 to135 kg pigs when ractopamine HCl (RAC; Paylean®; Elanco Animal Health, Greenfield, IN) is added to the diet, there is not a similar increase in the Zn requirement estimate. Recent research by Gowanlock et al. (2013) reported that dietary Zn levels at or below those estimated by the NRC (2012) are sufficient to support growth of pigs from 20 to 80 kg and pigs fed RAC diets from 80 to 115 kg. Others studies have suggested that added Zn above that of the NRC (2012) in finishing pig diets containing RAC may improve growth performance (Fry et al., 2013; Paulk et al., 2013b). The inconsistency in the response to added Zn in RAC diets may be due to levels of stressors present in the environment. Pigs raised in a commercial environment are subject to higher animal densities and different environments, which result in lower growth rates and feed intakes than those that occur in a university setting (Koketsu, 1997). Therefore, the response to added Zn needs to be tested in a commercial environment to determine if the Zn requirement is different than that of the NRC (2012).

One consequence of feeding higher levels of Zn if not utilized by the pig is increased excretion and impact on the environment. Increasing dietary Zn levels above the requirement results in increased Zn excreted in swine waste (Creech et al., 2004).Therefore, it is important to define the duration of feeding added Zn to maximize performance will minimizing Zn excretion.

The first objective of this study was to determine the effect of added Zn from ZnO or Zn AA complex (**ZnAA** ; Availa Zn, Zinpro, Eden Prairie, MN) in diets with RAC on growth performance and carcass characteristics of finishing pigs reared in a commercial environment. The second objective was to determine if added Zn was required to be fed throughout the grower period, and with or without RAC in the finishing period to achieve an improvement in growth performance and its influence on plasma and fecal Zn concentrations.

MATERIALS AND METHODS

General

The Kansas State University Institutional Animal Care and Use Committee approved the protocol used in these experiments. All 4 experiments were conducted off-campus at commercial research-finishing barns with pigs (Line 337 × 1050: PIC Hendersonville, TN) housed with approximately 25 pigs per pen. The barns were naturally ventilated and double-curtain-sided with completely slatted flooring and deep pits for manure storage. Each barn was equipped with a 4-hole stainless steel dry self-feeder and a cup 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. All experimental diets were in meal form and were prepared at a commercial feed mill. A subsample of experimental diets was collected and analyzed for dietary Zn (Ward Laboratories, Inc., Kearney, NE). Samples were prepared using the method outlined by AOAC int. (2012) and

analyzed using an iCAP 6000 series ICP Emission Spectrometer (Thermo Electron Corporation, Marietta, OH). Diet samples for experiment 2 and phase 1 of experiment 4 were lost in transition; therefore, Zn analysis was not conducted on those diets.

Experiment 1

A total of 1,234 pigs (initially 103.7 kg) were used in a 28-d study. Pens consisted of 23 to 28 pigs per pen with either all barrow, all gilt, or mixed-sex pens. Pens of pigs were blocked by BW and gender and were randomly assigned to diets with 12 pens per treatment. Dietary treatments consisted of (1) a corn-soybean meal–based negative control diet (0.70% SID Lys); (2) a positive control diet (0.92% SID Lys) containing 10 ppm RAC; (3) treatment 2 plus 50 ppm added Zn from ZnO, and (4) treatment 2 plus 50 ppm added Zn from ZnAA. All diets contained 80 ppm Zn from ZnO provided by the trace mineral premix (Table 6.1).

Pigs and feeders were weighed weekly to determine ADG, ADFI, and G:F. On d 14, the 6 heaviest pigs from each pen (determined visually) were sold according to the normal marketing procedures of the farm. These pigs were included in the growth data up to day 14, but were not included in the carcass data. On d 28, the remaining pigs were tattooed by pen and transported to a USDA-inspected processing plant (JBS Swift and Company,Worthington, MN) for processing and carcass data collection. Hot carcass weight was collected immediately following evisceration. Backfat and loin depth were collected using an optical probe (Fat-O-Meter, SFK Technology A/S, Denmark) inserted between the third and fourth last rib (counting from the ham end of the carcass) at a distance approximately 7 cm from the dorsal midline and percentage lean was calculated (NPPC, 2000). Percentage carcass yield was calculated by dividing HCW at the packing plant by the live weight obtained at the farm.

All data were analyzed as a randomized complete block design using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC) with pen as the experimental unit and BW and gender as blocking factors. Hot carcass weight was used as a covariate for analyses of backfat thickness, loin depth, and percentage lean. Contrast statements consisted of: (1) control vs. RAC diet, (2) RAC diet vs added Zn, and (3) added Zn from ZnO vs. ZnAA. Statistical significance was determined at $P < 0.05$ and trends at $P < 0.10$.

Experiment 2

The experiment was implemented on d 75 of a 102-d study designed to determine the effects of 0, 7.5, and 15% dietary bakery by-product on performance of finishing pigs. The procedures are described by Paulk et al. (2013b).

A total of 1,234 pigs (average BW 101.9 kg) were used in a 27-d study. Pens of pigs were randomly assigned to diets with and without 50 ppm added Zn from ZnO and blocked by BW, bakery by-product level, and gender. All diets contained 5 ppm RAC and 83 ppm Zn from ZnO provided by the trace mineral premix (Table 6.2). There were 24 pens per treatment.

All growth performance and carcass data were collected as determined for Exp. 1. On d 9, the 5 heaviest pigs from each pen (determined visually) were sold according to the normal marketing procedures of the farm.

All data were analyzed as a randomized complete block design using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC) with pen as the experimental unit and BW, bakery by-product, and gender as blocking factors. Hot carcass weight was used as a covariate for analyses of backfat thickness, loin depth, and percentage lean. Statistical significance was determined at $P < 0.05$ and trends at $P < 0.10$.

Experiment 3

A total of 1,197 pigs (initially 58.8 kg) were used in a 72-d study to determine the effects of added Zn from ZnO fed during the grower (d 0 to 45) and finisher (d 45 to 72) in diets with or without RAC on growth performance, carcass characteristics, and plasma and fecal Zn concentrations. There were 25 pigs per a pen and a total of 24 pens per treatment for the whole plot and 12 pens per treatment for the sub plot. Pens were randomly assigned to a 2 × 2 × 2 factorial arrangement in a split-plot design. The whole plot consisted of diets with or without 75 ppm added Zn from ZnO from d 0 to 45 and the subplots were diets with or without 75 ppm added Zn from ZnO and with or without 10 ppm RAC from d 45 to72. All diets contained 50 ppm Zn supplied from the premix (Table 6.3).

Pigs and feeders were weighed on d 0, 23, 45, 52, 57, 65, and 72 to determine ADG, ADFI, and G:F. Subsamples of 1, 2, 4, and 4 pigs were bled from each pen on d 0, 45, 52, and 63, respectively. On d 0, the median weight barrow from each pen was ear tagged to allow for bleeding on subsequent collection dates. On day 52 and 63, four median weight barrows, including the previously selected pig were selected from each pen for blood collection. Samples were collected via jugular venipuncture into heparinized (143 USP unite of NA heparin) vacutainer tubes (Tyco Healthcare Group LP, Mansfield, MA), inverted, and immediately placed on ice until samples were processed. On d 45 and 63, fecal grab samples were collected on 3 random pigs per pen for determination of Zn concentrations. On d 57, the 6 heaviest pigs from each pen (determined visually), and on d 72, the remaining pigs were tattooed by pen and transported 1 h to a USDA-inspected processing plant (JBS Swift and Company, Worthington, MN) for processing and carcass data collection as described in Exp. 1.

For plasma Zn analysis, *w*hole blood was centrifuged (2000 × g, 15 min, 4°C) and plasma removed and frozen at -20°C. Plasma was deproteinized by diluting 1:4 in 12.5% trichloroacetic acid followed by centrifugation at 2,000 × g for 15 min (GS-6KR, Beckman-Coulter, Brea, CA) and collection of the supernatant for analysis. The ashed fecal samples were placed in 10 mL of 6N HCl and boiled for 10 min (AOAC, 1995). Zinc concentrations were determined by flame atomic absorption spectrophotometry (Perkin Elmer 3110 AA Spectrometer, PerkinElmer, Waltham, MA).

Data were analyzed as a split-plot design using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC) with dietary grower treatment (0 or 75 ppm added Zn from d 0 to 45) as the whole plot and dietary finisher treatment (diets with or without RAC × 0 or 75 ppm added Zn from d 45 to 72) as the subplot. Pen served as the experimental unit. For plasma Zn concentration analysis, the statistical structure was the same except day of bleeding and treatment × d of bleeding served as a fixed effects in addition to dietary treatment. Day of bleeding also served as the repeated measure with animal as the subject. The covariance structure compound symmetry was used. Hot carcass weight was used as a covariate for analyses of backfat thickness, loin depth, and percentage lean. Statistical significance was determined at $P < 0.05$ and trends at $P < 0.10$.

RESULTS

Experiment 1

Diet samples from experiment 1 were not available for analysis due to being lost in transit; therefore, analyzed Zn concentrations were not determined. From d 0 to 14, pigs fed the RAC diet had improved ($P < 0.05$) ADG (1.115 vs 0.924) and G:F (0.398 vs 0.337) compared to pigs fed the control diet (data not shown). Pigs fed RAC diets with added Zn from ZnO or

ZnAA tended to have improved (P = 0.058; 1.160 & 1.154 vs 1.115) ADG and improved (P = 0.044; 0.421 & 0.399 vs 0.398) G:F compared to pigs fed RAC diets, respectively. When comparing Zn sources, pigs fed RAC diets with 50 ppm added Zn from ZnO did not differ in ADG, but had reduced (P = 0.003; 2.759 vs 2.907 kg) ADFI, resulting in increased (P = 0.003; 0.421 vs 0.399) G:F compared to pigs fed RAC diets with 50 ppm added Zn from ZnAA.

Overall (d 0 to 28), pigs fed the RAC diet had increased (P < 0.05) ADG, G:F, final BW, HCW, loin depth, and percentage lean, and reduced (P = 0.001) backfat thickness compared with those fed the control diet (Table 4). Added Zn or Zn source did not influence overall growth performance.

Experiment 2

Analyzed Zn levels for experimental diets are reported in Table 2. Analyzed concentrations were within acceptable limits for analytical variation according to the Association of American Feed Control Officials (2013). Overall (d 0 to 27), no differences in growth performance or carcass characteristics were observed when pigs were fed diets with 50 ppm added Zn from ZnO compared with those fed the RAC diet (Table 5).

Experiment 3

Analyzed Zn levels for experimental diets are reported in Table 3. Analyzed concentrations were within acceptable limits for analytical variation according to the Association of American Feed Control Officials (2013). From d 0 to 45, there were no differences in ADG but increased (P = 0.016) ADFI, which resulted in decreased (P = 0.008) G:F compared to pigs fed the control diet. For the finisher phase (d 45 to 72), there were no interactive effects of Zn grower × Zn finisher × RAC or Zn grower × Zn finisher for growth performance and carcass characteristics (Table 6). There was an added Zn during the grower phase × RAC interaction (P

= 0.034) for ADG of finishing pigs. This resulted from pigs fed RAC and diets with added Zn during the grower phase having increased ADG compared to pigs fed RAC diets without added Zn during the grower phase. Added Zn during the grower phase did not influence ADG of pigs fed the control diet without RAC during the finisher phase. There was a tendency for an added Zn during the finisher phase × RAC interaction (P = 0.066) for ADG of finishing pigs. This resulted from pigs fed control diets with added Zn during the finisher phase having decreased ADG compared to pigs fed control diets without added Zn. Pigs fed RAC diets with or without added Zn had similar ADG. There was an added Zn during the finisher phase × RAC interaction (P = 0.025) for ADFI. Pigs fed RAC diets with added Zn during the finisher phase had increased ADFI compared to those fed added Zn diets without RAC. However, pigs fed RAC diets without added Zn during the finishing phase had similar ADFI to pigs fed control diets without added Zn. Pigs fed RAC diets had improved (P < 0.05) ADG and G:F compared to those fed diets without RAC for the 27 d finishing period. Pigs fed diets with added Zn during the finisher phase had decreased (P < 0.05) ADG and G:F compared to those fed diets without. Overall (d 0 to 72), there were no dietary treatment interactions for growth performance and carcass characteristics. Pigs fed RAC for the last 27 d of the experiment had improved (P < 0.05) ADG, G:F, final BW, HCW, loin depth and percentage lean, and reduced (P = 0.002) backfat thickness compared to pigs fed diets without RAC. Added Zn did not influence overall growth performance or carcass characteristics of pigs.

From d 0 to 45, pigs fed added Zn had increased (P = 0.001) average daily Zn intake (Table 7). From d 45 to 72, there was an added Zn during the finisher phase × RAC interaction (P = 0.004). Either added Zn during the finishing period or RAC caused an increase in average

daily Zn intake; however, average daily Zn intakes were further increased when both added Zn during the finisher period and RAC were fed to pigs.

For plasma Zn concentrations, there was no 4, 3, or 2-way interactions among dietary treatment and day. Added Zn during the grower phase did not influence plasma Zn concentrations on d 45. However, pigs fed diets with 75 ppm added Zn during the finisher phase had increased ($P < 0.05$) plasma Zn levels on d 52 and 63 compared to those fed diets without added Zn during the finisher phase. There was no effect of the RAC diet on plasma Zn concentration. For fecal analysis, pigs fed added Zn during the grower period had increased ($P < 0.05$) fecal Zn concentrations on d 45 compared to those fed diets without added Zn. For d-63 fecal Zn concentrations, there was an added Zn during the finishing period × RAC interaction ($P = 0.032$). Either added Zn during the finishing period or RAC caused an increase in d 63 fecal Zn concentration; however, concentrations were further increased when both added Zn during the finisher period and RAC were fed to pigs.

DISCUSSION

Two of the three experiments consisted of a control diet without RAC and a RAC diet without added Zn. This allowed us to confirm the effects of the RAC diet on finishing pig growth performance and carcass characteristics independent of added Zn. Apple et al. (2007) summarized 23 publications determining the effects of RAC on growth performance and carcass characteristics of finishing pigs. They concluded that adding 10 ppm RAC to finishing pig diets resulted in a 11.7%, 13.3%, 2.4 kg, and 3.5 cm^2 average increase in ADG, G:F, HCW, and LM area, respectively, and a 1.4 mm reduction in 10^{th} rib fat depth. In the experiment conducted herein, improvements in ADG, G:F, and HCW were greater than the average improvement determined in the meta-analysis; however, values fell within the range of differences observed.

Although Apple et al. (2007) determined that RAC reduces 10th rib backfat thickness, but it is not a consistent response with the change ranging from -16.1 to 6.6%. Data from Exp. 1 and 3 observed reductions in backfat thickness. They also observed an increase in LM area in pigs fed RAC diets. Similarly, RAC diets increased loin depth in Exp. 1 and 3.

Previous research has reported inconsistent results determining the effects of added Zn on growth performance of finishing pigs fed RAC. Fry et al. (2013) observed a tendency for improved G:F in finishing pigs fed RAC diets with 40 ppm added Zn compared to those fed RAC diets without added Zn. They observed no difference in performance when Zn was added to RAC diets in a second experiment. They conducted a third experiment to determine if the effects of added Zn were dependent upon inclusion rate of RAC. They concluded that added Zn to diets containing 5 or 7.5 ppm RAC did not influence growth performance of finishing pigs. Therefore, RAC dosage does not explain the variability in the response. Paulk et al. (2013b) determined that adding 150 ppm Zn from ZnO or 50 ppm Zn from ZnAA to RAC diets led to a 2.7 and 5.1% numerical increase in ADG, respectively, and a 3.7 and 3.1% numerical increase in G:F, respectively. In a second experiment, they observed no difference in growth performance of finishing pigs fed RAC diets with added Zn (Paulk et al., 2014). In addition, Rambo et al. (2013) determined that pigs fed RAC diets with 50 ppm added Zn had a 2.2% numerical increase in G:F compared to those fed the RAC diet without added Zn. In a second experiment, they did not observe an improvement pig performance when fed RAC diets with added Zn. In Exp. 2, pigs fed added Zn from d 0 to 14 had improved ADG and G:F; however, added Zn to RAC diets did not improve overall performance.

Researchers have also evaluated the effects of added Zn source on finishing pig performance. Patience et al. (2011) observed that pigs fed ZnAA had improved ADG compared

to pigs fed ZnO and pigs fed ZnAA for a portion of the experiment had intermediate ADG to pigs fed either $ZnSO_4$ or ZnAA for the entire experiment. Rambo et al. (2012) observed no difference in performance between in Zn source (ZnO vs. ZnAA) from d 0 to 35; however, from d 35 to 56, pigs fed ZnAA diets had improved ADG and a trend for improved G:F compared to those fed ZnO diets. Patience et al. (2013) conducted an experiment to determine if the Lys:calorie ratio of a finishing pig diet with RAC could affect the response to Zn source. Dietary treatments were organized as a 2 × 2 factorial with 2 levels of Lys (2.69 g vs 3.14 g SID Lys per Mcal ME) and 2 sources of zinc supplementation (50 ppm Zn from either $ZnSO_4$ or ZnAA). Respective Zn source was fed prior to initiation of the experiment. The authors reported no difference in growth performance of pigs fed added Zn independent of the Lys:calorie ratio. Therefore, 2 out of the 3 experiments demonstrate improvements in performance when Zn is added from ZnAA vs inorganic Zn sources for the grower and finisher periods. In Exp. 1 herein, we did not observe a difference in growth performance and carcass characteristics among finishing pigs fed added Zn from ZnO vs ZnAA for the last 28 d before harvest. This is similar to previous studies that only fed added Zn from varying Zn sources in the final phase diets (Fry et al., 2013, Paulk et al., 2013b; Paulk et al, 2014). Therefore, the response to Zn source may be dependent on the duration of feeding; however, this has not been demonstrated through appropriate experimental designs.

Previous research has determined that endogenous Zn within corn-soybean meal diets is sufficient to support maximum growth performance in growing pig diets (Wedekind et al., 1994; Gowanlock et al., 2013). In Exp. 3, it was determined that feeding 75 ppm added Zn increased ADFI and reduced G:F when fed to growing pigs. Although added Zn during the grower phase did not improve growing pig performance, 75 ppm added Zn from d 0 to 45 increased the pig's

ADG response to RAC by 3.7% during the finisher phase (d 45 to 72). To the best of our knowledge, there is no published data that determines the effects of dietary Zn level during the growing period on the response to RAC.

In Exp. 3, added Zn during the grower phase did not influence plasma Zn concentrations on d 45. However, pigs fed added Zn during the finisher phase had increased plasma Zn levels on d 52 and 63 compared to those fed diets without added Zn during the finisher phase. Paulk et al. (2014) observed similar increases in plasma Zn levels when finishing pigs where fed RAC diets with added Zn. A majority of Zn excreted is unabsorbed Zn; however, a small amount results from endogenous losses (NRC, 1980). Pigs fed added Zn had increased fecal Zn concentrations. In addition, pigs fed the RAC had increased fecal Zn concentrations. The amount of unabsorbed and endogenous Zn secreted in the feces is dependent upon the needs of the pig and are increased with increasing Zn intake (McDowell, 1992). The finisher phase Zn × RAC interaction and the effects of RAC fecal Zn concentration can possibly be explained by increases in daily Zn intake.

Conclusion

As expected, pigs fed RAC diets had improved growth performance and carcass characteristics but added Zn with RAC did not. However, added Zn to diets of growing pigs may increase the ADG response to RAC fed during the finishing phase. In addition, pigs fed diets with 75 ppm added Zn during the finisher phase had increased plasma Zn levels. Pigs fed added Zn during the grower and finisher period and pigs fed RAC diets during the finisher phase had increased fecal Zn concentrations. However, concentrations were further increased when both added Zn during the finisher period and RAC were fed to pigs.

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Table 6.4 Diet composition, Exp. 1 (as-fed basis)[1,2]

Item	Control	RAC[3]
Ingredient, %		
Corn	54.68	45.83
Soybean meal, (46.5% CP)	13.63	22.39
Bakery meal	15.00	15.00
DDGS[4]	15.00	15.00
Limestone	1.03	1.08
Salt	0.35	0.35
Vitamin trace mineral premix[5]	0.08	0.08
L-Lys sulfate	0.23	0.23
Phytase[6]	0.005	0.005
Ractopamine HCl[7]	–	0.05
Calculated analysis, %		
ME, kcal/kg	3,408	3,402
NE, kcal/kg	2,313	2,277
Standardized ileal digestible (SID) Lys, %	0.70	0.92
Total Lys, %	0.83	1.07
SID Lys: ME, g/Mcal	2.05	2.70
Ca, %	0.48	0.52
Total P, %	0.39	0.43
Available P, %	0.21	0.21

[1]Diets were fed in meal form for the final 28 d prior to slaughter. Basal diets contained 80 ppm Zn from ZnO provided by the trace mineral premix.

[2]Dietary treatments were obtained by replacing corn in the RAC diet to achieve 50 ppm of added Zn from ZnO or from ZnAA (Availa-Zn; Zinpro, Eden Prairie, MN).

[3]Ractopamine HCl (RAC; Paylean®; Elanco Animal Health, Greenfield, IN)

[4]Dried distillers grains with solubles.

[5]Provided per kg of premix: 4,509,409 IU vitamin A; 701,464 IU vitamin D3; 24,050 IU vitamin E; 1,402 mg vitamin K; 12,025 pantothenic acid; 18,037 mg niacin; 3,006 mg vitamin B2 and 15,031 mg vitamin B12, 40,084 mg Mn from manganese oxide, 90,188 mg Fe from iron sulfate, 100,209 Zn from zinc oxide, 10,021 mg Cu from copper sulfate, 501 mg I from Ethylenediamin dihydroiodide, and 300 mg Se from sodium selenite.

[6]OptiPhos 2000 (Enzyvla LLC, Sheridan, NJ) provided 250 FTU per kg of diet.

[7]Provided 10 ppm of ractopamine HCl (Paylean; Elanco Animal Health, Greenfield, IN).

Table 6.5 Diet composition, Exp. 2 (as-fed basis)[1,2]

	No added Zn		
Bakery, %	0	7.5	15
Ingredient,%			
Corn	63.25	56.28	49.22
Soybean meal, (46.5% CP)	18.99	18.46	18.03
Bakery by-product	—	7.50	15.00
DDGS[2]	15.00	15.00	15.00
Choice white grease	0.70	0.70	0.70
Limestone	1.15	1.12	1.09
Salt	0.35	0.35	0.35
Vitamin and trace mineral premix[3]	0.08	0.08	0.08
L-Lys sulfate	0.40	0.43	0.45
L-Thr	0.05	0.05	0.05
Phytase[4]	0.007	0.007	0.007
Ractopamine HCl[5]	0.025	0.025	0.025
Calculated analysis, %			
ME, kcal/kg	3,392	3,415	3,437
NE, kcal/kg	2,307	2,312	2,316
Standardized ileal digestible (SID) Lys, %	1.03	0.95	0.85
Total Lys, %	1.16	1.07	0.96
SID Lys: ME, g/Mcal	3.10	2.85	2.56
Ca, %	0.65	0.62	0.59
Total P, %	0.61	0.56	0.53
Available P, %	0.39	0.35	0.33

[1]Dietary treatments were obtained by replacing corn in diets to achieve 50 ppm added Zn from zinc oxide (ZnO). Analyzed total Zn concentrations were 103, 109, and 124 ppm in the 0, 7.5 and 15% bakery diets without added Zn, respectively, and 150, 168, and 148 ppm in 0, 7.5 and 15% bakery diets with added Zn, respectively.

[2]Dried distillers grains with solubles.

[3]Provided per kg of premix: 4,509,409 IU vitamin A; 701,464 IU vitamin D3; 24,050 IU vitamin E; 1,402 mg vitamin K; 12,025 pantothenic acid; 18,037 mg niacin; 3,006 mg vitamin B2 and 15,031 mg vitamin B12, 40,084 mg Mn from manganese oxide, 90,188 mg Fe from iron sulfate, 100,209 Zn from zinc oxide, 10,021 mg Cu from copper sulfate, 501 mg I from Ethylenediamin dihydroiodide, and 300 mg Se from sodium selenite.

[4]OptiPhos 2000 (Enzyvla LLC, Sheridan, NJ) provided 350 FTU per kg of diet.

[5]Provided 10 ppm ractopamine HCl (Paylean, Elanco Animal Health, Greenfield, IN).

Table 6.6 Diet composition of Exp. 3 (as-fed basis)[1,2]

	Phase 1	Phase 2	Phase 3	
Item	Control	Control	Control	RAC[3]
Ingredient, %				
Corn	54.60	58.10	72.50	62.76
Soybean meal, 46.5% CP	12.95	9.78	10.58	20.26
DDGS[4]	30.00	30.00	15.00	15.00
Monocalcium phosphate, 21 % P	0.20	–	0.05	–
Limestone	1.33	1.25	1.08	1.03
Salt	0.35	0.35	0.35	0.35
Vitamin premix[5]	0.08	0.08	0.05	0.05
Trace mineral premix[6]	0.05	0.05	0.05	0.05
L-Lys sulfate	0.44	0.40	0.33	0.33
Met hydroxy	—	—	—	0.09
L-Thr	—	—	—	0.03
Phytase[7]	0.005	0.004	0.010	0.010
Ractopamine HCl[8]	—	—	—	0.05
Calculated analysis, %				
ME, kcal/kg	3,318	3,329	3,333	3,327
NE, kcal/kg	2,396	2,416	2,447	2,467
Standardized ileal digestible (SID) Lys, %	0.83	0.73	0.66	0.90
Total Lys, %	1.01	0.90	0.79	1.06
SID Lys:ME,g/Mcal	2.50	2.19	1.98	2.70
Ca, %	0.59	0.52	0.46	0.46
Total P, %	0.46	0.40	0.37	0.40
Available P, %	0.28	0.22	0.21	0.21

[1]Diets were fed in meal form from d 0 to 72. Basal diets for all 3 phases contained 55 ppm Zn from ZnO provided by the trace mineral premix.

[2]Dietary treatments were obtained by replacing corn to achieve 50 ppm of added Zn from ZnO. Analyzed total Zn concentrations were 88 and 131 ppm in the phase 2 control and added Zn diet diets, respectively, and 96, 155, 117, and 180 for the control, control + added Zn, RAC, and RAC + added Zn diets, respectively.

[3]Ractopamine HCl (RAC; Paylean®; Elanco Animal Health, Greenfield, IN)

[4]Dried distillers grains with solubles.

[5]Provided per kilogram 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.

[6]Provided per kilogram 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, 330 mg I from calcium iodate, and 299 mg Se from sodium selenite.

[7]OptiPhos 2000 (Enzyvla LLC, Sheridan, NJ) provided 250, 200, and 500 FTU per kg of diet for phase 1, 2, and 3, respectively.

[8]Provided 10 ppm of ractopamine HCl (Paylean; Elanco Animal Health, Greenfield, IN).

Table 6.7 Effects of added zinc on growth performance and carcass characteristics of finishing pigs fed ractopamine HCl, Exp. 1[1]

			Added Zn, 50 ppm			Probability, *P* <		
Item	Control	RAC[2]	ZnO[2]	ZnAA[2, 3]	SEM	Control vs. RAC	RAC vs. added Zn	ZnO vs. ZnAA
d 0 to 28								
ADG, kg	0.918	1.097	1.115	1.110	0.014	0.001	0.326	0.784
ADFI, kg	2.923	2.896	2.865	2.920	0.040	0.577	0.930	0.255
G:F	0.316	0.379	0.390	0.381	0.004	0.001	0.184	0.135
BW								
d 0	103.6	103.7	103.7	103.8	0.81	0.812	0.941	0.855
d 14	116.6	119.3	119.9	119.9	0.88	0.001	0.256	0.937
d 28	126.1	130.6	131.2	130.9	1.06	0.001	0.546	0.684
Carcass characteristics								
HCW, kg	94.5	97.8	98.8	97.9	0.91	0.001	0.409	0.277
Carcass yield,[4]%	74.94	74.85	75.34	74.87	0.355	0.870	0.565	0.352
Backfat thickness,[5] mm	16.69	15.05	15.33	15.16	0.313	0.001	0.564	0.663
Loin depth,[5] mm	69.10	71.24	71.33	71.32	0.602	0.013	0.894	0.994
Lean,[5,6] %	53.40	54.49	54.34	54.43	0.189	0.001	0.605	0.702

[1]A total of 1,234 pigs (Line 337 × 1050: PIC Hendersonville, TN; initially 103.7 kg) were used in a 28-d study with 23 to 28 pigs per pen and 12 pens per treatment.

[2]Diets contained 10 ppm of ractopamine HCl (Paylean; Elanco Animal Health, Greenfield, IN).

[3]Zn AA complex (Availa-Zn; Zinpro, Eden Prairie, MN)

[4]Calculated by dividing HCW by live weight obtained at the farm.

[5]Adjusted using HCW as a covariate.

[6]Calculated using NPPC (2001) equation.

Table 6.8 Effects of added zinc on growth performance and carcass characteristics of finishing pigs fed ractopamine HCl, Exp. 2[1]

Item	Control	50 ppm added Zn	SEM	*P* <
d 0 to 27				
ADG, kg	1.072	1.079	0.009	0.548
ADFI, kg	3.063	3.046	0.024	0.608
G:F	0.350	0.355	0.003	0.274
BW, kg				
d 0	102.0	101.8	0.71	0.851
d 9	112.1	111.6	0.76	0.635
d 27	127.9	128.7	0.83	0.525
Carcass characteristics				
HCW, kg	96.3	96.8	0.59	0.573
Yield, [2]%	75.31	75.24	0.220	0.828
Backfat thickness, [3]mm	15.83	15.66	0.169	0.468
Loin depth, [3]mm	70.30	70.95	0.354	0.187
Lean, [3,4]%	54.00	54.15	0.108	0.319

[1]A total of 1,263 pigs (Line 337 × 1050: PIC Hendersonville, TN; initially 101.9 kg) were used in a 27-d study with 25 to 27 pigs per pen and 24 pens per treatment.

[2]Percentage yield was calculated by dividing HCW by live weight obtained at the farm before transport to the packing plant.

[3]Adjusted using HCW as a covariate.

[4]Calculated using NPPC (2001) equation.

Table 6.9 Effects of added zinc during the grower and/or finisher phase on growth performance and carcass characteristics of finishing pigs fed diets with or without ractopamine HCl (RAC; Exp. 3)[1]

Added Zn d 0-45:	-	-	-	-	+	+	+	+		Probability[2], *P* <				
Added Zn d 45-72:	-	-	+	+	-	-	+	+						
Added RAC d 45-72:	-	+	-	+	-	+	-	+	SEM	Zn grower × RAC	Zn finisher × RAC	Zn grower	Zn finisher	RAC
d 0 to 45														
ADG, kg	0.887	—	—	—	0.889	—	—	—	0.006	—	—	0.859	—	—
ADFI, kg	2.496	—	—	—	2.584	—	—	—	0.024	—	—	0.016	—	—
G:F	0.356	—	—	—	0.344	—	—	—	0.0030	—	—	0.008	—	—
d 45 to 72														
ADG, kg	0.898	1.066	0.859	1.069	0.900	1.116	0.852	1.100	0.014	0.034	0.066	0.134	0.015	0.001
ADFI, kg	2.897	2.823	2.846	2.887	2.907	2.912	2.859	2.996	0.048	0.103	0.025	0.299	0.647	0.305
G:F	0.310	0.378	0.302	0.370	0.310	0.383	0.299	0.368	0.005	0.595	0.737	0.927	0.003	0.001
d 0 to 72														
ADG, kg	0.887	0.949	0.880	0.949	0.892	0.966	0.878	0.956	0.012	0.450	0.698	0.549	0.293	0.001
ADFI, kg	2.647	2.599	2.602	2.633	2.688	2.705	2.676	2.717	0.047	0.301	0.164	0.225	0.879	0.565
G:F	0.336	0.365	0.338	0.361	0.332	0.357	0.329	0.352	0.005	0.793	0.439	0.174	0.338	0.001
BW, kg														
d 0	58.7	—	—	—	58.7	—	—	—	—	—	—	0.996	—	—
d 45	99.0	98.9	98.9	99.0	99.1	99.1	99.0	99.1	2.48	0.985	0.946	0.966	0.989	0.972
d 72	118.7	122.9	118.0	123.6	119.4	124.0	118.0	124.2	2.70	0.754	0.400	0.865	0.700	0.001
Carcass Characteristics														
HCW, kg	86.0	88.2	86.0	90.0	85.4	88.7	87.1	89.5	2.21	0.950	0.873	0.960	0.404	0.026
Yield,[3] %	74.09	74.64	74.12	75.35	73.09	73.98	75.52	74.08	1.349	0.534	0.657	0.719	0.383	0.740
Backfat thickness,[4] mm	16.75	13.81	15.69	14.86	16.29	14.13	16.28	13.67	0.627	0.565	0.345	0.720	0.785	0.001
Loin depth,[4] mm	62.64	64.59	61.71	63.12	61.99	65.52	61.58	66.06	1.127	0.158	0.898	0.386	0.485	0.002
Lean,[4,5] %	53.13	55.13	53.69	55.13	53.52	55.01	53.33	55.94	0.572	0.644	0.697	0.737	0.375	0.001

[1]A total of 1,197 pigs (Line 337 × 1050: PIC Hendersonville, TN) were used in a 72-d study with 25 pigs per pen and 6 pens per treatment.
[2]No interactive effects ($P > 0.154$) of Zn grower × Zn finisher × RAC or Zn grower × Zn finisher.
[3]Calculated by dividing HCW by live weight obtained at the farm.
[4]Adjusted using HCW as a covariate.

[5]Calculated using NPPC (2001) equation.

Table 6.10 Effects of added Zn during the grower and/or finisher phase on plasma and fecal Zn concentrations of finishing pigs fed diets with or without ractopamine HCl (RAC; Exp. 3)[1]

Added Zn d 0-45:	-	-	-	-	+	+	+	+		Probability[2,3,4], *P* <			
Added Zn d 45-72:	-	-	+	+	-	-	+	+		Zn finisher	Zn	Zn	
Added RAC d 45-72:	-	+	-	+	-	+	-	+	SEM	× RAC	grower	finisher	RAC
Zn intake, mg/d													
d 0-45	252	—	—	—	382	—	—	—	4.0	—	0.001	—	—
d 45-72	278	330	441	520	279	341	443	539	6.8	0.002	0.281	0.001	0.001
d 0-72	263	279	321	354	343	366	400	447	5.9	0.004	0.001	0.001	0.001
Plasma Zn, μg/mL													
d 0	1.33	—	—	—	1.38	—	—	—	0.043	—	0.422	—	—
d 45	1.34	—	—	—	1.34	—	—	—	0.029	—	0.980	—	—
d 52	1.23	1.29	1.36	1.31	1.22	1.25	1.25	1.33	0.038	0.571	0.254	0.013	0.204
d 63	1.25	1.34	1.37	1.34	1.34	1.32	1.39	1.41	0.037	0.428	0.269	0.011	0.560
Fecal Zn, ppm													
d 45	591	—	—	—	1,038	—	—	—	17.7	—	0.001	—	—
d 63	854	988	1,197	1,460	922	941	1,262	1,533	59.1	0.032	0.371	0.001	0.001

[1]A total of 1,197 pigs (PIC 337 × 1050) were used in a 72-d study with 25 pigs per pen and 6 pens per treatment. On d 0 and 45 the median weight barrow from each pen was ear tagged to allow for bleeding on subsequent collection dates. On day 52 and 63, four median weight barrows, including the previously selected pig were selected from each pen for blood collection. On d 45 and 63, fecal grab samples were collected on 3 random pigs per pen

[2]For Zn intake, no interactive effects ($P > 0.073$) of Zn grower × Zn finisher × RAC, Zn grower × Zn finisher, or Zn grower × RAC.

[3]For plasma Zn, no interactive effects ($P > 0.083$) of Zn grower × Zn finisher × RAC, Zn grower × Zn finisher, or Zn grower × RAC.

[4]For fecal Zn, no interactive effects ($P > 0.477$) of Zn grower × Zn finisher × RAC, Zn grower × Zn finisher, or Zn grower × RAC.

Chapter 7 - Effect of dietary zinc and ractopamine-HCl on pork chop muscle fiber type distribution, tenderness, and color characteristics

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ABSTRACT

A total of 320 finishing pigs (PIC 327 × 1050; initially 98 kg) were used to determine the effects of adding Zn to diets containing Ractopamine HCl (**RAC**) on muscle fiber type distribution, fresh chop color and cooked meat characteristics. Dietary treatments were fed for approximately 35-d and consisted of: a corn-soybean meal–based negative control (**CON**); a positive control diet with 10 ppm of RAC (**RAC+**); and the RAC+ diet plus 75, 150, or 225 ppm added Zn from either ZnO or Availa-Zn. Loins randomly selected from each treatment (n = 20) were evaluated using contrasts: CON vs RAC+, interaction of Zn level × source, Zn level linear and quadratic polynomials, and Zn source. There were no Zn source effects or Zn source × level interactions throughout the study ($P > 0.10$). Pigs fed RAC+ had increased ($P < 0.02$) percentage type IIX and a tendency for increased ($P = 0.10$) percent type IIB muscle fibers. Increasing added Zn decreased (linear, $P = 0.01$) percentage type IIA and tended to increase ($P = 0.09$) IIX muscle fibers. On d 1, 2, 3, 4, and 5 of display, pork chops from pigs fed the RAC+ treatment had greater ($P < 0.03$) L^* values compared to the CON. On d 0 and 3 of display, increasing added Zn tended to decrease (quadratic, $P = 0.10$) L^* values and decreased (quadratic, $P < 0.03$) L^* values on d 1, 2, 4, and 5. Pigs fed RAC+ had decreased ($P < 0.05$) a^* values on d 1 and 4 of display and tended to have decreased ($P < 0.10$) a^* values on d 0 and 2 compared to CON pork chops. RAC+ treated pork chops had a tendency for increased ($P < 0.08$) oxymyoglobin percentage

compared to CON pork chops on d 1, 2, 4, and 5. On d 0, as dietary Zn increased in RAC+ diets, there was a decrease (linear, $P < 0.01$) in the formation of pork chop surface oxymyoglobin percentage. RAC+ decreased ($P < 0.001$) metmyoglobin reducing ability (**MRA**) of pork chops on d 5. Chops from pigs fed added Zn had increased (quadratic, $P < 0.03$) MRA on d 3 and 5 of the display period. There was a trend for increased (linear, $P = 0.07$) cooking loss with increasing Zn in RAC diets and treatments did not affect tenderness as measured by Warner-Bratzler shear force ($P > 0.07$). In conclusion, RAC+ diets produced chops that were lighter and less red, but maintained a greater percentage of surface oxymyoglobin throughout a 5-d simulated retail display. RAC+ reduced MRA at the end of the display period, but supplementing Zn to RAC diets restored MRA to near CON treatment levels at the end of the display period.

INTRODUCTION

Ractopamine-HCl (**RAC**; Paylean®, Elanco Animal Health, Greenfield, IN) is a beta-adrenergic agonist that is approved to be fed to finishing swine during the final 20 to 40 kg of weight gain prior to harvest. Utilizing this growth promoting technology in pigs improves live performance and carcass characteristics (Apple et al., 2007). The trace-mineral Zn has been suggested to increase the RAC response based on studies showing that added Zn will improve ADG and G:F (Patience and Chipman, 2011; Rambo et al., 2012).

While these studies provide justification to increase the amount of Zn in RAC containing diets, no research has demonstrated the effect of these diets on fresh meat characteristics including color and cooked meat characteristics. Color is the single most important attribute consumers evaluate when making a purchasing decision (Hedrick et al., 1994; Mancini and Hunt, 2005). Feeding RAC can produce LM that is darker and less red (Apple et al., 2008). Tenderness

constitutes the most important attribute consumers evaluate during their eating experience (Sanders et al., 2007). Meta-analysis indicates RAC increases shear force by 0.5 kg (Dunshea et al., 2005). In a subsequent meta-analysis, decreases in tenderness occurred in a dose dependent manner as RAC inclusion level was increased (Apple et al., 2007). Both of these characteristics are influenced by muscle fiber type and the metabolic profile associated with these fibers (Ryu and Kim, 2005; Lee et al., 2010). Because RAC possesses a history of altering muscle fiber types (Aalhus et al., 1992; Depreux et al., 2002), the possible synergistic effect that RAC and Zn could elicit on muscle hypertrophy may alter the muscle's fiber type distribution to affect shelf-life and tenderness. Therefore, the objective of this study was to evaluate the effects of adding Zn to RAC containing diets on muscle fiber type distribution, fresh chop color, and cooked meat characteristics.

MATERIALS AND METHODS

Live Animal Management

The Kansas State University Institutional Animal Care and Use Committee approved the protocol used in this experiment.

A total of 320 finishing pigs (PIC 327 × 1050) with an average initial BW of 98 kg were housed at the Kansas State University Swine Teaching and Research Center. The finishing barn was an environmentally controlled facility with 1.5 m^2 slatted-floor pens. Each pen was equipped with a dry self-feeder and a nipple waterer to provide ad libitum access to feed and water. Two replications of the same barn were used. Within each replication, two 40 pen groups (24 barrow pens and 16 gilt pens or 16 barrow pens and 24 gilt pens per group) were subjected to

the experimental treatments. Therefore, 4 groups of pigs were subjected to the following experimental procedures separately from one another.

Pens of pigs were allotted to 1 of 8 dietary treatments, with 2 pigs housed in each pen with a total of 20 pens per treatment. Dietary treatments consisted of: a corn-soybean meal–based negative control diet formulated to 0.66% standardized ileal digestible (SID) Lys (**CON**); a positive control diet formulated to contain 0.92% SID lysine and 10 ppm of RAC (**RAC+**); the RAC+ diet plus 75, 150, or 225 ppm added Zn from ZnO; or the RAC+ diet plus 75, 150, or 225 ppm added Zn from Availa-Zn (Zinpro, Eden Prairie, MN; Table 1). All diets contained 55 ppm Zn from $ZnSO_4$ provided by the trace mineral premix. Experimental diets were fed in meal form, and ZnO or Availa-Zn was added to the RAC diet at the expense of corn. Diets were fed for the last 41 days prior to slaughter for group 1 of the first barn replication and the last 35 days for the remaining groups of pigs in the barn replications.

Harvest and Sample Collection

At the completion of the feeding period, pigs were transported to the Kansas State University Meats Laboratory for harvest under Federal Inspection. After a 24 h post-slaughter chilling period, a 30.48 cm portion of the *longissimus lumborum* muscle (beginning at the 10^{th} rib) from the left side of one randomly selected pig from each pen was collected for immunohistochemistry and fresh pork quality analysis. Additionally from this sample, 24-h pH was measured using a Hanna HI 99163 meat pH probe (Hanna Instruments, Smithfield, RI) inserted into the 11^{th}-12^{th} rib interface, and a 2.54 cm thick chop was collected from this location to be used for immunohistochemical analysis. The remainder of the muscle sample was vacuum packaged and stored at 2 ± 3°C for 13 d postmortem.

Immunohistochemistry

A 1 cm^2 portion of muscle was collected from the geometric center of each chop designated for immunohistochemistry . After collection, the muscle was embedded in Optimal Cutting Temperature (OCT) tissue embedding media (Fisher Scientific, Pittsburgh, PA), frozen by submersion in supercooled isopentane, and stored at -80°C until analysis. For each sample, two 10 µm cryosections were collected on frost resistant slides (Fisher Scientific) and the methods of Gonzalez et al. (2008) were followed for immunodetection with modifications. Non-specific antigen binding sites were inhibited by incubating cryosections in 5% horse serum and 0.2% TritonX-100 in phosphate buffered saline (**PBS**) for 30 min. All sections were incubated with the following primary antibodies in blocking solution for 60 min: 1:50 α-dystrophin (Thermo Scientific, Waltham, MA); 1:10 supernatant myosin heavy chain, slow, IgG2b (BA-D5, Developmental Studies Hybridoma Bank, University of Iowa, Iowa City, IA); 1:10 supernatant myosin heavy chain type 2A, IgG1 (SC-71, Developmental Studies Hybridoma Bank); and 1:10 supernatant myosin heavy chain type 2B, IgM (BF-F3, Developmental Studies Hybridoma Bank, University of Iowa, Iowa City, IA). After incubation, sections were washed with PBS 3 times for 5 m, followed by incubation in the following secondary antibodies (1:1000) in blocking solution for 30 m: Alexa-Fluor 488 goat anti-mouse IgM for BF-F3 (Invitrogen, San Diego, CA); Alexa-Fluor 594 goat anti-mouse IgG1for SC-71 (Invitrogen); Alexa-Fluor 633 goat anti-mouse IgG2b for BA-D5 (Invitrogen); and Alexa-Flour 594 goat anti-rabbit H&L for α-dystrophin (Invitrogen). Additionally, 1:1000 Hoechst Dye 33342 (Invitrogen) was utilized to identify all fiber associated nuclei. Finally, sections were washed for 3 five min periods in PBS and then covered with 5 µL of 9:1 glycerol in PBS and were coverslipped for imaging.

Cryosections were imaged using a Nikon Eclipse TI-U inverted microscope with 10× working distance magnification (Nikon Instruments Inc., Melville, NY). Four representative

photomicrographs per section were captured using a Nikon DS-QiMc digital camera (Nikon Instruments Inc.) that was calibrated to the 10× objective. For myosin heavy chain fiber type data collection, a minimum of 2 photomicrographs per section (minimum 500 fibers per animal) were analyzed for isoform distribution utilizing NIS-Elements Imaging Software (Basic Research, 3.3; Nikon Instruments Inc.). Fibers that were positive for the BA-D5 antibody were counted as type I fibers. Fibers strongly stained only for SC-71 or BF-F3 were labeled as type IIA and type IIB fibers, respectively. Fibers stained weakly for both SC-71 and BF-F3 were labeled as type IIX fibers.

Chop Cutting and Simulated Retail Display

At the conclusion of the 13 d aging period, loin muscles were removed from their packages and cut into five 2.54 cm thick chops. The first 4 chops were utilized for simulated retail display. Of these chops, the first three chops were used for d 0, 1, and 3 metmyoglobin reducing ability analysis and the fourth chop was used for 5-d intact packaged chop surface color attributes including the collection of *L**, *a**, and *b**values and spectral data for the calculation of surface myoglobin redox forms. The fourth chop was also utilized for d 5 metmyoglobin reducing ability analysis. The final chop was immediately subjected to mechanical tenderness analysis by Warner-Bratzler shear force.

All chops allocated to simulate retail display were placed on white 1S Styrofoam trays (Genpack, Glens Falls, NY) with a Dri Loc (Dri-Loc 50, Cryovac Sealed Air Corporation, Duncun, SC) absorbent pad and overwrapped with PVC film (MAPAC M [1,450 cc/0.06m^2/24 h, 72 gauge], Bordon Packaging and Industrial Products, North Andover, MA). Chops were placed in coffin-style retail cases (Model DMF 8, Tyler Refrigeration Corporation, Niles, MI) at 3 ± 2˚C. Cases were constantly illuminated with fluorescent lights (32 W Del-Warm White

3000˚K, Philips Lighting Company, Somerset, NJ) that emitted a constant 24 h case average intensity of 2,143 ± 113 lx. Every 12 h, chops were rotated from left to right and front to back in the cases to account for variation in temperature and light intensity. Absolute CIE *L**, *a**, and *b**, and spectral reflectance (400 to 700 nm) readings were taken at 3 locations on each 5-d retail display chop through the PVC packaging using a Hunter Lab Miniscan EZ spectrophotometer (Illuminant A, 2.54 cm diameter aperture, 10˚ observer; Hunter Associates Laboratory, Reston, VA). Absolute and spectral values from the 3 scans were averaged and reflectance at 473 nm, 525 nm, 572nm, and 700 nm were used to calculate surface percentages of metmyoglobin and oxymyoglobin using the equations from Krzywicki (1979) as published in the AMSA Color Guidelines (AMSA, 2012).

Metmyoglobin Reducing Ability

The procedures of Gonzalez et al. (2009) and Watts et al. (1966) were followed for metmyoglobin reducing ability (**MRA**) with modifications. On the day of analysis, chops were pulled from the retail display case and cut into 5 cm x 5 cm portions that were indicative of the discoloration pattern for the entire chop. Each section was placed in a 400 mL beaker and oxidized in 100 mL of 0.3% sodium nitrite at 25 ± 2˚C for 20 min. After the samples were blotted of excess solution, they were vacuum packaged in 25.4 × 30.5 cm Prime Source Vacuum Pouches (3 mil standard barrier, Bunzl Processor Division, Koch Supplies, Kansas City, MO) that possess an oxygen transmission rate of 4.5 cc/1002/24 h/23˚C/65% relative humidity. Reflectance measurements (400 nm to 700 nm) were collected initially after vacuum packaging and every 30 min for 2 h using a Hunter Lab Miniscan EZ spectrophotometer (Illuminant A, 2.54 cm diameter aperture, 10˚ observer; Hunter Associates Laboratory). Metmyoglobin was

calculated as described above. Metmyoglobin reducing ability was calculated as (observed decrease in metmyoglobin concentration ÷ initial metmyoglobin concentration) × 100.

Warner-Bratzler Shear Force Analysis

The AMSA (1995) guidelines for instrumental cooked meat tenderness were followed for shear force analysis. Fresh cut chops were weighed and a thermocouple wire (30-gauge and constantan, Omega Engineering, Stamford, CT) was inserted into the geometric center of each chop for internal temperature monitoring using a Doric Minitrend 205 monitor (VAS Engineering, San Francisco, CA). Chops were cooked on electric, open-hearth Farberware grills (Model 450-A, Yonkers, NY) to an internal temperature of 35°C, then flipped and cooked to a final internal temperature of 71°C. After cooked chops were chilled overnight at 7 ± 1°C, six 1.27 cm diameter cores were extracted from each chop parallel to the muscle fiber orientation. Each core was sheared once through the center of the core perpendicular to the muscle fiber orientation using an Instron Model 5569 Testing Machine (Instron, Canton, MA) with a Warner-Bratzler shear head attached (100 kg compression load cell, crosshead speed of 250 mm/min). Cooking loss was determined by measuring the difference in chop weight before and after cooking and dividing by precooked chop weight.

Statistics

All data were analyzed as a generalized randomized complete block design using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC) with pen as the experimental unit and animal/chop as the observational unit. For cooked meat analysis and fiber isoform distribution, dietary treatment served as the fixed effect while gender within group within barn was included as random effects. Contrast statements consisted of: (1) negative control vs. positive control RAC diet, (2) interaction between increasing Zn level and Zn source, (3) increasing Zn linear

and quadratic polynomials, and (4) added Zn from ZnO vs. Availa-Zn. For shelf-life analysis, the statistical structure was the same except for day of display and the day by treatment interaction served as fixed effects in addition to dietary treatment. Day of display by dietary treatment interaction was evaluated by the following contrast: (1) interaction between negative control vs. positive control RAC diet and day of display, (2) interaction between increasing Zn level and day of display, (3) interaction between Zn source and day of display, and (4) interaction between increasing Zn level, Zn source, and day of display. Day of display also served as the repeated measure with chop as the subject. Statistical significance was determined at $P < 0.05$ and trends or tendencies were determined at $0.05 > P < 0.10$.

RESULTS

For all dependent variables observed in the study, there was no Zn source effect or an interaction between Zn source and level ($P > 0.10$). Therefore, all data presented will combine the Zn treatment groups by the level of supplementation which consists of 75 ppm (**75Zn**), 150 ppm (**150Zn**), and 225 ppm (**225Zn**) Zn.

Utilizing immunohistochemical techniques, the effect of the dietary treatments on myosin heavy chain isoform distribution was evaluated (Figure 1). Our data indicated that the percentage of type I muscle fibers were not affected by RAC+ or RAC diets with added Zn ($P > 0.10$). There was no difference ($P = 0.16$) in the percentage of type IIA fibers when comparing CON and RAC+ treated muscles. However, there was a decrease (linear, $P = 0.01$) in percentage of type IIA fibers as Zn concentration increased. Loin muscle samples from pigs fed the RAC+ treatment had a decreased ($P = 0.02$) percentage of type IIX muscle fibers compared to the CON. There was only a tendency for an increased (linear, $P = 0.09$) percentage of type IIX fibers when supplemental Zn was added to the RAC diet. For type IIB fibers, pigs in the RAC+ treatment

had a tendency to possess more ($P = 0.10$) fibers than CON pigs. Finally, adding supplemental Zn to the RAC diet did not affect ($P > 0.10$) the percentage of type IIB muscle fibers in the loin.

Pork chops from all treatment groups were displayed under simulated retail conditions for 5 d and daily objective measures of pork color were collected. For L^*, a^*, and b^* values (Figure 2), only a^* and b^* values were affected by day of display (quadratic, $P < 0.05$). Pork chops of pigs from the RAC+ treatment did not differ ($P > 0.10$) in L^* values compared to CON pork chops on d 0 of the display period. On d 1, 2, 3, 4, and 5, pork chops from pigs fed the RAC+ treatment had greater L^* values compared to the CON pork chops ($P < 0.03$). On d 0 and 3 of display, increasing the Zn content of the diet resulted in a trend for lower L^* values (quadratic, $P = 0.10$). In addition, on d 1, 2, 4, and 5 of the display period, increasing Zn reduced the L^* values (quadratic, $P < 0.03$). Of the Zn treatment groups, pork chops from pigs in 150Zn treatment possessed the lowest L^* values over the entire display period. Pork chops from RAC+ pigs possessed lower a^* values compared to CON pork chops on d 1 and 4 of display ($P < 0.05$). However, RAC+ chop a^* values tended to be lower on d 0 and 2 of display ($P < 0.10$). On d 4 of display, a^* values were greater (quadratic, $P = 0.04$) as Zn was added to the diet, with 150Zn chops obtaining in the greatest a^* value. For all other display days, increasing dietary Zn in the diet did not affect a^* values ($P > 0.10$). On d 0 of display, b^* values of pork chops from RAC+ pigs were not different ($P > 0.10$) from CON pigs. However, for the remainder of the display period chops from RAC+ pigs possessed lower b^* values than CON pigs ($P < 0.04$). On d 1 of display, adding Zn to the diet decreased (quadratic, $P = 0.03$) b^* values, with the lowest response detected in the Zn75 group. On d 2 and 4, supplemental Zn tended to decrease b^* values, with Zn150 exhibiting the lowest value ($P < 0.09$).

The objective measures of chop surface oxymyoglobin and metmyoglobin percentages indicate that there was a day effect on both redox forms ($P < 0.001$; Figure 3). For oxymyoglobin, there was an increase (quadratic, $P < 0.001$) in chop surface percentages from d 0 to d 1, but thereafter the surface percentage of oxymyoglobin decreased. On d 0 of the display period, the percentage of oxymyoglobin formed on the surface of chops was not different ($P = 0.63$) between CON and RAC+ treated pork chops. However on the same day of display, as dietary Zn increased from 0 to 225 ppm in RAC diets, there was a decrease (linear, $P < 0.01$) in the formation of pork chop surface oxymyoglobin percentage. For the remaining days of display, RAC+ treated pork chops had a tendency for greater surface oxymyoglobin percentage compared to CON pork chops on d 1, 2, 4, and 5 ($P < 0.08$). For metmyoglobin surface percentage, as the day of display increased, the surface percentage of metmyoglobin increased (quadratic, $P < 0.001$); however, inclusion of a dietary RAC or supplemental Zn did not affect chop surface metmyoglobin accumulation ($P > 0.10$).

As expected, all chops exhibited a reduction ($P < 0.0001$) in MRA as the day of display increased (Figure 4). At the beginning of the display period, chop MRA ranged from 52% to 56%. When compared to the CON treatment, RAC+ did not affect MRA on this day or d 1 and 3 of the display period ($P > 0.10$). By d 5 of display, chop MRAs ranged from 31% to 42%. Inclusion of RAC in the diet reduced ($P < 0.001$) MRA compared to the CON pork chops. While supplemental Zn did not affect MRA on d 0 and 1 ($P > 0.10$) of display, as the level of dietary Zn was increased, there was an increase (quadratic, $P < 0.03$) in MRA on d 3 and 5 of the display period. Seventy-five ppm of added Zn was sufficient to maximize the MRA of RAC-treated pork chops on d 3 of display while 150 ppm of added Zn resulted in the greatest MRA on d 5.

Chops from RAC+ pigs did not differ in pH, cooking loss, or shear force values when compared to CON chops (Table 2; $P > 0.10$). However, there was a trend for increased (linear, $P = 0.07$) cooking loss as dietary Zn increased from 0 to 225 ppm in RAC diets.

DISCUSSION

Our method of muscle fiber type assignment is in agreement with Lefaucheur et al. (2002), who utilized the same set of antibodies in the LM of the pig. Similar to our findings, the authors reported that SC-71 recognized both type IIA and IIX fibers, with the IIA fibers staining more intensely than the IIX fibers. In addition, our fiber isoform distribution pattern was similar to the distribution reported by the authors with a greater percentage of type IIB fibers (46 to50%), a moderate percentage of type IIX fibers (25 to 32%), and low percentages of type I (8%) and IIA (14 to11%) fibers.

Numerous reviews document the effect that muscle fiber distribution can have on fresh meat quality and color (Klont et al., 1998; Lee et al., 2010). In our study, we found that feeding 10 ppm of RAC to pigs during the final 35 d of feeding did not have an effect on type I or type IIA fiber percentage, but decreased the percentage of type IIX fibers while tending to increase the percentage of type IIB fibers. Using histological techniques, Aalhus et al. (1992) reported that RAC supplemented at 20 ppm did not affect the percentage of red (type I) fibers, but the percentage of intermediate fibers (type IIA/X) were reduced while white fibers (type IIB) were increased. Depreux et al. (2002) also found that type I and IIA fibers in the LM were unaffected by either 20 or 60 ppm of RAC supplementation as detected by ELISA. In agreement with the current study, the researchers reported that there was a strong significant correlation ($R = -0.768$) between decreases in type IIA/X fibers and increases in type IIB fibers. Gunawan et al. (2007) also reported that RAC supplemented at 20 ppm increased the mRNA expression of type IIB

fibers at the expense of type IIX fibers, while type I expression was unaffected. Interestingly, the authors found that type IIA expression decreased 96 h after initial RAC administration and continued to decrease for approximately one week. However by the end of the 4 week trial, type IIA expression returned to pre-supplementation levels. In the current study, we demonstrate that increasing Zn in the RAC containing diets decreased the type IIA isoform throughout the feeding period, which tended to correspond to increases in type IIX fibers. Therefore, this data indicate that Zn supplementation could have inhibited the reestablishment of the pre-supplementation type IIA fiber pool, as seen by Gunawan et al. (2007), which catalyzed a decrease in the percentage of type IIA while increasing type IIX fibers.

The literature documents many of the live production and carcass characteristic advantages of feeding RAC and these advantages serve as the main incentive for pork producers to utilize RAC in their operations (Apple et al., 2007; Bohrer et al., 2013). However, the literature contains mixed and variable results when examining the effect of RAC on cooked chop tenderness and other fresh meat quality characteristics. In the present study, we observed that neither RAC nor Zn supplementation elicited an effect on 24-h pH. Others have demonstrated that increasing the percentage of type IIB fibers in the muscle negatively impacts the extent of pH decline (Ryu et al., 2006) and that this decline is due to significantly greater glycogen and lactate concentrations produced by the type IIB fibers in the early postmortem period (Choe et al., 2008). Therefore, the effect of the RAC induced fiber shift toward more type IIB fibers and this effect on pH development becomes a major concern. Since we did not experience ultimate pH development differences between treatments, we attribute this finding to our RAC+ treatment only increasing the percentage of type IIB fibers by less than 5%. The lack of a RAC effect on ultimate pH reported here is in agreement with studies examining the RAC response on

numerous variables including breed (Stroller et al., 2003), heavy weight/late finishing pigs (Fernandez-Duenas et al., 2008; Kutzler et al., 2011), or pigs raised under commercial conditions (Athayde et al., 2012). Of the numerous studies that have found a significant effect of RAC on ultimate pH (Carr et al., 2005; Apple et al., 2008; James et al., 2013), pH values were increased by 0.07 to 0.08. The lack of 24-h pH effects indicates that our treatments did not affect postmortem ultimate pH; therefore, this demonstrates that the meat quality attributes measured in the study are independent of treatment catalyzed ultimate pH differences.

In a survey of consumers, Sanders et al. (2007) found that pork tenderness was important to 57 percent of consumers and these consumers were willing to pay a $0.82/kg premium for a guaranteed juicy and tender product. Hence, efficiently producing meat products that possess the peak palatability attributes is the goal of livestock producers. There are no data available that demonstrate the effect of Zn on tenderness and the available data evaluating the impact of beta-agonists are inconsistent. When feeding beta-agonists, the poor tenderness of products harvested from animals fed these compounds is attributed to two mechanisms. The first mechanism is the lack of postmortem proteolytic activity (Wheeler and Koohmaraie, 1992); while the second is large increases in muscle fiber hypertrophy (Carr et al., 2005). Numerous studies in swine indicate that RAC supplementation can decrease tenderness as measured by WBSF by as much as 29% (Uttaro et al., 1993; Herr et al., 2001; Athayde et al., 2012), while other studies report no RAC effect on tenderness (Stoller et al., 2003; Apple et al., 2008; Kutzler et al., 2011). Stroller et al. (2003) reported that while they detected a significant RAC effect on WBSF, their trained sensory panel was unable to identify a significant RAC influence on tenderness and juiciness. Carr et al. (2005) found that when feeding RAC at 10 or 20 ppm, both WBSF and sensory panel tenderness scores indicated that RAC increased chop toughness. Our data falls into the category

of studies that were unable to detect a RAC effect on tenderness. Even though RAC+ increased the cross-sectional area of type IIA and IIX fibers (Paulk et al., 2014), aging the loins for 2 wk may have negated the effect these larger fibers had on tenderness. Xiong et al. (2006) reported that subjecting pork from RAC fed animals for extended aging duration improved pork tenderness which suggests that there is a sufficient amount of postmortem proteolytic activity in RAC supplemented pigs to improve tenderness.

A concerning trend we detected in our study was that when Zn was added to RAC diets, moisture retention during cooking tended to be reduced. As dietary Zn increased, cook loss increased by up to 2.09 percent in the 225Zn treatment group. Therefore, this increase in moisture loss during cooking could have a negative effect on consumer palatability due to the reduction of moisture in the cooked product and the loss of the benefits associated with increased juiciness. Our major concern is that with this loss of moisture, an increased percentage an increased percentage of pork from pigs fed both RAC and Zn could be perceived by the consumer as not being tender.

The literature contains a limited amount of comprehensive studies that document the effect that RAC or Zn elicit on fresh chop color during a simulated retail display period. Apple et al. (2008) conducted a 5-d retail shelf-life study and reported that there was no interaction between RAC, day of display, and dietary fat source. Therefore, the authors reported only the main effects, which indicated that over the 5-d retail display period, RAC chops were slightly darker, and less red and yellow than control chops. Our study produced RAC+ chops that were divergent in fresh color characteristics when compared to CON chops. Compared to the data of Brewer et al. (1998) and Joo et al. (1995), who utilized different illuminants that the current study (F and D65, respectively), all chops in the current study had greater L^* values than chops

that were categorized as pale soft and exudative (**PSE**). Specifically compared to Brewer et al. (1998), chops from RAC+ pigs had *L** values that were near values of PSE chops (65.91), while CON chop values were more near normal chop values (53.09). Chops from RAC+ pigs were lighter and less yellow in color on d 1-5 of display, and less red on d 0, 1, 2 and 4 of display. Additionally, RAC+ chops possessed more surface oxymoyglobin on all days of display except d 0 and 3. These findings are consistent with studies which indicate that RAC supplemented chops are lighter than non-supplemented chops when color is measured at 24 h postmortem (Armstrong et al., 2004, Leick et al., 2010). Bergstrom (2011) did not find a RAC induced reduction in the lightness of chops, but found that RAC supplementation reduced chop *a** and *b** values over a 6-d shelf life period. However, Rickard et al. (2012) found that RAC increased redness values during 7-d of display when products were stored at 4°C for 30 d or -20°C for 60 d. But when chops were immediately displayed after a 24 h chill, RAC decreased chop redness compared to the non-RAC chops. Because our chops were aged between the 24-h and 30-d storage period and RAC decreased redness, it appears that the RAC effect on objective color characteristics is dependent on length of storage.

Carr et al. (2005) hypothesized that the LM of RAC supplemented pigs possessed lower *a** values because of the shift of intermediate fibers to white fibers. The authors also concluded that the lower *a** values were an indication of reduced oxymyoglobin formation on the surface of the chops which was a result of rapid fiber hypertrophy and dilution of myoglobin in the muscle. In our study, we also found that RAC+ chops tended to possess more type IIB fibers than CON chops. However, we found that surface oxymyoglobin content was greater on the surface of RAC+ chops. This could be due to RAC+ chops containing less mitochondria because of their greater type IIB isoform distribution. When muscle possesses a copious amount of

mitochondria, myoglobin must compete with these organelles for oxygen to consume which results in less oxymyoglobin formation (Klont, 1998). Therefore, since the RAC+ chops possessed a fiber type distribution that favored the presence of less mitochondria, more oxygen was available for consumption by the myoglobin in the muscle which increased the formation of oxymyoglobin. This hypothesis is supported by work in beef that demonstrates that the inside *Semimembranosus* muscle (greater surface oxymyoglobin content) possesses a lower oxygen consumption rate when compared to the outside *Semimembranosus* (Sammel et al., 2002).

An interesting trend we detected is that addition of Zn to the RAC diets seemed to shift chop color characteristics away from RAC+ values and toward CON chops values. Quadratic Zn effects were detected on d 3 for *a** values, d 1-5 for *L** values, and d 1, 2, and 4 for *b** values. For d 0 oxymyoglobin percentage, there was a linear Zn effect on this day, with increasing Zn in the diet resulting in a decrease in oxymyoglobin percentage. Ma et al. (2012) reported that when Zn was deleted from the swine diet, *a** and *b** values were reduced. While the authors did not give an explanation behind these value drops, our results show that Zn supplementation to RAC diets can have a restorative effect on objective color values. Additionally, since Zn supplementation decreased the amount of type IIA fibers resulting in more IIX and IIB fibers, we believe this finding is the result of the same mechanism described previously.

The same trend of Zn supplementation restoring the color characteristics of RAC+ chops to that of CON chops can also be seen in the MRA data. The RAC effect on MRA was not detected until d 5 of the display period when RAC+ chops possessed 11.6 percent less MRA than CON chops. This finding can be a function of the type IIB fiber type shift and reduction of mitochondria that also reduces the amount of NADH in the muscle (Howlett and Willis, 1998). Thus, the MRA of the muscle is limited because there is less NADH to reduce metmyoglobin

formation (Mancini and Hunt, 2005). Gonzalez et al. (2008) conducted a RAC study in beef that looked at the same shelf-life characteristics as the current trial. In contrast to this study, the authors did not find a RAC effect on oxymoyglobnin and metmyoglobin formation, or MRA of LM steaks displayed for 5-d. However as was seen in our study, at the end of the display period, steaks supplemented with RAC began to have reduced MRA. While the difference between RAC+ and CON MRA detected at d 5 did not translate to increases in chop surface metmyoglobin formation, extending the display period could demonstrate that the RAC induced reduction in MRA may result in greater metmyoglobin formation on the surface of these chops. When additional Zn was added to the diet, there was a quadratic Zn effect in which adding 150 ppm of Zn maximized MRA by 9.2 percent over RAC+ chops. This same effect was seen at d 3, where adding 75 ppm of Zn to the diet increased MRA by 6.3 percent over RAC+ chops. While the exact mechanism is unknown, we hypothesize that the IIA fiber type shift could play a crucial role in establishing the optical oxygen consumption rate and MRA as hypothesized by McKenna et al. (2005). Additionally, if extending the display period proves that RAC reduces color stability during extended display, Zn supplementation can serve as a countermeasure to these effects as indicated by the ability of the Zn treatments to restore MRA and *a** values close to control values.

CONCLUSION

Feeding pigs 10 ppm of RAC during the final 35 d before slaughter decreased the amount of type IIX fibers while tending to increase the percentage of type IIB fibers in the *Longissimus lumborum*. Supplementing the RAC diets with dietary Zn above the NRC requirement decreased the percentage of type IIA fibers and tended to increase the percentage of type IIX fibers. These fiber shifts had effects on meat color characteristics. Ractopamine-HCl produced chops that

were lighter and less red, but maintained a higher percentage of surface oxymyoglobin throughout a 5-d simulated retail display. While RAC improved these shelf-life characteristics, it reduced MRA at the end of the display period. Supplementing Zn to RAC diets restored MRA to near CON treatment levels at the end of the display period which is most important to retailers. Zinc supplementation tended to increase chop cook loss, which may impact sensory attributes of the chops and should be explored further.

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Table 7.1 Diet composition (as-fed basis)[1,2]

Item	Control	RAC
Ingredient, %		
Corn	83.06	74.24
Soybean meal, (46.5% CP)	15.22	23.97
Monocalcium P, (21% P)	0.25	0.20
Limestone	0.75	0.78
Salt	0.35	0.35
Vitamin premix[3]	0.075	0.075
Trace mineral premix[4]	0.075	0.075
L-Lys HCl	0.15	0.15
DL-Met	—	0.015
L-Thr	—	0.025
Phytase[5]	0.075	0.075
Ractopamine HCl[6]	—	0.05
Total	100	100
Calculated analysis, %		
Standardized ileal digestible (SID) amino acids, %		
Lys	0.70	0.92
Ile:Lys	71	70
Leu:Lys	179	158
Met: Lys	31	30
Met & Cys: Lys	65	60
Thr: Lys	63	64
Trp: Lys	19	19
Val: Lys	84	79
Total Lys, %	0.79	1.03
CP, %	14.3	17.6
ME, Mcal/kg[7]	3.362	3.358
NE, Mcal/kg[7]	2.301	2.269
SID Lys: ME (g/Mcal)	2.08	2.74
Ca, %	0.41	0.44
P, %	0.39	0.42
Available P, %	0.21	0.21

[1] Diets were fed in meal form for the duration of the experiment.

[2] Dietary treatments were obtained by replacing corn in the Ractopamine-HCl diet to achieve 75, 150, and 225 ppm added Zn from ZnO (Zinc Nacional S.A., Monterrey, Mexico) or Availa-Zn (Zinpro, Eden Prairie, MN).

[3]Vitamin premix provided 3,307 IU Vitamin A, 413 IU Vitamin D_3, 13 IU Vitamin E, 1.32 mg Vitamin K, 11.6 μg Vitamin B_{12}, 14.9 mg niacin, 8.27 mg pantothenic acid, and 2.48 mg riboflavin per kilogram of the complete diet.

[4] Trace mineral premix provided 16.53 mg Mn, 55.06 mg Fe, 55.06 mg Zn, 8.25 mg Cu, 0.15 mg I, and 0.15 mg Se per kilogram of the complete diet.

[5]Phyzyme 600 (Danisco Animal Nutrition, St. Louis, MO) provided 450.4 phytase units (FTU)/kg, with a release of 0.1% available P.

[6] Provided 10 ppm of Ractopamine HCl (Paylean; Elanco Animal Health, Greenfield, IN).
[7]Values for ingredients were derived from NRC (1998).

Table 7.2 LSMEANS of pork *Longissimus lumborum* chop cooked meat characteristics from pigs supplemented Ractopamine-HCl and three levels of dietary zinc

			Zinc, ppm[1,2]				*P* - Value		
Item	Control	RAC+[1]	75	150	225	SEM	RAC	Zn Linear	Zn Quadratic
Cooking loss, %	24.74	23.54	25.06	24.60	25.63	0.98	0.30	0.07	0.70
Shear force, kg	3.56	3.55	3.76	3.63	3.73	0.14	0.97	0.44	0.59
pH[3]	5.44	5.43	5.44	5.46	5.44	0.02	0.89	0.67	0.43

[1]10 ppm of Ractopamine-HCl (Paylean; Elanco Animal Health, Greenfield, IN) fed during the experiment.
[2]Dietary treatments were obtained by replacing corn in the Ractopamine-HCl diets to achieve 75, 150, and 225 ppm added Zn from ZnO (Zinc Nacional S.A., Monterrey, Mexico) or Availa-Zn (Zinpro, Eden Prairie, MN).
[3]pH collected at 24-h postmortem.

Figure 7.1 Tenth rib *Longissimus lumborum m*yosin heavy chain isoform distribution of pigs fed a basal diet containing 0 ppm Ractopamine-HCl (CON), pigs supplemented 10 ppm Ractopamine-HCl (RAC+), and pigs supplemented 10 ppm Ractopamine-HCL and 75 ppm (75Zn), 150 ppm (150Zn), or 225 ppm (225Zn) of zinc.

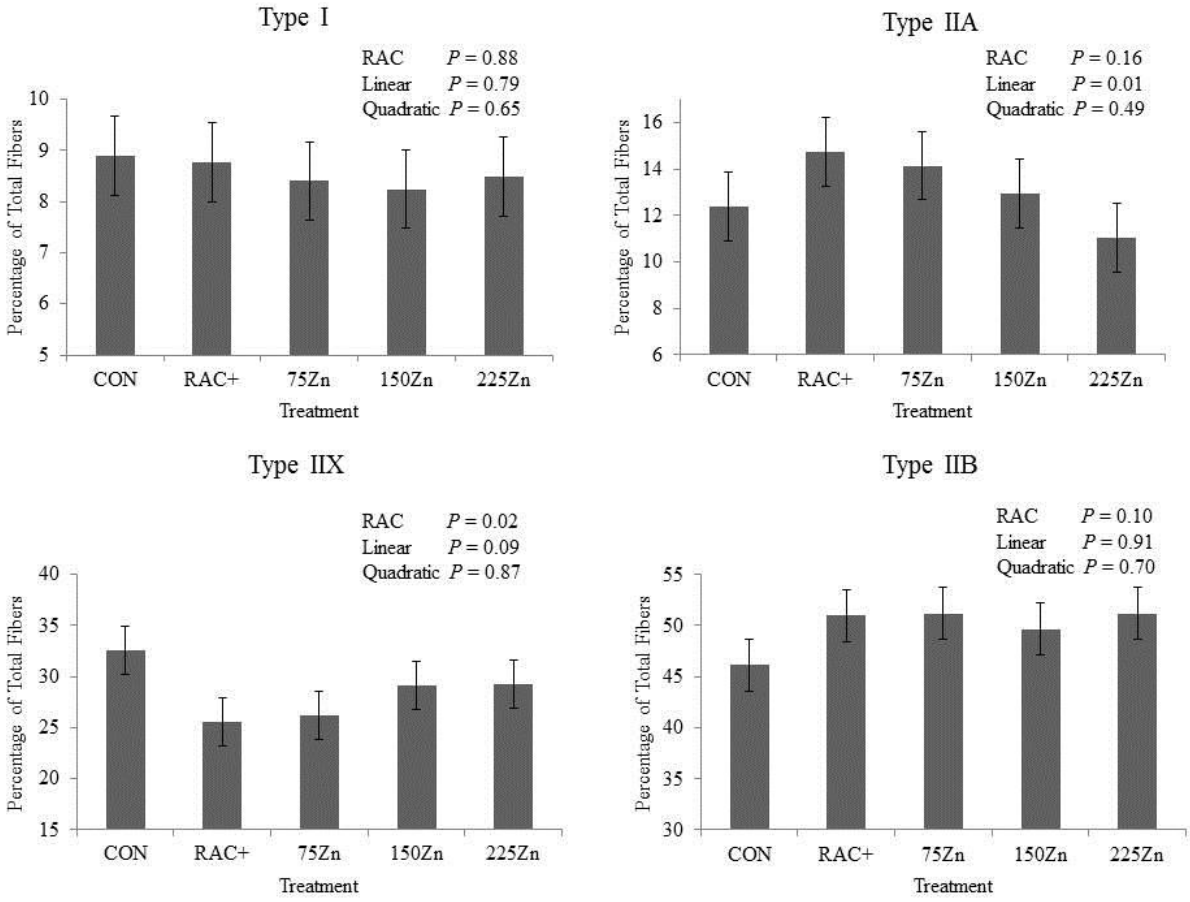

Figure 7.2 Surface *L* a* b values of loin chops from pigs fed a basal diet containing 0 ppm Ractopamine-HCl, pigs supplemented 10 ppm Ractopamine-HCl, and pigs supplemented 10 ppm Ractopamine-HCl and 75 ppm (75Zn), 150 ppm (150Zn), or 225 ppm (225Zn) of zinc.**

*L** = Lightness (0 = Black; 100 = White), *a** = Redness (-60 = Green; 60 = Red), and *b** = Yellowness (-60 = Blue; 60 = Yellow). R designates a Ractopamine-HCl effect and Q designates a quadratic zinc effect. The superscript * indicates a significant effect ($P \leq 0.05$), while the superscript # indicates marginal significance ($P \leq 0.10$).

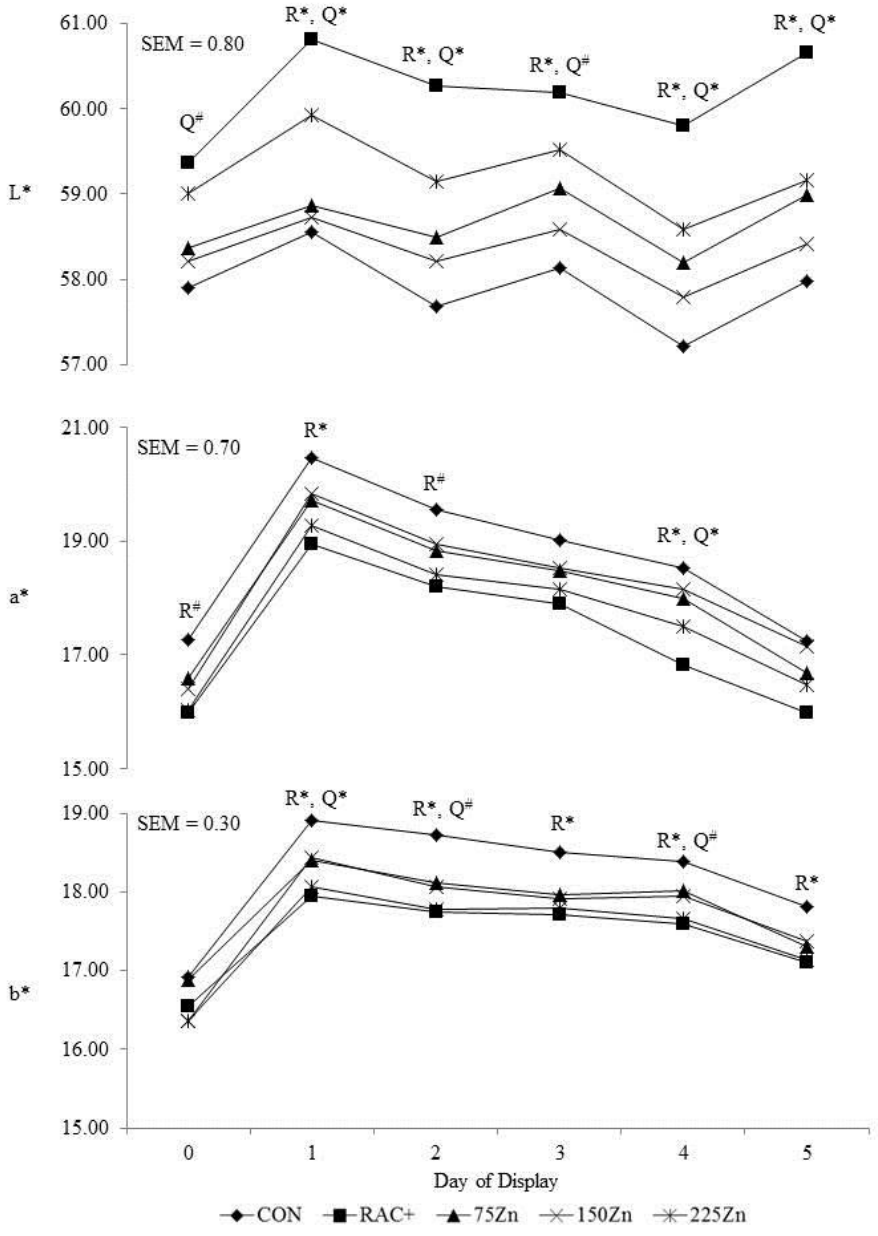

Figure 7.3 Surface oxymyoglobin and metmyoglobin percentages of loin chops from pigs fed a basal diet containing 0 ppm Ractopamine-HCl, pigs supplemented 10 ppm Ractopamine-HCl, and pigs supplemented 10 ppm Ractopamine-HCL and 75 ppm (75Zn), 150 ppm (150Zn), or 225 ppm (225Zn) of zinc.

R designates a Ractopamine-HCl effect and L designates a linear zinc effect. The superscript * indicates a significant effect ($P \leq 0.05$), while the superscript # indicates marginal significance ($P \leq 0.10$).

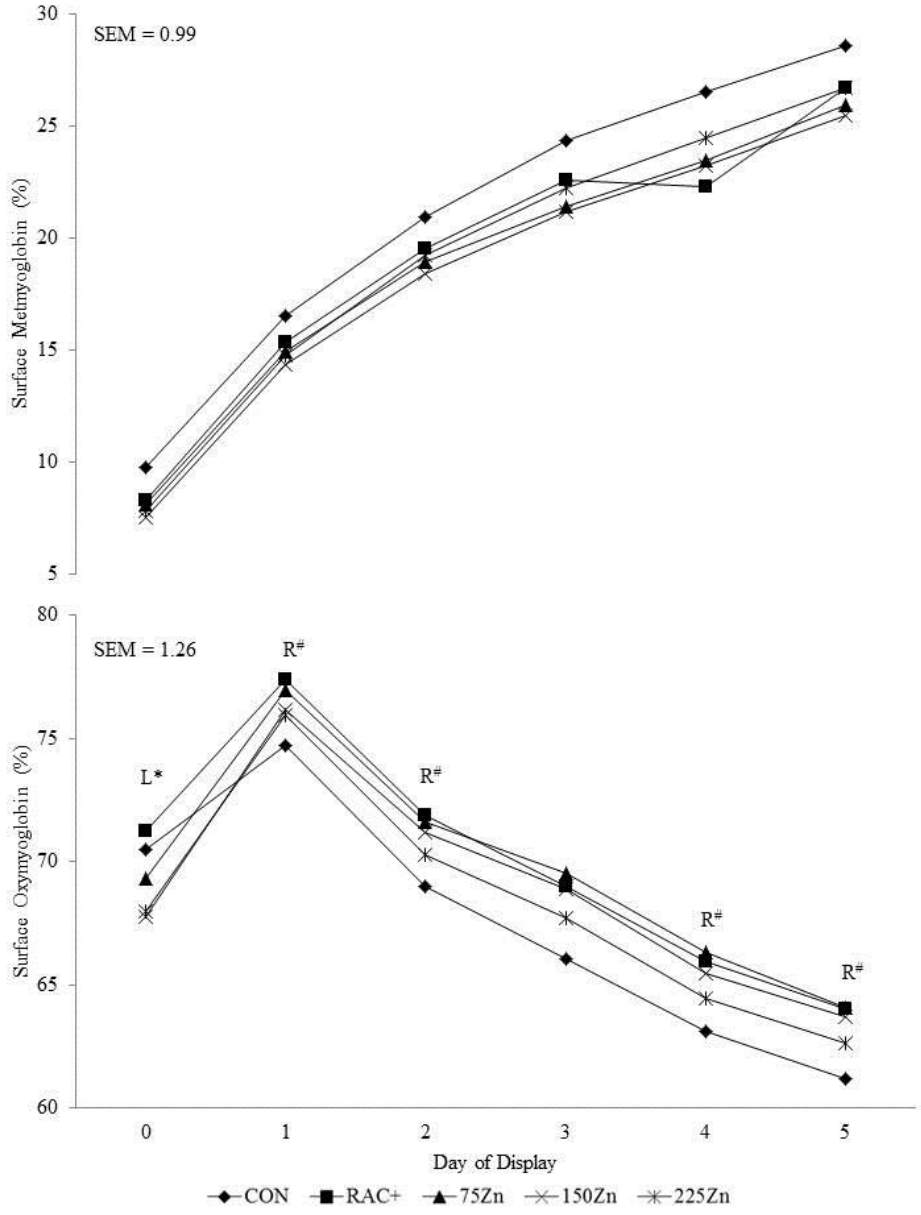

Figure 7.4 Metmyoglobin reducing ability of loin chops pigs fed a basal diet containing 0 ppm Ractopamine-HCl, pigs supplemented 10 ppm Ractopamine-HCl, and pigs supplemented 10 ppm Ractopamine-HCl and 75 ppm (75Zn), 150 ppm (150Zn), or 225 ppm (225Zn) of zinc.
R designates a Ractopamine-HCl effect and Q designates a quadratic zinc effect. The superscript * indicates a significant effect ($P \leq 0.05$).