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## The utilization of red blood cells in diets for swine and poultry

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# THE UTILIZATION OF RED BLOOD CELLS IN DIETS FOR SWINE AND POULTRY

A Thesis

Submitted to the Graduate Faculty of the  
Louisiana State University and  
Agricultural and Mechanical College  
in partial fulfillment of the  
requirements for the degree of  
Master of Science

in

The Interdepartmental Program in Animal Sciences

by  
Emily D. Frugé  
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## **ABSTRACT**

The purpose of this research was to determine if increasing levels of RBC would affect growth performance and carcass characteristics of finishing pigs and growth performance of broilers. Three experiments were conducted to determine the effect of incremental levels of red blood cells (RBC; 0 to 4% and 0 to 2%), and thus increasing levels of dietary Leu on growth performance and linear carcass measurements of finishing pigs. Our results suggest that feeding 3 or 4% RBC causes a decrease in growth performance. However, feeding 1 or 2% RBC in the diets of finishing pigs had no detrimental effects on growth performance. Three experiments were conducted to determine the effect of incremental levels of RBC (0, 0.5, 1, 2, 3, 4, 5, 6, and 7%) on growth performance of broilers fed diets with supplemental L-Arg and L-Ile (adequate) and diets with no supplemental L-Arg and L-Ile (deficient). The results of this research indicate that up to 6% RBC can be added to a broiler diet without affecting growth performance as long as the diet is supplemented with L-Arg and L-Ile. Furthermore, up to 3% RBC can be added to broiler diets without supplemental Arg and Ile with no detrimental effects on growth performance. Broilers respond quite differently in growth performance to increasing levels of RBC compared with finishing pigs.

# CHAPTER 1

## INTRODUCTION

Increasing feed costs and the need to improve the efficiency of livestock has caused nutritionists to depend on alternative protein sources and increasing use of supplemental amino acids (AA). Blood products (blood meal and blood cells), which are by-products of the slaughter industry, are commercially available to producers and are widely utilized in nursery pig diets (Wahlstrom and Libal, 1977; Kats et al., 1994; DeRouchey et al., 2002; and Kerr et al., 2004b) but not in poultry diets (Tyus II et al., 2008). The main disadvantage of utilizing blood products is a deficiency of Ile. DeRouchey et al. (2002) analyzed blood meal and red blood cells (RBC) and reported that blood meal contains 0.96% Ile; whereas, blood cells contain 0.37% Ile. As the dietary levels of RBC increase, soybean meal (SBM) decreases, which results in a further decrease in dietary Ile. Thus, Ile supplementation to a diet containing RBC is critical to maintain growth performance of both swine and poultry (Parr et al., 2003; Hale et al., 2004; Kerr et al., 2004a; Kidd et al., 2004; Parr et al., 2004; and Dean et al., 2005).

One possible advantage of utilizing blood products is that they contain relatively high levels of Leu. DeRouchey et al. (2002), reported that blood meal contains 11.86% Leu and that blood cells contain 12.60% Leu. Researchers recently reported that infusion of rats (Garlick and Grant, 1988 and Garlick, 2005) and neonatal pigs (Escobar et al., 2005 and Escobar et al., 2007) with Leu resulted in increased muscle protein synthesis. Researchers also have evaluated the effects of excess dietary Leu on carcass characteristics and fat content of muscle of finishing pigs (Cisneros et al., 1996; Hyun and Baker, 2003; and Yu et al., 2007). Because RBC are very high in Leu

(DeRouchey et al., 2002) and readily accessible to producers, RBC may be utilized as a more economical alternative to crystalline Leu in swine and poultry diets.

Therefore, the purpose of this research was to determine the effects of incremental levels of RBC in a corn-soybean meal (C-SBM) diet on growth performance and carcass characteristics of finishing pigs and on growth performance of broiler chicks.

## **CHAPTER 2**

### **REVIEW OF LITERATURE**

#### **INTRODUCTION**

The need to increase efficiency of livestock without increasing the cost of the diet or reducing meat quality has become more important due to recent increases in feed costs. One possible way to increase efficiency is to increase lean gain of livestock. Leucine has been linked to increases in muscle protein synthesis in rats and neonatal pigs (Garlick and Grant, 1988; Escobar et al., 2005; Garlick, 2005; and Escobar et al., 2007). Crystalline Leu also has been evaluated in diets of finishing pigs because of the suggestion that excess levels of Leu result in the production of lipid precursors, which in turn, could affect carcass characteristics (Cisneros et al., 1996; Hyun and Baker, 2003; and Yu et al., 2007). Additionally, blood products such as blood meal and blood cells have a high concentration of Leu but are deficient in Ile (DeRouchey et al., 2002). Blood products are widely used in the diets for nursery pigs because of improvements in growth performance (Kats et al., 1994 and DeRouchey et al., 2002).

#### **LEUCINE AND MUSCLE PROTEIN SYNTHESIS**

Researchers suggest that Leu stimulates muscle protein synthesis, but the mechanism is not clear. Utilizing rat diaphragms, Buse and Reid (1975) reported that Leu was responsible for the uptake of radio-labeled precursors into muscle protein and that it plays an important role in protein synthesis and regulation of protein turnover. Fulks et al. (1975) also reported that a mixture of branched-chain amino acids (BCAA) increased protein synthesis and decreased protein degradation in rat diaphragms. McNurlan et al. (1982) reported that infusing rats with Leu failed to stimulate protein synthesis. They concluded that the high levels of insulin and free AA found in the

muscle represented maximal synthesis. Furthermore, they suggest that Leu stimulates muscle synthesis mostly in catabolic states (fasting/starvation, *in vitro* studies). Garlick and Grant (1988) reported that when young fasted rats were infused with a mixture of AA and insulin, the BCAA had a major impact on the sensitivity of muscle protein to insulin, which resulted in an increase in muscle protein synthesis. Anthony et al. (2001) reported that in post-absorptive rats when insulin is held constant, there is no lasting response to feeding Leu on muscle protein synthesis. Thus, the research of Anthony et al. (2001) supports the hypothesis that Leu acts in conjunction with insulin. In a review on Leu regarding the regulation of protein metabolism, Garlick (2005) suggested that Leu does not act alone, but acts with insulin in the regulation of protein synthesis.

Many studies have been conducted examining the role of insulin and AA in neonatal pigs. Wray-Cahen et al. (1998) and Davis et al. (2001) suggested that when glucose and AA levels were near fasting, insulin stimulated muscle protein synthesis in 7 d old neonatal pigs, and this response to insulin decreases with age (d 26). Davis et al. (2002) also reported that increases in insulin or AA increased muscle protein synthesis and this response declined with age. When insulin and AA were infused together, there was an increase in protein synthesis, but this increase was not greater than the responses found with either insulin or AA alone. Davis et al. (2002) suggests that insulin and AA, though responsive by themselves, may be interacting within the same skeletal muscle pathways. O'Connor et al. (2002) reported similar results. When AA are infused alone with no rise in plasma insulin, there is an increase in protein synthesis. O'Connor et al. (2002) suggested that AA and insulin work independently of each other in the neonatal pig. Escobar et al. (2005) reported that when neonatal pigs were infused with Leu, without stimulating an increase in circulating insulin, there was

increased muscle protein synthesis. However, after 60 min of infusion, there was no response to Leu in skeletal muscle. Recently, Escobar et al. (2007) evaluated the same effects of Leu as before, but instead they infused a mixture of additional AA to determine if prolonged (120 min) Leu infusion would increase protein synthesis in the neonatal pig. They reported that when additional AA were infused with Leu, muscle protein synthesis increased but not when Leu was infused alone. This response is greater in 7 d old pigs and decreases in older neonates (d 26). Thus, the response to Leu is dependent upon AA availability and age of animal. Additionally, Escobar et al. (2007) reported that in neonatal pigs, Leu acts on protein synthesis through specific translation initiation factors.

#### **SUPPLEMENTAL LEUCINE ADDITION IN DIETS FOR SWINE**

Supplemental Leu has been utilized in swine finishing diets to examine the effects on growth performance and carcass characteristics. Cisneros et al. (1996) evaluated the effects of feeding interval, Leu supplementation, and AA deficiency on growth and carcass measurements. Cisneros et al. (1996) were particularly interested in evaluating the fat content of the carcass with supplemental Leu because dietary Leu and Ile in excess could possibly supply lipid precursors. They reported no effect of Leu supplementation on growth performance or carcass characteristics. Hyun and Baker (2003) reported that a relatively high level of crystalline Leu (2.0%) supplemented to finishing pig diets decreased growth performance. However, Leu supplementation resulted in increased intramuscular fat and marbling of the longissimus muscle. Recently, Yu et al. (2007) fed a combination of conjugated linoleic acid and Leu. They reported no effects of Leu or conjugated linoleic acid on growth performance, but intramuscular fat was increased with Leu supplementation.

## **UTILIZATION OF BLOOD PRODUCTS IN DIETS FOR SWINE AND POULTRY**

Blood products, such as blood cells and blood meal, are used when evaluating the Ile requirement for swine (Parr et al., 2003; Kerr et al., 2004a; Parr et al., 2004; and Dean et al., 2005) and poultry (Hale et al., 2004 and Kidd et al., 2004), because blood products are deficient in Ile; however, they are relatively high in Leu. Other researchers evaluated the effects of blood cells and blood meal on growth performance of swine. DeRouchey et al. (2002) compared blood meal and blood cells in diets of nursery pigs, and reported an increased growth response to both blood products compared with the control, which contained no blood products. There was a greater response to blood meal during the first week with no responses in growth to either blood product during the second week. Kerr et al. (2004b) evaluated the effects of blood cells and Ile in nursery pig diets. Red blood cell inclusion up to 4% did not affect growth performance; however, inclusion of 5% or greater RBC resulted in decreased growth performance. Kerr et al. (2004b) also reported that performance was restored to that of the control with supplemental Ile. Kats et al. (1994) evaluated the effects of blood meal of different species on nursery pig performance. Regardless of specie, blood meal addition to the diets improved growth performance up to d 28 post-weaning; however, there was no response to blood meal after d 28 post-weaning. Wahlstrom and Libal (1977) evaluated the effects of blood meal as a protein source in growing and finishing pigs. They reported that when 4% drum-dried blood meal replaced an equivalent amount of protein from SBM, there was reduced growth performance of growing pigs. When the diet was supplemented with Lys, growth performance was restored to that of the control. Inclusion of 8% blood meal greatly reduced growth performance. Furthermore, feeding 6% rotary steam-dried blood meal to growing pigs and 5.5% to finishing pigs resulted in

equal performance compared with pigs fed the control. Growth performance was not affected for pigs fed supplemental protein in the form of equal blood and meat meal, SBM and blood meal, and SBM and meat meal compared with pigs fed the control.

The effects of blood products on growth performance in poultry have been recently evaluated. Tyus II et al. (2008) reported that single comb white leghorn chicks fed 16.8% blood meal (100% protein source) experienced a reduction in growth performance compared with a diet with no blood meal. Birds fed an equal amount of protein from SBM and blood meal (11.2%) experienced increased growth performance compared with chicks fed blood meal alone (16.8%) or a mixture of blood meal and alfalfa meal (14.4%). Tyus II et al. (2008) concluded that replacing up to 50% of SBM in the diet with blood meal supplemented with Ile does not negatively affect growth performance. Kidd et al. (2004) reported that 18- to 30- d old broilers fed a C-SBM diet with 6% RBC and supplemental L-Arg·HCl and L-Ile had similar growth performance compared with the control. However, when diets were not supplemented with L-Ile, there was a significant decrease in growth performance compared with a diet containing no RBC.

## **CHAPTER 3**

# **EFFECT OF INCREMENTAL LEVELS OF RED BLOOD CELLS (RBC) ON GROWTH PERFORMANCE AND LINEAR CARCASS MEASUREMENTS OF FINISHING PIGS**

### **INTRODUCTION**

Researchers have shown that Leu increases muscle protein synthesis in rats and neonatal pigs (Garlick and Grant, 1988; Escobar et al., 2005; Garlick, 2005; and Escobar et al., 2007). Recently, the industry has moved to producing market hogs that have a higher fat content because of meat quality concerns (NPPC, 2000). The utilization of supplemental Leu has been evaluated in finishing pig diets due to the suggestion that excess Leu levels result in increased amounts of lipid precursors. The increased precursors could in turn affect the lean and fat content of the carcass (Cisneros et al., 1996; Hyun and Baker, 2003; and Yu et al., 2007). Additionally, blood products such as blood meal and blood cells have a high concentration of Leu but are deficient in Ile (DeRouchey et al., 2002). Blood products are widely used in nursery pig diets because they result in improved growth performance (Kats et al., 1994 and DeRouchey et al., 2002).

The objective of this research was to determine the effect of increasing incremental levels of RBC (total dietary Leu) on growth performance and carcass characteristics of finishing pigs.

### **MATERIALS AND METHODS**

All methods used in these experiments were approved by the LSU Agricultural Center Animal Care and Use Committee. Yorkshire, Yorkshire × Landrace, or Yorkshire × Landrace × Duroc pigs from the LSU Agricultural Center Swine Unit were used in all

experiments. Pigs were housed in an open-sided building with 1.5 × 3.0-m pens and concrete slotted floors. Pigs were allotted to dietary treatments based on sex, initial weight, and ancestry in a randomized complete block design. Dietary treatments were formulated to meet or exceed the nutrient requirements of barrows and gilts gaining 350 g of lean gain per day (NRC, 1998) and were formulated to contain 0.52% apparent ileal digestible (aid) Lys for barrows and 0.59% aid Lys for gilts. Diets were formulated on an aid basis because AA digestibility coefficients for RBC were only available on an aid basis (APC Inc., Ankeny, IA). All diets were formulated to contain 0.50% Ca and 0.45% total P (NRC, 1998). Feed in mash form and water were available *ad libitum*. All pigs and feeders were weighed every 14 d until completion of the trial.

#### Experiment 1

Experiment 1 was conducted to evaluate the level of supplemental RBC to a diet that would elicit a growth response and change linear carcass measurements in barrows and gilts. Forty barrows and 40 gilts with an average initial body weight (BW) of  $84.6 \pm 4.9$  and  $82.42 \pm 4.8$  kg and final BW of  $118.7 \pm 6.5$  and  $120.0 \pm 9.6$  kg, respectively were used. The 5 dietary treatments for barrows and gilts were a corn-soybean meal (C-SBM) control with 0, 1, 2, 3, or 4% RBC (Table 3.1). Each dietary treatment contained 2 replicates of barrows and 2 replicates of gilts with 4 pigs per replicate pen. Two barrows and 2 gilts per treatment replicate were randomly selected and slaughtered for collection of linear carcass measurements on d 43 and 50, respectively. Pigs were killed by jugular puncture after electrical stunning at the LSU Agricultural Center Meat Laboratory. Linear carcass measurements and fat and lean content from total body electrical conductivity (TOBEC; Model MQI-27; Meat Quality Inc., Springfield, IL) were determined as described by Matthews et al. (2001). Kilograms

of fat-free lean and percentage muscling were also determined as described by NPPC (2000).

## Experiment 2

Experiment 2 was similar to Experiment 1, except 0, 1, and 2% RBC were used (Table 3.1). Forty-eight barrows and 48 gilts with an average initial BW of  $82.5 \pm 6.4$  and  $79.2 \pm 7.0$  kg and final BW of  $125.5 \pm 6.2$  and  $119.8 \pm 8.8$  kg, respectively were used. Each dietary treatment contained 4 replicates of barrows and 4 replicates of gilts with 4 pigs per replicate pen. Before the start of the experiments, all pigs received the same finishing diet and blood was collected on d 0 (baseline) at 0700 and again on d 34. Blood was collected via vena cava puncture and placed in 10-mL tubes containing sodium heparin (BD Vacutainer, Franklin Lakes, NJ). Samples were placed on ice before centrifugation at  $3,000 \times g$  at  $0^{\circ}\text{C}$  for 20 min. Plasma was collected and samples were frozen until analysis for plasma urea nitrogen (PUN). One barrow and one gilt per treatment replicate were randomly selected and slaughtered for collection of linear carcass measurements on d 38 for 2 replicates, and on d 62 for the other 2 replicates. The pigs were killed and slaughtered as in Experiment 1, except TOBEC data were collected on 2 replicates due to mechanical complications. On the day of slaughter, viscera from each carcass was collected for full and empty viscera weights. For the empty viscera weights, the gastrointestinal tract was separated into the stomach, small intestine, large intestine, spleen, and colon. The remaining mesentery, bladder, and fat were weighed together. The remaining organs consisting of the heart, lungs, gall bladder, and liver were weighed together and recorded.

### Experiment 3

Experiment 3 was similar to Experiment 2 except only barrows were used. Thirty-six barrows with an average initial and final BW of  $86.0 \pm 5.7$  and  $133.4 \pm 8.4$  kg, respectively, were used. The 3 dietary treatments were 0, 1, and 2% RBC (Table 3.1). Each dietary treatment contained 4 replicates with 3 pigs per replicate pen. Pigs were bled, slaughtered, and data collected were the same as in Experiment 2, except that all pigs remaining on trial (total of 33) were slaughtered on d 49, and 2 pigs per treatment replicate were selected for collection of empty viscera weights.

### Statistical Analysis

Data were analyzed by ANOVA procedures using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC) as randomized complete block designs in a  $2 \times 5$  factorial arrangement for Experiment 1 and a  $2 \times 3$  factorial arrangement for Experiment 2 with the factors being sex and RBC level. Experiment 3 was analyzed as a randomized complete block design with weight as the blocking factor. The pen of pigs was the experimental unit for all data. The initial PUN was used as a covariate for final PUN values for Experiments 2 and 3. Orthogonal contrasts were used in all 3 experiments to determine linear and quadratic effects of RBC. An alpha level of 0.10 was used to determine treatment differences.

## **RESULTS**

In Experiment 1 (Table 3.2), final BW, ADG, and G:F decreased linearly ( $P < 0.10$ ) as RBC addition increased from 0 to 4%. Average daily feed intake was not affected by increasing RBC addition. Hot carcass weight decreased ( $P < 0.05$ ) linearly and quadratically with increasing RBC. There were no effects of RBC addition on loin

Table 3.1. Composition of corn-soybean meal diets with incremental levels of red blood cells in Experiments 1, 2, and 3, as-fed basis.<sup>1</sup>

Ingredient	Barrows					Gilts				
	Red blood cells, %									
	0	1	2	3	4	0	1	2	3	4
Corn	83.02	85.46	87.91	90.33	92.69	82.92	85.34	87.76	90.13	92.47
Soybean meal (47.5%)	14.02	10.51	7.00	3.49	---	14.02	10.51	7.00	3.50	---
Blood cells <sup>2</sup>	---	1.00	2.00	3.00	4.00	---	1.00	2.00	3.00	4.00
Monocalcium phosphate	0.58	0.64	0.71	0.77	0.84	0.58	0.64	0.71	0.77	0.84
Limestone	0.88	0.88	0.89	0.89	0.89	0.88	0.88	0.89	0.89	0.89
Salt	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Sodium bentonite	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Vitamin mix <sup>3</sup>	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.38
Mineral mix <sup>4</sup>	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
L-Lys·HCl	0.028	0.028	0.028	0.028	0.028	0.118	0.118	0.118	0.118	0.118
L-Trp	---	---	---	0.002	0.007	0.002	0.007	0.011	0.016	0.020
L-Ile	---	---	---	0.013	0.067	---	---	---	0.053	0.108
L-Thr	---	---	---	---	0.013	0.010	0.024	0.038	0.052	0.066
DL-Met	---	---	---	---	---	---	---	---	---	0.019
Calculated composition										
ME, kcal/kg	3,314	3,322	3,329	3,337	3,346	3,314	3,322	3,329	3,338	3,346
CP, %	13.57	13.03	12.49	11.95	11.46	13.66	13.13	12.60	12.11	11.62
Ca, %	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
P, %	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45
Ile, %	0.54	0.47	0.41	0.36	0.35	0.54	0.47	0.41	0.40	0.39
Leu, %	1.34	1.36	1.38	1.40	1.43	1.33	1.36	1.38	1.41	1.42
aid Lys, %	0.52	0.52	0.52	0.52	0.52	0.59	0.59	0.59	0.59	0.59
aid Met, %	0.20	0.19	0.19	0.18	0.17	0.20	0.19	0.18	0.18	0.19
aid TSAA <sup>5</sup> , %	0.41	0.39	0.37	0.35	0.33	0.41	0.39	0.37	0.35	0.35
aid Thr, %	0.37	0.35	0.34	0.32	0.32	0.38	0.38	0.38	0.38	0.38
aid Trp, %	0.11	0.10	0.10	0.09	0.09	0.11	0.11	0.11	0.11	0.11
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aid Ile, %	0.44	0.38	0.33	0.29	0.29	0.44	0.38	0.33	0.33	0.33
aid Val, %	0.51	0.54	0.56	0.59	0.62	0.51	0.54	0.56	0.59	0.62
aid Leu, %	1.15	1.19	1.23	1.26	1.30	1.15	1.19	1.23	1.26	1.30
aid His, %	0.31	0.35	0.38	0.42	0.45	0.31	0.35	0.38	0.42	0.45
aid Arg, %	0.694	0.627	0.560	0.493	0.426	0.694	0.627	0.560	0.493	0.426
aid Phe + Tyr, %	0.939	0.910	0.881	0.852	0.823	0.939	0.910	0.880	0.851	0.822

<sup>1</sup>A basal diet was mixed for each sex to contain the minimum of all ingredients except for RBC, soybean meal, L-Trp, L-Ile, L-Thr, DL-Met, and additional ingredients were added to each diet as needed.

<sup>2</sup>Innomax™ Porcine RBC, provided by Innovative Proteins: A division of PMI Nutrition International LLC, Brentwood, MO.

<sup>3</sup>Provided the following per kilogram of diet: vitamin A, 8,267 IU; vitamin D<sub>3</sub>, 2,480 IU; vitamin E, 66 IU; menadione, 6.2 mg; riboflavin, 10 mg; Ca-d-pantothenic acid, 37 mg; niacin, 66 mg; folic acid, 2.5 mg; pyridoxine, 3.3 mg; thiamin, 3.3 mg; and vitamin C, 83 µg.

<sup>4</sup>Provided the following per kilogram of diet: zinc, 127 mg; iron, 127 mg; manganese, 22 mg; copper, 12.7 mg; iodine, 0.80 mg; and selenium, 0.3 mg.

<sup>5</sup>TSAA = total sulfur amino acids.

<sup>6</sup>Amino acid values for corn and soybean meal (NRC, 1998) are on an apparent ileal digestible basis using coefficients from NRC, 1998. Amino acid values for blood cells are on apparent ileal digestible basis using coefficients from (APC, Inc., Ankeny, IA).

muscle area, 10<sup>th</sup> rib back fat, carcass length, or percent muscle; however, average back fat increased both linearly and quadratically ( $P < 0.08$ ) as RBC increased from 0 to 4%. There was a quadratic effect ( $P < 0.03$ ) on dressing percentage as RBC inclusion increased. Dressing percentage was increased by the 2% RBC addition and decreased by the 3 and 4% addition. There was a linear decrease ( $P < 0.03$ ) in weight of fat-free lean calculated from both NPPC and TOBEC with increased RBC addition. There was no effect ( $P > 0.10$ ) of RBC inclusion on total fat, lean:fat, percent lean, or percent fat determined by TOBEC. There was a significant sex effect ( $P < 0.06$ ) on initial BW, hot carcass weight, 10<sup>th</sup> rib backfat, average backfat, dressing percent, percent muscling, fat-free lean, lean:fat and percent fat (Table 3.3). No other responses were affected by sex ( $P > 0.10$ ).

In Experiment 2 (Table 3.4), there was a quadratic effect ( $P < 0.06$ ) of RBC addition on average backfat. Average backfat decreased at the 1% RBC addition, but returned to the level of the control at the 2% addition. Increasing RBC addition from 0 to 2% did not affect ( $P > 0.10$ ) final BW, ADFI, G:F, hot carcass weight, loin muscle area, 10<sup>th</sup> rib back fat, carcass length, dressing percent, percent muscle, fat-free lean from both NPPC and TOBEC, TOBEC total fat, lean:fat, percent lean, and percent fat, or percentages of full viscera, organs, and empty viscera sections. Plasma urea N decreased linearly ( $P < 0.02$ ) with increasing levels of RBC. There was a significant sex effect ( $P < 0.10$ ) on initial and final BW, ADFI, G:F, hot carcass weight, 10<sup>th</sup> rib backfat, organ weight, stomach percentage, percent muscling, total fat, lean:fat, percent lean, percent fat, and PUN. There was no effect ( $P > 0.10$ ) of sex on the remaining response variables (Table 3.5).

In Experiment 3 (Table 3.6), there were no effects ( $P > 0.10$ ) of RBC addition on final BW, ADG, ADFI, G:F, hot carcass weight, loin muscle area, 10<sup>th</sup> rib backfat, carcass length, dressing percent, organ weight, full viscera weight, stomach weight and percent, small intestine weight and percent, large intestine weight, colon weight and percent, spleen weight and percent, intestinal fat weight and percent, percent muscling, fat-free lean calculated from both NPPC and TOBEC equations, TOBEC total fat, lean:fat, percent lean, percent fat, and PUN. Average backfat linearly decreased ( $P < 0.03$ ), and full viscera and large intestine percent increased ( $P < 0.09$ ) as RBC increased in the diet.

## **DISCUSSION**

The objective of this research was to evaluate the effect of adding incremental levels (0 to 4%) of RBC to finishing pig diets. Previous research suggests a relationship between Leu addition and an increase in muscle protein synthesis as well as Leu increasing the production of lipid precursors. The relatively high Leu levels present in RBC may have the same positive effect on growth performance and carcass traits of finishing pigs. However, in Experiment 1 increasing levels of RBC decreased final BW, ADG, and G:F. Due to the linear decrease in final BW, there was a linear and quadratic decrease in hot carcass weight, which resulted in a linear decrease in fat-free lean calculated from both NPPC and TOBEC equations. There was an increase in average back fat and an increase in dressing percentage with increasing RBC levels. There were no other effects of RBC addition on the remaining linear carcass measurements. The increase in average backfat is consistent with Leu effects on increased lipid precursors (Cisneros et al., 1996; Hyun and Baker, 2003; and Yu et al., 2007), but we

Table 3.2. Growth performance and carcass measurements of barrows and gilts fed incremental levels of red blood cells in Experiment 1.

Growth response <sup>1</sup>	Red blood cells, %					SEM
	0	1	2	3	4	
Initial BW, kg	82.68	84.31	83.61	83.17	83.81	0.65
Final BW, kg <sup>2</sup>	121.08	121.25	119.66	120.39	114.35	1.60
ADG, kg <sup>2, 3</sup>	0.83	0.81	0.79	0.82	0.68	0.03
ADFI, kg	3.02	2.85	2.86	3.14	2.67	0.18
G:F, kg/kg <sup>2</sup>	0.28	0.29	0.28	0.27	0.25	0.01
Carcass measurements <sup>4</sup>						
Final BW, kg <sup>2</sup>	125.28	125.30	122.28	122.79	117.76	1.77
Hot carcass wt, kg <sup>2, 3</sup>	92.99	93.58	92.70	91.00	87.34	1.17
Loin muscle area, cm <sup>sq</sup>	49.49	51.09	47.80	46.40	46.15	2.44
10 <sup>th</sup> rib backfat, cm	2.16	2.02	2.36	2.36	1.92	0.17
Average backfat, cm <sup>2, 3</sup>	2.55	2.76	2.86	2.79	2.77	0.08
Carcass length, cm	86.04	84.54	84.22	84.38	84.54	0.64
Dressing percent <sup>3</sup>	74.23	74.66	75.82	74.15	74.15	0.41
Percent muscling <sup>5</sup>	52.88	53.71	51.75	51.40	53.68	1.22
Fat-free lean, kg <sup>2, 5</sup>	49.14	50.33	47.89	46.79	46.76	1.11
TOBEC analysis <sup>6</sup>						
Fat-free lean, kg <sup>2</sup>	49.34	49.59	47.55	47.18	46.46	1.00
Total fat, kg	27.28	25.62	27.64	27.13	24.37	1.53
Lean:fat	1.84	1.99	1.80	1.77	1.96	0.15
Lean, %	53.15	52.94	51.39	51.89	53.23	1.18
Fat, %	29.32	27.44	29.70	29.82	27.75	1.15

<sup>1</sup>Data are means of 2 replicates of barrows and 2 replicates of gilts with 4 pigs per replicate. Sex × treatment interaction was not significant ( $P > 0.10$ ); therefore, it was removed from the model.

<sup>2</sup>Linear effect, ( $P < 0.10$ ).

<sup>3</sup>Quadratic effect, ( $P < 0.08$ ).

<sup>4</sup>Data are means of 2 replicates of barrows and 2 replicates of gilts with 2 pigs per pen killed on d 43 and 50, respectively, for each treatment replicate.

<sup>5</sup>Calculated using the equation for ribbed carcasses described by the NPPC (2000).

<sup>6</sup>Calculated using total body electrical conductivity (TOBEC) analysis with equations from Higbie et al. (2002).

Table 3.3. Sex effects in Experiment 1.

Growth response <sup>1</sup>	Barrows	Gilts	SEM	P value
Initial BW, kg	84.61	82.42	0.41	0.01
Final BW, kg	118.65	120.04	1.01	0.35
ADG, kg	0.81	0.77	0.02	0.15
ADFI, kg	3.03	2.79	0.12	0.17
G:F, kg/kg	0.27	0.28	0.01	0.51
Carcass measurements <sup>2</sup>				
Final BW, kg	121.89	123.48	1.12	0.33
Hot carcass wt, kg	90.22	92.83	0.74	0.03
Loin muscle area, cm <sup>sq</sup>	46.34	50.03	1.54	0.12
10 <sup>th</sup> rib backfat, cm	2.49	1.83	0.11	0.01
Average backfat, cm	3.04	2.45	0.05	0.01
Carcass length, cm	84.33	85.16	0.41	0.18
Dressing percent	74.02	75.18	0.26	0.01
Percent muscling <sup>3</sup>	50.91	54.46	0.77	0.01
Fat-free lean, kg <sup>3</sup>	45.90	50.47	0.70	0.01
TOBEC analysis <sup>4</sup>				
Fat-free lean, kg	46.70	49.35	0.64	0.01
Total fat, kg	27.36	25.46	0.97	0.19
Lean:fat	1.73	2.01	0.09	0.06
Lean, %	51.74	53.30	0.75	0.16
Fat, %	30.35	27.25	0.91	0.03

<sup>1</sup>Data are means of 10 replicates of barrows and 10 replicates of gilts with 4 pigs per replicate.

<sup>2</sup>Data are means of 10 replicates of barrows and 10 replicates of gilts with 2 pigs per replicate killed on d 43 and 50, respectively, for each treatment replicate.

<sup>3</sup>Calculated using the equation for ribbed carcasses described by the NPPC (2000).

<sup>4</sup>Calculated using total body electrical conductivity (TOBEC) analysis with equations from Higbie et al. (2002).

Table 3.4. Growth performance and carcass measurements of barrows and gilts fed incremental levels of red blood cells in Experiment 2.

	Red blood cells, %			
Growth response <sup>1</sup>	0	1	2	SEM
Initial BW, kg	80.86	80.74	81.01	0.64
Final BW, kg	122.08	123.30	122.55	1.72
ADG, kg	0.84	0.86	0.85	0.03
ADFI, kg	3.16	3.12	3.17	0.11
G:F, kg/kg	0.27	0.28	0.27	0.01
Carcass measurements <sup>2</sup>				
Final BW, kg	126.14	123.66	125.23	2.28
Hot carcass wt, kg	96.47	93.78	95.25	1.76
Loin muscle area, cm <sup>sq</sup>	44.01	45.23	44.39	1.63
10 <sup>th</sup> rib backfat, cm	2.21	2.18	2.21	0.18
Average backfat, cm <sup>3</sup>	2.57	2.30	2.57	0.11
Carcass length, cm	85.09	84.11	83.53	0.92
Dressing percent	76.41	75.71	76.09	0.52
Organ wt, kg	3.93	3.71	3.57	0.15
Organ, %	3.12	3.02	2.85	0.12
Full viscera wt, kg <sup>4</sup>	7.81	7.93	8.25	0.38
Full viscera, %	6.21	6.43	6.58	0.32
Stomach wt, kg	0.61	0.59	0.57	0.02
Stomach, %	0.49	0.48	0.45	0.01
Small intestine wt, kg	1.13	1.40	1.42	0.19
Small intestine, %	0.90	1.13	1.13	0.15
Large intestine wt, kg	0.94	0.86	0.86	0.05
Large intestine, %	0.75	0.70	0.69	0.04
Colon wt, kg	0.31	0.30	0.29	0.02
Colon, %	0.24	0.24	0.24	0.02
Spleen wt, kg	0.21	0.22	0.20	0.01
Spleen, %	0.17	0.17	0.16	0.01
Intestinal fat, kg	2.57	2.57	2.68	0.13
Intestinal fat, %	2.02	2.08	2.13	0.09
Percent muscling <sup>5</sup>	51.39	51.97	51.59	1.06
Fat-free lean, kg <sup>5</sup>	49.10	48.52	48.94	1.00
TOBEC analysis <sup>6</sup>				
Fat-free lean, kg	44.89	43.00	46.82	2.25
Total fat, kg	22.69	20.41	22.95	2.11
Lean:fat	2.21	2.31	2.14	0.35
Lean, %	49.92	49.89	50.53	1.95
Fat, %	24.77	23.66	24.51	2.15
Plasma urea N (PUN)				
PUN, mg/dL <sup>7</sup>	11.28	10.95	9.88	0.59

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<sup>1</sup>Data are means of 4 replicates of barrows and 4 replicates of gilts with 4 pigs per replicate. Sex × treatment interaction was not significant ( $P > 0.10$ ); therefore, it was removed from the model.

<sup>2</sup>Data are means of 4 replicates of barrows and 4 replicates of gilts with 1 pig per pen killed on d 38 for replicates 3 and 4 and on d 62 for replicates 1 and 2. TOBEC data were collected on 2 replicates due to mechanical complications.

<sup>3</sup>Quadratic effect, ( $P < 0.07$ ).

<sup>4</sup>Full viscera weights are the weights of the whole viscera after removal from the carcass.

<sup>5</sup>Calculated using the equation for ribbed carcasses described by the NPPC (2000).

<sup>6</sup>Calculated using total body electrical conductivity (TOBEC) analysis with equations from Higbie et al. (2002).

<sup>7</sup>Linear effect, ( $P < 0.02$ ).

Table 3.5. Sex effects in Experiment 2.

Growth response <sup>1</sup>	Barrows	Gilts	SEM	P value
Initial BW, kg	82.50	79.24	0.52	0.01
Final BW, kg	125.50	119.79	1.40	0.01
ADG, kg	0.87	0.83	0.02	0.16
ADFI, kg	3.32	2.99	0.09	0.02
G:F, kg/kg	0.26	0.28	0.01	0.06
Carcass measurements <sup>2</sup>				
Final BW, kg	127.22	122.80	1.86	0.11
Hot carcass wt, kg	97.13	93.21	1.44	0.07
Loin muscle area, cm <sup>sq</sup>	43.60	45.49	1.33	0.33
10 <sup>th</sup> rib backfat, cm	2.47	1.93	0.15	0.02
Average backfat, cm	2.54	2.42	0.09	0.35
Carcass length, cm	84.55	83.94	0.75	0.57
Dressing percent	76.29	75.86	0.42	0.48
Organ wt, kg	3.89	3.58	0.12	0.10
Organ, %	3.06	2.93	0.10	0.37
Full viscera wt, kg <sup>3</sup>	8.03	7.96	0.31	0.88
Full viscera, %	6.33	6.48	0.26	0.68
Stomach wt, kg	0.58	0.60	0.01	0.41
Stomach, %	0.46	0.49	0.01	0.07
Small intestine wt, kg	1.36	1.27	0.15	0.68
Small intestine, %	1.08	1.03	0.12	0.79
Large intestine wt, kg	0.89	0.88	0.04	0.97
Large intestine, %	0.70	0.72	0.03	0.60
Colon wt, kg	0.29	0.31	0.02	0.52
Colon, %	0.23	0.25	0.01	0.26
Spleen wt, kg	0.21	0.21	0.01	1.00
Spleen, %	0.16	0.17	0.01	0.45
Intestinal fat, kg	2.55	2.66	0.10	0.43
Intestinal fat, %	2.00	2.16	0.07	0.13
Percent muscling <sup>4</sup>	50.20	53.10	0.86	0.03
Fat-free lean, kg <sup>4</sup>	48.62	49.29	0.81	0.57
TOBEC analysis <sup>5</sup>				
Fat-free lean, kg	43.74	46.07	1.84	0.40
Total fat, kg	25.99	18.04	1.72	0.01
Lean:fat	1.71	2.73	0.28	0.04
Lean, %	47.11	53.12	1.59	0.03
Fat, %	27.93	20.70	1.75	0.02
PUN, mg/dL	11.32	10.08	0.48	0.01

<sup>1</sup>Data are means of 12 replicates of barrows and 12 replicates of gilts with 4 pigs per replicate.

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<sup>2</sup>Data are means of 12 replicates of barrows and 12 replicates of gilts with 1 pig per replicate killed on d 38 for replicates 3 and 4 and d 62 for replicates 1 and 2.

<sup>3</sup>Full viscera weights are the weights of the whole viscera after removal from the carcass.

<sup>4</sup>Calculated using the equation for ribbed carcasses described by the NPPC (2000).

<sup>5</sup>Calculated using total body electrical conductivity (TOBEC) analysis with equations from Higbie et al. (2002).

Table 3.6. Growth performance and carcass measurements of barrows fed incremental levels of red blood cells in Experiment 3.

	Red blood cells, %			
Growth response <sup>1</sup>	0	1	2	SEM
Initial BW, kg	86.19	85.68	86.13	0.58
Final BW, kg	134.88	134.27	131.07	2.26
ADG, kg	1.01	1.00	0.93	0.05
ADFI, kg	3.73	3.65	3.59	0.16
G:F, kg/kg	0.27	0.28	0.26	0.01
Carcass measurements <sup>2</sup>				
Final BW, kg	134.88	133.99	131.07	2.30
Hot carcass wt, kg	99.77	98.51	97.48	1.48
Loin muscle area, cm <sup>sq</sup>	50.83	48.53	48.17	2.14
10 <sup>th</sup> rib backfat, cm	2.15	2.32	2.31	0.13
Average backfat, cm <sup>3</sup>	3.20	3.11	2.94	0.07
Carcass length, cm	85.62	86.52	85.94	0.61
Dressing percent	73.98	73.58	74.38	0.41
Organ wt, kg	3.53	3.65	3.65	0.14
Organ, % <sup>5</sup>	2.62	2.72	2.79	0.06
Full viscera wt, kg <sup>4</sup>	8.41	8.78	8.90	0.33
Full viscera, % <sup>3</sup>	6.24	6.54	6.80	0.19
Stomach wt, kg	0.58	0.56	0.61	0.02
Stomach, %	0.43	0.42	0.47	0.02
Small intestine wt, kg	1.60	1.63	1.59	0.06
Small intestine, %	1.19	1.21	1.21	0.03
Large intestine wt, kg	1.39	1.56	1.53	0.07
Large intestine, % <sup>3</sup>	1.03	1.16	1.17	0.05
Colon wt, kg	0.22	0.20	0.24	0.03
Colon, %	0.16	0.15	0.19	0.03
Spleen wt, kg	0.15	0.18	0.18	0.02
Spleen, %	0.11	0.13	0.14	0.01
Intestinal fat, kg	1.67	1.51	1.61	0.09
Intestinal fat, %	1.24	1.13	1.23	0.06
Percent muscling <sup>5</sup>	52.81	51.70	51.73	0.79
Fat-free lean, kg <sup>5</sup>	52.63	50.88	50.35	1.15
TOBEC analysis <sup>6</sup>				
Fat-free lean, kg	46.41	43.16	43.74	1.28
Total fat, kg	31.28	33.23	32.96	1.27
Lean:fat	1.53	1.31	1.35	0.10
Lean, %	46.51	43.83	45.04	1.44
Fat, %	31.30	33.68	33.68	1.03
Plasma urea N (PUN)				
PUN, mg/dL	12.79	11.24	10.72	1.65

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<sup>1</sup>Data are means of 4 replicates with 3 pigs per replicate.

<sup>2</sup>Data are means of 4 replicates with 3 pigs per replicate killed on d 49.

<sup>3</sup>Linear effect, ( $P < 0.10$ ).

<sup>4</sup>Full viscera weights are the weights of the whole viscera after removal from the carcass.

<sup>5</sup>Calculated using the equation for ribbed carcasses described by the NPPC (2000).

<sup>6</sup>Calculated using total body electrical conductivity (TOBEC) analysis with equations from Higbie et al. (2002).

would also expect an increase in 10<sup>th</sup> rib backfat and total fat of the carcass, which did not occur. The increase in dressing percentage is also consistent with the positive effects of Leu, which led us to believe that Leu might have increased the yield of the carcass.

Experiment 2 was conducted to re-evaluate the effects of RBC on the increase in dressing percentage that was observed in Experiment 1. Because of the decreased growth performance of pigs fed diets containing 3% RBC or greater in Experiment 1, the highest inclusion level of RBC used in Experiment 2 was 2%. Increasing RBC addition from 0 to 2% did not affect growth performance, conventional linear carcass measurements, or responses calculated from NPPC and TOBEC equations except for a quadratic effect on average backfat thickness. The increase in average backfat is consistent with Leu effects on increased lipid precursors and the results of Experiment 1, but again, there was no effect on 10<sup>th</sup> rib backfat and total fat of the carcass.

The results of Experiment 3 were similar to Experiment 2. Increasing RBC addition from 0 to 2% did not affect growth performance, conventional linear carcass measurements, or responses calculated from NPPC and TOBEC equations except for the following: a linear decrease on average backfat and a linear increase in full viscera and large intestine percent. These results are similar to those of Experiment 2 except for the decrease in average backfat, which was not observed in previous experiments.

The sex effects for both experiments followed the general trend. For Experiment 2, in which gilts were leaner and grew more efficiently than barrows, barrows were heavier and consumed more feed. In Experiment 1, the sex effects for feed intake and gain were not observed.

Plasma urea N is widely used as a tool for determining protein utilization (Knowles et al., 1997 and Guzik et al., 2002). In Experiments 2 and 3, PUN was used to determine the effect of the addition of RBC. In Experiment 2, plasma urea N was decreased for pigs fed 2% RBC compared with the control. This change in PUN was due to a decrease in the level of CP as the level of RBC increased.

Dean et al. (2005) fed a diet containing 5% RBC with supplemental Ile and reported that growth performance as well as 10<sup>th</sup> rib back fat and loin muscle area was not affected by 5% RBC + Ile compared with pigs fed the control. Our results show a linear decrease in ADG and G:F with up to 4% RBC and with Ile supplementation. The Ile:Lys ratio used in our experiment was 0.56. The results from Dean et al. (2005) indicate that the ideal Ile:Lys ratio to maximize growth performance in finishing pigs fed 5% RBC is greater than 0.56. Therefore, in Experiment 1, the decrease in growth performance for 3 and 4% RBC was probably due to a deficiency of Ile.

Cisneros et al. (1996) reported that supplementing a finishing diet with 2% crystalline Leu (1.03% vs. 3.03% total Leu) had no affect on growth performance, carcass length, 10<sup>th</sup> rib backfat, and muscle fat content. Hyun and Baker (2003) also reported that 2% supplemental Leu added to diets of barrows and gilts did not affect hot carcass weight, dressing percent, carcass fat depths, and loin muscle area. However, 2% Leu supplementation decreased weight gain and increased intramuscular fat and marbling. Recently, Yu et al. (2007) reported that feeding a combination of Leu and conjugated linoleic acid had no affect on growth performance, carcass length, average backfat, and loin muscle area. Based on the results from these studies, there is an indication that 2% Leu (total Leu > 3%) addition to the diet of finishing pigs does not affect loin muscle area, hot carcass weight, 10<sup>th</sup> rib backfat, carcass length, and

dressing percent. In Experiment 1 we had increased dressing percentage, but this effect was not seen in the other 2 experiments. There was increased average backfat in Experiments 1 and 2, but this was not observed in Experiment 3. These positive effects of Leu were not consistent throughout all 3 experiments; therefore, our data are in agreement with previous research in that feeding up to 1.43% Leu from RBC does not affect carcass traits.

## **CHAPTER 4**

# **EFFECT OF INCREMENTAL LEVELS OF RED BLOOD CELLS (RBC) ON GROWTH PERFORMANCE OF BROILERS**

### **INTRODUCTION**

Recent increases in the cost of feedstuffs have caused nutritionists and producers to explore ways to reduce the cost of protein sources in broiler diets. Many producers utilize supplemental AA to reduce diet cost. Another option is to utilize alternative protein sources, such as animal by-products. Recently, researchers have shown that blood products can be successfully used in the diets for poultry without detrimental effects on growth performance (Kidd et al., 2004 and Tyus II et al., 2008). However, blood products are deficient in Ile, which may limit their use. Our research conducted with finishing pigs indicated that RBC addition greater than 2% decreased growth performance (see chapter 3). Therefore, the objectives of this research were to evaluate growth performance of broilers fed incremental levels of RBC with and without supplemental AA, and to determine if broilers respond in the same manner as finishing pigs to increasing levels of RBC.

### **MATERIALS AND METHODS**

All methods used in these experiments were approved by the LSU Agricultural Center Animal Care and Use Committee. Three experiments were conducted with male (Experiment 1) or male and female (Experiments 2 and 3) Ross × Ross 708 broilers. On d 0 post-hatching, broilers were sexed, weighed, wing banded, and randomly allotted to dietary treatments. All broilers were housed in Petersime starter batteries with fluorescent lighting. Feed in mash form and water were available *ad libitum*. All experiments were conducted for 18 d, at which time the feeders and birds were weighed

for determination of ADG, ADFI, and G:F. All Diets were formulated to contain 1.36% Lys and TSAA:Lys of 0.75, and all other nutrients (except for Arg and Ile in the deficient diets) were formulated to meet or exceed the NRC requirements (NRC, 1994).

#### Experiment 1

Experiment 1 was conducted to evaluate the effect of incremental levels of RBC on growth performance of broilers. Each treatment had 6 replicates with 6 birds per replicate. Diet 1 was a conventional C-SBM control starter diet with no added RBC. Diet 2 was the control with 0.5% RBC (Table 4.1). This level of RBC was used because it required no supplemental L-Arg·HCl and L-Ile. The other 3 treatments were 1, 2, and 3% RBC with supplemental L-Arg·HCl and L-Ile.

#### Experiment 2

Experiment 2 was conducted to evaluate the effect of incremental levels of RBC on growth performance of broilers fed diets supplemented with needed AA or with no AA supplemented other than Met, Thr, and Lys. Each treatment had 7 replicates (3 female and 4 male) with 6 birds per replicate. Diet 1 was a conventional C-SBM diet with no added RBC. Diet 2 was the control with 0.5% RBC. Diets 3 to 9 were supplemented with RBC in 1% increments from 1 to 7%. Diets 10 to 16 also contained 1, 2, 3, 4, 5, 6, and 7% RBC, but supplemental AA (L-Arg·HCl and L-Ile) were not added, other than Met, Thr, and Lys. Diets 11 to 16 were calculated to be deficient in Arg, and diets 10 to 16 were calculated to be deficient in Ile. Because of an error in diet formulation, diets 3 to 9 contained excess L-Arg·HCl.

Table 4.1. Diet composition for male and female broilers fed incremental levels of red blood cells in Experiments 1, 2, and 3, as-fed basis.<sup>1</sup>

Diet	Red blood cells (RBC), %								
	1	2	3 & 10	4 & 11	5 & 12	6 & 13	7 & 14	8 & 15	9 & 16
Ingredient	0	0.5	1	2	3	4	5	6	7
Corn	53.38	54.27	55.11	56.70	58.30	59.90	61.49	63.09	64.69
Soybean meal (47.5%)	36.92	35.43	33.95	31.00	28.04	25.08	22.13	19.17	16.22
Soy oil	5.05	5.11	5.16	5.28	5.40	5.52	5.63	5.75	5.87
Monocalcium phosphate	1.52	1.52	1.53	1.54	1.55	1.56	1.57	1.58	1.59
Limestone	1.49	1.50	1.51	1.53	1.55	1.57	1.59	1.61	1.63
Salt	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
LSU trace minerals <sup>2</sup>	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Nutra Blend vitamins <sup>3</sup>	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Choline chloride	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
DL-Met	0.302	0.313	0.324	0.346	0.368	0.390	0.412	0.434	0.456
Biolys	0.200	0.200	0.200	0.200	0.200	0.200	0.200	0.200	0.200
L-Thr	0.108	0.116	0.124	0.140	0.157	0.173	0.189	0.206	0.222
L-Arg·HCl <sup>4</sup>	-----	-----	-----	0.064	0.135	0.207	0.279	0.350	0.422
L-Arg·HCl <sup>5</sup>	-----	-----	0.038	0.155	0.272	0.389	0.507	0.624	0.741
L-Ile	-----	-----	0.011	0.067	0.123	0.178	0.234	0.289	0.345
Calculated nutrient composition <sup>6</sup>									
ME, kcal/kg	3,200	3,200	3,200	3,200	3,200	3,200	3,200	3,200	3,200
CP, %	22.36	22.21	22.11	22.02	21.92	21.83	21.74	21.64	21.55
Ca, %	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
P, %	0.72	0.72	0.71	0.70	0.69	0.68	0.67	0.66	0.65
Non-phytate P, %	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45
Lys, %	1.36	1.36	1.36	1.36	1.36	1.36	1.36	1.36	1.36
Thr, %	0.95	0.95	0.95	0.95	0.95	0.95	0.95	0.95	0.95
Met, %	0.64	0.64	0.65	0.66	0.68	0.69	0.70	0.71	0.73
TSAA, %	1.01	1.01	1.01	1.01	1.01	1.01	1.01	1.01	1.01
Ile, %	0.95	0.92	0.90	0.91	0.91	0.91	0.91	0.91	0.91
Leu, %	1.88	1.90	1.92	1.95	1.99	2.02	2.06	2.09	2.12
Arg, % <sup>7</sup>	1.48	1.46	1.43	1.42	1.42	1.42	1.42	1.42	1.42

Continued on the next page

Arg, % <sup>8</sup>	1.48	1.45	1.46	1.49	1.53	1.57	1.61	1.64	1.68
Gly + Ser, %	2.05	2.03	2.01	1.98	1.95	1.92	1.89	1.86	1.82
Ile:Lys	0.70	0.68	0.67	0.67	0.67	0.67	0.67	0.67	0.67
Arg:Lys <sup>9</sup>	1.09	1.07	1.05	1.04	1.04	1.04	1.04	1.04	1.04
Arg:Lys <sup>10</sup>	1.09	1.07	1.07	1.10	1.13	1.15	1.18	1.21	1.24
sid Lys, %	1.23	1.23	1.23	1.22	1.22	1.21	1.21	1.21	1.20
sid Thr, %	0.84	0.84	0.84	0.85	0.85	0.85	0.86	0.86	0.86
sid TSAA, %	0.92	0.92	0.92	0.93	0.93	0.93	0.93	0.94	0.94
sid Ile, %	0.87	0.85	0.83	0.84	0.84	0.84	0.84	0.84	0.85
sid Arg, % <sup>8</sup>	1.36	1.33	1.33	1.38	1.42	1.46	1.50	1.54	1.58
Calculated nutrient composition <sup>11</sup>									
ME, kcal/kg	3,200	3,200	3,200	3,201	3,202	3,203	3,204	3,205	3,206
CP, %	22.36	22.21	22.04	21.72	21.39	21.06	20.74	20.41	20.09
Ile, %	0.95	0.92	0.89	0.84	0.78	0.73	0.68	0.62	0.57
Arg, %	1.48	1.46	1.43	1.37	1.30	1.25	1.19	1.13	1.07
Ile:Lys	0.70	0.68	0.66	0.62	0.58	0.54	0.50	0.46	0.42
Arg:Lys	1.09	1.07	1.05	1.01	0.96	0.92	0.88	0.83	0.79

<sup>1</sup> A basal diet was mixed for diets 1 and 2 for Experiments 1 and 2, and a basal diet was mixed for all diets in Experiment 3. The basal diet contained the minimum of all ingredients except for RBC, and additional ingredients were added when needed to each diet. Diets 4 through 8 were mixed by blending different proportions of diets 3 and 9 to achieve the desired percent of RBC for Experiments 1 and 2. Diets 11 through 15 were mixed by blending different proportions of diets 10 and 16 to achieve the desired percent of RBC for Experiments 1 and 2; these diets contained cornstarch in the place of L-Arg·HCl and L-Ile.

<sup>2</sup> Provided per kilogram of diet: copper (copper sulfate·5H<sub>2</sub>O), 4 mg; iodine (potassium iodate), 1.0 mg; Iron (ferrous sulfate·7H<sub>2</sub>O), 60 mg; manganese (manganese sulfate·H<sub>2</sub>O), 60 mg; selenium (sodium selenite), 0.1 mg; zinc (zinc sulfate·7H<sub>2</sub>O), 44 mg; calcium (calcium carbonate), 723 mg.

<sup>3</sup> Provided per kilogram of diet: vitamin A, 8,003 IU; vitamin D<sub>3</sub>, 3,004 IU; vitamin E, 25.00 IU; menadione, 1.50 mg; vitamin B<sub>12</sub>, 0.02 mg; d-biotin, 0.10 mg; folacin, 1.00 mg; niacin, 50.00 mg; d-pantothenic acid, 15.00 mg; pyridoxine, 4.00 mg; riboflavin, 10.00 mg; thiamin, 3.00 mg.

<sup>4</sup> Quantity of L-Arg·HCl that was fed in Experiment 3.

<sup>5</sup> Quantity of L-Arg·HCl that was fed in Experiment 2 (excess).

<sup>6</sup> Nutrient composition for diets 1 to 9.

<sup>7</sup> Percentage Arg that was fed in Experiment 3.

<sup>8</sup> Percentage Arg that was fed in Experiment 2 (excess L-Arg·HCl).

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<sup>9</sup>Arg:Lys ratio that was fed in Experiment 3.

<sup>10</sup>Arg:Lys ratio that was fed in Experiment 2 (excess L-Arg·HCl).

<sup>11</sup>Nutrient composition for diets 10 to 16. L-Arg·HCl and L-Ile were replaced with cornstarch in diets 10 to 16. Diets 10 to 16 calculate to be deficient in Ile and diets 11 to 16 calculate to be deficient in Arg.

### Experiment 3

Because of the formulation error in diets 3 to 9 in Experiment 2, Experiment 3 was conducted to re-evaluate the effect of incremental levels of RBC on growth performance of broilers fed diets supplemented with needed AA without excess L-Arg·HCl. Each treatment had 5 replicates (2 female and 3 male) with 6 birds per replicate. Diet 1 was a conventional C-SBM diet with no added RBC. Diet 2 was the control with 0.5% RBC. Diets 3 to 9 were supplemented with RBC in 1% increments from 1 to 7%.

Data were analyzed by ANOVA procedures using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC) as a completely randomized design (Experiment 1) or as a randomized complete block design (Experiments 2 and 3) with sex as the blocking factor. The experimental unit was the pen of chicks. In all experiments, orthogonal contrasts for unequally spaced treatments were used to determine linear and quadratic effects of RBC. Contrasts were obtained from the PROC IML procedure of SAS. In Experiment 2, the data were analyzed 2 ways. The first analysis was as a  $2 \times 7$  factorial arrangement of treatments; the factors were AA supplemented or not supplemented and level of RBC (without the 0% or 0.5% levels included in this analysis). The contrast statements of source (with or without AA supplementation), linear, and quadratic, and source by linear, and source by quadratic interactions were evaluated. In the second analysis, the linear and quadratic effects of RBC within source (with or without AA supplementation) were evaluated. An alpha level of 0.10 was used to determine treatment differences.

## **RESULTS**

In Experiment 1, there were no effects ( $P > 0.10$ ) of RBC addition on ADG, ADFI, and G:F compared with broilers fed the control diet (Table 4.2).

In Experiment 2, treatment  $\times$  sex interaction was not significant ( $P > 0.10$ ) and was removed from the statistical model. In the 1<sup>st</sup> statistical analysis, there was an overall source effect ( $P < 0.01$ ) (AA adequate vs. AA deficient), and source  $\times$  linear ( $P < 0.01$ ) and source  $\times$  quadratic ( $P < 0.08$ ) interactions for ADG, ADFI, and G:F (Table 4.3, Figure 4.1). With increasing levels of RBC, there was decreased growth performance for birds fed the AA-adequate diets, but the effect was greatest at 7% RBC addition. However, there was a more severe decrease in growth performance for birds fed AA-deficient diets. In the 2<sup>nd</sup> statistical analysis, broilers fed AA-adequate diets had a quadratic decrease in ADG, a linear and quadratic decrease in ADFI, but a linear increase in G:F. Generally, the RBC addition tended to increase or not affect ADG up to the 6% addition with supplemental AA, but ADG was decreased by the 7% RBC addition. The response in ADFI was similar, but the decrease in ADFI at 7% RBC addition was different ( $P < 0.05$ ) from the 0% RBC addition. Feed efficiency was increased by the addition of RBC. Broilers fed AA-deficient diets with incremental levels of RBC had a linear and quadratic decrease in ADG, ADFI, and G:F ( $P < 0.01$ ).

Red blood cell addition up to 6% can be added with supplemental AA (L-Arg·HCl and L-Ile), and up to 3% RBC can be added without supplemental AA with no detrimental effects on growth performance.

In Experiment 3, treatment  $\times$  sex interaction was not significant ( $P > 0.10$ ) for any response variable and was removed from the statistical model. With increasing levels of RBC, there was a linear and quadratic decrease in ADG and ADFI, but no effect on G:F

(Table 4.4, Figure 4.1). Generally, the RBC addition tended to increase or not affect ADG up to the 7% addition. The response in ADFI was similar, but the decrease in ADFI at 7% RBC addition was different ( $P < 0.04$ ) from the 0% RBC addition. Feed efficiency was not affected by the addition of RBC. Red blood cell addition up to 6% can be added with supplemental AA (L-Arg·HCl and L-Ile) with no detrimental effects on growth performance.

## **DISCUSSION**

The objective of Experiment 1 was to evaluate the effect of incremental levels of RBC on growth performance of broilers from d 0 to 18. There were no significant effects of RBC addition up to 3% on ADG, ADFI, and G:F when compared with broilers fed the control. The diet containing 0.5% RBC was formulated to mimic a commercial diet that would contain no supplemental L-Arg·HCl and L-Ile due to the lack of commercial availability of these AA. The results of this experiment indicate that 1 to 3% RBC can be added to the diets of d 0 to 18 broilers with supplemental Arg and Ile with no negative effects on growth performance.

Because there were no negative effects on growth performance with the addition of up to 3% RBC, Experiment 2 was conducted to evaluate the effect of incremental levels of RBC (0, 0.5, and 1 to 7%) on growth performance of broilers fed diets with supplemental L-Arg·HCl and L-Ile (adequate) and diets with no supplemental L-Arg·HCl and L-Ile (deficient). The diets with supplemental AA contained excess L-Arg·HCl. The diets containing 2 to 7% RBC were calculated to be deficient in Arg, and diets containing 1 to 7% RBC were calculated to be deficient in Ile. Red blood cell addition from 1 to 6% with supplemental AA resulted in no negative effects on growth

performance. In addition, up to 3% RBC can be added without supplemental AA with no negative effects on growth performance.

Because the diets with supplemental AA contained excess L-Arg·HCl in Experiment 2, Experiment 3 was conducted to re-evaluate the effect of incremental levels of RBC (0, 0.5, and 1 to 7%) on growth performance of broilers fed adequate L-Arg·HCl and L-Ile. The results of Experiment 3 are similar to those of Experiment 2, in that red blood cell addition from 1 to 6% with supplemental AA resulted in no negative effects on growth performance.

Kidd et al. (2004) reported that 18- to 30- d old broilers fed a C-SBM diet with 6% RBC and supplemental L-Arg·HCl and L-Ile had similar growth performance compared with broilers fed the control diet with no RBC. When the diets were not supplemented with L-Ile, there was a significant decrease in growth performance compared with a diet containing no RBC. Though our research was conducted using d 0 to 18 broilers, we had similar results in that broilers fed the diet containing 6% RBC with supplemental Arg and Ile had similar growth performance compared with broilers fed the control. Furthermore, there was a decrease in growth performance of broilers fed diets containing 6 or 7% RBC with no supplemental Arg and Ile. Kidd et al. (2004) fed broilers diets that contained supplemental Arg without supplemental Ile. Their results indicate that an Ile deficiency may have a greater impact on growth rather than a deficiency in Arg.

Tyus II et al. (2008) fed d 0 single comb white leghorn chicks equal amounts of SBM and blood meal at an inclusion of 11% blood meal and 11% SBM with supplemental Ile. During the first 2 weeks, the chicks fed this diet had decreased feed consumption and body weight gain compared with a diet containing no blood meal.

Though the results of our experiment and Tyus II et al. (2008) are not comparable because of the differences in diets and type of bird, the results are similar. Increasing levels of blood products over 6% with Ile supplementation in broiler or white leghorn chick diets results in a decrease in growth performance.

Table 4.2. Growth performance of male broilers fed incremental levels of red blood cells in Experiment 1.<sup>1</sup>

Diet	Red blood cells, %					SEM	Linear	Quad
	1	2	3	4	5			
Variable	0	0.5	1	2	3			
ADG, g	30.54	30.82	29.54	30.58	30.27	0.58	0.80	0.63
ADFI, g	38.13	37.96	37.31	37.44	37.18	0.67	0.30	0.69
G:F, g/g	0.80	0.81	0.79	0.82	0.81	0.01	0.39	0.87

<sup>1</sup>Data are means of 6 replicates with 6 birds per replicate.

Table 4.3. Growth performance of male and female broilers fed incremental levels of red blood cells with adequate and deficient levels of arginine and isoleucine in Experiment 2.<sup>1</sup>

Diet	1	2	3	4	5	6	7	8	9			
Variable <sup>2</sup>	Red blood cells, %									SEM	Linear <sup>3</sup>	Quad <sup>3</sup>
	0	0.5	1	2	3	4	5	6	7			
Diets 3 to 9												
ADG, g <sup>4</sup>	32.73	32.77	32.51	33.64	34.10	33.41	32.82	32.87	31.83	0.67	0.50	0.02
ADFI, g <sup>4</sup>	40.85	41.78	40.38	41.84	41.79	41.21	40.41	39.16	37.99 <sup>a</sup>	1.01	0.01	0.03
G:F, g <sup>4</sup>	0.80	0.78	0.81	0.80	0.82	0.81	0.81	0.84 <sup>a</sup>	0.84 <sup>a</sup>	0.01	<.0001	0.45
Diets 10 to 16 <sup>5</sup>												
Diet	1	2	10	11	12	13	14	15	16	SEM	Linear <sup>3</sup>	Quad <sup>3</sup>
ADG, g <sup>4</sup>	32.73	32.77	33.46	32.26	31.64	29.94 <sup>a</sup>	27.61 <sup>a</sup>	23.45 <sup>a</sup>	18.64 <sup>a</sup>	0.67	<.0001	<.0001
ADFI, g <sup>4</sup>	40.85	41.78	42.07	40.93	40.34	40.00	37.90 <sup>a</sup>	32.94 <sup>a</sup>	28.23 <sup>a</sup>	1.01	<.0001	<.0001
G:F, g <sup>4</sup>	0.80	0.78	0.80	0.79	0.79	0.75 <sup>a</sup>	0.73 <sup>a</sup>	0.71 <sup>a</sup>	0.66 <sup>a</sup>	0.01	<.0001	<.0001

<sup>1</sup>Data are means of 7 replicates (3 female and 4 male) with 6 birds per replicate. Diets supplemented with AA contained excess L-Arg·HCl.

<sup>2</sup>The sex × treatment interaction was not significant ( $P > 0.10$ ) and was removed from the model.

<sup>3</sup>The linear and quadratic effects within AA-adequate and AA-deficient diets.

<sup>4</sup>In the overall statistical analysis, there were source effects, and source × linear and source × quadratic interactions in ADG, ADFI, and G:F ( $P < 0.01$ ), except for a source × quadratic interaction in ADFI, which was ( $P < 0.08$ ).

<sup>5</sup>Diets 10 to 16 are calculated to be deficient in Ile, and diets 11 to 16 are calculated to be deficient in Arg.

<sup>a</sup>Significantly different from control ( $P < 0.05$ ).

Table 4.4. Growth performance of male and female broilers fed incremental levels of red blood cells with adequate levels of arginine and isoleucine in Experiment 3.<sup>1</sup>

Diet <sup>2</sup>	1	2	3	4	5	6	7	8	9			
Red blood cells, %												
Variable <sup>3</sup>	0	0.5	1	2	3	4	5	6	7	SEM	Linear	Quad
ADG, g	31.71	32.35	33.49 <sup>a</sup>	33.26	32.36	31.69	31.56	31.14	30.27	0.67	0.01	0.06
ADFI, g	39.91	40.21	42.07 <sup>a</sup>	41.34	40.42	39.95	39.57	38.50	37.40 <sup>a</sup>	0.79	0.01	0.02
G:F, g	0.79	0.80	0.79	0.80	0.80	0.79	0.80	0.81	0.81	0.01	0.26	0.45

<sup>1</sup>Data are means of 5 replicates (2 female and 3 male) with 6 birds per replicate.

<sup>2</sup>These diets are identical to the diets fed in Experiment 2, except they do not contain excess levels of L-Arg·HCl.

<sup>3</sup>The sex × treatment interaction was not significant ( $P > 0.10$ ) and was removed from the model.

<sup>a</sup>Significantly different from control ( $P < 0.08$ ).

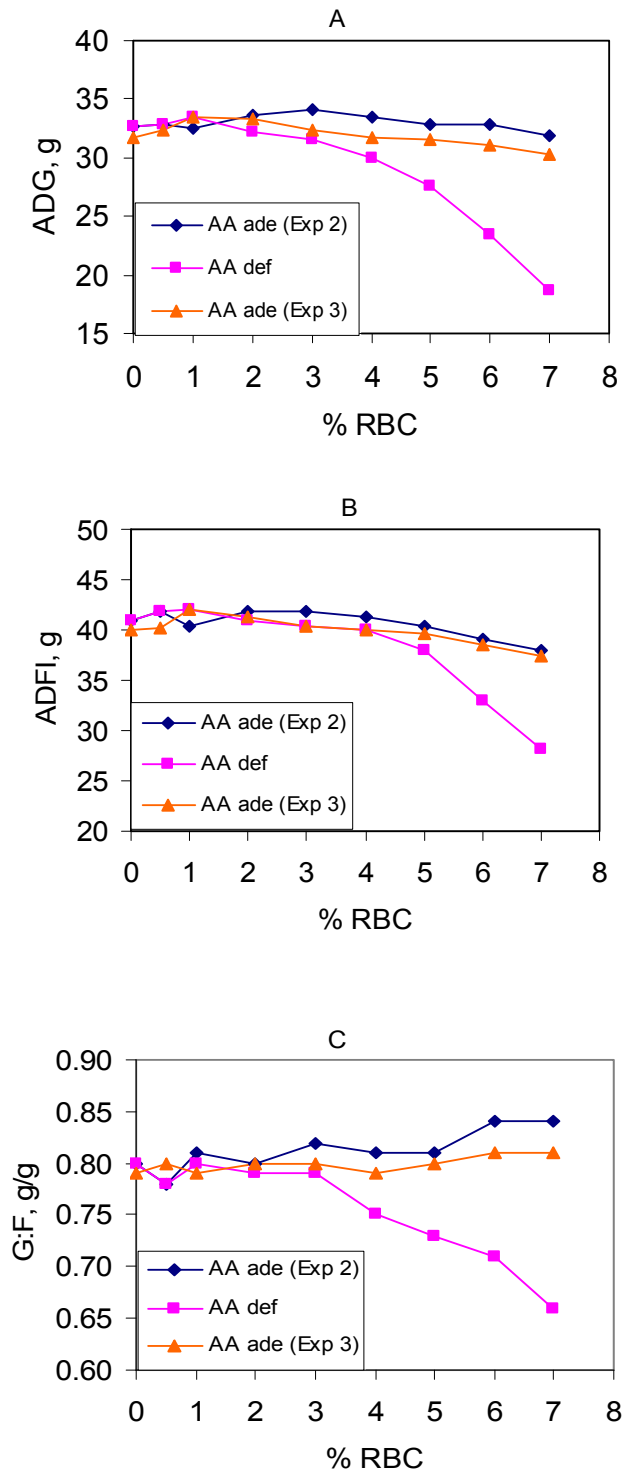


Figure 4.1. Average daily gain (A), average daily feed intake (B), and gain:feed (C) for male and female broilers fed incremental levels of red blood cells with adequate and deficient levels of arginine and isoleucine in Experiment 2, and adequate arginine and isoleucine in Experiment 3. In the overall statistical analysis, for Experiment 2, there were source effects, and source  $\times$  linear and source  $\times$  quadratic interactions in ADG, ADFI, and G:F ( $P < 0.01$ ), except for a source  $\times$  quadratic interaction in ADFI, which was ( $P < 0.08$ ).

Our results indicate that a starter broiler diet can contain up to 6% RBC with supplemental AA and can contain up to 3% RBC without supplementation of AA with no effect on growth performance. Broilers respond differently to increasing levels of RBC compared with swine. These results indicate that there is no growth depression in poultry with up to 6% RBC inclusion; however, in finishing pigs there was a growth depression with 3% RBC inclusion. Though the diets of finishing pigs were supplemented with needed AA, there was still a decrease in growth performance at 3% RBC addition. This response was not observed in broilers until the addition of 7% RBC with supplemented AA.

## **CHAPTER 5**

### **SUMMARY AND CONCLUSIONS**

This research was conducted to determine if increasing levels of dietary Leu (as RBC) would affect growth performance and carcass characteristics of finishing pigs and growth performance of broilers.

Three experiments were conducted to determine the effect of incremental levels of RBC (0 to 4% and 0 to 2%) on growth performance and linear carcass measurements of finishing pigs. Our results suggest that feeding 3 or 4% RBC causes a decrease in growth performance. However, feeding 0, 1, or 2% RBC in the diets of finishing pigs causes no detrimental effects on growth performance.

Three experiments were conducted to determine the effect of incremental levels of RBC (0, 0.5, and 1 to 7%) on growth performance of broilers fed diets with supplemental L-Arg·HCl and L-Ile (adequate) and diets with no supplemental L-Arg·HCl and L-Ile (deficient). The results indicate that up to 6% RBC can be added to a broiler diet without reducing growth performance if the diets are supplemented with L-Arg·HCl and L-Ile. Also, up to 3% RBC can be added with no AA supplementation with no detrimental effects on growth.

Increasing levels of RBC in a diet does not have the same effect on broiler growth performance compared with finishing pig growth performance. In finishing pigs, when 3 or 4% RBC were included in the diet with supplemental Ile, there was a decrease in ADG and G:F. This was not observed with broilers. The reason that broilers respond in this manner to higher RBC diets is unclear; therefore, further research is needed to determine why these 2 non-ruminant species respond so differently to increasing RBC addition.

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## APPENDIX

### LIST OF ABBREVIATIONS

Item	Abbreviation
Apparent ileal digestible	aid
Amino acid (s)	AA
Analysis of variance	ANOVA
Average daily feed intake	ADFI
Average daily gain	ADG
Body weight	BW
Branched-chain amino acid (s)	BCAA
Corn	C
Crude protein	CP
Gain:feed	G:F
Day	d
General linear model	GLM
National pork producers council	NPPC
Nitrogen	N
Plasma urea nitrogen	PUN
Red blood cells	RBC
Statistical analysis software	SAS
Soybean meal	SBM
Total body electrical conductivity	TOBEC
Total sulfur amino acids	TSAA

## **VITA**

Emily Dawn Frugé, daughter of Ronald (Fudge) and Bertha Frugé, was born in Lake Charles, Louisiana, in September, 1983. Emily is the youngest of three children, and was raised on a small show pig farm operation in Bell City, Louisiana. After graduating from Bell City High School in 2001, she attended Louisiana State University. In May of 2006, she completed a Bachelor of Science degree with a concentration in animal, dairy, and poultry sciences. In June 2006, she began pursuing her Master of Science degree with a concentration in animal, dairy, and poultry sciences at Louisiana State University.