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Effects of Crystalline Amino Acid Supplementation of Reduced Crude Protein (RCP) Diet on Net Energy Basis on Growing-Finishing Swine

Effects of Crystalline Amino Acid Supplementation of Reduced Crude Protein (RCP) Diet on Net Energy Basis on Growing-Finishing Swine

A thesis submitted in partial fulfillment of
the requirements for the degree of
Master of Science in Animal Science

by

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Truman State University
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This thesis is approved for recommendation to the Graduate Council.

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ABSTRACT

Barrows and gilts (n=210) were used to test the effects of crystalline amino acid (AA) supplementation of reduced crude protein (RCP) diets formulated on a net energy basis on quality characteristics of the LM from growing-finishing pigs. Pigs were blocked by weight, and pens (3 barrows and 3 gilts/pen) within each block were assigned randomly to either corn-soybean meal diets (C) or 1 of 3 RCP diets and added crystalline AA levels for the dietary treatments during each feeding phase. During the last 3-wk feeding phase, 10 ppm of ractopamine were included in all diets. At slaughter and after a 24-h rapid chilling period, a subsample of whole pork loins (3/pen) was collected during carcass fabrication and further processed into LM chops for quality data collection. A 2.5-cm-thick slice was removed from the anterior end of randomly selected fresh pork bellies (3 bellies/pen). Belly slices were further dissected into the outer s.c. (OSC) and middle s.c. (MSC) fat layers, as well as intermuscular fat (INT). Jowl, belly and LM samples were freeze-dried and analyzed for fatty acid composition. As CP decreased in swine diets, Japanese ($P < 0.01$), American ($P = 0.032$), and fat color scores decreased (linear, $P = 0.017$), whereas LM drip loss increased (linear, $P = 0.015$) with decreasing CP diets. Lightness (L*) value increased ($P = 0.015$) linearly with decreasing CP diets; yet, neither a* nor b* values were affected ($P \geq 0.414$) by RCP treatments. Furthermore, marbling and firmness scores, cooking loss percentage, and shear force values were not ($P \geq 0.503$) affected by dietary CP levels. Color was detrimentally affected by reducing dietary CP and adding crystalline AA in diets formulated on a net energy basis. There were no ($P \geq 0.132$) dietary treatment × fat layer interactions; however, proportions of all SFA and all MUFA increased (linear, $P \leq 0.006$), and proportions of all PUFA decreased (linear, $P = 0.010$), as CP was reduced in the diet. Also, belly fat IV decreased linearly ($P < 0.001$) with decreasing dietary

CP. Total SFA were greatest ($P < 0.05$) in the INT, and SFA percentage was greater ($P < 0.05$) in the MSC than OSC. The OSC and MSC had greater ($P < 0.05$) proportions of all MUFA than INT, whereas OSC had greater ($P < 0.05$) proportions of PUFA than MSC and INT. The OSC had the greatest ($P < 0.05$) IV (74.0), and IV of the MSC was greater ($P < 0.05$) than that of the INT (70.0 vs. 68.1). Similarly, total PUFA percentages in the jowl and LM samples decreased (linear, $P \leq 0.0003$), whereas MUFA percentages in the jowl and LM measured (linear, $P \leq 0.0062$) with decreasing dietary CP. In particular, in swine diets oleic acid (18:1c9) increased (linear, $P \leq .0142$) and linoleic acid (18:2n6) decreased in the LM and the jowl fat as CP was decreased. Similar to 18:2n6, linolenic acid (18:3n3) in the jowl fat and LM had decreased (linear, $P \leq .0001$) 27% and 17.8% in the jowl fat and LM respectively, among RCP treatments. Results indicate that fatty acid composition differs greatly among the fat layers of fresh pork bellies, MUFA composition of pork belly fat was increased at the expense of PUFA by reducing dietary CP, suggesting enhanced *de novo* synthesis in pigs fed RCP diets supplemented with crystalline AA.

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DEDICATION

This Master's Thesis is dedicated to both my grandfathers, Neil R. Cook and Robert H. Hooks. Both my grandfathers imprinted on me at a very young age the dedication and hard work that is required in the agriculture field. Each of them had their own agricultural practices, Neil having his own cattle herd in Vilonia, AR, and Robert having his flock of chickens in the backyard. It was these practices and gentlemen that made me fall in love with animals and want to continue working with animals every day. My grandfather's gave me the support and advice that was needed to continue my education and acquire a Master's Degree in Animal Science. I love both of you very much and am grateful for everything that you have taught me.

"The Lord is my strength and my shield; in him my heart trusts; so I am helped, and my heart exults, and with my song I give thanks to him." Psalms 28:7

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LIST OF ABBREVIATIONS

All abbreviations are within standard of use from the Journal of Animal Science and Meat Science.

LYS	Lysine
THR	Threonine
ADG	Average daily gain
ADFI	Average daily feed intake
G:F	Gain-to-feed
AA	Amino acids
RCP	Reduced crude protein
N	Nitrogen
CP	Crude protein
GE	Gross energy
DE	Digestible energy
ME	Metabolizable energy
NE	Net energy
ER	Energy restriction
HI	Heat increment
Arg	Arginine
Cys	Cystine
Gly	Glycine
Ser	Serine
Mcal	Mega-calories
IMF	Intramuscular fat

Met	Methionine
Trp	Tryptophan
LT	Longissimus thoracic
LM	Longissimus muscle
Ile	Isoleucine
Val	Valine
BW	Body weight
UFA	Unsaturated fatty acids
SFA	Saturated fatty acids
MUFA	Monounsaturated fatty acids
PUFA	Polyunsaturated fatty acids
PPAR-γ	Peroxisome proliferator-activated receptor gamma
GLUT-4	Glucose transporter protein 4
SCD	Stearoyl-CoA desaturase

CHAPTER 1: REVIEW OF LITERATURE

Introduction

Today, there is an increase in N_2 pollution in the world. One of the major contributors is N_2 excretion in animal waste, especially in the affluent from confined animals (Canh et al., 1998; Prince et al., 2000). With an increasing problem in N_2 excretion, research has focused on formulating swine diets to reduce N_2 excretion into the environment. Kerr and Easter (1995) estimated that each one percentage unit reduction in dietary protein (when accompanied by appropriate AA supplementation) resulted in 8% less N_2 excreted in manure, and subsequent research demonstrated that feeding reduced-CP, AA-supplemented diets reduced the amount of N_2 excreted from swine (Kerr and Easter, 1995; Kendall, 2000; Figueroa et al., 2002). However, the environmental advantages of feeding AA-supplemented, reduced CP diets has resulted in considerable variability in live pig performance and pork carcass characteristics (Dourmad et al., 1993; Kerr et al., 1995; Figueroa et al., 2002).

Reduction of 2 or 3 percentage units of protein is possible with no reduction in ADG or feed efficiency when AA are supplemented to growing-finishing swine (Cromwell, 1996; Tuitoek et al., 1997b). Furthermore, reducing CP to 3 percentage units, or more, also has produced no reduction in ADG or G:F (Hahn et al., 1995; Kerr et al., 1995). Yet others have found that reducing CP more than 3 percentage units retards ADG of swine (Hansen et al., 1993; Gomez et al., 2002).

Most studies indicated that feeding swine lower levels of CP results in increased carcass fatness (Canh et al., 1998, Kerr et al., 1995). Interestingly, intramuscular fat (IMF) content is also increased by feeding reduced CP and (or) lysine-deficient diets, especially during the finishing phases (Castell et al., 1994; Kerr et al., 1995; Cisneros et al., 1996; Szabo et al., 2001; Apple et

al., 2004). Cromwell and his colleagues (1996) observed equal growth rate, but greater backfat depth and lower percent carcass lean in pigs fed low CP diets fortified with L-lysine, DL-methionine and L-threonine compared to pigs fed higher CP diets with no synthetic AA. Zhang et al. (2008) observed that feeding reduced CP diets with supplemental AA also increased moisture loss of the LM and decreased the yellowness of the meat. Overall, there are still mixed results on whether feeding reduced CP diets supplemented with feed-grade AA produces similar carcass characteristics as feeding high CP diets. Therefore, one objective of this study was to test the live growth performance and carcass characteristics of swine that were fed reduced CP diets supplemented with crystalline AA.

Energy Systems

Diets for swine can be formulated on different energy systems. Dietary energy can be broken down into gross energy (GE), digestible energy (DE), metabolizable energy (ME), and net energy (NE). Gross energy, or heat of combustion, is the energy released by burning a sample of feed in excess oxygen, whereas DE is the GE of feed minus the GE of feces. Metabolizable energy is the DE minus energy excreted in urine and combustible gases (methane). Lastly, NE is the ME minus heat increment, produced during digestion of feed, metabolism of nutrients, and excretion of waste (Moehn et al., 2005). Growing pigs rarely retain 50% of ingested energy, and, even though 80 to 90% of GE is digested, some energy is lost in urine and as methane (van Milgen and Noblet, 2003). ME content of a diet is the difference between DE and these 'material' energy losses. The ME not retained is lost as heat (van Milgen and Noblet, 2003). Dietary CP or AA level, energy density, and the ratio of lysine-to-energy primarily determines the deposition rate of protein and lipid (Szabo et al., 2001). It has been shown that excess CP intake increases energy expenditure (Buttery and Boorman, 1976) and impacts organ size and

energy metabolism (Yen, 1997; Nyachoti et al., 2000). Energy restriction (ER) has biological effects on improved antioxidant defenses (Sreekumar, R. 2002), reduced oxidative damage (Drew, 2003; Lee, 1999), increased protein turnover, and increased fatty acid metabolism.

Amino Acids

In 1935, the first individual AA was discovered and developed in crystalline form in a laboratory by W.C. Rose. Since then, crystalline AA have became more readily available with the addition of methionine and lysine in the 1960's, tryptophan and threonine in the 1980's and isoleucine and valine in the 1990's (Kerr, 2006). With discovery of these AA and a plethora of nutritional studies in swine, diets are formulated to include the essential AA, including: lysine (Lys), threonine (Thr), tryptophan (Trp), methionine (Met), cysteine (Cys), isoleucine (Ile), histidine (His), valine (Val), arginine (Arg), phenylalanine (Phe), and tyrosine (Tyr) (Powell et al., 2011). If one of these AA is lessened or missing in the diet -meaning it is limiting- the performance of the pig will be jeopardized. There are a total of 20 AA that make up the building blocks of proteins. The decrease in growth performance may presently limit the degree to which CP can be reduced in diets for pigs. If the low-CP diets have essential AA in the correct ratios to Lys, growth performance should be the same unless the low-CP diet is deficient in total nitrogen; however N_2 deficiency will limit the capacity of pigs to produce nonessential AA. Conditionally essential AA namely, Arg and Cys along with the precursor glycine (Gly) plus serine (Ser) would be the first AA affected by a N_2 deficiency because they are present in reduced quantities in low-CP diets and are dependent on de novo synthesis to optimize growth. Production of these AA could be the limiting factor in optimizing the utilization of low-CP diets supplemented with AA (Powell et al., 2011).

Reduced Crude Protein

Performance traits

Diets initially started changing by reducing the CP to reduce N_2 excretion, without adding any AA. Performance traits, such as ADG, ADFI, G:F were measured to test the performance of the pig. When pigs were fed a 20% CP diet, they grew faster than pigs fed a 12% CP diet for 140 d, but there was no difference in ADG when fed to 196 d (Davey & Morgan, 1969). Hale and Southwell (1969) reported that ADG was similar when CP decreased from 15 to 18% to 11 to 14%, but G: F increased when pigs were fed the higher CP diets. These results were replicated again by Davey (1976), when pigs were fed either a 16% or 11% CP diet, and G:F was 22% greater for the pigs fed the 16% CP diet. However, ADG and G:F can be suppressed because the diets are deficient in several essential AA (Castell et al., 1994; Goerl et al., 1995; Kerr et al., 1995). Chen and collegues (1995) found that pigs fed diets formulated with 10-25% CP affected ADG and G:F quadratically, even though ADFI was not influenced by CP alone.

Carcass characteristics

Carcass characteristics can be visual color scores, firmness, marbling, hot carcass weight (HCW), slaughter weight, lean percentage, LM area, instrumental color (L*, a*, and b*), moisture, ash, fat, and ultrasound depths (backfat and LM). Chen and colleagues (1995) observed increasing the protein level from 10 to 25% decreased backfat depths, dressing percentage, and LM area. When pigs were fed a 10% CP diet, the IMF content was greater than that of pigs fed 25% CP diets, but LM moisture and protein contents were greater in the pigs fed 25% than 10% CP (Goerl et al., 1995). Color is an important factor, and has an influenced on consumers when purchasing meat. Longissimus muscle color “a*” and “b*” values decreased

linearly, while "L*" values declined with increased dietary CP levels (Goerl et al., 1995). Karlsson and colleagues (1993) also accredited higher reflectance measure to IMF at lower CP levels. Goerl et al. (1995) also reported that water-holding capacity, or drip loss rate, did not differ across dietary CP concentration.

Reduced AA

Performance traits

Dietary Lys, the first limiting AA in swine nutrition, can have regressive effects on performance when it is deficient in the diet. Next limiting essential AA in order are Thr, Trp, Met, Cys, Ile, His, Val, Arg, Phe, and Tyr. If any AA is not balanced for pig's requirement, it will have reduced performance characteristics even if other AA are adequate in the diet. Alternatively, whole-body protein turnover can be increased with high AA diets (Reeds et al., 1980). Pigs fed 0.85% Lys, which is below the NRC (1998) recommended level, had lower ADG and G:F compared to pigs fed 1.05 and 1.25% during the grower phase (Reynolds & O'Doherty, 2006). Providing adequate amounts of Lys in the diet will increase ADFI, which will impact ADG and G:F (Fabian, 2002). Similarly, Chiba and colleagues (1999) demonstrated improved ADG in pigs fed a high-AA diet when compared to pigs fed on a low-AA diet; however, G:F of pigs was not affected by dietary AA content in the diets (Chiba et al., 1999). In general, pigs fed diets deficient in any limiting-AA will have depressed growth performance compared to those that are fed recommended AA levels.

Carcass Characteristics

When different levels of Lys are fed to finishing swine, no differences in backfat were observed among the varying dietary Lys levels (Reynolds and O'Doherty, 2006). Pigs fed a high-amino acid diet had an increase in backfat. Also, Chiba et al. (1999) reported that LM area was

greater in pigs fed reduced AA diets when compared to pigs fed high-AA diets. Research has also shown that LM marbling decreased with the increasing dietary Lys, but LM color was not altered by Lys control of swine diets (Fabian et al., 2002). Conversely, when leucine was increased 2% in swine finishing diet, IMF content and marbling scores of the LM increased by supplemental leucine (Hyun et al. 2003). In addition, Hyun and colleagues (2003) found that supplemental leucine had no effect on L* or a * values of the LM; however, yellowness (b*) of the LM increased by supplemental leucine.

Reduced Crude Protein with AA supplementation

Performance traits

With the reduction of dietary CP and the addition of AA, pigs can perform at the same level as high-CP diets, while limiting N_2 excretion. When the low-CP diets were supplemented with Lys, Trp and Thr to the levels of the high-CP diets, pig performance was improved to a level equivalent to that of the high-CP diets (Kerr et al., 1995). Reduction of 2 or 3 percentage units of CP is possible with no reduction in ADG or feed efficiency when AA are supplemented (Cromwell, 1996; Tuitoek et al., 1997b). Decreasing dietary CP 3 percentage units, or more, has also produced no reduction in ADG or feed efficiency (Hahn et al., 1995; Kerr et al., 1995). Researchers have also observed dietary CP can be reduced in the final stages of growth with only minor adverse effects on growth rate and feed conversion efficiency (Kerr et al., 1995; Le Bellego, van Milgen & Noblet, 2002), as long as dietary essential AA intakes and net energy (NE) are maintained. However, at the lowest levels of dietary CP, a tendency for increased backfact has been observed by Canh et al. (1998) and Kerr et al. (1995). Overall, ADG, ADFI, and G:F will remain similar in pigs when dietary CP is reduced no more than 2 to 3 percentage

units and swine diets are fortified with AA; however, reducing dietary CP more than 3 percentage units results in considerable variation in live pig performance.

Carcass traits

Marbling is the last adipose tissue to be deposited in finishing animals, although adipose tissue starts to accumulate in the early weaning periods (Hauser et al., 1997; Harper and Pethick, 2004). It is generally accepted that intramuscular fat (IMF) positively influences flavor, juiciness, tenderness and/or firmness and the overall acceptability of meat in different species (Hodgson et al., 1991; Hovenier et al., 1993; Fernandez et al., 1999; Wood et al., 2008). Intramuscular fat content was increased by feeding Lys-deficient diets (Castell et al., 1994; Kerr et al., 1995; Cisneros et al., 1996; Szabo et al., 2001; Apple et al., 2004). Cisneros and colleagues (1996) reported that feeding pigs reduced CP to DE ratio (CP:DE) diets increased IMF levels in the LM, whereas Bonnet and colleagues (2007) reported that reducing dietary CP by 45 from starter to finisher phases caused increased backfat thickness, 10^{th} rib fat depth, leaf fat weight, and total lipid in the LM.

Along with the increase in carcass fatness associated with reductions in dietary CP, Kerr and colleagues (1995) observed a decrease in LM area, whereas Cromwell and his colleagues (1996) observed equal growth rate, but greater backfat depth and lower lean percentage in pigs fed low-CP diets fortified by Lys, Met and Thr compared to pigs fed higher-CP diets with no supplemental AA. Ward and Southern (1992) and Kerr and Easter (1995) also demonstrated decreases in carcass leanness in pigs fed low-CP diets supplemented with Lys, Thr, Trp and (or) Met compared with those fed intact protein. Synthesize fat in the muscle results from the balance between uptake, synthesis and degradation of triglycerols, which involve many metabolic pathways in both intramuscular adipocytes and myofibers (Hocquette et al., 2010). Color is one

of the major characteristics in which consumers judge quality of pork. Lowering dietary Lys content and nutrient level increased moisture water losses from the LM, as well as decreased yellowness of the meat (Zhang et al., 2008).

Fatty Acids

Deposited lipids originate from dietary FA and *de novo* synthesized fatty acids (FA). Fatty acids are composed of saturated (SFA; no double bonds between the carbons) monounsaturated (MUFA; a single double bond between 2 carbon molecules), or polyunsaturated (PUFA; double bonds between the carbon atoms). With increase fat deposition, fat accumulation in the body tends to have increase saturated fats (Wood et al., 1985), whereas feed-restricted pigs have decreased adipocyte volume, backfat thickness, and lipogenic capacity (Mersmann et al., 1981; Leymaster and Mersmann, 1991). Key lipogenic genes are decreased in the adipose tissue of starved pigs compared to well-fed pigs (McNeel and Mersmann, 2000), but only minor changes have been reported in chronically restricted pigs (McNeel et. al., 2000). Increased growth rates result in reduced fat deposition and higher degrees of unsaturation (Cliplef & McKay, 1993; Schinkckel et al., 2002). Dietary Lys plays a crucial role in regulating energy metabolism in the porcine muscle. Katsumata et al. (2001, 2003) indicated that reductions in Lys intake upregulates the gene expression of glucose transporter protein 4 (GLUT4) and peroxisome proliferator-activated receptor gamma (PPAR-γ) in porcine muscle, and is associated with a higher activity of mitochondrial oxidative enzymes.

Threonine is an indispensable AA and one of the major limiting AA in pig diets. Feeding a low-Thr diet to growing pigs enhanced the abundance of the mRNA of GLUT4 in skeletal muscle (Katsumata et al., 2004). Dietary lipid content and degree of polyunsaturation can cause

elevated deposition of PUFA in pork fat depots, leading to increased fat softness, enhanced risk of oxidation, greater IMF content and reductions in SFA and/or MUFA composition (Katsumata et al., 2004). Leucine and isoleucine are branched-chain AA which are metabolized within the muscle, and, if supplied in excess, can release lipid precursors, thereby increasing fat levels (Rodwell, 1993). Leucine in particular can be a ketogenic AA, and, after going through catabolism, can yield acetate and acetoacetate, which are lipid precursors (Rodwell, 1993).

Even though other factors, such as genotype, sex, age, slaughter weight and environmental temperature, also affect lipid and the FA content, nutrition is the main factor through which the lipid and FA deposition in pigs may be altered (Wood, 1984; Lebret and Mourot, 1998; Le Dividich et al., 1998). One possible explanation for this could be that lower dietary CP levels stimulate the expression of stearoyl- CoA desaturase (SCD1), which catalyses the cellular biosynthesis of MUFA (Doran et al., 2006). Oleic and palmitoleic acids, the main products of SCD, are the primary MUFA in fat depots and membrane phospholipids. A different pattern of SCD gene isoforms may exist between muscle and subcutaneous fat tissues (Doran et al., 2006). Similarly, Da Costa and colleagues (2004) have shown that a low-Lys (low CP) diet increases SCD transcriptional rate in pig muscles. Lipogenic enzymes, such as acetyl-CoA carboxylase (one key lipogenic enzyme), and NADH-producing enzymes, such as glucose-6-phosphate dehydrogenase or malic enzyme, have been related to IMF (Mourot and Kouba, 1999; Chartrin et al., 2006b; Bonnet et al., 2007). Intramuscular adipocytes have relatively low activity levels of enzymes of lipogenesis compared to subcutaneous adipocytes in pigs (Gardan et al., 2006).

Ractopamine

Ractopamine HCl is a phenethanolamine β- adrenergic agonist used as a feed supplement that redirects nutrients from lipogenesis to increase protein synthesis and muscle protein

accretion without deleteriously affecting pork quality (Apple et al., 2007). It is used primarily to improve lean growth rate and carcass lean percentage, as well as feed efficiency (Boler et al. 2010; Rikard-Bell et al. 2009; Hinson et al. 2011). For ractopamine to be used at maximum potential, low energy intake is needed to repartition to protein deposition. If energy intake is increased above 8.3 and 7.7 Mcal of ME/d for barrows and gilts respectively, the energy will be deposited as fat (Williams et al. 1994). It has also been observed that after the first 3 wks, the effects that ractopamine provides will not respond (Mills 2002a). Paylean is the commercial feed ingredient for ractopamine HCl, and it needs to be fed to finishing swine at 4.5 to 9.0g/ton in a complete ration containing at least 16% CP. The lysine content needs to be between .90% and 1.20% for the last 45 to 90 lbs. of gain or last 35 days before closeout of barn (Elanco Animal Health).

Conclusion

One of the major environmental issues focusing confined animal production is N_2 excretion is pig urine and manure (Prince et al., 2000; Canh et al., 1998). However, there has been major studies in swine nutrition to mitigate N_2 excretion, without detrimentally impacting growth rate and efficiency of growing-finishing swine. Feeding reduced-CP, AA-supplemented diets has shown to reduce the amount of N_2 excreted from swine (Kerr and Easter, 1995; Kendall, 2000; Figueroa et al., 2002). In fact, Kerr and Easter (1995) estimated that each 1 percentage unit reduction in dietary protein (when accompanied by appropriate AA supplementation) resulted in 8% less N_2 excreted in manure. On the other hand, growth performance and carcass characteristics of pigs fed reduced-CP diets have varied considerably (Dourmad et al., 1993; Kerr et al., 1995; Figueroa et al., 2002). To date, there is little research on the effect of AA-supplementaed, reduced CP diets, formulated on a NE basis, on pig performance and carcass characteristics of swine.

Moreover, few studies have focused the possible changes in FA composition among a number of fat deports associated with feeding reduced CP diets, fortified with feed-grade AA.

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Chapter 2

Effects of Crystalline Amino Acid Supplementation of Reduced Crude Protein (RCP) Diets Formulated on Net Energy Basis on Growing-Finishing Swine: 1. Growth Performance, Carcass Characteristics, and Longissimus Muscle Quality

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ABSTRACT

Barrows and gilts (n=210) were used to test the effects of crystalline AA supplementation of reduced CP (**RCP**) diets (formulated on NE basis) on growth performance, carcass composition, and LM quality characteristics. Pigs were blocked by initial BW (20.7 ± 0.7 kg) before allotment to pens (3 barrows and 3 gilts/pen), and, within each block, pens (4 pens/block) were assigned randomly to either corn-soybean meal diets (**Ctrl**) or 1 of 3 RCP diets supplemented with crystalline AA to meet SID AA ratios during each of the 5 feeding phases (23 to 41, 41 to 59, 59 to 82, 82 to 104, and 104 to 127 kg BW). Ractopamine hydrochloride (10 mg/kg) was included in all diets during the last 3-wk feeding phase. At a mean weight of 127.0 kg, all pigs were humanely slaughtered at a commercial pork packing plant, and, after a 24-h rapid chilling period, a subsample of whole, bone-in pork loins (2/pen) was captured during carcass fabrication for LM quality data collection. Across the entire feeding trial, ADG and ADFI decreased (linear, $P \leq 0.001$) with decreasing dietary CP, but ADG and ADFI were only reduced ($P < 0.05$) by feeding the RCP3 diets compared to feeding Ctrl, RCP1, or RCP2 diets. Conversely, G:F tended to be increased (linear, $P = 0.059$) across the entire feeding trial with decreasing dietary CP. Both slaughter weight and HCW decreased (linear, $P \leq 0.007$) as CP was reduced in the diets. At the end of the feeding trial, ultrasonically measured fat depth was reduced ($P < 0.05$) in pigs fed RCP3 when compared to those fed RCP1 and RCP2 diets, but neither FOM 10th rib fat depth ($P \geq 0.619$) nor LM depth ($P \geq 0.236$) were affected by dietary CP content. Visual color of the LM decreased (linear, $P \leq 0.032$), and L* values increased (linear, $P = 0.015$), with decreasing dietary CP, but there was no ($P \geq 0.172$) effect of dietary CP on a* or b* values. Although LM drip loss percentage increased (linear, $P = 0.015$) as CP was reduced in swine diets, LM firmness and marbling scores, as well as cooking loss percentage and shear

force values, were similar ($P \geq 0.301$) among dietary treatments. Results suggest that CP can be replaced with crystalline AA to meet the requirement of the first 5 limiting AA (RCP2) without impacting growth performance, carcass composition, or LM quality negatively; however, further CP reductions resulted in more variable pig performance and pork quality characteristics.

Keywords: amino acid supplementation, growth performance, net energy, pork quality, reduced crude protein, and swine.

Introduction

Animal waste is one of the major contributions to N_2 pollution in world, and has become more evident with the concentration of animals in confined housing (Prince et al., 2000; Canh et al., 1998). With an increasing N_2 excretion into the environment, research has focused on formulating more environmental friendly swine diets. Research indicated that feeding pigs reduced-CP, AA-supplemented diets can reduce the amount of N_2 excreted into the environment (Kendall, 2000; Figueroa et al., 2002). In fact, Kerr and Easter (1995) estimated that each 1 percentage unit reduction in dietary protein (when accompanied by appropriate AA supplementation) resulted in 8% less N excreted in manure. However, live pig performance and pork carcass characteristics are quite variable when reduced-CP diets were fed to growing and finishing swine (Dourmad et al., 1993; Kerr et al., 1995; Figueroa et al., 2002). Reductions of 2 to 3 percentage units CP is possible without impacting ADG or G:F when feed-grade AA were added to meet AA requirements of growing-finishing swine (Hahn et al., 1995; Kerr et al., 1995; Cromwell, 1996; Tuitoek et al., 1997b). Conversely, reducing dietary CP more than 3 percentage units had detrimental effects on ADG of swine (Hansen et al., 1993; Gomez et al., 2002).

Most research has indicated that reducing dietary CP results in fatter carcasses (Canh et al., 1998, Kerr et al., 1995). Yet, one advantage of feeding growing-finishing pigs reduced CP diets has been the repeated observation of increased marbling and/or intramuscular fat deposition in the LM (Castell et al., 1994; Kerr et al., 1995; Cisneros et al., 1996; Szabo et al., 2001; Apple et al., 2004). In addition, Zhang et. Al (2008) reported that feeding reduced CP diets supplemented with AA, increased moisture loss and decreased the yellowness of pork. Because of the considerable variation in growth performance and the increased fat deposition in pigs fed AA-supplemented, reduced CP diets, the objective of this study was to test the effects of AA supplementation of

reduced CP diets formulated on a NE basis on the growth performance, pork carcass composition and LM quality of growing-finishing swine.

Materials and Methods

Pig husbandry and all experimental protocols were in accordance with standard operating procedures for swine experiments and approval (protocol no.11023) issued by the University of Arkansas Interdepartmental Animal Care and Use Committee prior to initiating this study.

Animals and Diets

Crossbred pigs (n = 210) from the mating of C29 females and line 380 sires (PIC North American, Hendersonville, TN) were blocked by BW (20.7 ± 0.7 kg) into 9 blocks of 24 pigs/block. Pigs within blocks were allotted randomly to mixed-gender pens (3 barrows and 3 gilts/pen), and pens were assigned randomly to 1 of 4 dietary treatments (Table 1): 1) conventional corn-soybean meal-based diets (20.64, 19.38, 16.75, 14.99, and 17.98% CP in feeding phases 1, 2, 3, 4, and 5, respectively), with industry-acceptable levels of supplemental crystalline LYS, MET, and THR to meet standardized ileal digestible (**SID**) LYS requirements for growing-finishing swine (**Ctrl**); 2) reduced CP diets (18.82, 16.85, 14.68, 13.05, and 16.60% CP in feeding phases 1, 2, 3, 4, and 5, respectively) formulated to meet SID VAL requirements without supplemental VAL, but supplemented with crystalline LYS, MET, THR, and TRP to ensure that all indispensable AA were not deficient (**RCP1**); 3) reduced CP diets (18.43, 16.50, 14.08, 12.61, and 16.20% CP in feeding phases 1, 2, 3, 4, and 5, respectively) formulated to meet SID ILE requirements, but supplemented with crystalline LYS, MET, TRP, THR, VAL, and ILE to ensure that indispensable AA were not deficient in these diets (**RCP2**); or 4) reduced CP diets (16.68, 14.70, 12.48, 11.11, and 14.60% CP in feeding phases 1, 2, 3, 4, and 5, respectively) formulated to meet SID HIS requirements without supplemental HIS, but supplemented with

LYS, MET, THR, TRP, VAL, and ILE to ensure that all indispensable AA were not deficient in these diets (**RCP3**). Pigs were fed a 5-phase diet with transition from grower-I (feeding-phase 1) to grower-II (feeding-phase 2), grower-II to finisher-I (feeding-phase 3), finisher-I to finisher-II (feeding-phase 4), and finisher-II to finisher-III (feeding-phase 5) at BW of 41, 59, 82, and 104 kg, respectively. Within each feeding phase, diets were isocaloric (2.53, 2.55, 2.59, 2.62, and 2.57 Mcal/kg NE for feeding phases 1, 2, 3, 4, and 5, respectively) and isolysinic (1.07, 0.90, 0.77, 0.70, and 0.95% LYS for feeding phases 1, 2, 3, 4, and 5, respectively). In addition, 10 mg/kg of ractopamine hydrochloride (Paylean; Elanco Animal Health, Greenfield, IN) was included in all of the 3-week finisher-III diets.

Pigs were housed in a curtain-sided building with completely slatted floors. Each 1.5 × 3.0-m pen was equipped with a single-hole feeder and cup water dispenser for ad libitum access to diets and water. Individual pig BW, as well as pen feed disappearance, were measured at the end of each feeding phase to calculate ADG, ADFI, and G:F. In addition, a trained and certified technician ultrasonically measured 10^{th} rib fat and LM depths on 1 randomly chosen pig from each pen (immediately after pen allotment), as well as on all pigs at the end of each feeding phase to monitor muscle growth and fat deposition.

Pig Slaughter and Carcass Collection

At an average BW of 127.0 kg, all pigs were transported from the University of Arkansas Swine Farm approximately 8 h (720 km) to a commercial pork packing plant (Cargill Meat Solutions, Beardstown, IL) and slaughtered according to humane, industry-accepted procedures after a 6-h lairage period. Tenth rib fat and LM depths were measured on-line with a Fat-O-Meater (FOM) probe inserted between the 10^{th} and 11^{th} ribs at a distance approximately 7 cm from the midline, and HCW and FOM- estimated fat-free lean yield were recorded. Then, the

carcasses were subjected to a 24-h conventional blast-chilling system. Approximately 4 h after entering the chilling system, the lateral processes of the backbone were identified with the individual pig's tattoo numbers, and identified whole, bone-in pork loins were captured during carcass fabrication, vacuum packaged, and subsequently transported under refrigeration back to the University of Arkansas in a refrigerated truck.

Loin Fabrication and Pork Quality Data Collection

Upon arrival at the University of Arkansas Red Meat Abattoir, loins were removed from the packaging material, blade and sirloin sections were removed from each loin, and color of the subcutaneous fat over the center of each loin was evaluated according to the Japanese fat color standards (1 = white to 4 = yellow; Japan Ham & Sausage Processors Coop. Assoc., Shibuya-ku, Tokyo, Japan). Then, center-cut loins were further processed into five 2.54-cm-thick, closely-trimmed (0.64-cm external fat thickness), deboned, LM chops for pork quality data collection. One chop from each loin was designated for measuring drip loss with EZ-DripLoss Tubes (Danish Meat Research Institute, Taastrup, Denmark) in accordance to the procedure of Correa et al. (2007), whereas a second chop was trimmed free of all external fat and used to visually estimate marbling (1 = 1% intramuscular fat content to 10 = 10% intramuscular fat content; NPPC, 1999) before being placed in Whirl-Pak bags and frozen at -20°C for quantifying ether extracted intramuscular fat content (AOAC, 1995). The third chop was allowed to bloom for 30 min before measuring visual color based on both the American (1 = pale, pinkish gray to 6 = dark purplish red; NPPC, 1999) and Japanese color standards (Nakai et al., 1975). After visual color data collection, instrumental color (L*, a*, and b* values) was determined from a mean of 3 random readings made with a Hunter MiniScan EZ model 4500L (Hunter Associate Laboratory, Reston, VA) using illuminant A and a 25-mm view diameter. The

spectrocolorimeter was calibrated against standard white and black tiles (Catalog # MSE70852: Hunter Associates Laboratory) before instrumental data collection. Firmness (1 = very soft/watery to 5 = very firm/dry; NPPC, 1991) was measured on the 4th chop before it was vacuum packaged and subsequently frozen at -20°C for Warner-Bratzler shear force (**WBSF**) determination at a later date. Two 5-g LM samples were removed from the 5th chop for quantifying moisture content according to the freeze-drying method of Apple et al. (2001).

Warner-Bratzler Shear Force Determination

Vacuum-packaged LM chops were thawed for 16 h at 2°C, removed from the packaging material, blotted dry on paper towels, and weighed before being cooked to an internal temperature of 71°C (AMSA, 1995) on electric, countertop griddles (National Presto Industries, Inc., Eau Claire, WI) set at 204°C. Chops were turned every 3 min and internal temperature was monitored with a hand-held digital thermometer (Foodcheck Thermometer; Comark Instruments, Inc., Hichin, Herefordshire, UK). At the internal endpoint temperature, chops were removed from the griddles, allowed to cool to room temperature, and re-weighed in order to calculate cooking loss percentage. Then, six to eight 1.27-cm-diameter cores were mechanically removed parallel with the muscle fiber orientation, and each core was sheared once with a Warner-Bratzler shear force (WBSF) device attached to an Instron Universal Testing machine (model 4466; Instron Corp., Canton, MA), with a 55-kg tension/compression load cell and a crosshead speed of 200 mm/s. Peak WBSF values of the cores from each chop were averaged for statistical analysis.

Statistical Analyses

Data were analyzed as a randomized complete block design, with blocks based on initial BW and pen as the experimental unit. Analysis of variance was generated using the mixed

models procedure of SAS (SAS Inst., Inc., Cary, NC). The lone fixed effect in all statistical models was dietary treatment, whereas block was the random effect. Least-squares means were computed and separated using a pairwise *t*-test (PDIFF option) when a significant *F*-test ($P \leq 0.05$) was observed. In addition, orthogonal contrasts were used to test the linear and quadratic effects of reducing dietary CP on growth performance, carcass compositional measures, and pork quality attributes. Because the dietary CP percentages were not spaced evenly, PROC IML (SAS Inst., Inc.) was used to generate the appropriate linear and quadratic contrast statements.

Results and Discussion

Diet Composition

Analyzed CP content of grower-I and grower-II diets exceeded the formulated CP between 0.37 (RCP2) and 0.78 (RCP1) and between 0.18 (RCP2) and 1.03 (Ctrl), respectively (Table 3). However, when comparing the analyzed and formulated total AA composition of diets, total AA was typically within the range of 0.01 to 0.10 percentage units greater than formulated AA composition. Among finisher diets, analyzed CP content was typically greater than formulated values, whereas total AA were typically within 0.05 percentage units of formulated levels in the finisher diets (Table 4). There were a few exceptions, however, including a greater than 0.10 percentage unit difference between analyzed and formulated LEU in RCP1 and RCP2 finisher-I diets and between analyzed and formulated PHE in all finisher-III diets. In addition, total LYS content was less than formulated values in finisher-II reduced CP diets, but no more than 0.10 percentage units (RCP1).

Growth Performance

During the first (grower I) and second (grower II) feeding phases, ADG ($P \geq 0.120$) and ADFI ($P \geq 0.058$) were similar among dietary treatments; however, pigs fed RCP2 had greater

($P < 0.05$) G:F than those fed either Ctrl or RCP3 diets during the first (quadratic, $P = 0.017$), but not ($P \geq 0.127$) during the second feeding phase (Table 5). Dietary CP did not ($P \geq 0.097$) affect ADG during the 4th feeding phase (finisher II), but ADG decreased (linear, $P \leq 0.025$) with decreasing dietary CP in finisher-I and III diets, with the greatest decrease ($P < 0.05$) observed between pigs fed RCP2 and RCP3 diets. On the other hand, ADFI was not ($P \geq 0.187$) affected by dietary treatments during the 3rd feeding phase (finisher I), but reductions in dietary CP decreased (linear, $P \leq 0.024$) ADFI during the last 2 feeding phases (finisher II and III), and, again, the greatest reduction in ADFI was between RCP2- and RCP3-fed pigs. Among finisher-I diets, G:F decreased (linear, $P = 0.046$) with decreasing dietary CP, but G:F did not ($P \geq 0.154$) differ among dietary treatments during the finisher-II and III phases. Across the entire feeding trial, both ADG and ADFI decreased (linear, $P \leq 0.001$) with decreasing dietary CP, but G:F tended to increase (linear, $P = 0.059$) as dietary CP was reduced in swine diets.

Even though pig BW were similar ($P \geq 0.060$) during the grower phases, BW declined (linear, $P \leq 0.024$) during the finisher phases with decreasing dietary CP, with the greatest reductions ($P < 0.05$) in BW between pigs fed RCP2 and RCP3 diets (Table 5). Ultrasonically measured LM depth was similar among dietary treatments at the end of each grower phase, as well as at the end of the finisher-I and III feeding phases; yet, LM depth tended to decrease (linear, $P = 0.056$) as CP was reduced in finisher-II diets. Interestingly, changes in 10th rib fat depth during the feeding trial were highly variable. For example, ultrasonically measured fat depth increased linearly with decreasing dietary CP during the grower-II ($P < 0.001$), finisher-I ($P < 0.001$), and finisher-II ($P = 0.038$) feeding phases; however, by the end of the last feeding phase, RCP3-fed pigs had less ($P < 0.05$) 10th rib fat than pigs fed RCP1 or RCP2 diets (quadratic, $P = 0.025$).

Reducing dietary CP by 2 to 3 percentage units had no effect on ADG when swine diets were supplemented with crystalline AA to correct any deficiencies (Kerr et al., 1995; Tuitoek et al., 1997; Knowles et al., 1998); however, others have shown that reductions in dietary CP of more than 3 percentage units decreased ADG (Smith et al., 1998; Figrueroa et al., 2002; Gomez et al., 2002). When finishing pigs were fed reduced CP diets fortified with crystalline AA, Apple et al. (2012) reported linear reductions in ADG with decreasing dietary CP. Conversely, in a later study, Apple et al. (2013) reported that overall ADG actually increased quadratically, with pigs fed the lowest CP levels (63 to 70% of the control diet) having lower ADG than pigs fed corn-soybean meal diets formulated to meet AA requirements without crystalline AA. Even though the reductions in dietary CP were similar between Apple et al. (2013) and the present study, diets in the former study were formulated on an ME basis, whereas the latter diets were formulated on an NE basis.

Even though Figueroa et al. (2002) observed a quadratic increase in ADFI as dietary CP was reduced from 16 to 11% in swine diets, other research has indicated that reductions in dietary CP alone (Tous et al., 2014; Madrid et al., 2012; Hinson et al., 2009) or with supplemental AA (Knowles et al., 1998; Smith et al., 1998). Cisneros et al. (1996) reported that ADFI was not altered by feeding reduced CP diets during the late finishing period, but Apple et al. (2012) noted linear decreases in ADFI, and especially when ractopamine hydrochloride was included in the last feeding phase, as CP was reduced in swine finishing diets.

Reducing dietary CP by less than 3 percentage units has no appreciable effects on G:F (Tuitoek et al., 1997; Cromwell, 1996); yet, reductions in dietary CP of more than 3 percentage units decreased G:F of growing-finishing pigs (Carpenter et al., 2004; Cisneros et al., 1996). Hahn et al. (1995), Kerr et al. (1995), and Knowles et al. (1998) noted similar G:F between

control diets and reduced-CP diets fortified with crystalline AA, whereas Gomez et al. (2002) reported that G:F was still reduced by feeding reduced-CP diets, even with the addition of crystalline AA. Apple et al. (2012) reported that G:F was only reduced during the earliest finishing phase when pigs were fed reduced-CP diets, whereas G:F increased quadratically in gilts as dietary CP was reduced, with gilts fed the lowest CP diets having similar G:F than those fed the control diets, but G:F was not affected by reductions in CP in barrows.

Pork Carcass Characteristics

Slaughter weight and HCW were decreased (linear, $P \leq 0.007$) by reducing the CP content of swine diets; yet, only the slaughter weights of RCP3-fed pigs were less ($P < 0.05$) than pigs fed Ctrl, RCP1, and RCP2 diets, whereas carcasses of pigs fed RCP3 were lighter ($P < 0.05$) than those from Ctrl- and RCP1-fed pigs (Table 6). Conversely, neither dressing percent ($P \geq 0.318$), 10^{th} rib fat depth ($P \geq 0.619$), LM depth ($P \geq 0.236$), nor calculated fat-free lean yield ($P \geq 0.121$) were affected by dietary CP.

Similar to the present results, Smith et al. (1998) reported that HCW decreased as dietary CP was decreased in swine diets; however, a number of studies have demonstrated that reducing dietary CP had on appreciable effects on either pig slaughter weights or HCW (D'Souza et al., 2012; Hinson et al., 2009; Knowles et al., 1998). Even though Apple et al. (2012) reported that reducing the CP content of finisher diets did not affect fat depth or percent dissectible fat from fresh hams, research has shown that pork carcasses become fatter when pigs are fed reduce-CP diets (Canh et al., 1998) or reduced-CP diets supplemented with feed-grade AA (Kerr et al., 1995). In addition, 10^{th} rib fat depth, along with the percent dissectible ham fat, increased and calculated fat-free lean yield and percent dissectible ham muscle decreased, with decreasing dietary CP when all diets were formulated on a ME basis (Apple et al., 2013); however, in the

present study, carcass fatness measures were not affected by similar reductions in dietary CP when diets were formulated on an NE basis.

Pork Quality Characteristics

Visual (Japanese and American) color scores for the LM were reduced (linear, $P \leq 0.032$) by decreasing dietary CP, whereas the LM became lighter (greater L* values; linear, $P = 0.015$) as CP was reduced in swine diets; however, dietary CP level had no ($P \geq 0.172$) effect on LM redness or yellowness (Table 7). Interestingly, the visual color of the subcutaneous fat over the center of the pork loin actually declined (became whiter; linear, $P = 0.017$) as CP was reduced in swine diets, which agrees with previous results from this laboratory when RCP diets were formulated on an ME basis (Young et al., 2014).

Subjective firmness of the LM was similar ($P \geq 0.304$) among dietary treatments, and, even though LM drip loss percentages increased (linear, $P = 0.015$) with decreasing dietary CP, LM moisture content was not ($P \geq 0.294$) affected by dietary treatments (Table 7). In addition, dietary CP did not affect LM marbling scores ($P \geq 0.404$) or ether-extracted intramuscular fat content ($P \geq 0.145$), nor did cooking losses ($P \geq 0.301$) and WBSF values ($P \geq 0.344$) change in response to reducing dietary CP.

Visual color scores of the LM do not appear to be affected by reducing the dietary CP content of swine diets (Young et al., 2013; Apple et al., 2012; Hinson et al., 2009). In contrast to the results of the present study, Goerl et al. (1995) observed no effect of reducing dietary CP on the lightness (L* values) of LM, but a* (Young et al., 2013; Goerl et al., 1995) and b* values (Goerl et al., 1995) increased as CP was reduced in swine diets. In addition neither LM drip loss percentages nor firmness scores were affected by reducing the dietary CP fed to growing-finishing swine (Young et al., 2013; Apple et al., 2012; Castell et al., 1994). Although results of

the present study indicated that marbling scores and IMF content were not altered by feeding reduced-CP diets, a number of studies have shown that the IMF content and/or marbling scores of the LM actually increased as CP was reduced in swine diets (Cisneros et al., 1996; Goerl et al., 1995; Castell et al., 1994), even when supplemented with feed-grade AA (Apple et al., 2012; Kerr et al., 1995). In addition, Young et al. (2013) reported that WBSF values increased quadratically as dietary CP was reduced in diets of gilts, but not barrows; however, others have noted no effect of feeding reduced-CP diets supplemented with crystalline AA on shear force values of cooked pork LM (Madeira et al., 2013; Apple et al. 2012).

In summary, as swine were fed dietary CP with AA supplementation growth performance in ADG and ADFI decreased with the decrease in dietary CP. However, G:F tended to increase as dietary CP was reduced in the diets. Increases in fat accumulation in backfat, 10^{th} rib fat, and leaf fat were not observed, as well as, IMF did not increase with decreases with dietary CP reduction. Therefore, leanness in pigs can be retained when decreasing CP in the diet. Visual color scores decreased with decreased dietary CP. In conclusion, pigs fed reduced dietary CP with AA supplementation can sustain lean carcasses with minor quality issues.

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Table 2-Composition (as-fed basis) of grower diets[1]

	Grower I (23 to 41 kg)				Grower II (41 to 59 kg)			
Ingredient, %	Ctrl	RCP1	RCP2	RCP3	Ctrl	RCP1	RCP2	RCP3
Corn	51.03	56.70	57.94	63.68	48.62	56.51	57.62	63.51
Soybean meal	23.63	18.25	17.05	11.50	15.90	8.48	7.40	1.70
DDGS	20.00	20.00	20.00	20.00	30.00	30.00	30.00	30.00
Yellow grease	2.60	1.93	1.78	1.00	2.85	1.93	1.80	1.00
Limestone	1.25	1.275	1.28	1.298	1.29	1.34	1.335	1.36
Salt	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Mono-calcium phosphate	0.16	0.20	0.22	0.28	---	0.03	0.05	0.12
Vitamin premix[2]	0.13	0.13	0.13	0.13	0.15	0.15	0.15	0.15
Mineral premix[3]	0.10	0.10	0.10	0.10	0.15	0.15	0.15	0.15
Copper sulfate	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
L-lysine	0.350	0.512	0.549	0.716	0.334	0.558	0.590	0.763
DL-methionine	0.032	0.077	0.087	0.134	---	0.017	0.026	0.075
L-threonine	0.082	0.156	0.172	0.248	0.029	0.131	0.145	0.224
L-typtophan	---	0.029	0.035	0.065	---	0.040	0.046	0.076
L-valine	---	---	0.020	0.112	---	---	---	0.094
L-isoleucine	---	---	---	0.095	---	---	0.018	0.115
Ethoxiquin	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Phytase[4]	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Tylan-40[5]					0.03	0.03	0.03	0.03
Calculated composition								
CP, %	20.64	18.82	18.43	16.68	19.38	16.85	16.50	14.70
SID lysine, %	1.07	1.07	1.07	1.07	0.90	0.90	0.90	0.90
NE, Mcal/kg	2.53	2.53	2.53	2.53	2.55	2.55	2.55	2.55
SID LYS:NE, g/Mcal NE	4.23	4.23	4.23	4.23	3.54	3.54	3.54	3.54
Total Ca, %	0.61	0.60	0.61	0.61	0.58	0.58	0.58	0.58
Total available P, %	0.31	0.30	0.30	0.30	0.29	0.28	0.28	0.28

Continued Table 1 Composition (as-fed basis) of grower diets

SID AA ratios								
MET:LYS	29.7	31.8	32.2	34.4	31.1	29.53	30.02	32.6
MET + CYS:LYS	55.0	55.0	55.0	55.0	60.0	55.0	55.0	55.0
THR:LYS	65.0	65.0	65.0	65.0	65.0	65.0	65.0	65.0
TRP:LYS	18.0	18.0	18.0	18.0	18.0	18.1	18.1	18.0
ILE:LYS	65.3	56.9	55.0	55.0	68.7	55.1	55.0	55.0
VAL:LYS	73.0	65.0	65.0	65.0	79.9	66.9	65.1	65.0
LEU:LYS	148.1	136.7	134.1	122.3	174.0	155.3	152.7	138.2
HIS:LYS	42.4	37.8	36.8	32.1	46.3	38.9	37.8	32.0
ARG:LYS	103.8	89.5	86.3	71.5	105.6	82.3	78.9	60.9
PHE:LYS	79.4	70.5	68.5	59.3	86.7	72.1	70.0	58.8
PHE + TYR:LYS	138.4	123.6	120.2	104.9	154.9	130.6	127.2	108.4

[1]Control (Ctrl) or reduced CP (RCP) dietary treatments.

[2]Vitamin premix supplied 6,614 IU of vitamin A, 827 IU of vitamin D_3, 26 IU of vitamin E, 2.7 mg of vitamin K, 16.5 mg of pantothenic acid, 30 mg of niacin, 5 mg of riboflavin, and 26 µg of vitamin B_{12} per kilogram of premix (NB-6508; Nutri Blend Corp., Neosho, MO).

[3]Mineral premix supplied 138 mg/kg of Fe from ferrous sulfate, 138 mg/kg of Zn from zinc sulfate, 33 mg/kg of Mn as manganous sulfate, 13.8 mg/kg of Cu from copper sulfate, 0.25 mg/kg of Se from sodium selenite, and 0.25 mg/kg of I from calcium iodate per kilogram of premix (NB-8534; Nutri Blend Corp, Neosho, MO).

[4]Ronozyme P CT (DSM Nutritional Products, LLC, Parsippany, NJ).

Table 3- Composition (as-fed basis) of finisher diets[1]

	Finisher I (59 to 82 kg)				Finisher II (82 to 104 kg)				Finisher III (104 to 127 kg)[2]			
Ingredient, %	Ctrl	RCP1	RCP2	RCP3	Ctrl	RCP1	RCP2	RCP3	Ctrl	RCP1	RCP2	RCP3
Corn	59.58	65.99	67.92	73.08	67.77	73.78	75.15	80.04	63.66	67.96	69.25	74.44
Soybean meal	12.93	6.88	5.04	---	11.75	6.08	4.75	---	21.63	17.53	16.30	11.25
DDGS	22.50	22.50	22.50	22.50	15.75	15.75	15.75	15.75	10.00	10.00	10.00	10.00
Yellow grease	2.65	1.90	1.65	1.00	2.50	1.80	1.65	1.00	2.35	1.85	1.68	1.00
Limestone	1.25	1.268	1.273	1.29	1.113	1.133	1.135	1.15	1.08	1.093	1.095	1.108
Salt	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Mono-calcium phosphate	---	0.08	0.10	0.16	0.06	0.13	0.14	0.20	---	0.05	0.07	0.13
Vitamin premix[3]	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13
Mineral premix[4]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Copper sulfate	0.50	0.50	0.50	0.50	---	---	---	---	---	---	---	---
L-lysine	0.285	0.468	0.523	0.675	0.253	0.424	0.464	0.607	0.300	0.424	0.462	0.614
DL-methionine	---	---	0.010	0.053	---	---	0.005	0.046	0.062	0.097	0.107	0.150
L-threonine	0.020	0.102	0.127	0.196	0.020	0.098	0.116	0.181	0.110	0.166	0.183	0.253
L-typtophan	---	0.032	0.042	0.069	---	0.031	0.038	0.063	---	0.023	0.029	0.056
L-valine	---	---	---	0.083	---	---	---	0.079	---	---	0.021	0.104
L-isoleucine	---	---	0.031	0.117	---	---	0.023	0103	---	---	---	0.086
Ethoxiquin	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Phytase[5]	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Tylan-40[6]	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Calculated composition												
CP, %	16.75	14.68	14.08	12.48	14.99	13.05	12.61	11.11	17.98	16.60	16.20	14.60
SID lysine, %	0.77	0.77	0.77	0.77	0.70	0.70	0.70	0.70	0.95	0.95	0.95	0.95
NE, Mcal/kg	2.59	2.59	2.59	2.59	2.62	2.62	2.62	2.62	2.57	2.57	2.57	2.57
SID LYS:NE, g/Mcal NE	2.98	2.98	2.98	2.98	2.67	2.67	2.67	2.67	3.70	3.70	3.70	3.70

Continued from Table 2 Composition (as-fed basis) of finisher diets

Total Ca, %	0.55	0.55	0.55	0.55	0.50	0.50	0.50	0.50	0.51	0.51	0.51	0.51
Total available P, %	0.26	0.26	0.26	0.26	0.25	0.25	0.25	0.25	0.24	0.24	0.24	0.24
SID AA ratios												
MET:LYS	32.2	28.9	29.2	31.9	32.2	28.8	28.7	31.6	32.8	34.6	35.1	37.3
MET + CYS:LYS	62.2	55.7	55.0	55.0	62.6	55.9	55.0	55.1	58.0	58.0	58.0	58.0
THR:LYS	65.0	65.0	65.0	65.0	65.0	65.0	65.1	65.0	68.0	68.0	68.0	68.1
TRP:LYS	18.1	18.0	18.0	18.0	18.0	18.1	18.1	18.1	18.0	18.1	18.0	18.0
ILE:LYS	69.1	56.0	56.0	56.0	68.6	55.0	55.1	55.0	64.4	57.2	55.1	55.1
VAL:LYS	81.2	68.8	65.1	65.0	80.9	68.0	65.0	65.0	71.9	65.0	65.0	65.0
LEU:LYS	179.0	161.2	155.9	140.9	178.2	159.8	155.5	139.9	144.4	134.5	131.6	119.4
HIS:LYS	47.3	40.2	38.0	32.1	47.3	39.9	38.2	32.0	42.0	38.1	36.9	32.0
ARG:LYS	106.5	84.2	77.5	58.8	106.7	83.6	78.3	58.8	103.9	91.6	87.9	72.8
PHE:LYS	87.8	73.9	69.7	58.1	87.2	72.8	69.4	57.3	78.0	70.4	68.1	58.6
PHE + TYR:LYS	155.6	132.5	125.5	106.1	152.8	128.7	123.2	102.9	133.8	121.0	117.1	101.4

[1]Control (Ctrl) or reduced CP (RCP) dietary treatments.

[2]0.025% (as fed basis), or 10 mg/kg, ractopamine hydrochloride (Paylean; Elanco Animal Health, a Division of Ely Lilly, Greenfield, IN) was included in all finisher-III diets.

[3]Vitamin premix supplied 6,614 IU of vitamin A, 827 IU of vitamin D_3, 26 IU of vitamin E, 2.7 mg of vitamin K, 16.5 mg of pantothenic acid, 30 mg of niacin, 5 mg of riboflavin, and 26 µg of vitamin B_{12} per kilogram of premix (NB-6508; Nutri Blend Corp., Neosho, MO).

[4]Mineral premix supplied 138 mg/kg of Fe from ferrous sulfate, 138 mg/kg of Zn from zinc sulfate, 33 mg/kg of Mn as manganous sulfate, 13.8 mg/kg of Cu from copper sulfate, 0.25 mg/kg of Se from sodium selenite, and 0.25 mg/kg of I from calcium iodate per kilogram of premix (NB-8534; Nutri Blend Corp, Neosho, MO).

[5]Ronozyme P CT (DSM Nutritional Products, LLC, Parsippany, NJ).

Table 4- Calculated and analyzed AA composition of grower diets[1]

	Grower I (23 to 41 kg)				Grower II (41 to 59 kg)			
Dietary component, %	Ctrl	RCP1	RCP2	RCP3	Ctrl	RCP1	RCP2	RCP3
Calculated								
CP	20.64	18.83	18.43	16.68	19.38	16.85	16.50	14.70
Lysine	1.24	1.23	1.22	1.21	1.08	1.06	1.06	1.05
Methionine	0.37	0.39	0.39	0.41	0.33	0.32	0.32	0.34
Threonine	0.85	0.84	0.84	0.82	0.75	0.73	0.73	0.72
Tryptophan	0.23	0.22	0.22	0.22	0.20	0.19	0.19	0.19
Isoleucine	0.82	0.72	0.70	0.69	0.75	0.61	0.61	0.60
Valine	0.94	0.85	0.84	0.83	0.89	0.76	0.74	0.73
Leucine	1.83	1.69	1.66	1.52	1.83	1.64	1.61	1.47
Histidine	0.53	0.48	0.46	0.41	0.50	0.42	0.41	0.36
Arginine	1.23	1.07	1.03	0.87	1.08	0.86	0.83	0.65
Phenylalanine	0.99	0.88	0.86	0.75	0.93	0.78	0.76	0.64
Analyzed								
CP	21.06	19.61	19.17	17.05	20.41	17.15	16.68	14.69
Lysine	1.27	1.26	1.24	1.23	1.10	1.09	1.05	1.04
Methionine	0.39	0.42	0.40	0.43	0.35	0.33	0.33	0.35
Threonine	0.89	0.87	0.85	0.82	0.77	0.73	0.70	0.71
Tryptophan	0.24	0.23	0.23	0.22	0.19	0.19	0.20	0.19
Isoleucine	0.85	0.78	0.77	0.75	0.79	0.65	0.64	0.65
Valine	1.01	0.94	0.95	0.94	0.96	0.82	0.80	0.78
Leucine	1.93	1.85	1.83	1.64	1.92	1.72	1.70	1.53
Histidine	0.55	0.50	0.49	0.43	0.52	0.43	0.42	0.36
Agrinine	1.29	1.16	1.14	0.95	1.16	0.92	0.86	0.70
Phenylalanine	1.04	0.96	0.95	0.81	0.99	0.82	0.80	0.68

[1]Control (Ctrl) or reduced CP (RCP) dietary treatments.

Table 5- Calculated and analyze AA composition of finisher diets[1]

	Finisher I (59 to 82 kg)				Finisher II (82 to 104 kg)				Finisher III (104 to 127 kg)[2]			
Ingredient, %	Ctrl	RCP1	RCP2	RCP3	Ctrl	RCP1	RCP2	RCP3	Ctrl	RCP1	RCP2	RCP3
Calculated												
CP	16.75	14.68	14.08	12.48	14.99	13.05	12.61	11.11	17.98	16.60	16.20	14.60
Lysine	0.92	0.91	0.90	0.89	0.83	0.81	0.81	0.80	1.09	1.08	1.08	1.07
Methionine	0.29	0.27	0.27	0.29	0.27	0.24	0.24	0.26	0.35	0.37	0.37	0.39
Threonine	0.64	0.63	0.62	0.61	0.57	0.56	0.56	0.55	0.78	0.77	0.76	0.75
Tryptophan	0.17	0.17	0.17	0.16	0.15	0.15	0.15	0.15	0.20	0.20	0.20	0.19
Isoleucine	0.64	0.53	0.53	0.52	0.57	0.47	0.47	0.46	0.72	0.64	0.62	0.61
Valine	0.77	0.66	0.63	0.62	0.69	0.59	0.56	0.55	0.82	0.74	0.74	0.73
Leucine	1.61	1.45	1.40	1.27	1.44	1.29	1.26	1.14	1.58	1.48	1.44	1.31
Histidine	0.43	0.37	0.36	0.31	0.39	0.33	0.32	0.27	0.46	0.42	0.41	0.36
Arginine	0.93	0.75	0.69	0.54	0.84	0.67	0.63	0.48	1.09	0.96	0.93	0.77
Phenylalanine	0.80	0.68	0.64	0.54	0.71	0.60	0.57	0.48	0.86	0.78	0.75	0.65
Analyzed												
CP	17.25	14.92	14.22	12.94	15.81	13.00	13.43	11.37	18.03	16.87	16.52	15.98
Lysine	0.94	0.93	0.94	0.92	0.85	0.71	0.80	0.77	1.18	1.18	1.14	1.15
Methionine	0.30	0.28	0.27	0.29	0.26	0.23	0.24	0.25	0.36	0.37	0.36	0.39
Threonine	0.66	0.63	0.63	0.71	0.59	0.55	0.56	0.53	0.79	0.79	0.74	0.75
Tryptophan	0.18	0.16	0.16	0.15	0.15	0.13	0.14	0.13	0.20	0.19	0.20	0.18
Isoleucine	0.70	0.58	0.59	0.51	0.63	0.50	0.45	0.44	0.76	0.68	0.67	0.84
Valine	0.84	0.74	0.71	0.69	0.75	0.64	0.61	0.60	0.87	0.79	0.80	0.81
Leucine	1.69	1.56	1.51	1.33	1.51	1.35	1.31	1.21	1.66	1.54	1.52	1.47
Histidine	0.45	0.39	0.38	0.33	0.41	0.35	0.34	0.29	0.48	0.45	0.43	0.39
Agrinine	0.98	0.78	0.74	0.60	0.89	0.72	0.68	0.53	1.13	1.03	0.98	0.85
Phenylalanine	0.86	0.73	0.70	0.60	0.79	0.66	0.64	0.55	0.96	0.88	0.89	0.75

[1]Control (Ctrl) or reduced CP (RCP) dietary treatments.

Table 6- Effects of AA supplementation of reduced CP (RCP) diets on growth performance and ultrasonically measured carcass traits of growing-finishing swine[1]

	Dietary treatments[1]					*P*-values[2]		
	Ctrl	RCP1	RCP2	RCP3	SEM	Trt	Lin	Quad
No. of pens	9	9	9	9				
Initial BW, kg	20.7	20.7	20.7	20.7	0.07	0.886	---	---
Initial UBF[3], cm	0.25	0.24	0.26	0.26	0.017	0.822	---	---
Initial ULMD[3], cm	1.71	1.64	1.65	1.75	0.081	0.729	---	---
Grower I (23 to 41 kg)								
ADG, kg/d	0.93	0.94	0.94	0.92	0.019	0.391	0.560	0.120
ADFI, kg/d	1.54	1.52	1.49	1.52	0.029	0.217	0.263	0.196
G:F	0.60^{y}	0.61^{xy}	0.64^{x}	0.61^{y}	0.008	0.032	0.441	0.017
BW, kg	46.8	47.0	47.2	46.5	1.1X	0.380	0.596	0.115
UBF[3], cm	0.78	0.79	0.83	0.84	0.030	0.211	0.067	0.974
ULMD[3], cm	3.24	3.32	3.34	3.19	0.061	0.253	0.540	0.061
Grower II (41 to 59 kg)								
ADG, kg/d	1.02	1.00	1.04	0.97	0.025	0.218	0.220	0.215
ADFI, kg/d	2.39	2.48	2.49	2.37	0.066	0.285	0.986	0.058
G:F	0.43	0.41	0.42	0.41	0.010	0.320	0.127	0.698
BW, kg	58.0	58.0	58.6	57.2	1.2X	0.089	0.247	0.060
UBF[3], cm	0.89^{y}	1.00^{x}	0.99^{x}	1.06^{x}	0.041	0.004	< 0.001	0.401
ULMD[3], cm	3.50	3.57	3.61	3.52	0.070	0.507	0.720	0.172
Finisher I (59 to 82 kg)								
ADG, kg/d	1.16	1.16	1.13	1.10	0.022	0.090	0.025	0.362
ADFI, kg/d	2.88	2.89	2.87	2.80	0.053	0.358	0.187	0.233
G:F	0.40	0.40	0.39	0.39	0.005	0.116	0.046	0.965
BW, kg	85.9^{x}	85.9^{x}	85.8^{x}	83.6^{y}	1.5X	0.048	0.024	0.084
UBF[3], cm	1.13^{y}	1.26^{x}	1.36^{x}	1.37^{x}	0.048	0.002	< 0.001	0.262
ULMD[3], cm	4.36	4.37	4.35	4.31	0.057	0.822	0.474	0.543
Finisher II (82 to 104 kg)								
ADG, kg/d	0.96	0.95	0.95	0.90	0.022	0.276	0.097	0.298
ADFI, kg/d	3.14	3.15	3.08	2.96	0.054	0.066	0.024	0.161

Continued Table 5. Effects of AA supplementation of reduced CP (RCP) diets on growth performance and ultrasonically measured carcass traits of growing-finishing swine

G:F	0.31	0.30	0.31	0.30	0.007	0.944	0.944	0.955
BW, kg	106.2^{x}	105.9^{x}	105.7^{x}	105.6^{y}	1.5X	0.011	0.004	0.061
UBF[3], cm	1.56^{y}	1.66^{xy}	1.74^{x}	1.67^{xy}	0.044	0.042	0.038	0.081
ULMD[3], cm	4.75	4.76	4.76	4.59	0.054	0.096	0.056	0.096
Finisher III (104 to 127 kg)								
ADG, kg/d	1.09^{x}	1.07^{x}	1.07^{x}	0.99^{y}	0.031	0.045	0.009	0.293
ADFI, kg/d	3.22^{x}	3.13^{xy}	3.06^{y}	2.84^{z}	0.052	< 0.001	< 0.001	0.349
G:F	0.33	0.34	0.35	0.35	0.007	0.347	0.154	0.346
BW, kg	134.5^{x}	133.8^{x}	133.6^{x}	128.3^{y}	1.9X	0.004	0.001	0.093
UBF[3], cm	1.96^{xy}	2.02^{x}	2.02^{x}	1.83^{y}	0.060	0.045	0.068	0.025
ULMD[3], cm	5.43	5.46	5.39	5.34	0.065	0.503	0.234	0.514
Overall (23 to 127 kg)								
ADG, kg/d	1.03^{x}	1.03^{x}	1.03^{x}	0.98^{y}	0.013	0.004	0.001	0.093
ADFI, kg/d	3.08^{x}	3.05^{x}	3.01^{x}	2.86^{y}	0.043	0.001	< 0.001	0.190
G:F	0.34	0.35	0.35	0.35	0.004	0.400	0.228	0.227

^{x,y}Within a row, least squares means lacking common superscripted letters differ, $P < 0.05$.

[1]Refer to Tables 1 and 2 for description of diets.

[2]Probability values for the main effect of dietary treatment (Trt), as well as for linear (Lin) and quadratic (Quad) effects of reductions in dietary CP.

[3]Ultrasonically measured backfat (UFt) and LM depth (ULMD) at the 10th rib on 2 randomly selected pigs (1 gilt and 1 barrow) from each pen.

Table 7- Effects of AA supplementation of reduced CP (RCP) diets on pork carcass characteristics

	Dietary treatments[1]					*P*-values[2]		
	Ctrl	RCP1	RCP2	RCP3	SEM	Trt	Lin	Quad
No. of pens	9	9	9	9				
Slaughter weight, kg	135.3^{x}	133.5^{x}	134.9^{x}	128.1^{y}	4.18	0.007	0.005	0.101
Dressing percent	72.9	73.3	73.2	73.9	0.73	0.775	0.318	0.880
HCW, kg	97.9^{x}	98.5^{x}	96.9^{xy}	94.6^{y}	1.01	0.017	0.007	0.133
10th rib fat depth, mm	19.5	19.3	20.9	19.0	0.55	0.619	0.811	0.810
LM depth, mm	63.7	63.4	63.4	62.7	0.78	0.236	0.454	0.951
Calculated FFLY[3], %	53.11	53.02	52.61	53.34	0.025	0.121	0.384	0.607

^{x,y}Within a row, least squares means lacking a common superscript differ, $P < 0.05$.
[1]Refer to Tables 1 and 2 for description of diets.
[2]Probability values for the main effect of dietary treatment (Trt), as well as for linear (Lin) and quadratic (Quad) effects of reductions in dietary CP.
[3]FFLY = fat-free lean yield.

Table 8- Effects of AA supplementation of reduced CP (RCP) diets on LM quality traits

	Dietary treatments[1]					*P*-values[2]		
	Ctrl	RCP1	RCP2	RCP3	SEM	Trt	Lin	Quad
No. of pens	9	9	9	9				
Japanese color score[3]	2.9^{x}	2.6^{xy}	2.6^{xy}	2.3^{y}	0.15	0.021	0.003	0.916
American color score[4]	3.3	3.1	3.1	2.9	0.13	0.132	0.032	0.888
Lightness (L*)[5]	53.16^{y}	52.67^{y}	54.55^{xy}	55.24^{x}	0.699	0.052	0.015	0.408
Redness (a*)[5]	17.56	17.59	17.52	17.16	0.206	0.414	0.172	0.343
Yellowness (b*)[5]	13.12	12.89	13.4	13.25	0.286	0.560	0.443	0.869
Subcutaneous fat color[6]	2.6^{xy}	2.7^{x}	2.4^{z}	2.4^{yz}	0.08	0.039	0.017	0.999
Firmness score[7]	3.4	3.4	3.6	3.3	0.16	0.503	0.901	0.304
Drip loss, %	1.17	1.37	2.02	2.37	0.431	0.094	0.015	0.841
Moisture content, %	73.61	73.84	73.66	73.88	0.160	0.584	0.294	0.957
Marbling score[8]	1.6	1.8	1.7	1.7	0.13	0.770	0.717	0.404
Intramuscular fat, %	6.23	6.74	7.46	7.34	0.574	0.374	0.145	0.513
Cooking loss, %	21.62	21.82	21.87	22.07	0.004	0.761	0.301	0.997
Shear force, kg	4.09	4.25	4.23	4.32	0.155	0.759	0.344	0.811

$^{x-z}$Within a row, least squares means lacking common superscripted letters differ, $P < 0.05$.

[1]Refer to Table 1 and 2 for description of diets.

[2]Probability values for the main effect of dietary treatment (Trt), as well as for linear (Lin) and quadratic (Quad) effects of reductions in dietary CP.

[3]1 = pale gray to 6 = dark purple (Nakai et al., 1975).

[4]1 = pale pinkish gray to 6 = dark purplish red (NPPC, 1999).

[5]L* = measure of darkness to lightness (larger number indicates a lighter color); a* = measure of redness (larger number indicates a redder color); and b* = a measure of yellowness (larger number indicates a more yellow color).

[6]1 = white to 4 = yellow (Japan Ham & Sausage Processors Coop. Assoc., Shibuya-ku, Tokyo, Japan).

[7]1 = very soft/watery to 5 = very firm/dry (NPPC, 1991).

[8]1 = 1% i.m. fat to 10 = 10% i.m. fat (NPPC, 1999).

Chapter 3

Effects of Crystalline Amino Acid Supplementation of Reduced Crude Protein (RCP) Diets Formulated on Net Energy Basis on Growing-Finishing Swine: 2. Fatty Acid Composition of the Jowl, Fresh Pork Belly, and Longissimus Muscle

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ABSTRACT

Barrows and gilts (n=210) were used to test the effects of crystalline AA supplementation of reduced crude protein (**RCP**) diets (formulated by net energy basis) on growth performance, carcass composition, and LM quality characteristics. Pigs were blocked by initial BW (20.7 ± 0.7 kg) before allotment to pens (3 barrows and 3 gilts/pen), and, within each block, pens (5 pens/block) were assigned randomly to either corn-soybean meal diets (**Ctrl**) or 1 of 3 RCP diets supplemented with crystalline AA to meet SID AA ratios during each of the 5 feeding phases (23 to 41, 41 to 59, 59 to 82, 82 to 104, and 104 to 127 kg BW). Ractopamine hydrochloride (10 mg/kg) was included in all diets during the last 3-wk feeding phase. At a mean weight of 127.0 kg, all pigs were humanely slaughter at a commercial pork packing plant, and, after a 24-h rapid chilling period, jowls and whole (bone-in) loins, as well as 2.5-cm-thick bellies slices, were collected for fatty acid analysis. Even though the proportion of all saturated fatty acids (SFA) in jowl s.c. fat ($P \geq 0.085$) and LM ($P \geq 0.344$) were not affected by dietary CP, the total monounsaturated fatty acids (MUFA) composition in jowl subcutaneous (s.c.) fat and LM increased (linear, $P \leq 0.008$) as CP reduction in swine diets. In addition, the proportion of all polyunsaturated fatty acids (PUFA), as well as the PUFA:SFA and calculated iodine value (IV), decreased in jowl fat (linear, $P < 0.001$) and LM (linear, $P < 0.001$) with CP was reduced in swine diets. Belly slices were further dissected into the outer s.c. (OSC), middle s.c. (MSC), and intermuscular (INT) fat layers. The INT layer was the most ($P < 0.05$) saturated, whereas the OSC layer had the most ($P < 0.05$) PUFA, as well as the greatest ($P < 0.05$) PUFA:SFA and IV compared to either MSC or INT layer. Moreover, the proportions of all SFA and MUFA increased (linear, $P \leq 0.006$) in belly fat with decreasing dietary CP, but the percentage of all PUFA, along with PUFA:SFA and calculated IV, decreased (linear, $P < 0.001$) as CP was

reduced in diets of growing-finishing swine. Results indicate that: 1) fatty acid composition differs considerably among the fat layers of fresh pork bellies, and 2) the fatty acid composition of pork muscle and fat were altered by reducing dietary CP, suggesting enhanced de novo synthesis in pigs fed reduced CP diets supplemented with crystalline AA.

Keywords: amino acid supplementation, belly fat, fatty acid composition, jowl fat, net energy, pork belly, and reduced crude protein

Introduction

Many factors such as genotype, sex, age, slaughter weight and environmental temperature can affect lipid and the FA content in swine, however nutrition is the main factor in which lipids and FA can be altered in pigs (Wood, 1984; Lebret & Mourot, 1998; Le Dividich et al., 1998). Oliveira and collegues (2006) observed nutrient manipulations in the diet can change the carcass composition of swine. Research has shown pigs that have restrictions in their energy intake, have decreased backfat thickness, adipocyte volume, and lipogenic capacity (Mersmann et al., 1981; Leymaster & Mersmann, 1991). Intramuscular adipocytes have different levels of enzyme activity in lipogenesis in those cells compared to subcutaneous adipocytes in pigs (Gardan et al., 2006). Little research has been conducted to analyze the process of lipid metabolism and deposition in the different layers in the belly for the FA deposition, and very little published research has been conducted to determine the FA composition of LM and jowl subcutaneous fat fed RCP diets. Overall, the objective of this study was to look at fatty acid concentrations in the outer and middle subcutaneous fat tissues, intermuscular fat tissue of the belly, along with the longissimus muscle and the jowl subcutaneous fat layer from pigs fed RCP diets.

Materials and Methods

Pig husbandry and all experimental protocols were in accordance with standard operating procedures for swine experiments and approval (protocol no.11023) issued by the University of Arkansas Interdepartmental Animal Care and Use Committee prior to initiating this study.

Animals and Diets

Crossbred pigs (n = 210) from the mating of C29 females and line 380 sires (PIC North American, Hendersonville, TN) were blocked by BW (20.7 ± 0.7 kg) and gender into 9 blocks of 24 pigs/block. Pigs within blocks were allotted randomly to mixed-gender pens (3 barrows and 3

gilts/pen), and pens were assigned randomly to 1 of 4 dietary treatments (Tables 1 and 2): 1) conventional corn-soybean meal-based diets (20.64, 19.38, 16.75, 14.99, and 17.98% CP in feeding phases 1, 2, 3, 4, and 5, respectively), with industry-acceptable levels of supplemental crystalline LYS, MET, and THR to meet standardized ileal digestible (**SID**) LYS requirements for growing-finishing swine (**Ctrl**); 2) reduced CP diets (18.82, 16.85, 14.68, 13.05, and 16.60% CP in feeding phases 1, 2, 3, 4, and 5, respectively) formulated to meet SID VAL requirements without supplemental VAL, but supplemented with crystalline LYS, MET, THR, and TRP to ensure that all indispensable AA were not deficient (**RCP1**); 3) reduced CP diets (18.43, 16.50, 14.08, 12.61, and 16.20% CP in feeding phases 1, 2, 3, 4, and 5, respectively) formulated to meet SID ILE requirements, but supplemented with crystalline LYS, MET, TRP, THR, VAL, and ILE to ensure that indispensable AA were not deficient in these diets (**RCP2**); or 4) reduced CP diets (16.68, 14.70, 12.48, 11.11, and 14.60% CP in feeding phases 1, 2, 3, 4, and 5, respectively) formulated to meet SID HIS requirements without supplemental HIS, but supplemented with LYS, MET, THR, TRP, VAL, and ILE to ensure that all indispensable AA were not deficient in these diets (**RCP3**). Pigs were fed a 5-phase diet with transition from grower-1 (feeding-phase 1) to grower-II (feeding-phase 2), grower-II to finisher-I (feeding-phase 3), finisher-I to finisher-II (feeding-phase 4), and finisher-II to finisher-III (feeding-phase 5) at BW of 41, 59, 82, and 104 kg, respectively. Within each feeding phase, diets were isocaloric (2.53, 2.55, 2.59, 2.62, and 2.57 Mcal/kg NE for feeding phases 1, 2, 3, 4, and 5, respectively) and isolysinic (1.07, 0.90, 0.77, 0.70, and 0.95% LYS for feeding phases 1, 2, 3, 4, and 5, respectively). In addition, 10 mg/kg of ractopamine hydrochloride (Paylean; Elanco Animal Health, Greenfield, IN) was included in all of the 3-week finisher-III diets. Pigs were housed in a curtain-sided building with

completely slatted floors, and each 1.5 × 3.0-m pen was equipped with a single-hole feeder and cup waters for ad libitum access to diets and water.

Pig Slaughter and Carcass Collection

At an average BW of 127.0 kg, all pigs were transported from the University of Arkansas Swine Farm approximately 8 h (720 km) to a commercial pork packing plant (Cargill Meat Solutions, Beardstown, IL) and slaughtered according to humane, industry-accepted procedures after a 6-h lairage period. Carcasses were subjected to a 24-h conventional blast-chilling system, and, at approximately 4 h after entering the chilling system, the lateral processes of the backbone, as well as the jowl and belly, were identified with the individual pig's tattoo numbers, and identified pork cuts were captured during carcass fabrication. From each identified belly, a 2.5-cm-thick slice was removed from the anterior end of each belly and placed in identified Whirl-Pak bags before being boxed for transport. Skin-on jowls were placed in lined-boxes, whereas whole, bone-in loins were vacuum packaged, and boxed (4 loins/box) before transport under refrigeration back to the University of Arkansas.

Sample Preparation

Upon arrival at the University of Arkansas Red Meat Abattoir, center-cut pork loins were manufactured and a 2.54-cm-thick chop was cut, trimmed of all external fat, and two 10-g samples were taken for freeze LM fatty acid analysis. In addition, the subcutaneous (**s.c.**) fat layer was dissected from each jowl, whereas each the s.c. fat layer of each belly slice was removed at the natural seam and further dissected into the outer (**OSC**) and middle (**MSC**) s.c. fat layers. The fat between the primary (latissimus dorsi) and secondary (cutaneous trunci) lean were removed, pooled, and designated as intermuscular fat (**INT**).

Fatty acid analysis

Duplicate 10-g fat samples from the jowl, belly OSC, belly MSC, and belly INT, as well as LM, were weighed and placed in 30-mL beakers, and reweighed. Beakers were then placed into vacuumed-flasks attached to the manifold of a Labconco freeze-dryer (Model 4.5, Laconco Corp., Kansas City, MO) with a temperature setting of -50°C and a vacuum of less than 10 mm of Hg. Fat and muscle samples were freeze-dried for 72 h before duplicate 30-mg, freeze-dried samples were subjected to direct transesterification by incubating in 2.0 mL of 0.2 *M* methanolic KOH in 16 x 125-mm screwcap tubes at 50°C for 30 min with vortex-mixing 2 to 3 times/min until dissolved (Murrieta et al., 2003). Tubes were allowed to cool to room temperature, and 1 mL of saturated NaCl was added to each tube. Then, 2 mL of hexane containing 0.5 mg/mL of an internal standard (methyl tridecanoic acid [13:0]) were added to tubes, tubes were vortexed, and subsequently centrifuged for 5 min at 1,100 x g at 22°C to separate phases.

Fatty acid methyl esters (FAME) were transferred to GLC vials that contained 1.0-mm bed of anhydrous sodium sulfate. Separation of FAME was achieved by GLC (Model 5890 Series II GC with automatic sample injector [HP-7673] with HP-3365 software; Hewlett-Packard, Avondale, PA) equipped with a 100-m capillary column (0.25-mm internal diameter; Model 2560 Fused Silica Capillary; Supelco Inc., Bellefonte, PA) and a He as the carrier gas (20 cc/sec). Oven temperature was maintained at 150°C for 5 min, ramped at 4°C/min to 194°C for 15 min, and then ramped at 2.5 °C/min to 235 °C for 16.25 min, whereas injector and detector temperatures were maintained at 250 °C. Qualification of peaks was accomplished using purified standards obtained from Supelco (Bellefonte, PA; 37 component mix) and individual acids from Nu-Check Prep (Elysian, MN) and Martreya (Pleasant Gap, PA).

The total proportion of all SFA was the sum of the weight percentages of capric (10:0), lauric (12:0), myristic (14:0), pentadecanoic (15:0), palmitic (16:0), margaric (17:0), stearic

(18:0), and arachidic (20:0) acids, whereas the total proportion of all MUFA was the sum of the weight percentages of myristoleic (14:1), palmitelaidic (16:1*t*), palmitoleic (16:1*c*), all 18-carbon fatty acids with a single trans double bond (18:1*t*), oleic (18:1*c*9), vaccenic (18:1*c*11), and gadoleic (20:1) acids. In addition, the total percentage of all PUFA was calculated by summing the weight percentages of linoleic (18:2*n*-6), total conjugated linoleic (CLA, including the particular isomer of 18:2*c*9*t*11), α-linolenic (18:3*n*-3), eicosadienoic (20:2), dihomo-γ-linolenic (20:3*n*-6), eicosatrienoic (20:3*n*-3), arachidonic (20:4), eicosapentaenoic (20:5), docospentaenoic (22:5), and docosahexaenoic (22:6) acids. The PUFA:SFA ratio was calculated by dividing the total proportion of PUFA by the total proportion of SFA, and iodine value (**IV**) was calculated according to the AOCS (1998) equation: $(0.95 \times [\Sigma 16:1]) + (0.86 \times [\Sigma 18:1]) + (1.732 \times [\Sigma 18:2]) + (2.616 \times [\Sigma 18:3]) + (0.785 \times [20:1])$, where brackets indicate the weight percentage.

Statistical Analysis

Data were analyzed as a randomized complete block design, with blocks based on initial BW and pen as the experimental unit. Analysis of variance was generated with the mixed models procedure of SAS (SAS Inst. Inc., Cary, NC). The statistical model for jowl and LM fatty acid composition included dietary treatment as the lone fixed effect, whereas the model for fresh belly fatty acid composition included dietary treatment, belly fat layer, and the 2-way interaction as fixed effects; block was included in the model as the lone random effect. Least-squares means were computed and separated using a pairwise *t*-test (PDIFF option) when a significant *F*-test ($P \leq 0.05$) was observed. In addition, orthogonal contrasts were used to test the linear and quadratic effects of reducing dietary CP on fatty acid composition of the jowl, belly, and LM; however, because the dietary CP percentages were not spaced evenly, PROC IML (SAS Inst., Inc.) was used to generate the appropriate linear and quadratic contrast statements.

Results

Fatty Acid Composition of the LM

Dietary CP had no effect on the proportions of all SFA ($P \geq 0.344$), and, in particular, weight percentages of lauric acid (12:0; $P \geq 0.152$) myristic acid (14:0; $P \geq 0.154$), pentadecanoic acid (15:0; $P \geq 0.324$), palmitic acid (16:0; $P \geq 0.171$), stearic acid (18:0; $P \geq 0.914$); and arachidic acid (20:0; $P \geq 0.621$) in the LM (Table 3). However, the proportion of capric acid (10:0) in the LM increased (linear, $P = 0.045$) with increasing dietary CP, whereas the proportion of margaric acid (17:0) in the LM decreased (linear, $P = 0.024$) with increasing dietary CP.

The weight percentage of all MUFA, as well as the percentages of palmitoleic (16:1*c*), oleic (18:1*c*9), and vaccenic (18;1*c*11) acids, increased (linear, $P < 0.001$) in the LM as dietary CP was reduced in swine diets (Table 3). In particular, the LM from pigs fed RCP3 diets had greater ($P < 0.05$) proportions of all MUFA, 16:1*c*, 18:1*c*9, and 18:1*c*11 than the LM from pigs fed Ctrl or RCP1 diets, and the LM of RCP2-fed pigs had greater ($P < 0.05$) levels of all MUFA, 16:1*c*, 18:1*c*9, and 18:1*c*11 than that of Ctrl-fed pigs. In contrast, the percentage of all 18:1*t* fatty acids decreased (linear, $P < 0.001$) with decreasing dietary CP, with the LM from Ctrl-fed pigs having the greatest ($P < 0.05$) proportion of 18:1*t* and the LM from pigs fed RCP1 diets had greater ($P < 0.05$) 18:1*t* than those fed either RCP2 or RCP3 diets. Interestingly, no palmitelaidic acid (16:1*t*) was detected in the LM.

The total proportion of PUFA in the LM, as well as the individual PUFA of linoleic (18:2*n*-6), α-linolenic (18:3*n*-3), eicosadienoic (20:2), dihomo-γ-linolenic (20:3*n*-6), eicosatrienoic (20:3*n*-3), arachidonic (20:4), eicosapentaenoic (20:5), docospentaenoic (22:5), and docosahexaenoic (22:6) acids, decreased (linear, $P \leq 0.050$) with decreasing dietary CP

(Table 3). More specifically, LM from pigs fed control diets had greater ($P < 0.05$) proportions of all PUFA, especially 18:2*n*-6 and 18:3*n*-3, than pigs fed the RCP2 or RCP3 diets. The proportion of all CLA in the LM was similar ($P \geq 0.094$) among the dietary treatments, but the weight percentage of the conjugated isomer 18:2*c*9*t*11 decreased (linear, $P < 0.001$) as CP was reduced in swine diets, with the LM from Ctrl-fed pigs having greater ($P < 0.05$) percentages of 18:2*c*9*t*11 than the LM from either RCP1- or RCP2-fed pigs; pigs fed RCP3 diets had the least ($P < 0.05$) 18:2*c*9*t*11 in the LM compared to the other dietary treatments.

The proportion of other, non-identifiable fatty acids in the LM was not ($P \geq 0.331$) affected by dietary CP (Table 3). Yet, PUFA:SFA and calculated IV decreased (linear, $P \leq 0.001$) as CP was reduced in swine diets, with the LM from Ctrl-fed pigs having greater ($P < 0.05$) PUFA:SFA and IV than the LM from RCP2- and RCP3-fed pigs and the LM from RCP1-fed pigs had greater ($P < 0.05$) PUFA:SFA and IV than that of pigs fed RCP3 diets.

Fatty Acid Composition of Jowl Subcutaneous Fat

Weight percentages of 10:0, 12:0, 14:0, and 16:0 were increased (linear, $P \leq 0.010$) with decreasing dietary CP, with jowl fat from pigs fed the RCP3 diets having greater ($P < 0.05$) proportions of 10:0, 12:0, 14:0, and 16:0 than jowl s.c. fat from Ctrl-fed pigs (Table 4). Conversely, levels of 15:0, 17:0, 18:0, and 20:0 in jowl s.c. fat did not ($P \geq 0.062$) differ among the dietary treatments. Moreover, reducing the CP in diets of growing-finishing pigs had no ($P \geq 0.085$) effect on the proportion of all SFA in jowl s.c. fat.

Reducing dietary CP resulted in linear increases in the proportions of all MUFA ($P = 0.008$), including 16:1*c* ($P = 0.006$), 18:1*c*9 ($P = 0.022$), and 18:1*c*11 ($P = 0.008$), in jowl s.c. fat, with weight percentages of all MUFA, 16:1*c*, 18:1*c*9, and 18:1*c*11 being greater ($P < 0.05$) in jowl fat from RCP3-fed pigs compared to jowl fat from pigs the Ctrl or RCP1 diets (Table 4).

Interestingly, the proportions of the *trans*-MUFA (16:1*t* and 18:1*t*) in jowl s.c. fat decreased (linear, $P < 0.001$) with decreasing dietary CP; however, the dietary treatments had no ($P \geq 0.533$) effect on the weight percentages of 20:1 in jowl fat.

Reducing dietary CP resulted in decreases (linear, $P < 0.001$) in the percentage of all PUFA in the jowl s.c. fat, with jowl fat from pigs fed RCP3 diets having less ($P < 0.05$) total PUFA than jowl fat from either Ctrl-fed or RCP1-fed pigs (Table 4). In addition, linear reductions in the proportions of 18:2*n*-6 ($P = 0.001$), 20:2 ($P = 0.009$), 20:3*n*-6 ($P = 0.001$), 20:3*n*-3 ($P < 0.001$), and 22:5 ($P < 0.001$) were observed in jowl fat with decreasing dietary CP. Total CLA percentages, and especially the proportion of the 18:2*c*9*t*11 isomer, were decreased (linear, $P < 0.001$) in jowl s.c. fat as CP was reduced in swine diets, with RCP3-fed pigs having the lowest ($P < 0.05$) levels of CLA compared to the other dietary treatments. Weight percentages of 20:4 and other non-identifiable fatty acids were similar ($P \geq 0.068$) among the dietary treatments, and, interesting, 18:3*n*-3, 20:5, and 22:6 were not detected in jowl s.c. fat samples.

The PUFA:SFA of jowl fat was reduced (linear, $P < 0.001$) as dietary CP was reduced in swine diets, with jowl fat from pigs fed Ctrl diets having the greatest ($P < 0.05$), and pigs fed RCP3 diets having the least ($P < 0.05$), PUFA:SFA (Table 4). Moreover, calculated IV decreased (linear, $P < 0.001$) with decreasing dietary CP, and jowl fat from Ctrl-fed pigs had greater ($P < 0.05$) IV than that of pigs fed the reduced CP diets.

Fatty Acid Composition of Fresh Pork Belly Fat

Weight percentages of 16:1*t* greater ($P < 0.05$) in the OSC layer of pigs fed Ctrl and RCP1 diets than those fed RCP2 and RCP3 diets, whereas the MSC layer from RCP3-fed pigs had the least ($P < 0.05$) quantity of 16:1*t* and pigs fed RCP2 diets had lower ($P < 0.05$) levels of

16:1*t* in the MSC layer than either Ctrl- or RCP1-fed pigs (dietary treatment × belly fat layer, $P = 0.024$; Figure 1). Within the belly INT fat, pigs fed Ctrl diets had the most ($P < 0.05$), and pigs fed RCP3 diets had the lowest ($P < 0.05$), percentages of 16:1*t*, with INT levels in RCP1-fed pigs greater ($P < 0.05$) than RCP2-fed pigs. Otherwise, there were no interactions between dietary CP and belly fat layer for any of the SFA ($P \geq 0.150$), MUFA ($P \geq 0.129$), PUFA ($P \geq 0.116$), PUFA:SFA ($P \geq 0.334$), or calculated IV ($P \geq 0.139$). Thus, results of the main effects of dietary treatments and fat layer are presented in Tables 5 and 6, respectively.

Effects of reduced CP

The sum of all SFA in belly fat increased (linear, $P = 0.006$) with decreasing dietary CP, with belly fat of pigs fed the reduced CP diets being more ($P < 0.05$) saturated than that of pigs fed the Ctrl diets (Table 5). More specifically, the proportions of 10:0, 12:0, 14:0, and 16:0 increased (linear, $P < 0.001$) with decreasing dietary CP, whereas the weight percentages of the odd-chain SFA (15:0 and 17:0) actually decreased (linear, $P \leq 0.002$) as CP was reduced in swine diets. Interestingly, the quantity of 18:0 increased in belly fat as dietary CP was reduced from Ctrl to RCP2 but levels of 18:0 declined as CP was further reduced ($P < 0.05$) from RCP2 to RCP3 (quadratic, $P = 0.014$); however, proportions of 20:0 were similar ($P \geq 0.251$) among the dietary treatments.

The proportions of all MUFA, as well as the weight percentages of the primary MUFA 16:1*c*, 18:1*c*9, and 18:1*c*11, increased (linear, $P < 0.001$) as dietary CP was reduced in swine diets (Table 5). More specifically, belly fat from pigs fed RCP2 and RCP3 diets had greater ($P < 0.05$) quantities of 18:1*c*9 and all MUFA than belly fat from pigs fed Ctrl and RCP1 diets, whereas RCP3-pigs had more ($P < 0.05$) 16:1*c* and 18:1*c*11 in belly fat samples than all other dietary treatments, with belly fat of pigs fed RCP2 diets having greater ($P < 0.05$) quantities of

16:1*c* and 18:1*c*11 than that of pigs fed Ctrl or RCP1 diets. On the other hand, percentages of all 18:1*t* fatty acids, as well as 20:1, were reduced (linear, $P \leq 0.022$) by decreasing the dietary CP fed to growing-finishing pigs.

The concentration of all PUFA in belly fat decreased (linear, $P < 0.001$) with decreasing dietary CP, with belly fat from pigs fed Ctrl being the most ($P < 0.05$), and that from pigs fed RCP2 and RCP3 diets being the least ($P < 0.05$), polyunsaturated (Table 5). The weight percentages of all identified PUFA (18:2*n*-6, 18:3*n*-3, 20:2, 20:3*n*-6, 20:3*n*-3, 20:4, and 22:5) decreased (linear, $P \leq 0.005$) as CP was reduced in the diets of growing-finishing pigs, with belly fat of Ctrl- and RCP1-fed pigs having greater ($P < 0.05$) quantities of 18:2*n*-6, 20:2, and 20:3*n*-6 than pigs fed RCP2 and RCP3 diets. In addition, weight percentages of all CLA (and the 18:2*c*9*t*11 isomer), 18:3*n*-3, 20:3*n*-3, and 22:5 were greatest ($P < 0.05$) in belly fat of Ctrl-fed pigs, whereas percentages of these individual PUFA were least ($P < 0.05$) in belly fat from RCP3-fed pigs, and belly fat from pigs fed RCP1 diets had greater ($P < 0.05$) levels of CLA, 18:3*n*-3, 20:3*n*-3, and 22:5 than that of RCP2-fed pigs. Proportions of other unidentified fatty acids also declined (linear, $P < 0.001$) with decreasing dietary CP. Additionally belly fat PUFA:SFA and IV decreased (linear, $P < 0.001$) in response to the concomitant increases in SFA and MUFA as PUFA decreased in fat from fresh pork bellies.

Belly fat layer

The INT layer had the greatest ($P < 0.05$) proportions of all SFA, as well as 12:0, 14:0, 16:0, and 18:0, than either s.c. fat layer, and the MSC layer had greater ($P < 0.05$) percentages of 12:0, 14:0, 16:0, and 18:0 than the OSC layer (Table 6). In addition, the INT and MSC layers had greater ($P < 0.05$) quantities of 10:0 and 17:0 than the OSC layer; however, the concentration of 15:0 was greater ($P < 0.05$) in the OSC than the MSC and INT layers, and the

MSC layer had the greatest ($P < 0.05$), whereas the INT layer had the lowest ($P < 0.05$), percentage of 20:0.

The proportion of all MUFA, and particularly the major MUFA 16:1*c* and 18:1*c*9, were greater ($P < 0.05$) in the s.c. belly fat layers than the INT layer (Table 6). Moreover, the OSC layer had the greatest ($P < 0.05$), and the INT layer had the lowest ($P < 0.05$), concentrations of 18:1*c*11 and 20:1, but the percentage of all 18:1*t* was similar ($P = 0.162$) among the 3 belly fat layers.

Similar to changes in MUFA levels among the belly fat layers, the OSC layer had greater ($P < 0.05$) proportions of all PUFA, as well as 18:2*n*-6, 18:3*n*-3, and 20:3*n*-6, than the MSC and INT layers (Table 6). In addition, weight percentages of all CLA, 18:2*c*9*t*11, 20:2, and 20:3*n*-3 were greatest ($P < 0.05$) in the OSC, whereas the MSC layer had greater ($P < 0.05$) quantities of CLA, 18:2*c*9*t*11, 20:2, and 20:3*n*-3 than the INT layer. Percentages of 20:4 were greater ($P < 0.05$) in the OSC and INT layers than the MSC layer, and the OSC layer had the most ($P < 0.05$), and the MSC layer the least ($P < 0.05$), amount of 22:5. The OSC layer had greater ($P < 0.05$) levels of other unidentifiable fatty acids and PUFA:SFA than the MSC and INT layers, and calculated IV was greatest ($P < 0.05$) in the OSC layer, with the MSC layer having a greater ($P < 0.05$) IV than the INT belly fat layer.

Discussion

Fatty acid compositions of swine differ between IMF and subcutaneous fat. Intramuscular adipocytes have relatively low activity levels of enzymes of lipogenesis in those cells compared to subcutaneous adipocytes in pigs (Gardan et al., 2006). In the present study, total SFA percentage, palmitic acid (16:0), stearic acid (18:0) did not differ among the dietary treatments in the LM. Total MUFA percentage along with oleic acid (18:1c9) increased linearly while dietary

crude protein decreased. Total PUFA percentage decreased with decreasing dietary crude protein. These results are similar to previous studies (Alonso et al., 2009; Madeira et al., 2012; Tous et al., 2014; Wood et al., 2013). Cameron and Enser (1991) showed greater IMF content in intramuscular fat also had greater concentrations of SFA and MUFA (which are mainly found in neutral lipids), but are prone to decrease the concentration of PUFAs (which are found in the phospholipids), which is also similar to our results. One possibility that could have made this occur is that lower dietary protein levels stimulate a muscle lipogenic enzyme (stearoyl Co-A desaturase), and increase de novo synthesis (Doran et al, 2006). Looking specifically at NE content as it increases in diet, the total SFA, MUFA, PUFA percentages did not change (Suarez-Belloch et al., 2012). Therefore, pigs on dietary crude protein diets will increase in SFA, MUFA percentages in the LM, while PUFAs decrease.

In the present study, weight percentages of 10:0, 12:0, 14:0, and 16:0 were increased with decreasing dietary CP. Moreover, reducing the CP in diets of growing-finishing pigs had no effect on the proportion of all SFA in jowl s.c. fat. As for MUFAs, reducing the dietary CP, caused a linear increase. Interestingly, the proportions of the *trans*-MUFA (16:1*t* and 18:1*t*) in jowl s.c. fat decreased with decreasing dietary CP; however, the dietary treatments had no effect on the weight percentages of 20:1 in jowl fat. With PUFAs, reducing dietary CP resulted in decreases in the percentage of all PUFA in the jowl s.c. fat. The PUFA:SFA of jowl fat was reduced as dietary CP was reduced in swine diets. Doran and colleagues (2006) saw increases in stearic and palmitic acid with reduction in crude protein, and also saw increases in oleic acid, which is similar to our study. Teye and colleagues (2006b) observed low protein diets increase concentrations of SFA (16:0 and 18:0) and reduce concentrations of PUFA (18:2 and 18:3n3) which resulted in a lower PUFA:SFA ratio, which is similar to our results. In contrast, Alonso

and colleagues (2010) did not observe a decrease in PUFA:SFA ratio and did not get differences in the fatty acids. Madeira and collegues (2013) only detected changes in 16:1c7, 18:2n6 and 18:3n3 in the subcutaneous adipose tissue with differences in the diet, which partially corresponds to our data. Tous and colleagues (2014) noticed increases in SFA and 18:0 but not 16:0 when dietary protein decreased, MUFAs increased while PUFAs decreased with dietary protein reductions, which follows the results shown in our study.

Belly fat layers were dissected into the outer subcutaneous layer (OSC), middle or inner subcutaneous layer (MSC), and the intermuscular layer (INT). The INT layer had the greatest proportions of all SFA, as well as 12:0, 14:0, 16:0, and 18:0, than either s.c. fat layer, and the MSC layer had greater percentages of 12:0, 14:0, 16:0, and 18:0 than the OSC layer. The proportion of all MUFA, and particularly the major MUFA 16:1*c* and 18:1*c*9, were greater in the s.c. belly fat layers than the INT layer. Moreover, the OSC layer had the greatest, and the INT layer had the lowest, concentrations of 18:1*c*11 and 20:1, but the percentage of all 18:1*t* was similar among the 3 belly fat layers.

Similar to changes in MUFA levels among the belly fat layers, the OSC layer had greater proportions of all PUFA, as well as 18:2*n*-6, 18:3*n*-3, and 20:3*n*-6, than the MSC and INT layers. Eggert and colleagues (2001) showed that the fatty acid composition of belly fat were similar in all fatty acids except three (18:1n–7, 18:3n– 6, and 18:3n–3) with different dietary treatments Belly fat had more total CLA, SFA, less MUFA, and UFA, resulting in lower PUFA:SFA and greater SFA:UFA ratios than belly fat. Similar results were shown for having different FA compositions in the different layers of the belly, where unsaturation decrease when getting closer to the intermuscular fat, same went for the MUFAs (Monzoils et al., 2007). In all, FA compositions differed between the layers of the belly, and could attribute to belly firmness.

In summary, the fatty acid composition of the intramuscular fat and subcutaneous fat differed among pigs. Increase in SFAs and MUFAs at the expense of PUFAs, suggest there is an increase in de novo synthesis if pigs are fed a reduced crude protein diet supplemented with amino acids. Belly fatty acid composition differed between the layers of outer subcutaneous, inner subcutaneous and intermuscular fat. Data suggest that the inner subcutaneous fat was most metabolically active, while the intermuscular fat was the most mature which is opposite of the backfat layers. This could attribute to belly firmness being associated to all fat layers.

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Table 1. Composition (as-fed basis) of grower diets[1]

	Grower I (23 to 41 kg)				Grower II (41 to 59 kg)			
Ingredient, %	Ctrl	RCP1	RCP2	RCP3	Ctrl	RCP1	RCP2	RCP3
Corn	51.03	56.70	57.94	63.68	48.62	56.51	57.62	63.51
Soybean meal	23.63	18.25	17.05	11.50	15.90	8.48	7.40	1.70
DDGS	20.00	20.00	20.00	20.00	30.00	30.00	30.00	30.00
Yellow grease	2.60	1.93	1.78	1.00	2.85	1.93	1.80	1.00
Limestone	1.25	1.275	1.28	1.298	1.29	1.34	1.335	1.36
Salt	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Mono-calcium phosphate	0.16	0.20	0.22	0.28	---	0.03	0.05	0.12
Vitamin premix[2]	0.13	0.13	0.13	0.13	0.15	0.15	0.15	0.15
Mineral premix[3]	0.10	0.10	0.10	0.10	0.15	0.15	0.15	0.15
Copper sulfate	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
L-lysine	0.350	0.512	0.549	0.716	0.334	0.558	0.590	0.763
DL-methionine	0.032	0.077	0.087	0.134	---	0.017	0.026	0.075
L-threonine	0.082	0.156	0.172	0.248	0.029	0.131	0.145	0.224
L-typtophan	---	0.029	0.035	0.065	---	0.040	0.046	0.076
L-valine	---	---	0.020	0.112	---	---	---	0.094
L-isoleucine	---	---	---	0.095	---	---	0.018	0.115
Ethoxiquin	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Phytase[4]	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Tylan-40[5]					0.03	0.03	0.03	0.03
Calculated composition								
CP, %	20.64	18.82	18.43	16.68	19.38	16.85	16.50	14.70
SID lysine, %	1.07	1.07	1.07	1.07	0.90	0.90	0.90	0.90
NE, Mcal/kg	2.53	2.53	2.53	2.53	2.55	2.55	2.55	2.55
SID LYS:NE, g/Mcal NE	4.23	4.23	4.23	4.23	3.54	3.54	3.54	3.54
Total Ca, %	0.61	0.60	0.61	0.61	0.58	0.58	0.58	0.58
Total available P, %	0.31	0.30	0.30	0.30	0.29	0.28	0.28	0.28

Continued Table 1

SID AA ratios								
MET:LYS	29.7	31.8	32.2	34.4	31.1	29.53	30.02	32.6
MET + CYS:LYS	55.0	55.0	55.0	55.0	60.0	55.0	55.0	55.0
THR:LYS	65.0	65.0	65.0	65.0	65.0	65.0	65.0	65.0
TRP:LYS	18.0	18.0	18.0	18.0	18.0	18.1	18.1	18.0
ILE:LYS	65.3	56.9	55.0	55.0	68.7	55.1	55.0	55.0
VAL:LYS	73.0	65.0	65.0	65.0	79.9	66.9	65.1	65.0
LEU:LYS	148.1	136.7	134.1	122.3	174.0	155.3	152.7	138.2
HIS:LYS	42.4	37.8	36.8	32.1	46.3	38.9	37.8	32.0
ARG:LYS	103.8	89.5	86.3	71.5	105.6	82.3	78.9	60.9
PHE:LYS	79.4	70.5	68.5	59.3	86.7	72.1	70.0	58.8
PHE + TYR:LYS	138.4	123.6	120.2	104.9	154.9	130.6	127.2	108.4

[1]Control (Ctrl) or reduced CP (RCP) dietary treatments.

[2]Vitamin premix supplied 6,614 IU of vitamin A, 827 IU of vitamin D_3, 26 IU of vitamin E, 2.7 mg of vitamin K, 16.5 mg of pantothenic acid, 30 mg of niacin, 5 mg of riboflavin, and 26 µg of vitamin B_{12} per kilogram of premix (NB-6508; Nutri Blend Corp., Neosho, MO).

[3]Mineral premix supplied 138 mg/kg of Fe from ferrous sulfate, 138 mg/kg of Zn from zinc sulfate, 33 mg/kg of Mn as manganous sulfate, 13.8 mg/kg of Cu from copper sulfate, 0.25 mg/kg of Se from sodium selenite, and 0.25 mg/kg of I from calcium iodate per kilogram of premix (NB-8534; Nutri Blend Corp, Neosho, MO).

[4]Ronozyme P CT (DSM Nutritional Products, LLC, Parsippany, NJ).

Table 2. Composition (as-fed basis) of finisher diets[1]

	Finisher I (59 to 82 kg)				Finisher II (82 to 104 kg)				Finisher III (104 to 127 kg)[2]			
Ingredient, %	Ctrl	RCP1	RCP2	RCP3	Ctrl	RCP1	RCP2	RCP3	Ctrl	RCP1	RCP2	RCP3
Corn	59.58	65.99	67.92	73.08	67.77	73.78	75.15	80.04	63.66	67.96	69.25	74.44
Soybean meal	12.93	6.88	5.04	---	11.75	6.08	4.75	---	21.63	17.53	16.30	11.25
DDGS	22.50	22.50	22.50	22.50	15.75	15.75	15.75	15.75	10.00	10.00	10.00	10.00
Yellow grease	2.65	1.90	1.65	1.00	2.50	1.80	1.65	1.00	2.35	1.85	1.68	1.00
Limestone	1.25	1.268	1.273	1.29	1.113	1.133	1.135	1.15	1.08	1.093	1.095	1.108
Salt	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Mono-calcium phosphate	---	0.08	0.10	0.16	0.06	0.13	0.14	0.20	---	0.05	0.07	0.13
Vitamin premix[3]	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13
Mineral premix[4]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Copper sulfate	0.50	0.50	0.50	0.50	---	---	---	---	---	---	---	---
L-lysine	0.285	0.468	0.523	0.675	0.253	0.424	0.464	0.607	0.300	0.424	0.462	0.614
DL-methionine	---	---	0.010	0.053	---	---	0.005	0.046	0.062	0.097	0.107	0.150
L-threonine	0.020	0.102	0.127	0.196	0.020	0.098	0.116	0.181	0.110	0.166	0.183	0.253
L-typtophan	---	0.032	0.042	0.069	---	0.031	0.038	0.063	---	0.023	0.029	0.056
L-valine	---	---	---	0.083	---	---	---	0.079	---	---	0.021	0.104
L-isoleucine	---	---	0.031	0.117	---	---	0.023	0103	---	---	---	0.086
Ethoxiquin	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Phytase[5]	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Tylan-40[6]	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Calculated composition												
CP, %	16.75	14.68	14.08	12.48	14.99	13.05	12.61	11.11	17.98	16.60	16.20	14.60
SID lysine, %	0.77	0.77	0.77	0.77	0.70	0.70	0.70	0.70	0.95	0.95	0.95	0.95
NE, Mcal/kg	2.59	2.59	2.59	2.59	2.62	2.62	2.62	2.62	2.57	2.57	2.57	2.57
SID LYS:NE, g/Mcal NE	2.98	2.98	2.98	2.98	2.67	2.67	2.67	2.67	3.70	3.70	3.70	3.70

Continued Table 2

Total Ca, %	0.55	0.55	0.55	0.55	0.50	0.50	0.50	0.50	0.51	0.51	0.51	0.51
Total available P, %	0.26	0.26	0.26	0.26	0.25	0.25	0.25	0.25	0.24	0.24	0.24	0.24
SID AA ratios												
MET:LYS	32.2	28.9	29.2	31.9	32.2	28.8	28.7	31.6	32.8	34.6	35.1	37.3
MET + CYS:LYS	62.2	55.7	55.0	55.0	62.6	55.9	55.0	55.1	58.0	58.0	58.0	58.0
THR:LYS	65.0	65.0	65.0	65.0	65.0	65.0	65.1	65.0	68.0	68.0	68.0	68.1
TRP:LYS	18.1	18.0	18.0	18.0	18.0	18.1	18.1	18.1	18.0	18.1	18.0	18.0
ILE:LYS	69.1	56.0	56.0	56.0	68.6	55.0	55.1	55.0	64.4	57.2	55.1	55.1
VAL:LYS	81.2	68.8	65.1	65.0	80.9	68.0	65.0	65.0	71.9	65.0	65.0	65.0
LEU:LYS	179.0	161.2	155.9	140.9	178.2	159.8	155.5	139.9	144.4	134.5	131.6	119.4
HIS:LYS	47.3	40.2	38.0	32.1	47.3	39.9	38.2	32.0	42.0	38.1	36.9	32.0
ARG:LYS	106.5	84.2	77.5	58.8	106.7	83.6	78.3	58.8	103.9	91.6	87.9	72.8
PHE:LYS	87.8	73.9	69.7	58.1	87.2	72.8	69.4	57.3	78.0	70.4	68.1	58.6
PHE + TYR:LYS	155.6	132.5	125.5	106.1	152.8	128.7	123.2	102.9	133.8	121.0	117.1	101.4

[1]Control (Ctrl) or reduced CP (RCP) dietary treatments.

[2]0.025% (as fed basis), or 10 mg/kg, ractopamine hydrochloride (Paylean; Elanco Animal Health, a Division of Ely Lilly, Greenfield, IN) was included in all finisher-III diets.

[3]Vitamin premix supplied 6,614 IU of vitamin A, 827 IU of vitamin D_3, 26 IU of vitamin E, 2.7 mg of vitamin K, 16.5 mg of pantothenic acid, 30 mg of niacin, 5 mg of riboflavin, and 26 µg of vitamin B_{12} per kilogram of premix (NB-6508; Nutri Blend Corp., Neosho, MO).

[4]Mineral premix supplied 138 mg/kg of Fe from ferrous sulfate, 138 mg/kg of Zn from zinc sulfate, 33 mg/kg of Mn as manganous sulfate, 13.8 mg/kg of Cu from copper sulfate, 0.25 mg/kg of Se from sodium selenite, and 0.25 mg/kg of I from calcium iodate per kilogram of premix (NB-8534; Nutri Blend Corp, Neosho, MO).

[5]Ronozyme P CT (DSM Nutritional Products, LLC, Parsippany, NJ).

Table 3. Effects of AA supplementation of reduced CP (RCP) diets on the fatty acid composition (weight percentage) of the LM

	Treatments[1]					*P*-value[2]		
Fatty acid	Ctrl	RCP1	RCP2	RCP3	SEM	TRT	LIN	QUAD
ΣSFA	35.66	36.14	36.20	36.29	0.447	0.757	0.334	0.647
10:0	0.094^{x}	0.102^{w}	0.101^{w}	0.101^{w}	0.0025	0.038	0.045	0.035
12:0	0.079	0.083	0.080	0.079	0.0018	0.365	0.868	0.152
14:0	1.18	1.25	1.25	1.25	0.031	0.302	0.154	0.215
15:0	0.057	0.053	0.053	0.060	0.0055	0.750	0.703	0.324
16:0	22.43	22.81	22.95	23.00	0.288	0.498	0.171	0.520
17:0	0.32^{w}	0.30^{wx}	0.28^{x}	0.28^{x}	0.013	0.089	0.024	0.533
18:0	11.44	11.45	11.38	11.43	0.187	0.994	0.952	0.914
20:0	0.166	0.167	0.164	0.162	0.0052	0.924	0.621	0.745
ΣMUFA	40.73^{y}	42.76^{xy}	44.57^{wx}	46.03^{w}	0.821	0.001	< 0.001	0.568
16:1*c*	2.56^{y}	2.76^{xy}	3.02^{wx}	3.13^{w}	0.099	0.001	< 0.001	0.493
Σ18:1*t*	0.36^{w}	0.34^{w}	0.29^{x}	0.26^{y}	0.009	< 0.001	< 0.001	0.807
18:1*c*9	32.92^{y}	34.57^{xy}	35.94^{wx}	37.03^{w}	0.676	0.002	< 0.001	0.539
18:1*c*11	4.28^{y}	4.47^{xy}	4.69^{wx}	4.96^{w}	0.096	< 0.001	< 0.001	0.893
20:1	0.60	0.62	0.63	0.65	0.017	0.244	0.046	0.894
ΣPUFA	22.10^{w}	19.61^{wx}	17.79^{xy}	16.25^{y}	1.025	0.003	< 0.001	0.513
18:2*n*-6	17.03^{w}	15.10^{wx}	13.65^{xy}	12.46^{y}	0.729	0.001	< 0.001	0.472
18:2*c*9*t*11	0.090^{w}	0.077^{x}	0.077^{x}	0.070^{y}	0.0021	< 0.001	< 0.001	0.062
ΣCLA	0.108	0.093	0.095	0.105	0.0076	0.392	0.852	0.094
18:3*n*-3	0.49^{w}	0.42^{x}	0.37^{y}	0.30^{z}	0.011	< 0.001	< 0.001	0.536
20:2	0.46^{w}	0.43^{wx}	0.38^{xy}	0.36^{y}	0.017	0.001	< 0.001	0.595
20:3*n*-6	0.39	0.35	0.33	0.31	0.023	0.140	0.025	0.611
20:3*n*-3	0.089^{w}	0.067^{x}	0.059^{x}	0.052^{x}	0.0075	0.005	0.001	0.216
20:4	3.09	2.79	2.57	2.40	0.239	0.234	0.050	0.714
20:5	0.109^{w}	0.091^{wx}	0.088^{wx}	0.074^{x}	0.0077	0.031	0.004	0.713

Continued Table 3

22:5	0.30^{w}	0.25^{wx}	0.22^{x}	0.19^{x}	0.021	0.010	0.001	0.627
22:6	0.110^{w}	0.099^{wx}	0.088^{wx}	0.078^{x}	0.0089	0.093	0.015	0.867
Other FA	1.56	1.52	1.48	1.47	0.063	0.773	0.331	0.770
PUFA:SFA	0.63^{w}	0.55^{wx}	0.49^{xy}	0.45^{y}	0.035	0.009	0.001	0.558
IV	65.8^{w}	64.0^{wx}	62.9^{xy}	61.9^{y}	0.67	0.003	< 0.001	0.431

$^{w-z}$Within a row, least squares means lacking common superscripted letters differ, $P < 0.05$.
[1]Refer to Tables 1 and 2 for description of dietary treatments.
[2]Probability values for main effect of RCP diets (TRT), as well as the linear (LIN) and quadratic (QUAD) effects of RCP diets.

Table 4. Effects of AA supplementation of reduced CP (RCP) diets on the fatty acid composition (weight percentage) of jowl subcutaneous fat

	Treatments[1]					*P*-value[2]		
Fatty acid	Ctrl	RCP1	RCP2	RCP3	SEM	TRT	LIN	QUAD
ΣSFA	30.55	31.46	31.16	31.47	0.338	0.214	0.085	0.359
10:0	0.057^{x}	0.059^{x}	0.063^{wx}	0.068^{w}	0.0022	0.005	< 0.001	0.643
12:0	0.060^{x}	0.063^{wx}	0.064^{wx}	0.068^{w}	0.0017	0.030	0.003	0.847
14:0	1.21^{x}	1.26^{wx}	1.25^{x}	1.31^{w}	0.020	0.008	0.001	0.922
15:0	0.069	0.065	0.062	0.062	0.0027	0.231	0.062	0.444
16:0	19.83^{x}	20.37^{wx}	20.29^{wx}	20.62^{w}	0.194	0.056	0.010	0.510
17:0	0.41	0.40	0.39	0.38	0.016	0.590	0.207	0.629
18:0	8.73	9.06	8.85	8.77	0.174	0.574	0.995	0.272
20:0	0.18	0.19	0.19	0.19	0.004	0.605	0.435	0.385
ΣMUFA	46.80^{x}	46.95^{x}	47.91^{wx}	48.46^{w}	0.442	0.041	0.009	0.757
16:1*t*	0.052^{w}	0.048^{w}	0.045^{x}	0.035^{y}	0.0013	< 0.001	< 0.001	0.063
16:1*c*	2.15^{x}	2.20^{x}	2.34^{wx}	2.57^{w}	0.103	0.035	0.006	0.446
Σ18:1*t*	0.58^{w}	0.55^{w}	0.49^{x}	0.40^{y}	0.015	< 0.001	< 0.001	0.178
18:1*c*9	39.27^{x}	39.37^{x}	40.13^{wx}	40.41^{w}	0.352	0.081	0.022	0.905
18:1*c*11	3.80^{x}	3.86^{x}	3.99^{wx}	4.11^{w}	0.080	0.048	0.008	0.794
20:1	0.94	0.92	0.92	0.93	0.024	0.922	0.789	0.533
ΣPUFA	21.26^{w}	20.28^{wx}	19.61^{xy}	18.77^{y}	0.406	0.002	< 0.001	0.712
18:2*n*-6	18.43^{w}	17.69^{wx}	17.11^{xy}	16.51^{y}	0.371	0.008	0.001	0.712
18:2*c*9*t*11	0.152^{w}	0.138^{x}	0.138^{x}	0.120^{y}	0.0033	< 0.001	< 0.001	0.734
ΣCLA	0.22^{w}	0.21^{w}	0.21^{w}	0.19^{x}	0.006	0.004	< 0.001	0.679
18:3*n*-3	0.96^{w}	0.83^{x}	0.78^{y}	0.65^{z}	0.016	< 0.001	< 0.001	0.726
20:2	0.94^{w}	0.91^{wx}	0.88^{x}	0.87^{x}	0.018	0.055	0.009	0.726
20:3*n*-6	0.144^{w}	0.136^{wx}	0.136^{wx}	0.128^{x}	0.0031	0.011	0.001	0.804
20:3*n*-3	0.153^{w}	0.135^{x}	0.125^{y}	0.106^{z}	0.0025	< 0.001	< 0.001	0.674
20:4	0.28	0.27	0.27	0.26	0.007	0.299	0.087	0.927
22:5	0.075^{w}	0.066^{x}	0.067^{x}	0.055^{y}	0.0016	< 0.001	< 0.001	0.531

Continued Table 4

Other FA	1.41	1.34	1.34	1.31	0.035	0.274	0.068	0.545
PUFA:SFA	0.70^{w}	0.65^{x}	0.63^{xy}	0.60^{y}	0.017	0.003	< 0.001	0.515
IV	74.9^{w}	73.3^{x}	73.0^{x}	72.0^{x}	0.48	0.003	< 0.001	0.443

$^{w-z}$Within a row, least squares means lacking common superscripted letters differ, $P < 0.05$.
[1]Refer to Tables 1 and 2 for description of dietary treatments.
[2]Probability values for main effect of RCP diets (TRT), as well as the linear (LIN) and quadratic (QUAD) effects of RCP diets.

Table 5. Effects of AA supplementation of reduced CP (RCP) diets on the fatty acid composition (weight percentage) of fresh pork belly fat

	Treatments[1]					*P*-value[2]		
Fatty acid	Ctrl	RCP1	RCP2	RCP3	SEM	TRT	LIN	QUAD
ΣSFA	34.11^{x}	34.79^{w}	35.31^{w}	35.00^{w}	0.255	0.003	0.006	0.023
10:0	0.060^{z}	0.065^{y}	0.072^{x}	0.078^{w}	0.0020	< 0.001	< 0.001	0.881
12:0	0.067^{z}	0.070^{y}	0.073^{x}	0.077^{w}	0.0011	< 0.001	< 0.001	0.682
14:0	1.24^{y}	1.30^{x}	1.33^{x}	1.36^{w}	0.016	< 0.001	< 0.001	0.221
15:0	0.072^{w}	0.067^{x}	0.062^{y}	0.063^{xy}	0.0020	< 0.001	< 0.001	0.055
16:0	21.13^{y}	21.61^{x}	21.99^{w}	22.06^{w}	0.147	< 0.001	< 0.001	0.091
17:0	0.44^{w}	0.43^{wx}	0.39^{y}	0.40^{xy}	0.114	0.002	0.002	0.211
18:0	10.88^{wx}	11.05^{wx}	11.19^{w}	10.75^{x}	0.138	0.057	0.412	0.014
20:0	0.20	0.20	0.21	0.21	0.002	0.494	0.251	0.714
ΣMUFA	43.03^{x}	43.26^{x}	44.25^{w}	44.99^{w}	0.327	< 0.001	< 0.001	0.514
16:1*t*	0.056^{w}	0.050^{x}	0.042^{y}	0.033^{z}	0.0014	< 0.001	< 0.001	0.519
16:1*c*	1.85^{y}	1.94^{y}	2.10^{x}	2.31^{w}	0.053	< 0.001	< 0.001	0.194
Σ18:1*t*	0.65^{w}	0.61^{x}	0.51^{y}	0.43^{z}	0.012	< 0.001	< 0.001	0.151
18:1*c*9	36.39^{x}	36.64^{x}	37.44^{w}	37.94^{w}	0.255	< 0.001	< 0.001	0.760
18:1*c*11	3.26^{y}	3.22^{y}	3.37^{x}	3.49^{w}	0.048	< 0.001	< 0.001	0.063
20:1	0.82	0.79	0.79	0.79	0.009	0.100	0.022	0.342
ΣPUFA	21.61^{w}	20.76^{x}	19.30^{y}	18.84^{y}	0.345	< 0.001	< 0.001	0.316
18:2*n*-6	18.96^{w}	18.33^{w}	17.05^{x}	16.78^{x}	0.314	< 0.001	< 0.001	0.336
18:2*c*9*t*11	0.141^{w}	0.128^{x}	0.121^{y}	0.109^{z}	0.0017	< 0.001	< 0.001	0.379
ΣCLA	0.19^{w}	0.17^{x}	0.16^{y}	0.14^{z}	0.004	< 0.001	< 0.001	0.114
18:3*n*-3	0.98^{w}	0.85^{x}	0.76^{y}	0.64^{z}	0.013	< 0.001	< 0.001	0.266
20:2	0.83^{w}	0.81^{w}	0.76^{x}	0.76^{x}	0.013	< 0.001	< 0.001	0.262
20:3*n*-6	0.13^{w}	0.12^{w}	0.12^{x}	0.11^{x}	0.002	< 0.001	< 0.001	0.834
20:3*n*-3	0.14^{w}	0.12^{x}	0.11^{y}	0.09^{z}	0.002	< 0.001	< 0.001	0.128
20:4	0.27^{w}	0.26^{wx}	0.26^{x}	0.25^{x}	0.004	0.030	0.005	0.379

Continued Table 5

22:5	0.071^{w}	0.062^{x}	0.058^{y}	0.051^{z}	0.0010	< 0.001	< 0.001	0.146
Other FA	1.27^{w}	1.21^{x}	1.16^{y}	1.18^{xy}	0.016	< 0.001	< 0.001	0.008
PUFA:SFA	0.64^{w}	0.61^{x}	0.55^{y}	0.55^{y}	0.013	< 0.001	< 0.001	0.135
IV	72.5^{w}	71.26^{x}	69.2^{y}	69.4^{y}	0.42	< 0.001	< 0.001	0.083

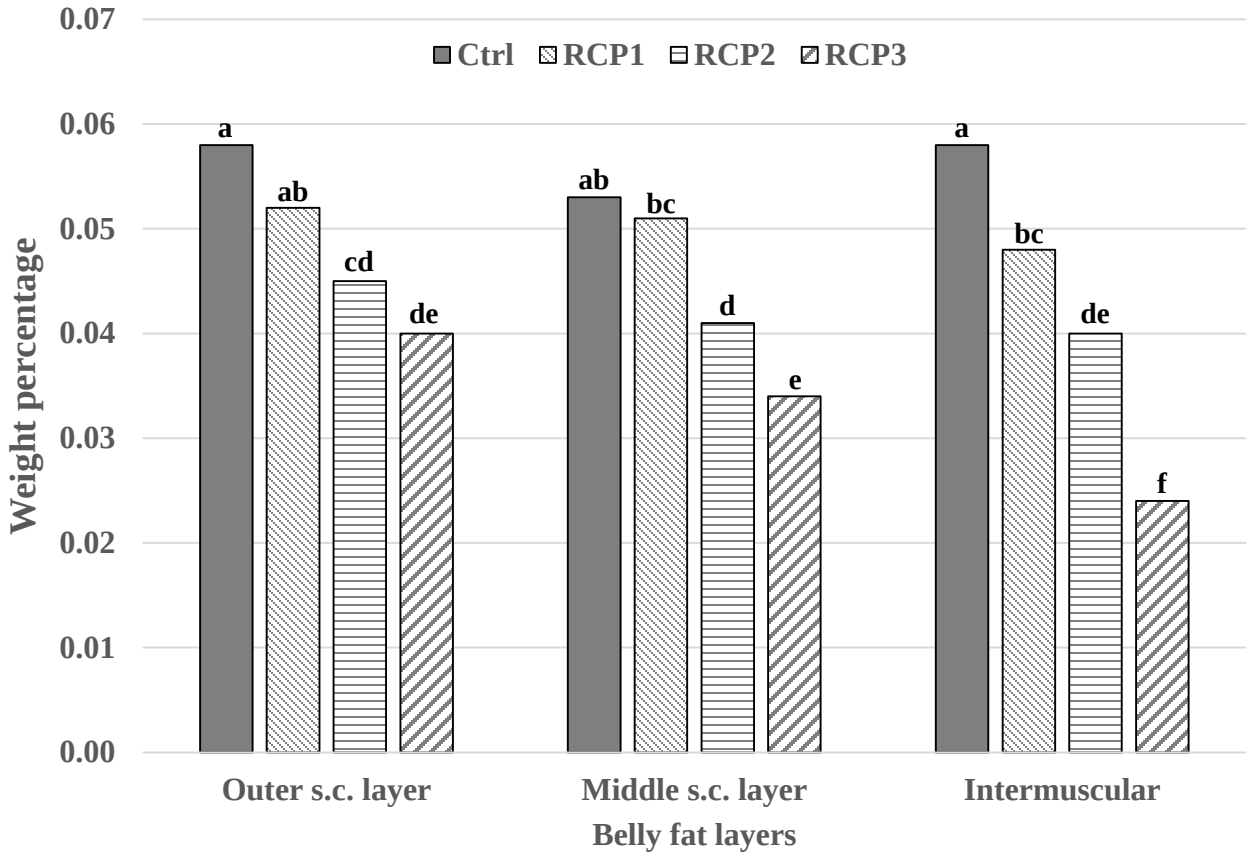
$^{w-z}$Within a row, least squares means lacking common superscripted letters differ, $P < 0.05$.
[1]Refer to Tables 1 and 2 for description of dietary treatments.
[2]Probability values for main effect of RCP diets (TRT), as well as the linear (LIN) and quadratic (QUAD) effects of RCP diets.

Table 6. Fatty acid composition (weight percentage) of fresh pork belly fat layers

	Belly fat layers[1]				
Fatty acid	OSC	MSC	INT	SEM	*P*-value
ΣSFA	31.93^{z}	34.83^{y}	37.65^{x}	0.227	< 0.001
10:0	0.056^{y}	0.075^{x}	0.077^{x}	0.0018	< 0.001
12:0	0.063^{z}	0.072^{y}	0.080^{x}	0.0010	< 0.001
14:0	1.26^{z}	1.29^{y}	1.38^{x}	0.015	< 0.001
15:0	0.070^{x}	0.062^{y}	0.066^{y}	0.0017	0.001
16:0	20.43^{z}	21.76^{y}	22.91^{x}	0.130	< 0.001
17:0	0.44^{x}	0.40^{y}	0.40^{y}	0.010	0.008
18:0	9.41^{z}	10.97^{y}	12.54^{x}	0.124	< 0.001
20:0	0.21^{y}	0.21^{x}	0.19^{z}	0.002	< 0.001
ΣMUFA	45.31^{x}	44.82^{x}	41.51^{y}	0.296	< 0.001
16:1*t*	0.049^{x}	0.045^{y}	0.042^{y}	0.0012	0.001
16:1*c*	2.11^{x}	2.08^{x}	1.96^{y}	0.051	0.002
Σ18:1*t*	0.56	0.54	0.55	0.010	0.162
18:1*c*9	38.18^{x}	38.04^{x}	35.09^{y}	0.227	< 0.001
18:1*c*11	3.54^{x}	3.33^{y}	3.15^{z}	0.044	< 0.001
20:1	0.88^{x}	0.80^{y}	0.72^{z}	0.008	< 0.001
ΣPUFA	21.47^{x}	19.20^{y}	19.72^{y}	0.310	< 0.001
18:2*n*-6	18.87^{x}	16.95^{y}	17.52^{y}	0.283	< 0.001
18:2*c*9*t*11	0.140^{x}	0.124^{y}	0.110^{z}	0.0016	< 0.001
ΣCLA	0.19^{x}	0.16^{y}	0.13^{z}	0.004	< 0.001
18:3*n*-3	0.85^{x}	0.78^{y}	0.79^{y}	0.012	< 0.001
20:2	0.91^{x}	0.76^{y}	0.70^{z}	0.011	< 0.001
20:3*n*-6	0.13^{x}	0.11^{y}	0.11^{y}	0.002	< 0.001
20:3*n*-3	0.14^{x}	0.11^{y}	0.10^{z}	0.003	< 0.001
20:4	0.27^{x}	0.25^{y}	0.26^{x}	0.004	0.003
22:5	0.064^{x}	0.057^{z}	0.060^{y}	0.0017	< 0.001
Other FA	1.31^{x}	1.16^{y}	1.14^{y}	0.014	< 0.001
PUFA:SFA	0.68^{x}	0.55^{y}	0.53^{y}	0.011	< 0.001
IV	74.0^{x}	70.0^{y}	68.1^{z}	0.372	< 0.001

$^{x-z}$Within a row, least squares means lacking common superscripted letters differ, $P < 0.05$.

Figure 1. Interactive effects of reduced CP diets and belly fat layer on the weight percentages of palmitelaidic acid (16:1t).



Chapter 4:
Conclusion

Environmental impact is an important consideration today for farming. Animal waste is a major contributor of N_2 excretion, especially in the affluent air from confining animals in houses. Since N_2 excretion is increasing, research has focused on formulating swine diets to limit environmental impact. One particular solution to limiting N_2 excretion is feeding animals RCP diets. However, the environmental advantages of feeding AA-supplemented, reduced CP diets have been variable in live pig performance and pork carcass characteristics. Due to the variation in growth performance and the increased fat deposition in pigs fed AA-supplemented, reduced CP diets, the objective of this study was to test the effects of AA supplementation of reduced CP diets formulated on a NE basis on the growth performance, pork carcass composition and LM quality of growing-finishing swine.

Growth performance did not vary greatly among the dietary treatments. The greatest effect overall was that ADG differed among the RCP2 and RCP3 diets, where RCP3 diet declined in ADG. ADFI showed to decrease with decrease in dietary CP. G:F tended to increase with decrease in dietary CP with AA-supplementation. Fat accumulation did not increase in backfat, 10^{th} rib fat or leaf fat and the IMF while dietary CP decreased. However, visual color scores decreased with decrease in dietary CP, but L*, or lightness, increased with decrease in dietary CP. Pigs can retain lean carcasses and have minor quality issues when decreasing CP in a RCP diet supplement with AA.

Research was continued to analyze the process of lipid metabolism and the deposition of FA in the belly fat layers, the LM and jowl s.c. fat. Results indicate FA composition differed greatly among the fat layers of fresh pork bellies and among the intramuscular fat in pigs. SFAs

and MUFAs compositions increased, while PUFAs decreased with a decrease in dietary CP. Suggesting *de novo* synthesis was increased in pigs fed a RCP diet supplemented with AA. In addition, there was a greater proportion of SFAs in the INT layer compared to outer subcutaneous fat, where there was more PUFAs and MUFAs. Data suggest that the inner subcutaneous fat was most metabolically active, while the intermuscular fat was the most mature, which is different than the backfat layers. This could attribute to belly firmness being associated to all fat layers.

Overall, feeding pigs a RCP diet on a net energy basis with feed-grade AA supplementation showed no detrimental effect on growth performance and carcass characteristics. Results indicated FA composition differed greatly among the fat layers of fresh pork bellies, with SFA being in greatest concentration in the INT compared to MUFA and PUFA when reducing dietary CP. This was similar to the results of the pork lean and fat, which also had increased MUFA compositions. This suggests enhanced *de novo* synthesis was involved in pigs fed RCP diets supplemented with crystalline AA.

APPENDIX

UNIVERSITY OF ARKANSAS

Research Compliance
Office of the Director

MEMORANDUM

TO: Charles Maxwell

FROM: Craig N. Coon, Chairman
Institutional Animal Care
And Use Committee

DATE: February 14, 2011

SUBJECT: IACUC PROTOCOL **APPROVAL**
Expiration date : **February 15, 2014**

The Institutional Animal Care and Use Committee (IACUC) has **APPROVED** Protocol **#11023- "INTEGRATED RESOURCE MANAGEMENT TOOL TO MITIGATE THE CARBON FOOTPRINT OF SWINE PRODUCED IN THE U.S.**" You may begin this study immediately.

In granting its approval, the IACUC has approved only the protocol provided. Should there be any changes in the protocol during the research, please notify the IACUC in writing **prior** to initiating the changes. If the study period is expected to extend beyond **04-15-2014,** you must submit a new protocol. By policy the IACUC cannot approve a study for more than 3 years at a time.

The IACUC appreciates your cooperation in complying with University and Federal guidelines for research involving animal subjects.

cnc/car

cc: Animal Welfare Veterinarian