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CARCASS, MEAT QUALITY AND BIOCHEMICAL TRAITS OF BERKSHIRE, AND YORKSHIRE PROGENY WITH OR WITHOUT PAYLEAN TREATMENT

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CARCASS, MEAT QUALITY AND BIOCHEMICAL TRAITS OF BERKSHIRE AND YORKSHIRE PROGENY WITH OR WITHOUT PAYLEAN® TREATMENT

By

MATTHEW JOHN RITTER

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ABSTRACT

CARCASS, MEAT QUALITY AND BIOCHEMICAL TRAITS OF BERKSHIRE AND YORKSHIRE PROGENY WITH OR WITHOUT PAYLEAN® TREATMENT

By

MATTHEW JOHN RITTER

Berkshire (B) and Yorkshire (Y) progeny were utilized to determine 1) if superior pork color and water-holding capacity (WHC) are associated with the activity of rate-limiting glycolytic enzymes, glycogen stored in the muscle and/or the proportion of Type I myosin heavy chain isoform (MHC). Additionally, two separate experiments (P1 and P2) were conducted to determine if Paylean® (P) fed at 10 ppm for the last four weeks improves growth and carcass traits without adversely affecting pork quality. In general, Y pigs had less backfat (tenth and average) and larger loin muscle areas (LMA) than B ($P<.01$), but loin muscle from B carcasses had lower d1 CIE L* values and less fluid loss ($P<.1$). Breed differences in color and WHC were not associated with glycolytic enzyme activity or MHC distribution. Paylean® tended to improve growth rate and feed efficiency ($P<.09$), but did not affect feed intake or live weight. In both experiments (P1 and P2), P improved LMA and ham weights ($P<.06$) but not carcass weights or backfat thickness. In P2, P was more effective at improving growth traits and carcass muscling in B than Y pigs. In P1, P did not affect color or WHC but BP had lower heme pigment concentrations than BC ($P<.05$). Also, P did not adversely affect subjective, color, firmness and marbling or WHC in P2. Therefore, Paylean® is an effective management tool that improves the inferior growth rate and carcass muscling of Berkshire progeny and does not adversely affect their superior pork quality attributes.

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LIST OF ABBREVIATIONS

ADG-Average Daily Gain
ADFI-Average Daily Feed Intake
ADP-Adenosine Diphosphate
AMG-Amyloglucosidase
AMP-Adenosine Monophosphate
AMPK-Adenosine Monophosphate-Activated Protein Kinase
AOAC-Association of Analytical Chemists
ATPase-Adenosine Triphosphate-Hydrolyzing Enzyme
ATP-Adenosine Triphosphate
AUAUC-All-University Committee on Animal Use and Care
β1-β-adrenergenic receptor subtype 1
β2-β-adrenergenic receptor subtype 2
β3-β-adrenergenic receptor subtype 3
B-Berkshire-sired Pigs
β-agonist-β-adrenergic agonist
BC-Berkshire-sired Control Pigs
BCA™-Bicinchonic Acid
BF10-Tenth Rib Backfat Thickness
BP-Berkshire-sired Pigs Fed Paylean® at 10 ppm for the Last 4 Weeks of the Finishing Phase
B x R-Breed by Room Interaction
°C-Degrees Celsius
CoA-CoEnzyme A
CIE - Commission International de l'Eclairage (CIE) L*, a* and b* measurement
CP-Dietary Crude Protein
CWHC-Loin Muscle Water-Holding Capacity Determined by High-Speed Centrifugation
Cyclic AMP-Cyclic Adenosine Monophosphate
EGTA-(Ethylene glycol-bis[β-aminoethyl ether]-N',N',N,N'-tetraacetic acid
DFD-Dark, Firm and Dry
DNA-Deoxyribonucleic Acid
DPS-Downer Pig Syndrome
Drip1-d 1 Drip Loss Determined by the Suspension Drip Method
F-1,6-Phosphate-Fructose-1,6-Phosphate
F-6-P-Fructose-6-Phosphate
Fe^{+2}-Iron in the Ferrous State
Fe^{+3}-Iron in the Ferric State
FE-Feed Efficiency
Filter Paper-Loin Muscle Fluid Loss Determined by the Filter Paper Method
FG-Fast, Glycolytic Muscle Fibers
FOG-Fast, Oxidative, Glycolytic Muscle Fibers
G-6-P-Glucose-6-Phosphate
GP-Glycolytic Potential
H^{+}-Hydrogen Ions

h^2-
HD
HD
HD
K-
KC
KH
LG
LM
LM
LS
Lys
Mg
MgC
MH
MH
MSU
Non
NPB
NPP
NN-
Nn-H
nn-H
Over
%FF
P-Pa
PFK
PK-H
PSE-
PSS-
RAC
RFN
RN-

m-T
RTN
SAS
SDS
SDS
SEM
SO-S
T x E

LIST OF ABBREVIATIONS

h^2-Heritability
HD#1-Harvest Day #1
HD#2-Harvest Day #2
HD x B-Harvest Date by Breed Group Interaction
K^+-Potassium Ions
KCl-Potassium Chloride
KH_2PO_4-Potassium Phosphate (Dibasic)
LG-Lean Gain per Day
LM-*Longissimus* Muscle
LMA-Loin Muscle Area
LS Means-Least Square Means
Lys-Dietary Lysine
Mg^{+2}-Magnesium Ions
$MgCl_2$-Magnesium Chloride
MH-Malignant Hyperthermia
MHC-Myosin Heavy Chain Isoforms
MSU-Michigan State University
Non-AMG-Not Treated with Amyloglucosidase
NPB-National Pork Board
NPPC-National Pork Producers Council
NN-Normal Genotype for the HAL-1843™ Mutation
Nn-Heterozygous Genotype for the HAL-1843™ Mutation
nn-Homozygous Genotype for the HAL-1843™ Mutation
Overall-Harvest Days 1 and 2
%FFL-Percentage of Fat-Free Lean
P-Paylean® Fed at 10 ppm for the Last 4 Weeks of the Finishing Phase
PFK-Phosphofructokinase
PK-Pyruvate Kinase
PSE-Pale, Soft and Exudative
PSS-Porcine Stress Syndrome
RAC-Ractopamine-Hydrochloride
RFN-Red, Firm and Non-exudative
RN^--The dominant and undesirable allele at Rendement Technological Napole Gene Locus
rn^+-The normal and recessive allele at the Rendement Technological Napole Gene Locus
RTN-Rendement Technological Napole Yield
SAS®-Statistical Analysis Software
SDS-Sodium Dodecyl Sulfate
SDS-PAGE- Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis
SEM-Standard Error of the Mean
SO-Slow, Oxidative Muscle Fibers
T x B-Treatment by Breed Interaction

LIST OF ABBREVIATIONS

T x R-Treatment by Room Interaction
Tris-(Tris[hydroxymethyl]aminomethane)
U.S-The United States of America
USDA-The United States Department of Agriculture
WHC-Water-holding Capacity
Y-Yorkshire-sired Pigs
YC-Yorkshire-sired Control Pigs
YP- Yorkshire-sired Pigs Fed Paylean® at 10 ppm for the Last 4 Weeks of the Finishing Phase
TCA-Tricarboxylic Acid Cycle

INTRODUCTION

In today's swine industry, packers pay premiums based on carcass composition not meat quality. Therefore, pigs that are faster growing, leaner and heavier muscled are more profitable for producers. It has been well documented that as the selection for muscling and leanness has increased, so has the risk of producing pale, soft and exudative (PSE) pork. This is evident in two pork quality surveys conducted in the early 1990s when packers started to demand leaner and heavier muscled pigs (Kauffman et al., 1992; Cannon et al., 1995). Although tremendous research efforts have been made to more effectively understand the mechanisms that cause inferior pork quality in high lean genotypes, little progress has been made in improving pork quality over the last 30 years (Cassens, 2000).

Meanwhile, little research has focused on the mechanism(s) responsible for producing superior quality pork. Several breed evaluations have established that Berkshire progeny consistently have the highest ultimate pH, lowest Commission International de l'Eclairage (CIE) L* values and lowest drip losses, whereas Yorkshire and Landrace progeny have inferior color and water-holding capacity (WHC; NPPC, 1995; Goodwin, 2000). Understanding the biochemical mechanism(s) responsible for superior pork quality in Berkshire progeny and inferior pork quality in other pigs that do not possess the HAL-1843™ and RN^- mutations may lead to new approaches to improve inferior quality pork of pigs with high lean genetics.

On the other hand, it is also well documented that Berkshire progeny are generally slower growing, fatter and lighter muscled than other genetic lines (NPPC, 1995). Therefore, Berkshire progeny have not been practical or profitable to produce and thus

the use of Berkshires in U.S. commercial operations has been limited. However, Paylean® (ractopamine-hydrochloride), a β-adrenergic agonist, was approved in December 2000 by the U.S. Food and Drug Administration to be fed up to 20 ppm for the last 41 kg of live weight (68-109 kg). It is well established that ractopamine improves growth and carcass traits, but does not adversely affect pork quality traits (Uttaro et al., 1993; Crome et al., 1996). Therefore, Paylean® should be an ideal tool to improve the lean yield of Berkshire-sired pigs, while maintaining their superior pork quality.

The objectives of the present work were to: 1) determine if the superior pork color and WHC of Berkshire progeny are associated with the amount of glycogen stored in the muscle at death, the in vitro activity of rate-limiting glycolytic enzymes, a higher proportion of myosin heavy chain isoform Type I and/or a higher concentration of myoglobin and 2) to determine if supplementation of Paylean® at 10 ppm to the diet for the last four weeks of the finishing phase improves the economically important traits of growth, muscling and leanness, without compromising the superior pork quality of Berkshire-sired pigs.

The present work will identify the biochemical mechanism(s) that are responsible for superior quality pork, and could lead to the development of new strategies to improve the pork quality of high lean genetics. Additionally, the present work will provide management strategies to improve the carcass composition of pigs with superior pork quality. Collectively, these data could lead to the consistent production of lean and muscular pigs with superior color and WHC.

LITERATURE REVIEW

Energy Metabolism During the Conversion of Muscle to Meat

In order to effectively understand why variations in pork quality exist, we must understand the postmortem conversion of muscle to meat. During antemortem conditions, muscle relies on oxidative metabolism (glycolysis, TCA cycle and electron transport chain) to produce ATP for muscle contraction. Oxidative metabolism involves glycogen being converted to glucose-1-phosphate by glycogen phosphorylase, glucose being converted to pyruvate via glycolysis and pyruvate entering the TCA cycle in the mitochondria (Voet et al., 1999). Oxidative metabolism can convert one molecule of glucose from glycogen to 37 ATP molecules (Aberle et al., 2001).

During the harvesting process, blood is removed from the body by exsanguination. After exsanguination, oxygen can no longer be supplied to the muscle. Therefore, there is a shift from oxidative metabolism to anaerobic metabolism. Anaerobic glycolysis is the primary supplier of ATP for postmortem muscle. In anaerobic glycolysis, glycogen is converted to glucose-1-phosphate by phosphorylase, glucose-1-phosphate is converted to pyruvate via glycolysis and pyruvate is converted to lactate by lactate dehydrogenase. The net reaction of anaerobic glycolysis is glucose + 2 ADP $\rightarrow$ 2 lactate + 2 ATP + 2 H^+ (Aberle et al., 2001; Voet et al., 1999). The more H^+ produced via anaerobic glycolysis, the lower the pH of the meat because pH is the –log $[H^+]$. This has important meat quality implications because the rate and extent of pH decline influence fresh pork color and WHC (Briskey, 1963).

Variations in Pork Quality

Red, Firm and Non-exudative Pork

Red, firm and non-exudative (RFN) pork is the ideal pork product. The National Pork Producers Council (NPPC) Pork Quality Solutions Team established pork quality targets to define the ideal quality. The acceptable subjective color score ranges from 3 to 5 on a 6 point scale (1 = pale pinkish gray to white and 6 = dark purplish red) and this is associated with CIE L* values between 37 and 49. Ultimate pH should be between 5.6 and 5.9. Additionally, ideal pork should have less than 2.5% drip loss when measured at 24 h postmortem (NPPC, 1999).

Dark, Firm and Dry Pork

Dark, firm and dry (DFD) pork occurs in pigs subjected to long-term stress such as transporting pigs across the country during the summer. Long-term stress may cause muscle glycogen stores to be depleted. When glycogen stores are depleted, there is little glycogen to be converted to lactate and H^+ via anaerobic glycolysis. Therefore, DFD pork has a high ultimate pH (>6.1), minimal protein denaturation, a dark purplish-red color, a reduced incidence of off-flavors and superior WHC (Kauffman et al., 1992; Prusa, 2000). However, the high ultimate pH of DFD pork makes this product more favorable for the growth of bacteria that can lead to a shorter shelf life (Johnson, 2001). Additionally, many consumers discriminate against DFD pork due to its unacceptable dark, purplish-red color (Kauffman et al., 1992).

Pale, Soft and Exudative Pork

Pale, soft and exudative (PSE) pork was first observed in the late 1950s in the U.S. (Briskey et al., 1959). It has been well established that as selection for leanness and

muscling has increased, so has the occurrence of PSE pork. Pale, soft and exudative pork was estimated to occur in 10% to 16% of all carcasses in the U.S. during the early 1990s (Kauffman et al., 1992; Cannon et al., 1996). The PSE condition is believed to be caused by many factors such as short-term stress, improper animal handling prior to harvest, slow carcass chilling and genetic mutations (HAL-1843™ and RN^- genes).

Pale, soft and exudative pork is characterized by its pale color and inferior WHC. The PSE condition is caused by an elevated carcass temperature and a rapid pH decline (HAL-1843™) or by low ultimate pH (RN^-). The combination of an elevated carcass temperature and a rapid pH decline early postmortem results in protein denaturation (Wismer-Pederson, 1959; Wismer-Pederson & Briskey, 1961; Bendall & Wismer-Pederson, 1962). Additionally, when meat has a very low ultimate pH, such as RN^- pigs, protein denaturation can occur (Lawrie et al., 1958; Lawrie, 1960). It is well established that denaturation of sarcoplasmic and myofibrillar proteins adversely affects pork color and WHC (Sayre and Briskey, 1963a; Warner et al., 1997; Joo et al., 1999). Joo et al. (1999) concluded that sarcoplasmic protein solubility is associated with pork color, while WHC is associated with myofibrillar protein solubility and myosin denaturation, which are influenced by ultimate pH (Offer, 1991). Additionally, Offer (1991) demonstrated that the denaturation of myosin prior to the onset of rigor is responsible for excessive fluid loss and poor texture properties of PSE pork. In PSE meat, myofilament spacing is decreased and myosin heads are smaller and thus water is expelled (Offer et al., 1989). Offer (1991) also concluded that myosin denaturation is influenced by the rate of pH decline, ultimate pH and carcass chilling.

The Effects of Genetics on Pork Quality

HAL-1843™ Gene

The HAL-1843™ gene is also referred to as the stress gene or halothane gene and causes malignant hyperthermia. The HAL-1843™ gene is a mutation in the sarcoplasmic reticulum calcium release channel (ryanodine receptor), which increases the probability that the channel will be in the open state. This in turn causes chronic calcium release from the sarcoplasmic reticulum and leads to an increased frequency of muscle tremors or rigidity. Increased contractile activity is thought to lead to an increase in muscle mass due to work-induced muscle hypertrophy (Berchtold et al., 2000), although evidence for this is limited.

Fujii et al. (1991) identified the mutation responsible for Porcine Stress Syndrome (PSS) and malignant hyperthermia (MH) in pigs. The PSS or MH condition, is caused by a C to T transition mutation at nucleotide 1843 that causes arginine to be replaced by cysteine at position 615. A DNA-based test has been commercially available since 1993 to determine genotypes for the HAL-1843™ mutation. Normal (NN), heterozygous (Nn) and homozygous (nn) are the three possible genotypes for the HAL-1843™ mutation (Fujii et al., 1991). This mutation is believed to have additive beneficial effects on carcass and additive deleterious effects on meat quality traits (Sather et al., 1991). Despite the known increase in muscle associated with the HAL-1843™ mutation, many commercial producers have eradicated this gene from their herds due to its higher death losses (O'Brien et al., 1992; Goodwin, 1995) and deleterious effects on fresh meat quality (Klont et al., 1993; NPPC, 1995; Sather et al., 1998; Stalder et al., 1998) and processing yields (Leach et al., 1996).

Pigs that have at least one copy of the HAL-1843™ mutation have larger loin muscle areas (NPPC, 1995; Stalder et al., 1998) or larger loin muscle depth (Dugan et al., 1997; Pommier et al., 1998; Sather et al., 1998), depending on the measure used. Additionally, increases in wholesale ham (Monin et al., 1981; Leach et al., 1996) and loin weights (Monin et al., 1981; Leach et al., 1996; Pommier et al., 1998) have been observed. Meanwhile, the efficacy of this mutation to reduce backfat has been inconsistent (Sather et al., 1991; Leach et al., 1996; Dugan et al., 1997; Pommier et al., 1998; Sather et al., 1998; Stalder et al., 1998).

However, HAL-1843™ gene increases the occurrence of pale, soft and exudative (PSE) pork (O'Brien et al., 1992; Goodwin, 1995; Sather et al., 1998). Halothane sensitive pigs and pigs with at least one copy of the HAL-1843™ mutation are more susceptible to increases in carcass temperatures in the early postmortem period than normal pigs (Warriss & Listen, 1982; Dugan et al., 1997). Elevated carcass temperatures increase the rate of glycolysis causing a rapid pH decline in stress-susceptible, halothane carrier and halothane positive pigs (Kocwin-Podsiadla et al., 1995; De Smet et al., 1996; Fisher & Mellett, 1997; Pommier et al., 1998; Sather et al., 1998; Stalder et al., 1998; Monin et al., 1999). As a result, some stress-susceptible pigs and pigs with the HAL-1843™ mutation are more likely to produce carcasses with a pale color (Sather et al., 1991; Klont et al., 1993; NPPC, 1995; Leach et al., 1996; Murray & Johnson, 1998; Pommier et al., 1998; Sather et al., 1998; Stalder et al., 1998) and excessive water loss (Sather et al., 1991; NPPC, 1995; De Smet et al., 1996; Leach et al., 1996; Dugan et al., 1997; Pommier et al., 1998; Sather et al.. 1998; Stalder et al., 1998).

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Rendement Technological Napole (RN^-) Gene

Abnormally high glycogen concentrations in muscle have been reported in the U.S. for Hampshire pigs as early as the 1960s (Sayre et al., 1963b; Kastenschmidt et al., 1968). In 1985, Monin and Sellier established that Hampshire progeny had the highest glycolytic potential (229 μmoles/g tissue) because of high muscle glycogen at death, which results in a relatively low ultimate pH. Monin et al. (1987) concluded that the "Hampshire Effect" of high glycolytic potential and low ultimate pH was inherited dominantly.

In 1986, Naveau et al. concluded that a gene other than the halothane gene was responsible for the variation observed in Rendement Technological Napole Yield (RTN). Naveau et al. (1986) referred to the proposed gene as RN^- due to its adverse effects on RTN. Le Roy et al. (1990) established that 2 alleles exist for the mutation that is responsible for the low RTN of Hampshire progeny. The two alleles were designated as rn^+ and RN^-, where RN^- is the dominant allele that has deleterious effects on pork quality.

Fernandez et al. (1992) examined glycolytic potential and RTN of two different Hampshire crossbred pig populations, one of which was known to carry the RN^- gene. The authors concluded that the RN^- gene that affects RTN was the same gene that was responsible for the high glycolytic potential and low ultimate pH observed in Hampshire progeny. Therefore, the RN^- gene is also referred to as the Rendement Napole Gene, the Acid Meat Gene or the "Hampshire Effect."

In 2000, the mutation for the RN^- gene was identified in the PRKAG3 (AMP-activated protein kinase) protein. Five alleles were found, but only one of these alleles is responsible for the RN^- mutation. This mutation is caused by a substitution of a

glutamine for an arginine at position 200 in the amino acid sequence of the PRKAG3 protein (Milan et al., 2000). It is believed that AMP-activated protein kinase activates ATP production and inhibits ATP utilization (Hardie et al., 1998). Additionally, it is believed that AMP-activated protein kinase (AMPK) inhibits glycogen synthesis and activates glycogen breakdown (Hardie et al., 1998; Hayashi et al., 1998; Kurth-Kraczek et al., 1998; Bergeron et al., 1999; Holmes et al., 1999). Milan et al. (2000) established that rn^+ pigs had three times more AMPK activity than RN^- pigs. Therefore, the authors hypothesized that AMP-activated protein kinase in RN^- pigs is not inhibiting glycogen synthesis or is not activating glycogen degradation to the same effect in pigs without the RN^- mutation (Milan et al., 2000).

A DNA-based test is now commercially available for the RN^- gene. It has been estimated that 85.1% of Swedish Hampshires and 87% of U.S. Hampshires are at least carriers of the RN^- mutation. This translates to an allele frequency of 0.61 for Swedish Hampshires and 0.63 for U.S. Hampshires (Enfalt et al., 1997; Miller et al., 2000). This is a concern since the poor meat quality traits associated with this mutation are inherited dominantly (Le Roy et al., 1990).

RN^- adversely affects fresh and processed pork quality attributes since RN^- pigs have approximately 70% more muscle glycogen than normal pigs that is ultimately converted to lactate and H^+ (Estrade et al., 1993). As a result, relatively low ultimate pH values of 5.26 (Hamilton et al., 2000), 5.40 (Monin & Sellier, 1985) and 5.43 (Miller et al., 2000) have been reported for RN^- pigs. These pH values approach the isoelectric point for myosin (5.1), at which the net charge is zero and the water-binding capacity is low (Wismer-Pedersen, 1971). Therefore, RN^- carcasses consistently have higher drip

losses (Enfalt et al., 1997a; Enfalt et al., 1997b; Hamilton et al., 2000; Miller et al., 2000), cooking losses (Monin & Sellier, 1985; Hamilton et al., 2000; Miller et al., 2000) and lower RTN yields (Naveau et al., 1986; Enfalt et al., 1997a; Enfalt et al., 1997b). However, the effect of the RN^- gene on fresh pork color has been inconsistent (Enfalt et al., 1997a; Lebret et al., 1999; Hamilton et al. 2000; Miller et al., 2000).

Variations in Pork Quality Across Breeds

Numerous studies have been conducted to compare pork quality traits across different genetic lines revealing a high degree of variation. Pigs of the Berkshire, Chester White and Duroc breeds consistently produce pork with superior color and WHC. On the other hand, pigs of Landrace and Yorkshire genetics are more likely to have inferior fresh pork color and WHC (NPPC, 1995; Goodwin, 2000). It is also well established that Hampshire pigs have inferior WHC, but this is primarily due to the high frequency of the RN^- mutation in this breed (Miller et al., 2000). It is interesting to note that pigs that are normal for the HAL-1843™ and RN^- mutations can still produce PSE pork. Therefore, it is important to identify the mechanism(s) responsible for producing PSE pork. Hypothetically, breed differences may be explained by differences in the activities of rate-limiting glycolytic enzymes and muscle fiber types.

Rate-Limiting Glycolytic Enzymes and Glycolytic Intermediates

Kastenschmidt et al. (1968) measured glycolytic intermediates from pigs with a rapid pH decline (Poland China) and from pigs with a more gradual pH decline (Chester White and Hampshire). Poland China pigs had higher levels of glucose-1-phosphate, glucose-6-phosphate and fructose-6-phosphate at 0, 0.25, 0.5 and 1 h postmortem, and higher levels of pyruvate at 0, 0.25 and 0.5 h postmortem. Additionally, Poland China

pigs had lower levels of fructose-1,6-diphosphate and phosphoenol pyruvate at 0.5, 1, 2 and 3 h postmortem. Therefore, the authors concluded that the rapid pH decline of Poland China pigs appeared to be caused by the coordinated stimulation of phosphorylase, phosphofructokinase (PFK) and pyruvate kinase (PK). The authors also suggested that phosphorylase, PFK and PK were potentially rate-limiting or controlled steps in anaerobic glycolysis, with the most control being exerted at the PFK step. The Poland China breed has been noted for having a high frequency of the HAL-1843™ mutation (Goodwin, 1995), whereas the Hampshire breed has been noted for having a high frequency of the RN^- mutation (Miller et al., 2000). Therefore, it would be of interest to conduct a study on today's pigs in order to biochemically determine the cause of accelerated rates of postmortem glycolysis in pigs that are normal for the HAL-1843™ and RN^- mutations.

Rate-limiting Glycolytic Enzymes (Phosphofructokinase and Pyruvate Kinase)

Phosphofructokinase and pyruvate kinase operate far from equilibrium in skeletal muscle (Kastenschmidt et al., 1968). Therefore, they are considered the two rate-limiting enzymes in glycolysis. Phosphofructokinase utilizes Mg^{+2} as a cofactor and catalyzes the reaction of fructose-6-phosphate + ATP to fructose-1,6-diphosphate + ADP. Phosphofructokinase has maximal activity around pH 8.0. Phosphofructokinase is a highly regulated enzyme that is inhibited by ATP, citrate, creatine phosphate, phosphoenolpyruvate, 2-phosphoglycerate, 3-phosphoglycerate, 1,3-diphosphoglycerate and cyclic AMP. Phosphofructokinase is activated by fructose-1,6-diphosphate, fructose-2,6-diphosphate, ADP, AMP, inorganic phosphate and fructose-6-phosphate (Connett & Sahlin, 1996; Dr. D. Romsos, personal communication; Voet et al., 1999). Meanwhile,

PK utilizes Mg^{+2} and K^{+} as cofactors and catalyzes the reaction of phosphoenolpyruvate + ADP to pyruvate + ATP. Also, PK is activated by ADP and inhibited by ATP (Connett & Sahlin, 1996; Voet et al., 1999).

The Relationships Between Rate-Limiting Glycolytic Enzymes and Pork Quality Traits

Sayre et al. (1963b) studied postmortem glycolysis of Chester White, Hampshire and Poland China pigs. Hampshire pigs had more total phosphorylase and PFK activity than Chester White and Poland China pigs. However, the authors concluded that total phosphorylase and PFK activity did not appear to be associated with the rate of pH decline. However, it is likely that some or all of the Hampshire pigs used in this study had at least one copy of the RN^{-} mutation since Hampshire pigs had 2 to 3-fold more muscle glycogen than Chester White or Poland China pigs, respectively. Furthermore, the Poland China pigs used in this study had a very rapid pH decline to 1 h postmortem (pH 5.85) and a high incidence of PSE pork. The rapid pH decline and high incidence of PSE pork indicates that the Poland China pigs might have had at least one copy of the HAL-1843™ mutation. Therefore, the relationship between PFK activity and the rate of pH decline might have been masked due to the potential presence of two major genes that are known to have adverse effects on pork quality traits.

Sayre et al. (1963c) also examined the effects of heat treating pigs at 42-45°C for 1 h prior to harvest, feeding a high sucrose diet or a high-fat and high-protein diet on fresh pork quality traits. The authors randomly assigned Chester White, Hampshire and Poland China pigs to the treatment groups described above. The authors concluded that PFK activity was not associated with the rate of pH decline and could not explain the rapid pH decline of the heat-treated pigs. However, the authors compared treatment

means for PFK activity rather than examining partial correlations for PFK activity and pH values. Correlations would have been more informative because Chester White pigs were unaffected by heat treatment as these pigs had an ultimate pH above 5.8, a darker color and better WHC than Hampshire and Poland China pigs subjected to heat-treatment. Their data suggests that treatment by breed interactions might have occurred and thus affected treatment means. However, Sayre et al. (1963c) did not statistically analyze treatment by breed interactions. Also, as mentioned earlier it is likely that the HAL-1843™ mutation was segregating in the Poland China breed, while it is likely that RN^- gene was segregating in the Hampshire breed.

Frucotse-6-phosphate substrate cycling refers to the conversion of fructose-6-phosphate (F-6-P) to fructose-1,6-diphosphate (F-1,6-P) by PFK and the reverse reaction catalyzed by fructose diphosphatase. Fructose-6-phosphate substrate cycling has been examined in halothane sensitive and normal pigs. Halothane sensitive pigs had higher resting body temperatures than normal pigs. Pigs were than subjected to the halothane challenge test. After exposure to halothane gas, the body temperature of halothane sensitive pigs increased up to 5°C, while the body temperature of normal pigs was slightly decreased. Additionally, halothane gas treatment resulted in a substantial decrease of muscle ATP, a 6 to 8 fold increase in substrate cycling and a 1.2 to 2-fold increase in the rate of glycolysis in halothane sensitive pigs. Meanwhile, ATP levels, substrate cycling and the rate of glycolysis in normal pigs were not affected by halothane gas treatment. Despite substantial differences between halothane sensitive and non-sensitive pigs in glycolytic rate and substrate cycling, PFK, fructose diphosphatase,

aldolase and triose phosphate isomerase activities did not differ between genetic lines (Clark et al., 1973).

Schwagele et al. (1996) studied PK activity in muscle from halothane sensitive and control pigs. Genotypes were determined by the halothane gas challenge test. Halothane sensitive pigs had a 45 minute pH less than 5.5 and the control pigs had a 45 minute pH greater than 6.2. Pyruvate kinase activity was assayed at pH 7.0 and at pH 5.5. Halothane sensitive pigs had approximately 4-fold more total and specific PK activity when assayed at pH 7.0 than control pigs. When assayed at pH 5.5, halothane sensitive pigs retained approximately 70% of their activity at pH 7.0, while muscle from control pigs had less had less than 10% of their activity at pH 7.0. It was also interesting to note that halothane sensitive pigs had 3 major isoforms (isoforms 1, 2 and 3) for PK, whereas muscle from control pigs only had two (isoforms 1 and 2). Pyruvate kinase from muscle of control pigs was comprised of 72% of isoform 1, 28% of isoform 2 and less than 1% of isoform 3. Whereas, PK from muscle of halothane sensitive pigs was comprised of 58% of isoform 1, 23% of isoform 2 and 19% of isoform 3. Isoforms 1, 2 and 3 were assayed at pH 7.0, 6.5, 6.0, 5.5 and 5.0. Isoform 3 retained approximately 40% of maximal activity at pH 5.5 and 15-20% at pH 5.0, whereas isoforms 1 and 2 had approximately 10% of maximal activity at pH 5.5 and almost no activity at pH 5.0. The authors conducted in vitro phosphorylation and dephosphorylation experiments and concluded that PK isoform 1 becomes phosphorylated and forms isoform 2, which in turn can become phosphorylated and form isoform 3. The authors concluded that halothane sensitive pigs have more PK activity due to phosphorylation, which allows PK to be more stable under acidic conditions (pH<5.5). However, it is unclear if isoform 3 is one of the

causes responsible for abnormal postmortem muscle metabolism or if isoform 3 is merely an effect of the abnormal postmortem muscle metabolism in halothane sensitive pigs (Schwagele et al., 1996).

Hypothesizing that higher PFK and PK activities would be associated with a more rapid pH decline, Allison et al. (2002) measured PFK and PK activity in Duroc- and Pietrain-sired pigs that were produced from HAL-1843™ normal parent seedstock. Correlations between PFK, PK and pork quality traits were examined. Enzyme activities were not related to the rate of pH decline or fresh pork color. However, PFK was negatively correlated with centrifuge WHC ($r = -0.384$, $P < 0.05$) and day 7 purge ($r = -0.484$, $P < 0.01$). These correlations imply that pigs with higher PFK activity had better WHC.

Muscle Fiber Types

Muscle fiber types have important implications for the meat industry in terms of lean quantity and quality. The various properties of muscle fiber types include myoglobin (pigment) concentration, speed of contraction, glycolytic and/or oxidative metabolism and fiber diameter. The combination of muscle contraction speed and muscle metabolism (glycolytic and/or oxidative) could influence the rate and extent of pH decline, which is known to influence color and WHC (Briskey, 1963). Additionally, differences in fiber diameter due to muscle fiber type could be responsible for differences in muscle mass.

Muscle fiber types can be classified by contraction speed as fast-twitch (Type IIA or Type IIB) or slow-twitch (Type I; Brooke & Kaiser, 1970). Additionally, muscle fiber types can be classified on the basis of muscle metabolism as glycolytic,

glycolytic/oxidative or oxidative (Ashmore & Doerr, 1971). Muscle fibers are often described as Type I or slow-twitch, oxidative (SO); Type IIA or fast-twitch, oxidative, glycolytic (FOG); and Type IIB or fast-twitch, glycolytic (FG; Solomon & Dunn, 1988).

Fiber Types I, IIA and IIB differ greatly in regards to contraction speed, muscle metabolism and fiber diameter. Type I fibers are associated with tonic muscles that are more resistant to fatigue than Type II fibers. Type I muscle fibers are more likely to utilize oxidative metabolism and thus have a higher number and larger mitochondria, more myoglobin, more oxidative enzyme activity, lower muscle glycogen stores and fewer glycolytic enzymes than Type IIB fibers. On the other hand, Type IIB muscle fibers are fast-twitch, glycolytic, phasic and are associated with muscles that need rapid bursts of energy for short durations. Thus, these fibers have a higher glycogen content and a greater amount of glycolytic enzymes, but small and few mitochondria and a more developed sarcoplasmic reticulum. Furthermore, Type IIB muscle fibers are lower in myoglobin content and thus have a paler color. Type IIA muscle fibers are usually referred to as intermediate muscle fibers because they are fast-twitch and utilize both glycolytic and oxidative metabolism. While Type IIA fibers are fast-twitch fibers, they are slower than Type IIB fibers but faster than Type I fibers. Type IIA fibers are high in glycogen and have an intermediate size and number of mitochondria. However, these fibers are high in myoglobin and are red in color. Finally, Type I fibers have the smallest muscle fiber diameter, while Type IIB have the largest (Aberle et al., 2001).

Implications of Muscle Fiber Types For Carcass and Meat Quality Traits

A histochemical comparison of wild boar, halothane susceptible Landrace and normal Landrace pigs suggests that genetic selection for leanness and/or muscling has

resulted in an increased percentage of Type IIB muscle fibers. The wild boar had a significantly higher proportion of Type IIA and a significantly lower proportion of Type IIB in the *longissimus* muscle. Additionally, the wild boar had a significantly higher proportion of Type I and Type IIA and a significantly lower proportion of Type IIB fibers in the *semitendinosus* and *gluteus* muscles than normal Landrace pigs (Essen-Gustavsson & Lindholm, 1984). Additional studies have suggested that selecting for leanness in pigs could result in a higher proportion of Type IIB muscle fibers (Rahelic & Puac, 1981; Weiler et al., 1995; Lefaucheur et al., 2002).

Larzul et al. (1997) established that Type IIB fibers had significant negative genetic correlations to 30 min pH and ultimate pH while being positively correlated to glycolytic potential and CIE L* values in pigs that were normal for the HAL-1843™ and RN^- mutations. They concluded that Type IIB muscle fibers have undesirable relationships to measures of pork quality and a high heritability (h^2=0.58). Therefore, a selection program for decreasing the percentage of Type IIB could be implemented to improve pork quality traits of pigs that are normal for the HAL-1843™ and RN^- mutations.

Additionally, a histochemical study conducted by Ruusunen and Puolanne (1997) established that Hampshire pigs had a higher proportion of Type I and a lower proportion of Type IIB muscle fibers than muscle from Landrace and Yorkshire pigs. However, the authors concluded that the variation within breeds for fiber type was larger than the variation across breeds. They speculated that pigs with same muscle fiber type profiles could be found in all breeds. Therefore, sires within each breed could be identified for

desirable muscle fiber types and fiber types could be implemented into a selection program.

Quantifying Muscle Fiber Types

The most commonly used methods for muscle fiber type classification are the methods developed by Brooke and Kaiser (1970) and Ashmore and Doerr (1971). The Brooke and Kaiser (1970) method utilizes the pH stability of myosin ATPase to determine slow twitch (Type I) and fast-twitch (Type IIA and Type IIB) muscle fibers. In the two-step method of Ashmore and Doerr (1971), myofibrillar ATPase and succinate dehydrogenase staining are used to determine contraction speed and muscle metabolism. Both of these methods are very tedius and time consuming. Solomon and Dunn (1988) combined these two methods into one procedure.

Another approach that has been used to classify muscle fiber types is the analysis of myosin heavy chain isoforms (MHC). Myosin, the most abundant protein in skeletal muscle, has four major myosin heavy chain isoforms (MHC) expressed in skeletal muscle. On the basis of contraction speed, the MHC are ranked: Type I, Type IIA, Type IIX and Type IIB from slow to fast and oxidative to glycolytic. Myosin heavy chain isoforms are associated with myofibrillar ATPase activity and contraction speed (reviewed by Schiaffino & Reggiani, 1994; Talmadge, 2000). Therefore, MHC are used as indicators of muscle fiber types.

Talmadge and Roy (1993) developed a method to separate MHC isoforms of rat skeletal muscle by SDS-PAGE. This method allows for the separation of MHC into four distinct bands (MHC Types IIA, IIX, IIB and I). Bee et al. (1999) compared the histochemical fiber typing with MHC composition using the Talmadage and Roy method.

The authors could only separate MHC into three bands, which they identified as Type IIA, Type II X/B and Type I based on the differing migration of the four MHC isoforms found in rat skeletal muscle. The relative proportions of *longissiumus* Types I, IIA and IIB muscle fibers were 12.2%, 16.5% and 71.3%, respectively. Meanwhile the relative proportions of *longissiumus* MHC Type I, IIA and IIX/B were 5.8%, 47.2% and 47.1%, respectively. The authors concluded that there was a significant correlation between the relative proportion of Type I muscle fibers and the relative proportion of MHC Type I ($r = 0.8$) as well as a significant correlation between the relative proportion of Type IIB muscle fibers and the relative proportion of MHC Type II X/B ($r = 0.76$). There was no correlation between the relative proportion of Type IIA and the relative proportion of MHC Type IIA ($r = -0.05$). Electrophoretic separation of MHC Type I and Type IIX/B can be used as an accurate indicator of the proportion of Type I and IIB muscle fibers, which have the most implications for pork quality.

Myoglobin

Myoglobin is the primary pigment responsible for fresh meat color (Seideman et al., 1984; Aberle et al., 2001), and its primary function is to distribute oxygen in muscle tissues (Voet et al., 1999). This globular protein has a porphyrin ring with a centrally located iron atom (Walters, 1975; Seideman et al., 1984; Hedrick et al., 1993; Voet et al., 1999). The valence state of the iron atom (Fe^{+2} or Fe^{+3}) and the compound (oxygen or water) that is bound to the free binding site of myoglobin determine the myoglobin state of fresh meat (oxymyoglobin, metmyoglobin and deoxymyoglobin). The amount of myoglobin present and the state of myoglobin are the two most important factors that determine fresh meat color (Walters, 1975; Seideman et al., 1984; Herrick et al., 1993).

It is well established that species, gender, age, amount of physical activity and muscle fiber type affect the amount of myoglobin in muscle. Gonadally intact males have more myoglobin than females and castarated males. Veal calves have substantially less myoglobin than older market and breeding cattle (Seideman et al., 1984). Furthermore, Type I muscle fibers, which utilize oxidative metabolism, have a higher myoglobin content. The higher myoglobin content in red muscle fibers contribute to the darker red color found when there is a higher proportion of Type I muscle fibers (Walters, 1975; Seideman et al., 1984; Aberle et al., 2001).

Myoglobin represents 80 to 90% of the total muscle pigment in an animal after exsanguination (Aberle et al., 2001) and is commonly measured spectrophotometrically as heme pigment concentration (Hornsey, 1956; Warriss, 1979). Larzul et al. (1997) calculated the heritability of heme pigment content to be medium ($h^2 = 0.39$), but it appears halothane genotype does not affect this parameter (Monin et al., 1980). However, significant differences between genetic lines have been reported for myoglobin (Allen et al., 1966) and total heme pigment (Monin & Sellier, 1985; Warriss et al., 1990a; Lindahl et al., 2001). Additionally, Lindahl et al. (2001) demonstrated that total heme pigment was correlated to CIE L* and a* values in fresh pork.

Ractopamine

Ractopamine is a phenethanolamine and a partial β-adrenergic agonist (β-agonist) that was developed by and is currently produced by ELANCO Animal Health and marketed under the name Paylean®. Ractopamine is similar in structure to natural (epinephrine and norepinephrine) and other synthetic (cimaterol, clenbuterol and salbutalmol) β-agonists (Smith & Paulson, 1994; Mills, 2001). Although β-agonists were

not yet approved for commercial use in the livestock industry, their effects on growth and carcass traits were studied extensively in the 1980s and 1990s.

In December of 2000, Paylean® (ractopamine-hydrochloride; RAC) became the first β-agonist approved by the U.S. Food and Drug Administration for commercial use in swine. Up to 20 ppm for the last 41 kg of live weight (68-109 kg) can be fed to improve growth and carcass traits. To maximize the potential benefits of Paylean®, it must be fed fed in a complete feed that contains a minimum of 16% crude protein.

Mode of Action

Ractopamine has a high affinity to bind to β-adrenergic receptors, which are located on the binding site in the middle of the 7-transmembrane domain proteins, which are embedded in the lipid bi-layer (Liu & Mills, 1989; Mills, 2001). The β-adrenergic receptor subtypes in porcine adipose tissue are 75% β1, 18% β2 and 7% β3. In porcine skeletal muscle, the β-adrenergic receptor composition is 60% β1, 39% β2 and 1% β3. Although β1 receptors are the most densely populated subtype found in porcine adipose tissue and skeletal muscle, β2 receptors have been shown to be more effective at activating adenylate cyclase (Mills, 2001).

Ractopamine decreases fat by increasing lipolysis and/or decreasing lipogenesis (Merkel et al., 1987; Liu et al., 1989; Mills et al., 1990; Peterla & Scanes, 1990). Ractopamine binds to the β-adrenergic receptors and activates the G-protein linked signaling system. Activation of the G proteins leads to the activation of adenylate cyclase, which catalyzes the conversion of ATP to cyclic AMP. Cyclic AMP is a second messenger that activates protein kinase A (cyclic AMP-dependent protein kinase). Protein kinase A stimulates lipolysis by activating hormone sensitive lipase and reduces

lipogenesis by inactivating acetyl CoA carboxylase. Hormone sensitive lipase is an important enzyme in lipolysis that converts triglycerides to fatty acids and glycerol. Acetyl CoA carboxylase is a key enzyme in lipogenesis that catalyzes the first reaction of fatty acid synthesis by converting acetyl CoA to malonyl CoA (Mersmann, 1998; Voet et al., 1999; Mills, 2001).

Ractopamine increases muscle mass by causing muscle fiber hypertrophy, which is an increase in muscle fiber diameter. Aalhus et al. (1992) reported that RAC increased the cross-sectional area of Type IIA and Type IIB muscle fibers and promoted a shift in fiber type from Type IIA to Type IIB. Additionally, Bark et al. (1992) reported that RAC significantly increased muscle accretion rates. It is speculated that RAC increases muscling by increasing the rates of protein accretion. This can be accomplished by increases in protein synthesis relative to protein degradation (i.e. increases in synthesis and decreases in degradation; large increases in synthesis and small increases in degradation; small decreases in synthesis and large decreases in degradation; increases in synthesis and no change in degradation, and no change in synthesis but decreases in degradation). Bergen et al. (1989) reported that RAC significantly increased fractional protein synthesis rates, which numerically increased protein accretion rates by 20%.

β-Adrenergic Receptor Regulation

The ability of RAC to improve growth and carcass traits decreases over time during administration. Maximal improvements in average daily gain (ADG) have been reported to occur between 0 to 7 days (Dunshea et al., 1993a), 7 to 21 days (Williams et al., 1994) and between 0 to 28 days (Dunshea et al., 1993b) of RAC treatment. However, ADG decreased in all of these studies after the maximal improvements were observed.

Herr et al. (2001a) speculated that the RAC response for growth and backfat traits decreases over time due to desensitization of β-adrenergic receptors.

Spurlock et al. (1994) concluded that long-term RAC treatments do not affect the affinity of RAC for β-adrenergic receptors, but instead decreased β-adrenergic receptor density. This phenomenon is known as down-regulation or desensitization and refers to the β-adrenergic receptor becoming saturated by the β-agonist, and thus decreasing the number of β-adrenergic receptors that are available to bind the β-agonist. Mills et al. (1990) concluded that adipocytes from pigs fed RAC are less responsive to in vitro administration of RAC than adipocytes from control pigs. Additionally, Liu et al. (1994) concluded that RAC was more effective at increasing carcass muscling than decreasing carcass fat. It was speculated that β-adrenergic receptors in adipose tissue are less sensitive to RAC or are more susceptible to desensitization than β-adrenergic receptors in skeletal muscle (Mills et al., 1990; Liu et al., 1994). Spurlock et al. (1994) confirmed that β-adrenergic receptors are more susceptible to desensitization or down-regulation in adipose tissue than skeletal muscle. β-adrenergic receptor density was decreased by approximately 53% in the middle layer and by 30% in the outer layer of subcutaneous fat after eight days of RAC treatment. However, β-adrenergic receptor density only decreased by approximately 23% in *longissimus* muscle after 24 days of RAC treatment. Therefore, RAC may be more effective at improving carcass muscling than reducing carcass fat. This has been demonstrated in several studies that have reported significant increases in loin muscle area and no changes in tenth rib backfat (Cline & Forrest, 1989; Mitchell et al., 1991; Stites et al., 1991).

Other Factors Affecting the Ractopamine Response

Genetics, dietary crude protein (CP) and dietary lysine (Lys) concentrations also influence the ability of RAC to improve growth and carcass traits (Gu et al., 1991b; Bark et al., 1992; Schinckel et al., 2001a; Schinckel et al., 2002a). Pietrain pigs have been shown to have a higher density of β-adrenergic receptors than Large White pigs (Bocklen et al., 1986). Also, ractopamine is more effective at improving growth and carcass traits in pigs that have high lean growth rates (Gu et al., 1991b; Bark et al., 1992). Therefore, it could be speculated that lean and heavy muscled pigs have more β-adrenergic receptors and thus more capacity to respond to RAC. However, high lean growth pigs require more dietary CP and Lys (Stahly et al., 1988) and RAC-fed pigs require 0.26% more Lys than control pigs (Weldon & Armstrong, 2001). Additionally, Webster et al. (2002) concluded that at least 1% Lys is necessary to maximize the response of RAC on growth and carcass traits. Furthermore, it is known that that if the need for higher dietary CP and Lys are not met in RAC fed pigs, the response will be limited (Weldon & Armstrong, 2001; Schinckel et al., 2002a).

Effects on Growth Traits

The efficacy of RAC to improve growth traits has been studied extensively and most concluded that that RAC does not affect feed intake (Yen et al., 1991, Gu et al., 1991a; Dunshea et al., 1993a; He et al., 1993). However in contrast, a few studies have reported a decrease in feed intake (Yen et al., 1990; Crome et al., 1996). However, RAC consistently increases average daily gains (Watkins et al., 1990; Bark et al., 1992; Willams et al., 1994) and feed utilization (Watkins et al., 1990; Bark et al., 1992; He et al., 1993). This improvement in feed efficiency is primarily due to the increases in

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average daily gains without changes in feed intake. Additionally, RAC has been reported to improve lean gain (Williams et al., 1994).

Effects on Carcass Traits

Ractopamine increases loin muscle area (Yen et al., 1990; Crome et al., 1996; Xiao et al., 1999) and the weights of the *longissimus dorsi* (Grant et al., 1993; Xia et al., 1999), *psoas major* (Crome et al., 1996), *biceps femoris* (Xia et al., 1999; Schinckel et al., 2002b), *quadriceps femoris* (Schinckel et al., 2002b), *semimebranosus* (Xiao et al., 1999; Schinckel et al., 2002a) and *semitendinosus* (Bergen et al., 1989; Xiao et al., 1999; Schinckel et al., 2002a) muscles. Additionally, RAC increases the weights of boneless wholesale boston butts, picnic shoulders, loins and hams (Crome et al., 1996). Improvements in trimmed wholesale boston butt (Yen et al., 1990; Crome et al., 1996), picnic shoulder (Yen et al., 1990; Crome et al., 1996), loin (Yen et al., 1990; Stites et al., 1991; Uttaro et al., 1993; Crome et al., 1996), belly (Uttaro et al., 1993) and ham (Stites et al., 1991; Uttaro et al., 1993; Crome et al., 1996) weights have also been observed for RAC-treated pigs. Additionally, improvements in dressing percentages (Yen et al., 1990; Crome et al., 1996: Ivers et al., 2000) and hot carcass weights (Yen et al., 1990; He et al., 1993; Crome et al., 1996) have been reported for RAC-treated pigs.

On the other hand, the efficacy of RAC to reduce carcass fat has been inconsistent. Schinckel et al. (2002a) fed two different diets varying in CP and Lys concentrations and concluded that the efficacy of RAC to improve carcass composition is dependent upon RAC doseage and dietary lysine. Phase feeding dietary CP and Lys for six weeks (18% CP and 1.08% Lys for weeks 1 and 4, 20% CP and 1.22% Lys for weeks 2 and 3, 16% CP and 0.94% Lys for week 5 and 16% CP and 0.82% Lys for week 6) with

ractopamine was more effective at improving carcass composition than feeding a constant 16% CP and 0.82% Lys diet with RAC for 6 weeks (Schinckel et al., 2002a). Reductions in tenth rib backfat have been observed in RAC-treated pigs. Crome et al. (1996) reported that 10 and 20 ppm of RAC reduced tenth rib backfat when a 16% CP and .82% Lys corn-soybean meal diet was fed. Dunshea et al. (1993a) reported that 20 ppm of RAC decreased tenth rib backfat when a 1.07% Lys wheat-soybean meal diet was fed. Additionally, Bark et al. (1992) reported that 20 ppm of RAC supplemented to a 17.7% CP and 1.08% corn-soybean meal diet decreased tenth rib backfat. However, Cline and Forrest (1989) and Yen et al. (1991) reported no changes in backfat thickness when 20 ppm of RAC was fed with a 16% CP corn-soybean meal diet. Furthermore, the efficacy of RAC to reduce average midline backfat has been inconsistent as Bark et al. (1992) reported that 20 ppm of RAC significantly decreased average backfat, but Yen et al. (1991) and Dunshea et al. (1993a) reported no change in average backfat when 20 ppm of RAC was fed.

Effects on Pork Quality

The effects of RAC on pork quality are of particular interest due how RAC improves carcass compostion. Because RAC reduces carcass fat by increasing lipolysis and decreasing lipogenesis (Merkel et al., 1987; Peterla and Scanes, 1990), there are concerns that it will reduce intramuscular fat content, which is important for meat flavor and juciness. Because of muscle hypertrophy and a potential increase in the proportion of Type IIB muscle fibers, there are concerns that RAC could adversely affect the rate and extent of pH decline and fresh pork color. Cimaterol, another β-agonist, has been shown to increase shear force values of loin chops in pork (Merkel, 1988). This is

presumably due to increased calpastatin activity (Kretchmar et al., 1988; Sensky et al., 1996) since calpastatin is a known inhibitor of the calpain proteases, which are responsible for the postmortem tenderization of meat. Therefore, RAC could potentially produce less tender pork.

Several studies utilizing different diets, genetics, environments and levels of RAC have been designed to examine the effects of RAC on loin muscle pH, color, water-holding capacity and tenderness. Despite the potentially negative impacts RAC might have on pork quality, several authors have concluded that RAC does not adversely affect fresh pork quality traits (Merkel, 1988; Dunshea et al., 1993a; Uttaro et al., 1993; McKeith & Ellis, 2001), processing yields (Stites et al., 1991; Jeremiah et al., 1994a) or sensory traits (McKeith et al., 1988a; Jeremiah et al., 1994a; Jeremiah et al., 1994b). Aalhus et al. (1990) concluded that RAC had minor negative effects on loin muscle 40 min postmortem pH, CIE a* values, drip loss and tenderness but no PSE or DFD pork was produced from RAC-treated pigs.

Future Concerns

Despite all of the potential benefits of feeding RAC, it is estimated that only 10-20% of all hogs in the U.S. are currently being fed RAC (Dr. A. Schroeder, personal communication). Many producers are hesitant to feed RAC due to the negative perceptions about altered animal behavior, increased lameness and an increased percentage of dead and downer pigs. Ractopamine-treated pigs appear to be more visibly excitable (personal observation), but Schaefer et al. (1992) concluded that RAC had no major effects on animal behavior. Additionally, He et al. (1993) concluded that 20 ppm of RAC had no effect on joint-cartilage soundness. Although, preliminary research

indicates that RAC does not affect behavior and soundness, the effects of RAC on behavior and soundness need to be further examined using different genetics, diets and facilities. Also, Ivers et al. (2002) examined downer pig syndrome (DPS) in control and RAC-treated pigs. The authors concluded that DPS was observed in RAC-treated and control pigs and is caused by metabolic acidosis. However, they did not state whether or not RAC-treated pigs were more susceptible to developing DPS than control pigs and thus more research is needed in this area.

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CHAPTER 1

BIOCHEMICAL CHARACTERIZATION OF SUPERIOR QUALITY PORK

ABSTRACT

Berkshire-sired (B; n=16) and Yorkshire-sired (Y; n=16) barrows and gilts were harvested at a commercial abattoir on two days. *Longissimus* muscle (LM) from B had lower pH at 180 min postmortem (P>.05), lower glycolytic potential (P<.06) and a higher ultimate pH (P<.02). Loin muscle (LM) from B pigs also had lower d 1 CIE L* values (P<.01) and lower d 7 purge (P<.05), and tended to have less fluid loss when subjected to high-speed centrifugation than Y (P<.09). Phosphofructokinase and pyruvate kinase activity, heme pigment concentration and myosin heavy chain isoform (MHC) distribution did not differ between breeds. Harvest day by breed interactions existed for 20 min LM temperature and fluid loss measured by the suspension drip and filter paper methods (P<.05). On harvest day 2, MHC Types IIA and IIX/B were correlated to 20 min (r = .61 and -.65, respectively; P<.05) and 24 h pH (r = .62 and -.58, respectively; P<.05) and drip loss (r = -.51 and .55, respectively P<.06). Additionally on harvest day 2, glucose-6-phosphate was correlated to 20, 45 and 180 min pH (r = -.83, -.68 and -.59, respectively; P<.03) and fluid loss measured by the suspension drip, high-speed centrifugation or the filter paper methods (r = .73, .76 and .74, respectively; P<.01). Breed differences in color and water-holding capacity (WHC) were not associated with glycolytic enzyme activity or the proportion MHC. However, a higher proportion of MHC Type IIX/B and higher quantities of glucose-6-phosphate may contribute to accelerated pH decline under less favorable antemortem or postmortem conditions.

Keywords: Glycolytic enzymes; Myosin heavy chain isoforms; Glycolytic potential

INTRODUCTION

Little research has focused on the mechanism(s) responsible for producing superior quality pork. Berkshire progeny consistently have the highest ultimate pH, lowest CIE L* values and lowest drip losses, whereas Yorkshire and Landrace progeny have inferior color and WHC (NPPC, 1995; Goodwin, 2000). Understanding the biochemical mechanisms responsible for superior pork quality in Berkshire progeny and inferior pork quality in pigs that do not possess the HAL-1843™ and RN^- mutations may lead to new approaches to improve quality pork.

The rate and extent of pH decline are associated with fresh pork color and WHC (Briskey, 1963). The combination of a high muscle temperature and a rapid pH decline early postmortem can result in denaturation of sarcoplasmic and myofibrillar proteins (Wismer-Pedersen, 1959; Bendall & Wismer-Pedersen, 1962; Sayre & Briskey, 1963a), which are associated with inferior color and WHC, respectively (Warner, Kauffman & Greaser 1997; Joo, Kauffman, Kim & Park, 1999; Offer, 1991). Additionally, low ultimate pH is caused by excessive glycogen in the muscle at death (Monin & Sellier, 1985). Low ultimate pH results in denaturation of myofibrillar proteins and reduction in the net protein charge, which can result in reduced WHC (Lawrie, Gatherum & Hale, 1958; Lawrie, 1960; Wismer-Pederson, 1959).

Kastenschmidt, Hoekstra and Briskey (1968) suggested that the coordinated stimulation of phosphorylase, phosphofructokinase (PFK) and pyruvate kinase (PK) was responsible for rapid pH declines. They also suggested that PFK and PK were rate-limiting steps in anaerobic glycolysis. Schwagele, Haschke, Honikel and Krauss (1996) examined PK activity in muscle from halothane sensitive and control pigs. Halothane

sensitive pigs had approximately 4-fold more total and specific PK activity when assayed at pH 7.0 than control pigs. When assayed at pH 5.5, PK from muscle of halothane sensitive pigs retained approximately 70% of the maximum activity (activity at pH 7.0), while muscle from control pigs had less than 10%. These authors concluded that halothane sensitive pigs have more PK activity due to enzyme phosphorylation, which allows PK to be more stable under acidic conditions.

A histochemical study established that Landrace pigs had lower proportions of Type I and Type IIA and a higher proportion of Type IIB muscle fiber types in the *semitendinosus* and *gluteus* muscles than the wild boar (Essen-Gustavsson & Lindholm, 1984). It appears that genetic selection for leanness and/or muscling results in an increased percentage of Type IIB fibers. However, Type IIB muscle fibers have negative genetic correlations to the rate and extent of pH decline and positive genetic correlations to CIE L* values (Larzul, Lefaucheur, Ecolan, Gogue, Talmant, Sellier, Le Roy & Monin, 1997).

Myoglobin is the primary pigment responsible for fresh meat color. The amount and oxidation state of myoglobin are the two most important factors that determine fresh meat color (Walters, 1975; Seideman, Cross, Smith & Durland, 1984). In fresh pork, heme pigment concentration is negatively correlated to CIE L* (Warris et al., 1990a; Lindahl et al., 2001) and positively correlated to CIE a* values (Lindahl et al., 2001).

The objective of this experiment was to determine if superior pork color and WHC of Berkshire progeny are associated with the amount of glycogen stored in the muscle, in vitro activity of rate-limiting glycolytic enzymes and/or a higher proportion of Type I myosin heavy chain isoforms and/or a higher concentration of myoglobin.

MATERIALS AND METHODS

Experimental Groups

The Michigan State University All-University Committee on Animal Use and Care (AUCAUC) approved the use of animals for this research project (AUF #11/00-152-00). Pigs were raised and handled according to guidelines approved by the AUCAUC. Sires from three high meat quality indexing lines of the Berkshire breed identified at the National Barrow Show Progeny Tests and four Yorkshire lines selected for muscling were used to artificially inseminate F_1 Yorkshire-Landrace sows maintained at the Michigan State University (MSU) Swine Teaching and Research Farm. Progeny were raised in uniform conditions at MSU facilities. When pigs weighed approximately 86 kg, pigs were blocked by gender, sire and live weight into two breed groups each containing 16 pigs (8 barrows and 8 gilts per group). Two 2.46 m by 4.87 m pens with slatted floors, stainless steel feeders and nipple drinkers were used to house each breed group. All pigs had ad libitum access to water and a 16% crude protein corn-soybean meal diet containing 0.9% lysine.

Carcass Trait Data Collection

One day before harvest, pigs were weighed, transported to a commercial abattoir and allowed a minimum of 12 h rest. The eight pigs with the heaviest live weights from each breed group were harvested on January 4, 2001 and the remaining eight pigs in each group were harvested on January 8, 2001. Breed groups were kept separate during transit and during the resting period prior to harvest to minimize stress and fighting. Carcass weights were recorded prior to chilling. At 24 h postmortem, midline backfat measures were taken adjacent to the first rib, last rib and last lumbar vertebra (average backfat).

Loin muscle area and 10th rib fat thickness were determined according to current National Pork Producers Council (NPPC) guidelines (NPPC, 2000). Additionally, percent fat-free lean and kg of fat-free lean were calculated using NPPC equations (NPPC, 2000). Carcass muscling scores were determined and U.S.D.A. Grades were calculated as follows: 4(last rib backfat) – muscle score. The right side of each carcass was fabricated into wholesale cuts, which were subsequently trimmed and weighed. The right loin from each carcass was transported to the MSU Meat Laboratory for analysis.

Meat Quality Data Collection

At 20, 45, 180 min and 24 h postmortem, *longissimus* muscle (LM) temperature and pH were measured adjacent to the last rib. Temperature was measured using a hand held temperature probe (Model Number 33032, Atkins Technical Inc., Gainesville, FL) and pH was determined with a portable pH meter equipped with a puncture-type electrode (Model 1140, Mettler-Toledo, Woburn, MA). At 20 min postmortem, LM samples were removed midway between the last rib and the cranial edge of the ilium on the left side of each carcass. These samples were cut into 0.5 cm^3 pieces, frozen in liquid nitrogen, and stored at –80°C until biochemical analyses were conducted.

At 24 h postmortem, a 2.54 cm thick loin chop located adjacent to the third lumbar vertebra, was removed for proximate composition and WHC determination. Duplicate 10 g samples from this chop were used to determine WHC by high-speed centrifugation (40,000 x g for 30 min; Honikel & Hamm, 1994). Immediately posterior to this sample, two 2.54 cm thick loin chops were removed, allowed to bloom 15 min and used to determine subjective color and marbling according to current NPPC guidelines (NPPC, 2000) and firmness/wetness on a 5 point scale (1= very soft and very watery and

5 = very firm and dry; NPPC, 1991). These chops were also used for objective color measurement by using Commission International de l'Eclairage (CIE) L*, a* and b* measurements (D_{65}, 2° standard observer and 50 mm-diameter measuring area) with a Minolta chromameter (CR-310 series; Ramsey, NJ) and drip loss by the suspension method from 24 to 48 h postmortem (Honikel & Hamm, 1994). The cut surfaces of the remaining loin sections (~520 g) were used to measure exudates by the filter paper test (NPPC, 2000), except that duplicate Whatman® #3, 4.25 cm diameter circles were used. This section was vacuum packaged at 24 h postmortem in Cryovac shrink bags using a MultiVac machine (Koch, Type AG 800) and held at 4°C until day 7 postmortem. The difference between d 7 and 24 h weight was divided by 24 h weight and expressed as percent purge.

Tenderness

The 32 pigs used in this study were also included in another study designed to determine the rate and extent of postmortem tenderization (Allison, Tempelman & Doumit, 2001). A boneless loin section from approximately the eleventh rib to the last rib was removed for the determination of tenderness. Loin sections were divided into four equal sections and were randomly assigned to aging treatments of 1, 3, 7 and 14 d at 4°C. Loin chop tenderness was determined by Warner-Bratzler shear force of cores from duplicate chops aged according to treatment, then cooked to 71°C. For the purposes of this study only d 7 tenderness is reported.

Proximate Composition

Longissimus samples were removed adjacent to the third lumbar vertebrae to determine proximate composition and were frozen at -20°C until analysis was conducted.

These samples were milled to a powder with Dry Ice. Carbon dioxide was allowed to evaporate for two days at –20°C. Moisture content was measured by air-drying (AOAC Method 950.46B). Total fat was determined using the Soxtec Fat Analyzer (Tecator, Höganäs, Sweden; AOAC Method 991.36 Solvent Extraction Method). Crude protein was determined using combustion method 992.15 (AOAC, 1995; Leco FP-2000, Leco Corp., St Joseph, MI).

Glycolytic Potential

Biochemical analyses were conducted on LM samples collected 20 min postmortem. Two g were homogenized in 10 mL of 0.6 N perchloric acid with a Polytron (Brinkman, Westbury, NY) at setting 4 for two 30 sec bursts. Glucose equivalents (glycogen, glucose and glucose-6-phosphate) were prepared according to Dalrymple and Hamm (1973). Triplicate 200 µL samples, 1 mg amyloglucosidase (AMG; 1 mg/mL) and 20 µL of 5.4 N KOH were mixed and incubated for 2 h at 37°C. Tubes were inverted every 20 min. Samples were cooled in an ice bath for 20 min followed by the addition of 100 µL of ice-cold 3 N perchloric acid to precipitate proteins. Samples were allowed to settle for 10 min at 4°C and were then centrifuged at 7,000 x g for 5 min at 4°C in an Eppendorf Centrifuge 5415 (Brinkman, Westbury, NY). Supernatant fluids were saved and stored at 4°C until subsequent analysis could be conducted.

The remainder of the homogenate was centrifuged at 40,000 x g for 20 min at 4°C (RC-5 superspeed centrifuge; DU PONT Company, Wilmington, DE). After centrifugation, 1.8 mL of the supernatant was mixed with 180 µL KOH to neutralize the supernatant and was allowed to settle for 20 min at 4°C. Following centrifugation at

7,000 x g for 5 min at 4°C (Eppendorf Centrifuge 5415; Brinkman, Westbury, NY), supernatant fluids were stored at 4°C until quantification of free glucose, glucose-6-phosphate and lactate.

Free glucose, glucose-6-phosphate and lactate assays were adapted to a 96-well microtiter plate format and were assayed in duplicate with absorbance measured at 340 nm on a VERSAmax™ plate reader (Sunnyvale, CA). For free glucose and lactate assays, 10 μL supernatant and 190 μL of glucose or lactate assay reagents were added to each well. Glucose-6-phosphate was quantified by using a modification of the method developed by Bondar and Mead (1974). For glucose-6-phosphate assays, 10 μL sample extract, 190 μL of glucose-6-phosphate reagent (100 mM Tris-hydrochloride, 1 mM magnesium acetate and 0.66 mM NAD^+) and 10 μL of 20 U/mL glucose-6-phosphate dehydrogenase were added to each well. Infinity™ Glucose Reagent (Sigma 18-20) and L(+)Lactate (Sigma 826-A) kits, glucose-6-phosphate dehydrogenase and components of the glucose-6-phosphate reagent were purchased from Sigma Chemical Co. (St. Louis, MO). For the determination of free glucose, glucose-6-phosphate was subtracted from the amount of glucose measured in the non-AMG treated supernatant. Glycogen was calculated by subtracting the amount of free glucose and glucose-6-phospate from the total amount of glucose equivalents (AMG treated sample). Glycolytic potential was calculated by the following formula: 2([glycogen] + [glucose] + [glucose-6-phosphate]) + [lactate] and is reported as μmoles of lactate equivalents/g tissue (Monin & Sellier, 1985).

Determination of Glycolytic Enzyme Activity

Separate 1g LM samples collected and frozen at 20 min postmortem were used to quantify the in vitro activity of PFK and PK as described by Allison, Bates, Booren, Johnson and Doumit (2002).

Myosin Heavy Chain Isoform Analysis

For myosin heavy chain analysis, the washed pellet (myofibrillar component) remaining from pyruvate kinase extraction was suspended in 10 mL of extraction buffer (75 mM KCl, 10 mM KH_2PO_4, 2 mM $MgCl_2$, 2 mM EGTA, pH 7.0). A 0.75 mL aliquot was diluted with 0.75 mL of 2X sample loading buffer (125 m*M* Tris, 4% SDS, 20% glycerol, pH 6.8), heated at 50°C for 20 min and mixed by inversion every 5 min. Samples were then heated to 95°C for 3 min and centrifuged at 14,000 x *g* at 22°C for 20 min to remove insoluble material. Samples were stored at –20°C until analyzed. Protein concentrations were determined by using the BCA™ (bicinchoninic acid) Protein Assay (Kit No. 23225; Pierce, Rockford, IL).

Myosin heavy chain isoform distribution (IIA, IIX/IIB, and I) was determined by SDS-PAGE using 8% polyacrylamide (50:1 acrylamide: bisacrylamide) resolving gels with 4% polyacrylamide stacking gels (Mini-Protean II PAGE system; BIORAD) as described by Talmadge and Roy (1993). Samples were diluted with 2X sample loading buffer containing 10% mercaptoethanol and 0.75 µg protein in 10 µL per lane and were electrophoretically separated at 150 V for 3 h, then 70 V for 21 h at 4°C. Upper and lower buffers were changed after 3 h. Proteins were stained with coomassie blue to allow visualization. Gel images were acquired using a BIO-RAD gel documentation system and protein bands were quantified using Quantity One 4.1.0 software (BIO-RAD).

Total Heme Pigment Quantification

Total heme pigments were quantified as described by Warriss (1979), with slight modifications. Two g of LM were collected 24 h postmortem and stored at –80°C. Samples were homogenized in 10 mL of 0.04 M phosphate buffer (pH 6.8) and centrifuged at 6500 x *g* for 10 min at 4°C. A 5 mL aliquot of the supernatant was mixed with 0.5 mL of 6.6 mM potassium ferricyanide and 8.8 mM sodium cyanide and held at 4°C for 1 h. Samples were centrifuged at 30,000 *g* for 1 h at 4°C, and absorbance of the supernatant was measured at 540 nm. Total heme pigment concentration expressed as mg of total pigment/g of tissue was calculated as follows:

A_{540}/11,300 $L*mol^{-1}*cm^{-1}$ [(17,500g/mol*0.012 L*1000 mg/g)/2 g]

11,300 is the molar extinction coefficient for cyanmetmyoglobin (Drabkin, 1950)
17,500 g/mol is the molecular weight of myoglobin (Low & Rich, 1973)
0.012 L is the equivalent volume of diluted aqueous sample
2 g is weight of sample used for extraction.

Statistical Analysis

Statistical analysis was conducted using the PROC MIXED procedure of SAS (1998). For carcass and all meat quality traits except Warner-Bratzler shear, gender, breed group and harvest day were included in the model statement. Interactions for gender by breed group, breed group by harvest day, and gender by harvest day were also evaluated. Breed group least squares means and standard errors of the means are reported for carcass and meat quality traits. For analysis of Warner-Bratzler shear data, the PROC MIXED procedure of SAS (1998) was used with breed group, day postmortem, location within loin, gender and animal included in the model.

Partial correlation coefficients were determined using the PROC GLM procedure of SAS (1998). Overall partial correlation coefficients were determined for the

relationships between biochemical and fresh pork quality traits for all 32 pigs and the model statement included breed group, harvest day, gender and all possible two-way interactions. Harvest days were analyzed separately in an attempt to determine the cause of the harvest day by breed interactions. Partial correlation coefficients were also determined for the relationship between biochemical and fresh pork quality traits within a given harvest date. When harvest days were analyzed separately, breed group and gender were included in the model statement.

RESULTS AND DISCUSSION

Yorkshire progeny have been reported to be leaner and heavier muscled than B progeny (NPPC, 1995). In the current study, live and hot carcass weights did not differ between breeds, but Y carcasses had less tenth rib and average backfat than B carcasses (Table 1.1; $P<.01$). Yorkshire carcasses produced heavier trimmed wholesale loins and hams, and more kg of fat-free lean ($P<.05$). Berkshire progeny produced heavier trimmed belly weights than Y (Table 1.2; $P<.01$) and this is presumably due to the significantly higher fat content of B. I conclude that Y pigs were leaner and heavier muscled than B pigs used in this study.

Harvest day by breed interactions existed for carcass length, loin muscle area (LMA) and percent fat-free lean (Table 1.1; $P<.05$). The harvest day by breed interaction for carcass length is due to Y pigs having shorter carcasses on the second harvest day compared to the first, whereas B pigs had similar carcass lengths on both days. The harvest day by breed interactions for LMA and percent fat-free lean (%FFL) are attributed to lighter weight Y pigs having larger LMA and higher %FFL on the second harvest day compared to the first, while lighter weight B pigs had similar LMA and

%FFL on both days. Hot carcass weight, tenth rib backfat and loin muscle area are factors used to calculate percent fat-free lean. Therefore, the harvest day by breed interaction for LMA is most likely the cause of the harvest day by breed interaction for %FFL.

Berkshire and Yorkshire sire lines were selected to produce two populations that exhibit distinct differences in fresh pork quality traits. Berkshire-sired pigs tended to produce carcasses with higher 20 and 45 min LM pH ($P<.1$), while having significantly higher 180 min and 24 h postmortem pH (Table 1.3; $p<.05$). The observed differences in ultimate pH, although statistically significant, are small and seem unlikely to account for practical differences in pork quality. Harvest day by breed interactions existed for 20 min and 24 h temperature ($P<.05$). Carcasses of Y pigs exhibited higher LM temperatures at 20 min postmortem than carcasses of Berkshire-sired pigs on the second harvest day, resulting in a harvest day by sire-breed interaction (Table 1.3). Meanwhile, 24 h LM temperature was higher in B pigs and lower in Y pigs on the second harvest day.

The rate and extent of pH decline and early postmortem temperature are known to affect fresh pork color and WHC (Briskey, 1963). In the present study, loins from B pigs had higher pre-rigor and higher ultimate pH than Y. This was associated with more desirable color and WHC in B LM (Tables 1.4 and 1.5, respectively). Loin chops from Berkshire-sired pigs were darker in color based on both subjective and objective (CIE L* values) measures, and had higher subjective marbling scores (Table 1.4; $P<.05$). Additionally, LM from Berkshire-sired pigs had significantly less fluid loss from vacuum packaged loin sections stored at 4°C for 7 d ($P<.01$) and tended to have less fluid loss when subjected to high-speed centrifugation ($P<.09$). These findings are consistent with

data collected from the National Genetic Evaluation and National Barrow Show Progeny Tests that has established that Berkshire progeny have superior color and WHC traits (NPPC, 1995; Goodwin, 2000).

Harvest day by breed interaction existed for drip loss and exudate measured by the filter paper method (Table 1.5). These interactions are caused by a disproportionate increase in fluid loss from LM of Y carcasses on the second harvest day. It seems likely that this increased fluid loss is associated with the elevated LM muscle temperature of Y pigs on harvest day 2 combined with the lower pre-rigor pH observed in Y LM. The reason for the elevated postmortem muscle temperature of Y pigs on day 2 is unclear, as all pigs were handled in a similar fashion on both harvest days.

There was no difference between breed groups for d 7 tenderness determined by Warner-Bratzler shear force (Table 1.5). Breed comparisons have previously reported that Berkshire progeny were significantly more tender than Yorkshire progeny. However, these studies used Instron and a trained sensory panel to determine tenderness (NPPC, 1995; Goodwin, 2000). Additionally, B pigs tended to have more dry matter ($P<.09$) than Y pigs. Although there was no difference in the percentages of LM fat or protein (Table 1.6), these values are similar to those previously reported for Berkshire and Yorkshire progeny (NPPC, 1995; Goodwin, 2000).

Kastenschmidt et al. (1968) reported that rapid postmortem pH declines were caused by high levels of glucose-6-phosphate and the coordinated stimulation of phosphorylase, phosphofructokinase (PFK) and pyruvate kinase (PK). Schwagele et al. (1996) examined pyruvate kinase activity in pigs that were sensitive to halothane gas and

control pigs at pH 7.0 and pH 5.5 and concluded that halothane sensitive pigs had higher PK activity at lower pH's due to enzyme phosphorylation.

In the current study, PFK activities ranged from approximately 81 to 159 μmoles/min/g tissue (Table 1.7) and are similar to values reported previously (Rosochacki, Konecka, Piekarzewska, Poloszynowicz & Klewiec, 2000; Rosenvold, Petersen, Laerke, Jensen, Therkildsen, Karlsson, Moller, & Andersen, 2001; Allison et al., 2002) and are approximately 8 fold higher than values reported by Ahn, Patience, Fortin and McCurdy (1992). Pyruvate kinase activities for B and Y pig LM ranged from approximately 250 to 570 μmoles/min/g tissue (Table 1.7) and are similar to values reported for PK by Ahn et al. (1992), Allison et al. (2002) and Schwagele et al. (1996) for control pigs.

The in vitro activity of PFK and PK did not differ between B and Y pigs (Table 1.7). Sayre, Briskey and Hoekstra (1963b & 1963c) examined the effects of breed, diet and antemortem heat treatments on fresh meat quality traits and PFK activity. Breed differences were reported for PFK as Hampshire pigs had 30% higher PFK specific activity than Chester White and Poland China pigs. However, PFK specific activity was not affected by 1 h heat treatment prior to harvest, feeding high sucrose diet or by feeding a high fat and high protein diet. Additionally, recent work has reported no difference in PFK or PK activity between Duroc and halothane sensitive Pietrain (Rosochacki et al., 2000) or between HAL-1843™ normal Duroc and Pietrain pigs (Allison et al., 2002). Furthermore, Ahn et al. (1992) supplemented drinking water of Yorkshire-Landrace crossbred barrows with sodium bicarbonate, low levels of ammonium chloride and high levels of ammonium chloride and found that PFK activity was not affected by sodium

bicarbonate or ammonium chloride treatments. Rosenvold et al. (2001) reported that PFK activity was unaffected by low carbohydrate diets fed the last 3 weeks prior to harvest even though muscle glycogen was decreased. However, Ahn et al. (1992) demonstrated that PK activity was significantly decreased by sodium bicarbonate supplementation. Therefore, these authors concluded that PK activity could be affected by supplementation.

Based on the findings of Schwagele et al. (1996), I hypothesized that increased PFK and PK activity would be associated with increased rates of postmortem glycolysis. Contrary to my hypothesis, Sayre et al. (1963b & 1963c) reported that PFK activity was not associated with the rate of pH decline or meat quality traits. However, more recent studies have reported that PFK activity is correlated with pH or WHC. Ahn et al. (1992) observed a pooled positive relationship between PFK activity and carcass pH both measured at 0.75, 2, 4 and 24 h postmortem ($r = .487$; $P<.01$) but no relationships between PFK activity and color or WHC. Allison et al. (2002) reported that PFK activity was not associated with the rate of pH decline but instead was negatively correlated to centrifuge WHC and d 7 purge. They hypothesized that PFK became denatured and inactivated due to acidic conditions early postmortem. In the current study, PFK activity tended to be positively correlated with 20 min LM pH ($r = 0.38$; $P<.06$) and was correlated with the quantity of glycogen ($r = 0.46$; $P<.02$) but was not correlated with other pork quality or biochemical parameters. The positive relationship between PFK activity and 20 min pH was unexpected, but suggests that the PFK enzyme is more active in samples with a higher pH. This provides further evidence that PFK may be denatured early postmortem under acidic conditions. The positive relationship between PFK and

glycogen is not fully understood. Sayre et al. (1963a) reported that Hampshire pigs had 2 to 3-fold more glycogen and 30% more specific PFK activity than Chester White and Poland China pigs. Therefore, it appears that a positive relationship may have existed between glycogen and PFK activity in their experiment, even though correlations were not determined. Additionally, Ahn et al. (1992) reported that PFK was positively related to muscle glycogen ($r = .305$; $P<.01$). This relationship is likely due to increases in PFK abundance when substrate (glycogen) quantity is high. I suspect that increases in PFK activity when the quantity of glycogen is high would facilitate greater glycogen metabolism. On the other hand, in our study the activity of PK was not associated with the rate of pH decline, color or WHC. This is consistent with previous studies (Ahn et al., 1992; Allison et al., 2002).

Glycogen, free glucose, glucose-6-phosphate (G6P), lactate and glycolytic potential (GP) were quantified to determine their relationships to pork quality traits (Table 1.7). These values are similar to values previously reported for LM from pigs free of the RN^- mutation (Monin & Sellier, 1985; Wittmann, Ecolan, Levasseur & Fernandez, 1994; Fernandez, Lefaucheur & Candek, 1995; Kastenschmidt et al., 1968; Ciobanu, Bastiaansen, Malek, Helm, Woollard, Plastow & Rothschild, 2001; Lebret, Le Roy, Monin, Lefaucheur, Caritez, Talmant, Elsen & Sellier, 1999). Berkshire-sired pigs tended to have less LM glycogen at 20 min postmortem and a lower GP than Y pigs (Table 1.7; $P<.09$ and $P<.06$, respectively). No breed group differences were observed for G6P or lactate content, but a harvest date by treatment interaction existed for free glucose ($P<.05$). This interaction is attributed to B pigs having approximately two-fold

more free glucose on harvest day 1 compared to day 2, while Y had more free glucose on the second harvest date than the first.

Partial correlations were determined between glycolytic intermediates and pork quality parameters (Table 1.8). Glycogen and free glucose were not related to postmortem muscle pH, color or measures of fluid loss (P>.1). Wittmann et al. (1994) reported that glycogen was negatively correlated with ultimate pH but there was no relationship between free glucose and ultimate pH. In this study, glucose-6-phosphate was negatively correlated with 20, 45 and 180 min pH (P<.05), and was positively correlated to fluid loss measured by d 1 drip loss, centrifuge WHC and the filter paper methods (P<.01). These relationships are in agreement with the work of Charpentier (1968) and Kastenschmidt et al. (1968), who reported that pigs with rapid pH declines had higher quantities of G6P. Monin, Sellier, Ollivier, Goutefongea and Girard (1981) reported that halothane sensitive pigs had significantly higher G6P and lactate and lower ATP at 1 h postmortem than normal pigs. Additionally, Ahn et al. (1992) observed that pigs supplemented with sodium bicarbonate had the highest 45 min pH, while having the least G6P.

In my study, lactate was negatively correlated to 20 and 45 min pH (P<.05; Table 1.8) and GP was negatively correlated with 45 min pH (P<.01). However, neither of these traits were correlated to ultimate pH (P>.1). This was unexpected since van Laack and Kauffman (1999) demonstrated that lactate and GP are negatively correlated to ultimate pH. However, I measured lactate and GP on samples that were collected 20 min postmortem, whereas van Laack and Kauffman (1999) collected samples on d1 postmortem. Wittmann et al. (1994) also observed a weak relationship between GP and

ultimate pH. They stated this was presumably due to a lack of variation in GP. In the present study, differences in glycolytic potential are attributed to greater glycogen stores in muscle of Y pigs, and appear to explain the slightly lower ultimate pH in pigs. However, it is doubtful that the statistical difference in 24 h pH in the current study was important in determining fresh pork color and WHC. The relationship between GP and ultimate pH was weak as there was a lack of variation in glycolytic potential and ultimate pH. I suspect that the 12 h feed withdrawal prior to harvest reduced pig to pig variation in muscle glycogen stores. Additionally, none of our GP values were above 150 μmoles of lactate equivalents/g tissue, and thus it appears that all pigs were free of the RN^- mutation. Lactate was positively correlated to d 1 drip loss and fluid loss measured by the filter paper ($P<.05$), but was not correlated to centrifuge WHC or d 1 CIE L* values (Table 1.8). Because lactate was quantified on LM samples collected 20 min postmortem, samples with high lactate values most likely had a more rapid pH decline. Additionally, samples that had high lactate contents were more likely to experience protein denaturation and subsequently fluid loss. These inconsistent relationships between lactate the different methods for WHC methods may reflect differences in the fraction from which water is released under centripetal force versus gravity. Glycolytic potential was positively correlated to all measures of d1 fluid loss (Table 1.8; $P<.05$) and tended to be positively correlated to d1 CIE L* values ($P<.1$). Lactate and GP have previously been shown to have a positive relationship with L* values and drip loss (van Laack & Kauffman, 1999).

Myosin heavy chain isoforms (MHC) are indicators of muscle fiber type as they are associated with myosin ATPase staining and contraction speed (reviewed by

Schiaffino & Reggiani, 1994; Talmadge, 2000). The MHC distribution in this study was approximately 9% Type I, 34% Type IIA and 56% Type IIX/B (Table 1.9). These values reflect a lower proportion of Type IIA and a higher proportion of Type IIX/B than those previously reported for porcine LM (Bee et al., 1999). The distribution of MHC did not differ between breed groups (Table 1.9). This is in agreement with Essen-Gustavsson and Fjelkner-Modig (1985) who found no difference between breeds in histochemical muscle fiber types for Hampshire, Landrace and Yorkshire pigs. Ruusunen and Puolanne (1997) observed that Hampshire pigs had a higher proportion of Type I and a lower proportion of Type IIB muscle fibers than Landrace and Yorkshire pigs. However, they concluded that the variation within breeds for fiber type was larger than the variation across breeds and speculated that pigs with the same muscle fiber type profiles could be found in all breeds.

Partial correlation coefficients were determined for MHC distribution and pork quality measures (Table 1.10). Overall (n = 32) MHC Type IIX/B tended to be negatively correlated with 20 min pH ($r = -.37$; $P<.07$) and positively correlated with d 1 drip loss ($r = 0.34$; $P<.09$). Larzul et al. (1997) observed that muscle fiber Type IIB had significant negative genetic correlations to 30 min and 24 h pH.

Although significant differences between genetic lines have been reported for myoglobin (Allen et al., 1966) and total heme pigment (Monin & Sellier, 1985; Warriss et al., 1990a; Lindahl et al., 2001), total heme pigment concentration did not differ between sire-breeds (Table 1.9). The average value of 0.87 mg/g in the current study (Table 1.9) is somewhat lower than heme pigment concentrations reported for Iberian pigs reared outside (Mayoral, Dorado, Guillen, Robina, Vivo, Vazquez & Ruiz, 1999)

and for pigs in studies reported in the late 1970s (Warriss, 1979). However, our values are in the expected range of published values in recent studies using various methods (van Laack, Solomon, Warner & Kauffman, 1996; Warriss et al., 1990a; Warriss, Brown, Rolph and Kestin; 1990b).

The lack of a relationship between heme pigment and CIE L* was unexpected since previous studies have demonstrated a strong relationship between these two measures of fresh pork color (Warriss et al., 1990a; Lindahl et al., 2001). However, heme pigment was correlated to CIE a* (redness; r = .61) and a similar relationship has been previously reported by Lindahl et al., (2001). It was interesting to note that total heme pigment concentration was related to MHC Type IIA (r = .43; P<.03) and Type IIX/B (r = -.55; P<.01) while tending to be related to Type I (r = .34; P<.10). One would expect the MHC IIX/B to be negatively correlated to pigment since this MHC is associated with white, fast-twitch and glycolytic muscle fibers. Additionally, one would expect MHC Types I and IIA to have positive relationships to heme pigment because these isoforms are associated with red muscle fibers, which are high in myoglobin. The weaker correlation between MHC Type I than MHC Type IIA to heme pigment is most likely due to the lack of variation in MHC Type I observed in the present study between B and Y pigs.

Although poorly documented, the effects of harvest day on pork quality are well known. Kempster, Evans and Chadwick (1984) collected data from 15,487 pigs from 20 different pig populations over an eight-year time span and demonstrated that slaughter day had a significant effect on the occurrence of carcasses classified as being pale and watery. Additionally, these authors concluded that harvest day effects had more

influence on pork quality traits than genetics, gender, diet or slaughter weight. Furthermore, Warriss, et al. (1990a) reached a similar conclusion that harvest day effects had more impact on pork quality than source, genetics, gender or diet.

Several studies have reported that slaughter day significantly affects early postmortem pH and/or ultimate pH (Kempster et al., 1984; Monin & Sellier, 1985; Warriss et al., 1990a; Fernandez, Lefaucheur, Gueblez & Monin, 1991). Warriss et al. (1990a) reported significant harvest day effects for L*, a*, b*, hue, saturation and total soluble protein. Furthermore, slaughter day effects have also been reported to have significant effects on technological yield (Monin & Sellier, 1985; Fernandez et al., 1991). Slaughter day affects on ultimate pH have been attributed to effects on antemortem glycogen (Monin & Sellier, 1985; Fernandez, Lefaucheur, Gueblez and Monin, 1991). The degree of pre-slaughter stress, method of stunning, scalding time and temperature, carcass temperature and cooling regime could all be factors in determining the harvest day effects on early postmortem pH and temperature, and fresh pork color and WHC.

In the present study, harvest day had a major effect on WHC. More variation in pork quality was observed on harvest day 2 than day 1, which is difficult to explain. Pigs were handled similarly on both harvest days and transport of pigs for one hour and a half to a commercial abattoir during winter weather (January, 2001). Pigs were allowed to rest for a minimum of 12 h prior to harvest. Pigs were not mixed during transportation or during resting periods. Additionally, the interval from exsanguination to cooler was approximately 22 min for both harvest days. On the first day research pigs were harvested first and carcasses went into an empty cooler. Whereas, on the second harvest

day, pigs were harvested last and carcasses went into a full cooler. However, cooler temperature was similar on both harvest days.

Despite all of these similarities between harvest days, harvest day by breed group interactions existed for 20 min LM temperature and for fluid loss measured by the suspension drip and filter paper methods (Table 1.5). Yorkshire-sired pigs were on average 0.5°C warmer at 20 min postmortem on the second harvest day compared to the first, whereas B pigs were on average 0.4°C cooler at 20 min postmortem on the second harvest day compared to the first. It is interesting to note there was a trend for carcasses to be 2°C cooler by 180 min postmortem on the second harvest date compared to the first ($P<.08$). The lighter weight pigs were harvested on the second harvest day and most generally these pigs produced leaner and lighter weight carcasses that would be expected to chill more rapidly than the heavier pigs that were harvested on the first day. Despite the trend for a colder carcass temperature at 180 min postmortem on harvest date 2, Y pigs had approximately twice the fluid loss measured by the suspension drip and filter paper methods, whereas the WHC of B pigs was unaffected by harvest day. It appears that the higher fluid loss of Y pigs was due to protein denaturation from the combination of the 0.5°C higher temperature and 0.15 lower pH values at 20 minutes postmortem. Because carcasses entered the cooler on an average of approximately 22 minutes after exsanguination, the unfavorable conditions that caused protein denaturation were probably present prior to the carcasses entering the cooler.

To determine potential explanations for the fore mentioned effects of harvest day on fluid loss, partial correlation coefficients were calculated for MHC distribution and pork quality measures (Table 1.10). It is interesting to note that on harvest day 1, MHC

distribution was not related to the rate and extent of pH decline or measures of fluid loss. However on harvest day 2, MHC IIA and IIX/B were correlated to 20 min pH (r = .61 and -.64, respectively; P<.02), 24 h pH (r = .61 and -.57, respectively; P<.03), suspension drip loss (r = -.51 and .54, respectively; P<.06) and centrifuge WHC (r = -.51 and . 55 respectively; P<.08). Thus, it seems that relationships between MHC distribution and muscle pH or WHC may only be manifested on harvest days when antemortem or postmortem conditions are less favorable for optimum meat quality.

The relationships between G6P and pH decline or WHC within a given harvest day are shown in Table 1.11. Glucose-6-phosphate was not correlated with pH or WHC on harvest day 1. However on harvest day 2, G6P was negatively correlated with 20, 45 and 180 min pH (P<.03), and was positively correlated with fluid loss measured by suspension drip, high-speed centrifugation and the filter paper methods (P<.01).

It is possible that breed interactions influenced the stress management by the animal prior to harvest. If pigs became "excited" during movement, epinephrine would be released, which binds to the β-adrenergic receptor and leads to the activation of adnenylate cyclase, which increases intracellular cyclic AMP. Cyclic AMP in turn signals a cascade of events that results in the activation of glycogen phosphorylase (Voet, Voet & Pratt, 1999). Therefore, antemortem stress could increase the activity of phosphorylase and lead to an accumulation of glucose-6-phosphate, which has been shown to be associated with a rapid rate of pH decline (Kastenschmidt et al., 1968). No measures of stress were made, but Y pigs may have responded more negatively to transportation, handling and movement prior to harvest on the second day.

When Warriss and Lister (1982) prevented epinephrine from binding to the β-adrenergic receptor by administering carazolol, a β-adrenergic blocker, to Large White and stress-susceptible Pietrain pigs approximately 5.5 h prior to slaughter, 15 min postmortem temperature was decreased by 2°C and lactate decreased by 42.6%, while glycogen increased by 38.7% and 45 min pH increased by 0.7 pH units in LM of Pietrain carcasses ($P<.05$). As a result, none of the nine carazolol-treated Pietrain carcasses were classified as PSE, whereas seven of the nine control Pietrain pigs produced PSE carcasses. Therefore, epinephrine release due to preslaughter stress activates glycogen phosphorylase, which in turn accelerates the rate of postmortem glycolysis and adversely affects color and WHC.

CONCLUSIONS

Yorkshire-sired pigs were leaner, heavier muscled and produced more fat-free lean than Berkshire progeny. However, loin chops from B pigs had more desirable color and better WHC. Yorkshire-sired pigs and/or carcasses responded adversely to conditions on harvest day 2 that resulted in elevated LM temperature and increased fluid loss. Differences in meat quality characteristics between sire-breeds do not appear to be associated with an increase in glycolytic enzyme activity or the proportion of fast myosin isoforms. However, a higher proportion of MHC Type IIB/X and higher amounts of glucose-6-phosphate appear to be associated with accelerated pH decline under less favorable antemortem or postmortem conditions.

Table 1.1: Carcass Traits of Berkshire and Yorkshire Progeny*

Trait	Berkshire		Yorkshire		P-Value
	LS Means	SEM	LS Means	SEM	
Live Harvest Weight (kg)	113.4	1.62	116.5	1.39	NS
Hot Carcass Weight (kg)	86.1	1.27	86.7	1.10	NS
Carcass Length (cm)	81.9	0.72	83.5	0.62	HD X B (P<.05)**
Loin Muscle Area (cm^2)	37.6	1.03	43.2	0.88	HD X B (P<.05)**
Tenth Rib Backfat (mm)	23.3	1.00	18.0	0.90	(P<.001)
Last Rib Backfat (mm)	26.4	1.15	23.5	0.99	(P<.06)
Average Backfat (mm)	30.1	0.96	25.9	0.82	P<.01
USDA Grade	2.2	0.22	1.6	0.19	(P<.05)
Fat-Free Lean (kg)	42.5	0.74	46.0	0.64	(P<.001)
Fat-Free Lean (%)	49.7	0.49	53.5	0.43	HD X B (P<.05)**

*Means with different superscripts differ at the corresponding probability levels.

**HD X B indicates a harvest day by breed interaction.

Table 1.2: Trimmed Wholesale Cut Yields of Berkshire and Yorkshire Progeny*

Trait	Berkshire		Yorkshire		P-Value
	LS Means	SEM	LS Means	SEM	
Boston Butt (kg)	4.09	0.12	4.21	0.10	NS
Picnic Shoulder (kg)	3.53	0.08	3.70	0.07	NS
Belly (kg)	5.83	0.12	5.35	0.10	(P<.01)
Trimmed Loin (kg)	8.97	0.19	9.47	0.16	(P<.05)
Ham (kg)	9.43	0.16	10.2	0.16	(P<.01)

*Means with different superscripts differ at the corresponding probability levels.

Table 1.3: Postmortem Loin Muscle pH and Temperature*

Trait	Berkshire		Yorkshire		P-Value
	LS Means	SEM	LS Means	SEM	
20 min pH	6.50	0.06	6.35	0.05	(P<.08)
45 min pH	6.36	0.08	6.18	0.07	(P<.1)
180 min pH	6.04	0.09	5.78	0.08	(P<.05)
24 h pH	5.50	0.02	5.44	0.02	(P<.02)
20 min Temp (°C)	37.9	0.24	38.5	0.20	HD X B (P<.05)**
45 min Temp (°C)	35.9	0.36	36.6	0.31	NS
180 min Temp (°C)	24.7	0.56	24.5	0.48	NS
24 h Temp (°C)	4.47	0.08	4.40	0.07	HD X B (P<.05)**

*Means with different superscripts differ at the corresponding probability levels.

**HD X B indicates a harvest day by breed interaction.

Table 1.4: Subjective Scores and Objective Color of Fresh Pork Loin Chops*

Trait	Berkshire		Yorkshire		P-Value
	LS Means	SEM	LS Means	SEM	
Subjective Color[a] (1-6)	3.0	0.19	2.5	0.16	(P<.05)
Subjective Firmness[b] (1-5)	2.8	0.17	2.4	0.15	(P<.08)
Subjective Marbling[c] (1-10)	2.5	0.19	1.7	0.17	(P<.01)
CIE L* Day 1	50.5	0.72	53.1	0.62	(P<.01)
CIE a* Day 1	16.2	0.31	15.6	0.27	NS
CIE b* Day 1	10.1	0.29	11.1	0.25	(P<.02)

*Means with different superscripts differ at the corresponding probability levels.

[a]For subjective color scores, 1=pale pinkish gray to white; 6=dark purplish red.

[b]For subjective firmness scores, 1=soft; 5=very firm.

[c]For subjective marbling scores, 1=1% intramuscular fat; 10=10% intramuscular fat.

Table 1.5: *Longissimus* Muscle Water-Holding Capacity and Tenderness*

Trait	Berkshire		Yorkshire		P-Value
	LS Means	SEM	LS Means	SEM	
Centrifuge Water-Holding Capacity (%)	14.7	1.27	17.6	1.09	(P<.09)
Filter Paper (mg)	17.1	4.94	31.5	4.26	HD X B (P<.05)**
24 h Suspension Drip (%)	1.27	0.29	2.31	0.25	HD X B (P<.05)**
Day 7 Purge (%)	2.57	0.26	3.64	0.22	(P<.01)
Warner-Bratzler Shear Force (kg)	3.3	0.11	3.5	0.11	NS

*Means with different superscripts differ at the corresponding probability levels.

**HD X B indicates a harvest day by breed interaction.

Table 1.6: Proximate Composition of Fresh Loin Chops*

Trait	Berkshire		Yorkshire		P-Value
	LS Means	SEM	LS Means	SEM	
Dry Matter (%)	26.9	0.22	26.4	0.19	(P<.09)
Fat (%)	2.72	0.27	2.29	0.23	NS
Protein (%)	23.2	0.16	23.4	0.14	NS

*Means with different superscripts differ at the corresponding probability levels.

Table 1.7: Biochemical Characteristics of *Longissimus* Muscle at 20 min Postmortem*

Trait	Berkshire		Yorkshire		P-Value
	LS Means	SEM	LS Means	SEM	
Phosphofructokinase (μmol/min/g)	132.7	5.18	132.3	4.46	NS
Pyruvate Kinase (μmol/min/g)	418.7	27.7	441.4	23.9	NS
Glycolytic Potential (μmol lactate equivalents/g)	103.9	3.89	113.6	3.35	(P<.06)
Glycogen (μmol/g)	31.5	1.86	35.6	1.60	(P<.09)
Free Glucose (μmol/g)	1.16	0.19	1.56	0.16	HD X B (P<.05)**
Glucose-6-Phosphate (μmol/g)	4.37	0.92	3.34	0.79	NS
Lactate (μmol/g)	29.8	1.86	32.5	1.60	NS

*Means with different superscripts differ at the corresponding probability levels.

**HD X B indicates a harvest day by breed interaction.

Table 1.8: Partial Correlation Coefficients for Biochemical Measures and Pork Quality Traits

Trait	20 min pH	45 min pH	180 min pH	24 h pH	d1 CIE L*	Drip 1[a]	CWHC[b]	Filter Paper[c]
Glucose-6-Phosphate	-.61***	-.55***	-.42**	-.24	.17	.57***	.52***	.57***
Lactate	-.46**	-.44**	-.33*	.06	.29	.59***	.24	.56***
Glycolytic Potential	-.39*	-.53***	-.34*	-.33	.38*	.40**	.47**	.47**

*(P<.1)

**(P<.05)

***(P<.01)

[a]d 1 loin muscle drip loss determined by suspension drip method.

[b]d 1 loin muscle water-holding capacity determined by high-speed centrifugation.

[c]d 1 loin muscle fluid loss determined by filter paper method.

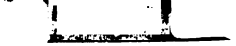

Table 1.9: Myosin Heavy Chain Isoforms and Heme Pigment Concentrations of *Longissimus* Muscle

Trait	Berkshire		Yorkshire		P-Value
	LS Means	SEM	LS Means	SEM	
Type I (% of Total MHC)	9.32	1.05	9.46	0.9	NS
Type IIA (%of Total MHC)	34.8	1.48	32.9	1.28	NS
Type IIB/X (% of Total MHC)	55.9	1.82	57.6	1.57	NS
Total Heme Pigment (mg/g)	0.89	0.03	0.85	0.03	NS

Table 1.10: Partial Correlation Coefficients for Myosin Heavy Chain Distribution and Pork Quality Traits

Trait	Myosin Heavy Chain Isofoms								
	Type I			Type IIA			Type IIX/B		
	HD#1[a]	HD#2[b]	Overall[c]	HD#1	HD#2	Overall	HD#1	HD#2	Overall
20 min pH	.30	.47*	.26	.04	.61**	.27	-.25	-.65**	-.37*
24 h pH	.16	.21	.17	-.20	.62**	.08	.03	-.58**	-.16
Drip 1[d]	-.02	-.42	-.12	-.04	-.51*	-.33*	.04	.55**	.34*
CWHC[e]	.09	-.28	.02	.07	-.49*	-.17	-.12	.48*	.13

*(P<.1)

**(P<.05)

[a]HD#1 is Harvest Day 1 (n = 16)

[b]HD#2 is Harvest Day 2 (n = 16)

[c]Overall is Harvest Days 1 and 2 (n = 32)

[d]d 1 loin muscle drip loss determined by suspension drip method.

[e]d 1 loin muscle water-holding capacity determined by high-speed centrifugation.

Table 1.11: Partial Correlation Coefficients for Glucose-6-phosphate and Fresh Pork Quality Measures

Trait	20 min pH	45 min pH	180 min pH	24 h pH	Drip 1[a]	CWHC[b]	Filter Paper[c]
Harvest Day #1	-.27	-.28	-.05	-.16	.18	.32	.06
Harvest Day #2	-.83**	-.68**	-.59*	-.41	.73**	.76**	.74**

*(P<.05)

**(P<.01)

[a]d 1 loin muscle drip loss determined by suspension drip method.

[b]d 1 loin muscle water-holding capacity determined by high-speed centrifugation.

[c]d 1 loin muscle fluid loss determined by filter paper method.

CHAPTER 2

THE USE OF PAYLEAN® TO INCREASE LEAN YIELD OF HIGH QUALITY PORK

ABSTRACT

Two experiments were conducted to determine the effects of dietary 10 ppm Paylean® (P) on growth, carcass, meat quality and biochemical traits of Berkshire- (B) and Yorkshire-(Y) sired progeny. In experiment 2, P tended to improve growth rate and feed conversion (P<.1), but not feed intake or live weight. In both experiments, P increased loin muscle area and ham weights (P<.06) but did not affect carcass weights or backfat thickness. In experiment 2, P was more effective at improving growth traits and carcass muscling in B than Y. In experiment 1, P did not affect fresh pork color or water-holding capacity (WHC) and did not affect *longissimus* glycolytic potential, glycolytic enzyme activity, proximate composition, Warner-Bratzler shear force or myosin heavy chain isoform distribution. However, P decreased heme pigment concentrations (P<.05). In experiment 2, P did not affect subjective color, firmness and marbling or WHC. Therefore, Paylean® is an effective management tool that improves the inferior growth rate and carcass muscling of Berkshire progeny and does not adversely affect their superior pork quality attributes.

Keywords: Paylean®, Ractopamine, Lean yield and Pork quality

INTRODUCTION

It is well established that Berkshire progeny have superior pork color and water-holding capacity (WHC). When compared to other genetic lines, Berkshire progeny have the highest ultimate pH, lowest CIE L* values, lowest drip and cooking losses (National Pork Producers Council, 1995; Goodwin, 2000). Due to their superior pork quality, Berkshire-sired pigs are in strong demand by Japanese export markets and they provide niche market opportunities for pork producers. However, breed comparisons have established that Berkshire progeny are generally slower growing, fatter and lighter muscled than other genetic lines (National Pork Producers Council, 1995; Goodwin, 2000; Ritter, Allison, Debar, Scheffler, Tempelman and Doumit, 2001). For these reasons, Berkshires have not been commercially accepted in the U.S. and thus their use in research has been limited.

Paylean® (ractopamine-hydrochloride (RAC); ELANCO Animal Health) is a β-adrenergic agonist that is approved by the U.S. Food and Drug Administration to be fed up to 20 ppm for the last 41 kg of live weight (68-109 kg) to improve growth and carcass traits. Ractopamine improves average daily gain and feed efficiency (Watkins, Jones, Mowrey, Anderson & Veenhuizen, 1990; Bark, Stahly, Cromwell & Miyat, 1992; He, Aherne, Thompson, Schaefer & Merrill, 1993). Ractopamine also increases loin muscle area and decreases tenth rib backfat (Yen, Mersmann, Hill & Pond, 1990; Bark et al., 1992; Crome, McKeith, Carr, Jones, Mowrey & Cannon, 1996). Furthermore, RAC increases trimmed wholesale Boston butt, picnic shoulder, loin and ham weights resulting in heavier hot carcass weights (Crome et al., 1996). Ractopamine does not adversely

affect fresh pork fresh pork quality traits, processing yields or sensory traits (reviewed by Merkel, 1988; McKeith & Ellis, 2001).

Therefore, Paylean® should be an ideal tool to improve the lean yield of Berkshire-sired pigs, while maintaining their superior pork quality. The objective of this study was to determine if supplementation of Paylean® at 10 ppm for the last four weeks of the finishing phase improves the economically important traits of growth, muscling and leanness, without compromising the superior pork quality of Berkshire-sired pigs.

MATERIALS AND METHODS

Two separate experiments (Experiment 1 and Experiment 2) were conducted to determine the effects of Paylean® on growth, carcass and meat quality traits. Experiment 1 served as pilot study that focused on the effects of Paylean® on carcass, meat quality and biochemical traits. Since pen numbers were limited, growth data were not statistically analyzed. Experiment 2 examined the effects of Paylean® on growth, carcass and meat quality traits. The Michigan State University All-University Committee on Animal Use and Care (AUCAUC) approved the use of animals and Paylean® for both of these experiments (AUF #11/00-152-00).

Experiment 1

Experimental Groups

Sires from three high meat quality-indexing families of the Berkshire breed identified at the National Barrow Show Progeny Tests and four Yorkshire lines selected for muscling were used to artificially inseminate F_1 Yorkshire-Landrace sows maintained at the Michigan State University (MSU) Swine Teaching and Research Farm. Progeny were raised under uniform conditions and when pigs weighed approximately 86 kg, three

groups of 16 pigs (8 barrows and 8 gilts per group) were blocked by gender, sire and live weight and assigned to the following groups: Berkshire-sired control (BC), Berkshire-sired and fed Paylean® (BP) and Yorkshire-sired control (YC). All pigs were fed a corn-soybean meal ration containing 16% crude protein (CP) and 0.9% lysine (Lys), without (BC, Y) or with (BP) 10 ppm of Paylean® for four weeks prior to harvest. To enable constant time on Paylean® and to equalize pig space per pen, 8 BP pigs were allotted to each of two pens and 8 additional weight-matched pigs were included in each pen. Therefore, four 4.87 m x 2.46 m pens with 16 pigs per pen were utilized in this experiment. Feed disappearance was recorded daily for each pen, and pigs were individually weighed at weekly intervals to determine average daily gain. Feed efficiency for each pen is expressed as gain (kg)/feed (kg).

Carcass, Meat Quality and Biochemical Traits Data Collection

Eight pigs from each treatment were harvested at a commercial abattoir on two harvest dates. Carcass, meat quality (pH, temperature, color, WHC and tenderness), proximate composition, myosin heavy chain isoforms, heme pigment, glycolytic metabolites and the activity of rate-limiting glycolytic enzymes were measured and quantified as described previously in Chapter 1.

Statistical Analysis

For experiment 1, statistical analysis was conducted by using the PROC MIXED procedure of SAS (1998). Gender, treatment group and harvest date along with all possible two-way interactions were included in the model statement for all carcass and meat quality traits except shear force. Interactions for gender by treatment group, treatment group by harvest date, and gender by harvest date were evaluated. Treatment

group least squares means and standard errors of the means were reported for carcass and meat quality traits. Harvest date by treatment group interactions existed ($P<0.05$). Data for these traits were plotted graphically and the interactions are described.

Samples used to quantify Warner-Bratzler shear data were also used in another study that randomly assigned four equal sized loin sections to various aging treatments to determine the effects of aging on the postmortem tenderization of pork (Allison, Tempelman & Doumit, 2001). Therefore, Warner-Bratzler shear data were analyzed using the PROC MIXED procedure of SAS (1998) with treatment group, day postmortem, location within loin, gender, and animal included in the model.

Experiment 2

Experimental Groups

Three high meat quality-indexing sires from the Berkshire breed identified at the National Barrow Show Progeny Tests and three Yorkshire boars were each artificially inseminated to F_1 Yorkshire-Landrace females maintained at the MSU Swine Teaching and Research Farm.

Pretreatment Period Live Animal Performance

When pigs weighed approximately 43 kg, 32 barrows were selected on the basis of weight and litter and moved to one of two rooms that each contained sixteen 2.15 by 2.15 m individual pens with partially slatted floors, stainless steel feeders and nipple drinkers. Litters were equally represented in each room and pigs were randomly assigned to pens within a room. During a two-week adjustment period, barrows were fed an 18% CP and 1.15% Lys corn-soybean meal diet that was supplemented with Tylan® for the first week, then pigs were fed a 16% CP and 0.9% Lys corn-soybean meal diet.

Throughout the entire trial, pigs had ad libitum access to feed and water, and feed disappearance was monitored daily. Tenth rib backfat and loin muscle area were measured by ultrasound and live weight was recorded every two weeks. Prior to Paylean® treatment, one pig was removed from the study due to an anatomical abnormality. Additionally, one barrow was removed due to lameness and consequently his littermate was removed. Also, one Berkshire-sired barrow was removed as an outlier (greater than 3 standard deviations away from the mean blocking weight) for live weight.

Treatment Period Live Animal Performance

At approximately 82 kg live weight, the remaining barrows (n = 28) were blocked by litter, live weight, ultrasound tenth rib backfat and loin muscle area and were assigned to one of four treatments in a 2 x 2 factorial design. The four treatments were (1) Berkshire-sired control; BC, (2) Berkshire-sired fed Paylean®; BP, Yorkshire-sired control; YC and Yorkshire-sired fed Paylean®; YP). Paylean® was fed at 10 ppm in a 16% CP and 0.9% Lys corn-soybean meal diet for the last four weeks of the finishing phase. Pens were reassigned to guarantee equal litter and treatment representation in each finishing room, but pigs were randomly penned within assigned rooms.

Loin Muscle Temperature and pH

All 28 barrows were harvested on one day at the same commercial abattoir used in experiment 1. Hot carcass weights were recorded prior to chilling. At 30, 60, 180 min and 24 h postmortem, *longissimus* muscle (LM) temperature and pH were measured adjacent to the last rib. Temperature was measured using a hand held thermocouple thermometer (Model no. 91100-50, Digi-Sense®, Vernon Hills, IL). A portable pH meter

equipped with a puncture-type pH electrode (Model 1140, Mettler-Toledo, Woburn, MA) was used to measure pH.

Carcass Characteristics

On day 1 postmortem, midline average backfat thickness was determined by measuring fat depth at the first rib, last rib and last lumbar vertebra. The right side of each carcass was ribbed between the 10^{th} and 11^{th} ribs. Loin muscle area (LMA) and 10^{th} rib fat thickness were determined according to current National Pork Producers Council (NPPC) guidelines (National Pork Producers Council, 2000). Additionally, percentage of fat-free lean, kg of fat-free lean and lean gain per day were calculated from equations developed by the NPPC (National Pork Producers Council, 2000). Carcass muscling scores were determined and U.S.D.A. Grades were calculated as follows: 4(Last rib backfat thickness) – carcass muscling score.

The right side of each carcass was fabricated into wholesale cuts, which were subsequently trimmed and weighed. A loin section from the 11^{th} rib to approximately midway between the last rib and the cranial edge of the ilium was removed from the right side of each carcass. These loin sections were transported to the MSU Meat Laboratory for analysis.

Fresh Pork Color and Water-Holding Capacity

Upon arrival at the MSU Meat Laboratory, loin sections were trimmed and bones were removed. A 1.27 cm-thick loin chop was removed from the loin section at approximately the 11^{th} rib. Then, a 2.54 cm thick loin chop was removed and duplicate 10 g samples free of subcutaneous fat were obtained to determine WHC by high-speed centrifugation (40,000 x **g** for 30 min; Honikel & Hamm, 1994). Immediately posterior

to this sample, two 2.54 cm thick loin chops were removed, allowed to bloom for approximately 15 min at 4°C and used to determine subjective color, firmness and marbling according to current NPPC guidelines (National Pork Producers Council, 2000). Subjective scores were assessed by an experienced evaluator, who was blinded to sample identity. These chops were also used for objective color (Commission International de l'Eclairage (CIE) L*, a* and b*; D_{65}, 2° standard observer and 50 mm-diameter measuring area) with a Minolta chromameter (CR-310 series; Ramsey, NJ) and drip loss by the suspension method (Honikel & Hamm, 1994). The remaining loin section was vacuum packaged at 24 h postmortem in Cryovac shrink bags using a MultiVac machine (Koch, Type AG 800) and held at 4°C until day 7 postmortem. The difference between d 7 and 24 h weight was divided by 24 h weight and expressed as percent purge.

Statistical Analysis

Statistical analyses were conducted using the PROC GLM procedure of SAS (2002). In this study, Paylean® treatment started at approximately 82 kg. However, pretreatment growth and ultrasound traits were evaluated. Since pigs were not treated with Paylean® during the pretreatment period, the four groups were evaluated independently. This was accomplished with breed group, room and breed group by room interactions included in the model statement. For all growth traits during the treatment period, breed, treatment, room and all possible two-way interactions were included into the model statement. Breed, treatment and breed by treatment interactions were included in the model statement for all ultrasound, carcass and meat quality traits evaluated during or after Paylean® treatment. Treatment group least squares means and standard errors of the means are reported for growth, carcass and meat quality traits.

RESULTS AND DISCUSSION

Paylean® was fed at 10 ppm with a 16% CP and 0.9% Lys corn-soybean meal diet for the last four weeks of the finishing phase. In experiment 1, average daily feed intake (ADFI), average daily gain (ADG), feed efficiency (FE) and lean gain per day (LG) were not statistically analyzed since pigs there were insufficient pen numbers. On the other hand, in experiment 2 pigs were individually penned and ADFI, ADG, and FE was measured four weeks prior to Paylean® treatment. Control and Paylean®–treated pigs had similar growth traits within breed at the beginning of the treatment period (Table 2.1).

In both experiments, ADFI was not affected by Paylean® treatment or by breed (Tables 2.2 & 2.3). My study like others demonstrated that RAC does not affect feed intake (Yen, Nienaber, Klindt & Crouse, 1991; Gu, Schinckel, Forrest, Kuei & Watkins, 1991a; Dunshea, King, Campbell, Sainz & Kim, 1993a; He et al., 1993). A few researchers have reported that RAC decreases feed intake (Yen et al., 1990; Crome et al., 1996).

It is well established that that RAC increases ADG (Watkins et al., 1990; Bark et al., 1992; Williams, Cline, Schinckel & Jones, 1994). In experiment 1, ADG of BP pigs was numerically similar to Y pigs and both of these groups had ADG that were approximately 20% greater than BC pigs (Table 2.2). In experiment 2, the ADG of BP, YC and YP were similar (Table 2.3), but Paylean®-treated (BP and YP) pigs tended to have higher ADG than control pigs (BC and YC; $P<.1$). It is unclear why ADG was not improved in YP pigs but other researchers have reported that RAC did not improve ADG (Aalhus, Jones, Schaefer, Tong, Robertson, Merrill & Murray, 1990; Yen et al., 1990; Gu et al. 1991a).

In experiment 1, apparent FE of BP and YC pigs were 18 and 27% higher than BC pigs, respectively (Table 2.2). Although a trend for a breed by room interaction existed for FE in experiment 2 (Table 2.3; P<.1), Paylean® (BP and YP) tended to improve FE (P<.09) when compared to control pigs (BC and YC). This is agreement with Watkins et al. (1990), Bark et al. (1992) and He et al. (1993) who reported that RAC improves FE, but is in contrast with reports by Dunshea, King, Campbell and Kim (1993b), Aalhus et al. (1990) and Mitchell, Solomon and Steele (1991).

Using the LG equation (National Pork Producers Council, 2000), Paylean® numerically increased LG of Berkshire progeny by 83% and 58% in experiments 1 and 2, respectively (Tables 2.2 & 2.3). It is interesting to note that Paylean® does not appear to improve LG of YP pigs when fed 10 ppm with a 16%CP and 0.9% Lys diet. Williams et al. (1994) reported that RAC improved lean gains of terminal crossbred barrows and gilts and this agrees with the LG of BP pigs but not YP pigs used in the current study.

In both experiments, Paylean® and breed did not influence live weight at harvest (Tables 2.2 & 2.3). No differences in live weights have been reported by Yen et al. (1991), Bark et al. (1992) and He et al. (1993) but Stites, McKeith, Singh, Bechtel, Mowrey and Jones (1991) and Crome et al. (1996) have reported that RAC increases live weight at harvest. In both experiments Paylean® treatment did not increase hot carcass weights (Tables 2.4 & 2.5). Watkins et al. (1990) also reported that RAC did not affect hot carcass weights, but my results conflict with those reported by Yen et al. (1990), He et al. (1993) and Crome et al. (1996).

Paylean® did not adversely affect carcass length in either experiment (Tables 2.4 & 2.5). However, in experiment 1, BP carcasses were shorter than Y carcasses (Table

2.4). In study 2 none of the four treatment groups differed from each other in carcass length (Table 2.5). The effects of RAC on carcass length have been inconsistent as Watkins et al. (1990), Yen et al. (1990) and Dunshea et al. (1993b) have reported decreases in carcass length while Stites et al. (1991), Yen et al. (1991), Dunshea et al. (1993a) have reported no change.

Although BP pigs appeared to become leaner along their loin edge in experiment 1, Paylean® did not reduce tenth rib, last rib or average backfat of BP (Table 2.4). To account for pig to pig variation in carcass composition and to observe the true efficacy of Paylean® to improve carcass traits, ultrasound backfat and LMA were recorded in experiment 2 during the pretreatment period and were used to block pigs into treatments. Prior to Paylean® treatment, ultrasound traits were not different within breeds (Table 2.6). During Paylean® treatment there were no difference between BC and BP or between YC and YP pigs for ultrasound tenth rib backfat or LMA (Table 2.6). Overall Paylean® (BP and YP) treatment did not reduce ultrasound backfat measures after 2 or 4 weeks of treatment but tended to increase ultrasound LMA after two weeks on treatment and continued to increase LMA after 4 weeks of treatment ($P<.1$ and $P<.06$, respectively) when compared to all of the control pigs (BC and YC). Ultrasound traits for Paylean®-fed pigs in the present study conflict with ultrasound traits reported by Williams et al. (1994). These authors demonstrated that RAC-fed pigs were significantly leaner at the tenth rib and had larger LMA after 23 kg of live weight gain on RAC-fed pigs while maintaining these significant advantages for an additional 14 kg of live weight gain. However, these authors fed higher concentrations of CP (16.3 to 21.9% vs. 16%), Lys

(1.00 to 1.33% vs. 0.9%) and RAC (44.7 mg/d vs. ~35.1 mg/d) for a longer duration (49 d vs. 28 d).

Paylean® did not reduce carcass backfat of Berkshire or Yorkshire progeny (Tables 2.4 & 2.5). These data agree with Cline and Forrest (1989), Mitchell et al. (1991), Stites et al. (1991) and Yen et al. (1991) who fed various concentrations of CP, Lys and RAC and concluded that RAC did not reduce carcass fat. However, Crome et al. (1996) reported that 10 and 20 ppm of RAC fed with a 16% CP diet decreased first rib, tenth rib and last lumbar vertebrae backfat but not reduce last rib backfat. Additionally, Bark et al. (1992) established that RAC reduced tenth rib and average backfat when 20 ppm of RAC was fed with a 17.7% CP diet. Other researchers have reported that RAC decreases tenth rib backfat but and does not affect first rib, last rib or average backfat thickness (Gu et al., 1991a; Dunshea et al., 1993a; Dunshea et al., 1993b).

Several studies have demonstrated that RAC increases LMA (Yen et al., 1990; Crome et al., 1996; Xiao, Xu & Chen, 1999). In both experiments, Paylean® increased LMA of BP, but did not increase LMA of YP in experiment 2 (Tables 2.4 & 2.5). It is unknown why LMA was not increased in YP pigs but Dunshea et al. (1993a) has reported that RAC does not increase LMA.

In both studies Paylean® improved or tended to improve kg of fat-free lean of BP pigs (Tables 2.4 & 2.5; $P<.05$ and $P<.07$, respectively). In experiment 1, BP and YC pigs had similar amounts of fat-free lean and both produced more kg of fat-free lean than BC ($P<.05$). Whereas in experiment 2, BP, YC and YP pigs had similar kg of fat-free lean and these groups tended to produce more kg of fat-free lean than BC pigs ($P<.07$). There was a harvest date by treatment group interaction for percentage of fat-free lean ($P<.05$).

This interaction is attributed to YC pigs generally having lighter weight carcasses with less fat and larger LMA on the second harvest day, whereas the percentage of fat-free lean was similar for BC and BP on both harvest days. Additionally, there were no differences in percentage of fat-free lean between breeds or treatments in experiment 2.

In experiment 1, Paylean® increased trimmed ham weights (Table 2.7; P<.05) but did not change trimmed weights of Boston butts, picnic shoulders, loins or bellies. While in experiment 2, BP carcasses had heavier trimmed loins than BC pigs (Table 2.8; P<.05), but YP pigs did not differ from YC pigs for trimmed wholesale cut weights. It is interesting to note that the overall effect of Paylean® (BP and YP) in experiment 2 was to increase trimmed loin weights (P<.02) while tending to improving the trimmed weights of picnic shoulders (P<.09) and hams (P<.06). The only consistencies between the two experiments is Paylean® increased or tended to increase ham weights, while having no effect on Boston butt or belly weights. My findings are consistent with previous reports that have demonstrated that RAC increases ham weights (Stites et al., 1991; Uttaro, Ball, Dick, Rae, Vessie & Jeremiah, 1993; & Crome et al, 1996) but does not affect Boston butt (Stites et al., 1991; Uttaro et al., 1993) or belly weights (Crome et al., 1996). However it should be noted that Yen et al. (1990) and Crome et al. (1996) reported RAC increased Boston butt weights while Uttaro et al. (1993) reported RAC increased belly weights. Additionally, in the present work RAC did not increase trimmed loin weights of BP in experiment 1 or YP pigs of experiment 2. This was unexpected since numerous studies have fed RAC and reported increases in trimmed loin weights (Yen et al., 1990; Stites et al., 1991; Uttaro et al., 1993; Crome et al., 1996). Furthermore, the efficacy of RAC to improve picnic shoulder weights is inconsistent between the two experiments and

in the literature. Yen et al., (1990) and Crome et al. (1996) have reported that RAC-treated pigs had heavier picnic shoulders, while Stites et al. (1991) and Uttaro et al. (1993) reported no change in picnic shoulder weights.

It appears in Experiment 2, that Paylean® was more effective at improving growth traits and carcass muscling of Berkshire progeny than Yorkshire progeny. It is well established that genetics, dietary CP and Lys also influence the ability of RAC to improve growth and carcass traits (Gu, Schinckel, Forrest, Kuei & Watkins, 1991b; Bark et al., 1992; Schinckel, Richert & Herr, 2001a; Schinckel, Herr, Kendall & Richert, 2002a). Ractopamine is more effective at improving growth and carcass traits in pigs that have high lean growth rates (Gu et al., 1991b; Bark et al., 1992). Yorkshire progeny traditionally have higher lean growth rates than Berkshire progeny (National Pork Producers Council, 1995). However, pigs with high lean growth rates require more dietary CP and Lys (Stahly, Cromwell & Terhune, 1988) and RAC-fed pigs require 0.26% more Lys than control pigs (Weldon & Armstrong, 2001). Additionally, Webster, Goodband, Tokach, Unruh, Nelssen, Dritz, Real, DeRouchey, Woodworth and Marsteller (2002) established that ADG and FE increase linearly in RAC-treated pigs as Lys is increased in the diet from 0.6% to 1.4%. They concluded that at least 1% Lys is necessary to maximize the response of RAC on growth and carcass traits with high lean pigs. Furthermore, if the need for higher dietary CP and Lys are not met in RAC-fed pigs, the response will be limited (Weldon & Armstrong, 2001; Schinckel et al., 2002a). Perhaps in my studies, the RAC response on growth and muscling traits was limited in Yorkshire progeny due to a limitation in dietary Lys, which was fed at 0.9% in the present study.

In Experiment 1, Paylean® did not adversely affect postmortem LM temperature or pH decline (Table 2.9). In fact, BP pigs had a lower 20 min temperature than BC pigs (P<.02) while tending to have a higher 180 min pH than BC pigs (Table 2.9; P<.07). This is in agreement with previous research that has concluded that RAC does not adversely affect the rate (Herr, Yake, Robson, Kendall, Schinckel & Richert, 2000; Herr, Hankins, Schinckel & Richert, 2001b) or extent of pH decline (Aalhus et al., 1990; Dunshea et al., 1993a; Herr et al., 2001b; McKeith & Ellis, 2001; Stoller, Zerby, Moeller, Baas, Johnson & Watkins, 2002). In experiment 2, BP tended to have a higher 30 min temperature than BC pigs (Table 2.10: P<.09) and there was a trend for a treatment group by breed interaction for 60 min pH (P<.06). This treatment group by breed interaction was unexpected and is attributed to Paylean® adversely affecting the rate of pH decline in BP pigs, but not the rate of pH decline of YP pigs. This conflicts with experiment 1, but Aalhus et al. (1990) also reported that feeding 10 ppm of RAC resulted in a significantly lower 40 min postmortem LM pH than control pigs. Although no other RAC experiments have observed a decrease in ultimate pH. BC pigs tended to have a higher ultimate pH than BP pigs in experiment 2 (P<.09).

Paylean® did not adversely affect NPPC subjective color, firmness or marbling in either experiment (Tables 2.11 & 2.12) and this is consistent with subjective color, firmness and marbling scores previously reported by Watkins et al. (1990), Crome et al. (1996), Herr et al. (2000) and McKeith & Ellis (2001). In experiment 1, Paylean® did not affect CIE L*,a*,b* values of BP pigs (Table 2.11) and this agrees with the findings of Dunshea et al. (1993a), Herr et al. (2000) and Stoller et al. (2002). However in experiment 2, a harvest date by treatment group interaction existed for CIE L* values

(Table 2.12; P<.05). This interaction is attributed to BP pigs having numerically higher CIE L* values than BC pigs, while YP pigs had lower numerical CIE L* values than YC pigs. To my knowledge no other RAC studies have reported adverse effects on CIE L* values. However, some studies have reported that RAC-fed pigs have significantly lower CIE a* and b* values (Aalhus et al., 1990; Uttaro et al., 1993; Ivers, Weldon, Muegge, Carr, England, McKeith, Anderson, 2000).

In both experiments, Paylean® did not affect fluid loss measures at d 1 (high-speed centrifugation & suspension drip) or d 7 purge (Tables 2.13 & 2.14). Several studies have established that RAC does not affect drip loss (Dunshea et al., 1993a; Uttaro et al., 1993; Xiao et al., 1999; Herr et al., 2000; McKeith & Ellis, 2001) or day 7 purge loss (Ivers et al., 2000; McKeith & Ellis, 2001; Herr et al., 2001b). However, inconsistent results for drip loss have also been reported (Aalhus et al., 1990; Herr et al., 2001b) for pigs fed RAC at various levels within a study.

My results are in agreement with others that Paylean® does not adversely affect loin chop tenderness determined by Warner-Bratzler shear force (Table 2.13; McKeith, Singh, Stites & Bechtel, 1988; Merkel, 1988; Dunshea et al., 1993a) and by Instron (Stoller et al., 2002). However, some studies have shown that loin chops from RAC-treated pigs are less tender measured by shear force (Aalhus et al., 1990; Uttaro et al., 1993; Ivers et al. 2000; Herr et al., 2001b) or by Kramer-Press-Ground-Analysis (Aalhus, Schaefer, Murray & Jones, 1992).

Paylean® had no effect on loin chop proximate composition (Table 2.15). This is in agreement with Adeola, Darko, He and Young (1990). However, RAC has been

reported to increase the percentage of protein and decrease the percentage of fat in LM (Uttaro et al., 1993; Xiao et al., 1999).

The BC and YC pigs from experiment 1 were also used in another experiment that was designed to biochemically explain why Berkshire progeny have superior pork quality traits (Chapter 1; Ritter et al., 2001). The objective of that experiment was determine if the superior color and WHC of Berkshire progeny is associated with glycogen stored in the muscle at death, the activity of rate-limiting glycolytic enzymes and/or a higher proportion of Type I myosin heavy chain isoforms (MHC). The relationships of glycolytic metabolites, glycolytic enzyme activity and MHC distribution with fresh pork quality traits are discussed in Chapter 1.

However, glycolytic metabolites and glycolytic enzyme activities are also of interest in muscle from Paylean®-treated pigs. β-adrenergic agonists bind to β-adrenergic receptors and this leads to the activation of adenylate cyclase and elevated levels of cyclic AMP. This in turn signals a cascade of events that leads to the activation of glycogen phosphorylase (Voet, Voet & Pratt, 1999). Warriss and Lister (1982) administered carazolol, a β-blocker, treatments one-half hour before transport and decreased 15 min LM temperature by 2°C and increased 45 min pH by 0.7 pH units in stress-susceptible Pietrain pigs. Treating pigs with β-adrenergic agonists could potentially increase the rate of glycogenolysis and accelerate the rate of pH decline. Therefore, glycolytic intermediates and rate-limiting glycolytic enzymes were examined in Paylean®-treated pigs.

Paylean® did not affect the quantities of loin muscle glycogen, glucose-6-phosphate, free glucose, lactate or glycolytic potential (Table 2.16). Likewise, the in

vitro activity of phosphofructokinase (PFK) and pyruvate kinase (PK) was not affected by Paylean® treatment (Table 2.16). My values for glycolytic metabolites are similar to those previously reported for LM from pigs free of the RN^- mutation (Monin & Sellier, 1985; Wittmann, Ecolan, Levasseur & Fernandez, 1994; Fernandez, Lefaucheur & Candek, 1995; Kastenschmidt et al., 1968; Ciobanu, Bastiaansen, Malek, Helm, Woollard, Plastow & Rothschild, 2001; Lebret, Le Roy, Monin, Lefaucheur, Caritez, Talmant, Elsen & Sellier, 1999). My PFK activity values are similar to values reported previously (Rosochacki, Konecka, Piekarzewska, Poloszynowicz & Klewiec, 2000; Rosenvold, Petersen, Laerke, Jensen, Therkildsen, Karlsson, Moller, & Andersen, 2001; Allison, Bates, Booren, Johnson & Doumit, 2002). Likewise my PK activity values are similar to those reported by Ahn et al. (1992) and Allison et al. (2002).

Since Aalhus et al. (1992) reported that RAC caused a shift in muscle fiber types, the relative proportion of muscle fiber types was determined by quantifying the distribution of MHC. Myosin heavy chain isoforms are associated with myosin ATPase staining and muscle contraction speed (reviewed by Schiaffino & Reggiani, 1994; Talmadge, 2000) and porcine Types I and IIB muscle fibers are correlated to MHC Types I and IIX/B, respectively (Bee, Solomon, Czerwinski, Long & Pursel, 1999). Paylean® treatment did not change the relative proportions of MHC in experiment 1 (Table 2.17). In contrast, Depreux, Grant, Anderson and Gerrard (2002) concluded that Paylean® affects MHC distribution when Paylean® is fed at 20 or 60 ppm with an 18.5% CP diet.

Decreases in heme pigment concentrations have been reported for LM from pigs fed salbutamol, a β-adrenergic agonist (Warriss, Brown, Rolph & Kestin, 1990b; Warriss, Kestin, Rolph & Brown, 1990c). Heme pigment concentrations are important for fresh

pork color since they are correlated to CIE L* (Warriss, Brown, Adams & Lowe, 1990a; Lindahl, Lundstrom & Tornberg, 2001) and a* values (Lindahl et al., 2001). However, to my knowledge heme pigment concentrations have not been examined in RAC-treated pigs. Loin muscle heme pigment concentrations in the current study are similar to those previously reported for porcine LM (Warriss et al., 1990a; Warriss et al., 1990b; van Laack, Solomon, Warner & Kauffman, 1996). In Experiment 1, heme pigment concentrations did not differ between breeds (BC and Y) but Paylean® significantly decreased heme pigment concentrations (Table 2.17; P<.05).

Paylean® increased LMA and carcass muscularity of BP pigs in both experiments. The increase in carcass muscling would be expected to be associated with a shift in muscle fiber type from Type IIA to Type IIB or an increased cross-sectional area of Type IIB (fast-glycolytic) muscle fibers (Aalhus et al., 1992). However, Type IIB muscle fibers are white, fast-twitch, glycolytic muscle fibers that have been shown to be negatively related to the rate and extent of pH decline and positively related to glycolytic potential and CIE L* values (Larzul, Lefaucheur, Ecolan, Gogue, Talmant, Sellier, Le Roy & Monin, 1997). In Experiment 2, BP pigs had a LM 60 min postmortem pH that was 0.15 pH units lower than BC pigs and BP pigs tended to have a lower ultimate pH than BC (Table 2.10; P<.09). Furthermore, BP pigs had higher CIE L* values than BC (Table 2.12). To complement this, BP had less heme pigment in Study 1 than BC pigs, but the MHC distribution did not differ (Table 2.17). The relative distribution of MHC is an indicator of the relative percentages of muscle fiber types (Bee et al., 1999). Beermann, Butler, Hogue, Fishell, Dalrymple, Ricks and Scanes (1987) established that cimaterol, a β-adrenergic agonist, increases muscle mass in sheep by increasing the cross-

sectional areas of Type I and Type II muscle fibers in LM. Therefore, Paylean® may increase carcass muscling and have minor effects on the rate and extent of pH decline, CIE L* values and heme pigment concentrations in BP due to increases in the cross-sectional area of Type IIB muscle fibers. However, the cross-sectional areas of Type IIB muscle fibers were not quantified in either experiment. Therefore, additional histochemical staining is necessary to determine the effects of Paylean® on the relative proportion of muscle fiber Types I, IIA and IIB and the effects of these fiber types on meat quality traits.

CONCLUSIONS

Collectively, these data suggest that Paylean® improves growth traits and carcass muscling of Berkshire progeny. It is interesting to note that Paylean® was more effective at improving growth traits and muscling of Berkshire progeny than Yorkshire progeny when Paylean® was fed at 10 ppm with a 16% CP diet for the last four weeks of the finishing phase. Additionally, BP pigs are similar to YC pigs in regards to ADG, FE, lean gain, LMA, ham weights and kg of fat-free lean. Therefore, Paylean® could be used as a management tool to improve the growth traits, lean yield and commercial acceptability of genetic lines that have superior pork quality traits. Additional research is necessary to quantify the relative percentage and cross-sectional areas of muscle fiber types I, IIA and IIB by histochemical staining and to determine the relationships between muscle fiber types and fresh pork quality traits in pigs fed Paylean®.

Table 2.1: Experiment 2 Performance Traits for Four Weeks Prior to Paylean® Treatment*

Trait	Berkshire		Yorkshire		SEM	P-Value
	Control	Paylean®	Control	Paylean®		
Initial Live Weight (kg)	55.5	57.5	54.4	55.9	1.41	NS
Final Live Weight (kg)	81.9	81.7	83.3	83.5	2.08	NS
ADFI[a] (kg/d)	3.13	3.08	3.20	3.30	0.09	NS
ADG[b] (kg/d)	0.94[ab]	0.87[a]	1.04[b]	0.98[ab]	0.06	(P<.07)
FE[c] (gain/feed)	0.14	0.13	0.15	0.13	0.01	NS

*Means with different superscripts differ at the corresponding probability levels.
[a]Average Daily Feed Intake.
[b]Average Daily Gain.
[c]Feed Efficiency.

Table 2.2: Experiment 1 Performance Traits With or Without Paylean®*

Trait	Berkshire Control		Berkshire Paylean®		Yorkshire		P-Value
	LS Means	SEM	LS Means	SEM	LS Means	SEM	
Initial Live Weight (kg)	91.7	1.64	90.7	1.47	89.3	1.49	NS
Live Weight at Harvest (kg)	114.3	1.90	117.8	1.71	116.8	1.73	NS
ADFI[a] (kg/d)	3.04	----	3.08	----	2.94	----	-------
ADG[b] (kg/d)	0.84	----	1.00	----	1.02	----	-------
FE[c] (gain/feed)	0.27	----	0.32	----	0.35	----	-------
Lean Gain (kg/d)	0.12	----	0.22	----	0.28	----	-------

*ADFI, ADG, FE and Lean Gain were not statistically analyzed since pen numbers were limited.
[a]Average Daily Feed Intake.
[b]Average Daily Gain.
[c]Feed Efficiency.

Table 2.3: Experiment 2 Performance Traits With or Without Paylean®*

Trait	Berkshire		Yorkshire		SEM	P-Value
	Control	Paylean®	Control	Paylean®		
Week 4 Live Weight (kg)	81.9	81.7	83.3	83.5	2.08	NS
Week 8 Live Weight (kg)	107.6	111.4	113.9	115.8	2.76	NS
ADFI[a] (kg/d)	3.49	3.46	3.60	3.63	0.13	NS
ADG[b] (kg/d)	0.96[a]	1.10[b]	1.14[b]	1.19[b]	0.05	P<.1
FE[c] (gain/feed)	0.13	0.14	0.14	0.15	0.01	B X R (P<.1)[d]
Lean Gain (kg/d)	0.24	0.38	0.38	0.42	0.04	B X R, T X R (P<.09)[d,e]

*Means with different superscripts differ at the corresponding probability levels.
[a]Average Daily Feed Intake.
[b]Average Daily Gain.
[c]Feed Efficiency.
[d]Breed by Room Interaction.
[e]Treatment by Room Interaction.

Table 2.4: Experiment 1 Carcass Traits With or Without Paylean®*

Trait	Berkshire Control		Berkshire Paylean®		Yorkshire		P-Value
	LS Means	SEM	LS Means	SEM	LS Means	SEM	
Hot Carcass Weight (kg)	86.8	1.49	90.0	1.34	87.0	1.36	NS
Carcass Length (cm)	82.4^{ab}	0.72	81.2^{a}	0.65	83.6^{b}	0.65	(P<.05)
Tenth Rib Backfat (mm)	23.0^{a}	1.07	23.7^{a}	0.96	17.9^{b}	0.97	(P<.001)
Last Rib Backfat (mm)	26.5^{a}	1.05	26.2^{a}	0.94	23.5^{b}	0.95	(P<.06)
Average Backfat (mm)	32.2^{a}	0.95	31.4^{a}	0.86	28.5^{b}	0.87	(P<.03)
USDA Grade	2.1^{a}	0.19	2.1^{a}	0.17	1.6^{b}	0.17	(P<.06)
Loin Muscle Area (cm^2)	38.3^{a}	1.32	43.4^{b}	1.19	43.4^{b}	1.21	(P<.01)
Fat-Free Lean (kg)	43.3^{a}	0.83	45.6^{b}	0.75	46.5^{b}	0.76	(P<.05)
Fat-Free Lean (%)	49.9	0.61	50.9	0.55	53.6	0.56	HDXT (P<.05)**

*Means with different superscripts differ at the corresponding probability levels.

**HD X T indicates a harvest day by treatment interaction.

Table 2.5: Experiment 2 Carcass Traits With or Without Paylean®*

Trait	Berkshire		Yorkshire		SEM	P-Value
	Control	Paylean®	Control	Paylean®		
Hot Carcass Weight (kg)	80.4[a]	84.2[ab]	85.9[b]	88.1[b]	2.13	(P<.08)
Carcass Length (cm)	79.7	79.6	81.2	80.8	0.97	NS
Tenth Rib Backfat (mm)	26.3	24.1	24.9	26.1	2.51	NS
Last Rib Backfat (mm)	33.2	33.0	33.0	32.3	1.81	NS
Average Backfat (mm)	34.0	33.2	32.3	32.8	1.76	NS
USDA Grade	3.2	3.2	3.1	2.9	0.27	NS
Loin Muscle Area (cm^2)	38.2[a]	44.8[b]	42.3[ab]	46.5[b]	2.29	(P<.05)
Fat-Free Lean (kg)	39.0[a]	43.1[b]	43.1[b]	44.5[b]	1.49	(P<.07)
Fat-Free Lean (%)	48.6	50.1	51.2	50.7	1.57	NS

*Means with different superscripts differ at the corresponding probability levels.

Table 2.6: Experiment 2 Ultrasound Traits Prior to and During the Treatment Period*

Trait	Berkshire		Yorkshire		SEM	P-Value
	Control	Paylean®	Control	Paylean®		
Week 0 BF10[a] (mm)	9.40[a]	8.08[ab]	6.11[c]	7.01[bc]	0.80	(P<.1)
Week 4 BF10[a] (mm)	17.0	17.2	15.7	16.9	1.63	NS
Week 8 BF10[a] (mm)	26.2	25.5	24.0	25.7	1.87	NS
Week 0 LMA[b] (cm^2)	26.7	28.6	26.9	28.3	0.91	NS
Week 4 LMA[b] (cm^2)	37.1	36.1	38.2	37.6	1.17	NS
Week 8 LMA[b] (cm^2)	39.7[a]	43.2[ab]	42.5[ab]	46.4[b]	1.84	(P<.02)

*Means with different superscripts differ at the corresponding probability levels.
[a]Tenth Rib Backfat Thickness.
[b]Loin Muscle Area.

Table 2.7: Experiment 1 Trimmed Wholesale Cut Weights With or Without Paylean®*

Trait	Berkshire Control		Berkshire Paylean®		Yorkshire		P-Value
	LS Means	SEM	LS Means	SEM	LS Means	SEM	
Boston Butt (kg)	4.15	0.11	4.26	0.10	4.22	0.10	NS
Picnic Shoulder (kg)	3.55	0.09	3.75	0.08	3.71	0.08	NS
Trimmed Loin (kg)	9.11	0.20	9.28	0.18	9.51	0.19	NS
Belly (kg)	5.90^{a}	0.16	5.96^{a}	0.14	5.37^{b}	0.15	(P<.05)
Ham (kg)	9.57^{a}	0.19	10.1^{b}	0.17	10.3^{b}	0.17	(P<.05)

*Means with different superscripts differ at the corresponding probability levels.

Table 2.8: Experiment 2 Trimmed Wholesale Cut Weights With or Without Paylean®*

Trait	Berkshire		Yorkshire		SEM	P-Value
	Control	Paylean®	Control	Paylean®		
Boston Butt (kg)	3.85^{a}	3.98^{ab}	4.08^{ab}	4.14^{b}	0.12	(P<.1)
Picnic Shoulder (kg)	3.21^{a}	3.37^{ab}	3.38^{ab}	3.63^{b}	0.11	(P<.02)
Loin (kg)	7.74^{a}	8.80^{b}	8.65^{b}	9.02^{b}	0.29	(P<.05)
Belly (kg)	4.65	4.88	5.05	4.95	0.24	NS
Ham (kg)	8.65^{a}	9.21^{ab}	9.60^{bc}	10.1^{c}	0.25	(P<.03)

*Means with different superscripts differ at the corresponding probability levels.

Table 2.9: Experiment 1 Postmortem Loin Muscle Temperature and pH*

Trait	Berkshire Control		Berkshire Paylean®		Yorkshire		P-Value
	LS Means	SEM	LS Means	SEM	LS Means	SEM	
20 min Temp (°C)	37.9[a]	0.22	37.1[b]	0.20	38.4[c]	0.20	(P<.06)
45 min Temp (°C)	35.9[ab]	0.36	35.4[a]	0.32	36.6[b]	0.33	(P<.05)
180 min Temp (°C)	24.7	0.47	25.2	0.42	24.5	0.43	NS
24 h Temp (°C)	4.49	0.07	4.36	0.07	4.41	0.07	HD X T (P<.1)
20 min pH	6.49[a]	0.06	6.50[a]	0.05	6.35[b]	0.05	(P<.07)
45 min pH	6.32[a]	0.07	6.46[a]	0.06	6.17[b]	0.06	(P<.09)
180 min pH	6.01[a]	0.08	6.20[b]	0.07	5.77[c]	0.07	(P<.07)
24 h pH	5.51	0.02	5.55	0.02	5.45	0.02	HD X T (P<.1)

*Means with different superscripts differ at the corresponding probability levels.

**HD X T indicates a harvest day by treatment interaction.

Table 2.10: Experiment 2 Postmortem Loin Muscle Temperature and pH*

Trait	Berkshire		Yorkshire		SEM	P-Value
	Control	Paylean®	Control	Paylean®		
30 min Temp (°C)	39.3[a]	39.9[b]	39.6[ab]	39.4[ab]	0.22	(P<.09)
60 min Temp (°C)	38.3	38.4	38.4	38.8	0.35	NS
180 min Temp (°C)	27.1	26.7	27.0	26.4	0.54	NS
24 h Temp (°C)	2.56	2.70	2.57	2.57	0.06	NS
30 min pH	6.28	6.30	6.21	6.32	0.07	NS
60 min pH	6.27	6.12	6.10	6.26	0.08	T x B (P<.06)**
180 min pH	5.84	5.69	5.65	5.78	0.10	NS
24 h pH	5.57[a]	5.47[b]	5.42[b]	5.42[b]	0.04	(P<.09)

*Means with different superscripts differ at the corresponding probability levels.

**T x B denotes a treatment by breed interaction.

Table 2.11: Experiment 1 Subjective Scores and Objective Color of Fresh Pork Loin Chops*

Trait	Berkshire Control		Berkshire Paylean®		Yorkshire		P-Value
	LS Means	SEM	LS Means	SEM	LS Means	SEM	
Subjective Color (1-6)[a]	3.0[a]	0.17	2.8[ab]	0.15	2.5[b]	0.15	(P<.05)
Subjective Firmness (1-5)[b]	2.9[a]	0.17	2.7[ab]	0.15	2.4[b]	0.15	(P<.05)
Subjective Marbling (1-10)[c]	2.5[a]	0.22	2.5[a]	0.20	1.7[b]	0.20	(P<.01)
CIE L* Day 1	50.7[a]	0.68	51.3[a]	0.61	53.1[b]	0.62	(P<.05)
CIE a* Day 1	16.1	0.27	15.8	0.24	15.5	0.24	NS
CIE b* Day 1	10.1	0.25	10.4	0.23	11.1	0.23	HD X T (P<.08)**

*Means with different superscripts differ at the corresponding probability levels.

**HD X T indicates a harvest date by treatment interaction.

[a]NPPC Subjective Color Scores, 1=pale pinkish gray to white; 6=dark purplish red.

[b]Subjective Firmness Scores, 1=soft; 5=very firm.

[c]NPPC Subjective Marbling Scores, 1=1% intramuscular fat; 10=10% intramuscular fat.

Table 2.12: Experiment 2 Subjective Scores and Objective Color of Fresh Pork Loin Chops*

Trait	Berkshire		Yorkshire		SEM	P-Value
	Control	Paylean®	Control	Paylean®		
Subjective Color (1-6)[a]	3.07	2.79	2.71	2.64	0.20	NS
Subjective Firmness (1-3)[b]	1.93	1.93	1.64	1.86	0.13	NS
Subjective Marbling (1-10)[c]	2.21	2.43	2.00	2.36	0.23	NS
CIE L* Day 1	52.46	53.89	54.78	53.04	0.77	T x B (P<.05)**
CIE a* Day 1	17.44	17.39	17.84	18.04	0.31	NS
CIE b* Day 1	6.07[a]	6.47[ab]	6.94[b]	6.75[b]	0.28	(P<.1)

*Means with different superscripts differ at the corresponding probability levels.

**T x B denotes a breed by treatment interaction.

[a]NPPC Subjective Color Scores, 1=pale pinkish gray to white; 6=dark purplish red.

[b]NPPC Subjective Firmness Scores, 1=soft; 2= firm; 3=very firm.

[c]NPPC Subjective Marbling Scores, 1=1% intramuscular fat; 10=10% intramuscular fat.

Table 2.13: Experiment 1 Loin Muscle Water-Holding Capacity (WHC) and Tenderness*

Trait	Berkshire Control		Berkshire Paylean®		Yorkshire		P-Value
	LS Means	SEM	LS Means	SEM	LS Means	SEM	
Centrifuge WHC (%)	14.9[a]	1.13	12.7[a]	1.02	17.7[b]	1.03	(P<.07)
Filter Paper (mg)	17.5	3.99	12.5	3.59	31.6	3.63	HD X T (P<.05)**
Day 1 Suspension Drip Loss (%)	1.20	0.25	1.03	0.22	2.28	0.23	HD X T (P<.05)**
Day 2 Suspension Drip Loss (%)	1.80	0.28	1.99	0.25	3.45	0.25	HD X T (P<.05)**
Day 7 Purge (%)	2.62[a]	0.24	2.87[a]	0.22	3.65[b]	0.22	(P<.05)
Day 7 Shear Force (kg)	3.3	0.11	3.5	0.11	3.6	0.11	NS

*Means with different superscripts differ at the corresponding probability levels.

**HD X T indicates a harvest day by treatment group interaction.

Table 2.14: Experiment 2 Loin Muscle Water-Holding Capacity (WHC)*

Trait	Berkshire		Yorkshire		SEM	P-Value*
	Control	Paylean®	Control	Paylean®		
Centrifuge WHC (%)	13.6[a]	15.2[ab]	18.3[b]	17.5[ab]	1.63	P<.06
Day 1 Suspension Drip Loss (%)	1.33[a]	1.64[a]	2.78[b]	2.05[ab]	0.36	P<.04
Day 2 Suspension Drip Loss (%)	2.43[a]	3.18[ab]	4.80[c]	3.82[bc]	0.53	P<.08
Day 7 Suspension Drip Loss (%)	5.62[a]	6.72[ab]	8.77[c]	7.95[bc]	0.79	P<.08
Day 7 Purge (%)	2.20[a]	2.99[ab]	3.45[b]	3.50[b]	0.38	P<.03

*Means with different superscripts differ at the corresponding probability levels.

Table 2.15: Experiment 1 Proximate Composition of Fresh Loin Chops

Trait	Berkshire Control		Berkshire Paylean®		Yorkshire		P-Value
	LS Means	SEM	LS Means	SEM	LS Means	SEM	
Dry Matter (%)	26.9	0.23	26.9	0.21	26.4	0.21	NS
Fat (%)	2.76	0.29	2.64	0.26	2.31	0.26	NS
Protein (%)	23.2	0.18	23.1	0.16	23.4	0.16	NS

Table 2.16: Experiment 1 Biochemical Characteristics of Loin Muscle at 20 min Postmortem*

Trait	Berkshire Control		Berkshire Paylean®		Yorkshire		P-Value
	LS Means	SEM	LS Means	SEM	LS Means	SEM	
Glycolytic Potential (μmol lactate equivalents/g)	104.0[a]	4.06	104.2[a]	3.65	113.6[b]	3.70	(P<.08)
Glycogen (μmol/g)	31.2[a]	1.95	33.4[ab]	1.75	35.5[b]	1.78	(P<0.1)
Glucose-6-Phosphate (μmol/g)	4.34	0.79	2.89	0.71	3.33	0.72	NS
Free Glucose (umol/g)	1.23	0.16	0.99	0.15	1.58	0.15	HD X T (P<.05)**
Lactate (μmol/g)	30.6	1.71	29.7	1.54	32.8	1.56	NS
Phosphofructokinase (μmol/min/g)	129.6	4.86	139.4	4.37	131.3	4.43	NS
Pyruvate Kinase (μmol/min/g)	421.9	25.5	423.7	22.9	442.4	23.2	NS

*Means with different superscripts differ at the corresponding probability levels.

**HD X T indicates a harvest day by treatment group interaction.

Table 2.17: Experiment 1 Myosin Heavy Chain Isoforms and Total Heme Pigment From Loin Muscle*

Trait	Berkshire Control		Berkshire Paylean®		Yorkshire		P-Value
	LS Means	SEM	LS Means	SEM	LS Means	SEM	
Type I (% of Total MHC)	9.36	0.85	10.4	0.77	9.48	0.78	NS
Type IIA (%of Total MHC)	35.6	1.27	35.6	1.14	33.2	1.16	NS
Type IIB/X (% of Total MHC)	55.0	1.58	54.0	1.42	57.3	1.44	NS
Total Heme Pigment (mg/g)	0.88[a]	0.03	0.77[b]	0.03	0.85[a]	0.03	(P<.07)

*Means with different superscripts differ at the corresponding probability levels.

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