RACTOPAMINE HYDROCHLORIDE AND THE ENVIRONMENTAL SUSTAINABILITY OF PORK PRODUCTION

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ABSTRACT

These experiments were conducted to determine if ractopamine hydrochloride (RAC) could improve nutrient utilization and decrease water use in hog operations. The growth experiment utilized a comparative slaughter technique that consisted of 120 barrows (95 ± 3 kg BW) including 12 assigned to an initial slaughter group; the remaining pigs were slaughtered at 108- or 120-kg. Growth performance and nutrient retention were determined. The 15 d metabolism experiment consisted of 54 pigs (95 ± 3 kg BW). Growth performance, feed, and water intake and urine and fecal output were measured. The metabolism experiment used 9 dietary treatments arranged as a 3 x 3 factorial: 3 levels of RAC (0, 5 and 10 ppm) and 3 standardized ileal digestible lysine: digestible energy (DE) ratios (1.75, 2.25 and 2.75 g/Mcal DE). The growth study was designed as a 3 x 3 x 2 factorial to include slaughter weight as an additional factor.

In the growth experiment, RAC had no effect ADG, ADFI, or G:F ($P > 0.10$). With increased Lys levels G:F improved ($P < 0.05$), but not ADG or ADFI ($P > 0.10$). Protein deposition rates numerically increased ($P = 0.11$), water deposition rates tended to increase ($P < 0.10$), whereas lipid deposition tended to decrease with RAC inclusion ($P < 0.10$). In the metabolism experiment, with greater levels of RAC and Lys the pigs had improved ADG ($P < 0.05$) and G:F ($P < 0.001$). Water intake ($P < 0.05$.) and urine output ($P < 0.05$) decreased with greater RAC inclusions. Lys inclusion did not alter water balance ($P > 0.10$). Urinary N excretion ($P < 0.05$), total N excretion ($P < 0.05$), and the urine N:fecal N ratio ($P < 0.001$) decreased with addition of dietary RAC; however fecal N ($P < 0.05$) increased with dietary RAC inclusion. Retention of N improved with addition of RAC to the diet ($P < 0.05$). With greater dietary Lys inclusion fecal N was reduced ($P < 0.001$). The pigs fed the 2.25 g/Mcal Lys tended to have the lowest urinary N and total N excretion and highest N retention ($P < 0.10$) and greatest urinary N:fecal N ratio ($P < 0.05$). A RAC x Lys interaction was observed for N digestibility, excretion, and retention and fecal and urinary N ($P < 0.05$). By improving N and water utilization in finishing pigs, RAC containing diets supplemented with sufficient Lys can reduce the environmental footprint of pork production.

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DEDICATION

I would like to dedicate this thesis to my family, my dad Bob, my mom Donna and my sister Angie. I want to thank you all for allowing me to continue my education even though it meant being away from my loved ones. There is no way I could have achieved this without the support and compassion from all three of you. Mom and Dad you have believed in me since I was born and gave me the means necessary to achieve whatever I set my heart to. Thank you for always supporting me and loving me unconditionally. Angie, I am glad that we became closer when I moved away. I love that I can always count on you for advice and I have someone who I can spend hours on the phone with talking about absolutely nothing, it really helped to clear my head when times were hectic.

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"When you are in love you can't fall asleep because reality
is better than your dreams."—Dr. Seuss

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LIST OF ABBREVIATIONS

AA	Amino acid
ADFI	Average daily feed intake
ADG	Average daily gain
ADWI	Average daily water intake
AIA	Acid insoluble ash
cAMP	Cyclic adenosine monophosphate
CF	Crude fat
CP	Crude protein
DE	Digestible energy
DM	Dry matter
GE	Gross energy
G:F	Gain-to-feed ratio
GIT	Gastrointestinal tract
HCW	Hot carcass weight
HPLC	High performance (pressure) liquid chromatography
ISG	Initial slaughter group
LM	Longissimus muscle
Mcal	Mega calorie
ME	Metabolizable energy
N	Nitrogen
NH_3	Ammonia
N_2O	Nitrous Oxide
NSP	Non-starch polysaccharide
P	Phosphorus
ppm	parts per million
RAC	Ractopamine hydrochloride
Rep	Replicate
SEM	Standard error of the mean
SID	Standardized ileal digestible
Trt	Treatment

WBSF Warner-Bratzler Shear Force

1 GENERAL INTRODUCTION

In recent years, swine production facilities have increased in size but facility numbers have decreased (Jongbloed and Lenis, 1998; Sherlock, et al., 2002). A larger production unit, with correspondingly more pigs, will increase the amount of manure produced from an individual facility. An increased amount of manure demands a larger land base to be able to sustain increased manure production (Sherlock et al., 2002). Manure contains large amounts of nutrients that maintain plant growth; however, excess quantities of nutrients applied per acre may harm certain aspects of the environment. Environmental concerns regarding manure are usually regarded in one of three categories that include: soil build-up of nutrients, water eutrophication and air quality (Jongbloed and Lenis, 1998).

The manipulation of swine diets has been a successful method for reducing nutrient excretion (NRC, 1998). Nitrogen (N) excretion has been reduced by decreasing dietary crude protein (CP) content and supplementing synthetic amino acids (AA) to ensure the pigs' AA requirements are met (Deng et al., 2007). Non-starch polysaccharides can increase microbial protein excretion, which is less volatile than N excreted as urea (O'Connell et al., 2006).

The addition of microbial phytases to swine diets can substantially decrease total phosphorus (P) output in manure (Beaulieu et al., 2007). In grain products, P is commonly bound to phytic acid. Swine and other monogastric species lack phytase enzymes to release P from phytic acid (NRC. 1998). Beaulieu et al. (2007) found the addition of phytase produced by *E.coli* can decrease total P excretion between 30 and 50 %.

Ractopamine hydrochloride (RAC; Paylean™; Elanco Animal Health, Guelph, ON) is a β-adrenergic agonist added to the diets of swine to improve growth performance, increase carcass lean and decrease carcass fat (Xiao et al., 1999; Patience et al., in press). Minor changes in pork quality with RAC supplementation have been reported (Patience et al., in press; Apple et al., 2007). To increase protein deposition in RAC supplemented pigs, dietary lysine concentration needs to be increased. Webster et al. (2007) concluded that RAC-fed pigs between 79 and 109 kg require ~0.80 % standardized ileal digestible (SID) lysine to optimize growth and carcass performance. The response to RAC, regarding performance and carcass benefits, has been noted in many research studies, including but not limited to those mentioned above.

A small number of studies have looked at RAC's impact on the environment. Carroll et al. (2001) developed a model that estimated that feeding 20 ppm RAC could potentially decrease manure output, the number of pigs required to sustain pork production and feed requirements. Sutton et al. (2001) and Decamp et al. (2001) utilized 20 ppm and 18 ppm RAC respectively, and found N excretion was decreased. In Canada, 5 and 10 ppm dietary RAC inclusion are the only currently approved dosages (Canadian Food Inspection Agency, 2008). Therefore, any reduction in N excretion at approved dosages could benefit the environment.

This project focused on the potential of RAC to reduce the environmental impact of pork production by improving nutrient utilization, reducing N excretion and increasing carcass leanness. Whole body growth, protein accretion rate and nitrogen retention in finishing barrows was measured. This was accomplished by conducting both comparative slaughter and metabolism experiments, allowing an assessment of indirect and direct measurements of nutrient utilization. This experiment also determined the lysine concentration required for an optimal response to RAC.

2 LITERATURE REVIEW

2.1 Pig production and the environment

Requirements for food production have changed with an increasing world population expanding the demand for animal products. With more animals being produced, disposal of manure is an increasing issue. Although all livestock are subjected to public scrutiny, it is especially prevalent within the swine industry. The nitrogen (N), phosphorus (P), ammonia (NH_3) and volatile fatty acid content of manure are the main concerns (O'Connell et al., 2006).

2.1.1 Nutrient excretion: Nitrogen

2.1.1.1 Impact on environment

The excretion of N by swine is a potential cause for concern because it is partly in the form of NH_3, which has environmental and odour implications. NH_3 becomes volatile at three different sites within pork production: 1) in the barn, 2) during manure storage and 3) during spreading of manure on farm land (Portejoie et al., 2004). Manure from swine facilities is used as a fertilizer for crops but in some locations more manure is produced than can be handled by the available land base (Sherlock et al., 2002). Ground water contamination is a concern when spreading manure on crops in excessive quantities. The ammonium N within the manure is converted into nitrate which causes release of NH_3 and nitrous oxide (N_2O). NH_3 and N_2O are known to contribute significantly to breakdown of the ozone layer and potentially cause climate change (Sherlock et al., 2002). NH_3 is also a concern for the welfare for animals and workers. Long term exposure to excessive NH_3 in the barn, combined with dust exposure, may cause lung tissue damage (Portejoie et al., 2004). Thus, it can be seen that the reduction of N excretion by the pig industry would have many benefits.

2.1.1.2 Reducing N excretion

The introduction of synthetic amino acids (AA) into swine nutrition has allowed for a reduction in N excretion by reducing the CP requirements in the diet, as well as reducing NH_3 production (Smith et al., 2004). A reduction in urinary N excretion with a lower CP diet would occur because when excess N is consumed the majority is excreted through the urine. O'Connell et al. (2006) found a 23 % reduction in total N excretion when dietary CP was reduced from 220 to 160 g CP/kg. Lowering dietary CP, while supplementing AA to requirement, does not pose

significant problems for pig performance (Portejoie et al., 2004) because a moderately lower CP diet does not significantly affect N retention (Smith et al., 2004). However, Deng et al. (2007) showed that a large decrease in dietary CP caused a reduction in retained N, therefore protein deposition and growth performance was reduced (Figure 2.1).

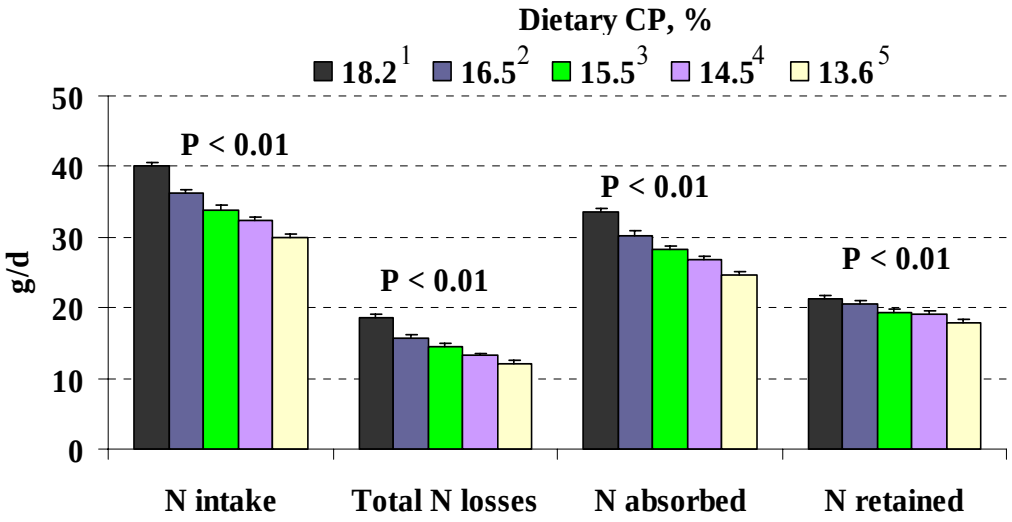

Figure 2.1 N balance in growing barrows fed low-protein diets supplemented with essential AA
[1]No supplemental AA
[2]L-lysine HCl (78.8%) supplemented at 0.18 % per kg of diet
[3]L-lysine HCL (78.8%) supplemented at 0.28 % per kg of diet, DL-methionine supplemented at 0.02 % per kg of diet, L-threonine 0.03 % per kg of diet
[4]L-lysine HCL (78.8%) supplemented at 0.37 % per kg of diet, DL-methionine supplemented at 0.04 % per kg of diet, L-threonine 0.08 % per kg of diet
[5]L-lysine HCL (78.8%) supplemented at 0.47 % per kg of diet, DL-methionine supplemented at 0.07 % per kg of diet, L-threonine 0.14 % per kg of diet
Error bars represent standard error of the mean (SEM).
Figure adapted from Deng et al. 2007.

Utilizing a diet that contains a larger fraction of fermentable non-starch polysaccharides (NSP) will help reduce the excretion of urea N which constitutes a large amount of total N excreted from the pig. The NSP portion of dietary fibre also can affect the N excretion patterns in pigs. Increased dietary NSP's can reduce N excretion in the faeces to a less volatile form of N, microbial protein, resulting in a decrease of the more volatile from of N (urea and ammonia) in the urine (O'Connell et al., 2006). N found in the faeces is usually in the form of dietary or

endogenous proteins. Proteinaceous N is less volatile than urea based N, which is found in the urine, because urea is easily converted into ammonia by urease enzymes present in the faeces (Portejoie et al. 2004). Dietary NSP inclusion can increase commensual bacteria which promote increased N utilization for microbial protein synthesis in the gut rather than urea synthesis in the liver (O'Connell et al., 2006). Lowering dietary electrolyte balance and increasing the NSP content of the diet will decrease manure pH (Clark et al., 2005). NH_3 is more soluble in an environment that has a low pH; therefore NH_3 emissions are reduced (Clark et al., 2005). Clark et al. (2005) found that the addition of NSPs or lowering the CP from 168 to 139 g CP/kg (as fed basis) had no effect on feed consumption, weight gain or feed conversion in growing pigs. Although lowering the CP of a diet can be cost effective, the addition of NSP's may not be commercially practical. In the Clark et al. (2005) study, they found the low and high CP diets were similar in price but the diet that contained NSPs increased diet cost by 30%. As can be seen from the above, reducing N excretion from pigs is possible through manipulation of the diet.

2.1.2 Nutrient excretion: Phosphorus

2.1.2.1 Impact on environment

In Canada, N is usually used as the rate-limiting nutrient when applying manure to farmland. Nevertheless, when excess quantities of manure are applied to the land, P may build-up in the soil and leach into surrounding water sources. A large amount of P in water stimulates growth of aquatic vegetation; upon decomposition, water quality is diminished or eutrophication occurs (NRC, 1998). The entire ecosystem is affected when eutrophication occurs because the death of many of the native species decreases oxygen in the water, therefore, negatively impacting surrounding ecosystems. Unlike N, P is not released as a gas into the environment, causing it to remain in the ecosystem and accumulate. In water ecosystems a large amount of P causes growth stimulation of cyanobacteria. These in massive amounts cause discolouration of water and development of "pond scum" (Iqbal et al., 2006). Certain cyanobacteria species can produce toxins which are dangerous to human and animal life. Microcystin, a hepatotoxin, produced by some cyanobacteria can cause neurological problems and liver failure in humans (Iqbal et al. 2006).

2.1.2.2 Reducing P excretion

The environmental complications that come with over-excretion of P as well as the expense associated with feeding inorganic P sources have stimulated research in developing products that will reduce excretion. The development of microbial phytase for use in swine diets is one of the most successful products. In most cereal and oilseed products, P is bound to phytic acid, making the P unavailable to monogastric animals. Monogastrics lack phytase enzymes in their gut making it difficult to release P from the tightly bound phytate molecule. When phytase is added to swine diets, it releases the P, making it available to the pig. When the pig is able to use a higher proportion of the dietary P, less is excreted in the faeces, therefore reducing P concentration in the environment. Harper et al. (1997) found that the commercially available phytase enzyme they used decreased faecal excretion of P by 21%. NRC (1998) stated that some phytases used can reduce P excretion up to 50 %.

Oilseeds and cereal grains have high total P levels but the majority of it is unavailable to pigs without phytase supplementation. A basic corn-soybean meal diet has over two-thirds of P bound as phytic acid (NRC, 1998). Animal products have a relatively high amount of available P, not in the form of phytic acid (Table 2.1). Microbial phytase supplementation is an effective way of providing more P to pigs, and reducing P excretion. Any reduction of P into the environment will reduce the risk of P accumulation in the soil and water surrounding swine facilities.

Table 2.1 Total and available P from selected feed ingredients

	Total P (%)	Bioavailability of P (%)
Corn	0.28	14
Barley (six row)	0.36	30
Soybean meal	0.65	31
Canola meal	1.01	21
Fish meal (Menhaden)	3.04	94
Meat and bone meal	4.98	90

Table adapted from NRC, 1998.

2.1.3 Nutrient excretion: other nutrients

N and P are the nutrients that have received the most attention regarding their environmental impact. Any mineral in excess can cause degradation of the environment; however, other than N and P, the nutrients causing the greatest concern are sodium, potassium, copper and zinc. To decrease excretion of these nutrients, it is important to develop swine diets that provide adequate but not excessive amounts of nutrients. Utilizing improved swine genetics, clean barn conditions, grinding and pelleting feed and use of high quality ingredients can improve nutrient utilization (NRC, 1998). Nutrient requirements change throughout a pig's life cycle; therefore, diets formulated to meet the pig's changing requirements will also reduce nutrient excretion.

2.1.4 Odour and gas emissions

Ammonia (NH_3), methane, nitrous oxide, and hydrogen sulphide are all emissions that are produced from swine facilities. Ammonia is a concern because it causes soil acidification and water eutrophication when manure is spread on land. Ammonia is volatilized in storage and when it is applied to the land (Portejoie et al., 2004). Ammonia application also acts as a secondary source of nitrous oxide that may contribute to climate change (Sherlock et al., 2002).

Dependent on the pig's status, methane is released at 0.9 to 21.1 kg per animal per year where 65% is released from the manure and the other 35% is expelled directly from the pig (Haeussermann et al., 2006). Factors which affect methane production are feed conversion, average daily gain and diet ingredients (Haeussermann et al., 2006). Methane is one of the major contributors to greenhouse gas emissions and the associated deterioration of the environment. Ruminants are one of the major sources of methane, estimated to produce 80-115 Tg (tera (10^{12}) grams) /year, but pig facilities also contribute and any reduction of methane emissions would benefit the environment (Haeussermann et al., 2006).

Nitrous oxide is released during dry crust formation when manure is stored in slurry form (Haeussermann et al., 2006). Like methane, nitrous oxide is a major contributor to the greenhouse gas effect (Burton et al., 1993).

Hydrogen sulphide is a toxic gas that is produced from anaerobic fermentation of manure. Concentrations in the air of greater than 280 mg/m^3 are toxic to humans and animals, causing serious health implications or death (Ni et al., 2002). Hydrogen sulphide is not released from

manure until it is disturbed; upon agitation, it is released and can cause dangerous hydrogen sulphide concentrations for humans and animals (Occupational Health and Safety, 2004). Human and animal safety is the major concern with hydrogen sulphide.

2.1.4.1 Causes of odours

Odour from manure is caused by the breakdown of undigested feed by anaerobic microorganisms (Gralapp et al., 2002) and the release of NH_3 from urinary excretion of urea. An increase in odour will occur when bacteria have increased substrate to ferment. This causes a release of odorous volatile organic compounds, short-chain volatile fatty acids and volatile carbon-, nitrogen-, and sulphur-containing compounds (Sutton et al., 1999). Excessive odour is a concern because it affects not only the workers within the barn; it also affects the community surrounding the swine facility. If unnecessary odour is not controlled within the barn and in lagoons, it can cause negative implications on the community and make it an undesirable place to live.

2.1.4.2 Mitigating gas and odour emissions

An economical way to reduce gas and odour emissions is prevention of nutrient overformulation. Formulating diets high in crude protein and non-fermentable carbohydrates increases excretion of NH_3 and allows more substrate for anaerobic bacteria to breakdown and create noxious odours. Utilizing synthetic AA in place of complete protein sources will reduce NH_3 emissions (Smith et al., 2004) but an excess of sulphur containing AA will increase odour emissions from the animal and manure (Gralapp et al., 2002).

Since NH_3 can serve as a secondary source of nitrous oxide (Sherlock et al., 2002), reducing nitrous oxide emissions will follow similar patterns as NH_3 reduction. Making N more available to the pig will prevent excessive N being excreted as either NH_3 or nitrous oxide. Methane production can be controlled by use of non starch polysaccharides because they stimulate the growth of commensual bacteria which do not produce methane to the same extent as methanogenic bacteria (Jensen and Jørgensen, 1994). Methane production can be reduced by lowering indoor temperatures of pig barns and addition of acids to manure (Haeussermann et al., 2006). An increase in cost with all of these methane reduction methods could potentially limit their use. Hydrogen sulphide may be controlled through management of manure systems, since it is released when manure is agitated (Occupational Health and Safety, 2004). If manure is

carefully handled with minimal agitation, hydrogen sulphide will be less of a concern. Managing gas and odour emissions from pig barns not only will benefit the environment but may reduce potential health hazards associated with inhalation of these gases.

2.2 Ractopamine

Ractopamine hydrochloride (RAC; Paylean™; Elanco Animal Health, Guelph, ON), a β-adrenergic agonist in the phenethanolamine class of chemicals, increases feed efficiency and growth performance in finishing swine diets (Xiao et al., 1999; Patience et al., in press). It causes partitioning of energy for increased lean yield rather than fat yield, resulting in increased protein accretion, muscle growth and increased muscle cell size. Figure 2.2 shows the six-membered aromatic ring, hydroxyl group, charged N and aromatic substitutions which are all required for binding to an adrenergic receptor (Smith. 1998). RAC has some characteristic structures which make this compound unique. Commercial RAC is sold as a racemic mixture, so not all isomers are biologically active within the pig (Mills et al., 2003). This is due to the chiral carbons that are marked with an asterisk in Figure 2.2.

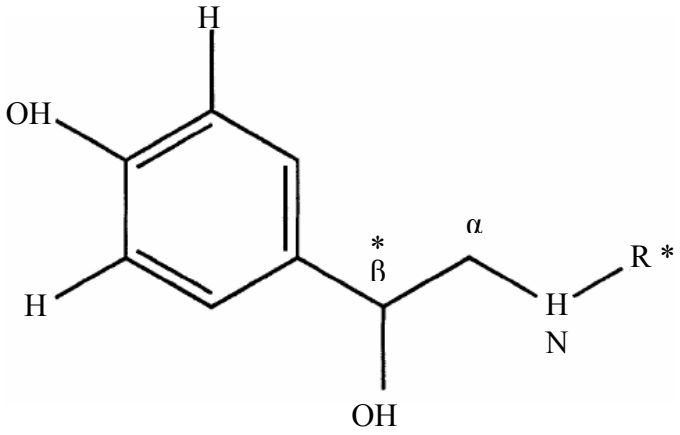

Figure 2.2 Generalized structure of the phenol category of β-adrenergic agonists
Figure adapted from Smith, D. J. 1998 and Mills et al., 2003.

2.2.1 Receptor binding

When a β-adrenergic agonist is ingested or a physiological one is released (epinephrine and norepinephrine), it binds to the receptor and activates the Gs protein (Mersmann, 1998), causing release of the enzyme, adenylyl cyclase, which induces cyclic adenosine monophosphate (cAMP). Cyclic adenosine monophosphate is important for a variety of cell functions but most importantly, it stimulates phosphorylation of intracellular proteins (Mersmann, 1998). It has also been shown that β-adrenergic agonists may decrease protease secretion within the cell which would cause a reduction in protein degradation (Mersmann, 1998). Figure 2.3 represents a model for the configuration of β-adrenergic receptors. There are seven transmembrane domains that anchor the receptor to the plasma membrane. In the center of the domains lies the ligand binding site which involves many amino acids from all 7 transmembrane domains. The ligand binding site is where activation of the Gs protein takes place (Mersmann, 1998).

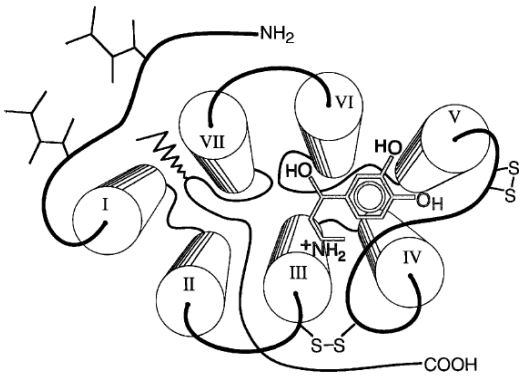

Figure 2.3 Projected Structure of β-Adrenergic receptor
Used by permission of Journal of Animal Science. Figure from Mersmann, 1998.

2.2.2 Cellular metabolism

RAC use in swine nutrition is known to increase protein accretion and reduce lipid accretion (Apple et al., 2004). The activity of RAC in the body is dependent on its activity at the receptor. Absorption, rate of metabolism and elimination of the compound can affect the outcome of physiological results (Smith, 1998). Unlike many other β-adrenergic agonists, RAC acts mainly on skeletal muscle cells, specifically binding to the β-1 and β-2 receptors. An initial dosage of RAC shows marked response to the agonist which gradually diminishes due to down-

regulation of the receptors (Bell et al., 1998). Stimulation of cAMP can affect various functions which include activation and inactivation of enzymes (Abney, 2006). The functions that RAC influences within skeletal muscle cell are: increased lactate dehydrogenase, decreased oxidative enzymes and changes in the contractile properties of the muscle (Depreux et al., 2002). An increase in lactate dehydrogenase indicates enhanced anaerobic glycolysis and with increased glycolytic capability, increased cell activity and protein turnover are more likely to occur (Vestergaard et al., 1994). This suggests that when RAC is fed, muscle metabolism shifts toward glycolytic rather than oxidative metabolism (Vestergaard et al., 1994). The change in contractile properties has been found to affect the hypertrophy of fast-twitch muscles in cattle (Vestergaard et al., 1994). This could be due to increased blood flow that has been noted in β-adrenergic agonists fed animals. Rats had increased blood flow to skeletal muscle following acute treatment of clenbuterol, however chronic treatment decreased blood flow (Rothwell et al., 1987). An increase in blood flow may deliver increased amounts of substrates required for protein synthesis (Mersmann, 1998).

2.2.3 Tissue effects

Protein tissue is the most influenced by RAC. An increase in carcass lean content and a decrease in the fat content are noted when RAC is incorporated into the diet (Xiao et al., 1999). RAC had the same effect on both lean and obese pigs in a study by Yen et al. (1990). They found that even in obese pigs, carcass lean increased, fat content decreased and feed efficiency improved. An increase in the diameter of the skeletal muscle fibres following RAC ingestion has been noted in several studies (Aalhus et al., 1992; Carr et al., 2005). The white fibres (Type IIb) increase in size and number within the muscle tissue. Intermediate fibres (Type IIa) will decrease in amount and the size of the red fibres (Type I) but the result of this change is unknown (Aalhus et al., 1992; Carr et al., 2005). The change in these fibres can cause increased toughness and higher shear force values, independent of connective tissue concentration or age of the animal (Carr et al., 2005).

The effect of RAC on adipose tissue is not as well documented as skeletal muscle. It was originally thought that RAC increased lipolysis and blocked the conversion of glucose into triglycerides (Liu et al., 1989). Liu et al., (1994) found that RAC had a profound effect on

skeletal muscle tissue causing a less significant response in adipose tissue. Ji et al., (1992) found that when RAC is fed, skeletal muscle retains more N and α-actin gene expression is increased. Liu et al. (1994) concluded that skeletal muscle is more responsive to RAC feeding than other tissues, including adipose. RAC feeding does decrease fatty acid synthesis which may alter the lipid deposition (Weber et al., 2006). In mouse adipose tissue, RAC increases apoptosis; this may explain the decrease in fat accretion in swine but more research is required (Weber et al., 2006).

2.2.3.1 Pork Quality

A high quality consumer product is one of the most important aspects of the livestock industry. The use of RAC in swine diets has led to some concerns regarding eating quality and appearance of pork. As shown in Table 2.2, increased RAC in the diet provided overall heavier ham, picnic, Boston butt, belly and loin weights. Heavier weights were achieved with 20 mg/kg of RAC and 10 mg/kg RAC showed significant improvement over the control group. Since protein accretion is more efficient in RAC fed pigs while fat accretion decreases, the lipid percentage in trimmings is reduced (Carr et al., 2005). Patience et al. (in press) found RAC increased lean yield by 1 percentage point while increasing carcass index.

Table 2.2 Trimmed wholesale cut weights (kg) and weights as a percentage of HCW (hot carcass weight) as influenced by dietary RAC concentrations

	RAC, mg/kg		
Variable[1]	0	10	20
No. of observations	60	60	60
401 Ham (collar off), % of HCW	22.83[a]	23.15[ab]	23.5[b]
401 Ham (collar off), wt, kg	8.54[a]	9.18[b]	9.57[c]
405 Picnic, % of HCW	10.84	10.76	10.71
405 Picnic, wt, kg	4.05[a]	4.27[b]	4.36[b]
406 Boston butt, % of HCW	7.75[a]	8.09[b]	8.2[b]
406 Boston butt, wt, kg	2.9[a]	3.22[b]	3.34[c]
409B Belly (skin on), % of HCW	9.94	9.88	10.14
409B Belly (skin on), wt, kg	3.72[a]	3.91[b]	4.13[c]
410 Loin, % of HCW	20.09	20.73	20.58
410 Loin, wt, kg	7.52[a]	8.23[b]	8.38[b]

[1]The number associated with the cut description is the Institutional Meat Purchase Specification, North American Meat Processors Association (NAMP, 1997) number of the cut most closely associated with actual cut specifications.
[a,b,c]Within a row, means without a common superscript letter differ, $P < 0.05$.
Table adapted from Carr et al., 2005.

Carcasses from RAC-fed pigs also have similar water holding capacity to control animals which helps prevent moisture loss during cooking (Patience et al., in press; Carr et al., 2005; Jeremiah et al., 1994). A meta-analysis of the literature was conducted by Apple et al. (2007) which showed that Warner-Bratzler Shear Force (WBSF) values were increased by 4.4, 10.9 and 8.6% when RAC was fed at levels of 5, 10 and 20 mg/kg. Taste panel evaluation detected lower initial and overall tenderness with RAC fed pigs which were consistent with higher WBSF values reported (Patience et al., in press). This increase in WBSF may not be due to RAC supplementation but the increased lysine levels typically associated with RAC containing diets (Apple et al., 2007). Patience et al. (in press) found that 5 ppm RAC had no effect on drip loss (24 and 48 hours), visual colour scores or marbling. There was a slight RAC effect on pork colour, CIE a* and b* values were lower, indicating a change in colour score with decreased red and yellow intensiveness. Even though a slight decrease in tenderness and colour has been reported with RAC use (Apple et al., 2007; Patience et al., in press), its overall benefits have encouraged increased use by the industry.

2.2.4 Practical applications

2.2.4.1 Time of delivery

RAC feeding is only beneficial during the 28 to 35 days prior to marketing (Armstrong et al., 2005). Increased live performance and improved carcass traits are seen during this period of the growth phase with RAC use (Armstrong et al., 2005). The response to RAC is not constant; it peaks at the beginning of the feeding period and begins to decrease due to down regulation of the β-adrenergic receptors (Bell et al., 1998). Bell et al. (1998) found that the response peaked at 14 days, but plateaued at 21 days and did not decrease until 21 days after initial feeding. It is believed that the β-adrenergic receptor becomes desensitized because density of the receptors within skeletal muscle decreases with prolonged RAC use (Bell et al., 1998).

Because of this response, different types of feeding programs have been developed to maximize protein accretion. These include step up and step down programs which change the level of RAC over time or constant feeding programs which provide the same amount throughout the finishing period. Step up programs increase RAC levels in the diet one or more times, step down programs decrease RAC levels one or more times and constant feeding programs provide the same amount of RAC. See et al. (2004) used a step up program with RAC fed at 5 ppm for

week 1 to 2, at 10 ppm for wk 3 to 4 and at 20 ppm for week 5 to 6. The step down program contained RAC at 20 ppm for week 1 to 2, at 10ppm for week 3 to 4 and at 5 ppm for week 5 to 6. The constant feeding program provided RAC at 11.7 ppm for week 0 to 6. They found that the step up and constant feeding program provided significant improvements in carcass leanness, ADG and feed conversion efficiency but the step down program did not contribute to increased performance. See et al. (2004) concluded either the constant or step up feeding program was beneficial but Armstrong et al. (2005) found the step up program to be the more valuable of the two. Utilizing a feeding program with 5 g RAC per tonne for 21 days and 10 g RAC per tonne for 14 days was the most favourable at improving live performance and carcass measurements. The same results were obtained with a feeding program of 5 g RAC per tonne for 14 days or 10 g RAC per tonne for 21 days so the first program would be more economical (Armstrong et al., 2005). The time of delivery of RAC is critical in maximizing a pig's response to RAC and utilizing a step up program is the most efficient way to maximize the economic returns accruing from RAC use.

2.2.4.2 Diet adjustments

When the body increases protein accretion, there is a higher demand for dietary protein or AAs. With an increased crude protein diet, RAC affects growth performance and carcass composition more consistently than with a low crude protein or amino acid diet (Xiao et al., 1999). Pigs that are fed a diet low in crude protein with RAC supplementation will not be capable of maintaining increased lean tissue production. Lysine is of greatest importance when RAC is first fed because growth is the most rapid during this phase (Neill et al., 2006). Webster et al. (2001) showed improved growth performance with levels up to 1.2 % total lysine, but 1.0 % total lysine was the most economical. Typical late finisher rations attempt to minimize essential amino acid levels to maximize profit, which generally do not support the increased lean gain provided by RAC (Apple et al., 2004). Not only is the lysine content of the diet important, the lysine:energy (Lys:ME) ratio is also important for maximizing performance. Growth performance and carcass composition increased up to an inclusion of 3.1 g lysine/Mcal ME (Apple et al., 2004). But feeding lysine ratios at levels higher than 3.0 g lysine/Mcal of ME can affect marbling and tenderness (Apple et al., 2004).

Gu et al. (1991) reported significant variation in requirements for dietary protein (especially lysine) among 5 different swine genotypes. Genotypes were segregated based on their genetic potential for high lean growth, providing genotypes with varying degrees of lean growth. They found that the effect of RAC on lean tissue was significantly higher within the lean genotype compared to that of the low lean yield genotype. Thus, the lean genotype appears to require increased dietary protein, including higher lysine levels (Gu et al., 1991). Utilizing a leaner genotype coupled with RAC use and sufficient protein will maximize carcass lean. Diet adjustments are critical whenever a feed additive is being used, but by maximizing the pig's nutritional potential, greater benefits from the product can be achieved.

2.2.4.3 Economic impact

Utilizing RAC in swine diets can have a variety of economic impacts on swine production. RAC improves carcass characteristics by increasing protein accretion while decreasing lipid accumulation in the tissue. With the increase in lean tissue accretion, the pig's requirement for dietary protein (lysine) increases. In normal finisher swine diets, producers decrease the amount of essential AA to reduce cost and maximize profit (Apple et al., 2004). Providing RAC into the diet will require an increase in the amino acid content of the diet that will raise ingredient cost. Increased lysine and RAC levels in the diet can cause a significant increase in the total cost of a diet, relative to a typical finisher diet, but the improvements in carcass composition will compensate for the increased cost.

Feeding RAC is the most valuable when producers are paid based on carcass weight because carcass weight increases (Main et al., 2001). RAC feeding is especially favourable to producers who utilize all-in-all-out management systems. One of the major advantages of RAC feeding is reduced variation of market weight. This is critical in all-in-all-out facilities because the value of lightweight pigs is lower. Patience et al. (in press) found that RAC feeding for approximately 28 days, reduced shipping light weight pigs from 7.5% to 0.8%, therefore, RAC has the potential to reduce penalties associated with carcass weights. This enables producers to ship the majority of their pigs within a defined period, potentially reducing the lightweight pigs that tie up barn space and continuing to consume feed. Improving the number of pigs sent to market at the target weight will reduce the penalties associated with shipping lightweight pigs. Shipping pigs in different weight categories causes issues at the slaughter plant when assembling

loads (i.e. equipment sizes/settings) (Morrow et al., 2005). Clearing out an entire grow/finish room will increase efficiency within the barn because lightweight pigs cause increased cleaning and labour and can pose a biosecurity risk.

2.3 RAC and the environment

The intensification of swine production has posed many environmental concerns like N and P excretion and odour and gas emissions. The length of time a pig spends in the barn, as well as the efficiency of nutrient utilization, determine the amount of nutrients that are excreted from the pig. The use of RAC has the potential to decrease the amount of nutrients excreted from the pig because lean yield is increased and ADG is improved; therefore pigs reach optimal market weight faster due to improved efficiency. This, in turn, reduces the number of days that each pig is required to stay in the barn to produce the final pork product. With reduced days in the barn, the pig will output less total manure (Sutton et al., 2001). RAC feeding increases protein accretion, which provides improved feed conversion, required to maintain the higher lean content of the carcass (Abney, 2006), which can decrease the manure output. Similarly, with decreasing nutrient excretion and manure output, the production of ammonia, greenhouse gases and odour can potentially be reduced because of a shorter feeding regimen provided by RAC supplementation (Sutton et al., 2001).

Carroll et al. (2001) developed a model to calculate potential benefits of feeding RAC at 20 ppm for 4 weeks to all finisher pigs within the USA (approximately 100 million pigs at that time) (Figure 2.4). They utilized the following data to determine potential environmental benefits which included: 9.8% increase in ADG, 12.7% increase in G:F, 5.7% increase in lean carcass percentage, 1.1% increase in dressing percentage and 4 fewer days for optimal market weight. An improvement in feed efficiency can decrease the time pigs require to reach market weight and fewer days in the barn could potentially reduce manure excretion by 2.12 billion litres. Carroll et al. (2001) concluded that the same amount of pork could be produced with 11.3% fewer pigs. There would also be a significant reduction in feed required for these pigs, due to improved G:F, which could potentially reduce corn requirements by 171,400 ha/yr and soybean requirements by 251,900 ha/yr. A reduction in crop requirements could also reduce chemical, fertilizer and pesticide use that could be needed to maintain these crops (Sutton et al., 2001).

Limited research has been conducted on the effect of RAC reducing nitrogen excretion from finishing pigs. With RAC fed pigs, protein accretion and carcass leanness are increased (Xiao et al., 1999); therefore there is potential for increased nitrogen retention and decreased nitrogen excretion. DeCamp et al. (2001) compared a typical finisher diet with 13.8 % CP with no RAC and an 18 ppm RAC diet containing 16.1 % CP and discovered a 51 % increase in nitrogen retention and a 14 % reduction in daily nitrogen excretion due to the addition of RAC. Sutton et al. (2001) compared nitrogen excretion between a 13.8 % CP control diet and a 16.1 % CP 20 ppm RAC supplemented diet. Urinary N excretion was reduced by 30 % and total manure output was decreased by 24.9 % (Sutton et al., 2001). Sutton et al. (2001) proposed that if a 16.1 % CP RAC supplemented diet was fed for 28 d, there would be 67.2 g less nitrogen excreted per pig marketed.

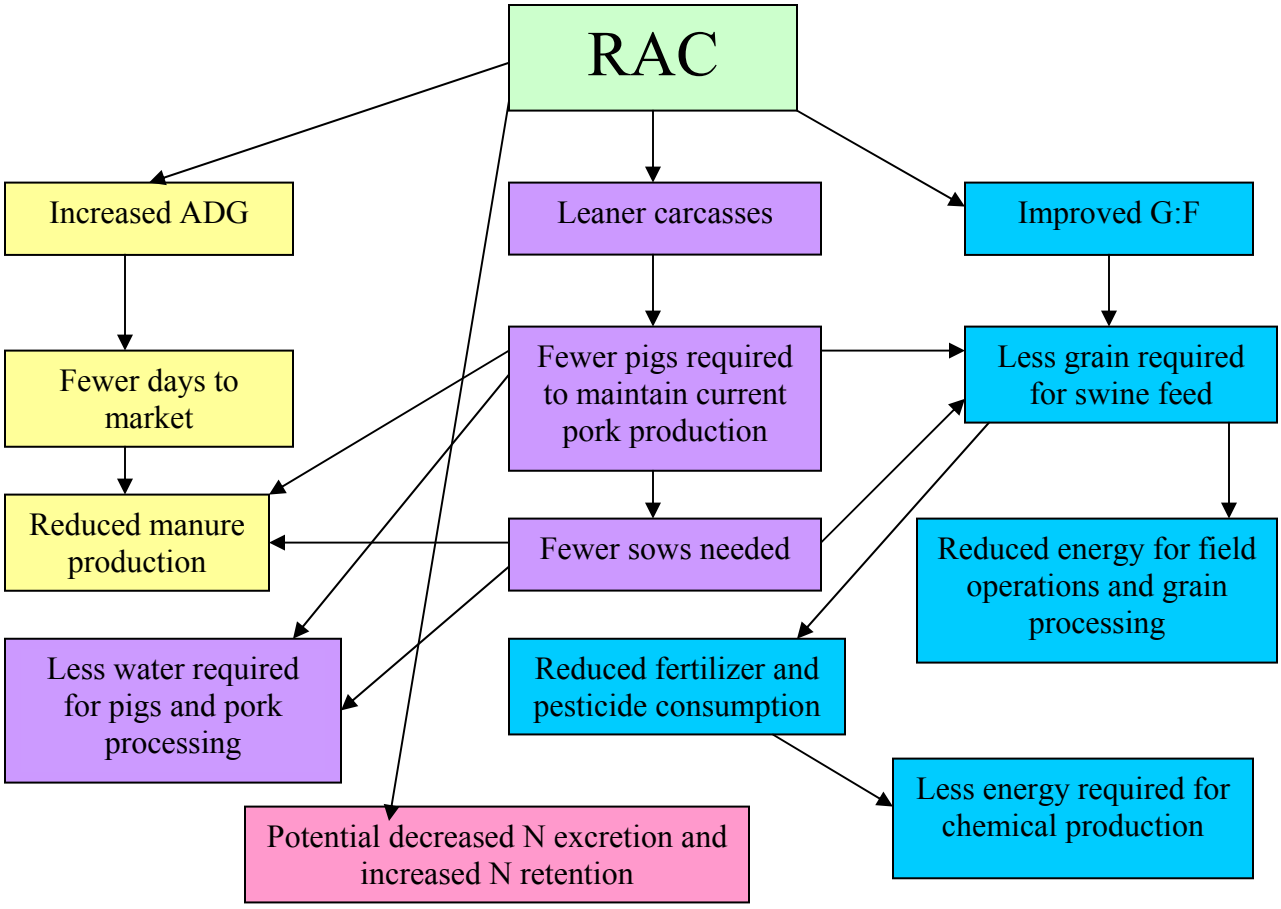

Figure 2.4 Proposed methods of reducing environmental concerns with RAC supplemented finisher swine diets
Figure adapted from Carroll et al., 2001.

RAC has already been established as a feed additive that can increase protein accretion, improve ADG and feed conversion (Xiao et al., 1999). There is the potential for RAC to decrease nutrient excretion, manure output, odour and greenhouse gas production from finishing swine facilities. By reducing the number of days required for pigs to reach market weight and improve the efficiency of nutrient utilization, RAC could reduce some of the environmental concerns associated with swine production. Determining the effect of RAC on the environmental impact of pork production will verify if the addition of RAC to diets is cost effective, in terms of pig performance, nutrient utilization and carcass quality.

3 RACTOPAMINE HYDROCHLORIDE AND THE ENVIRONMENTAL SUSTAINABLILITY OF PORK PRODUCTION

3.1 Introduction

Ractopamine (RAC) is a ß-adrenergic agonist that belongs to the phenethanolamine class of chemicals that includes other leanness enhancing substances i.e. cimaterol, clenbuterol, salbutamol and zilpaterol (Abney, 2006). RAC use in the swine industry has become important due to the benefits associated with adding it to swine diets. RAC inclusion can increase growth rate, feed efficiency and increase carcass lean, while decreasing carcass fat (Xiao et al., 1999; Patience et al., in press). RAC is considered a repartitioning agent, due to the ability to partition energy from fat accretion to protein accretion (Xiao et al., 1999, Patience et al., in press). The growth performance and carcass improvements are well noted in the literature but there is limited research on other benefits of dietary RAC inclusion.

A small number of studies have looked at RAC's impact on the environment. Carroll et al. (2001) and Sutton et al. (2001) utilized 20 ppm RAC and Decamp et al. (2001) used an inclusion level of 18 ppm RAC to determine any reduction in nutrient excretion. However, the Canadian Food Inspection Agency (2008) only approves 5 and 10 ppm dietary RAC inclusion, therefore, lower levels of RAC may not have similar nutrient reduction capabilities as 20 ppm RAC.

This project combines a comparative slaughter experiment (growth) and a metabolism experiment to measure nutrient retention. This provides a comparison of the two methods and also determines the accuracy of nutrient retention, both by direct and indirect measures.

Our hypothesis is that RAC will increase the utilization and retention of nutrients which will reduce excretion of nutrients into the environment. These pigs will have faster growth rates, which will reduce the number of days required to reach market weight. As well, reduced feed and water requirements will decrease manure output. The overall objective of this project is to define in very precise terms, the impact of RAC on the efficiency of pork production with a view to reduce the environmental footprint of pork production. The specific objectives are to determine the effect of RAC on the efficiency of N utilization leading to a reduction in N output, to evaluate the effect of RAC on the efficiency of animal performance, including improvements in carcass quality and improvements in rate and efficiency of gain and to determine the impact of

dietary lysine inclusion level on pigs' response to RAC. The final objective is to compare the direct (comparative slaughter method) and indirect (intake and excretion method) methods in determining nutrient retention.

3.2 Materials and Methods

All procedures used in this experiment were approved by the University of Saskatchewan Committee on Animal Care and Supply (Protocol #1970019) and adhered to principles established by the CCAC (1993). This study combined both a growth experiment and a metabolism experiment with dietary treatments arranged as a 3 X 3 factorial: 3 levels of ractopamine HCl (RAC) (Paylean™, Elanco Animal Health, Guelph, ON) (0, 5 g/tonne and 10 g/tonne) and 3 standardized ileal digestible (SID) lysine:DE ratios (1.75 g/Mcal, 2.25 g/Mcal and 2.75 g/Mcal) (Table 3.1). In the growth experiment, slaughter weight was an additional factor; therefore the growth experiment was designed as a 3 x 3 x 2 factorial. A total of 174 crossbred barrows (Camborough Plus females x L42 sire, PIC Canada, Winnipeg, MB) were selected, 120 and 54 for the growth and metabolism experiments, respectively, at a mean initial weight of 95 kg with a range of ± 3 kg.

Both feed and animal scales were checked against check weights prior to each use to ensure the integrity of the data.

3.2.1 Treatments

Table 3.1 shows diet ingredients and the formulated analysis for the nine dietary treatments. Nutrient composition is shown in Table 3.2 and AA content is presented in Table 3.3. Diets were based on wheat, barley, and soybean meal and also contained canola oil, vitamin/mineral premix, synthetic amino acids and RAC. All diets were formulated to contain 3,300 kcal DE/kg (Table 3.1) and formulated to meet or exceed the nutrient requirements of the finisher pig (NRC, 1998). Celite was added as a digestibility marker (Table 3.1). To ensure consistency of diet composition in both experiments, all experimental diets were prepared at the same time, pelleted and bagged, and if not used immediately stored at -20 °C until required.

Table 3.1 Ingredient composition of experimental diets (% as fed)

RAC (ppm)	**0**	**0**	**0**	**5**	**5**	**5**	**10**	**10**	**10**
SID Lys (g/Mcal DE)	**1.75**	**2.25**	**2.75**	**1.75**	**2.25**	**2.75**	**1.75**	**2.25**	**2.75**
Ingredient, %									
Wheat	59.27	54.52	47.93	59.24	54.50	47.90	59.22	54.47	47.88
Barley	30.00	31.00	32.00	30.00	31.00	32.00	30.00	31.00	32.00
Soybean Meal	6.40	10.00	15.50	6.40	10.00	15.50	6.40	10.00	15.50
Limestone	0.750	0.750	0.750	0.750	0.750	0.750	0.750	0.750	0.750
Dicalcium Phosphate	0.550	0.500	0.450	0.550	0.500	0.450	0.550	0.500	0.450
Salt	0.500	0.500	0.500	0.500	0.500	0.500	0.500	0.500	0.500
PSC Mineral Premix[1]	0.500	0.500	0.500	0.500	0.500	0.500	0.500	0.500	0.500
PSC Vitamin Premix[2]	0.500	0.500	0.500	0.500	0.500	0.500	0.500	0.500	0.500
Lysine HCl	0.135	0.250	0.310	0.135	0.250	0.310	0.135	0.250	0.310
dL-Methionine	-	0.010	0.050	-	0.010	0.050	-	0.010	0.050
L-Threonine	-	0.070	0.115	-	0.070	0.115	-	0.070	0.115
Canola Oil	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Celite	0.400	0.400	0.400	0.400	0.400	0.400	0.400	0.400	0.400
Paylean[3]	0.000	0.000	0.000	0.025	0.025	0.025	0.050	0.050	0.050
Formulated Analysis									
DE, kcal/kg	3,300	3,300	3,300	3,300	3,300	3,300	3,300	3,300	3,300
Crude Protein, %	16	17	19	16	17	19	16	17	19
Total Lysine, %	0.650	0.840	1.030	0.650	0.840	1.030	0.650	0.840	1.030
SID Lysine, %	0.580	0.750	0.850	0.580	0.750	0.850	0.580	0.750	0.850

[1] Provided per kg of diet: zinc, 100 mg as zinc sulphate; iron, 80 mg as ferrous sulphate; copper, 50 mg as copper sulphate; manganese, 25 mg as manganous sulphate; iodine, 0.50 mg as calcium iodate; selenium, 0.10 mg as sodium selenite.

[2] Provided per kg of diet: Vitamin A, 8250 IU; Vitamin D, 825 IU; Vitamin E, 40 IU; niacin, 35 mg; D-pantothenic acid, 15 mg; menadione, 4 mg; folacin, 2mg; thiamine, 1 mg; D-biotin, 0.2 mg; Vitamin B_{12}, 25 ug.

[3] Provided 20 g ractopamine hydrochloride per kg of diet.

Table 3.2 Composition of experimental diets (% as fed)

RAC (ppm)	**0**	**0**	**0**	**5**	**5**	**5**	**10**	**10**	**10**
SID Lys (g/Mcal DE)	**1.75**	**2.25**	**2.75**	**1.75**	**2.25**	**2.75**	**1.75**	**2.25**	**2.75**
Item									
Dry matter, %	86.94	86.85	87.58	88.14	87.11	86.82	87.21	87.03	87.57
Gross energy, kcal/kg	3,779	3,805	3,837	3,793	3,827	3,815	3,807	3,810	3,843
Acid insoluble ash, %	0.79	0.81	0.81	0.83	0.81	0.77	0.76	0.79	0.79
Ash, %	4.21	4.31	4.46	4.21	4.24	4.48	4.19	4.30	4.58
Nitrogen, %	2.28	2.46	2.72	2.29	2.58	2.77	2.32	2.41	2.70
Crude fat, %	2.55	2.86	2.44	2.70	2.58	2.64	2.49	2.72	3.01
Calcium, %	0.55	0.61	0.59	0.62	0.55	0.60	0.60	0.57	0.61
Phosphorus, %	0.41	0.37	0.38	0.41	0.44	0.43	0.39	0.38	0.34
Potassium, %[1]	0.40	0.46	0.57	0.41	0.49	0.59	0.42	0.47	0.57
Magnesium, %[1]	0.15	0.15	0.17	0.15	0.16	0.17	0.15	0.15	0.16
Sodium, %[1]	0.20	0.21	0.22	0.20	0.22	0.22	0.22	0.21	0.23
Salt, %[1]	0.52	0.54	0.55	0.50	0.55	0.56	0.56	0.54	0.58
Sulphur, %[1]	0.15	0.16	0.18	0.16	0.16	0.19	0.18	0.15	0.17
Zinc, ppm[1]	154.00	123.00	128.00	116.00	122.00	124.00	122.00	116.00	120.00
Manganese, ppm[1]	77.10	59.90	63.60	57.00	64.30	61.00	60.20	58.40	56.80
Copper, ppm[1]	51.50	49.20	43.10	44.80	48.60	46.10	41.70	39.90	43.70
Iron, ppm[1]	233.00	203.00	231.00	205.00	31.10[3]	209.00	197.00	210.00	233.00
Molybdenum, ppm[1]	<1.00	<1.00	<1.00	<1.00	<1.00	<1.00	<1.00	<1.00	<1.00
RAC, ppm[2,4]	<2.50	<2.50	<2.50	4.50	4.40	4.10	8.50	9.30	9.00

[1]Lab assays completed by Norwest Labs, Lethbridge, AB Canada T1J 4H1

[2]Lab assay completed by Laboratory Services, University of Guelph, Guelph, ON Canada N1H 8J7

[3]Iron content low due to a potential lab error at Norwest Labs. This diet was reanalyzed at Central Testing Laboratory LTD., Winnipeg, MB Canada R2J 3K4 and an iron value of 200 ppm was obtained.

[4]The initial RAC assay is presented in table. The analyzed values of RAC throughout experiment are as follows: The 0 ppm RAC diets had values < 2.5 ppm, the 5 ppm RAC diets had values between 4.1-4.6 ppm RAC and the 10 ppm RAC diets had values between 8.6-8.9 ppm RAC.

Table 3.3 Assayed amino acid content of experimental diets (as-fed basis), (g/100g sample)[1]

RAC (ppm)	**0**	**0**	**0**	**5**	**5**	**5**	**10**	**10**	**10**
SID Lys (g/Mcal DE)	**1.75**	**2.25**	**2.75**	**1.75**	**2.25**	**2.75**	**1.75**	**2.25**	**2.75**
Nutrient									
Essential AA									
Histidine	0.32	0.36	0.41	0.36	0.34	0.40	0.31	0.35	0.38
Isoleucine	0.50	0.59	0.67	0.59	0.55	0.67	0.51	0.59	0.64
Leucine	0.95	1.07	1.22	1.07	1.00	1.20	0.93	1.04	1.13
Lysine	0.65	0.83	1.05	0.73	0.82	1.04	0.63	0.84	0.98
Methionine	0.19	0.22	0.30	0.23	0.21	0.28	0.19	0.22	0.27
Phenylalanine	0.65	0.73	0.82	0.73	0.68	0.81	0.64	0.71	0.76
Threonine	0.43	0.55	0.67	0.48	0.52	0.66	0.42	0.52	0.61
Tryptophan	0.17	0.20	0.21	0.18	0.19	0.21	0.16	0.19	0.22
Conditionally Essential AA									
Arginine	0.71	0.84	0.99	0.80	0.78	0.96	0.69	0.80	0.91
Cysteine	0.25	0.27	0.31	0.28	0.26	0.29	0.24	0.26	0.28
Tyrosine	0.37	0.43	0.50	0.42	0.40	0.48	0.37	0.41	0.45
Non Essential AA									
Alanine	0.52	0.60	0.68	0.58	0.55	0.66	0.50	0.57	0.62
Aspartic acid	0.86	1.08	1.34	0.99	0.99	1.28	0.84	1.02	1.21
Glutamic acid	3.45	3.59	3.84	3.85	3.41	3.85	3.38	3.57	3.61
Glycine	0.56	0.64	0.70	0.62	0.58	0.68	0.54	0.60	0.64
Proline	1.19	1.20	1.25	1.29	1.14	1.27	1.15	1.19	1.19
Serine	0.55	0.61	0.70	0.59	0.55	0.66	0.52	0.54	0.61
Valine	0.62	0.70	0.78	0.71	0.66	0.79	0.62	0.70	0.75
Total	12.94	14.51	16.44	14.50	13.63	16.19	12.64	14.12	15.26

[1]Amino acid assays completed by Experiment Station and Chemical Laboratories, University of Missouri, Columbia, MO USA 65211

3.2.2 Growth Experiment

3.2.2.1 Animal selection, identification and care

All the barrows were individually weighed and sorted by weight and the experiment commenced when the barrows reached a starting weight of 95 ± 3 kg. Forty barrows were selected for each replicate; the 10 barrows with the smallest range in weight were selected for each of the four blocks, within replicate. Pigs were blocked according to weight and replicate was defined as the experiment repeated over time. Within each block, the pigs were randomly assigned to one of the 9 dietary treatments, and one pig in each block was assigned to the initial slaughter group (ISG). In total, this experiment was replicated 3 times, providing a total of 12 barrows per treatment (108 treatment pigs), 12 ISG pigs, and 120 barrows in total.

The experiment ended when the barrows reached a final live weight of 108 ± 3 kg or 120 ± 3 kg; selection of slaughter weight within treatment and replicate was random. Therefore, at each selection point, mid-point (108 ± 3kg) and end of the experiment (120 ± 3 kg), 6 pigs per treatment were in each weight group. The ISG pigs were randomly selected from all available pigs within a rep, to ensure they were representative of the pigs that were placed on test. The ISG pigs were sacrificed at the start of the experiment to provide initial baseline carcass composition.

Barrows were housed in individual pens measuring 0.91 m x 1.83 m (total 1.67 m^2). Each pen was equipped with a single-space dry feeder and a nipple drinker. Floors were made of slatted concrete and penning was made of PVC planking. The temperature was set to 18 °C, consistent with the thermoneutral zone for pigs of this size housed individually (Stanier et al., 1984). The lights were set on an automatic timer to turn on at 0700 h and off at 1900 h. Logbooks were kept to record daily observations and activities within the experimental room. Room temperature, room observations and health records of all pigs were recorded daily

Pigs received their dietary treatments, as well as water, ad libitum. Weekly pig weights and feeder weigh backs were recorded to calculate ADG, ADFI and G:F.

3.2.2.2 Slaughter procedure

When pigs reached the preselected slaughter weight, they were taken off test and all feed was removed the day prior to slaughter. As well, a live body weight was recorded on the morning of slaughter. Pigs were euthanized by captive bolt stunning, followed by exsanguination; all blood was collected into individual poly bags (6 mil Poly Bags, Uline, Waukegan, IL) and returned to the carcass after procedure completion. The carcass was split down the midline from the groin to the chest cavity and the entire gastrointestinal tract (GIT) was removed, emptied of digesta and patted dry. The gall and urinary bladders were also drained of contents. The emptied GIT was then returned to the carcass and an empty body weight recorded. The carcasses were placed in poly bags (6 mil Poly Bags, Uline, Waukegan, IL), labelled and frozen at –20 ºC for later grinding.

Carcasses were ground using an Autio GHP Grinder (model 801, Autio Company, Astoria, OR). The carcass was passed through a 12 mm die once and then a 6 mm die three times. A subsample was obtained on the final mix by taking random grab samples as the mince passed from the grinder. The grab samples were thoroughly mixed and 3 subsamples collected and frozen at -20 ºC. One subsample was freeze-dried in a model 25 LE Virtis freeze dryer (Virtis Co. Ltd.; Gardiner, NY) for later analyses while the 2 archive samples were stored at –20 ºC.

3.2.3 Metabolism experiment

3.2.3.1 Animal selection, identification and care

Animals required for the metabolism experiment were selected in a manner identical to that employed for the growth experiment. However, the pigs were grouped within weekly outcome groups to provide 18 barrows, 2 per treatment, at each start-up. Three replicates were employed, providing a total of 6 pigs per treatment; therefore, 54 pigs were required in total. Replicate was defined as the experiment repeated over time.

Pigs were housed in metabolism pens (1.5 m x 1.5 m) that allowed free movement and thus encouraged feed intake similar to that of pigs housed in growth pens. The metabolism pens had walls made of PVC planking with Tenderfoot® (Tandem Products Inc, Minneapolis, MN USA) floor and windows in each crate which allowed for visual but not physical, contact between pigs. These pens were equipped with a single-space dry feeder which were designed to

minimize feed wastage. Pigs were provided feed twice daily, as much as they could consume within 1 h. Feed was weighed before and after feeding periods to determine daily feed intake. Water was weighed and presented to the pigs after the feeding periods, ad libitum. Before the next feeding, remaining water was weighed back for calculation daily water intake. Daily logbooks were kept in the same manner as the growth experiment.

Animals remained in the crates for a period of 20 days; a 5-day acclimation period was employed, then they were placed on their assigned experimental diet (day 0) for a period of 15 days, with collection occurring on days 6 to 8 and 13 to 15, inclusive. Pigs were weighed at the beginning of the study (day 0) and the end of the experiment (day 15), allowing calculation of ADG. The average starting weight for all pigs was 95 ± 3kg, to correspond with the growth experiment. ADFI, ADWI and G:F were recorded for the experimental period.

3.2.3.2 Sample Collection

The pens contained urine collection trays located under the floor, to facilitate quantitative collection of urine. A screen was placed above the collection trays and glass wool above the funnel to provide a barrier for prevention of contamination of urine with faeces. Total faecal collection was accomplished by collection after every meal and throughout the day and early evening. Faeces were collected directly when the pig voided and off the floor of the pen; therefore, constant monitoring of test pens occurred. Any faeces that fell through the penning were collected off the screen. An indigestible marker, a source of insoluble ash (Celite™, World Minerals, Santa Barbara, CA USA,) was added to these diets for digestibility calculations, in the event that any faeces were lost. Faeces were pooled, weighed and frozen at -20 ºC after each day of the collection period.

Urine was collected quantitatively into ~4 litre collection vessels placed under the metabolism pens. Thirty ml of 6 N HCl was added to each container every day to maintain a low pH and thus minimize nitrogen losses due to volatilization. pH strips were used to randomly test the urine ensuring it remained below pH 2. Total urine produced was weighed and a 10 % aliquot was measured and stored at -20 °C. The samples were pooled within pig within the collection period.

3.2.4 Sample Preparation

Feed samples were collected during the course of the experiment, pooled and homogenized at the end of the experiment. The faecal samples, within pig and period, were thawed and homogenized using a tilt head mixer (model Accolade 400 series, Kitchen Aid, Mississauga, ON). Approximately 250 g of carcass and faecal samples were weighed, refrozen and freeze dried in a model 25 LE and 25 ES, Virtis freeze-dryer, respectively (Virtis Co. Ltd.; Gardiner, NY). Samples were reweighed after freeze drying. Carcass samples were blended in a Moulinex DPA2 800 Watt food processor (Group SEB Canada; Scarborough, ON**).** Carcass, faecal and feed samples were then ground through a 1.0 mm screen in a rotor mill (Model ZM1, Retsch, Newtown, PA). Urine samples, within pig and period, were thawed and mixed; two 170 ml urine samples were measured and retained.

3.2.5 Chemical Analysis

Feed was assayed before being fed to experimental animals for complete amino acid profile (Table 3.3) by the University of Missouri according to method 982.30 E (a,b,c) (AOAC. 2006). A commercial lab (Norwest Labs, Lethbridge, AB) analyzed crude protein and sulphur by the combustion method (method 990.30; AOAC. 1990), moisture by the gravimetric method (method 935.29; AOAC. 1990), and calcium, phosphorus, potassium, magnesium, manganese, sodium, zinc, copper, iron and molybdenum by ICP using method 985.01 (AOAC. 1990) (Table 3.2). The assay for RAC was also conducted before the commencement of the experiment, and repeated 4 times throughout the course of the experiment, to ensure activity remained constant. The assay was conducted by HPLC (method B04372, University of Guelph, Guelph, ON).

After the experimental periods, moisture content of freeze dried carcass, freeze dried faecal and feed samples was determined by drying at 135 °C in an airflow oven for 2 hr (method 930.15: AOAC. 1990). Two 5 g wet faecal samples were weighed and dried at 135 ± 3 °C in an airflow type oven for 4 hours to determine faecal DM. Gross energy on feed and faecal samples was determined using a bomb calorimeter (model C5003, IKA Calorimeter, Wilmington, NC). Benzoic acid was utilized as the standard for calibration. Nitrogen was determined on carcass, feed, urine and faecal samples by combustion (method 968.06; AOAC. 1990) using a Leco protein/nitrogen apparatus (Model FP-528, Leco Co.; St. Joseph, MI) and CP was calculated as nitrogen × 6.25. EDTA was used daily as a calibration standard. Crude fat in feed and carcass

samples were determined using a Soxhlet apparatus and with ethyl ether for feed and petroleum ether for carcass samples (method 920.39; AOAC. 1990). Ash content of feed and carcass samples was determined by incineration in a muffle furnace at 600°C for 12 h (method 942.05; AOAC. 1990). Acid Insoluble Ash (AIA) was determined for feed and faecal samples by insoluble ash gravimetry after treatment with 4N HCl (modified method; McCarthy et al., 1974). Calcium and phosphorus were determined by the methods described by Ryan and Estefan (2002). Atomic absorption spectrophotometry was utilized to determine calcium content (Perkin-Elmer Model 4000, Norwalk, CT). Phosphorus was determined spectrophotometrically at 405 nm in a Pharmacia LKB-Ultrospec III spectrophotometer model 80-2097-62 (Pharmacia Corp, Uppsala, Sweden). All samples were analysed in duplicate and repeated if coefficient of variation (CV) values were higher than the predetermined percentage. The CV values less than 1 % were used for GE in feed and faeces, less than 3 % for CP (feed, carcass and faecal samples), moisture (feed, faeces and carcass), ash in feed and faecal AIA. The CV values less than 5 % were taken for CP in urine, carcass ash, AIA in feed, calcium and phosphorus in feed and crude fat in carcass and a CV value of < 10 % was used for crude fat in feed.

3.2.6 Statistical Analysis

Individual pig was considered the experimental unit in both the metabolism and growth experiments. All data were analyzed by ANOVA using the MIXED procedure of SAS (SAS Inst. Inc.; Cary, NC). The metabolism experiment was designed using a randomized complete block design, with initial weight as the blocking factor. For the growth experiment, dietary treatment and slaughter weight were the main effects arranged as a 3 x 3 x 2 factorial design, which allowed for determination of main effects of RAC and lysine levels and slaughter group and any interactions. An initial body weight covariate was utilized for growth performance parameters.

Growth performance for the metabolism experiment was analyzed as a completely randomized design with treatments arranged as a 3 x 3 factorial, with initial body weight as a covariate. Chemical analyses of samples were analyzed as a repeated measure with sampling period in days and the Toeplitz model was used as the covariance structure. For the metabolism experiment main effects were dietary treatment, arranged as a 3 x 3 factorial which allowed for determination of main effects of RAC and lysine levels, as well as their interaction. Data are

reported as least square means. In all cases $P < 0.05$ was considered significant and trends were reported with P values between $0.05 < P < 0.10$.

3.3 Results

3.3.1 Growth Experiment

For the entire experimental period, there were neither feed refusals nor health issues. The only significant treatment interactions were: percent ash (SID lysine x Slaughter Weight; Figure 3.1) and percent water in the carcass (RAC x replicate (rep); Figure 3.2) and water deposition rate (RAC x rep; Figure 3.3). Significant interactions are denoted by a footnote in the appropriate tables, graphed, and presented as figures; otherwise, only main effects are shown.

3.3.1.1 Growth Performance

RAC had no effect on ADG, ADFI or G:F (Table 3.4; $P > 0.10$). Lysine had no effect on ADG or ADFI ($P > 0.10$). However, G:F increased with high dietary lysine concentration ($P < 0.05$). ADFI was higher in the 120 kg slaughter weight treatment ($P < 0.05$) when compared to the 108 kg slaughter weight. Slaughter weight did not affect ADG or G:F ($P > 0.05$).

Table 3.4 Effect of RAC, lysine and slaughter weight on growth rate, feed intake and feed conversion in finishing barrows[1]

Item	Initial Body Weight, kg	ADG, kg/d	ADFI, kg/d	G:F, kg/kg
RAC (ppm)				
0	96.5	1.4	4.0	0.35
5	95.9	1.4	3.9	0.36
10	96.0	1.5	3.8	0.38
Lysine (g/Mcal)				
1.75	95.9	1.4	4.0	0.35
2.25	96.3	1.4	3.9	0.35
2.75	96.3	1.5	3.9	0.39
Rep				
1	97.1	1.3	3.7	0.36
2	96.2	1.5	4.1	0.36
3	95.1	1.5	4.0	0.37
SEM[2]	*0.56*	*0.06*	*0.11*	*0.01*
Slaughter Weight (kg)				
108	95.9	1.4	3.8	0.37
120	96.4	1.5	4.0	0.36
SEM	*0.52*	*0.05*	*0.10*	*0.01*

Statistics		P-value		
RAC	-[3]	0.775	0.277	0.164
Lysine	-	0.232	0.783	0.029
RAC x Lysine	-	0.636	0.108	0.756
Rep	-	0.167	0.005	0.752
Slaughter Weight	-	0.307	0.009	0.636

[1] Data expressed as least square means. Data analyzed with initial body weight as a covariate. The covariate was not significant for any measurement: ADG (P=0.559), ADFI (P=0.666) or G:F (P=0.741).

[2] n=36 for RAC, lysine and rep, and therefore SEM values are presented in one row.

[3] (–) indicates no statistics were calculated on that parameter

3.3.1.2 Nitrogen Balance

There was a numerical increase in N retention with greater RAC inclusion (Table 3.5), whether expressed as amount per day ($P = 0.111$) or as percent of intake ($P=0.109$). Lysine improved N intake ($P < 0.001$), and N retention, when expressed as grams per day ($P < 0.05$) but not when expressed as a percent of intake ($P > 0.10$) and tended to increase N excretion when expressed as grams per day ($P < 0.10$), but not when expressed as a percent of intake ($P > 0.10$). The pigs slaughtered at 120 kg retained more N than the 108 kg slaughter weight treatment pigs when expressed as grams per day ($P < 0.05$) and tended to have higher N retention and excretion, expressed as a percent of intake ($P < 0.11$).

Table 3.5 The effect of RAC, lysine and slaughter weight on nitrogen balance in finishing barrows[1]

Item	N Intake,	N Retention,	N Excretion[2],	N Retention,	N Excretion,
		g/d		% intake	
RAC (ppm)					
0	91.0	25.9	65.0	28.8	71.2
5	92.4	29.7	62.7	32.3	67.7
10	89.5	30.3	59.3	34.1	66.0
Lysine (g/Mcal)					
1.75	84.1	25.6	58.6	30.7	69.3
2.25	91.5	28.6	62.9	31.5	68.5
2.75	97.2	31.7	65.6	32.9	67.1
Rep					
1	87.6	29.7	58.0	33.8	66.7
2	91.8	26.2	65.6	28.7	71.3
3	93.4	30.1	63.4	32.6	67.4
SEM[3]	*1.94*	*1.72*	*2.51*	*1.98*	*1.98*
Slaughter Weight (kg)					
108	89.7	26.6	63.1	30.0	70.0
120	92.2	30.7	61.6	33.4	66.6
SEM	*1.65*	*1.47*	*2.17*	*1.70*	*1.70*

Statistics	P values				
RAC	0.532	0.111	0.183	0.109	0.109
Lysine	<0.001	0.027	0.083	0.675	0.675
RAC x Lysine	0.227	0.786	0.479	0.933	0.933
Rep	0.063	0.159	0.046	0.107	0.107
Slaughter Weight	0.224	0.027	0.573	0.108	0.108

[1]Data expressed as least square means.
[2]N excretion calculated as the difference between N intake and N retention.
[3]n=36 for RAC, lysine and rep, and therefore SEM values are presented in one row.

3.3.1.3 Carcass Composition

Body composition as a percent of empty body weight (EBW) of the ISG pigs and the treatment pigs is shown in Table 3.6. In RAC fed pigs, CP and water content of the carcass was higher (Table 3.6; $P < 0.05$), and CF was lower ($P < 0.05$) than controls. RAC-fed pigs tended to have higher EBW than controls ($P < 0.10$). Ash content was similar among all treatments ($P > 0.10$). Carcass CP numerically increased ($P = 0.103$) and water content tended to increase ($P < 0.10$) with greater dietary lysine concentration but carcass ash, EBW and CF content remained unchanged among lysine treatments ($P > 0.10$). Carcass CF ($P < 0.05$), CP, ash and water contents and EBW ($P < 0.001$) were higher in the pigs slaughtered at 120 kg.

There was an interaction between dietary lysine concentration and slaughter weight on percent ash in the empty carcass (Figure 3.1; $P < 0.05$). The pigs slaughtered at 108 kg had small differences in percent ash among lysine treatments. However, with increased dietary lysine the percent ash in the empty carcass declined in the pigs slaughtered at 120 kg. There was also an interaction between RAC and rep on percent carcass water content (Figure 3.2; $P < 0.05$). The pigs in rep 1 had higher carcass water content with increased RAC levels, but this did not occur with the pigs in rep 2 and 3.

Table 3.6 Effect of RAC, lysine and slaughter weight on body composition of treatment finishing barrows[1]

	EBW, kg	CP	CF	Ash[2]	Water[3]
		Body Composition, % of EBW			
ISG Barrows[4]					
Rep					
1	90.5	12.5	16.1	2.1	40.1
2	89.6	13.0	13.4	2.3	39.1
3	88.6	13.0	14.0	2.2	40.3
Treatment Barrows[5]					
Item					
RAC (ppm)					
0	107.2	15.0	22.8	2.6	66.8
5	107.5	15.4	20.7	2.6	68.8
10	108.7	15.5	21.9	2.6	68.6
Lysine (g/Mcal)					
1.75	107.4	15.2	22.5	2.6	67.4
2.25	107.9	15.3	22.0	2.6	67.9
2.75	108.0	15.5	21.0	2.5	68.9
Rep					
1	108.3	15.2	22.7	2.5	67.8
2	106.8	15.2	20.7	2.6	68.3
3	108.3	15.6	22.1	2.6	68.2
SEM[6]	*0.56*	*0.12*	*0.52*	*0.03*	*0.52*
Slaughter Weight (kg)					
108	103.2	14.6	20.8	2.5	65.3
120	112.4	16.1	22.9	2.7	70.8
SEM	*0.49*	*0.10*	*0.42*	*0.02*	*0.44*

Statistics			P values		
RAC	0.050	0.005	0.019	0.384	0.007
Lysine	0.624	0.103	0.117	0.363	0.079
RAC x Lysine	0.660	0.990	0.798	0.470	0.680
Rep	0.028	0.013	0.029	0.057	0.745
Slaughter Weight	<0.001	<0.001	0.001	<0.001	<0.001

[1]Data expressed as least square means. Any column denoted with (–) indicates no statistics were calculated on that parameter

[2]The interaction between Lysine x Slaughter weight was significant (P=0.022, Figure 3.1)

[3]The interaction between RAC x rep was significant (P=0.001, Figure 3.2).

[4]Data expressed as arithmetic means.

[5]Statistics calculated for treatment barrows only.
[6]n=36 for RAC, lysine and rep, and therefore SEM values are presented in one row.

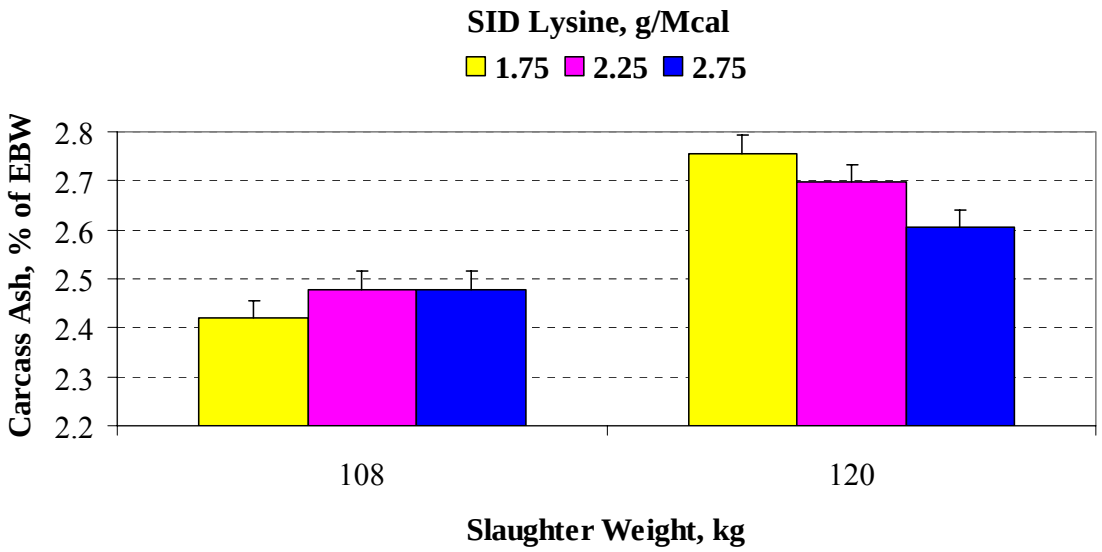

Figure 3.1 Interaction between dietary SID lysine and slaughter weight on percent of ash in the empty pig carcass (P=0.022).
Y error bars represent the SEM.

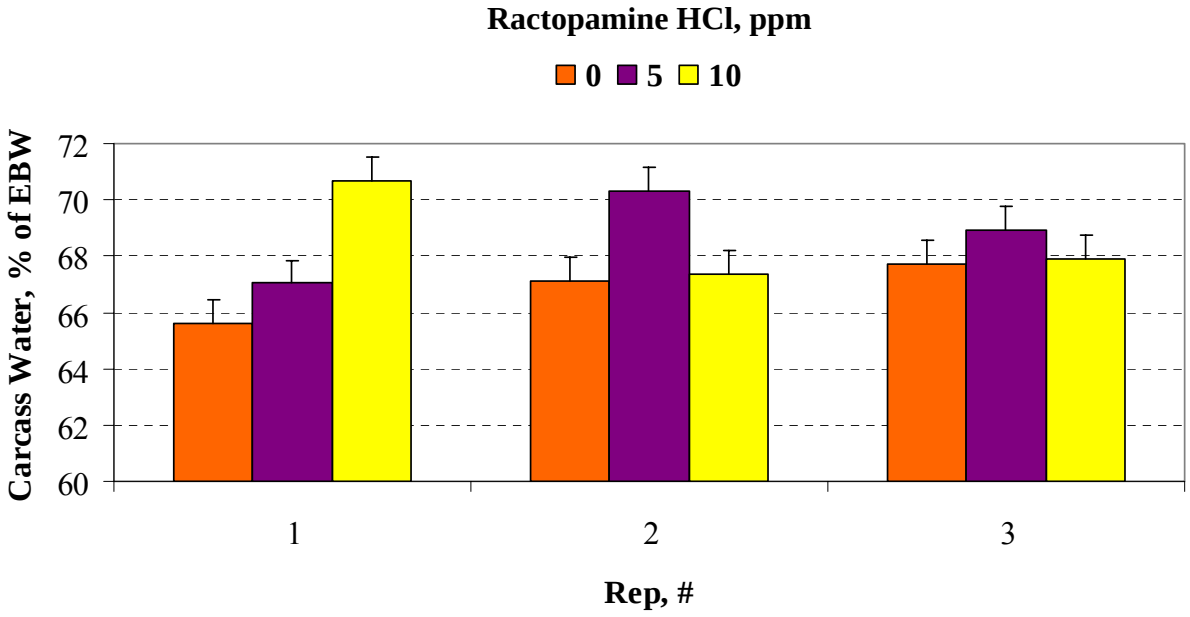

Figure 3.2 The interaction between RAC and rep on percent of water in the empty pig carcass (P=0.001).
Y error bars represent the SEM.

3.3.1.4 Nutrient Deposition Rates

RAC numerically increased protein deposition rates ($P = 0.111$), tended to increase water deposition rate ($P < 0.10$), and tended to reduce fat deposition rate ($P < 0.10$; Table 3.7). Protein deposition rate increased in response to higher lysine concentrations ($P < 0.05$). Dietary lysine concentration did not result in any differences in the deposition rate of crude fat or ash ($P > 0.10$) but tended to increase the water deposition rate ($P < 0.10$). The 120 kg slaughter weight pigs had increased deposition rates of protein, fat and water ($P < 0.05$) compared to the barrows slaughtered at 108 kg. There was also an interaction between RAC and rep on water deposition rates (Figure 3.3; $P < 0.05$). The pigs in rep 1 had higher water deposition rates with increased RAC levels, but this did not occur with the pigs in rep 2 and 3.

Table 3.7 The effect of RAC, lysine and slaughter weight on carcass nutrient deposition rates in finishing barrows[1]

	CP	CF	Ash	Water[2]
Item		g/d		ml/d
RAC (ppm)				
0	162.1	619.8	26.3	466.3
5	185.4	461.6	25.2	608.7
10	189.2	542.3	27.1	572.5
Lysine (g/Mcal)				
1.75	160.0	574.2	24.1	479.0
2.25	178.8	553.3	28.7	548.9
2.75	198.0	496.2	25.8	619.7
Rep				
1	185.4	475.9	26.6	527.3
2	163.5	562.6	22.9	551.4
3	187.8	585.2	29.1	568.9
SEM[3]	*10.77*	*49.68*	*2.18*	*45.80*
Slaughter Weight (kg)				
108	166.1	600.1	24.2	476.7
120	191.7	482.4	28.3	621.7
SEM	*9.20*	*42.22*	*1.78*	*38.97*

Statistics	P values			
RAC	0.111	0.055	0.837	0.050
Lysine	0.027	0.461	0.329	0.066
RAC x Lysine	0.786	0.754	0.338	0.726
Rep	0.159	0.209	0.141	0.782
Slaughter Weight	0.027	0.029	0.109	0.004

[1]Data expressed as least square means.
[2]The interaction between RAC x rep was significant P=0.013. See Figure 3.3.
[3]n=36 for RAC, lysine and rep, and therefore SEM values are presented in one row.

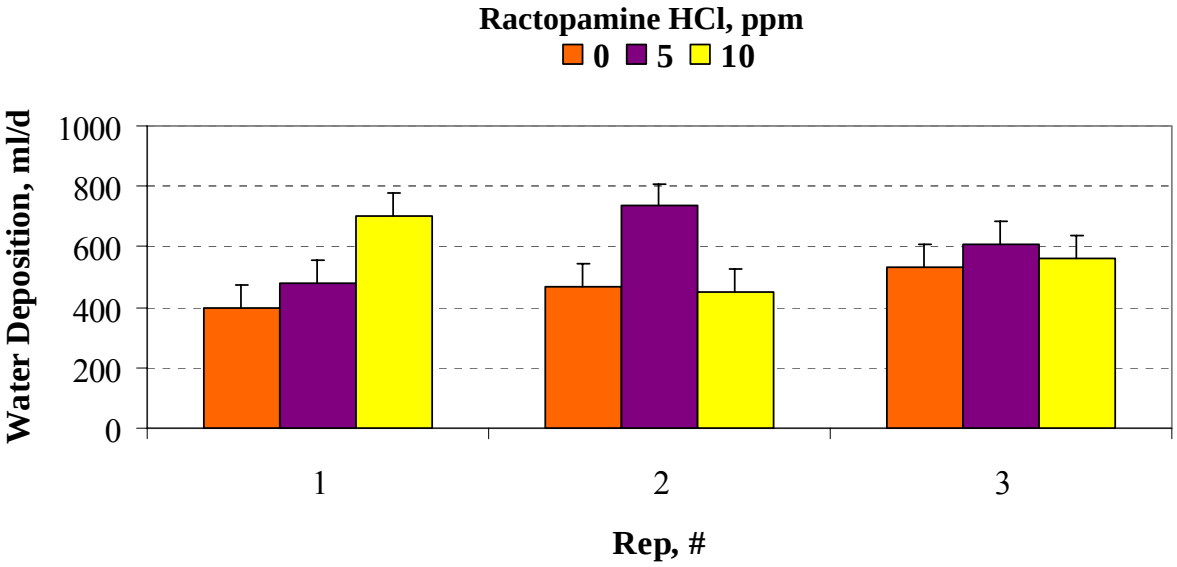

Figure 3.3 The interaction between RAC and rep on the water deposition rate in finishing barrows (P=0.013).
Y error bars represent the SEM.

3.3.2 Metabolism Experiment

One pig (on RAC 5 ppm and lysine 2.25 g/Mcal treatment) was removed from the experiment due to a leg injury. There were no other health issues or feed refusals.

Only main effects of treatment, sampling period and replicate (rep) are presented in Tables 3.8 and 3.9. A RAC x lysine interaction was observed ($P < 0.05$) for many N balance parameters (Table 3.10 and 3.11) so all significant interactions are presented as Figures (Figure 3.4 to 3.9). Rep was included in the majority of the tables due to its high statistical significance ($P < 0.05$).

3.3.2.1 Growth performance and water balance

For the overall experiment (Table 3.8), final body weight, ADG ($P < 0.05$) and G:F ($P < 0.001$) increased and ADFI tended to decrease ($P < 0.10$) as RAC concentration increased. Overall average daily water intake (ADWI) was not different among RAC treatments ($P > 0.10$). Final body weight, ADG ($P < 0.05$) and G:F ($P < 0.001$) increased and ADFI ($P < 0.05$) decreased with higher lysine levels. Lysine concentration showed no effect on ADWI ($P > 0.10$).

Table 3.9 describes ADFI, water balance and urine and faecal outputs during the sampling periods. Collection of these data occurred on days 6-8 and 13-15; therefore two sampling periods were included in statistical analysis. During the sampling period, a decrease in daily water intake, urine output, and total water excretion (urine output and faecal moisture) ($P < 0.05$) was observed with increased RAC levels. RAC numerically decreased apparent water retention ($P = 0.102$). The 10 ppm RAC level tended to decrease ADFI ($P < 0.10$). Dry faecal output was highest for the 5 ppm RAC-fed pigs when compared to the 0 and 10 ppm groups ($P < 0.05$). Higher lysine concentrations decreased daily faecal output ($P < 0.001$) and tended to decrease ADFI ($P < 0.10$). Dietary lysine levels showed no effect on water intake, excretion, retention or urine output ($P > 0.10$).

During the 13-15 d sampling period the pigs had higher ADFI (Table 3.9, $P < 0.001$), urine output, dry faecal output and water excretion ($P < 0.05$) when compared to the day 6-8 d period. The day 13-15 pigs tended to have higher water intake ($P < 0.10$). There was no sampling period effect on water retention ($P > 0.10$).

Table 3.8 The effects of RAC and lysine on final body weight, growth rate, feed intake, feed efficiency and water intake in finishing barrows[1]

	Body Weight, kg		ADG,	ADFI,	G:F	ADWI,
Item	Initial	Final	kg/d[2]	kg/d[2,3]	kg/kg[2,3]	l/d[2]
RAC (ppm)						
0	93.8	110.2	1.1	3.2	0.34	7.6
5	93.8	112.9	1.3	3.2	0.39	7.2
10	94.1	112.7	1.3	3.0	0.41	6.8
SEM	*0.65*	*0.54*	*0.04*	*0.06*	*0.01*	*0.29*
Lysine (g/Mcal)						
1.75	93.5	110.9	1.1	3.3	0.35	7.2
2.25	94.2	112.9	1.3	3.1	0.40	6.9
2.75	94.0	112.0	1.2	3.0	0.40	7.5
SEM	*0.65*	*0.54*	*0.04*	*0.06*	*0.01*	*0.29*
Rep						
1	94.0	113.8	1.3	3.4	0.40	7.1
2	96.9	113.8	1.3	3.3	0.40	7.4
3	90.8	108.2	0.96	2.7	0.35	7.1
SEM	*0.65*	*0.65*	*0.04*	*0.07*	*0.01*	*0.35*

Statistics		P-value				
RAC	-[4]	0.002	0.002	0.051	<0.001	0.144
Lysine	-	0.039	0.039	0.027	<0.001	0.311
RAC x Lysine	-	0.654	0.650	0.918	0.579	>0.999
Rep	-	<0.001	<0.001	<0.001	0.003	0.758

[1]Data expressed as least square means. Data analyzed with initial body weight as a covariate. The covariate was significant for ADFI (P<0.001), feed conversion (P=0.034) and final body weight (P < 0.001) but not for ADG (P=0.318) or ADWI (P=0.809).
[2]Calculated based on 15 d experimental period.
[3]As-fed basis.
[4](–) indicates no statistics were calculated on that parameter
.

Table 3.9 The effect of RAC and lysine on feed and water intake, faecal and urine output, water excretion and retention in finishing barrows[1]

Item	ADFI (dry basis), kg/d[2]	Water Intake, l/d[2,3]	Faecal Output (dry basis), kg/d[4]	Urine Output, l/d	Water Excretion, l/d[5]	Apparent Water Retention, l/d[6]
RAC (ppm)						
0	2.8	8.3	0.4	3.5	3.9	4.4
5	2.9	7.9	0.5	3.2	3.6	4.4
10	2.7	7.3	0.4	2.9	3.2	4.1
SEM	*0.05*	*0.25*	*0.01*	*0.18*	*0.18*	*0.12*
Lysine (g/Mcal)						
1.75	2.9	7.9	0.5	3.2	3.6	4.4
2.25	2.8	7.5	0.5	3.0	3.3	4.2
2.75	2.7	8.1	0.4	3.4	3.7	4.4
SEM	*0.05*	*0.25*	*0.01*	*0.18*	*0.18*	*0.12*
Rep						
1	3.1	7.8	0.5	3.3	3.6	4.1
2	3.1	8.0	0.5	3.3	3.7	4.4
3	2.3	7.8	0.4	3.1	3.3	4.4
SEM	*0.05*	*0.23*	*0.01*	*0.17*	*0.17*	*0.11*
Sample Period (days)						
d 6-8	2.7	7.7	0.4	3.0	3.4	4.3
d 13-15	2.9	8.0	0.5	3.3	3.7	4.3
SEM	*0.04*	*0.15*	*0.01*	*0.12*	*0.12*	*0.09*

Statistics	P values					
RAC	0.057	0.017	0.018	0.031	0.033	0.102
Lysine	0.053	0.186	<0.001	0.221	0.276	0.337
RAC x Lysine	0.846	0.994	0.060	0.840	0.769	0.125
Rep	<0.001	0.582	<0.001	0.602	0.264	0.087
Sample Period	<0.001	0.051	0.025	0.022	0.014	0.828

[1]Data expressed as least square means. Data analyzed as repeated measures with sampling periods and the Toeplitz model used for the covariance structure.
[2]ADFI and water intake calculated based on d 4-8 and d 11-15 to account for N and water retention before sampling periods.
[3]Water intake included water consumption and diet moisture.
[4]Faecal output calculated using indigestible marker data.
[5]Water excretion is the sum of faecal water output and urine output.
[6]Apparent water retention calculated as the difference between water intake and urine and faecal excretion and other moisture losses (i.e. respiration) are not accounted for.

3.3.2.2 N balance: grams per day

N intake, N digestibility, urinary N excretion, faecal N excretion and total N excretion decreased and N retention increased ($P < 0.05$) when dietary RAC concentrations were increased (Table 3.10).

N intake, N digestibility ($P < 0.001$), urinary N excretion, total N excretion and N retention ($P < 0.05$) increased with higher dietary lysine concentration (Table 3.10, $P < 0.001$) but faecal N excretion was unaffected ($P > 0.10$).

During d 13-15 the barrows had higher N intake (Table 3.10, $P < 0.001$), urinary N excretion, faecal N excretion, total N excretion and N retention ($P < 0.05$) when compared to d 6-8. N digestibility was unaffected by sampling period ($P > 0.10$).

A RAC x lysine interaction occurred ($P < 0.05$) for N digestibility and N retention (grams per day). The barrows fed 2.25 or 2.75 g/Mcal lysine had improved N retention over the control pigs (Figure 3.4). At the 1.75 g/Mcal lysine level, pigs fed 0 and 10 ppm RAC had improved N retention compared to the pigs receiving 5 ppm RAC. N digestibility increased in control pigs fed 1.75 and 2.25 g/Mcal lysine (Figure 3.5), however pigs fed 2.75 g/Mcal lysine had improved N digestibility with RAC inclusion.

Table 3.10 The effect of RAC and lysine concentration on nitrogen balance in finishing barrows[1]

Item	N Intake, g/d	N Digestibility, %[2]	Urinary N Excretion, g/d	Faecal N Excretion, g/d	Total N Excretion, g/d	N Retention, g/d[3]
RAC (ppm)						
0	80.5	84.4	28.5	12.6	41.1	39.4
5	84.1	83.2	25.5	14.1	39.6	44.5
10	77.0	83.8	23.3	12.6	35.9	41.1
SEM	*1.43*	*0.26*	*0.95*	*0.37*	*1.12*	*1.03*
Lysine (g/Mcal)						
1.75	76.0	83.0	24.6	13.0	37.6	38.4
2.25	80.4	83.7	24.1	13.2	37.3	43.0
2.75	85.2	84.8	28.6	13.1	41.7	43.6
SEM	*1.44*	*0.26*	*0.96*	*0.37*	*1.13*	*1.07*
Rep						
1	87.3	84.1	27.9	13.9	41.8	45.5
2	87.4	82.8	30.0	15.0	45.0	42.4
3	66.9	84.6	19.5	10.4	29.8	37.1
SEM	*1.41*	*0.26*	*0.94*	*0.36*	*1.10*	*1.03*
Sample Period (days)						
d 6-8	77.1	83.7	24.1	12.7	36.8	40.3
d 13-15	89.0	83.9	27.4	13.5	41.0	43.0
SEM	*1.10*	*0.20*	*0.74*	*0.27*	*0.84*	*0.79*

Statistics	P values					
RAC	0.003	0.008	0.001	0.003	0.004	0.003
Lysine	<0.001	<0.001	0.002	0.907	0.010	0.001
RAC x Lysine	0.441	0.001	0.137	0.080	0.072	0.002
Rep	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Sample Period	<0.001	0.412	0.002	0.021	0.001	0.015

[1]Data expressed as least square means. Data analyzed as repeated measures with sampling periods and the Toeplitz model used for the covariance structure.
[2]N digestibility calculated using the indigestible marker data.
[2]N retention calculated as the difference between N intake and excretion.

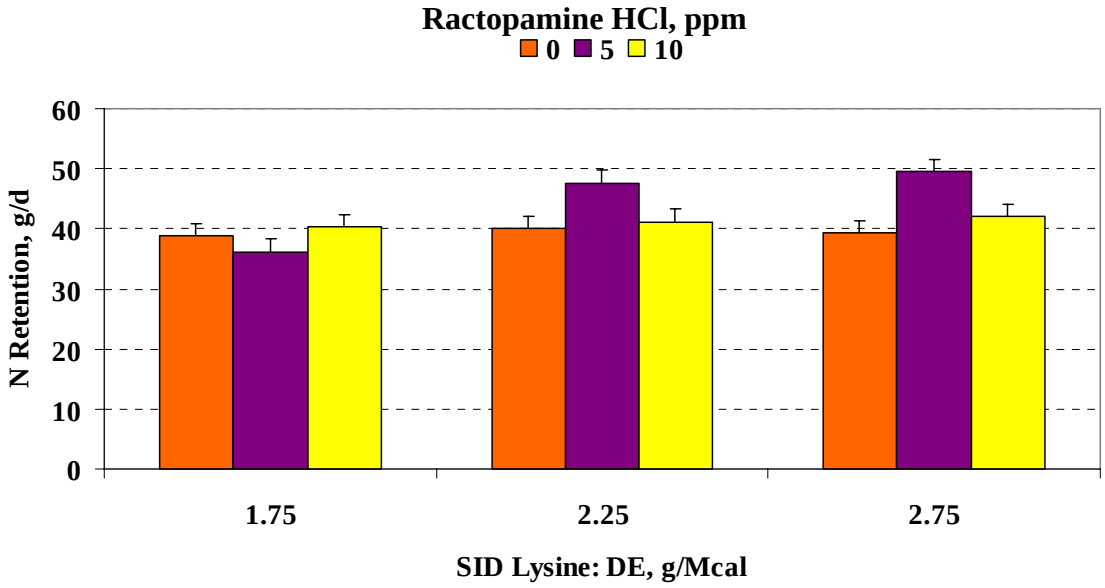

Figure 3.4 Interaction between dietary SID lysine and RAC on N retention (P=0.002). Y error bars represent the SEM.

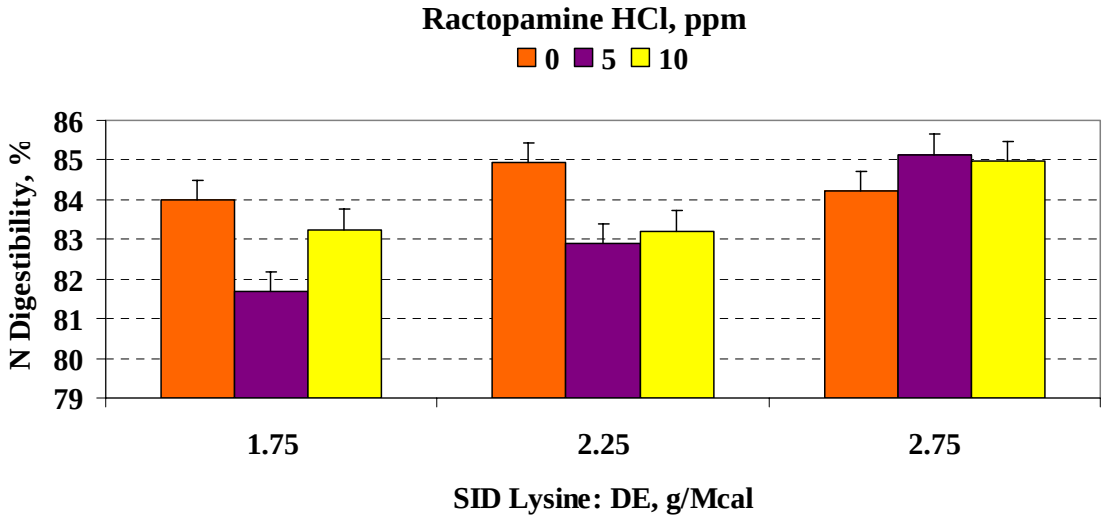

Figure 3.5 Interaction between dietary SID lysine and RAC on N digestibility (P=0.001). Y error bars represent the SEM.

3.3.2.3 N balance: percent of N intake

Urinary N and N excretion ($P < 0.05$), decreased, while N retention and faecal N ($P < 0.05$) increased with higher RAC levels (Table 3.11). The ratio of urinary N: faecal N decreased in the RAC containing treatments ($P < 0.001$).

Faecal N ($P < 0.001$) decreased and N excretion tended to decrease ($P < 0.10$) with higher lysine levels (Table 3.11). Urinary N, tended to be highest for the highest lysine fed barrows and N retention, tended to be highest for the mid level lysine fed barrows ($P < 0.10$). The ratio of urinary N:faecal N was highest for the 2.75 g/Mcal lysine level barrows ($P < 0.05$). The sample period had no affect on N balance ($P < 0.10$).

Faecal N, urinary N, N excretion and N retention, had significant RAC x lysine interactions. RAC increased faecal N, in pigs fed 1.75 and 2.25 g/Mcal lysine but it decreased in pigs fed 2.75 g/Mcal (Figure 3.4). In Figure 3.7, urinary N decreased in RAC fed pigs supplemented with higher lysine levels. However, at the low lysine levels only the pigs fed 10 ppm RAC had decreased urinary N. Pigs fed 10 ppm RAC, regardless of lysine level had decreased N excretion. At the lowest lysine level, pigs receiving 5 ppm RAC had the highest N excretion but at 2.25 and 2.75 g/Mcal the control pigs had the highest N excretion (Figure 3.8). N retention, shown in Figure 3.9, was higher in RAC fed pigs at all lysine levels, except the 5 ppm RAC pigs receiving the lowest lysine level which had decreased N retention.

Table 3.11 The effect of RAC and lysine concentration on urinary N: faecal N ratio, faecal and urinary N, N excretion and retention, all as a percent of N intake in finishing barrows[1]

Item	Urinary N: Faecal N	Faecal N,	Urinary N,	N excretion,	N retention,
		% of N intake			
RAC (ppm)					
0	2.3	15.6	35.1	50.7	49.3
5	1.8	16.8	30.2	47.0	53.0
10	1.9	16.2	29.8	46.0	54.0
SEM	*0.07*	*0.26*	*1.01*	*0.99*	*0.97*
Lysine (g/Mcal)					
1.75	1.9	17.0	32.2	49.2	50.8
2.25	1.9	16.3	29.9	46.2	53.8
2.75	2.2	15.2	33.1	48.3	51.7
SEM	*0.07*	*0.26*	*1.02*	*1.00*	*1.00*
Rep					
1	2.0	16.0	31.9	47.9	52.1
2	2.0	17.2	34.2	51.5	48.5
3	1.9	15.4	29.0	44.4	55.6
SEM	*0.07*	*0.26*	*1.00*	*0.97*	*0.97*
Sample Period (days)					
d 6-8	1.9	16.3	30.9	47.2	52.8
d 13-15	2.1	16.1	32.5	48.6	51.4
SEM	*0.06*	*0.22*	*0.79*	*0.75*	*0.75*

Statistics	P values				
RAC	<0.001	0.008	<0.001	0.003	0.003
Lysine	0.009	<0.001	0.069	0.086	0.086
RAC x Lysine	0.466	0.001	0.036	0.002	0.002
Rep	0.360	<0.001	0.002	<0.001	<0.001
Sample Period	0.139	0.412	0.129	0.154	0.155

[1]Data expressed as least square means. Data analyzed as repeated measures with sampling periods and the Toeplitz model used for the covariance structure.

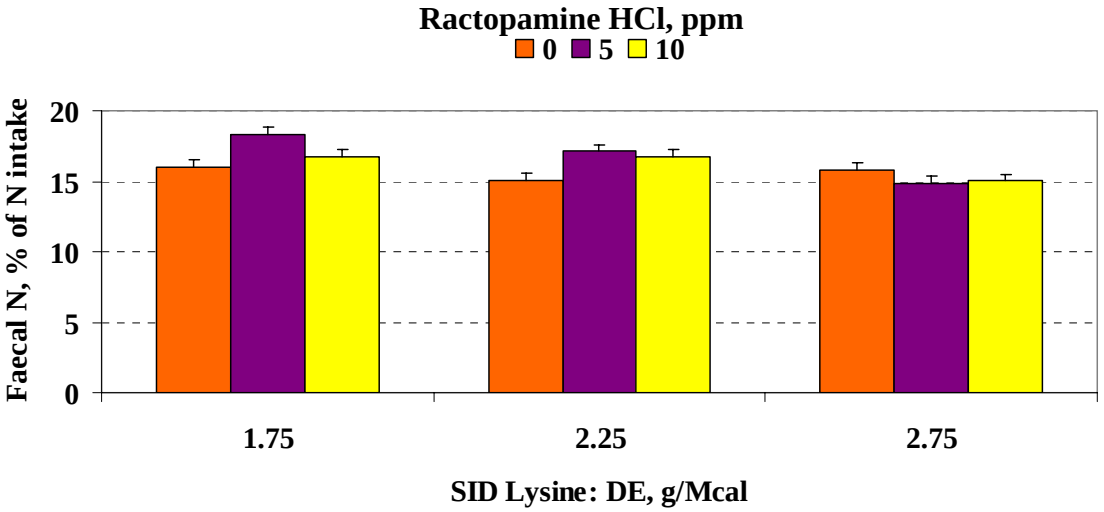

Figure 3.6 Interaction between dietary SID lysine and RAC on faecal N, expressed as a percent of N intake (P=0.001).
Y error bars represent the SEM.

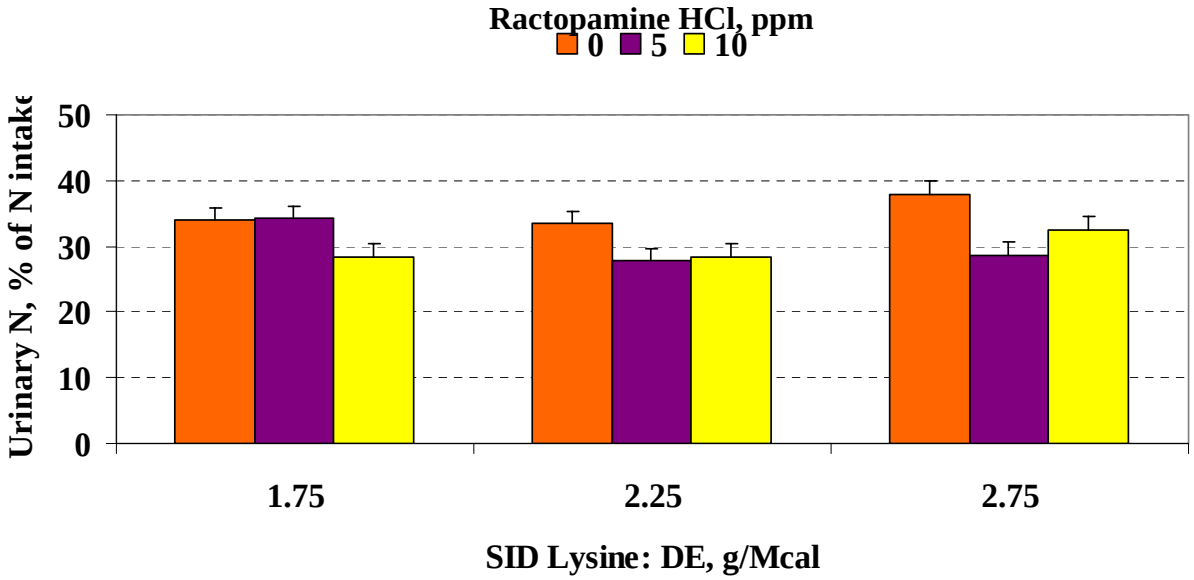

Figure 3.7 Interaction between dietary SID lysine and RAC on urinary N, expressed as a percent of N intake (P=0.036).
Y error bars represent the SEM.

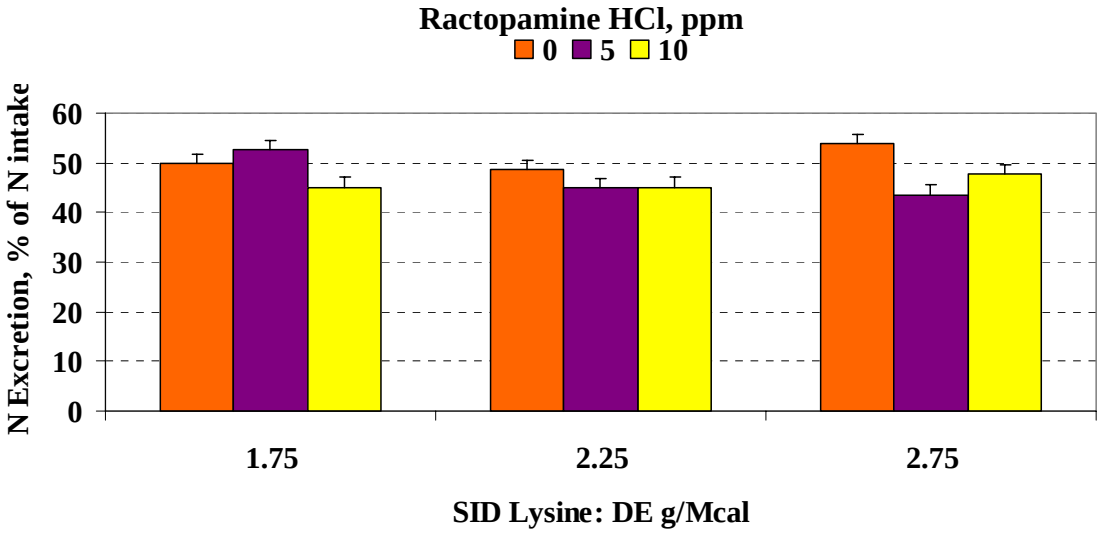

Figure 3.8 Interaction between dietary SID lysine and RAC on total N excretion, expressed as a percent of N intake (P=0.002).
Y error bars represent the SEM.

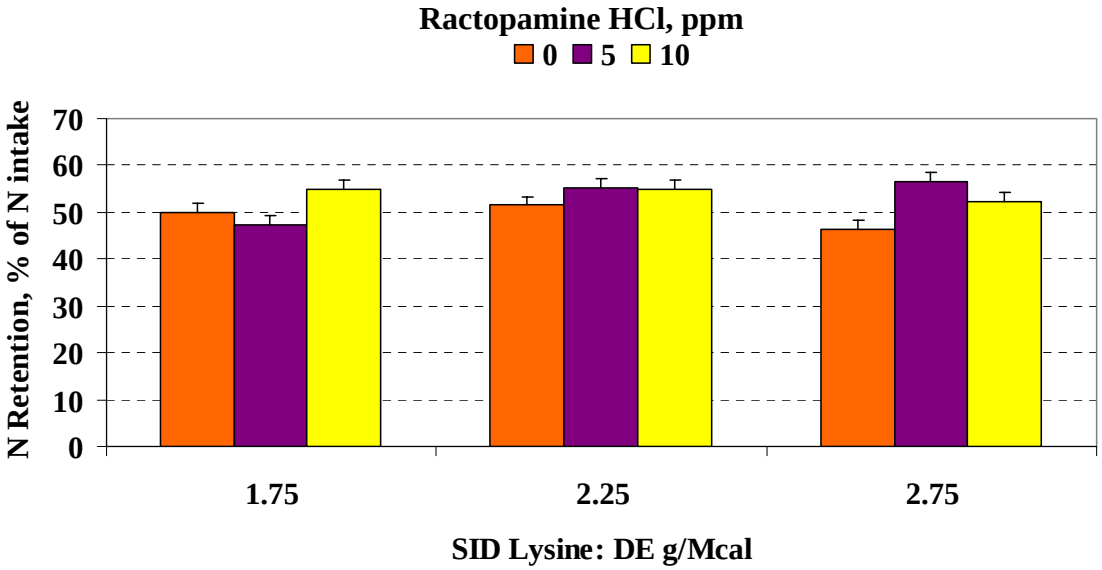

Figure 3.9 Interaction between dietary SID lysine and RAC on N retention, expressed as a percent of N intake (P=0.002).
Y error bars represent the SEM.

3.4 Discussion

The growth and metabolism experiments were conducted to determine if RAC and/or lysine addition to finishing swine diets have any potential environmental benefits.. Dietary treatments were made from the same batch of ingredients and prepared at the same time to limit ingredient variation. Formulation of these diets ensured that differences in diets were due only to different lysine and RAC levels; all other ingredients were kept similar to limit diet variability.

In the metabolism experiment, a large rep effect was noted for some parameters. Rep was left in the model due to the large variation in means from rep 3 when compared to rep 1 and 2. Some of this effect could be due to the high temperatures during the month that rep 3 took place. Rep 1 and 2 had an average morning barn temperature of 15.8 and 18.2 ^{o}C and the average daily high temperature was 20.4 and 21.6 ^{o}C, respectively. On the other hand, rep 3 had an average morning temperature of 22.3 ^{o}C and an average daily high temperature 27.2 ^{o}C (See Appendix A for daily temperatures throughout all 3 reps). Quiniou et al. (2000) reported that voluntary feed intake decreased when the ambient temperature increased. A substantial drop in feed intake occurred in the 90 kg weight group. The highest temperature Quiniou et al. (2000) recorded was 28 ^{o}C but in the present experiment, rep 3 had seven days with temperatures that exceeded 30 ^{o}C. The barn temperatures (Appendix A, Figure 6.2) are lower than the daily maximum temperatures (Appendix A, Figure 6.1) because recording of barn temperature occurred in the morning.

When ADG decreases it is primarily due to a reduction in ADFI but G:F tends to not change (Noblet et al., 2001). However, in the metabolism experiment G:F was decreased in rep 3 to 0.35 compared to 0.40 for rep 1 and 2. The rep effect was noted in many of the measurements but the effect on N balance was especially important. Since ADFI was decreased, N intake was lower, which decreased N excretion and retention.

3.4.1 Response to RAC

3.4.1.1 Growth Performance

In the growth experiment, the effect of RAC on growth performance of the barrows was not significant. The variation in growth performance between the metabolism and growth experiments could be due to intake differences. The pigs in the growth experiment had ad

libitum access to feed and the pigs in the metabolism experiment were allowed to eat as much as possible within two 1 hour periods each day. In the growth experiment, ADG, G:F and ADFI were not affected by dietary RAC inclusion. In the metabolism experiment, ADG, and G:F increased while ADFI decreased with 10 ppm RAC, which corresponds with data found in the literature. In a meta-analysis of 23 published reports, Apple et al. (2007) concluded that RAC supplemented at 5, 10 or 20 ppm significantly improved ADG and G:F in finishing pigs when compared to pigs fed no RAC. With only 5 ppm RAC supplementation and ad libitum feed, Patience et al. (in press) found ADG and G:F increased by 13 % compared to control pigs.

Some reports suggested that RAC does not improve ADG in swine. Carr et al. (2005), Mimbs et al. (2005) and Brumm et al. (2004) reported no effect of 10 ppm RAC on ADG when compared to control pigs; G:F however, was significantly improved. Conversely, See et al. (2005) reported no increase in G:F when 5 and 10 ppm RAC were supplemented. Apple et al. (2007) reported that high dietary inclusion of RAC (20 ppm) significantly decreased ADFI. In the present experiments, no dietary treatments contained 20 ppm RAC; therefore, no response to RAC on ADFI was expected. This was true in the growth experiment but the pigs in the metabolism experiment tended to have reduced ADFI with RAC inclusion. Since dietary treatments were not changed between experiments, the differences in growth performance between the growth and metabolism experiment must have been due to the feeding methods between experiments. The pigs in the growth experiment had higher feed intakes; therefore these pigs potentially reached their maximum performance without responding to RAC.

3.4.1.2 Carcass Composition

The repartitioning effect of RAC on protein and lipid tissue is well known. Pigs fed 20 ppm RAC had decreased lipid content in the empty carcass (Dunshea et al., 1993, Dunshea et al., 1998) and longismuss muscle (LM) (Xiao et al., 1999 and Uttaro et al., 1993). There is an inverse relationship with lipid and water content within swine tissue; therefore it would be assumed that a leaner pig would require more water to sustain the increase in protein deposition (Apple et al., 2007). In the growth experiment, the addition of either 5 or 10 ppm RAC increased the protein and water content of the carcasses, while lipid content decreased and ash content remained unchanged. Similar results using 20 ppm RAC were reported by Xiao et al. (1999) and Uttaro et al. (1993) in the LM and Dunshea et al. (1993) and Dunshea et al. (1998) in the whole

carcass (EBW). Conversely, Patience et al. (in press) found no effect of 5 ppm RAC on proximate analysis of loin muscle.

3.4.1.3 Carcass Nutrient Deposition

RAC inclusion in the growth experiment improved the pigs' water deposition rates, numerically improved protein deposition rates, while lipid deposition rates tended to decrease. Similar results have been found in the literature. Webster et al. (2007) found that RAC inclusion increased protein and water deposition rates and decreased lipid deposition rates in pigs. In the growth study, the pigs had improved daily nutrient deposition rates; furthermore overall nutrient deposition was improved in RAC fed pigs. Even in the treatments without RAC in the present study, protein deposition was high (~160 g/d) compared to the 0 ppm RAC (145 g/d) treatments used by Webster et al. (2007). This could be due to differences in the environment between facilities or due to the selection in slaughter procedures. They defined the whole carcass as every part of the pig except the head, leaf fat and viscera; conversely the present study included the whole pig (including head, leaf fat and emptied viscera). These carcass fractions are not usually consumed but they do contain a large amount of protein, fat and moisture which could alter carcass tissue deposition rates when not accounted for.

3.4.1.4 N Balance

N intake values collected in the metabolism experiment agree with Smith et al. (2004) who had a range of N intake from 77.9–90.9 g/day for ad libitum fed finisher pigs. In the metabolism experiment, pigs were fed as close to ad libitum as possible to achieve comparable values between the growth and metabolism experiment concurrently while allowing measurement of water intake. The pigs in the growth experiment had higher N intake (90-92 g/d) and did not respond to RAC. On the other hand, the pigs in the metabolism experiment had reduced N intake with 10 ppm RAC; however, their N intake increased when they received diets supplemented with 5 ppm RAC. This result could be due to the ADFI these pigs had on the various RAC levels. The pigs fed 5 ppm RAC had the highest ADFI; therefore N intake would be expected to increase.

In the metabolism study we expected to have no change in N digestibility (Sutton et al., 2001 and DeCamp et al., 2001) with RAC inclusion, but a reduction in N digestibility occurred. When β-agonists are fed to cattle they can cause a decrease in mobility of feedstuffs throughout

the GIT by decreasing intestinal contractions and increasing retention time of feed, resulting in improved digestibility (Walker et al., 2007); however this has not been observed in swine. There is no current data in the literature which shows RAC decreases N digestibility.

An increase in protein deposition with RAC feeding (Xiao et al., 1999) indicates that N retention would improve. A reduction in total N excretion could be due to a reduction in urinary N because in mammals, urine is the key vehicle for N excretion (Smith et al., 2004). The results obtained for N balance in the metabolism experiment agree with this. Total N excretion decreased by 5.2 g/day (4.7 %) with RAC supplementation. Urinary N decreased as much as 5.4 % when pigs were fed 10 ppm, RAC compared to the control group. On the other hand, faecal N increased as much as 1.2 % with RAC inclusion. Faecal N excretion was higher in the RAC-fed pigs, but NH_3 emissions would be reduced because the volatilization of faecal N into NH_3 is slower than urinary N volatilization (Aarnink and Verstegen, 2007). The urinary:faecal N ratios were reduced in the metabolism experiment in RAC-fed pigs due to the large reduction of urinary N. The control pigs had a urinary N:faecal N ratio of 2.3, the 5 ppm RAC pigs had a ratio of 1.8 and the 10 ppm RAC pigs had a ratio of 1.9.

3.4.1.5 Desensitization to RAC

The typical target market weight currently employed in western Canada is 115-120 kg. In this experiment the 108 kg slaughter weight was utilized along with 120 kg because carcass differences may occur with prolonged RAC use due to desensitization of β-adrenergic receptors (Bell et al., 1998). Therefore, a response to RAC may initially rise and then decline as the test period progresses, so an estimation of protein and lipid deposition at a 108 kg weight was necessary. This is particularly important in the determination of the efficiency of nutrient utilization as influenced by RAC treatment. The pigs slaughtered at 120 kg had higher CP and water deposition rates than the pigs slaughtered at 108 kg. These results indicate that the pigs did not experience desensitization to RAC. The desensitization may not have occurred in these pigs since the average time required to reach 120 kg was only an average of19 days for control animals and an average of 17 days for RAC treated pigs. Armstrong et al. (2005) found that desensitization to RAC occurs when fed at a constant level for 35 days and treatment is initiated at 78.5 kg. The pigs in this growth experiment were not supplemented with RAC until 95 ± 3 kg and were fed no longer than 25 days.

3.4.1.6 Water Balance

Previous research has not determined if RAC affects water balance in finisher pigs. In the metabolism study, water balance was measured and RAC decreased both water intake and excretion. RAC increases lean tissue accretion in swine; logically an increase in water consumption would be expected, due to the high water content (~80%) of lean tissue (Burrin, 2001). In this experiment, apparent water retention numerically decreased when 10 mg/kg RAC was supplemented. Previous research concluded that direct injection of β-adrenergic agonists into the hypothalamus stimulated thirst in rats (Leibowitz. 1971). However, the low dosage of RAC used in this experiment may not stimulate thirst the same way as direct stimulation on the hypothalamus. Shaw et al. (2006) stated that when CP is in excess in the diet, urine output may increase, potentially due to increasing excretion of excess N. It is possible that the RAC-fed pigs consumed less water since they did not need to excrete as much N because more was being retained. As a result, they consumed enough water to maintain the increase in lean deposition but did not require extra water to remove additional N. Alternatively, lysine had no effect on water intake or consumption so the amount of dietary lysine could not have shifted water consumption patterns enough to show a significant decrease in the RAC-fed pigs. The reduction in water intake and excretion due to RAC supplementation is probably due to a multitude of factors and without further research cannot be explained at this time.

3.4.2 Response to Lysine

3.4.2.1 Dietary treatments

The dietary treatments for both experiments were arranged in a 3 × 3 factorial so the impact of under-or over-feeding of amino acids for control and RAC fed pigs could be evaluated. Diets were formulated based on a SID lysine:energy (DE) ratio. This ratio is important (Apple et al., 2004); however the energy density of the diet was consistent among all treatments and met the energy requirements of the pig, so the results of the present experiment were due to the level of lysine inclusion. The lysine levels in each diet were based on previous research that indicates RAC-fed pigs require increased lysine for increased protein accretion (Xiao et al., 1999; Neill et al., 2006; Webster et al., 2001; Apple et al., 2004). The lowest lysine level (0.65 % total lysine /19.6 g total lysine per day, 0.58 % SID lysine/17.5 g SID lysine per day) was presumed adequate for control pigs (NRC, 1998 and Wei and Zimmerman, 2003) and the middle lysine

level (0.84 % total lysine/25.2 g total lysine per day, 0.75 % SID lysine/22.5 g SID lysine per day) was presumed adequate for the pigs receiving 10 ppm RAC based on expected improvements in body weight gain (Apple et al., 2004). The third level (1.03 % total lysine/32.4 g total lysine per day, 0.85 % SID lysine/27.5 g SID lysine per day) was included to determine if increased lysine would support increased nitrogen retention or protein accretion in pigs receiving the highest level of RAC. According to Elanco Animal Health (2007), RAC supplementation requires a minimum dietary lysine content of at least 0.85-0.95 % total lysine.

Pigs received 18.9, 23.6 and 25.8 g SID lysine per day and 21.8, 26.1 and 30.9 g total lysine per day for the low, medium and high lysine diets, respectively. All of these lysine levels exceed NRC (1998) lysine requirements. However, there has been recent research that today's leaner pigs require more lysine than the NRC (1998) recommendations. The literature does not state all lysine requirements in the same form; therefore, grams per day SID and total lysine were calculated from percent lysine values and ADFI from the appropriate journal article. According to Webster et al. (2007), finisher pigs fed no RAC require 0.80 % total (25.8 g total lysine per day) or 0.70 % SID lysine (22.6 g SID lysine per day), to improve G:F and achieve desirable carcass traits. Srichana et al. (2004) utilized a large commercial study with 865 barrows and determined the SID lysine requirement to be between 0.61-0.68 % SID lysine (18.5 to 22.5 g SID lysine per day). Wei and Zimmerman (2003) concluded that barrows weighing between 100 and 120 kg require 0.61 % (18.1 g/d) SID lysine or 0.68 % (20.2 g/d) total lysine.

The finishing pig's requirement for lysine increases when RAC is added to the diet because of the improvement in lean growth (Schinckel et al., 2003). Webster et al. (2007) concluded that a 1.0 % total lysine (32.3 g total lysine per day)/0.88 % SID lysine (28.4 g SID per day) and 1.2 % total (38.0 g total lysine per day)/1.06 % SID lysine (33.6 g SID per day) maximizes performance and carcass traits in pigs fed 5 ppm and 10 ppm RAC, respectively. Apple et al. (2004) fed 10 ppm RAC and found maximum growth performance with 1.02-1.08 % total lysine (21.6 to 22.9 g total lysine per day). Both of these studies recommended higher lysine levels than used in the present study.

The absence of a RAC x lysine interaction for stimulating growth is puzzling but the pigs in the growth study had extremely high protein deposition rates, which are reflected in the high ADG seen in this experiment. The pigs in the present study receiving 1.03 % total lysine were depositing 198 g/d of protein which is similar to Webster et al. (2007) who had pigs depositing

191 g/d of protein. On the other hand, de Lange et al. (2001) stated that protein deposition rates for growing-finishing hogs fall between 90 to 160 g/d but intact males can deposit > 200 g/d. The pigs used in this study and by Webster et al. (2007) must have had a high lean gain potential which was close to the lean gain potential of the boar. However, if any of the pigs were deficient in lysine, a RAC x lysine interaction would have occurred because RAC requires sufficient lysine to improve protein deposition (Cline and Richert, 2001). In the case of a significant RAC x lysine interaction, the sufficient lysine level(s) would have caused higher protein deposition rates but the deficient level(s) would have caused decreased protein deposition rates. If higher levels would have been used in the current study an increase in ADG and protein deposition rate would have most likely occurred.

3.4.2.2 Carcass Composition and Nutrient Deposition

When lysine concentration was increased in the growth experiment, there was a numerical increase in carcass protein and tendency for increased water content. These results agree with research conducted by Webster et al. (2007) who also found that RAC had a tendency to affect protein and water content of the carcass. Pigs on higher lysine treatments would be expected to have higher protein content and higher water content because an increase in dietary lysine can help sustain an increase in lean deposition. The associated higher water content is due to lean tissue containing ~ 80 % water while adipose only contains ~15 % (Burrin, 2001). Lysine is the most limiting amino acid in most finishing swine diets (Lewis, 2001) and without sufficient dietary lysine, synthesis of new protein tissue cannot occur at high enough rates to keep up with normal protein tissue turnover (Cline and Richert, 2001).

Although lysine concentration did not significantly affect ash content, a lysine x slaughter weight interaction was observed. The 108 kg slaughter weight pigs did not have different ash contents among lysine treatments but the 120 kg slaughter weight pigs had a stepwise decrease in ash content as dietary lysine increased. The lipid content of a pig carcass will increase as they mature which causes a decrease of percent carcass protein, water and ash (deLange et al., 2001). Therefore, since the 120 kg slaughter weight pigs had a decrease in lipid content with higher dietary lysine concentrations, one could assume that ash content would increase. However, in this instance, ash content decreased. A potential explanation for this effect is not apparent.

3.4.2.3 N Balance

The concentration of dietary lysine also influenced N balance. As lysine levels in the diet increased, N intake and N digestibility improved, even though ADFI tended to be lower with higher lysine concentrations. With an increase in N intake, daily urinary N excretion, total N excretion and N retention increased; however faecal N excretion was unaffected by lysine treatments.

The results presented for N balance, expressed as a percent of N intake, show that the urinary:faecal N ratio, urinary N and total N excretion were the lowest for pigs receiving 2.25 g/Mcal SID lysine. This suggests that the 2.25 g/Mcal SID lysine level met the pigs' requirements to maximize CP deposition given that this group had the highest percent N retention and highest ADG. Notably, the pigs receiving the lowest lysine level tended to have the highest percent N excretion which is unexpected since other literature has found lowering the CP level of the diet decreases N excretion (Deng et al., 2007 and Figueroa et al., 2002). Conversely, these pigs had the lowest N retention which does agree with Deng et al. (2007) and Figueroa et al. (2002). As a result, the pigs supplemented with higher dietary lysine excreted less N because they retained more. In the metabolism experiment the composition of the manure contained higher faecal N with higher dietary lysine. However urinary N excretion was lower and a reduction in urea N can lower the rate of NH_3 volatilization (Jongbloed and Lenis, 1998).

3.4.3 Comparison of Growth and Metabolism Experiments

N balance in the growth experiment differed from that obtained in the metabolism experiment. The percent N retention values were ~ 20 percentage points higher in the metabolism experiment. The determination of N retention by comparative slaughter has been shown to be more accurate than by balance study; however, when gaseous N losses are measured the two methods are equivalent (Quiniou et al., 1995). The N excretion values obtained in this metabolism study appear to be underestimated, which may be in part due to volatilization of ammonia N during urine collection. The urine collection trays used in the metabolism experiment have a large surface area (0.91 m x 1.83 m), so there may have been volatilization of N before the urine reached the collection bottles. Since all the collection trays were the same size, the bias would logically be the same for all. The urinary N excretion values may be underestimated, as well, Quiniou et al. (1995) reported N losses can also occur when faeces are

dried which could also result in overestimated N retention. Therefore, the N retention values reported herein are higher than other N balance studies using finisher pigs (Fabian et al., 2004 and Lynch et al., 2007). Fabian et al. (2004) obtained values between 33-35 % of N intake and Lynch et al. (2007) obtained values between 32-47 % of N intake. On the other hand, O'Connell et al. (2006) had comparable values (N retention values between 53-55 % of N intake) to the present metabolism experiment but the authors report that their retention values were higher due to inevitable loss of volatized N.

The flow of dietary N in the pig is presented in Figure 3.10. As an average, the N retention of finisher pigs is ~ 30% and N excretion is ~70 % (faecal N 20 % and urine N 50 %) (Aarnink and Verstegen, 2007). In the growth experiment reported herein the N retention ranged from 28-34 % and excretion ranged from 65-71 % of intake, which is very close to the model presented in Figure 3.10. However, the metabolism experiment gave N retention values of 49-56 % and excretion values of 46-51 %. The faecal values (15-17 %) were close to the values suggested by Aarnink and Verstegen (2007) but the urine values (29-35 %) were comparatively low. When results of N retention are compared between the growth and metabolism experiment the SEM of the growth experiment are higher (Table 3.5), indicating larger variation between pigs. Everts and Dekker (1994) stated the estimation of initial body composition becomes more difficult in larger pigs, and this can cause an increase in variation among pigs. In the present experiments, the metabolism experiment had overestimated N retention and the growth experiment had larger variation due to difficulty obtaining a representative initial body composition. It appears that these pigs true N retention are between the values obtained in the metabolism and growth experiments.

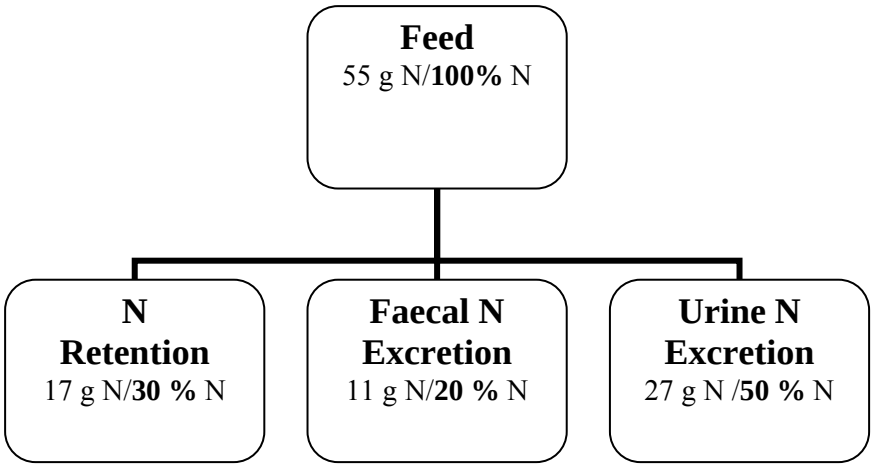

Figure 3.10 Nitrogen flow in finisher pigs†
†Figure adapted from Aarnink and Verstegen, 2007.

There were no significant interactions for N balance between RAC and lysine in the growth experiment, but the metabolism experiment had a few noteworthy interactions. In Figure 3.4, lysine concentration did not improve N retention in pigs fed 0 or 10 ppm RAC. The pigs fed 5 ppm RAC and the lowest lysine had the lowest N retention but when dietary lysine increased N retention improved. This could be due to the high N intake associated with the 5 ppm RAC pigs, which may have caused the improvement in N retention (Figure 3.9). As expected, an increase in N retention caused a decrease in urinary and total N excretion in the RAC pigs supplemented with higher dietary lysine (Figure 3.7 and 3.8). Therefore, the pigs fed 5 ppm RAC had enhanced N retention and reduced N excretion when supplemented with dietary lysine greater than 2.25 g/Mcal.

An interaction between RAC and lysine on N digestibility was surprisingly observed (Figure 3.5). RAC fed pigs had decreased N digestibility when fed the low and mid level of lysine but digestibility improved at the highest lysine level. The diet compositions (Table 3.1) were not different enough to cause a decrease in digestibility. There was only a ~4 percentage point difference between the lowest and highest N digestibility which were all in the normal range of N digestibility (> 80 %) (Zervas, 2001).

3.4.4 Environmental Impact of RAC

Calculations based on the present thesis data were applied to a commercial situation to define the potential impact of RAC on the environment. The values obtained in the metabolism

study were utilized to calculate nutrient balance in a 1000 head finishing barn (Table 3.12). In these calculations, pigs were started on treatment diets at 95 kg and finished at 120 kg. Based on average days on test in the present growth experiment, pigs fed no RAC averaged 19 d to reach market and RAC fed pigs required 17 d.

When 10 ppm RAC is supplemented at 95 kg and fed for 17 days, feed intake and water consumption was reduced by 7,530 kg (7.5 kg per pig) and 33,070 litres (33.1 litres per pig), respectively. Water excretion was reduced by 18,580 litres (18.6 litres per pig), faecal output (dry basis) declined by 880 kg (0.9 kg per pig), N intake was reduced by 220 kg (0.2 kg per pig) and N excretion declined by 171 kg (0.2 kg per pig). Even feeding 5 ppm, RAC reduced nitrogen and water excretion. Five ppm RAC reduced feed intake by 4,640 kg (4.6 kg per pig) and water intake by 22,700 litres (22.7 litres per pig). Water excretion was reduced by 12,800 litres (12.8 litres per pig), N intake was 100 kg (0.1 kg per pig) less and N excretion was reduced by 109 kg (0.1 kg per pig). When comparing the 5 ppm RAC pigs to the 10 ppm RAC pigs the 10 ppm RAC fed pigs had the most substantial reduction in intake and excretion of both water and nitrogen. Utilizing the results obtained in this experiment and applying them to a commercial situation proves that RAC can have a significant impact on reducing the environmental footprint from pork production. Therefore, feeding either 5 or 10 ppm RAC can improve environmental sustainability of market hogs by reducing feed requirements, decreasing water consumption and excretion and improving utilization of dietary N.

Table 3.12 Calculated water and nutrient balance for the finishing period (95-120 kg BW)[1].

	RAC (ppm)			Differences among RAC Treatments		
Item	**0[2]**	**5[2]**	**10[2]**	**0 vs. 5**	**0 vs. 10**	**5 vs. 10**
	Per 1000 pigs					
Feed Intake (As-fed), kg	60,800	54,400	51,000	-6400	-9800	-3400
N Intake, kg	1529.5	1429.0	1309.7	-100	-220	-119
Water Intake, litres	157,510	134,810	124,440	-22700	-33070	-10370
Water Excretion, litres[3]	73,150	60,350	54,570	-12800	-18580	-5780
Urine Output, litres	66,880	54,060	48,790	-12820	-18090	-5270
Faecal Output (Dry Basis), kg	8360	8330	7480	-30	-880	-850
N excreted, kg	781	673	610	-109	-171	-62
N retained, kg	748	756	699	8	-49	-56
	Per pig basis					
Feed Intake (As-fed), kg	60.8	54.4	51.0	-6.4	-9.8	-3.4
N Intake, kg	1.5	1.4	1.3	-0.1	-0.2	-0.1
Water Intake, litres	157.5	134.8	124.4	-22.7	-33.1	-10.4
Water Excretion, litres[3]	73.2	60.4	54.6	-12.8	-18.6	-5.8
Urine Output, litres	66.9	54.1	48.8	-12.8	-18.1	-5.3
Faecal Output (Dry Basis), kg	8.4	8.3	7.5	-0.1	-0.9	-0.9
N excreted, kg	0.8	0.7	0.6	-0.1	-0.2	-0.1
N retained, kg	0.8	0.8	0.7	0.0	-0.1	-0.1

[1]Calculations based on results obtained in the metabolism experiment except days to market were obtained from the growth experiment.
[2]Pigs fed RAC were considered to reach market weight (120 kg) in 17 days from 95 kg and pigs fed no RAC were considered to reach market weight in 19 days from 95 kg.
[3]Water excretion is the sum of urine output and faecal moisture.

3.4.5 Conclusions

Supplementation with RAC in finishing swine diets improved the N utilization of barrows. Total N excretion was reduced by 5.2 g/day in pigs fed 10 ppm RAC when compared to the control pigs. The reduction of total N excretion was due to RAC reducing urinary N excretion, as well as improving N retention. Interestingly, the 10 ppm RAC-fed pigs had consumed 1 l less water per day and excreted 0.7 l/d less water per day than control pigs. RAC improved growth performance in the metabolism experiment but not in the growth experiment. However, pigs in the growth experiment, regardless of treatment, had high efficiencies of gain. Dietary RAC inclusion of 10 ppm RAC increased carcass protein by 0.5 % and carcass water by 1.8 % and decreased the lipid content by 0.9 % when compared to control pigs. Daily deposition rates of lipid tended to decrease while protein deposition rates numerically improved. Previous research has shown lysine inclusion level influences growth performance or carcass

characteristics but this did not occur in this experiment, which was unexpected. When dietary lysine levels increased, RAC-fed pigs did not demonstrate improved growth performance or carcass characteristics. The urinary N excretion in the metabolism experiment was lower than previously published reports perhaps due to volatilization of N as ammonia. This may have caused differences in results of N balance between the growth and metabolism experiments. Based on balance studies found in the literature, the urinary excretion data obtained in the present metabolism experiment were higher than other published reports. Future studies using the N balance method must ensure that volatilization losses are accounted for.

3.4.6 Implications

RAC feeding has the potential to improve the environmental footprint associated with marketing hogs. Results from these experiments indicate that supplementing either 5 or 10 ppm RAC in finishing swine diets can improve N utilization. A decrease in urinary N excretion from 35.1 % to 29.8 % and improvement in N retention from 49.3 to 54.0 % in control and 10 ppm RAC-fed pigs, respectively, can reduce excess N being released in soil and water when manure is spread on land. RAC also improved protein deposition rates to 189.2 g/d in the 10 ppm RAC-fed pigs, while lipid deposition rates decreased to 542.3 g/d. Supplementing RAC produced a leaner carcass with improved nutrient utilization. As well, RAC feeding reduced water intake by 1 l/d and water excretion was reduced by 0.7 l/d associated with 10 ppm RAC inclusion level. As a result, RAC-feeding can reduce the water consumption requirements for finishing hogs.

4 GENERAL DISCUSSION

Addition of RAC to swine diets has the potential to lessen environmental problems associated with pork production; as well it can improve carcass composition and nutrient deposition rates. An increase in carcass CP and decrease in CF improves the pork quality that consumers currently demand. An improvement in nutrient utilization due to RAC supplementation can produce a leaner carcass; therefore these pigs can retain more N and excrete less. When manure is spread on the land excess N can accumulate in soil and water, causing environmental degradation. A decrease in the N content of the manure can prevent over application of N on farmland. The effect of RAC on water consumption was surprising because it caused a decrease in water intake and excretion without altering water retention. Other studies measuring N balance and the effect of RAC utilized constant water intake values; therefore they were unable to study any impact of RAC on water usage (Sutton et al., 2001 and DeCamp et al., 2001). Nevertheless, the effect of RAC on water balance requires more research to determine the exact cause of the reduction in water intake and excretion.

Quiniou et al. (1995) conducted a similar experiment comparing nitrogen balance technique to the comparative slaughter technique to determine which method was better at predicting N balance in pigs. They concluded that results obtained in a balance experiment were comparable to the comparative slaughter technique as long as gaseous N losses are measured or accounted for. Unfortunately, due to large surface area of collection trays used in this metabolism experiment, there may have been losses of urinary and faecal N and without access to respiration chambers measurement of N gas losses was not possible. In the metabolism experiment, the positive effect RAC had on N balance would not change because volatilization losses would not be different among treatments but retention values may be slightly higher. Based on the majority of N balance literature, the pigs in the metabolism experiment had higher than normal N retention and lower N excretion; therefore when comparing the two experiments one would have more confidence that the results obtained in the growth study are the closest to the true N balance levels in the pig.

Other research is required to see if other nutrients are affected by RAC inclusion, i.e. phosphorus, potassium, copper and zinc. The research conducted by Sutton et al. (2001) and DeCamp et al. (2001) found RAC improved P retention but did not significantly alter total P excretion. RAC could have a significant effect on other minerals because of the profound effect

of RAC on N balance. Another potential area of research could be the RAC supplementation effect on greenhouse gas and odour emissions from finishing swine facilities that could accrue from the improvement in nutrient utilization and growth performance associated with RAC-feeding determined in these experiments.

5 REFERENCES

Aalhus, J.L., A.L. Schaefer, A.C. Murray and S.D.M. Jones. 1992. The effect of ractopamine on myofibre distribution and morphology and their relation to meat quality in swine. Meat Sci. 31: 397-409.

Aarnink, A.J.A. and M.W.A. Verstegen. 2007. Nutrition, key factor to reduce environmental load from pig production. Livest. Sci. 109: 194-203.

Abney, C.S. 2006. Effects of Optaflexx (Ractopamine Hydrochloride) on performance, rate and variation of intake, and acid-base balance in feedlot cattle. PhD Diss. Texas Tech Univ., Lubbock,TX.

AOAC. 1990. Official Methods of Analysis. 15th ed. Assoc. Offic. Anal. Chem., Arlington, VA.

AOAC. 2006. Official Methods of Analysis. 18th ed. Assoc. Offic. Anal. Chem., Arlington, VA.

Apple, J.K., C.V. Maxwell, D.C. Brown, K.G. Friesen, R.E. Musser, Z.B. Johnson and T.A. Armstrong. 2004. Effects of dietary lysine and energy density on performance and carcass characteristics of finishing pigs fed ractopamine. J. Anim. Sci. 82: 3277-3287.

Apple, J.K., P.J. Rincker, F.K. McKeith, S.N. Carr, T.A. Armstrong and P.D. Matzat. 2007. Review: Meta-analysis of the ractopamine response in finishing swine. Prof. Anim. Sci. 23:179-196.

Armstrong, T.A., B.T. Kremer, T.A. Marsteller and D. Mechler. 2005. Effects of ractopamine step-up use programs on finishing pigs fed under commercial conditions. J. Swine Health Prod. 13: 66-71.

Beaulieu, A.D., M.R. Bedford and J.F. Patience. 2007. Supplementing corn or corn-barley diets with an *E.coli* derived phytase decreases total and soluble P output by weanling and growing pigs. Can. J. Anim. Sci. 87: 353-364.

Bell, A.W., D.E. Bauman, D.H. Beermann and R.J. Harrell. 1998. Nutrition, development and efficacy of growth modifiers in livestock species. J. Nutr. 128: 360S-363S.

Brumm, M. C., P. S. Miller and R. C. Thaler. 2004. Response of barrows to space allocation and ractopamine. J. Anim. Sci. 82: 3373-3379.

Burrin, D. G. 2001. Nutrient requirements and metabolism. Pages 309-389 in Biology of the domestic pig. W. G. Pond and H. J. Mersmann, ed. Cornell University Press, Ithaca, NY.

Burton, C.H., R.W. Sneath and J.W. Farrent. 1993. Emissions of N oxide gases during aerobic treatment of animal slurries. Bioresour. Technol.. 45: 233-235.

CCAC. 1993. Guide to the care and use of experimental animals. 2nd ed. Canadian Council on Animal Care, Ottawa, ON.

Canadian Food Inspection Agency. 2008. Ractopamine Hydrochloride – MIB #82. http://www.inspection.gc.ca/english/anima/feebet/mib/mib82e.shtml Accessed Sept. 15, 2008.

Carr, S.N., D.J. Ivers, D.B. Anderson, D.J. Jones, D.H. Mowrey, M.B. England, J. Killefer, P.J. Rincker and F.K. McKeith. 2005. The effects of ractopamine hydrochloride on lean carcass yields and pork quality characteristics. J. Anim. Sci. 83: 2886-2893.

Carroll, A.L., D.B. Anderson, T.E. Elam and A.L. Sutton. 2001. Environmental benefits of Paylean® in finisher swine - an example based on adoption in the United States. http://www.ces.purdue.edu/extmedia/AS/AS-551-W.pdf. Accessed July 13, 2008.

Clark, O.G., S. Moehn, I. Edeogu, J. Price and J. Leonard. 2005. Manipulation of dietary protein and nonstarch polysaccharides to control swine manure emissions. J. Environ. Qual. 34: 1461-1466.

Cline, T.R. and B.T. Richert. 2001. Feeding growing-finishing pigs. Pages 717-718 in Swine Nutrition. 2nd ed. A. Lewis and L.L. Southern eds. CRC Press. Boca Raton, FL.

DeCamp, S.L., A. Hankins, A. Carroll, D.J. Ivers, B.T. Richert, A.L. Sutton and D.B. Anderson. 2001. Effect of ractopamine and dietary crude protein on nitrogen and phosphorus excretion from finishing pigs. Swine Research Report. Purdue University. West Lafayette, IN.

de Lange, C.F.M., S.H. Birkett and P.C.H. Morel. 2001. Protein, fat, and bone tissue growth in swine. Pages 66-70 in Swine Nutrition 2nd Ed. A. Lewis and L.L. Southern eds. CRC Press. Boca Raton, FL.

Deng, D., R.L. Huang, T.J. Li, G.Y. Wu, M.Y. Xie, Z.R. Tang, P. Kang, Y.M. Zhang, M.Z. Fan, X.F. Kong, Z. Ruan, H. Xiong, Z.Y. Deng and Y.L. Yin. 2007. Nitrogen balance in barrows fed low-protein diets supplemented with essential amino acids. Livest. Sci. 109: 220-223.

Depreux, F.F.S., A.L. Grant, D.B. Anderson and D.E. Gerrard. 2002. Paylean alters myosin heavy chain isoform content in pig muscle. J. Anim. Sci. 80: 1888-1894.

Ding, S.T., R.L. McNeel, E.O. Smith and H.J. Mersmann. 2000. Modulation of porcine adipocyte β-adrenergic receptors by a β-adrenergic agonist. J. Anim. Sci. 78: 919-926.

Dunshea, F.R., R.H. King, R.G. Campbell, R.D. Sainz and Y.S. Kim. 1993. Interrelationship between sex and ractopamine on protein and lipid deposition in rapidly growing pigs. J. Anim. Sci. 71: 2919-2930.

Dunshea, F.R., R.H. King, P.J. Eason and R.G. Campbell. 1998. Interrelationship between dietary ractopamine, energy intake, and sex in pigs. Aust. J. Agric. Res. 49: 565-574.

Elanco Animal Health. 2007. Paylean™ Technical Reference Guide. Elanco Animal Health Canada. Guelph, ON.

Environment Canada. 2007. Daily data report for Saskatoon Diefenbaker International Airport, Saskatoon Saskatchewan April to August 2007. http://www.climate.weatheroffice.ec.gc.ca/climateData/dailydatae.html?timeframe=2&Prov=XX&StationID=3328&Year=2007&Month=4&Day=25. Accessed Aug. 26, 2008.

Everts, H. and R.A. Dekker. 1994. Balance trials and comparative slaughtering in breeding sows: description of techniques and observed accuracy. Livest. Prod. Sci. 37: 339-352.

Fabian, J., L.I. Chiba, L.T. Frobish, W.H. McElhenney, D.L. Kuhlers and K. Nadarajah. 2004. Compensatory growth and nitrogen balance in grower-finisher pigs. J. Anim. Sci. 82: 2579-2587.

Figueroa, J.L., A.J. Lewis, P.S. Miller, R.L. Fischer, R.S. Gómez and R.M. Diedrichsen. 2002. Nitrogen metabolism and growth performance of gilts fed standard corn-soybean meal diets or low-crude protein, amino acid-supplemented diets. J. Anim. Sci. 80: 2911-2919.

Gralapp, A.K., W.J. Powers, M.A. Faust and D.S. Bundy. 2002. Effects of dietary ingredients on manure characteristics and odorous emissions from swine. J. Anim. Sci. 80: 1512-1519.

Gu, Y., A.P. Schinckel, J.C. Forrest, C.H. Kuei and L.E. Watkins. 1991. Effects of ractopamine, genotype, and growth phase on finishing performance and carcass value in swine: II. Estimation of lean growth rate and lean feed efficiency. J. Anim. Sci. 69: 2694-2702.

Haeussermann, A.E. Hartung, E. Gallmann and T. Jungbluth. 2006. Influence of season, ventilation strategy, and slurry removal on methane emissions from pig houses. Agric. Ecosyst. Environ. 112: 115-121.

Harper, A.F., E.T. Kornegay and T.C. Schell. 1997. Phytase supplementation of low-phosphorous growing-finishing pig diets improves performance, P digestibility, and bone mineralization and reduces P excretion. J. Anim. Sci. 75: 3174-3186.

Iqbal, M.Z., E.J. Brown, M.E. Clayton. 2006. Distribution of P in a biologically restricted lake in Iowa, USA. J. Hydrol. 326: 349-366.

Jensen, B.B. and H. Jørgensen. 1994. Effect of dietary fibre on microbial activity and microbial gas production in various regions of the gastrointestinal tract of pigs. Appl. Environ. Microbiol. 60: 1897-1904.

Jeremiah, L.E., R.O. Ball, J.K. Merrill, P. Dick, L. Stobbs, L.L. Gibson and B. Uttaro. 1994. Effects of feed treatment and gender on the flavour and texture profiles of cured and uncured pork cuts. I. Ractopamine treatment and dietary protein level. Meat Sci. 37: 1-20.

Ji, S.Q. 1992. Mode of action of β-adrenergic agonists on muscle protein metabolism. PhD Diss. Purdue Univ., West Lafayette, IN.

Johnson, L.F and M.E. Tate. 1968. Structure of "phytic acids". Can. J. Chem. 47:63-74.

Jongbloed, A.W. and N. P. Lenis. 1998. Environmental concerns about animal manure. J. Anim. Sci. 76: 2641-2648.

Leibowitz, S.F. 1971. Hypothalamic alpha- and beta-adrenergic systems regulate both thirst and hunger in the rat. Proc. Natl. Acad. Sci. U.S.A. 68: 332-334.

Lewis, A.J. 2001. Amino acids in swine nutrition. Pages 138-139 in Swine Nutrition 2nd ed. A. Lewis and L.L. Southern eds. CRC Press. Boca Raton, FL.

Liu, C.Y., A.L. Grant, K.H. Kim, S.Q. Ji, D.L. Hancock, D.B. Anderson and S.E. Mills. 1994. Limitations of ractopamine to affect adipose tissue metabolism in swine. J. Anim. Sci. 72: 62-67.

Liu, C.Y., J.L. Boyer and S.E. Mills. 1989. Acute effects of beta-adrenergic agonists on porcine adipocyte metabolism in vitro. J. Anim. Sci. 67: 2930-2936.

Lynch, M.B., T. Sweeney, J.J. Callan and J.V. O'Doherty. 2007. The effect of dietary crude protein concentration and inulin supplementation on nitrogen excretion and intestinal microflora from finisher pigs. Livest. Sci. 109: 204-207.

Main, R.G., S.S. Dritz, M.D. Tokach, R.D. Goodband and J.L. Nelssen. 2001. Effects of feeding graded levels of ractopamine (Paylean) on pig performance in a commercial finishing facility. Pages 74-76 in Proc. Swine Day Kansas State Univ., Manhattan, KS.

McCarthy, J.F., F.X. Aherne and D.B. Okai. 1974. Use of HCl insoluble ash as an index material for determining apparent digestibility with pigs. Can. J. Anim. Sci. 54:107-109.

Mersmann, H.J. 1998. Overview of the effects of β-adrenergic receptor agonists on animal growth including mechanisms of action. J. Anim. Sci. 76: 160-172.

Mills, S.E., J. Kissel, C.A. Bidwell and D.J. Smith. 2003. Stereoselectivity of porcine β-adrenergic receptors for ractopamine stereoisomers. J. Anim. Sci. 81: 122-129.

Mimbs, K.J., T.D. Pringle, M.J. Azain, S.A. Meers and T.A. Armstrong. 2005. Effects of ractopamine on performance and composition of pigs phenotypically sorted into fat and lean groups. J. Anim. Sci. 83: 1361-1369.

Morrow, W.E.M., S. Miller, R.E. Meyer, J. Roberts and D. Lascelles. 2005. The humane euthanasia-on-farm applications. North Carolina State University Animal Science Departmental Report .http://mark.asci.ncsu.edu/swinereports/2004-2005/Swine/Welfare_Behavior/Morrow.htm. Accessed July 18, 2008.

Neill, C.R., S.S. Dritz, M.D. Tokach, R.D. Goodband, J.L. Nelssen, J.M. DeRouchey, C.W. Hastad and J.L. Usry. 2006. Effect of increased dietary lysine on growth performance of gilts fed ractopamine HCL (Paylean) in a commercial facility. Pages 146-152 in Proc. Swine Day Kansas State Univ., Manhattan, KS.

Ni, J-Q., A.J. Heber, T.T. Lim, C.A. Diehl, R.K. Duggirala and B.L. Haymore. 2002. Hydrogen sulphide emission from two large pig-finishing buildings with long-term high-frequency measurements. J. Agric. Sci. 138: 227-236.

Noblet, J., J. Le Dividich and J. Van Milgen. 2001. Thermal environment and swine nutrition. Pages 529-535 in Swine Nutrition 2nd ed. A. Lewis and L.L. Southern eds. CRC Press. Boca Raton, FL.

NRC. 1998. Nutrient requirements of swine. 10th ed. National Academy Press, Washington, DC.

Occupational Health and Safety. 2004. Health and safety practices in pork production: technical modules. http://www.labour.gov.sk.ca/safety/porkproduction/index.htm. Accessed Jan. 2, 2007.

O'Connell, J.M., J.J. Callan and J.V. O'Doherty. 2006. The effect of dietary crude protein level, cereal type and exogenous enzyme supplementation on nutrient digestibility, N excretion, faecal volatile fatty acid concentration and ammonia emissions from pigs. Anim. Feed Sci. Technol. 127:73-88.

Patience, J.F., P. Shand, Z. Pietrasik, J. Merrill, D.A. Gillis, G. Vessie and A.D. Beaulieu. 2008. Effect of ractopamine in finishing swine diets on growth performance, carcass measurements and pork quality. Can. J. Anim. Sci. *In Press*.

Portejoie, S., J.Y. Dourmad, J. Martinez and Y. Lebreton. 2004. Effect of lowering dietary crude protein on N excretion manure composition and ammonia emission from fattening pigs. Livest. Prod. Sci. 91:45-55.

Quiniou, N., S. Dubois and J. Noblet. 1995. Effect of dietary crude protein level on protein and energy balances in growing pigs: comparison of two measurement methods. Livest. Prod. Sci. 41: 51-61.

Quiniou, N., S. Dubois and J. Noblet. 2000. Voluntary feed intake and feeding behaviour of group-housed growing pigs are affected by ambient temperature and body weight. Livest. Prod. Sci. 63: 245-253.

Rothwell , N.J., M.J. Stock and D.K. Sudera. 1987. Changes in tissue blood flow and β-receptor density of skeletal muscle in rats treated with the β_2-adrenoceptor agonist clenbuterol. Br. J. Pharmac. 90: 601-607.

Ryan, J. and G. Estefan. 2002. Soil and plant analysis laboratory manual. 2nd Ed. www.icarda.org/Publications/Lab_Manual/read.htm. Accessed Nov. 27, 2008.

Schinckel, A.P., N. Li, T. Richert, P.V. Preckel and M.E. Einstein. 2003. Development of a model to describe the compositional growth and dietary lysine requirements of pigs fed ractopamine. J. Anim. Sci. 81: 1106-1119.

See, M.T., T.A. Armstrong and W.C. Weldon. 2004. Effect of a ractopamine feeding program on growth performance and carcass composition in finishing pigs. J. Anim. Sci. 82: 2474-2480.

See, M.T., T.A. Armstrong, P.D. Matzat, B.A. Belstra, F.K. McKeith, P.J. Rincker, L. Xi, J. Odle, M. Culbertson, W. Herring and J. Hansen. 2005. Effect of ractopamine feeding level on growth performance and carcass composition. J. Anim. Sci. 83(Suppl. 2): 27. (Abstr.).

Shaw, M.I., A.D. Beaulieu and J.F. Patience. 2006. Effect of diet composition on water consumption in growing pigs. J. Anim. Sci. 84: 3123-3132.

Sherlock, R.R., S.G. Sommer, R.Z. Khan, C.W. Wood, E.A. Guertal, J.R. Freney, C.O. Dawson and K.C. Cameron. 2002. Ammonia, methane, and nitrous oxide emissions from pig slurry applied to a pasture in New Zealand. J. Environ. Qual. 31:1491-1501.

Smith, D.J. 1998. The pharmacokinetics, metabolism, and tissue residues of β-adrenergic agonists in livestock. J. Anim. Sci. 76: 173-194.

Smith, L.F., S.P. Lemay, J.F. Patience and R.T. Zijlstra. 2004. Effects of dietary crude protein level and sugar beet pulp inclusion on N excretion patterns in grower and finisher pigs. Can. J. Anim. Sci. 84: 717-720.

Srichana, P., A.M. Gaines, B.W. Ratliff, G.L. Allee and J.L. Usry. 2004. Evaluation of the true ileal digestible (TID) lysine requirement for 80-100 kg barrows and gilts. J. Anim. Sci. 82 (Suppl. 1): 578 (Abst.).

Stanier, M.W., L.E. Mount and J. Bligh. 1984. Energy balance and temperature regulation. Pages 45-47 in Cambridge texts in the physiological sciences. Cambridge University Press. Cambridge, England.

Sutton, A.L., B.T. Richert, S.L. Hankins, S.A. DeCamp, and A.L. Carroll. 2001. Potential impact of ractopamine on environmental stewardship. J.Anim. Sci. 79 (Suppl. 1): 239 (Abstr.).

Sutton, A.L., K.B. Kephart, M.W.A. Verstegen, T.T. Canh and P.J. Hobbs. 1999. Potential for reduction of odourous compounds in swine manure through diet modification. J. Anim. Sci. 77: 430-439.

Uttaro, B.E., R.O. Ball, P. Dick, W. Rae, G. Vessie and L.E. Jeremiah. 1993. Effect of ractopamine and sex on growth, carcass characteristics, processing yield, and meat quality characteristics of crossbred swine. J. Anim. Sci. 71: 2439-2449.

Vestergaard, M., P. Henckel, N. Oksbjerg and K. Sejrsen. 1994. The effect of cimaterol on muscle fiber characteristics, capillary supply, and metabolic potentials of longissimus and semitendinosus muscles from young Friesian bulls. J. Anim. Sci. 72: 2298-2306.

Walker, D.K., E.C. Titgemeyer, E.K. Sissom, K.R. Brown, J.J. Higgins, G.A. Andrews and B.J. Johnson. 2007. Effects of steroidal implantation and ractopamine-HCl on nitrogen retention, blood metabolites and skeletal muscle gene expression in Holstein steers. J. Anim. Physiol. Anim. Nutr. 91: 439-447.

Weber, T.E., B.T. Richert, M.A. Belury, Y. Gu, K. Enright and A.P. Schinckel. 2006. Evaluation of the effects of dietary fat, conjugated linoleic acid, and ractopamine on growth performance, pork quality, and fatty acid profiles in genetically lean gilts. J. Anim. Sci. 84: 720-732.

Webster, M.J., R.D. Goodband, M.D. Tokach, J.L. Nelssen, S.S. Dritz, J.A. Unruh, K.R. Brown, D.E. Real, J.M. Derouchey, J.C. Woodworth, C.N. Groesbeck and T.A. Marsteller. 2007. Interactive effects between ractopamine hydrochloride and dietary lysine on finishing pig growth performance, carcass characteristics, pork quality, and tissue accretion. Prof. Anim. Sci. 23: 597-611.

Webster, M.J., R.D. Goodband, M.D. Tokach, J.A. Unruh, J.L. Nelssen, S.S. Dritz, D.E. Real, J.M. DeRouchey, J.C. Woodworth and T.A. Marsteller. 2001. Interactive effects between Paylean (ractopamine HCL) and dietary lysine on finishing pig growth performance, carcass characteristics and tissue accretion. Pages 77-85 in Proc. Swine Day Kansas State Univ., Manhattan, KS.

Wei, R. and D. R. Zimmerman. 2003. An evaluation of the NRC (1998) growth model in estimating lysine requirements of barrows with a lean growth rate of 348 g/d. J. Anim. Sci. 81: 1772-1780.

Xiao, R-J., Z-R. Xu, H-L. Chen. 1999. Effects of ractopamine at different dietary protein levels on growth performance and carcass characteristics in finishing pigs. Anim. Feed. Sci. Technol. 79: 119-127.

Yen, J.T., H.J. Mersmann, D.A. Hill and W.G. Pond. 1990. Effects of ractopamine on genetically obese and lean pigs. J. Anim. Sci. 68: 3705-3712.

Zervas, S. 2001. Effects of dietary protein and fibre on nitrogen excretion patterns in swine. M.Sc. Diss., Univ. of Saskatchewan, Saskatoon, SK.

6 APPENDIX A

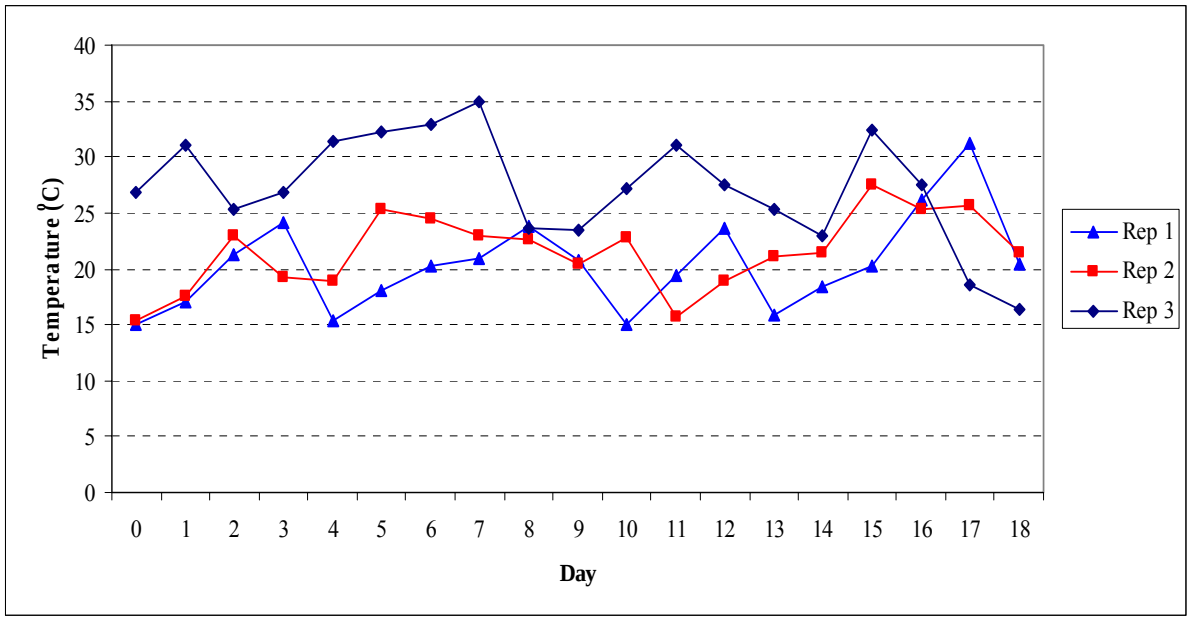

Figure 6.1 Maximum temperature recorded for Saskatoon, SK during rep 1, 2 and 3 of the metabolism experiment
Data from Environment Canada. 2007

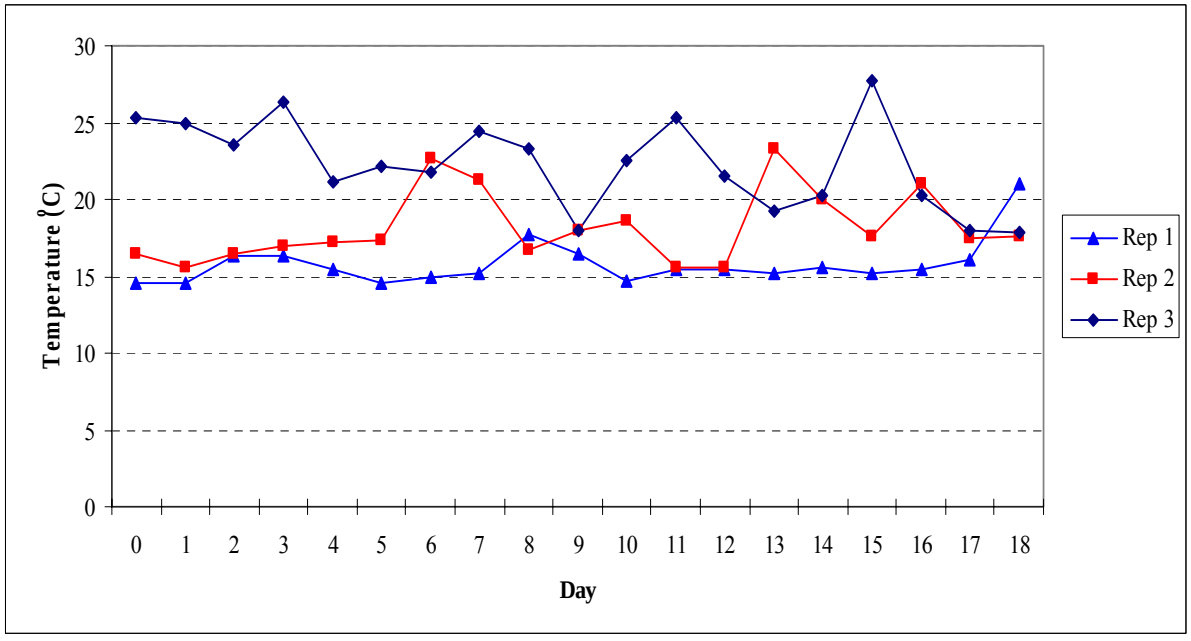

Figure 6.2 Morning barn room temperatures recorded during rep 1, 2 and 3 of the metabolism experiment