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The effect of two levels of dietary ractopamine hydrochloride (Paylean®) supplementation on growth performance, feed efficiency and quantitative carcass traits in finisher gilts

by

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Submitted in partial fulfilment of the requirements for the degree

Magister Scientiae Agriculturae
in
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DECLARATION

I declare that the dissertation/thesis, which I hereby submit for the degree Magister Scientiae at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

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Date:

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Signature

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ABSTRACT

The effect of two levels of dietary ractopamine hydrochloride (Paylean®) supplementation on growth performance, feed efficiency and quantitative carcass traits in finisher gilts

by

Paul David Teague

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Ractopamine hydrochloride (RAC) has been used in the pig production industry for over 30 years. RAC is a beta-adrenergic agonist which is supplemented in the feed during the last 28 days prior to harvesting in finisher pigs to modify the pig's metabolism such that nutrients are redirected to favour muscle accretion rather than adipose deposition, and hence improve growth efficiencies, feed utilisation and carcass revenues. The objective of this study was to investigate the effect of dietary ractopamine (Paylean®) supplementation at levels of 0, 5, and 10 mg.kg^{-1} (hereafter referred to as 0-RAC, 5-RAC, and 10-RAC respectively) on animal growth performance, efficiency and carcass characteristics including daily voluntary feed intake, feed efficiency, absolute daily growth rate (ADG) and daily live weight gain, and backfat thickness for the last 27 days in finishing gilts. In this 27-day study, a homogenous group of 71 grower gilts (LW = 43 ± 1 kg) were pre-selected at a source farm. The gilts were then housed in similar and equally sized group pens at the Hatfield experimental facility of the University of Pretoria and fed a standard maize-soya oilcake based grower ration formulated to contain 0.94% standardised ileal digestible Lys (1.05% total Lys) and 14.01 MJ ME kg^{-1} during the 28 day pre-adaptation phase. From these gilts, individuals were weighed and 58 gilts selected (average LW = 68.7 ± 4.3 kg), and placed into individual pens and the same diet for 7 days (adaptation phase), afterwhich they were assigned to 1 of 3 treatments in a completely randomized block design with 19, 19, and 20 replicate pens per treatment. The pigs were then fed a standard maize-soya oilcake finisher (treatment) diet containing either 0-RAC, 5-RAC or 10-RAC for 27 d before harvesting. All treatment diets were formulated to contain 1.02% standardised ileal digestible Lys (1.13% total Lys) and 13.96 MJ ME kg^{-1}. Individual pig LW, P2 thickness and pen feed disappearance were recorded weekly to determine LW changes, ADG, ADFI, and G:F. After 27 d on trial, gilts were slaughtered and carcass measurements were recorded at 24 h post-mortem. Overall, RAC supplementation did not affect ADFI or P2 (P > 0.05) but did influence LW (P = 0.049) and overall G:F (P = 0.012) after d27. At d15-d21 and d22-27, only a tendency (P = 0.169, 0.104 respectively) for a linear decrease in G:F with RAC supplementation was found. RAC also affected HCY (P

= 0.045) and CCY (P = 0.045) but not fat depth, meat depth or fat % (P > 0.05). These results indicate that RAC may have small but beneficial effects in modern pig production, but further research is required to optimize concentrations and duration of supplementation in modern lean pig genotypes.

Key words:
β-adrenergic agonist, ractopamine, growth, carcass, efficiency

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ABBREVIATIONS/ACRONYMS

ADFI	Average daily voluntary feed intake
ADG	Average daily gain
ATP	Adenosine triphosphate
βAA	Beta adrenergic agonist. Synonyms: β-agonist, beta agonist
βAR	Beta-adrenergic receptor. Subscripts referring to different types of receptors
cAMP	Cyclic adenosine monophosphate
CCY	Cold carcass yield. Synonyms: Cold carcass weight (CCWt)
CNS	Central nervous system
CP	Crude protein
d	Day
Dr%	Dressing percentage
EIF	Elongation initiation factor
ERK	Extracellular signal-regulated kinases
EU	
FCR	Feed conversion ratio
F:G	Feed to gain ratio (Also feed efficiency; inverse of G:F)
g	gram
G:F	Gain to feed ratio (Also feed efficiency; inverse of F:G)
GHIH	Growth hormone-inhibiting hormone. Synonyms: somatotropin release-inhibiting factor (SRIF), somatotropin release-inhibiting hormone, somatostatin
GHRH	Growth hormone-releasing hormone. Synonyms: growth hormone-releasing factor (GRF, GHRF), somatocrinin, somatoliberin
Gs	Glutamine synthetase
HCY	Hot carcass yield. Synonyms: Hot carcass weight (HCWt)
HGP	Hennessy Grading Probe
IGF-I	Insulin-like growth factor I
IMF	Intramuscular fat
JAK-STAT	Janus kinase-signal transducer and activator of transcription
Kg	kilogram
LM	Loin eye muscle
Lys	Lysine
m	metre
MAPK	Mitogen-activated protein kinases
ME	Metabolisable energy
mm	millimetre

P2	Position 65mm from the dorsal midline at the level of the posterior edge of the head of the last rib
PKA	Protein kinase A
PUFA	Poly-unsaturated fatty acid
RAC	Ractopamine hydrochloride (Paylean® Elanco Animal Health, Greenfield, IN)
SM	Semimembranosus muscle
STH	Somatotropin. Synonyms: growth hormone (GH), somatropin
WAT	White adipose tissue
WMSF	Warner-Bratzler shear force

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The effect of two levels of dietary ractopamine hydrochloride (Paylean®) supplementation on feed intake, feed efficiency, growth, and quantitative carcass traits in finisher gilts

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CHAPTER 1 INTRODUCTION

1.1 Contemporary market trends

In South Africa, pig carcasses are graded and classified according to the PORCUS classification system (Department of Agriculture, 2006) whereby weight, fat content and conformation are scored, and combined with other factors such as sex, damage, and age to provide an overall classification (Bruwer, 1992; Department of Agriculture, 2006; Siebrits *et al*., 2012). Carcasses are marketed and sold according to this relatively crude classification, with leaner and lighter carcasses being favoured for the fresh meat market, whilst more conditioned and heavier carcasses tend to be directed to the processed market.

Increasing health awareness and market demands for affordable high lean animal protein sources result in higher prices for leaner carcasses (Siebrits *et al*. 2012). Consequently, pig meat producers aim to meet these market trends by producing leaner, heavier carcasses.

1.2 Carcass components

A typical pig carcass consists of 65% prime cuts and 35% low value cuts. Lean tissue (protein) is the major component of these cuts per weight basis. Since lean tissue may contain >70% water (Table 1.1), and has a higher efficiency of synthesis (15 MJ balanced DE per kg lean synthesis) compared to adipose, it can be concluded that the lean component is the major contributor toward carcass value, whilst any excessive lipid deposition resulting in lowered carcass classification is energetically and economically inefficient. Consequently, limiting excessive subcutaneous lipid deposition and simultaneously maximising protein accretion remain the primary focus of pig meat production objectives, the benefits of which may clearly be seen in Table 1.2. It should be noted that although lipid synthesis may seem unfavourable, a certain level of adipose tissue in an animal is desired as this tissue has a specialised fatty acid-providing role, provides thermal insulation and mechanical protection, aids with immune function, acts as storage of lipid soluble vitamins, and has other key homeostatic functions.

In order to maximise economic gain from the growth potential of a pig, the entire biology of growth rather than one particular phase should be understood. A brief discussion of growth shall thus be provided in this literature review.

Table 1.1 Typical composition of a 76 kg carcass (adapted from English *et al*., 1988b)

Carcass component	Weight of tissue (kg)	Protein (kg)	Lipid (kg)	Carbohydrate (kg)	Water (kg)	Ash (kg)
Lean	42	8.40	1.30	0.5	31.53	0.27
Fat	17	0.95	11.90	.	4.12	0.03
Skin	4	0.69	0.91	.	2.40	.
Bone	13	1.12	5.50	.	4.48	1.90
Total (kg)	76	11.16	19.61	0.50	42.53	2.20
%	100	14.69	25.80	0.66	55.96	2.89

Table 1.2 Relationship between lean meat and carcass water content (SPESFEED, 2010)

Lean type	Live weight (kg)	Lean meat %	Water kg/carcass
Low lean	100	52	28
Medium lean	100	58	31
High lean	100	64	35

1.3 Growth

1.3.1 Definition

"Growth" is a term used to describe the progressive change in body tissue composition as an animal matures from conception to senescence. Involving both anabolic and catabolic pathways, these changes are primarily facilitated (or compromised) through nutritional excesses (or shortfalls) relative to the individual's maintenance requirements. For our purposes, the term growth will be used to describe the net summative effect of all anabolic and catabolic pathways in the body tissue leading to the accretion/deposition or catabolism of cellular material.

This simplistic concept is governed by numerous genetic factors, endocrine cascades, metabolic conditions, and environmental factors and consequently may be quantified according to any singular or combinations of these aspects.

1.3.2 Biology

Growth is comprised of two components, 1) hyperplasia (increase in cell number) and 2) hypertrophy (increase is cell size) (McGlone & Pond, 2003). Hyperplastic growth is responsible for the majority prenatal growth, whereas hypertrophic growth is responsible for the majority of postnatal growth. It is generally accepted that there are three sequential phases of growth: hyperplasia, hyperplasia-hypertrophy, and hypertrophy (McGlone & Pond, 2003).

With increasing age and feed intake, macroscopic observations of overall weight changes indicate growth follows a sigmoidal curve (Figure 1.1). However, such weight changes are actually the result of

allometric growth. Allometric growth is essentially the term coined to describe the sequential maturation of tissues which is brought on about by dissimilar rates growth within and between tissues (bone, fat, protein, and ash) with subsequent effects on cytosolic volumes and hence leading to a dissimilar growth rates of carcass components (lean, fat skin, and bone) (Figure 1.2).

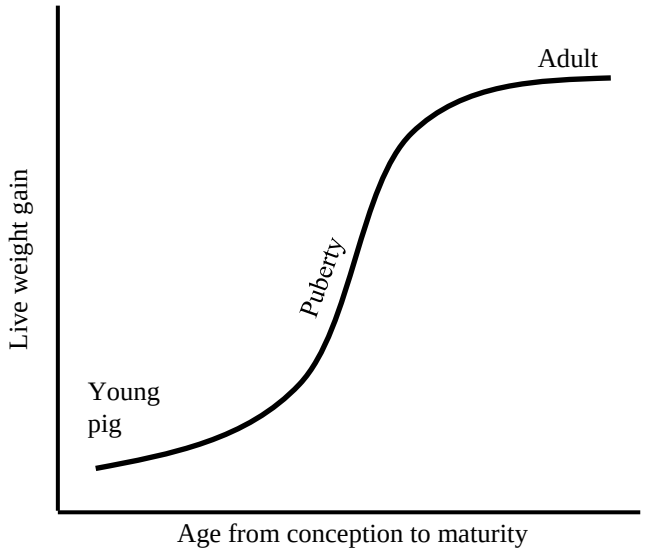

Figure 1.1 Typical cumulative growth a pig based on live weight gain from conception to maturity (adapted from English *et al*., 1988a)

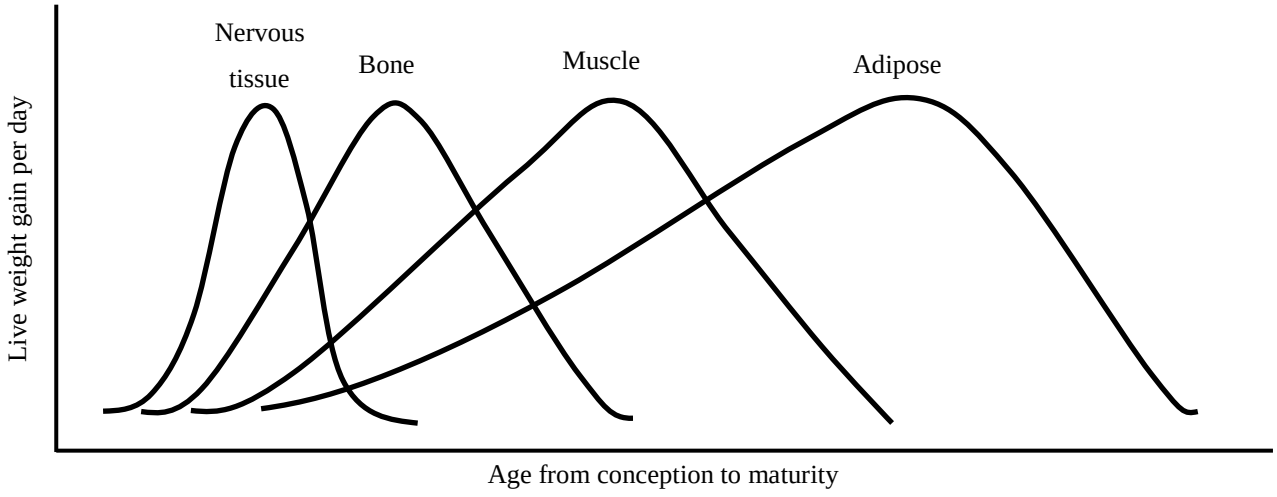

Figure 1.2 The allometric growth rate of nervous tissue, bone, muscle and fat as a function of age from conception to maturity (adapted from SPESFEED, 2010)

From this representation of allometric growth, it is apparent that as the animal ages, adipose tissue deposition accelerates whilst muscle development slows. This is essentially the end or fattening phase of

production wherein producers endeavour to manipulate growth by extending muscle growth and delaying the onset of or restricting adipose tissue growth.

1.3.3 Relative growth rates

Although intermediary metabolites may be used in either pathway, lipid metabolism and protein metabolism are non-antagonistic and independent processes. Protein metabolism is comprised of two processes, namely protein synthesis and protein degradation (proteolysis). The relative rates of these two processes determine the net rate of protein accretion or degradation. Similarly, lipid metabolism is also composed of two components, the degradation (lipolysis) and synthesis (esterification or lipogenesis), the relative rates of which determining the rate of lipid accretion or degradation.

1.3.4 Regulation

Growth is fundamentally regulated at a neurophysiological level whereby hypothalamic and pituitary secretions have cascading events at tissue levels, but is also regulated by a multitude of hormones. These regulatory mechanisms occur simultaneously and form intricately interlinked physiological pathways. Although it cannot be expected for all to be addressed in a single discussion, the major relative pathways will be discussed in this literature review.

Neurophysiological regulation

Growth is regulated on a neurophysiological level at the hypothalamic and pituitary regions through a variety of neurotransmitters including histamine, dopamine, acetylcholine, serotonin, gamma-aminobutyric acid (GABA), epinephrine, norepinephrine, endogenous opioid peptides and several neuropeptides. These neurotransmitters influence the pulsatile release of somatotropin (STH). Upon stimulation (or inhibition) the hypothalamus produces somatocrinin (GHRH) (or somatostatin (GHIH)) in an episodical manner, which enters and is transported through the hypophyseal portal system. GHRH stimulates the release of STH by the pituitary to systemic circulation, whilst GHIH inhibits STH secretion (Buonomo & Baile, 1992). This neurophysiological pathway is under constant regulation in the form of three distinct STH negative feedback pathways including 1) a GHIH mediated ultra-short loop inhibitory feedback system within the CNS, 2) a STH autoregulatory short-loop inhibitory feedback system, and 3) long-loop IGF-I negative feedback system (Bermann *et al*., 1994).

STH is a mitogen and is produced by somatotropic cells located in the anterior pituitary. Its mitogenic effects are achieved by two mechanisms: 1) activation of the MAPK/ERK pathway; and 2) stimulation of the JAK-STAT signalling pathway. In the MAPK/ERK pathway, after binding of STH to specific membrane receptors, A MAPK cascade is induced, and a complex route of at least three enzymes are activated in series and signal transduction ensued, eventually stimulating cell proliferation, especially of chondrocytes in cartilage (Zhang & Liu, 2002). In the JAK-STAT signalling pathway, activation inevitably stimulates gene

transcription, particularly in hepatocytes. These cells are stimulated to produce insulin-like growth factor 1 (IGF-1) which in turn stimulates growth directly and indirectly by a vast array of mechanisms, including but not limited to increased calcium retention, stimulation of sarcomere hypertrophy, promotion of lipolysis, increased protein synthesis, reduction in liver glucose uptake, promotion of gluconeogenesis, and increased deiodination of thyroxine (T4) to triiodothyronine (T3). Amongst other effects, these collectively result in an increased metabolic rate and potentiation of catecholamines (Aaronson & Horvath, 2002).

Other factors

Although the growth potential of any animal is determined by their underlying genetic constitution, several environmental and physiological limitations may restrict or result in deviations from this potential (McGlone & Pond, 2003). Nutritional status is considered to be a major determinant in the STH-IGF-1 axis. Sub-optimal nutrition has been found to elevate systemic STH concentrations, STH pulse amplitude and pituitary sensitivity to hypothalamic regulatory factors, but have no significant effect on either GHRH or GHIH secretion. Plasma metabolites such as glucose and volatile fatty acids, which are elevated and reduced respectively, shortly after ingesting a meal, also enhance STH secretion. These effects on STH, GHRH and GHIH are generated through insulin and glucagon pathways.

1.4 Efficiency of growth

1.4.1 Significance and quantification

Since the domestication of animals and adoption of pastoral lifestyles, mankind has endeavoured to quantify the absolute growth rate of their domesticated animals as well as the efficiency thereof to evaluate food source supply over a particular time frame. Similarly, modern commercial pig meat production systems are efficiency driven enterprises, aiming to gain the most financial reward for the least input cost and hence maximise profit margins.

Efficiency measures are largely a management tool and usually correlate to the particular producer's objectives. Consequently, they are represented in temporal (e.g. ADG) and/or economic (per unit of resource) used (e.g. F:G). As feed may constitute up to 70-80 % of total costs (English *et al*., 1988b, SPESFEED, 2010), and remuneration being a function of total carcass mass and PORCUS classification (Bruwer, 1992), the unit mass of edible lean meat produced per unit cost of feed consumed may be considered a fundamental, although crude, measure of efficiency.

However, in order to measure this efficiency, one needs to quantify growth. Apart from direct weight measurements, the most common approach to accomplish this is to use easily obtainable, practical and inexpensive linear carcass measurements, which combined with regression equation(s) based upon established growth patterns, provides an indication of the extent of maturity and/or growth of a particular tissue component (Topel & Kauffman, 1988). The difference in these linear measurements over a given time period provides the basis from which the absolute growth rate may be estimated.

Fortunately in swine, the numerous localised adipose depots throughout the body enlarge to such an extent that they become contiguous with other depots, such as the case for subcutaneous backfat. The depth of carcass fat over the *longissimus dorsi* (AUS-MEAT, 2000) at the P2 position (Figure 1.3), provides the most accurate objective indicator of total body fat over a wide range of live weights of crossbred swine (Rossouw, 1982; Bruwer, 1984; Bruwer, 1992), and has been accepted as the industry standard for carcass classification for many years. This P2 position is located approximately "65mm from the dorsal midline at the level of the posterior edge of the head of the last rib" (Greer *et al.*, 1987). A measurement (in millimetres) is taken from both left and right sides of the animal and their mean used.

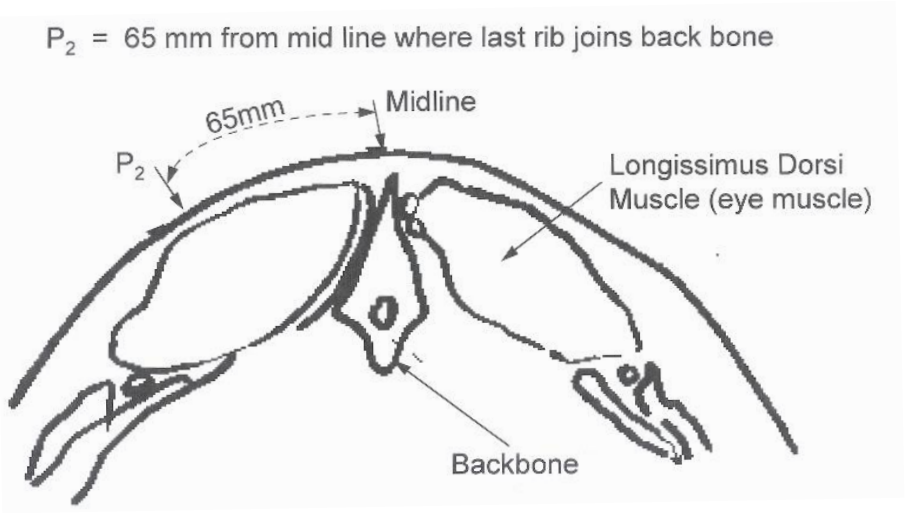

Figure 1.3 Transverse plane of a pig carcass at the last rib, indicating the P2 position relative to *longissumus dorsi*, dorsal midline, and backbone (Elizabeth *et al.*, 2004)

Accurate P2 measurements and carcass classification are considered a necessity, not only to correctly value the carcass, but also for the following reasons:

- *In vivo* P2 measurements may be used determine the optimum time to market animals or monitor dry-sow productivity to optimise breeding and maximise sow productivity.
- On-farm and abattoir P2 measurements may be used to evaluate breeder performance and/or identify any environmental and/or nutritional inadequacies leading to lowered productivity.
- Fat depth may be used to determine market destination

1.4.2 Measurement instruments

In the live animal P2 thickness may be measured by a variety of non-invasive probes including the Renco Lean-Meater® (RLM), SFK Pig Scan-A-Mode backfat scanner (SFK) and Meritronics A-Mode Pulse Echo scanner (MT), Sonalyser Pig Monitor (SON), Medata Backfat Grader (MED). These devices utilise ultrasonic waves and based upon the delay of the echo of the sound wave which is caused by the differing

densities of fat and lean tissue, P2 thickness is estimated. With the exception of MT, all these are hand-held devices. The RLM and MED were found to be the most user friendly due to their portable probes and lack of need to interpret an oscilloscope (as in the case of the MT), and hence rapid measurement (Greer *et al.*, 1987). Regarding accuracy, MT was found to be the most accurate, followed by SON, RLM, and MED. However, the differences between MT, SON, and RLM are negligible Greer *et al*. (1987). Nevertheless, all devices were found to underestimate carcass P2 compared to Introscrope® (INT) measurements (Greer *et al.*, 1987). Accuracies of the digital devices are most often compromised due to the inability to detect the 3rd layer of fat, ineffective coupling fluid contact, and mislocation of the P2 site, and very low P2 thicknesses (Greer *et al.*, 1987, Elizabeth *et al.*, 2004).

In abattoirs, although ultrasonic probes may also be used for carcass P2 measurement, optical devices such as the INT and the Hennessy Grading Probe (HGP) are preferentially used as they provide a far more accurate measurement of backfat depth. Although the INT is simplistic in design and easily cleaned, its accuracy largely relies on the operator's ability to use the device at the correct measurement site as well as correctly align a black line in the viewer and correctly read the backfat measurement. Unlike the INT, the HGP is more complex, relying on reflectance spectroscopy whereby liquid density variations and solid material surface finish variations cause differing back scattered light patterns, which are subsequently detected by a sensor. This allows the device to objectively measure a variety or parameters including meat and fat thickness, colour, water holding capacity, intramuscular marbling and tissue structure. A high level of precision is afforded through the HGP's ability to record up to 10 measurements per millimetre. Additionally, the HGP is less prone to operator-induced errors than the INT, and allows integration of automatic recording, which is not possible with the INT (AUS-MEAT, 2000). For these reasons, in most commercial abattoirs, the INT has been replaced by the HGP.

1.5 Manipulation of growth

Although optimization of management in terms of housing and biosecurity, as well as rapid genetic progression in lean lines and optimisation of nutrition through provision of high quality balanced rations allows one to improve production efficiencies greatly, without intervention, the full growth potential of modern lean genetic lines cannot be fully exploited. Consequently, many producers use metabolic-modifying xenobiotics or feed additives, a field that has undoubtedly dominated production animal research. Although xenobiotics and feed additives cannot substitute for optimal management, nutrition, or biosecurity, their use does significantly complement optimal management.

1.5.1 Metabolic growth modifiers

Metabolic modifiers act to increase feed efficiency, improve growth rate, and alter carcass composition by increasing lean tissue accretion and/or supressing fat deposition (Meisinger, 1989; Mitchell *et al.*, 1994).

By these carcass improvements, processing (deboning) efficiencies at meat packing facilities is also indirectly improved.

Metabolic modifiers may be categorised as: (1) somatotropin, (2) phenethanolamines (or β-adrenergic agonists), (3) anabolic steroids (e.g. estrogenic and androgenic implants), (4) vitamins or vitamin-like compounds (including specific minerals) fed in supra-nutritional levels, (5) 'designer' lipids (e.g. CLA) and (6) other modifiers (Dikeman, 2007). One of the more recent approaches in the field of xenobiotics has focussed on the use of β-adrenergic agonists (βAAs).

CHAPTER 2 LITERATURE REVIEW

2.1 History of β-adrenergic agonists in meat animal production

For more than 30 years, βAAs have been used in the treatment of chronic lung disease (bronchitis, obstructive pulmonary disease, asthma), as tocolytics in animals, and as heart tonics in both human and veterinary application (Anderson *et al.*, 2005; Pleadin *et al.*, 2012a; Pleadin *et al.*, 2012b). Beyond their therapeutic effects, βAAs have also been demonstrated to be able to redirect nutrients away from adipose and towards muscle, resulting in three metabolic actions: 1) stimulation of metabolic rate, 2) reduced adipose lipid deposition and 3) increased muscle protein accretion. These result in improved feed utilisation efficiency, increased dressing percentage, increased leanness, and increased rate of weight gain (Moody *et al.*, 2000; Anderson *et al.*, 2005) and have been demonstrated in most production animal species including cattle, sheep, chickens, turkeys, and swine. βAAs are also referred to as repartition agents due to their ability to reduce and increase body fat lean content respectively without altering organ or bone mass. Additional to their direct effects, the time taken for an animal to reach ideal slaughter weight is also reduced, reducing overall maintenance costs for each animal and hence improving profit margins.

2.2 Structure

βAAs are a group of xenobiotic catecholamine analogues. Structurally resembling the endogenous adrenocorticoids epinephrine and norepinephrine (Beermann, 2001; Mills, 2002), βAAs are typically characterised by a substituted aromatic ring, ethanolamine side chain, with various substitutions on the aliphatic nitrogen (Figure 2.1). The biological activity of βAAs is afforded by the alkaline pKa of the aliphatic amine group which allows the molecule to exist in the ionized form in blood and tissues at physiological pH (7.4). To an extent, this analogous chemical structure allows βAAs to mimic catecholamines. However, epinephrine and norepinephrine have broad-spectrum effects whereas βAAs are receptor specific and have selective effects.

2.2.1 Stereoisomerism

Chirality in βAAs produces optically active isomers which occur in a racemic mixture. RAC has two chiral centres, and hence a possible of four stereoisomers, RR, RS, SR, and SS (Mills, 2002). These isomers express different binding affinities, with the RR isomer having a 3- to -600 fold higher affinity for β_1AR and β_2AR than the other isomers (Mersmann, 1998; Mills, 2002). Thus, the RR isomer accounts for the majority of the response observed in RAC-supplemented pig diets.

2.2.2 Hydroxylated agonists

Some β-adrenergic agonists such as cimaterol and clenbuterol include a halogen atom that is substituted for the hydroxyl group (Smith, 1998). Although this halogen atom does not inhibit receptor binding, it does create a resistance to rapid metabolic deactivation by enzymes active toward aromatic hydroxyl groups

(Morgan, 1990; Smith, 1998), and hence giving these substances long half-lives and high oral potencies. The hydroxyl-halogen substitution also causes the aromatic portion of the agonists to be more lipophilic compared to the hydroxylated agonist, allowing the molecule to readily partition into adipocytes. Resultantly, these halogenated βAAs have very aggressive metabolic modifying effects with adverse effects on marbling and tenderness and visual appraisal parameters (colour scores) (Moody *et al*., 2000), and foodborne poisoning in humans due to the consumption of animal tissue containing residues of these βAAs (Salleras *et al*., 1995; Dikeman, 2007; Pleadin *et al*., 2010; Pleadin *et al*., 2012a; Li *et al.*, 2013).

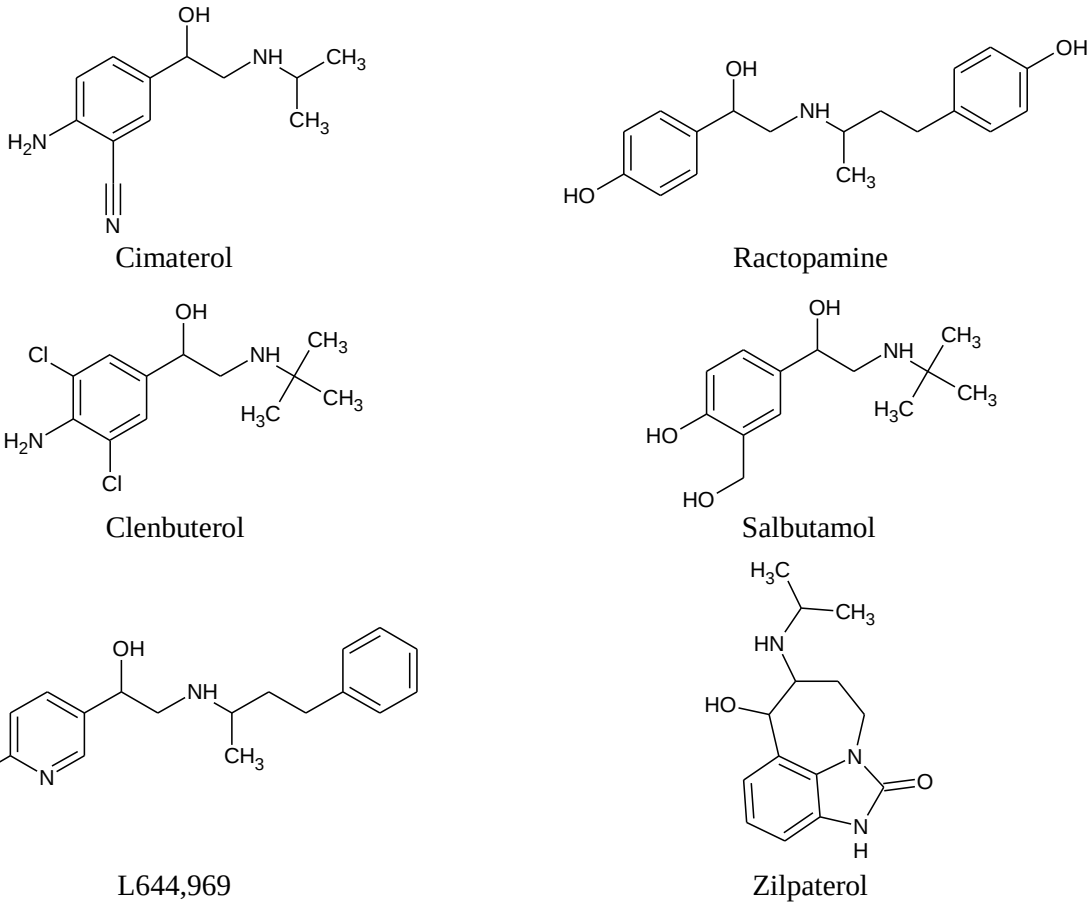

Figure 2.1 Structure of several βAAs (adapted from Anderson *et al.*, 2005)

2.3 Trade restrictions

Although effective doses of RAC in swine and Zilpaterol in cattle have negligible to undetectable residue levels, the negative publicity in the early 1990's resulting from the cases where the indiscriminate misuse of clenbuterol in Spain and France leading to acute food-poisoning in humans has resulted in the outright ban of all βAAs in all livestock in the EU (Directive 96/22/EC) as well as many other countries with

the subsequent establishment of national monitoring systems and international trade restrictions to eliminate their misuse.

Despite this, the use of ractopamine hydrochloride (RAC; Paylean®, Elanco Animal Health, Greenfield, IN) in finishing pigs as well as several other species has been approved in 21 countries including South Africa (Anderson *et al.*, 2005). RAC is labelled for use at doses of 5 to 10 $mg.kg^{-1}$ in complete pig diets with a minimum CP content of 16% for the final 20.5 to 40.9 kg weight gain before slaughter in pigs initially weighing at least 68 kg (live weight).

2.4 Elimination and withdrawal period

Although some βAA residues remain detectable 7 days after withdrawal (Pleadin *et al.*, 2012b), any consumption of residue containing tissues would pose a negligible risk to humans (Smith, 1998) since it is rapidly excreted via the urine in pigs (Dalidowicz *et al*., 1992; cited in Smith, 1998) resulting in low tissue residues, and has a low oral potency in humans (Smith, 1998). Thus, no withdrawal period is prescribed for RAC in pigs.

2.5 Pharmacokinetics of RAC

Like other βAAs, RAC appears to selectively enhance skeletal muscle growth, and hence increase carcass dressing percentages (Mills, 2002). This effect is facilitated through binding with cell-membrane bound β-adrenergic receptors (βAR) with ensuing physiological cascades.

2.5.1 β-adrenergic receptors

β-adrenergic receptors (βAR) belong to a subset of a large family of 7-transmembrane domain proteins known as G-protein coupled receptors (Mills, 2002). Three subtypes of βAR are recognised (β_1, β_2, β_3), and although present on almost every mammalian cell type including adipose and muscle cells, their relative abundances may vary between tissue types as well as within tissue types between ages (Mersmann, 2002). In pigs, the proportion of β_1- and β_2AR subtypes are dominant, with proportions approximately 80:20, 72:28, 65:35 and 50:50 in adipose, heart, lung and skeletal muscle respectively (McNeel & Mersmann, 1999; Liang & Mills, 2002; Mersmann, 2002). Normally these receptors are physiologically stimulated by the adrenal medullary hormone, epinephrine and the neurotransmitter, norepinephrine (Mersmann, 1998; Mills, 2002), but may also bind with and are stimulated by βAAs.

Although β_1AR and β_2AR are co-expressed in most tissues, and RAC is considered non-selective in binding to either receptor subtype (Mills, 2002), their differing relative ratios between tissues causes differences in tissue responses to RAC. Further, since βAR's are not only present on adipose and skeletal muscles, the specific effects manifested in growth and carcass compositional changes is complicated by other secondary effects such as modification of blood flow, alteration of hormones concentrations (e.g. lower plasma

insulin may lead to increased lipolysis and decreased lipogenesis), or alterations in CNS control of feed intake (Mersmann, 1998; Mersmann, 2002; Mills, 2002).

Since RAC exhibits a different response in adipose compared to muscle, the pharmacokinetics of RAC in these tissues will the discussed separately.

2.5.2 Adipose tissue

In pigs, white adipose tissue (WAT) is the primary site of *de novo* fatty acid synthesis. Pigs fed RAC fortified diets exhibit increased triglyceride hydrolysis and decreased fatty acid and triglyceride synthesis (Mills, 2002) which inevitably results in the decreased hypertrophy of adipocytes in WAT and hence decreased fat deposition (Mersmann, 2002). These are the summative effects of a decreased expression of lipolytic genes by cAMP-directed mechanisms (Halsey *et al*., 2011) which are resultant from a βAA-receptor initiated signal transduction pathway. Upon RAC-βAR binding, a Gs protein is activated, whose α-subunit then activates adenylyl cyclase (AC), which in turn converts adenosine triphosphate (ATP) to the intracellular signalling molecule, cyclic adenosine monophosphate (cAMP) (Moody *et al*., 2000; Mills, 2002; Anderson *et al*., 2005). This signal transduction mechanism is presented in Figure 2.2. cAMP then binds to the regulatory subunit of protein kinase A (PKA), releasing its catalytic subunit which phosphorylates several intracellular proteins (and enzymes) resulting in 1) the activation of rate limiting lipolytic enzymes and 2) inactivation of lipogenic enzymes involved in *de novo* fatty acid and triglyceride synthesis (Fain and Garcia-Sainz, 1983; Mersmann *et al*., 1987; Moody *et al*., 2000). These result in the activation and translocation of hormone-sensitive lipase which in turn initiates lipolysis (Mersmann, 2002). The liberated fatty acids are then largely exported to other tissues for use as oxidative fuels.

Through phosphorylation and inactivation of glucose transport and acetyl-CoA carboxylase, activation of PKA is also known to reduce expression of lipogenic genes and hence indirectly have antilipogenic activity (Mersmann, 1998; Mills, 2002). RAC also depresses lipogenesis in the adipogenic cell line TA1, inhibits adipocyte responsiveness to insulin (Hausman *et al*., 1989) and inhibits stromal vascular cells.

Therefore, the summative result of these effects is a reduced rate of lipogenesis and increased rate of lipolysis (triglyceride hydrolysis) (Mills, 2002), which is further potentiated by a reduced responsiveness to lipogenic cues such as insulin and reduced proliferation of adipocytes from its progenitors.

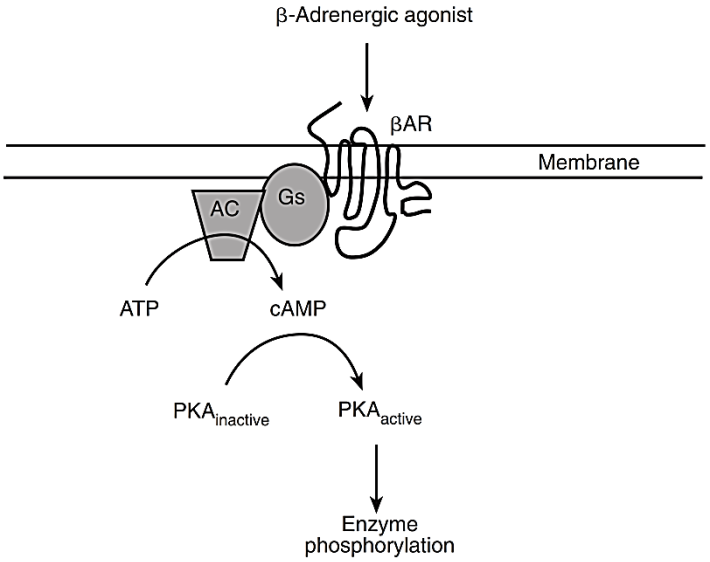

Figure 2.2 Mechanism of signal transduction from beta-adrenergic receptors (βAR) (Moody *et al*., 2000)

2.5.3 Skeletal muscle

Direct effects on muscle include myocyte hypertrophy, muscle fibre type frequency changes, and differential muscle RNA, DNA, and protein accretion rates (Moody *et al*., 2000; Beermann, 2001). Elucidation of the effect of RAC in muscles has involved quantification of key enzymes in muscle metabolism including the intracellular cathepsins (calcium-independent, lysosomal cysteine proteinases) and calpains (calcium-dependent, non-lysosomal cysteine proteases), and calpastatin (an endogenous calpain inhibitor), as well as specific muscle proteins such as actin and myosin.

In contrast to β2-specific βAAs (such as cimaterol, clenbuterol and L644.969), whose supplementation results in an up-regulation of cathepsin B & L, calpastatin, and decrease in calpain I (also known as μM calpain), resulting in an increase in protein synthesis as well as inhibition of protein degradation (Koohmaraie *et al*., 1991; Moody *et al*., 2000), RAC supplementation has been shown to increase α-actin mRNA abundance (Bergen *et al*., 1989; Grant *et al*., 1993; Helferich *et al*., 1990 cited by Beerman, 2001) as well as calpain II (also known as mM calpain) activity without either affecting calpain I or calpastatin activities, or calpastatin mRNA (Ji *et al*., 1991a, cited by Beerman, 2001; Ji, 1992, cited by Moody *et al*., 2000). Although RAC is predominantly β1-specific, it does express some β2 affinity (Hossner, 2005), which may be the reason for contradictory results of Bergen *et al*. (1989) who also found RAC supplementation to increase cathepsin L activity, but not cathepsin B and H, nor either calpains, and Sainz *et al*. (1993a) who found calpain II and calpastatin to be unaffected by RAC supplementation. Excluding discrepancies of Bergen *et al*. (1989) and Sainz *et al*. (1993a), these results are indicative that RAC predominantly increases the rate of protein synthesis, without affecting the rate of protein degradation (Moody *et al*., 2000).

Also, dose- and time-dependent changes in muscle fibre distribution (Depreux *et al*., 2002) and size (Sainz *et al*.,1993a) have been found with RAC supplementation. RAC increases the proportion of type IIb muscle fibres and decreases the proportion of type IIa muscle fibres (Aalhus *et al*., 1992) as well as increases the average fibre diameter of both IIa and IIb fibres (Aalhus *et al*., 1992; Dunshea *et al*., 1993b), without affecting either proportion or diameter of type I fibres (Aalhus *et al*., 1992). Hypertrophy of type II fibres accounts for the increase in muscle mass without change in muscle length (Aalhus *et al*., 1992; Beerman *et al*., 2001).

2.6 Factors influencing response to RAC

Owing to the complex pharmacokinetic and physiological mechanisms, the efficacy of RAC supplementation is affected by a number of factors including the animals' physiological state, species, diet, dosage, duration of supplementation, age, weight and genetics. The understanding of these is critical for a successful implementation of RAC supplementation.

2.6.1 Species

Differences in responses have been found between different species. These differences are partly due to differences in receptor selectivity (β1 or β2) and distribution, as well as the closeness of the species to its maximal growth rate resulting from differing selection intensities. These differences directly influence the response to RAC (*vide* β-adrenergic receptors).

2.6.2 Diet

Although RAC-induced growth and carcass improvements are independent of dietary energy content (Hinson *et al*., 2011), RAC-fortified fed swine require altered elevated dietary protein and lysine:energy contents in order to supply sufficient precursors for the elevated protein synthesis and deposition associated with RAC feeding (Mitchell *et al*., 1991; Dunshea *et al*., 1993a; Moody *et al*., 2000; Apple *et al*., 2004c). Typically, ≥167 g kg^{-1} CP (as-fed basis) is required to achieve a significant response to RAC (Dunshea *et al*., 1993a). However, optimal growth performance response achieved at 160-200 g kg^{-1} CP or lysine equivalent (Anderson *et al*., 1987, cited by Mitchell *et al*., 1994; Adeola *et al*., 1990; Jones *et al*., 1988, cited by Moody *et al*., 2000) and 13.8 MJ of ME/kg (as fed basis) with Lys:ME in the region of 0.574-0.741 g/MJ ME (as-fed basis) (Apple *et al*., 2004c). Despite these recommendations, diets to the higher end of the Lys:ME range have been found to have adverse effects on marbling and tenderness meat characteristics (Apple *et al*., 2004c). Additionally, increased carcass leanness may be achieved at lower CP and energy levels (Ji *et al*., 1991b, cited by Moody *et al*., 2000; Jones *et al*., 1992, cited by Moody *et al*., 2000) when feeding RAC-fortified diets. The higher CP% specification is augmented with RAC doses >20 $mg.kg^{-1}$ due to the RAC-induced depression in average daily voluntary feed intake (ADFI).

Also, dietary background prior to βAA supplementation has also been found to compound the CP% requirements, with greater RAC-induced responses in animals who receive higher CP% diets prior to and during βAA supplementation as compared to those who receive diets with restricted CP% prior to βAA supplementation (Mitchell *et al*., 1994).

Interestingly, Smith *et al*. (1995) found that although carcass composition improvements were noticeable in females under *ad. lib*. feeding, RAC-induced improvements in growth rate, growth efficiency and carcass composition were more pronounced under restricted feeding regimens rather than *ad. lib*. feeding in both sexes.

2.6.3 Age or weight

RAC-induced improvements in growth are observable in all animals irrespective of starting weight (Rikard-Bell *et al*., 2007; 2009a). However, with appetite and live-weight being correlated, and older animals tending to be more predisposed to adipose deposition, the net growth rate and feed efficiency responses to RAC is far more pronounced in older, heavier animals (Sainz *et al*., 1993b; Rikard-Bell *et al*., 2007; 2009a).

2.6.4 Genetics

RAC has been shown to be effective in divergent genotypes (Mills *et al*., 1990; Yen *et al*., 1990; Gu *et al*., 1991a,b; Goerl *et al*., 1995; Herr *et al*., 2001). Whilst both Bark *et al*. (1992) and Gu *et al*.(1991b) found that RAC had greater lean growth benefits in lean lines, Stoller *et al*. (2003) found RAC to reduce adiposity to a greater extent in lines with a greater propensity for fat deposition.

2.6.5 Sex

There is limited literature comparing the differences between sexes in response to RAC supplementation. Although RAC improves growth performance, growth efficiency and carcass traits regardless of sex, Rikard-Bell *et al*. (2009b) found that, regardless of RAC concentration, carcass lean, G:F and ADG were higher in boars than immunocastrates which were in turn greater than gilts; whilst carcass weights and ADFI was greater in immunocastrates than boars which were in turn greater than gilts. Also, the authors found changes in P2 were greater in immunocastrates than gilts, which were in turn greater than boars.

2.6.6 Treatment duration

As a result of βAR down-regulation in both adipose (Dunshea *et al*., 1998a) and muscle tissues (Williams *et al*., 1994) with chronic RAC supplementation, the effects of RAC diminish over time, and in other words are transient. Since urinary RAC elimination rate has been found to increase dramatically from 4-16% to 36-85% when repeat doses are given compared to single doses (Dalidowicz *et al*., 1992 (cited by Smith, 1998)), elimination rate of RAC may also promote transient effects.

Peak effects on different growth performance indicators occur at different times. For example, weight gain characteristics such as ADG begin to plateau after approximately 21 days (Williams *et al*., 1994), whereas, carcass composition benefits continue with increasing RAC durations (Williams *et al*., 1994). Although economic implications need to be considered to determine the optimum treatment duration, RAC is normally supplemented for the last 28 days prior to slaughter at a constant rate of 10 mg.kg^{-1} feed (DM basis).

To offset the desensitisation and reduced ADFI associated with chronic constant-dose RAC supplementation, alternative RAC feeding regimens (including step-up, intermittent, alternating, and step-down) have been explored. Step-up regimens have been demonstrated to prolong RAC-induced effects (See *et al*., 2004; Rikard-Bell *et al*., 2009b), whilst step-down regimens have been found to be less effective than constant doses (See *et al*., 2004). Intermittent and alternating RAC-supplementation has also been found to promote RAC-induced effects above constant doses, with intermittent being superior to alternating regimens (Sainz *et al*., 1993a,b). Furthermore, in alternating regimens, growth benefits are greater where RAC is supplemented in the latter half rather than initial half of the feeding period (Sainz *et al*., 1993a,b).

2.6.7 Dosage

Across literature, most studies include RAC within a range from 0 to 20 mg.kg^{-1}. Doses toward the lower end of the spectrum improve weight gain and feed efficiency with lesser carcass parameter improvements, however doses toward the higher end of the spectrum improve weight gain, feed efficiency, and carcass parameters. Although weight gain is optimised at low doses and diminishes at doses >20 mg.kg^{-1} due to the RAC-induced appetite depression, leanness and dressing percentage improvements continue through the highest doses tested (30 mg.kg^{-1}) (Moody *et al.*, 2000).

2.7 Effects of RAC

RAC has direct tissue effects which are quantifiable according to growth parameters, linear carcass measurement, carcass composition and meat quality characteristics. Additionally, RAC may potentiate these effects through an increased blood flow such that more substrate and energy may be delivered to skeletal muscle and non-esterified fatty acids away from adipose depots, and hence promoting protein synthesis and restricting lipogenesis (Mersmann, 1998).

2.7.1 Live animal growth performance

Average daily gain (ADG)

Although some authors have reported a 0.9% (Mimbs *et al*., 2005) and 1.9% (Mitchell *et al*., 1991) reduction in ADG in finishing swine when fed diets containing 10 and 20 mg.kg^{-1} RAC respectively, a meta-analysis by Apple *et al*. (2007b) revealed that across 23 publications, supplemental feeding of RAC at 5 mg.kg^{-1} improved ADG by 12% with a range of 6.4% (Stites *et al*., 1991) to 25.9% (Armstrong *et al*, 2004) compared to non-supplemented pigs, with no difference in improvement between pigs fed 5, 10 or 20 mg.kg^{-1} RAC.

Subsequently, Rikard-Bell *et al*. (2007, 2009a,b) found a linear increase in ADG in both immunocastrates, boars and gilts with RAC supplementation up to 20 mg.kg^{-1} for up to 28 days regardless of starting weight.

Average daily feed intake (ADFI)

The meta-analysis by Apple *et al*. (2007b) found that although ADFI was numerically reduced in 5 and 10 mg.kg^{-1} RAC supplemented diets, ADFI was only reduced at 20 mg.kg^{-1} RAC compared to control animals. Armstrong *et al*. (2004) remains the only author to report an increase in ADFI at 5 mg.kg^{-1} RAC supplementation.

Feed efficiency

Feed efficiency is often represented as a ratio such as F:G or G:F. Regardless, RAC supplementation results in a linear decrease in F:G (in other words increased G:F) with increasing RAC concentrations (Apple *et al*., 2007b). Improvements in F:G are quantifiable from as early as 6 d, and peaks around 6-13d. Although improvements are still significant for up to 34 d, the difference between supplemented and non-supplemented individuals diminishes over time. This diminishing effect may be explained by the rapid down-regulation of βAR's.

F:G decreases linearly with RAC up to at least 20 mg.kg^{-1} RAC regardless of sex, however F:G of intact boars remains superior to that of barrows which were in turn superior to gilts (Rikard-Bell *et al*., 2007, 2009a,b). At 5 mg.kg^{-1} RAC, F:G improves by approximately 10% (Apple *et al*., 2007b) with a range of 6.3% (See *et al*., 2005, cited by Apple *et al*., 2007b) to 17.2% (Stites *et al*., 1991), whilst G:F is improved by 13.3% and 16.7% (Apple *et al*., 2007b) with corresponding ranges of 5.6% (Mimbs *et al*., 2005) to 25.9% (Watkins *et al*., 1990), and 3.2% (Gu *et al*., 1991a) to 26.7% (Armstrong *et al*., 2004) at 10 and 20 mg.kg^{-1} RAC supplementation respectively.

The change in F:G is also directly correlated with the immediate decrease in ADFI, especially at higher RAC supplementation levels, which essentially mimics a restricted feeding-type response. This suggests that gut-throughput and other factors contributing to the digestibility and bioavailability of feed components would also contribute to the observed improvement in G:F.

2.7.2 Linear carcass measurements

RAC increases carcass cutability and primal yields of finisher pigs (Bohrer *et al*., 2013a) without adversely affecting processing characteristics of further processed products from either hams (Fernández-Dueñas *et al*., 2008; Boler *et al*., 2011), bellies (Scramlin *et al*., 2008), loins (Stites *et al*., 1991; Apple *et al*., 2004a; Carr *et al*., 2005a,b; Rincker *et al*., 2005, 2009) or shoulders (Tavárez *et al*., 2012).

Hot carcass yield (HCY)

Through a meta-analysis over multiple RAC trials, Apple *et al*. (2007b) found that, provided adequate dietary CP, HCY improved by an average of 2.3%, 3.1% and 2.7% with ranges of 1.4% (Watkins *et al*., 1990) to 7.7% (Armstrong *et al*., 2004), 0.1% (Watkins *et al*., 1990) to 6.5% (Armstrong *et al*., 2004) and 0.3% (Watkins *et al*., 1990) to 10.7% (Armstrong *et al*., 2004) for 5, 10, and 20 $mg.kg^{-1}$ RAC supplementation, respectively.

Some authors have found a linear relationship between HCY and RAC supplementation concentration continuing through RAC concentrations of 20 $mg.kg^{-1}$ RAC, however, Apple *et al*. (2007b) indicated that no further benefit in HCY improvement was found above 5 $mg.kg^{-1}$ RAC supplementation.

Dressing percentage (Dr%)

Apple *et al*. (2007b) found that Dr% was not radically affected by RAC treatment. Compared to control animals, 5, 10 and 20 $mg.kg^{-1}$ RAC supplementation rates only increased Dr% by a mean of 0.3%, 0.8%, and 1.2% with corresponding ranges of 0.3% (Stites *et al*., 1991) to 2.0% (See *et al*., 2005, cited by Apple *et al*., 2007b), 0.7% (Watkins *et al*., 1990) to 2.2% (See *et al*., 2005, cited by Apple *et al*., 2007b) and 0.9% (Yen *et al*., 1990) to 2.9% (Armstrong *et al*., 2004).

Fat depth

Throughout literature, there are conflicting results regarding the effect of RAC on subcutaneous fat depots. Although being a potent stimulator of adipose mobilisation, the combination of rapid adipocyte βAR down-regulation (Dunshea & King, 1995; Dunshea *et al*., 1998a), lack of effect on lipogenesis (Liu *et al*., 1994; Dunshea *et al*., 1998b), and relative insensitivity of adipocytes to βAAs (Pethick *et al*., 2005) results in RAC often not decreasing fat deposition (Fernández-Dueñas *et al*., 2008). Rikard-Bell *et al*. (2009b) further reported that the effects of RAC on fat deposition were influenced by genotype, dietary, and environmental factors and interactions. When considering the available studies, Apple *et al*. (2007b) found RAC to decrease 10^{th} rib fat depth by approximately 0.04 cm, 0.14 cm, and 0.23 cm with corresponding ranges of -10.0% to 15.3%, -16.1% to 6.6% and -27.5% to 17.2% for 5, 10 and 20 $mg.kg^{-1}$ RAC respectively. P2 fat thickness, like 10^{th} rib fat depth, is strongly positively correlated with overall carcass fatness, and hence can be expected to react in a similar pattern with RAC supplementation.

Loin eye muscle (LM) area and depth

LM area and depth are often used as indicators of total carcass lean growth. Apple *et al*. (2007b) found that LM area and depth increase linearly with increasing RAC supplementation concentrations. At 5 $mg.kg^{-1}$ RAC, LM area increased approximately 2.3 cm^2 (Apple *et al*., 2007b) with a range of 6.6% (Watkins *et al*., 1990) to 13.7% (Armstrong *et al*., 2004), whilst LM depth increased between 3.6% (Herr *et al*., 2001) to 8.9% (See *et al*., 2005 cited by Apple *et al*., 2007b). At 10 $mg.kg^{-1}$ RAC, LM area increased approximately 3.5 cm^2

(Apple *et al*., 2007) with a range of 3.3% (Stoller *et al*., 2003) to 20.7% (Crome *et al*., 1996), whilst LM depth increased between 5.4% (See *et al*., 2005 cited by Apple *et al*., 2007b) and 9.4% (Apple *et al*., 2004a, cited by Apple *et al*., 2007b). At 20 mg.kg^{-1} RAC, LM area increased approximately 4.5 cm^2 (Apple *et al*., 2007b) with a range of 4.8% (Schinckel *et al*., 2003) to 30.1% (Crome *et al*., 1996), whilst LM depth increased between 5.6% (Dunshea *et al*., 1993b) and 8.2% (Adeola *et al*., 1990).

2.7.3 Carcass composition

Further investigation by Apple *et al*. (2007b) revealed that calculated muscle yield, or estimated fat-free lean yield, increased on average of 0.9, 1.3, and 2.4 percentage units upon RAC supplementation at 5,10, and 20 mg.kg^{-1} concentrations respectively. Carcass dissection further demonstrated 3.1 to 5.1% (Watkins *et al*., 1990), 1.1 (Stites *et al.*, 1991) to 7.9% (Watkins *et al*., 1990), and 6.2 (Xiao *et al.*, 1999) to 11.8% (Watkins *et al*., 1990) increases in lean muscle composition, and 1.7 to 7.0% (Watkins *et al*., 1990), 6.2 (Aalhus *et al*., 1990) to 9.0% (Watkins *et al*., 1990) and 10.0 (Gu *et al*., 1991a) to 22.6% (Xiao *et al*., 1999) decrease in dissected carcass fat content with 5, 10, and 20 mg.kg^{-1} RAC supplementation respectively when compared to non-supplemented pigs.

10 and 20 mg.kg^{-1} RAC resulted in 3.4 (Herr *et al.*, 2001) to 17.2 % (Crome *et al.*, 1996) and 4.6 (Bark *et al.*, 1992) to 18.3% (Crome *et al.*, 1996) increases in dissected lean from hams respectively, whilst dissectible fat in hams may decrease by up to 45%. This more pronounced response to RAC in hams compared to the overall animal body suggested that the improvement to yields of particular high-value cuts brought about by RAC supplementation may be more responsible for the added carcass value, and should be emphasised during carcass valuation.

2.7.4 Meat quality

Fatty acid composition

With faster growing lean lines, there is an increase in unsaturation of subcutaneous fat depots. Despite health benefits associated with the consumptions of poly-unsaturated fatty acids (PUFA), this increased unsaturation may lead to handling and processing problems and reduced shelf life of pork products (Larrick *et al*., 1992, Wood *et al*., 1999). Apple *et al*. (2007b) found RAC supplementation to decrease subcutaneous adipose saturation largely through the increase in both monounsaturated fatty acids (MUFA) and PUFAs with more pronounced changes in PUFA proportions which were primarily the result of an increase in proportion of linoleic (Carr *et al*., 2005b; Xi *et al*., 2005 (cited by Apple *et al*., 2007b); Weber *et al*., 2006) and α-linolenic acids. Compared to non-supplemented pigs, RAC supplementation at 5, 10 and 20 mg.kg^{-1} has been reported to decrease saturated fatty acids by 3.8%, 2.8% and 1.0%, respectively, whilst simultaneously PUFA content increased by 10.2%, 7.3% and 9.5% respectively (Perkins *et al*., 1992). These changes in saturation were found to be more pronounced with increasing duration of supplementation, especially beyond 28 days.

Contrastingly, in muscle, changes in fatty acid profile may only be evident at 20 mg.kg^{-1} RAC supplementation (Apple *et al*., 2007b).

Dietary fat sources have also been implicated in altering saturation content, with animal-based sources increasing saturation, and plant based fat sources increasing poly-unsaturation (Apple *et al.*, 2007a). This may be used to mediate adverse meat quality attributes associated with RAC supplementation.

pH, firmness, and water-holding capacity

Muscle pH, measured 45 min and 24 h post-mortem, is known to directly affect colour, firmness and water holding capacity characteristics of meat. According to the majority of research, neither measurement have been found to be affected by RAC supplementation (Aalhus *et al*., 1990; Dunshea *et al.*, 1993b; Stites *et al*., 1994 (cited by Apple *et al*., 2007b); Herr *et al*., 2001; Stoller *et al*., 2003; Carr *et al*., 2005a,b; Rinker *et al*., 2005 (cited by Apple *et al.*, 2007b)). Similarly, firmness is reported to not be affected by RAC supplementation.

Although firmness and water-holding capacity of muscle are closely associated, the response of drip loss is somewhat inconsistent throughout literature. Uttaro *et al*. (1993), Stoller *et al*. (2003), Apple *et al*. (2004b) (cited by Apple *et al*., 2007b), Rincker *et al*. (2005) (cited by Apple *et al.*, 2007b) and Carr *et al*. (2005a) found RAC supplemented at 20 mg.kg^{-1} to reduce drip loss percentage by 6.5%, 7.6%, 15.6%, 20.3% and 33.2%, respectively, compared to non-supplemented pigs. However, when considering all available literature, Apple *et al*. (2007b) determined drip loss to not be significantly affected by RAC supplementation. Subsequently, Athayde *et al*. (2012) found RAC supplementation reduced SM drip loss, but did not affect LM drip loss.

Colour

Since pork colour is a critical characteristic influencing the perception of freshness, any adverse effect on colour caused by RAC would most likely render the use of RAC unfavourable. In some instances, 10 mg.kg^{-1} RAC supplementation has been implicated to increase American (Watkins *et al*., 1990; Apple *et al*., 2004b (cited by Apple *et al*., 2007b)) and Japanese (Apple *et al*., 2004b (cited by Apple *et al*., 2007b)) colour scores. However, the majority of research including Aalhus *et al*. (1990), Watkins *et al*. (1990), Stites *et al*. (1994) (cited by Apple *et al*., 2007b), Crome *et al*. (1996), Herr *et al*. (2001), Stoller *et al*. (2003), Carr *et al*. (2005a,b), Rincker *et al*. (2005) (cited by Apple *et al.*, 2007b) and (Patience *et al*., 2009) have been unable to find any detrimental effect of RAC supplementation on visual pork colour.

Throughout literature there are some inconsistencies in the effect of RAC on instrumental colour scores of lightness (**L***), redness (**a***) and yellowness (**b***). According Herr *et al*. (2001) and Armstrong *et al*. (2004), pork from RAC supplemented pigs had higher L* values and was hence lighter in colour than pork from non-supplemented pigs. However, the majority of research including indicated RAC to have no significant effect on lightness colour scores (Aalhus *et al*., 1990; Uttaro *et al*., 1993; Stoller *et al*., 2003; Apple *et al*., 2004b

(cited by Apple *et al*., 2007b); Carr *et al*., 2005a,b; Rincker *et al*., 2005, cited by Apple *et al*., 2007b; Athayde *et al*., 2012). Conversely, RAC supplementation was found to lead to subtle but significantly lower a* (Aalhus *et al*.,1990; Uttaro *et al*., 1993; Apple *et al*., 2004b (cited by Apple *et al*., 2007b); Armstrong *et al*., 2004; Carr *et al*., 2005a,b; Rincker *et al*., 2005, cited by Apple *et al*., 2007; Patience *et al*., 2009; Athayde *et al*., 2012) and b* scores (Aalhus *et al*., 1990; Uttaro *et al*., 1993; Herr *et al*., 2001; Apple *et al*., 2004b (cited by Apple *et al*., 2007b); Carr *et al*., 2005a,b; Patience *et al*., 2009 ; Athayde *et al*., 2012), and hence leading to less red and less yellow pork colourations. These differences in instrumental red and yellow colour scores may however not be visible to customers (Apple *et al*., 2007b).

Palatability

Palatability is largely measured by taste panel evaluations. As with other meat-types, marbling (or intramuscular fat; IMF) is known to contribute significantly to pork palatability characteristics such as flavour and juiciness, and may be measured subjectively or objectively through dissection or indirectly via Warner-Bratzler shear force (WMSF) values.

Barring a minority of studies that have found RAC either elevated (Watkins *et al*., 1990; Apple *et al*., 2004b (cited by Apple *et al*., 2007b)) or reduced (Aalhus *et al*., 1990) subjective marbling scores in RAC-fed pigs, RAC supplementation was found to not alter subjective marbling scores (Watkins *et al*., 1990; Stites *et al*., 1991; Crome *et al*., 1996; Stoller *et al*., 2003; Carr *et al*., 2005b; Rincker *et al*., 2005, cited by Apple *et al*., 2007b). However, subjective marbling scores have been found to be inconsistent with dissected fat content (Aalhus *et al*., 1990) and WBSF values in particular genetic lines (Rincker *et al*., 2009) and therefore any inferences based upon marbling scores would be ill-advised.

WBSF is a measure of the meat tenderness, and is inversely correlated with marbling. Compared to non-supplemented pigs, WBSF values were found to increase by 4.4, 10.9, and 8.6% when feeding 5, 10, and 20 $mg.kg^{-1}$ RAC supplemented diets respectively (Apple *et al*., 2007b). Similarly, Aalhus *et al*. (1990), Uttaro *et al*. (1993), Xiong *et al*. (2006), Patience *et al*. (2009), and Athayde *et al*. (2012) also found RAC to increase WBSF values. Kutzler *et al*. (2007) however did not detect differences in WBSF values between different RAC treatments. Although a reduced post-mortem protease activity potentially resulting from selection of high-lean lines (Lonergan *et al*., 2001) may account for some of this change in tenderness (Wang and Beermann, 1988; Beermann *et al*., 1990; Kretchmar *et al*., 1990; Xiong *et al*., 2006), RAC-induced changes in muscle fibre distribution and diameter (*vide*: Skeletal muscle) are associated with an increase in WBSF (Aalhus *et al*., 1992), and is likely the major contributor to decreased tenderness. Since pigs are not susceptible to cold shortening, and decreased tenderness has been associated with increased fibre diameters independent of secondary factors such as age and connective tissue strength (Swatland, 1984 (cited in Patience *et al*., 2009), these factors are unlikely to contribute to RAC-induced changes in shear force values (Aalhus *et al*., 1992). However, similar muscle fibre changes and WBSF increases are commonly found with diets with higher CP (Goerl *et al*., 1995), lysine (Goodband *et al*., 1990) and lysine:energy (Castell *et al*., 1994; Cameron *et al*.,

1999 (cited by Apple *et al*., 2007b)). Since these are typical characteristics of RAC-fortified diets, the increase in WBSF may perhaps simply be a symptom of higher CP and Lys diets rather than a response to RAC.

Regardless, sensory panel tests could not detect differences in chewiness, tenderness, juiciness, and flavour intensity (Stites *et al*., 1994(cited by Apple *et al*., 2007b); Stoller *et al*., 2003; Rincker *et al*., 2005, cited by Apple *et al*., 2007b; Fernández-Dueñas *et al*., 2008; Patience *et al*., 2009). Further, any potential negative effects of RAC supplementation on tenderness may effectively be counteracted by proper electrical stimulation and ageing or other mechanical means (Dikeman 2007), or although impractical, carcasses may be infused with calcium chloride (Koohmaraie *et al.*, 1991).

Thus, provided adequate post-mortem procedures and carcass handling protocols are in place, any negative RAC-induced effects on meat quality and tenderness may be rectified, and thus, any negative effects of ractopamine supplementation are effectively negligible.

2.7.5 Welfare considerations

RAC supplementation has been found to increase aggressiveness (Poletto *et al*., 2014) as well as hoof lesions and enterobacteriaceae shedding (Poletto *et al*., 2010) across all sexes in pigs. These effects are compounded by social status, with dominant individuals being more affected than subordinates, and should be considered when RAC-fortified diets are used.

Also, pigs on RAC-fortified diets have been found to be more difficult to handle or move and are therefore at greater risk of being subjected to rough handling and increased stress during transportation (Marchant-Forde *et al*., 2003)

2.8 Potential impact on meat industry

In addition to improving feed utilisation, growth performance, and carcass composition, RAC has secondary benefits to the pork producer including reducing required days to reach marketable weight (Moody *et al*., 2000), and decreasing the total environmental footprint by improving nitrogen, phosphorus and water retention, and hence reducing total waste (Moody *et al*., 2000; Ross *et al*., 2011).

2.9 Hypotheses

Since ractopamine fortified diets have been extensively researched. Hypotheses (or rather expectations) are based upon previous similar studies, and are as follows:

- No significant differences in appetite and hence ADFI between ractopamine treatment groups, irrespective of treatment duration.
- A linear increase in ADG and live weight with increasing ractopamine inclusion levels.
- A linear decline in FCR with increasing ractopamine treatment levels.
- Due to the compounding effects in ADG and FCR, an increase in live weight with increasing ractopamine inclusion levels is expected.

- Due to repartitioning effects, a linear decrease in P2 (relative to body weight) with increasing RAC inclusions is expected.
- A linear increase in dressing percentage with increasing ractopamine inclusion levels.

Null hypothesis: ADFI, ADG, FCR, P2, and live weights will not differ significantly between ractopamine inclusion levels irrespective of treatment duration.

CHAPTER 3 MATERIALS AND METHODS

All experimental procedures, care, and handling of animals were approved by the University of Pretoria Animal Ethics Committee.

The trial was conducted in three distinct phases: acclimatisation, adaptation, and experimental phase. Although some conditions remained constant, many were altered between phases, and as such, each phase is described separately.

3.1 Biosecurity

One week prior to the arrival of animals, the grower facility was thoroughly rinsed with municipal water, high-pressure washed with a chlorine-based disinfectant, and thereafter rinsed with municipal water again. All the slats were lifted and the slurry channels cleaned in the same manner. The house was left to air dry, and then fumigated. Upon conclusion of the trial, the entire facility was cleaned, disinfected and fumigated in the same manner as before the trial commenced.

To restrict access, all entrances and external doors were secured by a 2-way lever lock and/or a hardened steel padlock. Any individuals entering the facility were required to not have had any contact with other pigs (including pig meat processing facilities) for at least 72 hours prior and after visitation, and a shower-in-shower-out protocol employed. Visitors and working personnel were required to use safety clothing which was provided by the facility in a changing room upon exiting the shower facility, whilst personal clothing remained in the shower facility. All safety clothing was washed and remained on site at all times. Any visitors were accompanied by myself or one of my assisting colleagues at all times.

In accordance with standard biosecurity practices, a boot-dip was provided at the entrance/exit of both grower and individual housing sections of the facility.

3.2 Limitations

No heating source nor cooling misters were used due to lack of equipment to do so and due to the lack in need for this equipment during the autumn season (March-May) in which this trial was conducted. Ambient and in house temperatures were not monitored due to the lack of equipment for such duties.

3.3 Acclimatisation (Day -34 to -6)

This period served as an adaptation period allowing the gilts to acclimatise to the new housing and probable micro-climatic differences compared to their source farm.

3.3.1 Source and number of animals

71 commercial crossbred grower gilts (F2) between 42-44 kg live weight were pre-selected according to live weight at 12 weeks of age at the predetermined source farm (Day -34). Of the potential source farms,

EDE farms was chosen due to their high herd health standards, strict biosecurity practices, and high level of homogeneity. These preselected gilts were then transported by truck with 100% shade, to the research unit on the Hatfield Experimental Farm, Pretoria. Unfortunately the shading did tear *en route* but was successfully mended. Upon delivery, the pigs appeared hyperthermic, stressed, and exhausted. To alleviate these symptoms, housing lights were dimmed and ventilation fans placed on maximum output whilst gilts were sprayed with and provided cool municipal water *ad libitum*. The gilts were monitored until they were sufficiently rested.

3.3.2 Housing

The 71 gilts were randomly assigned to one of four 17 m^2 (3.4 m x 5.0 m) pens with partial (1.2 m) long slats running along the longer edge of the pen (i.e. ¼ slats). The resulting 0.94-1 m^2 floor area per gilt is in accordance with RSPCA welfare standards for pigs (RSPCA, 2012). Pens were separated by 1.1 m vertical steel bars, with 0.1 m gaps between bars to allow for socialisation.

A variable speed fan mounted to the southern wall provided ample ventilation by running continuously. The louvre windows on the southern and northern sides of the building were manually adjusted to manipulate air flow direction such as to avoid direct drafts on pigs directly adjacent to the windows.

Lighting was provided using fluorescent lights which were operated manually such as to maintain a 12 hour daylength (06h00 to 18h00).

Windows along the entire northern and southern walls provided additional ambient lighting throughout the day.

3.3.3 Feeding

Gilts received a standard balanced grower ration *ad libitum* in dry meal form for a period of 28 days. A table with detailed diet composition and theoretical analysis is reported (Appendix I). Feed was provided using a single 4-spaced feeding trough (100 cm x 20 cm), allowing 111-118 cm^2 feed space per animal, in accordance with RSPCA welfare standards for pigs (RSPCA, 2012). Municipal water was provided *ad libitum* through two bite nipples per pen.

3.3.4 Handling

On day -33, all animals received a numerically unique ear tag in the left ear for identification purposes. The ear tags were applied as quickly as possible using a steel cable snout snare to maintain control over the pig such that any potential injuries to handler and pig were minimised. Gilts were monitored twice daily and live weights measured weekly throughout this period. Pens were cleaned every morning.

3.4 Adaptation (Day -6 to 0)

During this phase, the trial individuals were selected and moved to the individual housing such that they adapted to the new housing conditions.

3.4.1 Selection

At 16 weeks of age (Day -6), all gilts were weighed. 58 gilts whose live weights were within 4.5 kg from the median (68.5 kg) were selected. 58 animals were selected as this was the maximum capacity of the individual housing pens. An uneven number of animals were used to fill the capacity of the test house, and was compensated for in the statistical analyses. The selected animals were blocked and randomly assigned into individual pens. Unselected animals were regrouped into a single group pen and remained in the group housing where they were fed any excess trial feed (grower and/or finisher rations) until termination of the experimental phase.

3.4.2 Housing

Pigs were housed in individual pens with 3.48 m^2 (2.9 m x 1.2 m) floor-area per gilt. These pens were constructed of partial concrete flooring, with 1.06 m long slats running along the breadth of the pen which covered a deep slurry canal. Pens were arranged side-on-side and back-to-back, such that each pen was surrounded by 3 other pens (excluding pens located at ends of rows). Pens were separated by 1.1 m vertical steel bars with 0.1 m gaps between bars to allow for socialisation. The spatial arrangement of the gilts in the house is shown in Appendix III.

Ventilation was provided using two variable speed fans which ran continuously, and louvre windows manually adjusted to manipulate air flow direction such as to avoid direct drafts on pigs directly adjacent to the windows. Lighting and cleaning of fouled pens were carried out in the same manner as in the acclimatisation phase.

3.4.3 Feeding

A control ration devoid of RAC was provided *ad libitum* in dry meal form for a period of 6 days using identical steel single-spaced trough feeders with 26 cm x 30 cm dimensions, thus allowing a feeder space of 780 cm^2 per gilt. The adaptation diet was formulated (DM basis) to contain approximately 13.38% CP and 0.94% lysine. A table with detailed diet composition and theoretical analysis for each treatment diet is reported (Appendix I). Each pen was equipped with a single bite nipple through which municipal water was provided *ad libitum*.

3.4.4 Handling

Feed was completely removed on the evening of day -1. Gilts and feeders were monitored twice daily for timeous detection of illness or feeder problems respectively.

3.5 Experimental phase (Day 0 to 27)

During this phase, selected animals were assigned to different treatment diets (0 mg.kg^{-1} RAC, 5 mg.kg^{-1} RAC and 10 mg.kg^{-1} RAC (DM basis); hereafter referred to as 0-RAC, 5-RAC, and 10-RAC respectively). Initially intended to be 28 days in length, this period was shortened to 27 days due to restricted abattoir availability.

3.5.1 Housing

Housing conditions were identical to that during the adaptation period.

3.5.2 Allocation of treatment variable

Using the random blocking method, pens (previously randomly assigned) were randomly assigned to one of three treatments levels of ractopamine hydrochloride enriched diets: 0-RAC, 5-RAC, and 10-RAC. Thus, the pigs were randomly assigned to both pen and treatment variable.

3.5.3 Feeding

Feed was provided *ad libitum* in dry meal form throughout this period. To ensure correct feeding, different coloured micro-grits (

Table 3.1) were included in the rations, different coloured feed bags were used to package the different rations (

Table 3.1), and the rations were stored in distinctly separate spaces in the feed store area. Apart from the coloured micro-grits and RAC inclusion level, all other feed ingredients remained identical across all rations. All diets were formulated (DM basis) to contain approximately 14.04% CP and 1.02% lysine. A table

with detailed diet composition and theoretical analysis for each treatment diet is reported (Appendix I). The premix composition is also reported (Appendix II)

On the seventh evening (18:00-22:00) of every week (sixth evening of the last week), any residual feed was collected and wet-weighed. Feed was withheld for approximately 8 hours to eliminate any bias from differences in gut-fill (2013, H. Bodenstein, Pers. Comm., Charles Street Veterinary Group, P.O. Box. 95315, Pretoria, 0145) and ensure strictly fasted body weights were measured during the following morning's measurements. Water was not withheld at any stage. Refused and/or fouled feed were discarded.

Table 3.1 Micro-grit and packaging colours for different treatment rations

	Micro-grit colour	Packaging colour
0-RAC*	Blue	Blue
5-RAC*	Green	Green
10-RAC*	Red	Red

*0-RAC, 5-RAC, 10-RAC refers to the experimental diets enriched with 0 $mg.kg^{-1}$, 5 $mg.kg^{-1}$ and 10 $mg.kg^{-1}$ RAC, respectively.

3.5.4 Handling

At 06h00 on the following morning after the feed withdrawal, gilts were removed from their pens and placed individually in a weighing crate where their fasted body live weights (LW) were measured to the nearest 100g. Whilst in the weighing crate, the medial and lateral P2 backfat depths were measured using a "Series 12 LEAN-MEATER® ultrasound probe" (Renco Corporation). The mean of medial and lateral P2 fat depths was recorded as the P2 depth. Paraffin was used as a coupling fluid for the ultrasound probe. Once all measurements were completed, gilts were returned to their respective pens and feeding recommenced.

On d27, all experimental animals were slap-tattooed on all four quarters with their respective pen number, and feed withheld from 20:00. On d28, at 04:00, all trial animals were weighed and transported via truck in compliance with the Animals Protection Act 1962 (Act No. 71 of 1962) to the Lynca abattoir in Meyerton where carcasses were harvested in compliance with the Meat Safety Act (Act No. 40 of 2000). Animals were harvested at 08:00, approximately 12 hours after final feed withdrawal.

3.5.5 Calculations

Average daily voluntary feed intakes (ADFI) were calculated by:

$$ADFI_i(g\ day^{-1}) = \frac{Feed\ intake}{\Delta time} = \frac{Feed\ allowed_i(g) - Feed\ remaining_i(g)}{t(days)}$$

Where i=1,…,4; t=7 for i=1,2,3 and t=6 for i=4

t= number of days

i= period of measurement, i.e. d1-7; d8-14; d15-21; d22-27

Feed remaining refers to the sum of unused and discarded/fouled feed

Average daily gain (ADG) was calculated by:

$$ADG_j\ (g\ day^{-1}) = \frac{\Delta LW}{\Delta time} = \frac{LW_j(g) - LW_{j-1}(g)}{t(days)}$$

Where j=1,…,4; t=7 for j=1,2,3 and t=6 for j=4

t= number of days

j= period of measurement, i.e. d1-7; d8-14; d15-21; d22-27

Feed conversion ratio (FCR) was calculated by:

$$FCR_j = \frac{Feed\ intake}{\Delta LW} = \frac{Feed\ allowed_j - Feed\ remaining_j}{LW_j - LW_{j-1}}$$

Where j=1,…,4

j= period of measurement, i.e. d1-7; d8-14; d15-21; d22-27

Feed remaining refers to the sum of unused and discarded/fouled feed

3.5.6 Abattoir measurements

At the Lynca abattoir, the carcass fat and meat depths were measured using a Hennessy Grading Probe. Hot carcass yields (HCY) were measured using the automated classification system coupled to the Hennessy Grading Probe. Cold carcass yields were calculated at 97% of HCY. P2 measurements were also measured by the HGP. To minimise operator errors, one trained operator probed all carcasses. This was carried out approximately 5 min after exsanguination, carcasses were probed 65 mm from the midline with an electronic probe (HGP4, Hennessy Grading Probe, Hennessy and Chong, Auckland, New Zealand) between the third and fourth ribs and anterior from the last rib. Once inside the cooling rooms, other measurements including the lengths and circumferences of the front limbs, lengths and circumferences of the hind limbs, and carcass lengths were recorded. Length and circumference measurements were limited by a inaccurate technique/apparatus and these results will not be analysed.

3.6 Statistical analysis

This study was analysed using a completely randomised design where pigs were randomly assigned a particular treatment, and then further randomly assigned a pen number. One-way ANOVA and a restricted maximum likelihood (REML) model was applied to all univariate measurements (ADG, LW, ADFI, FCR, P2) to test for differences between treatment effects and linear trend over treatments, and model the correlation over 27 days in a repeated measurements analysis. All assumptions of normality and homogenous and independent treatment variances were satisfied for this model. The fixed effects were specified as day, treatment and the day by treatment interaction, while the random effects were specified as the pen and pen by day interaction. A power model of order 1 and modelling for unequal day variances was found to best model the correlation over days. All analyses were executed using SAS, Version 9.2 (SAS, 2008).

The null and alternative hypotheses were:

$$H_0: \mu_1 = \mu_2 = \mu_3$$

$$H_a: not\ all\ \mu_i (i = 1, 2, 3)\ are\ equal$$

Where 1, 2, and 3 refer to RAC treatments 0-RAC, 5-RAC and 10-RAC respectively.

The Fischer's protected least significant difference test (FPLSD) and studentized range was applied to compare means at the 5% level, and the null hypothesis was rejected where $P < 0.05$.

3.6.1 Exclusion of individuals and adjustment of treatment group sizes

To facilitate statistical analyses, four individuals (ID 9, 12, 25, 52) were excluded from statistical analyses, reducing the treatment group sizes to 17 per treatment. Rationale for their exclusions are as follows:

- An extremely high FCR for ID12 was observed during the first week of the experimental phase. This may either be due to a poor feed intake (caused by either poor adaptation to the new feeder system, or erroneous feed intake measurement) or erroneous live weight measurement, or a combination of the two.
- ID25 was found dead upon delivery to the abattoir, and excluded from analyses due to missing slaughter data.
- ID9 and ID52 were eliminated to equalise treatment group sizes and facilitate statistical analyses without significantly reducing the statistical power of the data. A preliminary analyses for ADG adaptation, ADG overall, DFI adaptation, DFI overall, FCR week 1, FCR overall, P2 adaptation, P2 week 4 was conducted coupled with a scoring system for their residual identified ID9 and ID52 to have the highest residuals across the categories.

CHAPTER 4 RESULTS

4.1 Live weight gain

There was a small numerical difference in fasted body live weights (LW) during the adaptation phase (73.61 kg, 73.65 kg, and 73.91 kg for 0-RAC, 5-RAC, and 10-RAC, respectively) between treatment groups over the adaptation period, however these differences were not significant (P = 0.819; Table 4.1), thus confirming correct blocking application and random assignment of treatments. Over the first 21 d of the study, RAC did not affect LW (P = 0.713, 0.485, 0.218 for d7, d14 and d21 respectively; Table 4.1). However, on d27, a significant difference (P = 0.049; Table 4.1) in LW was found between 10-RAC and 0-RAC treatments but not between 5-RAC and either 0-RAC or 10-RAC treatments. Over the entire experimental phase, LW increased by 23.9 kg, 25.23 kg, and 27.41 kg for 0-RAC, 5-RAC, and 10-RAC treatments respectively.

A linear relationship between LW and treatment duration was not found to be significant at d7 and d14 (P = 0.747, 0.310, respectively). However, at d21 a tendency (P = 0.084) towards significance was found for a linear trend and at d27 a linear trend was found to be significant (P = 0.015). Hence, although only detectable at d27, live weight was found to linearly increase with increasing RAC treatment. LW change over the experimental phase for the three treatment groups is shown in Table 4.1 and Figure 4.1.

Table 4.1 Mean (± SD) fasted body live weight (kg) over a 27 day period of RAC supplementation at 0, 5, and 10 mg.kg^{-1} in gilts

	RAC treatment				
	0-RAC	5-RAC	10-RAC	Mean	p-value
Adaptation (d0)	73.61 (±2.444)	73.65 (±2.341)	73.91(±2.535)	73.72(±2.399)	0.819
d7	79.37 (±3.169)	80.28(±3.066)	79.73(±3.733)	79.79(±3.294)	0.713
d14	86.76(±3.617)	88.01(±2.889)	87.96(±3.943)	87.57(±3.493)	0.485
d21	94.39(±4.295)	95.83(±3.506)	96.80(±4.456)	95.67(±4.151)	0.218
d27	103.27^{a}(±4.679)	105.51^{ab}(±3.841)	107.14^{b}(±5.206)	105.31(±4.798)	0.049

$^{a, b}$ Means in the same row with different superscripts differ (P < 0.05)

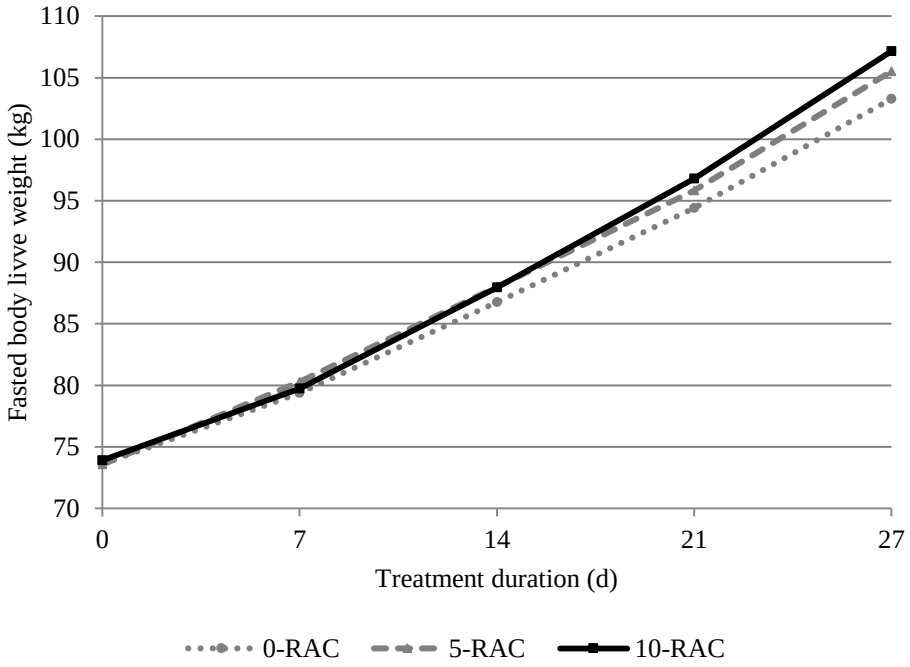

Figure 4.1 The effect of three levels of RAC supplementation on LW (kg) over a 27 day period of supplementation in gilts

4.2 Average daily gain

Average daily gain (ADG) per week as well as the overall ADG throughout the experimental phase was analysed by applying a linear mixed model. Although p-values for ADG at d0-d7, d8-d14 and d15-d21 were low or showed tendencies (P = 0.260, 0.217, 0.086, respectively; Table 4.2), a significant difference in ADG between 0-RAC and 10-RAC treatments was found only at d27 (P = 0.026; Table 4.2), with 5-RAC treatment intermediate between the two. At d22-d27, ADG for 0-RAC, 5-RAC and 10-RAC treatments were 1109.72 $g.d^{-1}$, 1210.42 $g.d^{-1}$, and 1292.36 $g.d^{-1}$, respectively (Table 4.2). Furthermore, when considering the overall ADG throughout the period, a significant difference (P = 0.005) was found between 0-RAC and 5-RAC treatments, but not between 5-RAC and 10-RAC treatments despite their numerical differences.

A linear trend was not found to be significant for ADG at d0-d7 (P = 0.917), whilst a tendency towards significance was found at d8-d14 (P = 0.083; Table 4.2), and at d15-d21 and d22-27 as well as overall ADG a linear trend was found to be significant (P = 0.040, 0.007, 0.001 respectively; Table 4.2). Hence, ADG was found to have a positive curvilinear relationship with RAC concentration.

The weekly change in ADG over the experimental phase and overall ADG throughout the experimental period for the three treatment groups are presented in Table 4.2. Weekly ADG and overall ADG throughout the experimental phase are presented in Figure 4.2 and 4.3, respectively.

Table 4.2 Mean (± SD) average daily gain (g day^{-1}) over a 27 day period of RAC supplementation at 0, 5, and 10 mg.kg^{-1} in gilts

	RAC treatment					
	0-RAC	5-RAC	10-RAC	Mean	p-value (treat)	p-value (lin)
d0-d7	823.02(±241.9)	946.83(±235.0)	831.75(±269.5)	867.20(±251.1)	0.260	0.917
d8-d14	1055.56(±168.8)	1104.76(±180.6)	1175.40(±251.4)	1111.90(±205.8)	0.217	0.083
d15-d21	1090.48(±247.1)	1116.67(±233.6)	1263.49(±258.1)	1156.88(±253.7)	0.086	0.040
d22-d27	1109.72^{a}(±177.4)	1210.42^{ab}(±179.4)	1292.36^{b}(±227.9)	1204.17(±206.8)	0.026	0.007
d0-d27	847.46^{c}(±80.0)	910.32^{d}(±84.3)	949.52^{d}(±102.0)	902.43(±97.3)	0.005	0.001

$^{a, b}$ Means in the same row with different superscripts differ (P < 0.05)

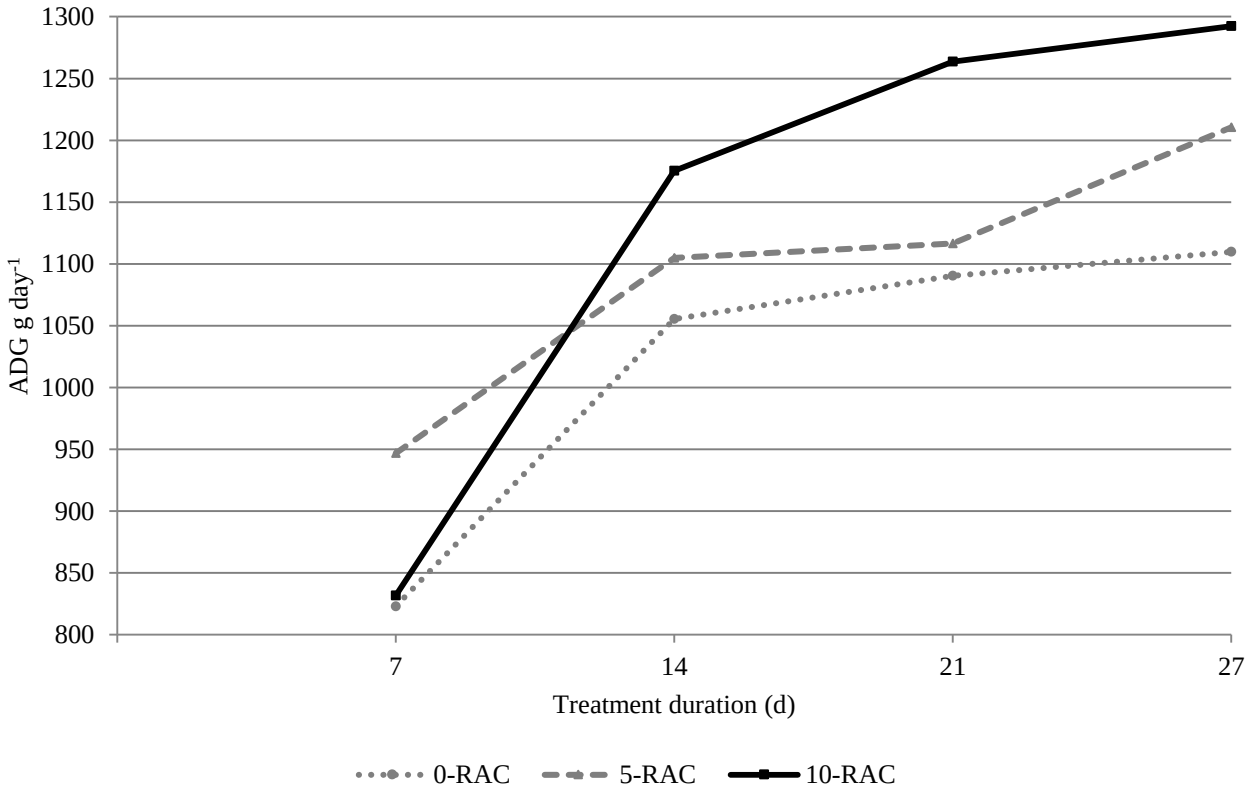

Figure 4.2 The effect of three levels of RAC supplementation on ADG (g day^{-1}) over a 27 day period of supplementation in gilts

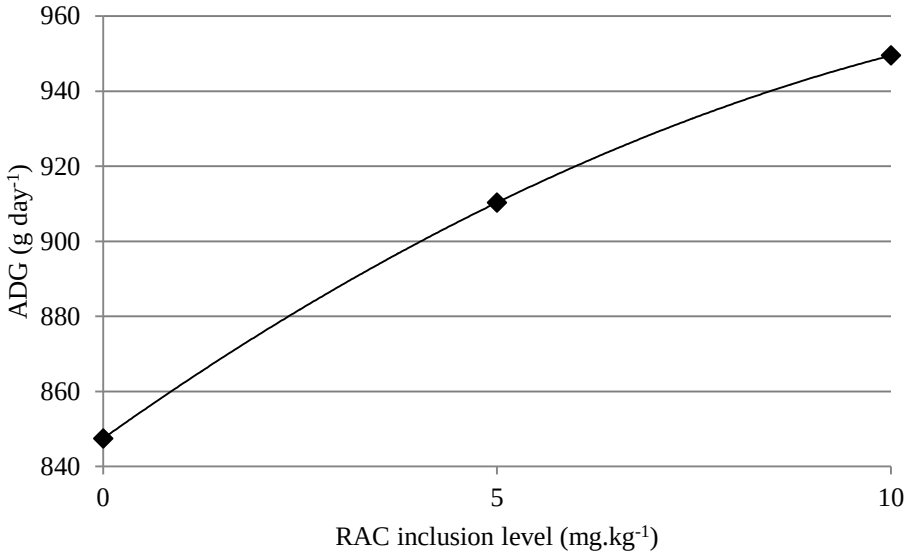

Figure 4.3 The effect of three levels of RAC supplementation on the overall ADG (g day^{-1}) throughout a 27 day period of supplementation in gilts

4.3 Average daily voluntary feed intake (ADFI) (kg.d^{-1})

The weekly and overall average daily voluntary feed intakes over the experimental period were analysed by applying a linear mixed model. Both the treatment main effect and treatment by day interaction were not found to be significant (P = 0.493, and P = 0.331 respectively) upon application of REML. This supports the null hypothesis, suggesting that ADFI's were not significantly different between RAC treatments over the 27 days of treatment. Despite this, the diminishing p-values between d0-d0-d7, d8-d14, d15-d21 and d22-d27 (P = 0.795 kg.d^{-1}, 0.516 kg.d^{-1}, 0.491 kg.d^{-1}, and 0.191 kg.d^{-1}, respectively;

Table 4.3) suggests a tendency toward significance for the treatment main effect. Treatment 0-RAC, 5-RAC and 10-RAC increased average daily voluntary feed intake by 1.154 kg.d^{-1}, 1.328 kg.d^{-1}, and 1.386 kg.d^{-1}, respectively. A linear trend was not found to be significant between RAC treatment and treatment duration at any week nor during the overall period. Weekly ADFI and overall ADFI throughout the experimental phase are presented in Figure 4.3 and 4.4, respectively.

Table 4.3 Mean (± SD) average daily voluntary feed intake (kg day^{-1}) over a 27 day period of RAC supplementation at 0, 5, and 10 mg.kg^{-1} in gilts

	RAC treatment				
	0-RAC	5-RAC	10-RAC	Mean	p-value
d0-d0-d7	2.446(±0.2497)	2.498(±0.3759)	2.431(±0.2975)	2.458(±0.3075)	0.795
d8-d14	2.854(±0.3305)	2.939(±0.2247)	2.959(±0.3033)	2.917(±0.2879)	0.516
d15-d21	3.082(±0.3606)	3.135(±0.3045)	3.214(±0.3308)	3.144(±0.3310)	0.491
d22-d27	3.600(±0.4497)	3.826(±0.3299)	3.817(±0.4541)	3.748(±0.4208)	0.191
Overall	2.892(±0.2839)	2.993(±0.2352)	2.998(±0.2609)	2.961(±0.2605)	0.395

[a, b] Means in the same row with different superscripts differ (P < 0.05)

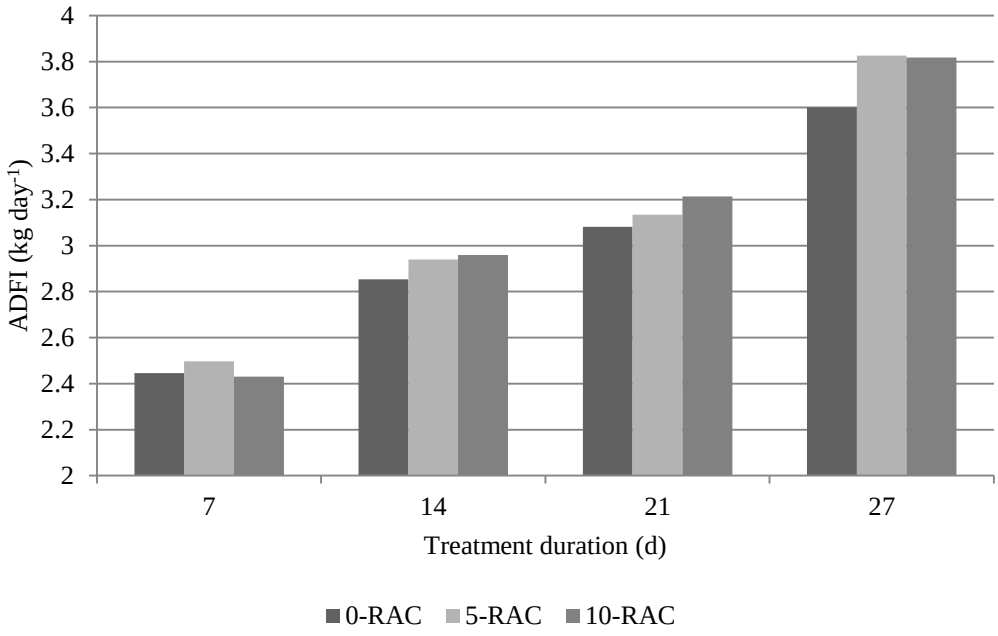

Figure 4.4 The effect of three levels of RAC supplementation on ADFI (kg day^{-1}) over a 27 day period of supplementation in gilts.

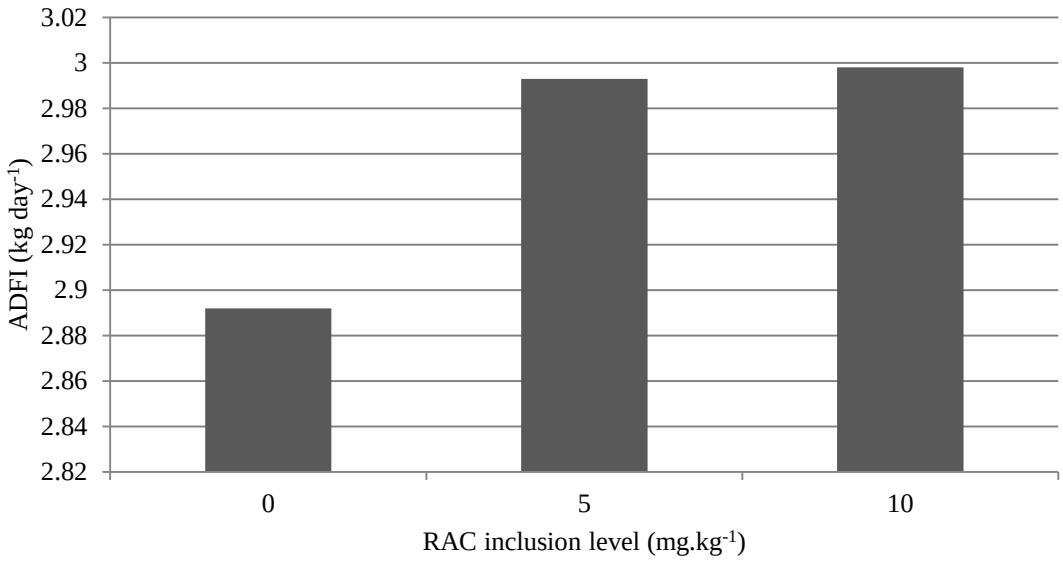

Figure 4.5 The effect of three levels of RAC supplementation on overall ADFI (kg day^{-1}) throughout a 27 day period of supplementation in gilts

4.4 Feed efficiency (F:G)

Weekly feed efficiency (F:G) as well as the overall F:G throughout the experimental phase was analysed by applying a linear mixed model. Upon application of REML, both day and treatment main effects as well as treatment by day interaction effects were not found to be significant (P = 0.498, 0.056 and 0.747 respectively), providing evidence that the feed efficiency was not affected by RAC treatment nor were differences found between different levels of RAC supplementation, and hence supporting the null hypothesis. Although no significant differences in F:G were found between treatments for each week (P = 0.785, 0.538, 0.169, and 0.104 for d0-d0-d7,d8-d14, d15-d21 and d22-d27 respectively; Table 4.4), when considering the overall F:G throughout the experimental phase, a significant difference (P = 0.012; Table 4.4) was found between 0-RAC and 10-RAC, but not between 5-RAC and either 0-RAC or 10-RAC treatments. Overall F:G throughout the entire treatment period were 2.836, 2.735 and 2.624 for 0-RAC, 5-RAC and 10-RAC respectively (Table 4.4).

Although a linear trend between F:G and RAC treatment was not found to be significant at d0-d0-d7 and d8-d14 (P = 0.996 and 0.296 respectively), at d15-d21 a tendency for significance for a linear trend was found (P = 0.094) and at d22-d27 the overall F:G a linear trend was found to be significant (P = 0.039 and 0.003 respectively). Hence, although only detectable at d22-d27 and when considering the entire experimental phase, F:G was found to linearly decrease with increasing RAC treatments. Weekly F:G and overall F:G throughout the experimental phase is presented in Figure 4.6 and Figure 4.7 respectively.

Table 4.4 Mean (± SD) F:G over a 27 day period of RAC supplementation at 0, 5, and 10 mg.kg^{-1} in gilts

	RAC treatment				
	0-RAC	5-RAC	10-RAC	Mean	p-value
d0-d7	2.862(±0.4314)	2.761(±0.5762)	2.861(±0.4681)	2.828(±0.4915)	0.785
d8-d14	2.766(±0.5250)	2.732(±0.5083)	2.593(±0.4361)	2.697(±0.4878)	0.538
d15-d21	2.922(±0.5703)	2.907(±0.5413)	2.620(±0.4795)	2.816(±0.5401)	0.169
d22-d27	2.885(±0.4241)	2.809(±0.3857)	2.622(±0.3012)	2.772(±0.3834)	0.104
Overall	2.836^{a}(±0.2468)	2.735^{ab}(±0.2170)	2.624^{b}(±0.1362)	2.732(±0.2196)	0.012

$^{a, b}$ Means in the same row with different superscripts differ (P < 0.05)

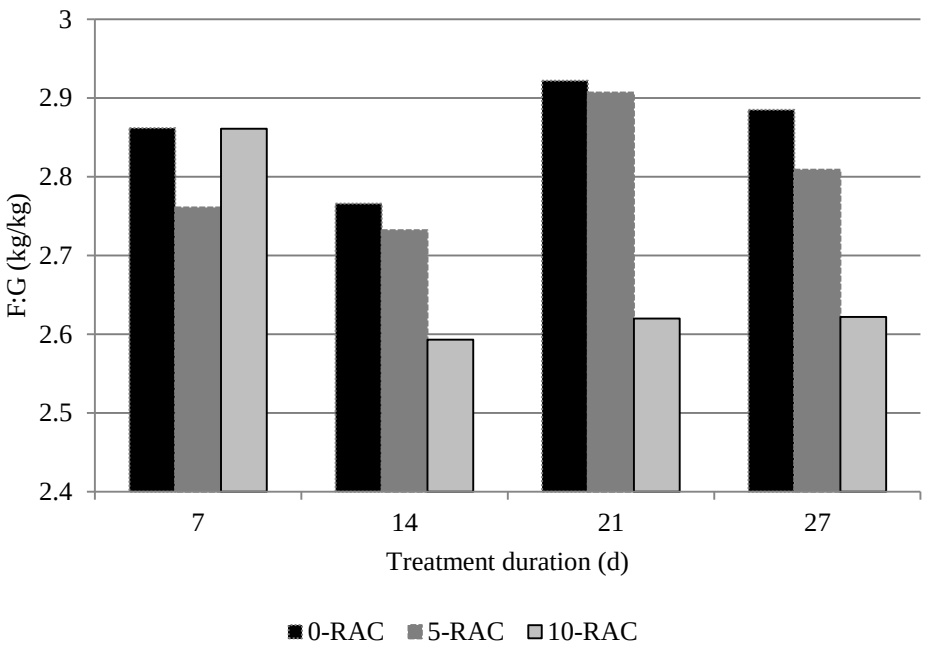

Figure 4.6 The effect of three levels of RAC supplementation on F:G throughout a 27 day period of supplementation in gilts

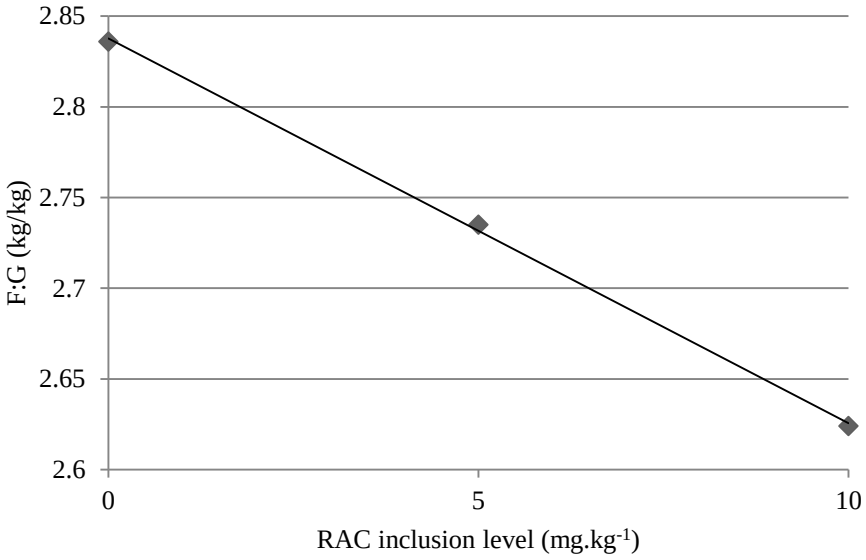

Figure 4.7 The effect of three levels of RAC supplementation on overall F:G throughout a 27 day period of supplementation in gilts

4.5 P2 backfat thickness

No significant difference in P2 backfat thickness ($P > 0.05$) was found between treatments during the adaptation phase, confirming correct blocking application and random assignment of treatments. P2 fat thickness increased significantly for all treatments over the experimental phase, however there were no significant differences between RAC treatments at any point in the trial. From a mean of 8.648 mm treatments 0-RAC, 5-RAC and 10-RAC increased by a mean of 1.472 mm, 1.722 mm, and 1.667 mm, respectively.

A linear trend was not found to be significant between P2 backfat thickness and treatment duration at any stage in the experimental phase (P = 0.347, 0.934, 0.459, 0.664 for d0, d0-d7, d8-d14 and d22-d27, respectively), however there was a tendency found at d15-d21 (P = 0.054). The weekly change of P2 backfat thickness over the experimental phase is presented in

Table 4.5 and Figure 4.8.

Table 4.5 Mean (± SD) P2 thickness (mm) over a 27 day period of RAC supplementation at 0, 5, and 10 mg.kg^{-1} in gilts

	RAC treatment					
	0-RAC*	5-RAC*	10-RAC*	Mean	p-value (treat)	p-value (lin)
Adaptation (d0)	8.778(±0.943)	8.722(±1.114)	8.444(±1.097)	8.648(±1.044)	0.600	0.347
d0-d7	8.667(±0.875)	8.556(±0.953)	8.639(±1.173)	8.620(±0.990)	0.943	0.934
d8-d14	8.694(±1.296)	8.944(±0.969)	8.972(±1.064)	8.870(±1.104)	0.715	0.459
d15-d21	9.222(±0.974)	9.528(±0.962)	9.889(±1.106)	9.546(±1.034)	0.154	0.054
d22-d27	10.139(±1.281)	10.278(±1.032)	10.306(±1.100)	10.241(±1.123)	0.896	0.664

[a, b] Means in the same row with different superscripts differ (P < 0.05)

*0-RAC, 5-RAC, 10-RAC different RAC treatments

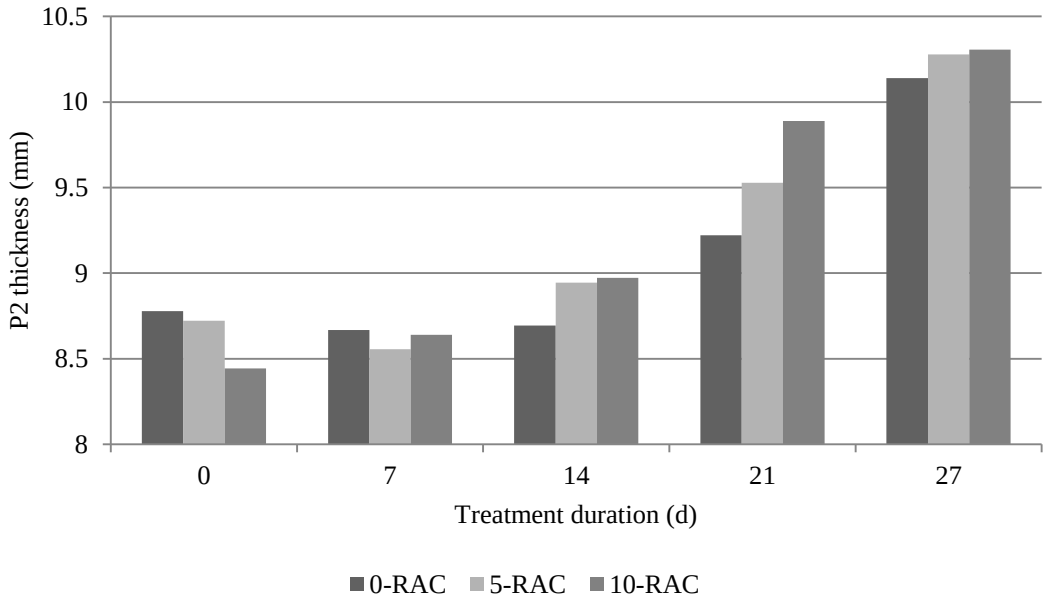

Figure 4.8 The effect of three levels of RAC supplementation on subcutaneous adipose depots as measured at the P2 location over a 27 day period of supplementation

4.6 Objective carcass measurements

Warm mass and cold mass for 10-RAC were significantly (P = 0.045, P = 0.045; Table 4.6) greater (86.32 kg; Table 4.6) than the control group (82.93 kg; Table 4.6). Cold carcass yield was not measured but calculated as a constant effect. Warm mass and cold mass for 5-RAC was not significantly different to either 0-RAC or 10 RAC treatment groups. No significant differences between treatments were found for fat depth, meat depth, meat %, or dressing % (P = 0.534, 0.452, 0.638, 0.612, respectively; Table 4.6).

A curvilinear trend was found to be significant for both warm mass and cold mass (P = 0.014 and 0.014, respectively) but not for fat depth, meat depth, meat % nor dressing percentage (P = 0.661, 0.415, 0.889, 0.436, respectively). Thus, warm mass and cold mass were found to linearly increase with increasing RAC treatments, whilst other objective carcass parameters were unaffected. Warm mass, cold mass, fat depth, meat depth, meat percentage and dressing percentage of the different RAC treatments are presented in, Figure 4.9, Figure 4.10, Figure 4.11, Figure 4.12, Figure 4.13 and Figure 4.14 respectively.

Table 4.6 Mean (± SD) warm mass (kg), cold mass (3%) (kg), P2 fat depth (mm), and P2 meat depth (mm) in gilts after 27 days of RAC supplementation at 0, 5, and 10 mg.kg^{-1}

	RAC treatment				
	0-RAC	5-RAC	10-RAC	Mean	p-value
Warm mass (kg)	82.93^{a}(±4.0763)	85.08^{ab}(±3.6294)	86.32^{b}(±4.2980)	84.77(±4.1807)	0.045
Cold mass 3% (kg)	80.44^{a}(±3.9543)	82.52^{ab}(±3.5223)	83.73^{b}(±4.1684)	82.23(±4.0558)	0.045
Fat depth (mm)	13.69(±2.4679)	14.53 (±2.3324)	14.02(±1.9667)	14.08(±2.2499)	0.534
Meat depth (mm)	99.89(±3.8006)	101.07(±2.2306)	100.67(±2.1597)	100.55(±2.8231)	0.452
Meat %	69.22(±1.0708)	68.87(±1.3894)	69.17(±1.0672)	69.09(±1.1731)	0.638
Dressing %	80.30 (±1.0047)	80.62(±1.0010)	80.57(±1.1244)	80.50(±1.0.349)	0.612

$^{a, b}$ Means in the same row with different superscripts differ (P < 0.05)

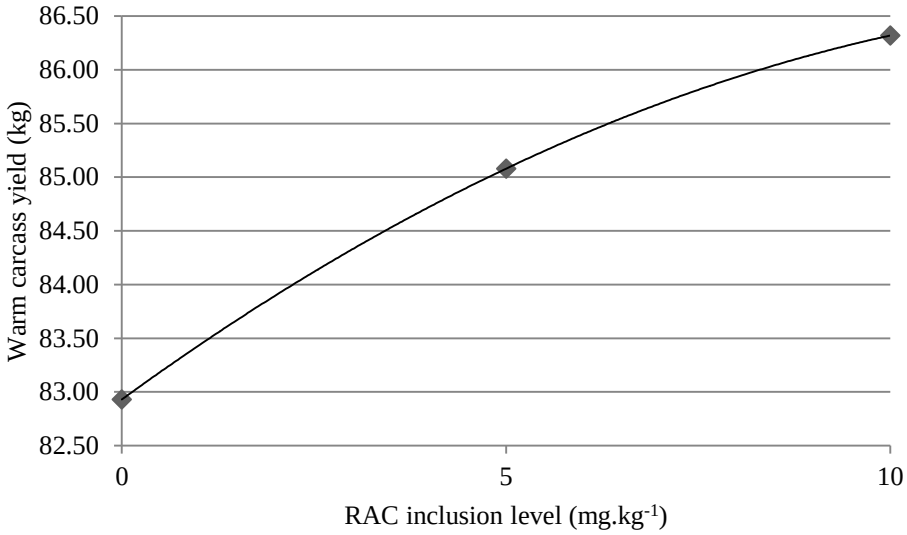

Figure 4.9 The effect of three levels of RAC supplementation on warm carcass yield (kg) following a 27 day period of supplementation in gilts

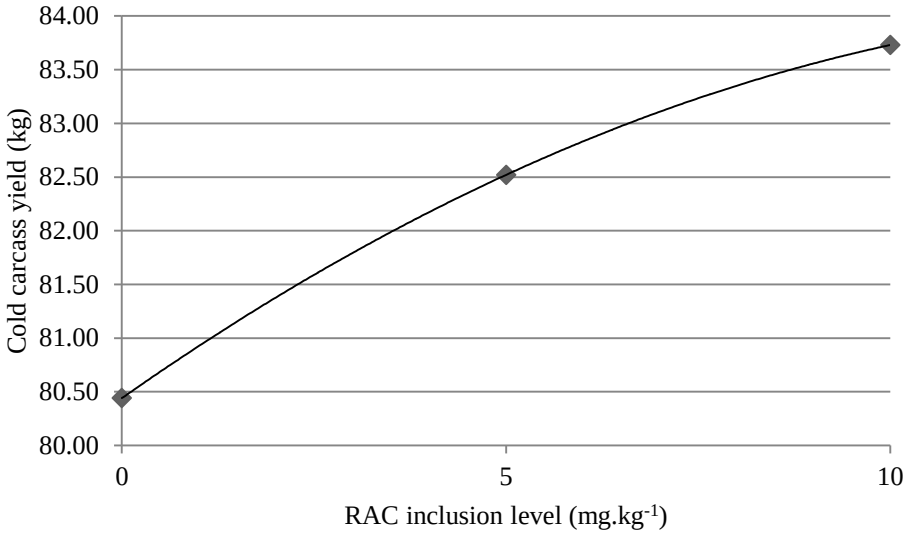

Figure 4.10 The effect of three levels of RAC supplementation on cold carcass yield (kg) following a 27 day period of supplementation in gilts

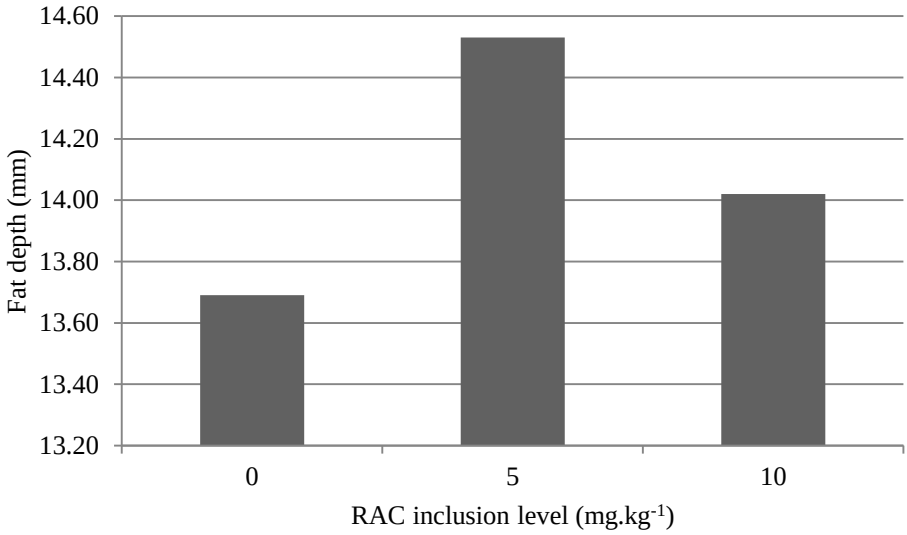

Figure 4.11 The effect of three levels of RAC supplementation on carcass fat depth (mm) following a 27 day period of supplementation in gilts

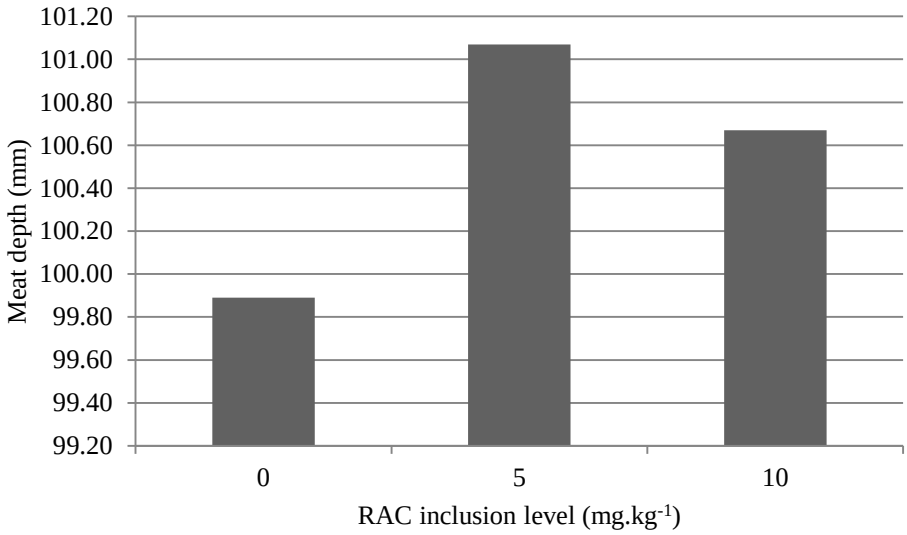

Figure 4.12 The effect of three levels of RAC supplementation on carcass meat depth (mm) following a 27 day period of supplementation in gilts

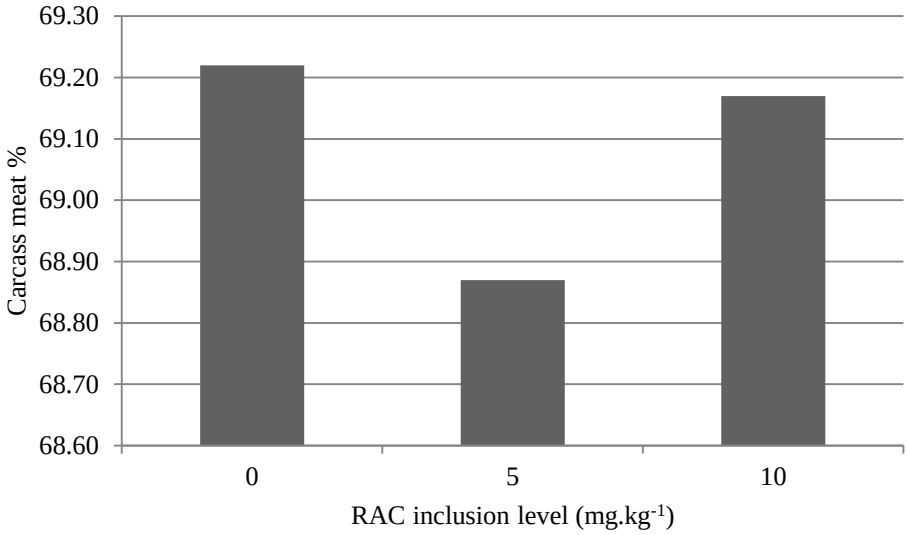

Figure 4.13 The effect of three levels of RAC supplementation on carcass meat percentage (%) following a 27 day period of supplementation in gilts

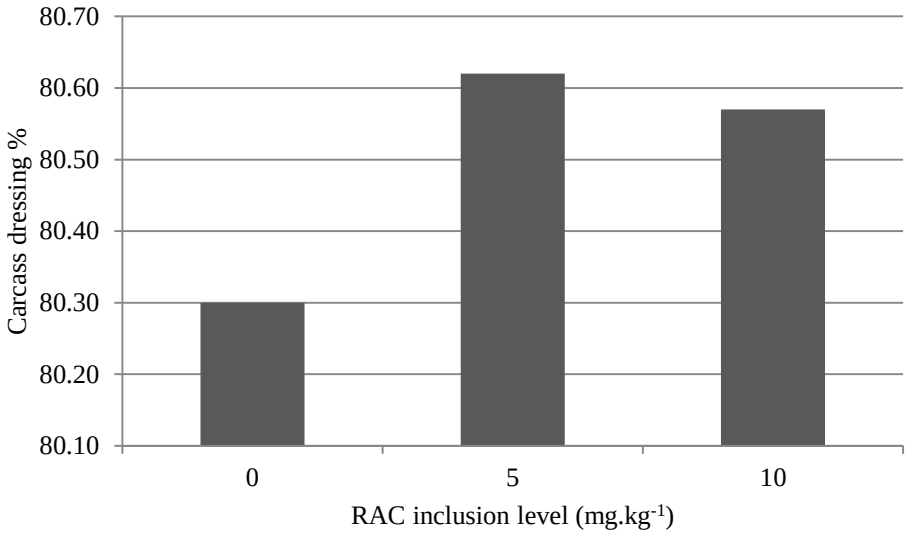

Figure 4.14 The effect of three levels of RAC supplementation on carcass dressing percentage (%) following a 27 day period of supplementation in gilts

CHAPTER 5 DISCUSSION

5.1 Live animal growth performance

5.1.1 Average daily gain (ADG) and fasted body live weight (LW)

A significant difference in fasted body live weight was only detectable at d27 between 0-RAC and 10-RAC treatments, with 5-RAC intermediate between the two. The declining p-values throughout the experimental phase suggests a weak response or insensitivity of detection. At d27, a significant difference in LW between 0-RAC and 10-RAC treatments was found, thus supporting the hypothesis that RAC improves LW gain in pigs. At d27, 10-RAC increased fasted body live weight by approximately 3.87 kg (or approximately 3.75%) compared to control individuals, whilst no difference was found between 5-RAC and either other treatment.

Significant differences in ADG between 0-RAC and 10-RAC was also only detectable after d27 or when considering the overall ADG throughout the experimental period. At d22-d27 10-RAC improved ADG by 182.64 gd^{-1} (or approximately 16%) above control individuals. When considering the overall ADG throughout the experimental period, 10-RAC improved ADG by 102.06 $g.d^{-1}$ (or approximately 12.04%) above control individuals. Similarly, Apple *et al.* (2007b) found ADG to be improved by 11.8% in RAC fortified diets at 5 $mg.kg^{-1}$ RAC over control counterparts (0.85 vs. 0.95 $kg.d^{-1}$), but no difference was found between diets containing any higher concentrations of RAC.

For both fasted body live weight and ADG, p-values steadily declined as the treatment duration increased. This is indicative of either a delayed or weak response or insensitivity of detection measures.

5.1.2 Average daily voluntary feed intake (ADFI)

Despite declining p-values which may indicate weak response level or insensitivity of feed disappearance measures, at no point in the experimental period were significant differences between any treatments found. From a mean intake of 2.46 $kg.d^{-1}$, ADFI increased linearly with treatment duration over the experimental period at a rate of approximately 322.5 $g.d^{-1}$. At 10-RAC, RAC-induced depression in ADFI was not yet observable. Similarly, Apple *et al.* (2007b) found no significant difference in ADFI between control and RAC-fortified diets (2.84, 2.83, 2.77 $kg.d^{-1}$ for 0, 5 and 10 $mg.kg^{-1}$ RAC respectively).

5.1.3 Feed efficiency (F:G)

Although feed F:G was not found to be significantly affected by RAC treatment at any singular point in the experimental phase, when considering the overall F:G throughout the experimental period, F:G was found to be significantly lower for 10-RAC compared to control individuals. Overall, at 10-RAC, F:G improved by 0.212 units (or approximately 7.48%) compared to control individuals. Although in principal, concurring with the meta-analysis of Apple *et al.* (2007b), these findings do not fall within the range as reported by the authors (3.33, 3.03, 2.94 for 0, 5, and 10 $mg.kg^{-1}$ RAC respectively; or alternatively a 10% and 13.3% improvement above control animals for 5 and 10 $mg.kg^{-1}$ RAC respectively). The range in improvement in F:G of RAC-

fortified diets above controls ranged from 6.3% (See *et al.*, 2005) to 17.2% (Stites *et al.*, 1991) at a level of 10 mg.kg^{-1} RAC.

Since F:G is essentially calculated from ADG and ADFI measurements, any delayed or weak responses in either of these parameters or imprecise measurements thereof would have a knock-on effect on F:G, and likely conceal differences in F:G.

5.2 Linear carcass measurements

5.2.1 Hot carcass yield (HCY) and Cold carcass yield (CCY)

After 27 days of treatment, RAC treatment was found to significantly improve HCY, with 10-RAC treatment yielding an improvement of approximately 4.09% (or 3.39 kg) above control animals. This is approximately midway in the range expected and reported by Apple *et al.* (2007b) and is most similar to that of Apple *et al.* (2004a) (cited by Apple *et al.*, 2007b) and Stites *et al.* (1991) who reported 4.09% and 4.04% improvements at 10-RAC, respectively. Since CCY was calculated using a linear regression and fixed at 97% of HCY, a similar trend can be observed in CCY.

Interestingly, meat depth was unaffected by RAC treatment. Since this measurement was measured using the HGP, it is inferred that the improved carcass yields observed were due to improvements in growth of muscle in the hams and/or shoulders of the animals.

5.2.2 Dressing percentage (Dr%)

After 27 days of treatment, dressing percentage was found to not be significantly affected by RAC supplementation. Improvements in Dr% with RAC supplementation found in previous literature are marginal (0.27% and 0.80% improvements above controls at 5 and 10 mg.kg^{-1} RAC inclusions respectively). The failure therefore of this study to detect such differences in Dr% could be indicative of the insensitivity of the data.

The mean Dr% of 80.50% achieved is far higher than that found in the majority of previous literature, as can be seen in Apple *et al.* (2007b) who report a range between 71.5% to 81.7%. This indicates that the lean genetic potential of current commercial animals is far higher than that previously reported, and warrants a review of the use of RAC on contemporary commercial lines. Since RAC recommended dosages (as labelled by manufacturer) are based upon trials conducted on animals with a much lower Dr%, re-validation of current RAC dosages should also be considered.

5.2.3 Fat depth

Across all RAC treatments, a mean fat depth of 14.08mm was achieved. Unlike Apple *et al.* (2007b), no significant differences in backfat depth were found between differing RAC treatments. Although this result is influenced by a number of factors and interactions between such factors (Rikard-Bell *et al.*, 2009b), these results are supported by evidence presented by Fernández-Dueñas *et al.* (2008) and Pethick *et al.* (2005) who indicated that RAC may potentially not affect fat deposition. If RAC did indeed not significantly affect backfat

depth, the positive correlation between backfat depth and carcass yield would lead one to expect significantly higher backfat depths at higher carcass yields. Contrary to this expectation and initial inferences, at significantly higher carcass yields, backfat depth was not significantly different from lower carcass yields. This indicates that RAC did significantly reduce backfat depth, concurring with evidence by Apple *et al.*, (2007b).

CHAPTER 6 CONCLUSION

Despite weak results in this particular study, the tendencies and significant findings found endorses the use of RAC up to an inclusion level of at least 10 mg.kg^{-1} to fortify diets for finishing gilts for at least the final 28 days prior to harvesting.

Although not entirely supported by the data in this study, the growth promoting effects of RAC, including suppression of adipose deposition and increased protein accretion, has been well documented throughout literature. These mechanisms occur via a cAMP cell signalling cascade. In contrast to other βAAs which have a more aggressive growth promoting effect tending to darker and firmer meat, ractopamine has a more moderate effect, and is widely used in the pork industry. However, the growth promoting effects of RAC are only fully realised when all other parameters including nutrition and environmental conditions are optimal, and the animal's full genetic potential cannot be realised by any other means.

The majority of RAC-centred research has focused predominantly around the crude growth enhancement in pigs in relation to the current pork classification system. It is pertinent that RAC research should be directed more to investigating the differential growth in specific muscles and how RAC would more accurately contribute to carcass value within a revised classification system (as recommended by Webb, 2015) which accounts for contemporary carcass types, emerging meat-quality orientated niche products (discussed in Stoller *et al*. 2003) or branded pork programs (Bohrer *et al*., 2013b), as well differentiates between carcass attributes that modern feedstuffs and growth enhancing technologies may contribute.

CHAPTER 7 CRITICAL EVALUATION

Inconclusive results obtained from this study may be attributed to a number of factors. Although under ideal conditions, a sample size of 57 (in other words 19 replicates per treatment) should provide sufficient statistical power, infrastructural limitations and disturbances lead to suboptimal conditions resulting in the exclusion of some individuals from statistical analyses (*vide* 3.6.1).

In retrospect, there were some limitations to the methods that could be improved in subsequent trials:

1. Residual feed should rather be dry-weighed to improve accuracies of feed intake measurements
2. Gut fill effects could be accounted for by statistical analyses, and although not withholding feed could eliminate any potential stress effects, this would certainly require more trial animals and significant costs to any trials. It would be far better to withhold both feed and water.

7.1 Infrastructure

7.1.1 Maintenance

The pig unit housing has remained in an unused and apparent state of disrepair for an extended period of time, with neglected routine maintenance leading to failure of key building elements. Maintenance was carried out, but only addressed superficial areas of concern.

Slurry canals

Originally the pig unit was fitted with bucket flushing type deep slurry canal systems. This system was kept for the group housing, and although required excessive water, worked very effectively. The individual housing slurry canal has been converted to a self-draining system, however key elements such as the number of animals per canal is not sufficient for this system to work effectively within the given time period of a grower trial. Also, outlet pipes are positioned slightly raised from the bottom of the canal, and consequently canals drain incompletely. Furthermore, roots from surrounding vegetation has damaged slurry canals to such an extent that all fluids leak rapidly through the cement, with remaining solids accumulating and unable to drain. This was apparent upon inspection of slurry canals before commencement of the trial as the canals has not been cleaned with dried faeces caked in, and needing to be removed by hand. Slurry canals should be re-sealed and either be shallowed with outlet pipes adjusted, or reverted to bucket flush systems.

Feed store roof

After personally inspecting and reporting the requirement for maintenance timeously, required maintenance was not completed for some time resulting in rainwater leaks leading to mould spoilage of at least 300 kg of feed. Mycotoxin loads in feed bags surrounding those which had visually spoiled was not carried out. Therefore, potentially, through the trial period, pigs could have been exposed to chronic mycotoxin loads which would consequentially influence growth performance. Leaks in the roofing were caused by sun-damage to corrugated fiberglass roof sheets and rusted steel corrugated roof sheets. Attempts to cover and protect feed

with plastic sheeting in the interim were also unsuccessful. All damaged roofing should be replaced as soon as possible to avoid further unnecessary wastages.

Water pipes

Copper water pipes are exposed and not securely fastened in place, resulting in movement on the pipe joints. This resulted in fatigue and breakage of a copper water pipe feeding the nipples to 20 pigs during the experimental phase. Fortunately this breakage occurred during a feeding session and was reported timeously. However, a delayed response of maintenance personnel lead to a restricted water allowance for at least 12 hours for these pigs. The exposed water pipes should be securely fastened to prevent future discrepancies in trial conditions.

7.1.2 Environmental control

Automated temperature (cooling or heating) and ventilation measures were not installed in the pig unit. Resultantly, fans were used on a 24 hour basis at minimum speed, with window louvers adjusted to prevent direct drafts on individual animals. Also, windows were not covered by curtains leading to the inability to restrict ambient lighting. As a result, in-house temperatures and daylength fluctuated with the external ambient weather conditions. Temperature, ventilation and daylength fluctuations likely caused differences in feed intake and growth performance.

An automated system panel (Mf-Net Master-1) is installed in the individual housing. This main controller is sufficiently capable, however needs to be integrated with variable speed fans, lighting, ventilation and heating systems. Curtains should be erected to allow for manipulation of daylength.

7.1.3 Feed dispensers

Prior to the commencement of the trial, the steel feed dispensers in the pig unit were found to be extremely corroded, with many being non-functional or spilling feed through their bottom. All dispensers were sandblasted, repaired, and repainted, but many remained in a non-adjustable state due to a severely corroded adjustment screw mechanism. Usage of some dysfunctional (jammed or over-dispensing) feed dispensers in the individual housing was unavoidable due to limited available feed dispensers. Also, the design of the feed dispensers encouraged fouling of feed and was prohibitive to excess feed removal. I would highly recommend replacing all feed dispensers with more modern commercial types such as to reduce feed wastage and man-hours operating the dispensers. Dispensers which are more practically user-friendly in terms of measuring feed intakes and cleaning would be beneficial to any feed-related trials.

7.2 Disturbances

Any disturbance would likely increase stress and reduce feed intake. Whilst observing pigs in the house, sounds and movement at the feed silo directly adjacent to the pig unit, which was operated multiple times

throughout a week, obviously caused some disturbance to the animals. Since the facility essentially lacks security measures such as fences and locks on entry points, there were occurrences when workers would walk up to and peer in to the windows of the pig house or enter the facility without permission. Apart from the disturbance caused, this also raised some biosecurity concerns. A perimeter fence isolating the facility should be erected, and all entry points secured. The use of curtains, as previously discussed, would also act to limit visual disturbances to the pigs.

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APPENDICES

Appendix I. Diet composition and theoretical analysis of diets

	Grower		Control (0 mg.kg^{-1} RAC)		Control +5 mg.kg^{-1} RAC		Control +10 mg.kg^{-1} RAC	
Ingredient	%							
Maize 7.5%	71.23		68.51		68.49		68.48	
Soya O/C 47%	17.48		19.00		19.00		18.99	
Wheat bran 15.5%	7.99		9.00		9.00		9.00	
Limestone 36	1.25		1.25		1.25		1.25	
Salt	0.50		0.50		0.50		0.50	
MCP	0.30		0.40		0.40		0.40	
L-Lysine HCl	0.40		0.45		0.45		0.45	
L-Threonine	0.15		0.20		0.20		0.20	
DL Methionine	0.12		0.17		0.16		0.16	
L-Tryptophan	0.05		0.06		0.06		0.06	
L-Valine	0.02		0.02		0.02		0.02	
Paylean® (RAC)	-		-		0.025		0.050	
Tylan 100	0.10		0.04		0.04		0.04	
Feed colourant	Green		Blue		Green		Red	
Premix	0.40		0.40		0.40		0.40	
	100.00		100.00		100.00		100.00	
Calculated analysis (DM basis)	Total	Avail	Total	Avail	Total	Avail	Total	Avail
DE (pig) (MJ kg^{-1})	14.01		13.97		13.96		13.96	
ME (pig) (MJ kg^{-1})	13.41		13.34		13.34		13.33	
SID M+C:Lys	0.61		0.62		0.62		0.62	
SID Thr:Lys	0.66		0.68		0.68		0.68	
SID Trp:Lys	0.21		0.21		0.21		0.21	
SID Val:Lys	0.72		0.70		0.70		0.70	
SID Ile:Lys	0.60		0.58		0.58		0.58	
SID His:Lys	0.41		0.40		0.39		0.39	
Lysine	1.05	0.94	1.13	1.02	1.13	1.02	1.13	1.02
Met + Cys %	0.66	0.58	0.71	0.63	0.71	0.63	0.71	0.63
Threonine %	0.72	0.62	0.79	0.69	0.79	0.69	0.79	0.69
Tryptophan %	0.22	0.20	0.24	0.21	0.24	0.21	0.24	0.21
Valine %	0.78	0.67	0.83	0.71	0.83	0.71	0.83	0.71
Crude protein	15.55	13.38	16.32	14.04	16.32	14.04	16.32	14.04
Crude fibre	3.24		3.36		3.36		3.36	
Crude fat	3.29	2.86	3.25	2.81	3.25	2.81	3.25	2.80
Ca:P	1.24		1.21		1.21		1.21	
Ca %	0.68		0.71		0.71		0.71	
P %	0.55	0.26	0.58	0.28	0.58	0.28	0.58	0.28
Na %	0.21		0.21		0.21		0.21	

Appendix II. Premix composition

Ingredient	Units	Content
Vitamin A	iu/kg	7,000,000.00
Vitamin D3	iu/kg	750,000.00
Vitamin E	mg/kg	50.00
Vitamin K3	mg/kg	2.00
Vitamin B1	mg/kg	1.50
Vitamin B2	mg/kg	4.50
Niacin (B3)	mg/kg mg/kg	25.00
Pantothenic acid (B5)	mg/kg	20.00
Vitamin B6	mg/kg	2.50
Vitamin B12	mcg/kg	0.030
Folic acid (B9)	mg/kg	0.50
Biotin	mcg/kg	0.15
Choline chloride	mg/kg	190.00
Iron	mg/kg	100.00
Manganese	mg/kg	80.00
Copper	mg/kg	125.00
Zinc	mg/kg	120.00
Iodine	mg/kg	1.00
Selenium	mg/kg	0.40
Phytase	FTU	750.00
Antioxidant	mg/kg	125.00

Appendix III. Individual housing layout