

TITLE

Mechanisms and effects of ractopamine hydrochloride on fat and muscle tissue deposition in finisher pigs.

By

Charles V Rikard-Bell,

BSc.Agr, MSc

A dissertation completed in partial fulfilment of the requirements for the Degree of

DOCTOR OF PHILOSOPHY

MURDOCH UNIVERSITY

School of Veterinary and Biomedical Sciences

JUNE 2012

DECLARATION

I declare that this is my own account of my research and contains as its main content work , which has not previously been submitted for a degree at any tertiary education institution. All experiments were planned and all results shared in full consultation with, and disclosure to, my supervisors Professor John Pluske and Professor Frank Dunshea.

Charles V. Rikard-Bell

June 2012

SUMMARY OF AMENDMENTS

General Response

The Examiners' Reports highlighted areas of this thesis that required clarification and areas well presented, and for this I would like to thank all the reviewers for their important suggestions and critique of the thesis. I found all phases of undertaking the degree of Doctor of Philosophy extremely rewarding including responding to the Examiners' comments, which allowed me to further refine the work presented. Please note that all the misprints and grammatical errors have been corrected in the thesis without notification below. My responses are as follows.

Specific Comments and Responses

Examiner: Professor John Patience

Chapter 1: General Introduction

Comment: Page 2, line16 Schinckel has some excellent modeling papers that are relevant in defining the lysine requirements.

Response: *The relevant paper has now been cited. Referencing this paper does not alter the wording of the paragraph, because the paper emphasizes that greater than 1.0 g/kg total lysine is required for finishing pigs supplemented with dietary RAC between 80 and 120 kg in order to optimize growth rate and carcass lean yield.*

Chapter 2: Review of the Literature

Comment: Reference throughout the thesis is made to limit feeding pigs. If this is the case then limit feeding is another difference between US and Australian conditions that probably should be mentioned.

Response: *Limit feeding is not practiced commercially in Australia. The reference to limit feeding in this thesis comes from the paper by Dunshea et al (2009) in which finishing pigs housed in individual pens were limit-fed to reflect typical energy intakes of commercial finisher pigs. Dunshea et al (2009) were investigating whether the combination of dietary RAC and betaine was additive.*

Comment: Define the term "available" lysine.

Response: *At first introduction of the term "available lysine", Section 2.5.4, p26 the term has been referenced as a footnote and defined in the footnote as standardized ileal digestibility of lysine.*

Comment: Given the interest in lysine level and ractopamine level, the student might wish to refer to Ross et al (2011) in which studied both these factors.

Response: *I have included the following paragraph in section 2.5.4 of the Literature Review: "A recent study in Canada by Ross et al (2011) observed no response in ADG or G:F for barrows fed dietary RAC across 3 levels of standardized ileal digestible (SID) lysine : DE ratios of 1.73, 2.14 and 2.63 g Mcal of DE (equivalent to 0.41, 0.51 and 0.63 g available lysine / MJ of DE). The expectation of a RAC x lysine interaction was not observed by Ross et al (2011) and the authors concluded that this may have been due to the excellent feed intakes (approximately 4.0 kg/day) providing greater than 22g of available lysine per day on the low lysine diet. Additionally Ross et al (2011) argued that lysine was not limiting in this experiment as a RAC x Lys interaction was*

not observed for any growth variable, and a 16% improvement in protein gain in RAC fed pigs over controls was observed.”

Chapter 3:

Comment: I do not think this is a 3 x 5 factorial experiment.

Response: *I have changed the description of the experimental design in section 3.2.1 to read as follows: “The study involved 120 pigs (40 gilts, 40 boars and 40 boars immunized against GnRF) and was carried out as a randomized complete block design consisting of three animal types (gilts, boars and boars immunized against GnRF) and five RAC dose regimes [(i) 0 mg/kg, (ii) 5 mg/kg and (iii) 10 mg/kg for 28 days, (iv) 5 mg/kg for 14 days and 10 mg/kg for 14 days (5/10 mg/kg RAC Step-up), (v) 5 mg/kg for 28 days plus daily pST (5mg/ml) injections (Reporcin®, OzBiofarm, Victoria) for the last 14 days] with 8 pigs per treatment group.”*

Comment: The initial and final weights should be noted either in the tables as an experiment outcome (final weight) or in footnotes to the table so the reader knows the initial and final weight.

Response: *Initial and final weights have been included in Table 3.2. I have also included initial and final live weights in Table 5.4 and initial weights in Table 6.3.*

Comment: The chapter discussion and conclusion does not specifically refer back to the hypothesis and in this instance state that the hypothesis was not supported.

Response: *I have altered the conclusion, section 3.5 as follows: “The results of the study presented do not support the hypothesis that diets supplemented with RAC over a 4-week period in single dose, step-up or single dose program with the addition of daily pST treatment in the final 2 weeks will improve feed efficiency as well as increase lean tissue deposition in boars, boars immunized against GnRF and gilts.”*

Chapter 4:

Comment: These data should be analyzed statistically using repeated measures analysis, since numerous repeated measures were reported.

Response: *The Examiner has commented he has concerns that repeated measures analysis was not used in Chapter 4, 5 and 7. In response, I would argue that the use of repeated measures analysis in these chapters is not required. Whilst measurements such as live weight was obviously repeated on the experimental units at day -7, 0, 7, 14, 21, and 28, one considers applying repeated measures when:*

- 1. There is an expectation of variability of the trait to differ between treatments. In all studies presented the variation of the trait measured was expected to be the same among treatments, and only the treatment mean for the trait was expected to differ.*
- 2. The repeated measure is likely to have a correlation with the adjacent measurements. This may have been the case if I had presented average daily gain (ADG) in the following manner 0-7; 7-14, 14-21, 21-28. However I have presented ADG for periods: 0 – 7; 0 – 14 and 0 – 21 which are considered independent estimates of the animals performance.*

Reference:

Kaps and Lamberson (2009) Biostatistics for animal science, an introductory text. CAB International, Wallingford Oxon, U.K.: 405 - 431.

Consultation with Professor Frank Dunshea.

Furthermore, the DXA information presented in Chapter 5 should not be analysed using repeated measures as each individual pig in the experiment only underwent a DXA measurement once (see Section 5.2.4, DXA live animal scanning). The body composition was determined for boars and gilts from one replicate from each dietary treatment at Days -1, 15 and 29, by using DXA as described by Dunshea et al. (2009). All pigs in the study were scanned once only.

Comment: The student indicates the danger of discussing sex effects since gilts and boars were studied in separate experiments, and then proceeds to compare sex responses. This should be corrected.

Response: In section 4.3.2, Experiment 2: Boars, I have excluded the references to Experiment 1: Gilts. The original paragraph was as follows: "As was seen with the gilts, the light initial-weight boars had a better FCR than either medium or heavy initial-weight boars (2.45 vs 2.58 and 2.71, respectively ($P < 0.001$)). Similar to the gilts, the ADFI increased with heavier pigs at the start (2954, 3153 and 3354 g/day for light, medium and heavy boars, respectively ($P < 0.001$)).". The paragraph has been altered to: "The light initial-weight boars had a better FCR than either medium or heavy initial-weight boars (2.45 vs 2.58 and 2.71, respectively ($P < 0.001$)). The ADFI increased with heavier pigs at the start (2954, 3153 and 3354 g/day for light, medium and heavy boars, respectively ($P < 0.001$))."

To improve clarity of section 4.4.3, Effects of start weight and dosage level of RAC on feed conversion ratio, I have altered the paragraph from: "In the present studies, dietary RAC improved FCR in gilts ($P = 0.023$) but not in boars ($P = 0.289$), although it is difficult to compare sexes in the present experiments as the studies were carried out at different times." The paragraph has been changed to: "In the present experiments, dietary RAC improved FCR in gilts, Experiment 1 ($P = 0.023$), but not in boars, Experiment 2 ($P = 0.289$). However and because the experiments were separate and carried out at different times, differences in responses to dietary RAC should be viewed with caution."

The intent of the discussion in section 4.4.3 was to highlight that although the boars did not improve FCR when fed dietary RAC in Experiment 2, the result cannot be compared to the significant improvement in FCR shown by gilts fed dietary RAC in Experiment 1 because the studies were separate. I then referred to the literature to highlight that no sex effects had been reported in studies measuring FCR in boars and gilts fed dietary RAC.

Comment: The explanation of no differences observed in PUN is not consistent with the literature, or our understanding of nitrogen metabolism in pigs.

Response: In section 4.4.2, Effects of dosage level of RAC on Plasma urea nitrogen, I have altered the following explanation from: "A possible explanation for this result could be that all the diets offered provided excess dietary protein (Table 4.1) for maximal protein deposition (King et al., 2000), and as such amino acid catabolism across all treatments resulted in similar levels of PUN.", to the following explanation: "The fact that RAC did not alter PUN may indicate disease challenge and pigs that do not efficiently utilize dietary lysine, and therefore amino acid catabolism occurred across all treatments resulting in similar levels of PUN.". The effect of disease is discussed further in Chapter 6.

Reference: van Heugten, E., Spears, J. W. & Coffey, M. T. (1994). The effect of dietary protein on performance and immune response in weanling pigs subjected to an inflammatory challenge. Journal of Animal Science 72(10): 2661-2669.

Chapter 5

Comment: I found this experiment perhaps a bit complex, given that the primary question being asked related to the adequacy of the lysine recommendations. Confounding the experiment with multiple levels of ractopamine seemed to detract from the ability to clearly answer the question and also reduced the precision of the experiment as well.

Response: *The complexity of the experiment was quite deliberate because the aim was to determine whether there were interactions between RAC dosage levels and dietary lysine on growth performance and carcass characteristics of finisher boars and gilts using dual energy X-ray absorptiometry (DXA) to measure body composition. The low level of lysine was chosen because it reflected commercial diets supplemented with RAC that were used in the Australian industry at that time. The high level of lysine was chosen to reflect recommendations from the literature which were primarily directed towards the US swine industry.*

Comment: Repeated measures should have been used, particularly the DXA data.

Response: *I have addressed this issue in Chapter 4 responses (above).*

Comment: The methods section states that pigs were fed *ad libitum* but later states that blood samples were collected 3 hours after feeding. Please address this apparent discrepancy.

Response: *In section 5.2.5, Determining Plasma Urea Nitrogen (PUN) content, the first paragraph has been altered from: "Blood samples were obtained by jugular venipuncture three hours after feeding on day -2 and day 9.", to: "Blood samples were obtained by jugular venipuncture three hours after the morning feed inspection on day -2 and day 9."*

Chapter 6

Comment: Table 6.1 needs an explanation as to how the diet were prepared.

Response: *In section 6.2.2, Experimental Animals and diets, I have included the following sentence to explain how the five dietary lysine levels were attained: "The composition of the control diets was formulated to meet the specified lysine:energy ratios (Table 1), by blending the two extreme lysine level diets A and E in specific ratios to produce control diets B (25% A and 75%E), C (50% A and 50% E) and D (75% A and 25%E)."*

Comment: What is meant by the term Total available lysine in Table 6.1.

Response: *I have included in the footnotes of Table 6.1 the definition of "Available Lysine" and "Total Available Lysine"*

Comment: Specify whether the broken line model or the quadratic model was used to define the lysine requirement, furthermore there is no explanation of how the model, and the model parameters were selected in terms for example of best fit.

Response: *The broken line model was used to define the lysine requirement. I have reworded and included additional lines in section 6.2.6 to further explain how the model and the model parameters were selected. The original explanation is as follows:*

“A break point analysis was applied to these data for ADG and FCR for each sex. The quadratic curves for ADG and FCR for each sex were fitted by a nonlinear regression computer model using GenStat 11th Edition (Payne et al., 2008).

$$a + b.(lysine) + c.(Lysine \geq d).(d-Lysine))$$

The estimates for a, b, c and d parameters were determined for each quadratic curve, where:

a = where the graph crosses the y axis at zero

b = (maximum gain – a) ÷ (d)

c = same as ‘b’

d = lysine concentration where maximum gain occurs

The model determined computed values for the parameters a, b, c and d. The computed values were then used to determine the maximum or minimum response for each trait using the straight line equation:

$$\text{Response of trait} = a + b (\text{lysine concentration})$$

The computed value of d is the lysine concentration where maximum gain occurs, and this is also where the break point occurs on the graph. To determine the minimum gain, the lowest level of lysine concentration is used in the above equation.”

This has been altered to:

“A break point analysis was applied to these data for ADG and FCR for each sex without and with dietary RAC. A nonlinear regression computer model (GenStat 11th Edition (Payne et al., 2008) was used to determine the critical lysine value for each data set. The regression model was defined as:

$$a + b.(lysine) + c.(Lysine \geq d).(d-Lysine))$$

where:

a = where the graph crosses the y axis at zero

b = (maximum gain – a) ÷ (d)

c = same as ‘b’

d = lysine concentration where maximum gain occurs

Prior values of the non-linear parameters ‘a’, ‘b’, ‘c’ and ‘d’ were estimated by fitting each ADG and FCR data set to the model. In order to obtain the best fitting segmented lines the model performs iterative numerical calculations to define the prior values. The computer model produced a nonlinear regression analysis for the response trait (ADG or FCR) and estimates of the non-linear parameters and corresponding standard errors were obtained. The computed values for a, b, c and d were then used to determine the maximum or minimum response for each trait using the straight line equation:

$$\text{Response of trait} = a + b (\text{lysine concentration})$$

The computed value of d is the lysine concentration where maximum gain occurs (critical value), and this is also where the break point occurs on the graph. To determine the minimum gain, the lowest level of lysine concentration is used in the above equation.

Comment: I would encourage the student to analyze the response to lysine in more biologically-based outcomes, such as g lysine/g lean tissue accretion.

Response: *I would certainly agree that the application of this type of analysis would have been invaluable had it been incorporated into the study, however the logistics of incorporating DXA scanning of live pigs or half carcasses was not possible and was also too expensive. DXA scanning was incorporated into studies reported in Chapter 5 and 7 where I was able to show differences in lean tissue accretion rate when differences were not apparent in ADG for pigs fed dietary RAC. To this extent I have clearly demonstrated the value of reporting lean accretion rates over ADG.*

Chapter 7

Comment: The issue of repeated measures analysis is relevant to this chapter.

Response: *I have addressed this issue in Chapter 4 responses (above).*

Comment: In the second experiment, is it possible that the absence of a response to ractopamine in boars was due to inadequate lysine? Also the quantity of added lysine HCL is very high; could this have been an issue as well?

Response: *The boars in the second experiment did respond to dietary RAC in the first 14 days, however this was expressed as a reduced ADFI rather than an improved ADG, the outcome being a significant ($P=0.033$) improvement in G:F. In this regard, I wonder whether the Examiner is referring to the first Experiment? If so, then I have proposed that lysine may have been limiting to boars in section 7.4 Discussion, p144.*

The quantity of Lysine HCL in the second experiment was not high. In this thesis addition rates of Lysine HCL for most diets was expressed as a % of the composition of the diet, however in Table 7.4 Lysine HCL is presented in g/kg, therefore the value of 0.9 g/kg is equivalent to 0.09% which is lower than additional Lysine HCL in experiments in Chapter 4 (Table 4.1; 0.16%); Chapter 5 (Table 5.1; 0.3%); Chapter 6 (Table 6.1; 0.26%) and Chapter 7 (Table 7.1; 0.23%).

Chapter 8

Comment: More details on biopsy collection methods would be helpful.

Response: *In section 8.2 I have included the following sentences: "Immediately prior to tissue sampling, the pig was anaesthetised as described in section 3.2.6 and a whole body DXA scan (Suster et al., 2004) was taken to determine fat, muscle and ash content. Specific to this chapter, immediately after DXA scanning, approximately 1 g of muscle (Gluteus Maximus) and 2 g of subcutaneous fat was taken from the same incision site on the hind leg. All samples were individually wrapped in foil and labelled: study number, date of sample, pig number. Samples were placed in liquid nitrogen and stored at -80°C until required for tissue analysis."*

Specific Comments and Responses

Examiner: Professor Martin Sillence

Declaration

Comment 1: The author is an employee of the company that markets ractopamine. As such I recommend that the issue of potential conflict of interest should be addressed and/or declared.

Response: *I have added the following statement to the original Declaration: "All experiments were planned and all results shared in full consultation with, and disclosure to, my supervisors Professor John Pluske and Professor Frank Dunshea"*

Summary:

Comment 2: The summary is too long and should be re-written with a focus on the main aims and conclusions of the work as a whole body.

Response: *I disagree with the Examiner's appraisal of the Summary. A total of seven separate experiments were conducted all of which required a brief description of experimental procedures and the main results and their significance highlighted. The thesis presents "a cogent description of a large body of work" and hence the Summary reflects the volume presented. The summary had also been read, reviewed and suggestions made by my supervisor, Professor John Pluske, prior to submission for examination.*

Comment 3: pIII. It is stated that RAC had no effect on ADG, but it is unclear whether this applies to RAC alone, RAC with pST, or both.

Response: *I have amended the sentence to include the treatment RAC+pST: "The results of Experiment 1 were surprising in the fact dietary RAC and RAC+pST had no effect on average daily gain (ADG) ($P=0.543$) or feed conversion ratio (FCR) ($P=0.255$) on these fast-growing, high-health-status finishing pigs."*

Comment 4: p6. The American terms epinephrine and norepinephrine should be replaced with English terms adrenaline and noradrenaline throughout the review.

Response: *The English terms have been used throughout the thesis.*

Comment 5: p6. Note that noradrenaline is both a neurotransmitter and a hormone released from the adrenal gland.

Response: *I have altered the sentence to read as follows: "Noradrenaline is both a neurotransmitter molecule and a hormone that is released from the adrenal gland and also the nerve endings of both the central nervous and sympathetic nervous systems, and is biosynthesised from tyrosine."*

Comment 6: p6. The term β -adrenergic is used throughout the thesis without regard to its precise meaning. 'ergic' refers specifically to a nerve, and so the term is appropriate when discussing 'adrenergic nerves, or neurotransmitters'. The correct term for the corresponding receptors is β -adrenoceptors (β -AR), and the term for a compound such as ractopamine is β -adrenoceptor agonist.

Response: *The term " β -adrenergic receptors" and " β -adrenergic agonist" have been replaced with " β -adrenoceptors" and " β -adrenoceptor agonist" respectively throughout the thesis.*

Comment 7: p12. Down regulation refers to long-term processes where there is significant receptor degradation and a decrease in the rate of receptor synthesis. Using the term desensitisation in a global sense to describe both short and long term events is not helpful.

Response: *I have altered the introductory paragraph in section 2.3.2, Desensitization of β -AR signalling, such that there is a clear distinction between the processes of 'desensitisation' and 'down regulation' of the β -adrenoceptors in the presence of a β -adrenoceptor agonist. The altered introductory paragraph is as follows: "Desensitisation is the reduction of response despite the continued presence of the stimulus. The mechanism that contributes to desensitisation involves the "uncoupling" of the receptors from G-protein, which is initiated in seconds to minutes of agonist exposure. The term down regulation refers to long-term processes and develops more slowly than uncoupling, taking hours to days (Mills, 2002b). Down regulation involves receptor degradation and a decrease in the rate of receptor synthesis. Both desensitisation and down regulation restricts the use of stimuli such as β -adrenoceptor agonists in animal production today, and research to circumvent these processes are discussed in section 2.6 of this review."*

Comment 8: p14. The discussion of theophylline shows good evidence of critical thinking. It is also worth mentioning that much of the work conducted on adipose tissue β -AR during the early 1990's led to erroneous conclusions based on flawed assumptions about sub-type selectivity in porcine tissue, of various compounds that had been used in the past to characterise these receptors in humans and other species.

Response: *In section 2.4.1, Lipolysis in adipose tissue, I have inserted a final paragraph: "Much of the work conducted on adipose tissue β -AR during the 1990s led to erroneous conclusions based on flawed assumptions about subtype selectivity in porcine tissue of the various compounds that had been used in the past to characterise these receptors in humans and other species (Sillence et al., 2005). β -ARs are well characterized in many mammalian species, however they have been difficult to define in porcine adipose tissue either through the adrenergic control of lipolysis (Liu et al., 1989) or direct ligand binding experiments (Coutinho et al., 1992; Mersmann, 1992). Researchers have reported that porcine β -AR do not show the degree of selectivity for classic β -AR ligands typical of other animal species (Coutinho et al., 1992; Liang and Mills, 2002; Mersmann, 2002). Furthermore the radioligands that have been proved to be ideal for labelling β -AR in some tissues and species, in swine can profoundly influence the characterization of the receptor (Mersmann and McNeel, 1992)."*

Comment 9: p18. Use isoprenaline throughout not the American "isoproterenol"

Response: *The English terms have been used throughout the thesis.*

Comment 10: p18. Provide some further interpretation or explanation of the results cited by Ding et al (2000). How do you reconcile the decrease in β -AR number caused by isoprenaline in the absence of any decrease in mRNA?

Response: *I have consulted the paper by Ding et al. (2000). The following paragraph has been inserted in section 2.4.3, Down regulation of β -AR in adipose tissue: "A plausible explanation for the decrease in β -AR number caused by isoprenaline in the absence of any decrease in β -AR mRNA is discussed by Ding et al. (2000). The β -AR have cAMP response elements (CRE) in the 5'-untranslated region that are stimulated by phosphorylated CRE binding proteins. The role of cAMP is to activate protein kinase A (see section 2.3.1), which then phosphorylates the CRE*

binding protein. Thus, increased cAMP can increase β -AR transcription. Nothing is known about the function of the CRE in porcine β -AR subtypes, however, the intracellular concentration of cAMP is increased when porcine adipocytes are incubated with isoprenaline (Hu et al., 1987). Speculatively, the modest isoproterenol-stimulated desensitization of porcine adipocyte β AR with no change in the β 1AR and β 2AR transcript concentrations may represent a relatively high rate of cAMP-stimulated transcription and translation coupled with modest rates of receptor phosphorylation and sequestration from the membrane. These experiments help explain the lack of response recorded in many studies measuring fat deposition in pigs treated with dietary RAC.”

Comment 13: p38. What instrument/detection system is was used for the amino acid analysis?

Response: *In section 3.2.3, Determining amino acid contents of each diet, I have included the following sentences to clarify the detection system used for the amino acid analysis: “Each separated amino acid was then mixed with the detection reagent ninhydrin and converted in a reaction loop (post-column) at 130°C to a ready measurable, characteristic blue violet or yellow colour. The amino acids were then detected by a photometer at a light of wavelength of 570 nm (or 440 nm for yellow proline derivatives) and through an integrated computer, a chromatogram was produced from which the concentration of the amino acid is determined.”*

Comment 14: p40. Presumably the RAC diets were specially formulated for this trial by adding a known quantity of RAC to a known quantity of feed? Explain why it is necessary to measure actual RAC content of the feed by chemical analysis. Note that chemical analysis is subject to error and provides an estimate of the actual concentration.

Response: *RAC diets were specially formulated for this trial by the addition of a known quantity of RAC to a known quantity of feed. It was necessary to measure actual RAC content (parts per million) to verify uniform distribution of RAC in the final feed. In section 3.2.4. I have added the following opening sentence: “In order to verify that RAC premix addition was uniformly distributed throughout each treatment diet at the expected concentration, samples from each treatment (and control) diet were taken for laboratory analysis of RAC concentration.”*

Comment 15: p42. The first paragraph should cite the relevant tables and figures that appear on the subsequent pages.

Response: *I have cited Table 3.2 in the first paragraph.*

Comment 16: p42. Define “empty body tissue”

Response: *The term has been defined in section 3.2.6, DXA live animal scanning.*

Comment 17: p48. Reproductive quality of the figures throughout the thesis is very poor, particularly the axis labels, which are hardly legible in my copy.

Response: *All figures checked – legibility is fine in both pdf version and Microsoft Word program. The unclear axis may have been a result of the printed copy. I consulted with Professor John Pluske in the construction and final presentation of each chapter, and all figures have been presented in the same format. The figures presented in Chapters 5 and 6 are from published papers and accepted by Animal Production Science.*

Comment 18: Figures 3.1 and 3.2 could easily fit on one page.

Response: *Figures 3.1 and 3.2 are now on one page.*

Comment 19: p50. There appears to be a problem with the reference management system as the citations appear with a # symbol, a citation number and curly brackets.

Response: *This simply means that Endnote references and citations require updating. This has been done and should now not occur at all in the thesis.*

Comment 23: p54. It is unclear if the 96 pigs were 96 gilts, or 96 gilts or boars. Rephrase.

Response: *I have described experimental numbers in section 4.2.1 as “A total of 96 gilts (Experiment 1) and 96 boars (Experiment 2) were allocated to 12 treatments with 8 replicates per treatment per experiment.”*

Comment 25: Statistics. In this chapter and all others there is no explanation of how means are separated i.e. was a multiple comparison test used such as least significant difference (LSD), or Tukey’s Test? This should be explained in the statistics sections and specified in the figure and table legends whenever multiple superscripts are used to denote differences.

Response: *Means have been separated by using least significant difference (LSD) procedures and statistical significance was accepted at $p < 0.05$. I have employed this method to separate the means in figures or tables in Chapters 4; 5; 7 and 8. Therefore in sections 4.2.5; 5.2.6; 7.2.5 and 8.2.4 headed ‘Statistical analysis’, I have included the following sentence: “Where appropriate, means were separated using the least significant difference (LSD) procedures and statistical significance was accepted at $P < 0.05$.”*

Comment 26: p62. I think it is stretching the imagination/misleading to state that the relationship between RAC and ADG was linear (even if linear equation was fitted to the data), as the effect of RAC shows a distinct plateau in light and medium pigs according to Fig 4.1.

Response: *The main effects of initial live weight and RAC dose are presented in Table 4.2. The main effect of RAC dose on ADG is significant ($p = 0.023$). Inspecting the ADG closer one notes that as RAC dose increases ADG increases, therefore checking for a linear relationship between RAC and ADG is very relevant as the relationship has been reported in early studies (see discussion, section 4.4.1). It is not misleading to state that the relationship between RAC and ADG is linear and this is supported by the strong probability value attached to the relationship ($P = 0.006$). As there were no significant dietary RAC x initial weight interactions in Experiment 1 (Gilts) there was no need to separate out the initial start weights for each RAC dosage levels as presented in Fig 4.1. However I did this because of the commercial relevance, to indicate graphically that responses to dietary RAC for ADG occur across all initial start weights.*

Comment 27: p68. In section 4.3.3. modify last sentence which currently reads “... at different conducted.”

Response: *Last sentence has been altered to read: “As both experiments were conducted separately and only included one sex, PUN values cannot be compared between sexes.”*

Comment 29: Discussion, Section 4.4. The discussion would benefit from more critical analysis in terms of providing possible reasons why the current results were not those expected or not consistent with some earlier reports:

- a. In particular there is no discussion on the strengths and weaknesses of the experimental design
- b. Discuss the likelihood that there was no real difference between light medium and heavy weight boars in ADG. The response to RAC of medium pigs?
- c. Given the amount of variation in this initial experiment, it is surprising that the subsequent studies were not designed with fewer treatment combinations and more replicates to ensure more robust conclusions.

Response: *Part a. and b. I have included the following as the second paragraph in the Discussion section 4.4: “It was unfortunate that the experimental design involved two separate experiments in succession with one sex per experiment, and only 8 replicates per treatment, which reduced the power of the experiments. Therefore, a reasonable argument for the observed high growth rate exhibited by the 5 mg/kg light-weight boars compared to control, 10 or 20 mg/kg groups may in fact be an anomaly, and the apparent lack of response to RAC by the medium start weight boars a consequence of their unusually fast ADG. However had each experiment included both sexes, the experiments could have been blocked by time and would have doubled the treatment size to 16 replicates and provided sufficient power to the study. Unfortunately combining boars and gilts into the one experiment was not possible due to their different source herds and disease status. Using the abbreviated equation described by Morris (1999) to determine approximate minimum replicate number required to achieve a significance between treatment means,*

$$n = 8 \times (CV\%)^2 / (d\%)^2$$

Where n = minimum number animals; CV% = (standard deviation) / (mean) x 100 ; and d = expected difference between treatment and control as a percentage.

and applying improvements in FCR of 8% and a coefficients of variation (CV) of 10% (Rikard-Bell et al., 2009a), a minimum of 12 replicates per treatment is calculated. However, it was argued that an analysis of the main effects would provide sufficient power to determine whether start weight (32 replicates per weight category) or RAC dosage level (24 replicates per dosage level) were factors that influence the magnitude of response to dietary RAC in finisher pigs.

Response: *Part c. The lessons learnt from the initial start weight x RAC dosage studies were incorporated into the lysine studies that followed. For example, both sexes were included in each lysine study that were blocked by time due to space allowances, and therefore increased the power of each study. Unfortunately as in many studies, I was having to compromise when designing each study. Referral to the unifying hypothesis enabled careful consideration, so whilst most studies were intricate in their factorial design, in all studies sufficient replication occurred when analysing main effects.*

Comment 30: p80 and p81. The introduction is presented in journal format. If the intended format of the thesis is ‘thesis by publication’ style and this is acceptable by the University, then this section can stand as is.

Response: *The formatting of this thesis was done in consultation with my supervisors, Professors John Pluske and Frank Dunshea. The style of formatting adopted is one that allows for publication in peer-reviewed journals.*

Comment 32: p81. As the lysine content was modified by altering the total protein mix, it would be helpful to acknowledge that this may have altered the level of amino acids in the diet and to comment on whether any of these other amino acids could be limiting.

Response: *I have altered the following sentence in section 5.2.2, Experimental Animals and Diets, from: “The concentrations of dietary lysine were altered by changing the protein content of the diets through adjustments to the amount of wheat, soybean and tallow.”, to the following: “The concentrations of dietary lysine were altered by changing the protein content of the diets through adjustments to the amount of wheat, soybean and tallow and formulated based on an ideal pattern of amino acids.” This discussion negates further comment regarding whether any other amino acid could have been limiting.*

Comment 35: p88. Suggest a comment to be included about why the gilts fed the high lysine diet appeared to grow more slowly than the gilts fed a low lysine diet. Was this difference real, and if so, what physiological explanation could there be? Or was it a likely artefact caused by too few animals or an outlying observation?

Response: *I have altered the paragraph in section 5.3.1, Live phase results, from: “A sex by lysine interaction was observed for the period 0 to 14 days for ADG ($P = 0.038$) such that boars fed high lysine diets grew more quickly than boars fed the low lysine diet, whereas gilts fed high-lysine diets grew more slowly than gilts fed low-lysine levels.”, to the following: “A sex by lysine interaction was observed for the period 0 to 14 days for ADG ($P = 0.038$) such that boars fed high lysine diets grew more quickly than boars fed the low lysine diet (1375 and 1278 g/day respectively), whereas gilts fed high-lysine and low-lysine diets grew at a similar rate (1170 and 1210 g/day, respectively).*

This suggests that the difference is a real effect. Furthermore, I have discussed the lysine x sex interactions in the discussion of section 5.4.5, Increasing dietary lysine: “An interesting outcome of this study were the observed sex x lysine interactions for ADG and lean tissue deposition indicating that the current recommendation of 0.56 g available lysine / MJ DE may limit the potential response of the boar in these traits. The present study showed that the rate of lean tissue deposition was not affected by increasing available lysine levels in gilts, however, boars showed increased lean deposition with the high-lysine diet (Fig 3), indicating that boars have a greater propensity for lean tissue deposition than gilt.”

Comment 37: p88. Paragraph 2 states that “over the study duration dietary RAC increased ADG” whereas Table 5.2 shows that ADG for the low lysine boars given 20 mg/kg RAC (1291 g/day) was less than that observed for the control boars (1330g/day).

Response: *Paragraph 2 implies that I am discussing main effects, however because there is an interaction between lysine and dietary RAC in the first 0 to 7 days, for ADG and FE, I am prohibited in discussing main effects of dietary RAC. Therefore I have deleted the references to ADG and FE over the first 0 to 7 days. As there are no observed lysine x dietary RAC interactions over the 28 days, I can refer to the main effects of dietary RAC. Table 5.2 presents a further partitioning of the data, with probabilities of the main effects of Sex, RAC and Lysine and their respective*

interactions. The examiner has highlighted a difference between two treatment means presented in Table 5.2. that are contrary to the probability of the main effects of dietary RAC. This is of course possible, however the main effect of dietary RAC in the population of pigs in the study presented was to increase ADG. However for greater clarity I have altered this paragraph to read as follows: “Over the study duration (0 to 28 days) the main effects of dietary RAC improved daily gain (P=0.026, Table 5.2). Additionally, as dosage of dietary RAC increased the ADG increased in a linear (P=0.072) and a quadratic (P=0.041) manner. However, FE was not altered (P=0.555) over the 28-day period. Further analysis of these data indicated there was a linear response for increased FE in the first 21-day period (P=0.015) (data not shown) with increasing levels of dietary RAC.”

Comment 38: p95. Stating that fat deposition was reduced by RAC in a quadratic manner (which implies a reduction leading to a nadir or low point) is misleading, when data show an apparent increase, then a decrease relative to controls. There is the likelihood that no real effect occurred here, and again the experiment might have suffered from a lack of statistical power.

Response: *I agree with the comment, and I am only reporting the results in this section. To address the issue that there may be no real effect of dietary RAC on adipose tissue deposition rate due to lack of statistical power, I have altered the following sentences in the discussion, section 5.4.4 Dietary RAC and adipose tissue, from: “Although fat tissue deposition as determined by DXA did not show a reduction after 14 days of treatment for RAC-fed pigs, fat tissue tended to be reduced (P = 0.074, quadratic) in the 20 mg/kg RAC-fed pigs after 28 days.”, to the following: “Although fat tissue deposition as determined by DXA did not show a reduction after 14 days of treatment for RAC-fed pigs, fat tissue tended to be reduced (P = 0.074, quadratic) in the 20 mg/kg RAC-fed pigs after 28 days compared to the control pigs. Although the fat tissue deposition rate of the 20 mg/kg RAC treatment group supports the corresponding carcass data, the DXA data is reporting the results of only 3 pigs per treatment and hence interpretation of the result needs to be treated with some caution.”*

Comment 39: p96. The following statement needs to be qualified “the study confirmed that increasing RAC dose improves FE and ADG in a linear manner” e.g. focusing on the high lysine groups, ADG does not appear to be linear with RAC during any of the 3 periods for either boars or gilts.

Response: *I have qualified this statement by referring specifically to the main effects of dietary RAC on FE and ADG after 21 and 28 day respectively, and as such I have altered the following sentence in section 5.4.1, Dietary RAC and ADG, FE and ADFI, from: “The study presented confirmed earlier observations that increasing RAC dose improves FE and ADG in pigs in a linear manner”, to the following: “This study reported that the main effects of dietary RAC improved FE and ADG and that increasing RAC dose improved FE and ADG in pigs in a linear manner after 21 and 28 days respectively, confirming the observations of earlier studies.”*

Comment 42: p99. Having a greater sensitivity to β -adrenoceptor agonists, or a different density of β -adrenoceptors is not a plausible explanation for why down regulation would be delayed.

Response: *Yes, I would concur. Firstly, this sentence does not make sense and I have changed the sentence in section 5.4.4, Dietary RAC and adipose tissue, from: “Dunshea et al. (2009) proposed several plausible reasons for this delay in down*

regulation.”, to read as follows: “Dunshea et al. (2009) proposed several plausible reasons for the observed reduction in the rate of fat deposition in the presence of dietary RAC.”

Comment 42 ctd: p99. The discussion about the different responsiveness of adipose tissue in the pigs over time to dietary RAC is focussed entirely on receptor numbers, which are not measured here. Although this is an important aspect, it is disappointing that other concepts such as potency, intrinsic activity and efficacy, and the factors that influence these, are not mentioned.

Response: *I would agree there is a strong focus on receptor numbers in the discussion, and the reason for this was because the study had been designed to measure the β -adrenoceptor gene expression in fat and muscle tissue (Chapter 8). I would also argue that I did discuss potency as the following sentence in the same paragraph indicates: “Second, the use of lower doses of RAC than used in the 1990s (5 or 10 mg/kg versus 20 mg/kg) may delay the down regulation of β -adrenoceptors, allowing the expression of a reduction in fat deposition.”*

Moreover, and as this thesis is presented in the format of “thesis by publication” and this chapter has now been published in Animal Production Science, I would propose that the argument suggested by the examiner not be included as it is out of the scope of this chapter. I have also addressed other factors such as age and gender throughout the thesis. I would point out that sex x RAC interactions were not observed in this chapter, and therefore I see no reason to put forward this as a possible factor.

Comment 43: p101. The conclusion is inaccurate (see comment 39 about ADG)

Response: *I have qualified this conclusion in comment 39 above, and I have therefore not altered this conclusion.*

Comment 45: p113. The statements concerning the critical level of lysine in gilts fed 5 and 10 mg RAC are confusing and appear contradictory. If the critical lysine level for 5 mg RAC was 0.51, and the level for 10 mg RAC was 0.50, then 10 mg did not increase the critical lysine level.

Response: *I have altered the following sentence from: “The 10 mg/kg RAC diet resulted in higher critical lysine levels compared to 5 mg/kg RAC diet of 0.50 and 0.52g available lysine/MJ DE for ADG and FCR respectively.”, to the following: “The 10 mg/kg RAC diet resulted in similar critical lysine levels when compared to 5 mg/kg RAC diet of 0.50 and 0.52g available lysine/MJ DE for ADG and FCR respectively.”*

Comment 45 ctd: Similarly, the statement concerning boars that “higher critical lysine levels of 0.56 versus 0.65 versus 0.65 and 0.54 versus 0.59 appears to be nonsense.

Response: *I appreciate the examiner’s confusion, however I found this section difficult to write clearly and succinctly.. I have therefore rewritten the following paragraph: “The boars offered the higher level RAC diet of 10 mg/kg had improved response plateaus for ADG and FCR compared to boars fed the 5 mg/kg RAC diet, however, the improvements required higher critical lysine levels of 0.56 versus 0.65 and 0.54 versus 0.59 g avail Lysine/MJ DE for ADG and FCR respectively.”, to the following: “The boars offered the higher level RAC diet of 10 mg/kg had improved response plateaus for ADG and FCR compared to boars fed the 5 mg/kg RAC diet, however the improvements required higher critical lysine levels. For example, in order to support the elevated responses observed when dietary RAC increased from 5 to 10 mg/kg, the critical lysine*

levels needed to increase from 0.56 to 0.65 g available lysine with respect to ADG and from 0.54 to 0.59 g available lysine with respect to FCR.”

Comment 46: p114. The figure legend is difficult to read. Formatting issue with graphs in this chapter.

Response: *Legends have been reformatted.*

Comment 47: p114. For this figure and several more the Y axis does not start at zero. This has the effect of exaggerating any difference between the treatment groups. While it can make a genuine difference more visible, it can also mislead the reader into believing there was a real difference when in fact the data just show random variation. I recommend reformatting all figures using a range commencing at 0.

Response: *In this instance I do not agree with the examiner’s comments. The range of the Y-axis has been selected to make a genuine difference visible. I would refer the examiner to King et al. (2000), Mullan et al. (2011), and Dunshea et al. (2009) in which similar graphs are presented with the Y-axis not starting at zero. In all cases the range of the Y-axis has been selected to make genuine differences more visible.*

Comment 48: p116. The results for 5 mg RAC at 0.64 g lysine do not seem to fit a logical pattern in boars. This suggests the presence of random variation caused by the undue influence of one or more outlying observations, again calling into question the power of the experimental design, which deserves some comment in the discussion.

Response: *I have included the following comment in the discussion section 6.4.3, Supplementation with dietary RAC: “The lack of response observed for boars fed 5 mg/kg RAC diet at 0.64 and 0.72 g available lysine were not as expected. A more logical response pattern to dietary RAC would have shown a continuance of improvement at these higher dietary lysine levels. These results can most likely be explained due to the random variation exhibited by the small number of 9 replicates per treatment group”*

Comment 49: p123. This page summarises the results nicely, but presents no critical analysis or any reference to other findings.

Response: *I have left this page as presented to the examiner. The introduction to the discussion was intended to briefly summarise the results of the study. I then critically addressed each of the major discussion points: increasing lysine, sex differences and supplementation with dietary RAC using references where appropriate. This chapter has been recently published in the journal Animal Production Science.*

Comment 50: p124. The Rikard-Bell citation should refer to an earlier chapter of the thesis rather than a paper “submitted for publication”

Response: *The citation has been deleted and replaced with “In Chapter 5 it was found that ...”*

Comment 51: p125. I disagree with the statement that RAC supplementation increased ADG linearly in gilts. The statement that “Pigs fed the higher lysine diets only responded to the higher RAC diets” is also misleading.

It should be pointed out that the ADG response of gilts to RAC was equally good at 5 and 10 mg/kg up to 0.56 g lysine (hence this is not a linear response), and that the response at 0.56g lysine was in fact equal to the response seen at the highest level of 0.72 g lysine.

Response: *The statement does not refer to gilts per se, but to both sexes and is drawn from Table 6.2 which indicates that as dietary RAC levels increase then ADG increases in gilts and boars; in this case, the linear relationship is supported by the p-value of 0.001. What is interesting in the analysis in Table 6.2 is the effect of increasing lysine on ADG, which shows not only a strong linear relationship ($P < 0.001$) indicating that as dietary lysine increases ADG increases, but also a quadratic relationship ($P = 0.001$). The presence of the quadratic relationship suggests that as dietary lysine increases the incremental increases in ADG become smaller (in gilts), whilst the linear relationship is probably more influenced by the boar responses.*

To incorporate the suggestions of the examiner and for better clarity I have changed the first paragraph in section 6.4.3 from: “Supplementation of diets with increasing levels of RAC increased ADG and FCR linearly ($P = 0.001$ and $P = 0.002$, respectively) and did not affect VFI ($P = 0.333$), which is in agreement with previous studies conducted at the same research institute (Rikard-Bell et al., 2007; Rikard-Bell et al., 2009d). Pigs fed the high lysine diets of 0.64 and 0.72 g available lysine/MJ DE only responded to the higher RAC diets, which is in agreement with the study of Webster et al. (2002)...”, to the following: “Supplementation of diets with increasing levels of RAC increased ADG and FCR linearly ($P = 0.001$ and $P = 0.002$, respectively) and did not affect VFI ($P = 0.333$) of the pigs in this experiment, which is in agreement with previous studies conducted at the same research institute (Rikard-Bell et al., 2007; Rikard-Bell et al., 2009d). However, the ADG response of gilts to RAC was similar at 5 and 10 mg/kg for the lower lysine diets of 0.40, 0.48 and 0.56g available lysine / MJ of DE, and therefore not suggestive of a linear response to dietary RAC. The lack of response observed for boars fed 5 mg/kg RAC diet at 0.64 and 0.72 g available lysine were not expected. A more logical response pattern to dietary RAC would have shown a continuance of improvement at these higher dietary lysine levels. These results are most likely be explained due to the random variation exhibited by the small number of replicates ($n = 9$) per treatment group. However, it is intriguing that gilts also exhibited a similar response when fed the 5 mg/kg diet which may highlight a dietary issue, although RAC and lysine levels were within specification for these diets. Pigs fed the high lysine diets of 0.64 and 0.72 g available lysine/MJ DE only responded to the higher RAC diets, which is in agreement with the study of Webster et al. (2002)”

Comment 52: p126. The notion that a higher RAC dose “stimulated a greater availability of β -adrenoceptors” is a radical one, even if there are data to support this. Although glucocorticoids are known to cause upregulation of their own receptors, as far as I am aware this is contrary to the observed effects of β -adrenoceptor agonists in every other system or species and should be acknowledged as such.

Response: *I have inserted the following sentence after the proposal of the notion: “This notion, however, is speculative and is contrary to the observed effects of β -adrenoceptor agonists in swine (Spurlock et al., 1994).”*

Comment 54: p126. The statement that “ β 1-AR may play an agonist or an antagonist role” is confusing as the terms agonist and antagonist refer to the properties of a drug not a receptor.

Response: *This was an oversight, and the sentence has been corrected to read: “Therefore, depending on dietary RAC concentration, RAC may play the role of an agonist or antagonist”.*

Comment 54 ctd: More likely explanations for the larger response caused by the 10 mg/kg RAC is that the higher dose caused a stronger stimulus by occupying more receptors, compensating for any desensitizing effect of RAC; showed less β_1/β_2 sub-type specificity and stimulated both types of receptors; or that the pigs were less able to metabolize or excrete the higher dose.

Response: *I have included these more likely suggestions in section 6.4.3, Supplementation with dietary RAC.*

Comment 57: p138. Specify means separation test.

Response: *See response to comment 25.*

Comment 58: p140, 145. Suggest modifying the stated objective. The experimental design allowed the author to test if the response to pST plus RAC was greater than the response to RAC alone. However, by excluding a 'pST alone' group the design did not allow him to determine if the response to pST and RAC was additive. In theory the response to the combined treatment could have been all due to pST.

Response: *The experiment was able to measure the additive effect of pST+RAC because the comparison was made to a RAC treatment and a control treatment. I do agree that without the 'pST alone' treatment I was not able to account for the pST effect without RAC, which I have discussed as a limitation in section 7.4.*

The application of pST was in the final 2 weeks of the treatment regimens, and I am therefore able to observe the dietary RAC response in the initial 2 weeks, followed by dietary RAC 'with pST' or 'without pST' for the final two weeks. To that extent I am able to comment on the additive effects of pST to a RAC regimen. I therefore have not modified the stated objective.

Comment 59: p142. The statement that pST is dose responsive is curious. In theory, all drugs that act through specific receptors should demonstrate a dose-response relationship, obeying the principles of binding kinetics.

Response: *I wanted to make the statement that studies have been conducted to show the dose response nature of pST, and that the low dose used in the experiment may have contributed to the small responses observed. In order to improve clarity I have added the following sentence to begin the paragraph: "Beermann et al. (1990) reported that pST increases skeletal muscle mass by muscle fibre hypertrophy and reduces muscle lipid concentration in a dose dependent manner. In general, my results have smaller responses to pST with respects to lean deposition rates when compared to the literature."*

Comment 60: p143. The term β -cell receptors is used several times instead of β -adrenoceptors.

Response: *All amendments have been made.*

Comment 61: p144. The discussion of possible interactions between pST and RAC is a little superficial and missing several key references such as Etherton and Walton, 1986; Watt, 1991 and Sillence, 2002.

Response: *I have included in the discussion section 7.4, Interactions between pST and RAC, and cited the key references. I have changed the original sentences from: "These results are typical of those cited in the literature (Campbell et al., 1991; Campbell et al., 1989; King et al., 2000). Likewise the addition of pST to RAC-treated*

pigs markedly reduced delta P2 back fat readings. This study also observed that in the final 14 days, dietary RAC also reduced the expected increase in P2 in boars ($P < 0.05$).”, to the following: “These results are typical of those cited in the literature (Campbell et al., 1991; Campbell et al., 1989; King et al., 2000). Likewise the addition of pST to RAC-treated pigs markedly reduced delta P2 back fat readings. It is possible that in the study presented the lipolytic effects of RAC are enhanced by the co-administration of pST. This is consistent with the observation that growth hormone has no direct lipolytic action in porcine adipose tissue, but increases the tissue’s catecholamine sensitivity (Etherton and Walton, 1986). Sillence et al. (2002) confirmed that growth hormone can attenuate the down regulation of β -adrenoceptors, seen in pigs treated with the β 2-adrenoceptor agonist clenbuterol.”

Comment 62: p147. Part 2 of this chapter presents a separate experiment with different aims to the experiment described in part 1. It is not clear why this is not presented as a separate chapter.

Response: *Part 1 and Part 2 of Chapter 7 refer to the two combination studies. In consultation with Professor John Pluske I decided to report the studies separately but under one chapter, as both technologies are metabolic modifiers and are used in combination with dietary RAC in the Australian pig industry.*

Comment 64: p152. All previous chapters have reported FCE values, whereas G:F values are reported here. This is doubtless due the requirements of the Journal of Animal Science, but altering the terminology here hinders comparisons with earlier data and disrupts the cohesiveness of the thesis.

Response: *I do agree. However, I would respectively request that the paper(s) remains as presented. My argument is that I requested to my supervisors Professor John Pluske and Professor Frank Dunshea that I would like to submit this thesis in the format of “Thesis by publication” with the intention of publishing in several journals. Unfortunately in doing so one has to follow the requirements of the specific journal. I believe this is an excellent way to present a thesis because the student becomes skilled in the practice of scientific writing whilst preparing the thesis. I would like to point out that the Journal of Animal Science requires reporting of G:F (Chapter 7), whereas Animal Production Science is satisfied with FE (Chapter 5); both are the same ratio. In all other chapters I have reported FCR.*

Comment 65: p164. β -adrenoceptors feature heavily throughout the thesis, yet the abbreviation β -AR is not introduced until chapter 8. For consistency and cohesiveness in presentation this needs to be amended.

Response: *I have introduced the abbreviation when the term β -adrenoceptor is first used in each chapter.*

Comment 67: p164. There are a few illogical statements. “ligand binding studies provide little evidence for functional receptors of the β 3 sub-type”

- a. Ligand binding studies are not intended to identify functional receptors by their nature – only the presence of specific binding.
- b. “Gunawan et al were not able to detect β 3-AR in muscle and concluded that β 3 were expressed at undetectable levels” If they were not detected, what evidence is there that they were expressed at all?

Response: *part a: I have deleted the word “functional”; part b: This was a direct quote from the paper by Gunawan et al. However, I have altered the sentence to read as follows: “Recently, Gunawan et al. (2007) did not detect β 3-AR in porcine skeletal muscle using real-time PCR and the primer was specifically designed to porcine sequences.” I wanted to cite the result obtained by Gunawan et al. because I was not able to observe β 3-AR gene expression in adipose or muscle tissue using PCR techniques.*

Comment 69: Chapter 9. No experiment is perfect and the chapter would be improved by acknowledgement or insight into the weaknesses inherent in the research in terms of experimental design, housing conditions, availability of pigs etc as well as some recommendations on how the methodology could be improved for future studies.

9.1 Response: *I have added the following to Chapter 9: “Weaknesses of this thesis*

The weaknesses of this thesis in my opinion were as follows:

- I. The dose response studies were conducted as separate studies for boars and gilts. This reduced the power of the study, as I was not able to compare interactions between sex and dosage rate. However, in later studies I was able to include sex as a factor and determine sex x RAC dose interactions.*
- II. The current gene expression data set and the corresponding DXA scan information only allows me to comment on effects at day 15, the first data point post-treatment. The greatest response to dietary RAC is within the first week. Animal numbers, and limitations of space in the finisher test station, restricted the number of data points. In particular DXA scans and tissue biopsies at Day 7 to coincide with live animal measurements would have been useful as there were RAC x lysine interactions for day 0-7 data.*
- III. The combination study involving porcine somatotropin and dietary RAC ideally should have included an additional treatment of pST without dietary RAC. However, availability of animals and space within the testing station influenced the experimental design. It has also been acknowledged, for the traits measured, that statistical significance ($p < 0.05$) for differences in treatment means may not have occurred due to a low number of replicates per treatment.*
- IV. The combination study involving boars immunized against GnRF did not have an acclimatisation period prior to commencement of treatment. The taking of measurements, allocation to treatment, and mixing of pigs on Day 0 may have affected responses to treatment. Additionally and due to pen numbers, I was restricted on treatment number, and therefore a straight 5 mg/kg RAC treatment was not included.*
- V. The incorporation of DXA scan measurements for the lysine titration study would have enabled measuring grams of available lysine/gram of tissue accretion in boars and gilts with or without dietary RAC. However, the logistics of transporting and handling carcasses and the related costs prevented incorporation of DXA scanning into this experiment.*

Specific Comments

Examiner: Professor Gary Allee

I would like to thank Professor Gary Allee for his comments regarding this thesis.

Comment: *The information in this dissertation would be more valuable to the Australian pig industry if there were an economic appraisal of dose and duration of RAC feeding and lysine levels*

Response: *Whilst I would agree with this comment it is out of the scope of this dissertation.*

Comment: How does dietary lysine level with RAC change in group housed pigs?

Response: *Interestingly the lysine titration study reported in this dissertation (Chapter 6) was extended to a group housed environment. Mullan et al (2011) reported that ADG in finisher gilts began to plateau at 0.56 g available lysine/MJ of DE, which was similar to the controls in chapter 6. However with the addition of dietary RAC the finisher gilts continued to respond with increasing dietary lysine levels up to 0.72 g available lysine. Mullan et al concluded that higher levels than 0.56g available lysine / MJ of DE are required to maximize performance in finisher gilts supplemented with dietary RAC. Similar to the individual pen study Mullan et al (2011) also reported responses to dietary RAC at low levels of dietary lysine (0.48 g available lysine / MJ of DE)*

SUMMARY

The series of experiments presented in this dissertation were conducted to evaluate the optimal responses to dietary RAC in the Australian pig industry. The unifying hypothesis was proposed in two parts: first, optimal responses to dietary ractopamine (RAC) depends on factors that include the level of dietary lysine, level of dietary RAC and gender, whilst the inclusion of specific metabolic modifiers porcine somatotropin (pST) and anti-GnRF immunization vaccine (Improvac) has synergistic effects on growth and performance. Second, the β -adrenoceptors (β) in adipose and skeletal muscle respond differently to RAC dose and therefore the down regulation of specific β s mediates the responses observed in fat and muscle tissue deposition rates.

Experiment 1 (Chapter 3) was conducted to compare current commercial applications of dietary RAC and dietary RAC+pST and their responses in growth performance as well as lean and fat tissue deposition at day 0, 14 and 28 in boars, gilts and boars immunized against GnRF. The study was also designed to confirm whether RAC decreases fat deposition in boars and boars immunized against GnRF. The results of Experiment 1 were surprising in the fact dietary RAC and RAC+pST had no effect on average daily gain (ADG) ($P=0.543$) or feed conversion ratio (FCR) ($P=0.255$) on these fast-growing, high-health-status finishing pigs. The effect of dietary RAC on tissue deposition rates were also unexpected as a reduction in fat deposition rate occurred ($P=0.064$) but no effect on lean deposition rate was observed ($P=0.642$). The results of experiment 1 influenced the design of the experiments that followed in which factors that affect responses in production and tissue deposition responses to dietary RAC were examined.

Experiment 2 (Chapter 4) comprised of two RAC dose studies to determine the response in light, medium and heavy initial-weight gilts (study 1) or boars (study 2) fed four levels of dietary RAC (0, 5, 10, and 20mg/kg) over a 28-day feeding regime. The hypothesis examined was that light, medium and heavy initial-weight pigs have similar responses to increasing levels of dietary RAC. The major findings were:

In gilts for all initial weight categories:

- Dietary RAC improved ADG, FCR compared to controls (P=0.023 and P=0.029, respectively).
- A linear relationship was observed for ADG (P=0.006) and FCR (P=0.003) with increasing dose of RAC
- Carcass weights improved linearly (P linear =0.006) and tended to improve dressing percentage (P=0.098) with increasing dose of dietary RAC, and

In boars for all initial weight categories,

- Incremental increases of RAC resulted in linear increases for ADG (P=0.003), HSCW (P=0.018) and dressing percentage (P=0.045).
- Dietary RAC did not alter FCR (P=0.289), however there was a tendency (P=0.082) for FCR to decrease linearly as dosage level of dietary RAC increased.

Experiment 3 (Chapter 5) was conducted to investigate whether there are interactions between RAC dosage levels and two levels of dietary lysine (low and high, 0.56 and 0.65 g of available lysine / MJ DE, respectively) on growth performance and carcass characteristics of finisher boars and gilts using dual energy X-ray absorptiometry (DXA) to measure body composition. The hypothesis examined was that low lysine diets of 0.56 g available lysine/MJ DE are sufficient to optimize the response in feed efficiency, growth rate and tissue deposition in boars and gilts fed high

(20 mg/kg) or low (5 mg/kg) levels of dietary RAC at initial weights of 65 kg. The major findings were:

- There were significant interactions ($P = 0.023$ and $P = 0.025$) between dietary RAC and lysine levels in the first seven days for ADG and feed efficiency (FE) respectively, such that pigs fed high-lysine diets supplemented with dietary RAC had improved ADG and FE, whereas pigs fed low-lysine diets did not respond to RAC supplementation in the first seven days.
- Over the study duration dietary RAC improved daily gain ($P=0.026$).
- As RAC dose increased ADG increased in a linear ($P = 0.072$) and a quadratic ($P = 0.041$) manner.
- In the first seven days dietary RAC improved FE ($P = 0.002$), but not over the study duration ($P = 0.555$).
- Dietary RAC reduced change in P2 backfat ($P=0.002$) as well as indicating a linear ($P = 0.033$) and quadratic ($P = 0.003$) reduction with increasing dose in both boars and gilts over the duration of the study.
- A lysine x sex interaction ($P=0.043$) indicated that lean deposition rate increased in boars but not gilts when fed the high lysine diet.
- Dietary RAC tended ($P < 0.1$) to increase lean deposition rate only in boars fed high lysine diets.
- Dietary RAC tended to alter lean tissue deposition rates over the initial 14-day period ($P = 0.067$) and as RAC dose increased lean tissue deposition increased over 14 ($P = 0.035$) and 28 days ($P = 0.044$).
- Fat deposition tended to be reduced in a quadratic manner ($P = 0.074$) as dietary RAC increased over 28 days.

Experiment 4 (Chapter 6) was conducted to investigate the responses of finisher pigs offered a wider range of dietary lysine levels (0.40, 0.48, 0.56, 0.64 and 0.72 g available lysine/MJ DE) and three levels of dietary RAC (0, 5 and 10 mg/kg) over 28 days duration, to determine the optimal level (critical value) of dietary lysine with or without RAC, after which the response to increasing dietary lysine is insignificant in male and female finisher pigs. The major findings were:

- The critical value of dietary lysine for gilts without RAC supplementation for ADG and FCR was 0.54 and 0.52 g available lysine/MJ DE, respectively.
- The critical value of dietary lysine for gilts offered 5 mg/kg RAC supplementation for ADG and FCR was 0.51 and 0.49 g available lysine/MJ DE, respectively. Increasing RAC supplementation to 10 mg/kg increased the critical values to 0.51 and 0.52 g available lysine/MJ DE for ADG and FCR respectively
- A response plateau was not calculated for control boars because the data set did not display diminishing responses for ADG or FCR over the range of dietary lysine concentrations offered.
- Boars offered the 10 mg/kg RAC diet had improved response plateaus for ADG and FCR compared to boars fed the 5 mg/kg RAC diet, however, the improvements required higher critical lysine levels of 0.65 versus 0.56 and 0.59 versus 0.54 g available Lysine/MJ DE for ADG and FCR respectively.

Experiment 5 (Chapter 7) consisted of two combination studies. The first study examined the combination of dietary RAC and porcine somatotropin (pST) and the second study examined the effect of anti-GnRF immunisation vaccine (Improvac) and dietary RAC. The hypothesis tested for the first study was that the combination of a 28-day dietary RAC regimen with the addition of daily injections of pST in the final 14

days will have additive effects on pig performance when compared to a 28 day dietary RAC regimen. The major findings were:

- Over the study duration FCR was reduced by the RAC + pST treatment ($P < 0.05$) and tended ($P < 0.09$) to be reduced by the RAC only treatment.
- The ADG of the RAC and the RAC+pST treated gilts increased ($P < 0.05$) compared to the control gilts, whereas the ADG of the treated boars did not differ from control boars (Treatment x sex interaction, $P = 0.025$)
- In the second half of the study both dietary RAC and the combination treatments increased ($P < 0.001$) lean tissue deposition in gilts by 165 and 286 g/day respectively.
- The RAC + pST treatment increased lean tissue deposition in boars by 202 g/day when compared to respective controls.
- The RAC + pST treatment also reduced ($P < 0.001$) fat tissue deposition by 87 g/day (gilts) and 118 g/day (boars) when compared to controls in the final 14 days.
- The dietary RAC treatment did not alter fat deposition significantly in gilts and boars.

The hypotheses to be tested for the second study were that 1) anti-GnRF immunization would increase average daily feed intake (ADFI) around 2 wk after secondary vaccination, and that 2) a simultaneous step-up in dietary RAC concentration would allow the additional energy intake to be deposited as lean tissue rather than fat.

The major findings were:

- Boars immunized against GnRF had greater ADG and ADFI, but a reduced Gain:Feed (G:F) than the entire boars ($P < 0.001$) over the study duration.
- Pigs fed RAC had greater ADG and Gain:Feed (G:F) ($P < 0.001$) and tended to eat less ($P < 0.076$) than the controls.

- The change in ultrasound P2 backfat during the study was greater ($P < 0.001$) in boars immunized against GnRF and tended to be reduced ($P = 0.076$) by RAC in boars immunized against GnRF.
- Percent lean in the half carcass was increased ($P = 0.006$) by dietary RAC, conversely, percent fat in the half carcass was decreased ($P = 0.004$) by dietary RAC.

Experiment 6 (Chapter 8) was conducted to examine the β gene expression using PCR techniques in adipose and skeletal muscle tissues of boars and gilts fed dietary RAC at either a low (5 mg/kg) or high (20 mg/kg) level compared to control pigs fed (0 mg/kg RAC).. The pigs used in this study were from Experiment 3. The hypotheses was that adipose and skeletal muscle tissue respond differently to dose level of RAC with respects to down (or up) regulation (as measured by the abundance of mRNA transcripts) of the specific β s in finisher boars and gilts. The major findings were:

Within adipose tissue:

- The $\beta 1$ -AR gene was not affected by duration of treatment ($P = 0.88$) or sex ($P = 0.54$), however the addition of dietary RAC reduced the expression ($P = 0.04$).
- The $\beta 2$ -AR gene was not affected by dietary RAC ($P = 0.66$), or sex ($P = 0.22$), however day of treatment increased $\beta 2$ -AR expression at day 29 of treatment compared to day 15 ($P = 0.06$).

Within skeletal muscle tissue:

- The $\beta 1$ -AR gene was not affected by sex ($P = 0.43$) or day of the study ($P = 0.69$) but increased expression with the addition of dietary RAC ($P = 0.04$).
- A sex x RAC dose x Day interaction was observed ($P = 0.12$). Only the boars fed the low RAC diet increased $\beta 1$ -AR expression at day 15 whereas gilts fed high RAC at

day 15 and the low RAC at day 29 had increased β 1-AR expression compared to controls.

- A sex effect was observed for β 2-AR expression ($P=0.05$), in that the control gilts had a greater expression of the β 2-AR gene after 29 days than control boars.
- The addition of dietary RAC tended to reduce the expression of the β 2-AR gene ($P=0.07$) particularly at the high inclusion level (20 mg/kg) of dietary RAC.

From the results obtained in this thesis, I conclude that:

1. RAC dose rates are effective in improving production indices and carcass traits in light (65 kg), medium (80 kg) and heavy (95 kg) initial-weight boars or gilts.
2. Dietary lysine levels are critical in the first 7 days of the dietary RAC regimen.
3. The lysine requirements for boars are higher than gilts between 65 and 95 kg live weight in order to maximise growth and lean tissue deposition.
4. The combination of pST in the final 14 days of a 28-day RAC feeding regime was synergistic in gilts for FCR, ADG and lean deposition, whereas boars further declined their P2 backfat levels with the addition of pST.
5. The additional growth in boars immunized against GnRF was as fat tissue, and that this could be attenuated by supplemental dietary RAC.
6. The β 2-AR has a more critical role as the age of the finisher pig advances as expression of the gene increases over time whereas the β 1-AR expression is constant.
7. The β 2-AR was less sensitive to dietary RAC in fat tissue than the β 1-AR gene which down regulated independent of RAC dose at day 15 and may explain the lipolytic response observed in Experiment 3, Chapter 5.

8. The down regulation of β 2-AR expression observed in muscle by pigs treated with RAC suggested that the β 2-AR may mediate the decline in response observed for ADG (8.6% to 3.7%) and FE (7.1% to 5.8%) in Chapter 5.
9. Low level of dietary RAC has a stimulatory effect on the expression of the β 1-AR gene. The up regulation of β 1-AR may explain why only small differences between responses in lean meat deposition rates occurred for pigs fed either a high (20 mg/kg) or low (5 mg/kg) RAC diet.

TABLE OF CONTENTS

DECLARATION	II
SUMMARY OF AMENDMENTS.....	III
SUMMARY	XXIII
TABLE OF CONTENTS.....	XXXI
LIST OF TABLES.....	XXXIV
LIST OF FIGURES	XXXVI
ACKNOWLEDGEMENTS	XXXVII
PUBLICATIONS AND CONFERENCE PAPERS	XXXVIII
ABBREVIATIONS USED IN THIS THESIS	XL
CHAPTER 1	1
<i>General Introduction</i>	1
CHAPTER 2	5
<i>A Review of the Literature</i>	5
2.1 A brief history of phenethanolamine repartitioning agents.....	6
2.2 Chemistry of ractopamine hydrochloride, a β -adrenoceptor agonist ligand.....	8
2.3 β -adrenoceptors (β -AR)	9
2.4 Metabolic effects of β -AR agonists	14
2.5 Factors affecting a response to ractopamine in finisher pigs	23
2.6 Methods to overcome down regulation.....	30
2.7 Combing dietary RAC regimens with other metabolic modifiers.	31
2.8 Conclusions.....	32
CHAPTER 3	36
<i>A comparison of the current applications of dietary ractopamine hydrochloride (Paylean®) in the Australian pig industry</i>	36
3.1 Introduction.....	37
3.2 Materials and Methods.....	38
3.3 Results.....	44
3.4 Discussion.....	51
3.5 Conclusion	55
CHAPTER 4	57
<i>Increasing Ractopamine Levels In Finisher Pig Diets Improves Growth Performances In Light, Medium And Heavy pigs</i>	57
4.1 Introduction.....	58
4.2 Materials and Methods.....	59

4.3	Results.....	63
4.4	Discussion.....	72
4.5	Conclusion.....	78
CHAPTER 5.....		79
<i>Current recommended levels of dietary lysine in finisher pig diets are sufficient to maximize the response to ractopamine over 28 days but are insufficient in the first 7 days.</i>		79
5.1	Introduction.....	80
5.2	Materials and Methods.....	81
5.3	Results.....	85
5.4	Discussion.....	96
5.5	Conclusion.....	101
CHAPTER 6.....		102
<i>Dietary ractopamine promotes growth, feed efficiency and carcass responses over a wide range of available lysine levels in finisher boars and gilts.</i>		102
6.1	Introduction.....	103
6.2	Materials and Methods.....	104
6.3	Results.....	109
6.4	Discussion.....	120
6.5	Conclusion.....	125
CHAPTER 7.....		127
<i>Combination Studies:</i>		127
<i>Part 1: Combining a Ractopamine Feeding Regime and Porcine Somatotropin Has Additive Effects on Finisher Pig Performance</i>		128
7.1	Introduction.....	128
7.2	Material and Methods.....	129
7.3	Results.....	132
7.4	Discussion.....	137
7.5	Conclusion.....	143
<i>Part 2: Ractopamine hydrochloride improves growth performance and carcass composition in immunocastrated boars, intact boars, and gilts.</i>		144
7.6	Introduction.....	144
7.7	Materials and Methods.....	145
7.8	Results.....	148
7.9	Discussion.....	155
7.10	Conclusion.....	159
CHAPTER 8.....		160

<i>Ractopamine hydrochloride affects β-1 and β-2 adrenoceptor gene expression in fat and muscle tissue of finisher boars and gilts.</i>	160
8.1 Introduction.....	161
8.2 Materials and Methods.....	163
8.3 Results.....	174
8.4 Discussion.....	179
8.5 Conclusion	184
CHAPTER 9	185
<i>The General Discussion.</i>	185
9.2 Introduction.....	186
9.3 The strengths of the thesis.....	186
9.4 Weaknesses of this thesis	190
9.5 Further research	191
CHAPTER 10	193
<i>Bibliography.</i>	193
APPENDIX I: Additional Information for Materials and Methods	205

LIST OF TABLES

1. Table 3.1	Composition of control diet (as fed, %)	43
2. Table 3.2	Effects of sex type, dietary RAC (Paylean) and dietary RAC and pST regimen on daily gain (g/d), feed intake (g/d) and feed conversion ratio (g/g).	47
3. Table 3.3	Effects of sex type and dietary RAC (Paylean) regime on growth performance over the first 2 weeks ¹	48
4. Table 3.4	Effects of sex type, dietary RAC (Paylean) and dietary RAC and pST regimen on tissue deposition and carcass characteristics	49
5. Table 4.1	Composition of control diet ^A (as fed, %)	62
6. Table 4.2	Effects of initial weight category and dietary RAC after 28 days of treatment on average daily gain (ADG), average daily feed intake (ADFI), feed conversion ratio and carcass traits in finisher gilts.	65
7. Table 4.3	Effects of initial weight category and dietary RAC after 28 days of treatment on average daily gain (ADG), average daily feed intake (ADFI), feed conversion ratio and carcass traits in finisher boars.	69
8. Table 4.4	Effects of start weight and dietary RAC dose on plasma urea nitrogen (PUN) levels in separate studies for finisher gilts (Experiment 1) and finisher boars (Experiment 2).	70
9. Table 5.1	Composition of low and high lysine content control diets (as fed, %).	83
10. Table 5.2	The effect of sex, dietary RAC and dietary lysine on average daily gain (ADG), feed efficiency (FE), average daily feed intake (ADFI), for the periods 0 to 7, 0 to 14 and 0 to 28 days.	87
11. Table 5.3	The effect of sex ^A , dietary RAC and dietary lysine on tissue composition ^B and P2 backfat ^C over the time periods of 0 to 14 and 0 to 28 days in finisher pigs.	91
12. Table 5.4	The effect of sex, dietary RAC and dietary lysine on Carcass weight and Carcass P2 backfat in finisher pigs.	94
13. Table 6.1	Composition of Control A to E and Treatment diets F to O (as fed, %).	106
14. Table 6.2	Effect of dietary lysine level and RAC dose on average daily gain (ADG), voluntary feed intake (VFI), and feed conversion ratio in finisher pigs after 21 days of treatment.	110
15. Table 6.3	Effect of dietary lysine level and RAC dose on carcass weight ^A and carcass P2 backfat in finisher pigs after 28 days of treatment.	116
16. Table 6.4	Effect of dietary lysine level and ractopamine (RAC) dose on Plasma Urea Nitrogen (PUN) levels in finisher gilts and boars.	119
17. Table 7.1	Composition of control diet (as fed).	131
18. Table 7.2	The effects of sex and dietary RAC for the first 14 ¹ days without pST and over the last 14 days with daily pST (RAC + pST) treatment on growth performance and carcass characteristics.	134
19. Table 7.3	The effects of sex ¹ and dietary RAC for 28 days without pST and with daily pST (RAC+pST) over the last 14 days of treatment on tissue composition in finisher pigs.	135
20. Table 7.4	Composition of control diet (as fed) ¹	147
21. Table 7.5	The effect of sex type (S) and Ractopamine (RAC) ¹ on growth performance of finisher pigs.	152
22. Table 7.6	The effect of sex type (S) and Ractopamine (RAC) ¹ on half carcass characteristics measured using dual x-ray absorptiometry.	153

23. Table 7.7 The effect of sex type (S) and Ractopamine (RAC)¹ on half carcass characteristics measured using dual x-ray absorptiometry.154

24. Table 8.1 Primer sequences used to measure gene expression with quantitative PCR167

25. Table 8.2 The effect of sex¹, dietary RAC and day on β -AR expression² in adipose and skeletal muscle tissue³ for gilts and boars.....177

LIST OF FIGURES

26. Fig 2.1 Ractopamine HCL.....	9
27. Fig 2.2 Model for the transmembrane topology of the β -adrenoceptor (β -AR).....	10
28. Fig 2.3 A schematic detailing the signaling process which occurs as a result of the binding of a β -adrenoceptor agonist with a β -adrenoceptor (β -AR).....	12
29. Fig 2.4 Weekly increase in body weight (kg) for pigs fed RAC diets of 5 mg/kg and 10 mg/kg over controls (0 mg/kg).	24
30. Fig 3.1 The effect of dietary RAC and sex on fat deposition in the first 2 weeks of the study.	50
31. Fig 3.2 The effect of dietary RAC and sex on P2 back fat after the first 2 weeks of the study.	50
32. Fig 3.3 The effect of dietary RAC, dietary RAC + pST and sex on P2 back fat over the final 2 weeks of the study.	51
33. Fig 4.1 The effects of start weight and RAC dose on average daily gain for finisher gilts after 28 days of treatment.....	66
34. Fig 4.2 The effect of start weight and dose of RAC on feed conversion ratio for finisher gilts after 28 days of treatment.	66
35. Fig 4.3 The effects of start weight and RAC dose on carcass weight (CW) for finisher gilts.	67
36. Fig 4.4 The effects of start weight and RAC dose on average daily gain for finisher boars after 28 days of treatment.....	71
37. Fig 4.5 The effects of start weight and RAC dose on carcass weight for finisher boars.. ..	71
38. Fig 4.6 The effects of start weight and RAC dose on dressing percentage for finisher boars.	72
39. Fig 5.2 The effect of low (\square 0.56 g) or high (\blacksquare 0.65 g) available dietary lysine and dosage level of RAC on lean tissue deposition after 28 days of the study for finisher boars.	93
40. Fig 5.1 The effect of low (0.56 g) or high (0.65 g) available dietary lysine and sex (\square gilt, \blacksquare boar) on lean tissue deposition after 28 days of the study.....	93
41. Fig 6.1 The effect of RAC dose at 0 (+), 5 (\diamond), or 10 (\blacklozenge) mg/kg and increasing available lysine (g/MJ DE) for average daily gain (ADG) in finisher gilts after 21 days. .	114
42. Fig 6.2 The effect of RAC dose at 0 (+), 5 (\diamond), or 10 (\blacklozenge) mg/kg and available lysine (g/MJ DE) for feed conversion ratio (FCR) in finisher gilts after 21 days.....	114
43. Fig 6.3 The effect of RAC dose at 0 (+), 5 (\square), or 10 (\blacksquare) mg/kg and available lysine (g/MJ DE) for average daily gain (ADG) in finisher boars after 21 days.....	115
44. Fig 6.4 The effect of RAC dose at 0 (+), 5 (\square), or 10 (\blacksquare) mg/kg and available lysine (g/MJ DE) for feed conversion ratio (FCR) in finisher boars after 21 days.	115
45. Fig 7.1 Effect of dietary RAC for 28 days without or with daily pST treatment for the final 14 days on Average Daily Gain (ADG) in gilts (\square) and boars (\blacksquare) in the final 14 to 28 day period.	136
46. Fig 7.2 Effect of dietary RAC for 28 days without or with daily pST treatment for the final 14 days on Average Daily Feed Intake (ADFI) in gilts (\square) and boars (\blacksquare) in the final 14 to 28 day period.	136
47. Fig 7.3 Effect of dietary RAC for 28 days without or with daily pST treatment for the final 14 days on Delta P2 Back Fat (Δ P2 Back Fat) in gilts (\square) and boars (\blacksquare) in the final 14 to 28 day period.	137
48. Fig 8.1 β 2-adrenoceptor expression (normalized to RPL19) as a percentage of controls (Day -1) in Adipose (\square) and skeletal muscle (\blacksquare) of boars (A) and gilts (B) fed 0 mg/kg RAC at Day -1 and 0, 5 or 20 mg/kg RAC at Day 15 and Day 29.	178

ACKNOWLEDGEMENTS

This dissertation reached fruition due to the patience and guidance of Professor John Pluske and Professor Frank Dunshea who compelled me to broaden my understanding.

I am also grateful to: the Pork CRC and Dr Roger Campbell for the provision of funds for the research presented; the technical officers Wayne Brown, Maree Cox, Doug Kertain, Paul Eason and Matt Borg at the Werribee Research Centre, and Wayne Morley at Rivalea Australia Pty Ltd for their assistance and experience in managing the research studies; Dr Greg Natrass for his expertise in guiding me through the laboratory techniques required for the gene expression work; Kristina Hacket and Fiona Selmes for the monitoring of the studies in the spirit of good clinical practices, a requirement for Elanco Animal Health, Pty Ltd.

Finally to my wife, Jennifer and family, Georgia, Hugo, Caitlin and Angus for their support, I would never have been able to complete this without you.

To them all I give my heartfelt gratitude.

PUBLICATIONS AND CONFERENCE PAPERS

Rikard-Bell, C., Curtis, M. A., van Barneveld, R. J., Mullan, B. P., Edwards, A. C., Gannon, N. J., Henman, D. J., Hughes, P. E. & Dunshea, F. R. (2009). *Ractopamine hydrochloride improves growth performance and carcass composition in immunocastrated boars, intact boars, and gilts*. **Journal of Animal Science** 87(11): 3536-3543.

Rikard-Bell, C. V., Pluske, J. R., Van Barneveld, R. J., Mullan, B. P., Edwards, A. C., Gannon, N. J., Henman, D. J. & Dunshea, F. R. *Current recommended levels of dietary lysine in finisher pig diets are sufficient to maximize the response to ractopamine over 28 days but are insufficient in the first 7 days*. **Animal Production Science** (submitted)

Rikard-Bell, C. V., Pluske, J. R., van Barneveld, R. J., Mullan, B. P., Edwards, A. C., Gannon, N. J., Henman, D. J. & Dunshea, F. R. *Dietary ractopamine promotes growth, feed efficiency and carcass responses over a wide range of available lysine levels in finisher boars and gilts*. **Animal Production Science** (submitted).

Rikard-Bell, C. V., Curtis, M. A., Van Barneveld, R. J., Mullan, B. P., Edwards, A. C., Gannon, N. J., Henman, D. J., Hughes, P. E. & Dunshea, F. R. (2005). *A step-up dietary ractopamine (Paylean) program improves growth performance and carcass traits in all sexes*. In ‘**Manipulating Pig Production X**’ (Ed. JA Paterson) p. 153. (Australasian Pig Science Association: Werribee)

Rikard-Bell, C. V., Csabo, C. s., Van Barneveld, R. J., Mullan, B. P., Edwards, A. C., Gannon, N. J., Henman, D. J. & Dunshea, F. R. (2007). *Increasing ractopamine levels in finisher pig diets improves growth performances in light, medium and heavy gilts*. In ‘**Manipulating Pig Production XI**’ (Ed. JA Paterson) p. 119. (Australasian Pig Science Association: Werribee)

Rikard-Bell, C. V., Pluske, J. R., Van Barneveld, R. J., Mullan, B. P., Edwards, A. C., Gannon, N. J., Henman, D. J. & Dunshea, F. R. (2009). *Increasing ractopamine levels in finisher pig diets improves growth performance in light, medium and heavy boars*. In ‘**Manipulating Pig Production XII**’ (Ed. RJ Van Barneveld) p. 69. (Australasian Pig Science Association: Werribee)

Rikard-Bell, C. V., Pluske, J. R., Van Barneveld, R. J., Mullan, B. P., Edwards, A. C., Gannon, N. J., Henman, D. J. & Dunshea, F. R. (2009). *Combining a ractopamine feeding regime and porcine somatotropin has additive effects on finisher pig performance*. In ‘**Manipulating Pig Production XII**’ (Ed. RJ Van Barneveld) p. 70. (Australasian Pig Science Association: Werribee)

Rikard-Bell, C. V., Pluske, J. R., Van Barneveld, R. J., Mullan, B. P., Edwards, A. C., Gannon, N. J., Henman, D. J. & Dunshea, F. R. (2009). *Responses of finisher boars and gilts to dietary lysine and ractopamine*. In ‘**Manipulating Pig Production XII**’ (Ed. RJ Van Barneveld) p. 71. (Australasian Pig Science Association: Werribee)

Rikard-Bell, C. V., Pluske, J. R., Van Barneveld, R. J., Mullan, B. P., Edwards, A. C., Gannon, N. J., Henman, D. J. & Dunshea, F. R. (2009). *Effects of dietary lysine on growth responses of pigs to increasing doses of ractopamine*. In '**Manipulating Pig Production XII**' (Ed. RJ Van Barneveld) p. 182. (Australasian Pig Science Association: Werribee)

Rikard-Bell, C. V., Natrass, G., Pluske, J. R., van Barneveld, R. J., Mullan, B. P., Edwards, A. C., Gannon, N. J., Henman, D. J. & Dunshea, F. R. (2011). *Ractopamine Effects β -1 and β -2 Adrenergic Receptor Gene Expression in Fat and Muscle Tissue of Boars and Gilts*. In '**Manipulating Pig Production XIII**' (Ed. RJ Van Barneveld) p. 90. (Australasian Pig Science Association: Werribee)

ABBREVIATIONS USED IN THIS THESIS

ADA	adenosine deaminase
ADFI	average daily feed intake
ADG:	average daily gain
ANOVA	analysis of variance
APP	actinobacillus pleuropneumonia
BW	body weight
cAMP	cyclic adenosine monophosphate
cDNA	complementary deoxyribonucleic acid
DE:	digestible energy
DNA	deoxyribonucleic acid
DXA	dual X-ray absorptiometry
EDTA	Ethylenediaminetetraacetic acid
FCE	feed conversion efficiency
FCR:	feed conversion ratio
FE:	feed efficiency
GDP	guanine diphosphate
GLDH	glutamate dehydrogenase
GnRF:	Gonadotropin releasing-factor
GTP	guanine triphosphate
HSCW	hot standard carcass weight
IRC	inter-run calibrator
mRNA	messenger ribonucleic acid
myHC	myosin heavy chain
NADH/NAD	nicotinamide adenine dinucleotide
NEFA	non-esterified fatty acid
OD	optical density
PCR	polymerase chain reaction
PKA	protein kinase A
pST:	porcine somatotropin
PUN	plasma urea nitrogen
RAC:	ractopamine HCl (Paylean , Elanco Animal Health)
REML	restricted maximum likelihood
TE	TE derived from components: <u>T</u> ris, a pH buffer, and <u>E</u> DTA
THEO	theophylline
VFI	voluntary feed intake
βAA	beta-adrenoceptor agonist
β	beta-adrenoceptor