

Effects of immunocastration on the nutrient responses, carcass traits and meat quality of growing pigs (*Sus scrofa domesticus*)

By

Tersia Needham

*Thesis presented in fulfilment of the requirements for the degree of
Master of Science in Animal Science in the Faculty of AgriScience in
at Stellenbosch University*



Supervisor: Prof L C Hoffman

Co-supervisor: Dr E Pieterse

December 2014

DECLARATION

By submitting this thesis electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the sole author thereof (save to the extent explicitly otherwise stated), that reproduction and publication thereof by Stellenbosch University will not infringe any third party rights and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

Date: December 2014

SUMMARY

Increased consumer awareness has motivated the industry to find alternative methods to surgical castration for controlling boar taint and aggression in male pigs. Immunocastration has been identified as a solution, however; little research has been done into the nutritional requirements of immunocastrated pigs. Thus the objective of this study was to determine the optimal protein levels for immunocastrated pigs with regards to growth performance, carcass traits and yields as well as meat quality when supplemented with or without ractopamine hydrochloride (RAC).

The study involved 120 male pigs following a 2 x 2 x 3 factorial design. The main effects evaluated were sex (immunocastrated versus entire), RAC supplementation (0 versus 10 mg/kg and dietary balanced protein level (7.50 {low}, 9.79 {medium} and 12.07 {high} g lysine/kg). Vaccination occurred at 16 and 20 weeks of age and from 20 weeks each pig was allocated to one of the balanced protein diets with RAC supplementation at either 0 or 10 mg/kg for the last 28 days of growth. Slaughtering occurred at 24 weeks at which time carcass traits were measured and carcasses were processed, commercial cuts were weighed, deboned and trimmed into muscle, bone and fat portions which were then weighed individually.

Immunocastration increased the average daily gain, average daily feed intake, feed conversion ratio (FCR) and backfat deposition after the second vaccination. The FCR was improved by RAC supplementation, with the best FCRs seen at the medium and high balanced protein diets. Immunocastration and RAC increased live weight at slaughter and calliper backfat thickness but no differences were seen for the dressed hot carcass weight or Hennessey Grading Probe backfat thickness. Supplementation of RAC increased the percentage of the hot carcass weight, comprising of the shoulder, hindquarter, loin and belly, as well as the shoulder muscle, hindquarter muscle and loin muscle percentages, while decreasing the fat percentage of the belly and hindquarter.

Chemical composition analysis of the *Longissimus thoracis* (LT) muscle indicated differences ($p \leq 0.05$) for crude protein content but they were considered biologically negligible. The cooking loss of the LT was decreased by immunocastration and feeding medium protein. Feeding RAC decreased the a^* and b^* colour values and increased the Warner Bratzler shear force (WBSF) values, resulting in less red and less tender meat. Entire males fed low dietary protein had the lowest L^* values, while entire males fed medium protein diets had the highest L^* values. Immunocastrates fed the low protein diet had the most tender meat, whereas immunocastrates fed the high protein diet had the least tender meat. Immunocastration decreased the androstenone levels to below 0.5 $\mu\text{g/g}$ fat and although it did not significantly affect the testicle size, it influenced the morphology and increased the lightness (L^*), yellowness (b^*) and decreased the redness (a^*) of the testicle's cut surface colour.

The results from this study indicated that the balanced dietary protein requirements for immunocastrates differ both with and without RAC supplementation and thus the correct dietary protein level needs to be provided so that growth performance and leanness is not compromised. The return per carcass can also be improved by supplementing RAC, owing to improved cutting yields and lean yields of carcasses. Together, immunocastration, RAC supplementation and the correct

balanced protein diet may allow pig producers to efficiently produce heavier male carcasses without boar taint while conforming to the animal welfare expectations of the consumer. However, an incentive for producers in terms of immunocastration needs to be provided by the possible modification of the current carcass classification system so that heavier immunocastrated male carcasses are not penalised.

OPSOMMING

Die toename in verbruiker bewustheid het die bedryf gemotiveer om alternatiewe metodes vir chirurgiese kastrasie te vind, terwyl beergeur en aggressie in manlike varke beheer word. Immunokastrasie is geïdentifiseer as 'n moontlike metode, maar min navorsing is egter tot dusver gedoen om die optimale proteïen vlakke vir hierdie immunokastrate te bepaal. Min is ook bekend oor die groei prestasie, karkas-eienskappe, opbrengs asook vleiskwaliteit van immunokastrate as raktopamien hidrochloried (RAC) tot die dieet bygevoeg word.

'n Studie is gevolglik gedoen met 120 manlike varke in 'n 2 x 2 x 3 faktoriaal ontwerp. Die hoof effekte wat geëvalueer was, is geslag (immunogekastreerd of intakt), RAC byvoeding (0 of 10 mg/kg) en gebalanseerde proteïenvlak (7.50 {lae}, 9.79 {medium} en 12.07 {hoë} g lisien/ kg). Inenting het plaasgevind op 16 en 20 weke ouderdom en van 20 weke was elke vark toegeken aan een van die gebalanseerde proteïen diëte met RAC byvoeding teen 0 of 10 dpm vir die laaste 28 dae van groei. Diere is geslag op 24 weke ouderdom, waartydens karkaseienskappe gemeet was. Die karkasse is vervolgens opgesny en die kommersiële snitte geweeg. Die snitte is verder verwerk en spier, been en vet is afsonderlik geweeg.

Immunokastrasie het die gemiddelde daaglikse toename, gemiddelde daaglikse voerinnname, voeromsetverhouding (VOV) en rugvet dikte verhoog na die tweede inenting. Die VOV is verbeter deur RAC byvoeding, met die beste VOVs waargeneem op die medium en hoë gebalanseerde proteïen diëte. Resultate het getoon dat beide immunokastrasie en RAC gelei het tot 'n toename in lewendemassa by slag asook vernier rugvetdikte, alhoewel daar geen verskil tussen warm karkasmassa of Hennessey Grading Probe (HGP) rugvetdikte was nie. Aanvulling van RAC verhoog die skouer, agterkwart, lende en pens uitgedruk as persentasie van die warm karkasmassa. Die massa, uitgedruk as persentasie van warmkarkasmassa, van die skouerspier, agterkwartspier en lendespier is ook verhoog terwyl pensvet verlaag het.

Chemiese analise van die *Longissimus thoracis* (LT) spier het aangedui dat die ru-proteïen inhoud verskil ($p \leq 0.05$) alhoewel die verskille wat gevind was waarskynlik nie biologiese waarde het nie. Die persentasie kookverlies van die LT is verlaag deur immunokastrasie en die middel proteïenvlak-dieet. Die byvoeding van RAC het die a^* en b^* kleurwaardes verlaag en die Warner-Bratzler-shear-force (WBSF)-waardes verhoog, wat gelei het tot minder rooi vleis asook minder sagtheid. Intakte varke wat 'n lae proteïen dieet gevoer was, het die laagste L^* waardes getoon terwyl intakte varke wat 'n middel proteïenvlak-dieet gevoer was die hoogste L^* waardes getoon het. Immunokastrate wat 'n lae proteïen-dieet gevoer was, het die sagste vleis gelever, maar immunokastrate wat 'n hoë proteïen-dieet gevoer was, het die taaiste vleis gelever. Immunokastrasie het die konsentrasie androstenoen tot onder $0.5 \mu\text{g/g}$ vet verlaag en alhoewel dit geen betekenisvolle effek op testikel grootte gehad nie, het dit die morfologie van die testikel geaffekteer asook die ligheid (L^*) en geelheid (b^*) verhoog en die rooiheid (a^*) van die gesnyde testikel oppervlakte verlaag.

Die resultate van hierdie studie dui daarop dat die gebalanseerde proteïen vereistes vir immunokastrate verskil vir immunokastrate met en sonder RAC byvoeding. Dit is dus belangrik dat die korrekte proteïenvlak en aminosuur samestelling gevoer moet word vir die spesifieke dier onder

beskouing ten einde groeiprestasie en maerheid te maksimeer. Die opbrengs per karkas kan ook verbeter word deur die byvoeding van RAC, as gevolg van die verbeterde snit-opbrengs en maer-vleis opbrengs van karkasse. Die kombinasie van immunokastrasie, RAC aanvullings asook die korrekte gebalanseerde proteïen-dieet skep die moontlikheid aan varkprodusente om swaarder beerkarkasse doeltreffend te produseer sonder die teenwoordigheid van beergeur terwyl daar terselfdertyd aan die verbruiker se dierewelsyn-wense voldoen word. Die insentief vir die produsent is egter nog afwesig en daarom is dit nodig dat die klassifikasie stelsel hersien moet word ten einde te verseker dat daar nie teen immunogekastreerde diere op die slaglyn gediskrimineer word nie.

ACKNOWLEDGEMENTS

I would like to thank the following people and institutions in no particular order, which made the completion of this thesis possible:

Prof. Louw Hoffman (Supervisor) and Dr Elsje Pieterse (Co-supervisor) at the Department of Animal Sciences, Stellenbosch University, for their continued support and guidance throughout my project.

National Research Foundation (NRF), South African Pork Producers' Organisation (SAPPO) and the South African Research Chair's Initiative for providing the financial support for the trial and my postgraduate studies.

Elanco™ and Zoetis™ for the sponsorship of their products Paylean® and Improvac® for my trial and the technical advice provided.

Prof Martin Kidd from the Centre for Statistical Consultancy and Gail Jordaan from the Department of Animal Sciences for the assistance with the statistical analysis and the designing of my experiments.

Agricultural Research Council (ARC) for the use of their Boar Testing facilities at Elsenburg for the duration of my growth trial.

Winelands Pork Abattoir for allowing me to collect data for my research on the slaughter line and deboning department. Thank you to Rudi Harmse, Lana Verster and Freddy Williams for your assistance, support and patience in the various roles which you played in making my project a success.

Nico Louw from Keibeas Piggeries who supplied the animals, their transport, equipment and feed for my trial as well as to Xaviera Nel who helped organise them and assisted in cleaning the facilities.

Dr Gary Burhmann for his assistance whenever I had a question regarding my pigs and the friendly visits to see how they were doing.

Joline van Zyl from Meadow Feeds who helped with the mixing of all my feeds for my growth trial.

Stefan Guizot from Tana Piggeries for the weekly use of their backfat scanner, which enabled me to collect valuable data for my study and Corne Botha from Nova Feeds for showing me how to use it and proving me with temperature loggers for the duration of my growth trial.

Konnie Plaatjies for helping me look after my pigs during my growth trial and the hard work you put in every day. Thank you for treating the animals with kindness and all the innovative ideas you had.

Prof Rob Gous, for his guidance throughout my study and for sharing his knowledge. Thank you for taking the time to comprehensively go through the various emails, questions and work which I sent you and providing crucial feedback to my project; I appreciate your leadership and mentorship.

John Morris at Stellenbosch University Experimental Farm, for his assistance, knowledge and patience with regards to my growth trial. Thank you for being there whenever I needed advice or assistance prior to and at any stage of my growth trial. The labour, equipment, knowledge and general support you provided was crucial to the success of my trial and I have learnt an incredible amount from you with regards to pig husbandry.

The students that formed part of the Meat Sciences Postgraduate Research team who assisted me with the data collection in the abattoir, deboning department and the laboratory work that followed.

All who assisted with the various stages of my experimental trial and lab analyses; especially Danie Bekker, Karla van Zyl and Megan North for the weekly herding and weighing of the pigs even when they were playful at over 100 kg. A special thanks to Karla for the encouragement and support throughout my growth trial and lab analyses, as well as Charlene Needham, who helped me clean pens on weekends and during her holidays.

Patrick Carroll for his positive attitude, patience and support. Thank you for stepping in whenever it was needed; for the use of your bakkie when my car could not get through the mud to the pig facilities or load pig feed; for the weekends you spent in pig pens and in the department helping me with lab work and meat processing.

My friends, family and the food and wine enthusiasts; for your encouragement, support, understanding and putting a smile on my face.

Mom and Dad, for your continued support throughout my studies both emotionally and financially. Thank you for always believing in my abilities and helping me make a success of my studies; whether I needed weighing devices built, shavings collected, man power or just someone to talk to.

LIST OF ABBREVIATIONS

FSH	Follicle stimulating hormone
LH	Luteinizing hormone
GnRH	Gonadotropin-releasing hormone
GH	Growth hormone
IGF-1	Insulin-like growth factor-1
EU	European Union
ADG	Average daily gain
ADFI	Average daily feed intake
FCR	Feed conversion ratio
DE	Digestible energy
ME	Metabolisable energy
NE	Net energy
CP	Crude protein
DFD	Dark, firm and dry
PSE	Pale, soft and exudative
LT	<i>Longissimus thoracis</i> muscle
LTL	<i>Longissimus thoracis et lumborum</i> muscle
IMF	Intramuscular fat
WHC	Water holding capacity
T1	Period from 16 to 20 weeks of age
T2	Period from 20 to 24 weeks of age
T1/2	Period from 16 to 24 weeks of age
C	Immunocastrated
E	Entire
RAC	Ractopamine hydrochloride
pH ₄₅	pH at 45 minutes <i>post mortem</i>
pH ₂₄	pH at 24 hours <i>post mortem</i>
pH ₄₈	pH at 48 hours <i>post mortem</i>
temp ₄₅	Temperature at 45 minutes <i>post mortem</i>
temp ₂₄	Temperature at 24 hours <i>post mortem</i>
temp ₄₈	Temperature 48 hours <i>post mortem</i>
HCW	Hot carcass weight
HGP	Hennessey Grading Probe
WBSF	Warner-Bratzler shear force
LSMeans	Least Squares Means
SEM	Standard error of the Mean
ANOVA	Analysis of Variance

NOTES

The language and style used in this thesis are in accordance with the requirements of the *Journal of Meat Science*. This thesis represents a compilation of manuscripts where each chapter is an individual entity and some repetition between chapters was therefore unavoidable. The opinions expressed and conclusions arrived at in this study are those of the author and are not necessarily to be attributed to the NRF.

The results from this study have been presented at:

The South African Pork Producers' Organisation (SAPPO) National Annual General Meeting and Premier Pork Producers' Organisation (PPP) Joint Symposium, 3 – 4 September 2014, Johannesburg, South Africa.

Swine Veterinary Society Continued Professional Development, 28 October 2014, Pretoria, South Africa.

LIST OF CONTENTS

DECLARATION.....	ii
SUMMARY	ii
OPSOMMING	iv
ACKNOWLEDGEMENTS	vi
LIST OF ABBREVIATIONS.....	viii
NOTES.....	ix
LIST OF CONTENTS.....	x
CHAPTER 1	1
General Introduction.....	1
1.1 Background	1
1.2 Research question, problem statement and hypotheses.....	2
1.3 Research aims and objectives	2
1.4 Significance of research.....	3
1.5 Brief chapter overview.....	3
1.6 References.....	4
CHAPTER 2	5
Literature Review	5
2.1 Background	5
2.2 Reproduction and boar taint in male pigs	6
2.3 Consumer acceptability of boar taint pork.....	7
2.4 An ethical alternative: immunological castration	8
2.4.1 Physiological and metabolic changes	9
2.4.2 Clearance of boar taint compounds	12
2.4.3 Consequences on performance, nutrient requirements and behaviour.....	14
2.4.4 Implications on carcass traits and meat quality	17
2.4.5 Reproductive system and functioning	20
2.5 Ractopamine hydrochloride: increased efficiency	21
2.5.1 Effect on performance and nutritional requirements.....	22
2.5.2 Influence on carcass composition and meat quality	23
2.6 Conclusion	25

2.7 References	25
CHAPTER 3	32
Growth responses of entire and immunocastrated male pigs to dietary protein with and without ractopamine hydrochloride	32
Abstract	32
3.1 Introduction	32
3.2 Materials and methods	34
3.2.1 Feed formulation	35
3.2.2 Animals, housing, feeding and experimental design	39
3.2.3 Statistical analysis	43
3.3 Results	44
3.4 Discussion	52
3.5 Conclusion	55
3.6 References	55
CHAPTER 4	58
Carcass traits and cutting yields of entire and immunocastrated pigs fed increasing protein levels with and without ractopamine hydrochloride supplementation	58
Abstract	58
4.1 Introduction	58
4.2 Materials and Methods	60
4.2.1 Animals, housing and feeding	60
4.2.2 Slaughter	61
4.2.3 Carcass fabrication and deboning	62
4.2.4 Statistical analysis	64
4.3 Results	64
4.3.1 Live weight, hot carcass weight, carcass scores and fat depth	64
4.3.2 Carcass cutting yields	66
4.4 Discussion	74
4.4.1 Live weight, hot carcass weight, carcass scores and fat thickness	74
4.4.2 Carcass cutting yields	76
4.5 Conclusion	78
4.6 References	79

CHAPTER 5	83
Physical meat quality and chemical composition of the <i>Longissimus thoracis</i> of entire and immunocastrated pigs fed varying dietary protein with and without ractopamine hydrochloride supplementation	83
Abstract	83
5.1 Introduction	83
5.2 Materials and Methods	85
5.2.1 Animals, housing and feeding	86
5.2.2 Slaughter and sampling	86
5.2.3 Physical meat quality	87
5.2.4 Chemical analyses	88
5.2.5 Statistical analysis	89
5.3 Results	89
5.3.1 Chemical composition	89
5.3.2 pH and temperature 45 minutes, 24 hours and 48 hours post-mortem	92
5.3.3 Surface colour	93
5.3.4 Drip loss, cooking loss and Warner-Bratzler shear force	94
5.4 Discussion	96
5.5 Conclusion	100
5.6 References	100
CHAPTER 6	104
Effects of immunocastration on boar taint compounds and testicle size, colour and histology on boars fed with or without ractopamine hydrochloride at varying balanced protein levels	104
Abstract	104
6.1 Introduction	104
6.2 Materials and methods	106
6.2.1 Animals, housing and feeding	106
6.2.2 Slaughtering and testicle measurements	107
6.2.3 Chemical analyses	108
6.3 Statistical analyses	109
6.4 Results	109
6.4.1 Androstenone, skatole and indole	109

6.4.2 Testicle measurements	110
6.5 Discussion	114
6.6 Conclusion	116
6.7 References	117
CHAPTER 7	120
General conclusions and recommendations	120

CHAPTER 1

General Introduction

1.1 Background

The South African pork industry contributes approximately 2.15 % to the primary agricultural sector with an estimated 4000 commercial producers, 100 smallholder farmers and 19 stud breeders which account for a total of 1 599 million pigs (DAFF, 2012). In 2011, over 2.4 million pigs were slaughtered, resulting in the production of over 2 million tons of pork. In South Africa, this pork is used in almost equal amounts for fresh pork products as well as processed pork products (DAFF, 2012).

Surgical castration has traditionally been practised as a means to control breeding, ease management, decrease aggressive and sexual behaviour as well as to improve the meat quality of various male livestock species. Surgical castration is an elective procedure and according to the South African Code for the Welfare of Pigs (NSPCA, 2014), male piglets may be surgically castrated with or without anaesthetic within seven days of birth. This can result in pain, infection, decreased growth performance, morbidity and mortality and thus surgical castration has raised various ethical and welfare issues (Tuytens *et al.*, 2012). Many European countries are considering implementing legislation which limits the application of surgical castration and since 2012, surgical castration without pain relief has been banned for organic farming in the European Union (EU) (Heid & Hamm, 2013). Surgical castration without anaesthesia has also been banned in Switzerland from 2009 and any form of surgical castration has been banned in Norway since 2009 (Font-i-Furnols *et al.*, 2012).

The production of entire, or uncastrated, male pigs is preferable over castrated animals due to the favourable anabolic influences of their male steroid hormones; however, castrates are often produced to prevent boar taint. Boar taint is an offensive smell and taste in the meat of entire male pigs and is caused by androstenone, skatole and indole which accumulate in the adipose tissue and decrease the eating quality of the pork (Bonneau, 1982). Due to the fact that surgical castration is becoming a more unacceptable means of controlling boar taint and the behaviour of male pigs, alternative methods need to be investigated, of which immunological castration is an attractive solution. Immunocastration involves immunising the animal against its own gonadotrophin releasing hormone (GnRH), using products such as Improvac[®] (Zoetis[™] Animal Health) in order to arrest testicular development and functioning. Although limited studies have been performed on the acceptance of the various castration methods, most of them reported high acceptance of immunocastration (Tuytens *et al.*, 2012). Since the production of entire male pigs can be beneficial in terms of a better feed efficiency and lower fat deposition when compared to surgical castrates, many producers in South Africa try to slaughter their pigs before sexual maturity in an attempt to decrease the prevalence of boar taint. Abattoirs also tend to discriminate against heavy male carcasses and usually penalise dressed carcasses over 100 kg. According to the PORCUS classification system (Agricultural Product Standards Act 119 of 1990) all male carcasses are marked with a "MD" stamp which indicates their sex and are often discriminated against when purchased by butchers. This

means that the return per carcass is limited when considering increased slaughter mass, which further supports the need to investigate alternatives such as immunocastration to control boar taint.

1.2 Research question, problem statement and hypotheses

Currently, there is a substantial body of literature on the effects of immunocastration which include behaviour, hormone levels, growth, and vaccination schedule amongst others. However, few studies have addressed the issue of how immunocastration affects the nutrient responses of immunocastrates, especially in terms of balanced protein. It is necessary to better understand the differences in potential rates of protein and lipid growth of entire males and those which have been immunocastrated in order to ensure the correct provision of the nutrients. Another aspect which has not been addressed in detail is the feeding of β -adrenergic agonists such as ractopamine hydrochloride (RAC) to immunocastrates; this intervention being used to increase weight gain, feed efficiency and leanness in entire males, but whose effects may be lost in immunocastrates due to metabolic changes resulting from this practise of castration. Such growth promoters may counteract the negative influences of immunocastration, including increased fat deposition and decreased feed efficiency.

This leads to the research question of how do the physical and metabolic changes associated with immunocastration and feeding ractopamine hydrochloride change the potential growth of body protein and lipid, hence altering the nutrient responses of immunocastrates during the period from second vaccination to slaughter? The problem statement is thus: immunocastration in pigs arrests testicular functioning after the second vaccination thereby decreasing the mature protein weight resulting in a reduction in protein requirements as well as lean yield. Feeding ractopamine hydrochloride to immunocastrates will counteract this decrease in lean yield but will influence the quality of the resulting pork meat.

The research hypotheses are as follows:

H₀: Immunocastration and ractopamine hydrochloride have no effect on the growth, carcass yield, leanness and meat quality of pigs.

H_a: Immunocastration and ractopamine hydrochloride have an effect on the growth, carcass yield, leanness and meat quality of pigs.

1.3 Research aims and objectives

Firstly, the aim of this research was to evaluate the growth performance and carcass traits of immunocastrates fed balanced protein diets of various digestible lysine levels at constant energy, fed with or without ractopamine hydrochloride (RAC). This research also aims to determine the effects of balanced protein diets of varying balanced protein (lysine) levels on immunocastrates fed with or without RAC. Thus the main objective of the study was to establish optimal balanced protein levels for immunocastrates, with and without RAC, in terms of their growth performance and carcass

characteristics and yields using a feeding and slaughter trial. The secondary objective was to measure the effect of RAC on the growth, fatness and meat quality of immunocastrates and lastly, to further investigate the influence of immunocastration on the reproductive functioning of the testicles using size measurements and histology.

1.4 Significance of research

The outcomes of this study will aid in further understanding the nutrient requirements of immunocastrated male pigs in terms of their digestible lysine and balanced protein requirements. This will enable pig producers to choose appropriate balanced protein (lysine) level(s) for immunocastrates to ensure optimal growth performance, leanness and carcass quality. The inclusion of RAC in the study aims to provide a means of counteracting the possible negative effects of immunocastration using a readily available product, Paylean[®], in South Africa. Thus the combination of feeding immunocastrates various balanced protein diets, with and without RAC provides insight in to which dietary balanced protein (lysine) level(s) are appropriate for immunocastrates fed RAC. This information can empower pig producers during the decision making process to switch from the production of surgical castrates or entire males to immunocastrates in order to ensure the pigs perform optimally while producing boar taint-free meat. The results from this study may also motivate the use of immunocastration in pig farming in South Africa to produce good quality carcasses as well as good eating quality pork.

1.5 Brief chapter overview

The various issues regarding surgical castration and the production of entire male pigs are discussed in Chapter 2: Literature review, which explores the application and effects of immunocastration as well as RAC supplementation. The need for further research into the nutrient requirements of immunocastrated male pigs and the potential use of RAC for counteracting the negative effects of immunocastration are highlighted within the literature review. The effect of feeding immunocastrates increasing levels of dietary balanced protein with and without RAC supplementation on the growth performance, which includes the effects on the average daily gain (ADG), average daily feed intake (ADFI), backfat thickness gain and feed efficiency, is evaluated in Chapter 3. This follows into the determination of immunocastration, dietary balanced protein and RAC supplementation on the carcass characteristics and cutting yields in Chapter 4 and the physical and chemical evaluation of the *Longissimus thoracis* (LT) muscle in Chapter 5 to establish the effect on meat quality characteristics. Chapter 6 investigates the effects of immunocastration on the compounds associated with boar taint as well as the various effects on the testicles, to further support current literature. Lastly, the outcomes of the research are combined into the general conclusions and recommendations within Chapter 7.

1.6 References

- Bonneau, M. (1982). Compounds responsible for boar taint, with special emphasis on androstenone: a review. *Livestock Production Science*, 9, 687-705.
- Department of Agriculture, Forestry and Fisheries (DAFF), Republic of South Africa. (2012). A Profile of the South African Pork Market Value Chain. Retrieved from: <http://www.nda.agric.za/docs/AMCP/Pork2012.pdf>
- Font-i-Furnols, M., Gispert, M., Soler, J., Diaz, M., Garcia-Regueiro, J., Diaz, I. & Pearce, M. (2012). Effect of vaccination against gonadotrophin-releasing factor on growth performance, carcass, meat and fat quality of male Duroc pigs for dry-cured ham production. *Meat Science*, 91, 148–154.
- Heid, A. & Hamm, U. (2013). Animal welfare versus food quality: Factors influencing organic consumers' preferences for alternatives to piglet castration without anaesthesia. *Meat science*, 95, 203–211.
- Republic of South Africa. (1990). *Agricultural Product Standards Act 119 of 1990*. Retrieved from www.nda.agric.za/docs/NPPOZA/APS%20Act.pdf
- South African Code for the Welfare of Pigs (2014). Retrieved from: <http://www.nspca.co.za/clientdata/10072/uploads/codes/code%20pig%20welfare.pdf> on 7 September 2014.
- Tuytens, F., Vanhonacker, F., Verhille, B., De Brabander, D. & Verbeke, W. (2012). Pig producer attitude towards surgical castration of piglets without anaesthesia versus alternative strategies. *Research in Veterinary Science*, 92, 524–530.

CHAPTER 2

Literature Review

2.1 Background

Improved production efficiency in order to maximise profit is important in all livestock production enterprises internationally and the South African pork industry is no exception. In order to increase the efficiency of meat production, the return per animal within the production unit needs to be maximised and one such method would be to increase the slaughter weight, carcass yield and carcass quality per animal. However, in the pork industry heavy male carcasses are associated with an offensive sensory attribute known as boar taint, which limits the producer to slaughter intact boars before they reach sexual maturity or surgically castrate in an attempt to control the prevalence of boar taint. Thus the return per animal is limited by having to produce smaller entire male carcasses or by producing less efficient physically castrated males. Previously, surgical castration has been used as a means of controlling boar taint and aggressive sexual behaviour; however, a more efficient feed conversion ratio as well as a lower fat percentage is sacrificed (Pauly *et al.*, 2009). Such aggressive and sexual behaviour distracts the animals from important activities such as feed intake, causes carcass damage and complicates animal management. The production of entire males may be preferable over castrated pigs (barrows) due to the favourable anabolic influences of their male steroid hormones. However, male hormones are not always beneficial; with boar taint being a prevalent issue in sexually mature male pigs. Androstenone (5 α -androst-16-en-3-one) and skatole (3-methylindole) are two major compounds involved in boar taint and are both lipophilic; therefore they accumulate in the adipose tissue (Bonneau *et al.*, 1982). Androstenone is a pheromone produced by the testes and skatole is a by-product of tryptophan breakdown by bacteria situated in the hindgut of all pigs (Patterson, 1968a). These compounds accumulate in the adipose tissue of mature boars, commencing during sexual development and consequently decrease the eating quality of the pork.

Approximately 79.3 % of the male pigs in Europe are surgically castrated (Fredriksen *et al.*, 2009) but by 2018 the EU aims to voluntarily ban the practise (Font-i-Furnols, 2012). Thus surgical castration is becoming a more unacceptable means of controlling boar taint due to welfare concerns since male piglets are typically castrated without the use of anaesthesia and are vulnerable to infection, morbidity, decreased growth performance and mortality (Rault *et al.*, 2011). Therefore, many European countries are considering implementing legislation which limits the application of surgical castration. Surgical castration without anaesthesia has been banned in Switzerland from 2009 and any form of surgical castration has been banned in Norway since 2009 (Font-i-Furnols *et al.*, 2012b). Thus alternative means of controlling boar taint need to be investigated, of which immunological castration is an attractive alternative. Limited studies have been performed on the producer acceptance of castration methods, however, most reported high levels of acceptance of immunocastration (Tuytens *et al.*, 2012).

Along with the investigation into techniques such as immunocastration comes the need to investigate what influences such practises will have on important production factors such as

nutritional requirements, feed efficiency, growth rate, carcass characteristics and meat quality. These factors are especially important in terms of maximising the margin between the cost of production and the value of the carcass. Since the primary reason for pig production is meat, one needs to consider how to optimally exploit the anabolic potential of immunocastrated male pigs in terms of muscle growth and using growth aids such as β -adrenergic agonists. This also includes the provision of the correct balanced dietary protein in terms of amino acids; of which digestible lysine is important in terms of muscle growth. Therefore, it is important to ensure such techniques do not negatively influence production while successfully eliminating boar taint, which is largely dependent on the sexual development and reproductive functioning of the male pig.

2.2 Reproduction and boar taint in male pigs

Together, the hypothalamus and the anterior pituitary gland control reproduction in the pig. The anterior pituitary gland is located below the hypothalamus and its function is to synthesize, store and secrete hormones involved in metabolism and reproduction. Hormones produced and secreted by the hypothalamus can be grouped into releasing hormones and inhibiting hormones which act on the pituitary gland, following transport by the hypophysial portal blood vessels. The pituitary releases its own hormones in response to hypothalamic hormones and of these pituitary hormones, follicle stimulating hormone (FSH), luteinizing hormone (LH) and prolactin are of reproductive importance in the male pig. Both LH and FSH secretion are governed by a neuropeptide secreted by the hypothalamus, known as gonadotropin-releasing hormone (GnRH), which binds to pituitary gland receptors and stimulates LH and FSH secretion. Testicle steroid production and release is governed by LH and FSH with LH playing a major role in the production of testosterone and FSH playing a minor role (Hughes & Varley, 1980). The Leydig cells and seminiferous tubules of the testes are responsible for the synthesis and secretion of male gonadal steroid hormones such as testosterone as well as spermatogenesis. The testosterone has a stimulatory effect on spermatogenesis and is essential in sperm maturation, along with the influence of FSH. The testes also produce oestrogen and androgens, however, oestrogen is mainly found in the conjugated form which is relatively inactive and androstenedione is a weakly androgenic precursor of testosterone (Hughes & Varley, 1980).

Androstenone is a pheromone also produced by the testes, which enters the blood plasma via the spermatic vein where it is deposited into fat for reversible storage or storage in the salivary glands (Bonneau *et al.*, 1982) and is secreted by the salivary glands during mating activity in order to increase the sexual interest of sows. It is a steroidal compound produced in the male piglet from birth (Booth, 1975) with maximal levels being reached during puberty (Zamaratskaia *et al.*, 2004a). When androstenone is stored in the fat of entire male pigs, it causes a characteristic smell or taste of their meat known as boar taint. Boar taint is a result of three compounds: androstenone (5 α -androst-16-en-3-one), skatole (3-methylindole) and indole, which are often found in combination with each other. Since androstenone exhibits both high levels and an intense odour in the fat, it has been the focus of many boar taint studies and since indole plays a minor role in the contribution of boar taint, it is generally neglected. These compounds associated with boar taint accumulate in the adipose tissue of

mature boars, commencing during sexual development and consequently decrease the sensory eating quality of the pork. It is important to note that the unpleasant odours of the preputial fluid caused by bacterial fermentations in the prepuce of the penis are not involved in boar taint (Patterson, 1968b).

Androstenone in pork from entire male pigs is described as an unpleasant smell and taste of urine or sweat by those sensitive to it. Skatole is produced from tryptophan breakdown by bacteria situated in the hindgut of all pigs (Patterson, 1968a) and is reabsorbed from the colon, causing a faecal smell and taste in the meat of all pigs. Skatole production is not limited to entire male pigs but is however more prevalent in entire males for reasons not fully understood. Theories for this include the influence of anabolic hormones on cell apoptosis (Claus *et al.*, 1994), which is a main source for tryptophan, as well as the effect of oestrogens and androgens on hepatic skatole metabolism (Babol *et al.*, 1999). In male pigs, high plasma skatole levels are found at a young age, after which they decrease significantly between 10 to 12 weeks of age; however, at 18 weeks of age, plasma skatole levels increase again (Zamaratskaia *et al.*, 2004a). Plasma androstenone levels also vary with age, with low levels being found at a young age and then increasing between 14 and 16 weeks. Zamaratskaia *et al.* (2004a) found that testosterone and androstenone increased simultaneously as the male pigs reached puberty and that skatole and androstenone levels in the adipose tissue are highly correlated for pigs between 20 and 24 weeks of age. The increase in skatole was preceded by testosterone and androstenone after 14 weeks of age, thus supporting the fact that they may inhibit skatole metabolism in the liver. Skatole and androstenone seem to have a synergistic effect, so that the odours associated with androstenone can be strengthened when skatole is present as well (Font-i-Furnols, 2012a).

2.3 Consumer acceptability of boar taint pork

The opinion of the consumer is important with regards to both the acceptability of a product as well as the production system of this product in terms of welfare concerns. The acceptability of pork from entire males depends on the androstenone and skatole levels within the meat cut, with skatole being more important as more consumers are sensitive to it than androstenone. Also, the risk of the consumer being able to perceive boar taint in pork increases when both skatole and androstenone are present (Font-i-Furnols, 2012a). Threshold values for boar taint detection by consumers are 0.5 to 1 µg/g fat for androstenone and 0.20 to 0.25 µg/g fat for skatole (Zamaratskaia *et al.*, 2004a). Approximately 45 % of consumers are sensitive to boar taint; however cooking methods can alter the perception of boar taint, with the effect of boar taint being higher in odour than flavour (Font-i-Furnols, 2012a).

Methodologies used to evaluate consumer acceptability of boar taint varies in terms of the type of product, location on the carcass, cooking method, use of tasting scale, methods to determine androstenone and skatole levels, etc. However, the review by Font-i-Furnols (2012a) grouped previous sensory studies into those done on fresh meat and then each different pork meat product and classified the results as:

- Meat from entire males was equally accepted as the other sexes
- Meat acceptability from entire males depended on the level of boar taint
- Meat acceptability from entire males depended on the level of androstenone and/or skatole
- Meat acceptability from entire males depended on the level of skatole
- Meat acceptability from entire males depended on the level of androstenone

With regards to fresh meat, the odour of entire male meat is less acceptable than the flavour according to the summarised results of 42 studies. However, in 11 out of the 42 studies, meat from entire males was equally accepted as that from the other sexes evaluated. These 11 studies were performed predominantly in the United Kingdom, Canada or the United States, which could indicate that people from these countries are less sensitive to androstenone since all the other studies showed that pork from entire males was less acceptable depending on the levels of boar taint or its individual compounds (Font-i-Furnols, 2012a). This indicates that boar taint is an existing problem in terms of consumer acceptability.

Bacon is currently the most frequently studied pork product in terms of boar taint ($n = 9$) (Font-i-Furnols, 2012a). In Canada, the United Kingdom and Ireland, bacon made from entire males was again deemed as acceptable as that from the other sexes evaluated. However, in Spain and Sweden, the acceptability of bacon from boars was less acceptable or depended on the level of boar taint or androstenone level. Cooked ham was the second most studied ($n = 5$) and in three studies the ham from entire males was accepted and in the rest the acceptability was less acceptable or depended on the level of boar taint or androstenone level. Deviation in the results between the acceptability of products, for example bacon and ham, can vary even though both undergo heat treatment, ham is served cold and thus boar taint may be more difficult to pick up within the product. In terms of dry cured ham, two out of the three trials indicated that the product from entire males was less acceptable or their androstenone levels exceeded 0.5 to 0.7 $\mu\text{g/g}$ fat (Font-i-Furnols, 2012a). These results indicate that curing and drying does not mask boar taint and thus such products made from entire males could pose huge problems in countries which produce dry-cured meats such as Italy and Spain.

It is clear that boar taint is a world-wide problem in both fresh and processed pork produced in various production systems using entire males and with the current movement away from surgical castration, it is likely that the consumer acceptability of commercially purchased pork will decrease. It is thus important to explore other alternatives to producing boar taint-free pork in an ethical manner, such as immunocastration.

2.4 An ethical alternative: immunological castration

Various methods of immunologically castrating pigs and other livestock have been investigated but targeting GnRH has been the most successful thus far. Currently, Improvac[®] (Improvast[®]/ Vivax[®]/ Innosure[®]) is approved for use in over 60 countries over the world and is the only commercially

available vaccine used for immunocastration purposes. It has been used in Australia and New Zealand since 1998 as a means of controlling boar taint (Product Overview: www.improvac.co.nz). Improvac[®] has been available in South Africa since 2006 and since the launch just under 700 000 boars have been vaccinated to date. The Improvac[®] (Zoetis[™] Animal Health) vaccine consists of a synthetic analogue of GnRH which is modified so that it cannot bind to the pituitary gland. This analogue is bound to a carrier protein which the immune system recognises as foreign and thus the GnRH analogue becomes immunogenic. In order to ensure male pigs are successfully immunocastrated two vaccines (2 mL) must be administered at least four weeks apart and four to six weeks prior to slaughter. The first vaccine primes the body for the second vaccination (booster) by initiating a primary immune response in which low levels of antibodies against GnRH, and an immunological memory, are produced (Product Overview: www.improvac.co.nz). The primary dose has no visible effect on testes function due to the lack of effect on testes size and testosterone concentrations measured at second vaccination (Dunshea *et al.*, 2001). The second vaccination initiates a large and rapid increase in circulating antibodies against GnRH (Claus *et al.*, 2007) which bind to GnRH, making it unable to attach to the receptors located on the pituitary gland thus disrupting the hypothalamic-pituitary-gonadal axis. This removes the stimulus for LH and FSH production and ultimately steroid production by the testes; which is essentially the same effect as surgical castration. However, one cannot simply assume that immunocastrated pigs are the same as surgical castrates in terms of their growth and nutritional requirements. Immunocastrates are different to surgical castrates in the way that surgical castrates still produce GnRH, LH and FSH however there are no testicles present to produce testosterone to negatively feedback on the pituitary gland.

2.4.1 Physiological and metabolic changes

Various studies have focused on the physiological and metabolic changes which immunocastrated male pigs undergo due to the unique change in their hormone production. The recommended vaccination protocol is based on providing sufficient clearance time for the boar taint compounds from the adipose tissue (Dunshea *et al.*, 2001). The timing of the second vaccination is of high practical relevance such that sufficient time is given for antibody formation, cessation of Leydig cell function and clearance of boar taint (Claus *et al.*, 2007). Thus the second vaccination should be given close to slaughter to maintain the anabolic effects of the male hormones as long as possible while still providing time for androstenone clearance (Claus *et al.*, 2007).

According to Zoetis[™], high levels of antibodies against GnRH are experienced 10 to 14 days post booster, while Claus *et al.* (2007) showed that after the booster, antibody levels increase within three to five days using the recommended schedule with the highest antibody levels experienced between four and six days after vaccination (Figure 2.1). Antibody levels subsequently decrease to approximately half the peak value for each individual animal and remained elevated until slaughter at 72 days. Brunius *et al.* (2011) also noted a rapid increase in antibody titre after the second vaccination followed by a gradual decay in pigs which they vaccinated early (at 10 and 14 weeks of age) as well as in those following a standard vaccination schedule (at 16 and 20 weeks of age). Even

though there may be a slight increase in antibody titre after the first priming vaccination, FSH and LH are not influenced significantly (Fuchs *et al.*, 2009b) and testosterone and oestradiol levels do not differ from controls (Kubale *et al.*, 2013). Dunshea *et al.*, (2001) also found that although the individual responses to vaccination vary, all immunocastrates showed a response in terms of GnRH antibodies production (titre >100). Prolonged effects have been observed by Brunius *et al.* (2011) and Zamaratskaia *et al.* (2008b) in which highly significant levels of antibodies remained 11 and 22 weeks after the booster, respectively.

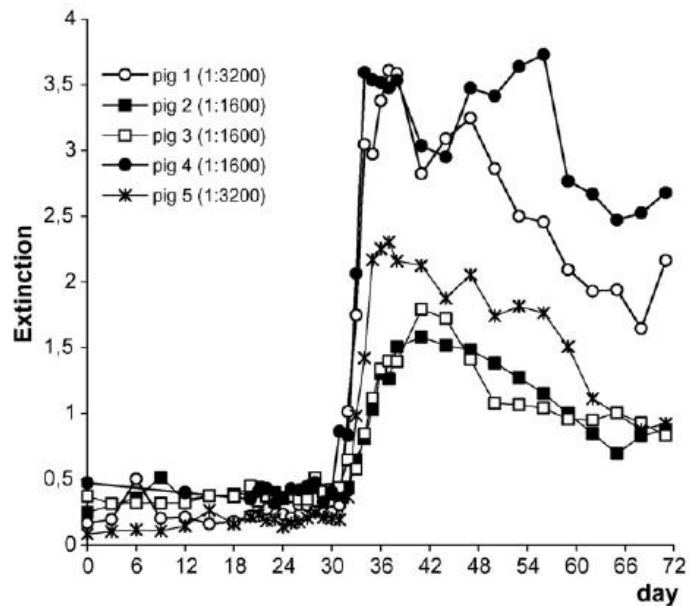


Figure 2.1. The GnRH antibody titre development in five individual immunocastrated Landrace boars, with first immunization at day 0 and the second on day 28 (Claus *et al.*, 2007)

The increase in antibody titre from approximately two days after the booster does not provide evidence of metabolic changes within immunocastrates, however, plasma LH concentrations provide an indication of the effects of vaccination on testes function and thus anabolic steroid production. According to Claus *et al.* (2007), plasma LH concentrations remained at a high level (0.158 ng/mL) after the first vaccination and then decreased (0.03 ng/mL), significantly without a lag after antibody development, within four to eight days post booster (Figure 2.2). The LH concentrations remained at the same decreased level until slaughter which indicates that although antibody titres decreased to half their peak values, this level was still sufficient to suppress LH production.

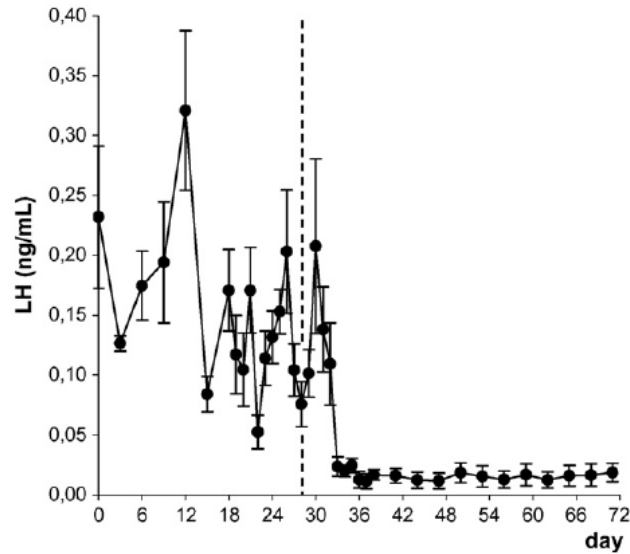


Figure 2.2. Mean luteinising hormone concentration (ng/mL) of 5 Landrace immunocastrates following the standard vaccination schedule at day 0 and day 28 (Claus *et al.*, 2007)

The Leydig cells within the testes undergo involution in response to a lack of LH and thus testosterone production is decreased. A similar pattern to LH was identified in plasma testosterone concentrations, which decreased (2.75 ng/mL to 0.25 ng/mL) after five to 10 days after the second vaccination and also remained stable until slaughtering. This decline is a result of decreased LH synthesis, which has an effect on the cholesterol side chain cleavage step in testosterone synthesis (Claus *et al.*, 2007). This decrease was also observed by Brunius *et al.* (2011) where testosterone levels in the immunocastrates decreased to that of the surgical castrates within two weeks of the second vaccination and by Dunshea *et al.* (2001) where testosterone levels decreased significantly two weeks post booster. The testes are also the site of androstenone production, which decreases (1.48 to 0.34 ng/mL) after the booster within four to eight days and did not increase until slaughter (Figure 2.4) (Claus *et al.*, 2007). The increase in antibody titre and the immediate response in terms of decreased LH and thus testosterone indicates that the potential anabolic growth of immunocastrates may be compromised after the second vaccination within a matter of days. However, immunocastration does not only influence androgen synthesis, it also influences oestrogen synthesis.

Brunius *et al.* (2011) measured the plasma oestradiol levels in immunocastrates, surgical castrates and boars the day prior to slaughter at 25 weeks of age and found that elevated levels were found in the boars whereas low levels were found in both the castrated sexes. Oestrogens are known to up-regulate hepatic growth hormone (GH) receptors and thus indirectly increase insulin-like growth factor-1 (IGF-1) expression (Brunius *et al.*, 2011). Bauer *et al.* (2009) found that GH levels were not significantly influenced by standard immunization, after the antibodies followed the general pattern also identified by Claus *et al.* (2007) and Brunius *et al.* (2011). Brunius *et al.* (2011) further demonstrated that GH levels do not change following the second vaccination and thus remain at the

same level as that within an entire male pig and did not fall to that found in a surgical castrate. Growth hormone stimulates the liver to produce IGF-1 which increases protein synthesis, along with the growth of other tissues. Brunius *et al.* (2011) also found that plasma IGF-1 levels were highest in boars, lowest in surgical castrates and intermediate in immunocastrates, as expected due to low oestradiol levels. According to Claus *et al.* (2007), plasma IGF-1 levels decrease (186 to 119 ng/mL) starting from five days after the booster and stabilising six to 10 days after. Plasma IGF-1 levels thus gradually decline after the booster vaccination and then stabilise after two to three weeks (Figure 2.3).

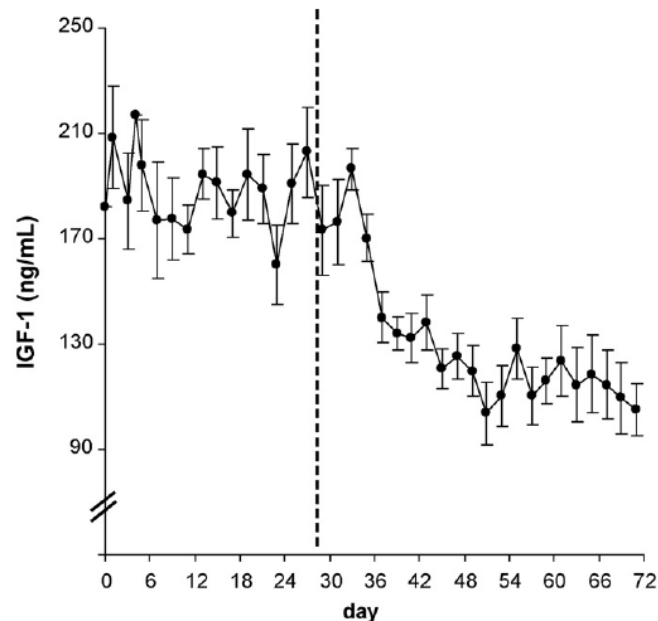


Figure 2.3. Mean insulin-like growth factor-1 concentration (ng/mL) of five Landrace immunocastrates following the standard vaccination schedule at day 0 and day 28, indicated by dashed line (Claus *et al.*, 2007)

2.4.2 Clearance of boar taint compounds

The disruption of the hypothalamic-pituitary-gonadal axis halts testosterone and androstenone production in the Leydig cells due to the absence of LH. Thus androstenone, which has been stored in the adipose tissue, is mobilised and enters the blood stream for metabolic clearance. Therefore the decrease in plasma androstenone does not represent lower levels of synthesis but rather a gradual clearance (Claus *et al.*, 2007). Provided the vaccination protocol is followed, the fat androstenone levels of immunocastrates are reduced at slaughter and skatole levels are also found to be low (Bonneau *et al.*, 1994; Kubale *et al.*, 2013). The mean androstenone values decrease significantly after the booster (1.48 to 0.34 ng/mL) and basal concentrations are reached four to eight days after the second dose, with no rise in concentration experienced (Figure 2.4).

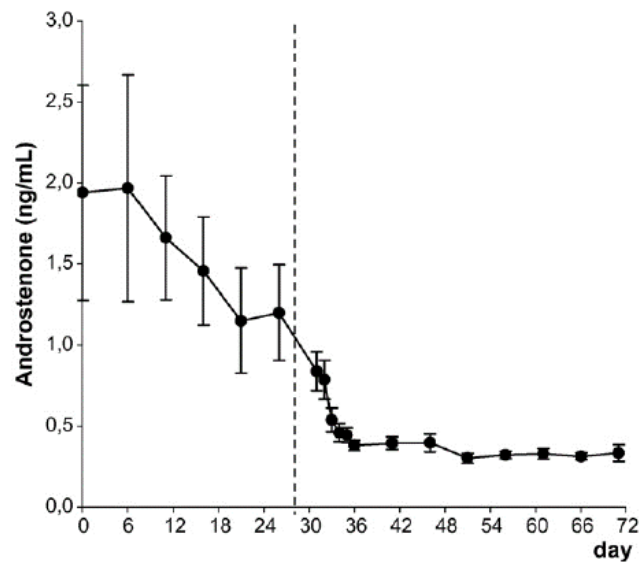


Figure 2.4. Mean plasma androstenone concentration (ng/mL) of five Landrace immunocastrates following the standard vaccination schedule at day 0 and day 28, indicated by dashed line (Claus *et al.*, 2007)

Metz *et al.* (2002) measured fat androstenone levels over time using biopsies of subcutaneous fat from immunocastrates (vaccinated at 10, 16 and 23 weeks; slaughtered 26 weeks) and found that within one week after the booster, androstenone levels were below 0.5 $\mu\text{g/g}$ in all but one immunocastrate and decreased further until slaughter where they were all below 0.15 $\mu\text{g/g}$. In the entire control boars, androstenone increased in a continuous and significant age-dependent fashion in control males and at 26 weeks of age, the mean concentration was 1.31 $\mu\text{g/g}$ fat. They also found that skatole levels in the immunocastrates were low at slaughter (14.4 ng/g fat) and were significantly less than that measured in the controls (109.2 ng/g).

The effects of the vaccination schedule on boar taint clearance was investigated by Lealiifano *et al.*, (2011) who showed that testes weights were reduced with increasing time between the booster and slaughter and that control pigs had testosterone levels three times higher and androstenone levels at least seven times higher than immunocastrates regardless of the vaccination schedule used. The standard vaccination protocol recommends four to six weeks be given for androstenone clearance from the adipose tissue, however, androstenone levels in the adipose tissue have been shown to decrease within two weeks after the second vaccination (Lealiifano *et al.*, 2011), but a larger gap between the booster vaccination and slaughter is still preferred. It was noted by Lealiifano *et al.* (2011) that although vaccinating at two weeks prior to slaughter allowed for the clearance of boar taint compounds and allowed an extended period of anabolic growth, it was difficult to handle the larger pigs for vaccination. Zamaratskaia *et al.* (2008b) noted a prolonged effect on the decreased androstenone and skatole levels observed up until 22 weeks after the second vaccination with the pigs remaining completely sexually inactive.

2.4.3 Consequences on performance, nutrient requirements and behaviour

Immunization against GnRH not only inhibits boar taint but also the anabolic effects of male steroid hormones on the growth potential. Due to the fact that immunocastration decreases testosterone production, one would expect immunocastrates to be less efficient than entire males after the second vaccination and start to perform similar to barrows. Although a decrease in testicular steroid production has a detrimental effect on muscle growth, the fact that immunocastrates have intermediate IGF-1 levels and normal GH levels implies that they could have a higher anabolic growth potential than barrows, provided their nutritional requirements are met. Such an example of how the anabolic potential of immunocastrates can be optimised by nutrition was demonstrated by Bauer *et al.* (2009), who compared two groups of standard vaccinated immunocastrates fed at two different intakes of 2 kg and 3 kg using high glycemic index carbohydrates (starch) in order to stimulate IGF-1 formation. The IGF-1 levels before the second vaccination were not significantly different but became significant after the second vaccination with those fed at a higher level having higher IGF-1 levels. The pigs fed at the higher level (3 kg) did not have an increased fat content after the second vaccination and had 32 % lower fat than barrows of the same weight and feeding regime. This indicates that higher feed intake could thus lead to increased growth without increased fat deposition and since GH inhibits lipogenesis, the surplus energy is possibly allocated to protein synthesis.

Boler *et al.* (2011) evaluated the effects of 7, 8, 9 and 10 g dietary lysine/kg on the performance of immunocastrates, described as low, low/medium, medium/high and high lysine. They found that immunocastrates should be fed higher lysine levels than traditionally given to surgical castrates in order to increase their cutting yields and that increasing levels of lysine decreased back fat thickness. It is important to ensure that the correct lysine levels are provided to immunocastrates since fat thickness has a large influence in the grading/classification, and thus price of carcasses. However, data is limited in terms of the responses of immunocastrates to various lysine levels, as well as other nutrients. Few studies have also evaluated the change in fat gain in immunocastrates during their growth.

Zeng *et al.* (2002) performed a digestibility study, using diets of varying energy content, comparing entire males and immunocastrates who received their second vaccination at 16 weeks of age. They found no difference in digestibility of proximate nutrients, but the digestibility of Ca and P was higher for entire males and immunocastrates than surgical castrates. The increased digestibility of Ca and P for immunocastrates compared to surgical castrates may be due to higher growth rates and a better feed conversion ratio (FCR) which means less P is excreted in faeces and (or) urine which has implications on environmental pollution caused by pig manure. Also, no interaction term was found between sex and dietary energy content, but there was a tendency for immunocastrates to perform better than surgical castrates, especially on a low energy diet (8.30 MJ NE/kg). When immunocastrates and entire males are compared in terms of growth performance. Dunshea *et al.* (2001) found that vaccinated boars performed better than those administered with a placebo, probably due to reduced aggressive and sexual behaviour. Thus immunocastrates spent more time eating and portioning energy towards growth rather than physical activity. The reduced testosterone

concentrations after the second vaccination did not have a detrimental effect on the growth of immunocastrates, which were also leaner and had a better feed conversion ratio than barrows. In fact, immunocastrates grew better than the entire males and barrows in the last four weeks before slaughter with average daily gains 30 % greater than entire males and 32 % better than barrows. Feed intake was 16 % higher in immunocastrates than entire males, while the intake of the barrows was 10% higher than entire males. Due to the absence of oestrogens and androgens, voluntary feed intake of immunocastrates increases rapidly after the second vaccination (Bauer *et al.*, 2009). However, the FCR in immunocastrates and entire males were similar, which were both less than that of the barrows (Dunshea *et al.*, 2001). Cronin *et al.* (2003) also found that immunocastrates had significant increased daily weight gains when compared to entire males for the last four weeks of the fattening period. However, Jaros *et al.* (2005) found no difference in average daily gain between surgical castrates and immunocastrates and Bonneau *et al.* (1994) found that the growth performance (rates) did not differ between intact males and immunocastrates.

Thus the results with regards to the growth performance of immunocastrates differ but this is to be expected since responses in growth are likely to differ depending on the vaccination schedule used. Since the first vaccination does not influence the growth performance of immunocastrates, the timing of the second vaccination with regards to physiological age and age at slaughter are important. Thus the second vaccination should be as close to slaughter to maintain the anabolic effects as long as possible while still providing time for androstenone clearance (Claus *et al.*, 2007). Therefore, the timing of second vaccination is of high practical relevance. For example, should the second vaccination be given before puberty, the production of male anabolic hormones will be disrupted earlier and should the delay until slaughter be relatively long, the more time there is for the drop in anabolic hormones to influence the growth and fat gains of the pig. This is supported by Lealiifano *et al.* (2011) who vaccinated pigs at two, three, four and six weeks prior to slaughter and found that the backfat thickness was significantly reduced when those vaccinated at six weeks were compared to the non-vaccinated control and those vaccinated two weeks prior to slaughter. However, vaccinating at four weeks prior to slaughter showed no significant difference in backfat thickness compared to controls. They also found that the adipose androstenone and blood testosterone levels were suppressed in all immunocastrated pigs regardless of schedule, but fat skatole levels were not different between treatments. It is thus likely that two weeks is adequate for clearance of boar taint compounds prior to slaughter but vaccinating earlier than six weeks prior to slaughter could significantly influence the fat gains of immunocastrates. An increase in average daily feed intake (ADFI) was apparent two weeks after immunisation regardless of the timing of the second vaccination and could be due to a number of things including the lack of testosterone limiting appetite, decreased distraction from aggressive activities as well as a change in nutrient requirements. The increase in ADFI can be seen within a week after the second vaccination, which can be seen by the results from Lealiifano *et al.* (2011) who administered the second vaccination at 2, 3, 4 and 6 weeks prior to slaughter (Figure 2.5).

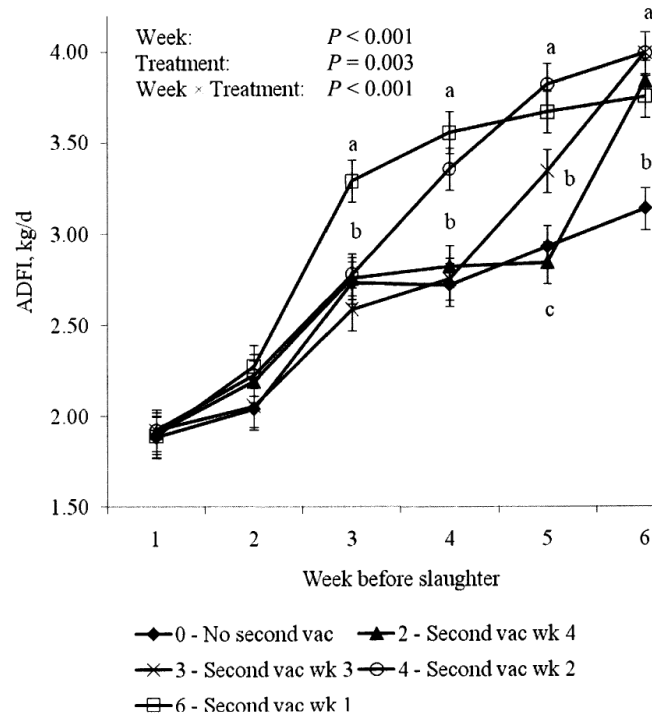


Figure 2.5 The ADFI of immunocastrates who received their vaccinations 2, 3, 4 and 6 weeks prior to slaughter. Those pigs vaccinated 2 weeks before slaughter were injected in Week 4; those at 3 weeks prior to slaughter in Week 3; those at 4 weeks were injected in Week 2 and those at 6 weeks were injected in Week 1. ^{ab}Means differ within individual weeks with different superscripts are significantly different (Lealiifano *et al.*, 2011).

Immunocastration increases voluntary feed intake by reducing male sexual and aggressive behaviour and thus entire male pigs tend to grow slower than castrated pigs at the end of the finishing phase possibly due to increased sexual activity and aggression. Cronin *et al.* (2003) measured the effects of immunocastration on behaviour and found that both entire and immunocastrated males were more active than surgical castrates before the second vaccination and displayed more social behaviour in a group housing situation. However, three weeks after the second vaccination, entire males spent more time displaying social behaviour than the immunocastrates of which aggressive behaviour was the predominant activity. Entire males also performed more mounting behaviour than immunocastrates after the second vaccination while immunocastrate behaviour was similar to that of surgical castrates. Brunius *et al.* (2011) also observed a decreased frequency of mounting in immunocastrated boars and Zamaratskaia *et al.* (2008b) reported that immunocastrates showed less social, aggressive and manipulating (nibbling or pushing) behaviour. Weiler *et al.* (2014) noted that immunocastrates increase their feed intake by increasing their meal duration rather than their number of meals per day. Out of the treatments groups consisting of gilts, immunocastrates, entire males and surgical castrates, immunocastrates had the highest growth rate due to their elevated feed intake (Weiler *et al.*, 2014). This is to be expected as testicular androgens and oestrogens suppress appetite as well as distract from feeding, replacing this activity with aggressive or sexual behaviour.

2.4.4 Implications on carcass traits and meat quality

The results for the influence of immunocastration on factors such as live body weight at slaughter, hot carcass weight (HCW) and backfat depth vary depending on the age at slaughter as well as the length of time between the second vaccination and slaughter. Other factors include gut fill and genital weights, since immunocastration increases feed intake and thus gut fill and decreases the genital weights which will influence the differences in dressing percentage when they are compared to entire males and surgical castrates. Dunshea *et al.* (2001) found that barrow carcasses tended to be the heaviest, immunocastrates intermediate and entire males were the lightest and that the dressing percentage of immunocastrates was less than boars. Gispert *et al.* (2010) agreed with this and also found that the live weight at slaughter and carcass weights of surgical castrates and immunocastrates was heavier than entire males and females. Most studies tend to agree with the conclusion that immunocastration decreases the dressing percentage when compared to barrows (Zamaratskaia *et al.*, 2008a; Fuchs *et al.*, 2009a; Pauly *et al.*, 2009; Gispert *et al.*, 2010).

Castration increases the fat deposition in males since the male hormones involved in stimulating muscle growth are no longer present. The effect of immunocastration on backfat thickness depends on the vaccination schedule since if the period between the booster and slaughter is prolonged, the more time the pig's metabolism has to adjust to the decrease in testosterone production. The effect of vaccination schedule on carcass traits was investigated by Lealiifano *et al.* (2011) who administered the booster vaccination at two, three, four and six weeks prior to slaughter. The results showed that vaccinating at four weeks showed no significant difference in hot carcass weight (HCW), dressing percentage and backfat depth. Vaccinating at two or three weeks prior to slaughter also had no influence on backfat depth and although the carcasses were lighter than those pigs vaccinated at four and six weeks, the dressing percentage was unaffected and the live weight at slaughter of the immunocastrates was unaffected by the vaccination schedule.

Backfat thickness is also influenced by diet, especially if the nutrient requirements are not being met by the supplied diet. Since the focus of pig production is lean meat production, dietary protein is of great importance and the amino acid lysine is especially important in muscle growth of the pig. If dietary digestible lysine is limiting for protein synthesis, the excess amino acids will be deaminated and stored as energy within adipose tissue. Therefore an increase in dietary lysine should decrease the backfat thickness as indicated by Boler *et al.* (2011), however, at a certain level of dietary lysine the requirements in terms of total protein will be oversupplied when maximal protein deposition rates are reached, if all amino acid ratios are maintained, and excess protein will again be deaminated and stored as fat. Boler *et al.* (2011) found that immunocastrates fed high dietary lysine (10 g lysine/kg) did not have significantly different backfat depths than entire males also fed high dietary lysine. These two treatments also had lower fat depths than the other immunocastrates fed at 7, 8 and 9 g lysine/kg, which may indicate that the requirements of immunocastrates do not differ from entire males and that 10 g lysine/kg is suitable for them. The dietary lysine levels also influenced the estimated carcass lean percentage, where entire males and immunocastrates fed high dietary lysine

had the highest lean yields, as well as the carcass cutting yields and lean cutting yields. Immunocastrates fed medium (9 g lysine/kg) and high (10g lysine/kg) lysine had greater carcass cutting yields and lean cutting yields than surgical castrates and their yields also increased as the dietary lysine increased. Immunocastration thus improves carcass yields, percentage fat free lean and did not influence meat quality when compared to surgical castrates. Although backfat measurement using the Hennessey Grading Probe (HGP) is common, carcass dissection yields more accurate results and thus commercial grading tends to underestimate the lean meat percentage (Dunshea *et al.*, 2001). The method of measurement should therefore be considered when results are being collected and compared.

Immunocastration tends to improve the lean meat percentage of the carcass when compared to surgical castration (Jaros *et al.*, 2005; Fuchs *et al.*, 2009a). For most carcass traits, immunocastrates seem to be intermediate to surgical castrates and entire males but more closely so to entire males, depending on the vaccination schedule (Bonneau *et al.*, 1994). Differences also exist between immunocastrates and surgical castrates in terms of carcass cutting yields, with immunocastration increasing the lean meat percentage in the belly (Dunshea *et al.*, 2001; Fuchs *et al.*, 2009a) but no differences have been seen for the loin, ham and shoulder cuts (Font-i-Furnols *et al.*, 2012b). Gispert *et al.* (2010) found that immunocastration increased the loin percentage but the belly cut percentage and the tenderloin percentage was not influenced when compared to entire males. Bonneau *et al.* (1994) found that immunocastrates had lighter heads, feet, loin, ham, shoulder but heavier fatty joints (kidney fat, backfat and belly) than entire males. Some studies investigating the carcass cutting yields of various sexes make use of the cut weights; however, it is more accurate to express the weight of each cut as a percentage of the carcass weight since variation in carcass weights will influence the cut weights, such that a pig that was heavier than the average weight will have heavier cuts. Although variation in body weight at the start of a trial should be minimized, this is not always possible with a large number of animals. It is thus important to block for body weight at the start of a trial or include initial weight as a covariate in the statistical analyses.

The quality of pork meat predominantly relies on the following properties; colour, lipid content and fatty acid composition, oxidative stability of these lipids, uniformity as well as expressible water holding capacity (WHC). These factors are important in terms of the consumer acceptance, processing and storage of pork but are a result of various aspects within pork production. Immunocastration seems to have little effect on the meat quality of the resultant pork (Gispert *et al.*, 2010) with consumer panels not being able to perceive any differences. Boler *et al.* (2011) found that immunocastration did not affect the shear force values or ultimate pH compared to surgical castrates and entire males while Pauly *et al.* (2009) found no differences on pH, colour and WHC, however, Braña *et al.* (2013) found greater loin drip losses in immunocastrates compared to barrows, however, this had no influence on the consumer panel evaluation. The consumer panel evaluation by D'Souza and Mullan (2002) found that immunocastrates had better eating quality compared to surgical castrates in one particular pig genotype and vice versa for another genotype. However, the trained sensory panel and consumer panel used by Font-i-Furnols *et al.* (2009) and Font-i-Furnols *et al.* (2008) respectively did not detect any differences between immunocastrates and surgical castrates in

terms of the eating quality of pork. Immunocastration may actually improve meat quality compared to entire males when group-housed pigs are evaluated since it decreases fighting lesions and stress. Dunshea *et al.* (2001) found that boars had the highest incidences of fighting lesions at slaughter and that immunocastration may prevent dark, firm and dry meat (DFD) due to glycogen exhaustion during physical exertion and pale, soft and exudative meat (PSE) caused by the stress of fighting.

In terms of the chemical composition, immunocastration does not influence the intramuscular fat (IMF) content of the *semimembranosus* muscle and *Longissimus thoracis* muscle compared to surgical castrates, entire males as well as female pigs (D'Souza & Mullan, 2002; Gispert *et al.*, 2010). However, feeding increasing levels of dietary lysine (7, 8, 9 and 10 g lysine/kg) decreases the extractable lipid content of the *Longissimus thoracis et lumborum* (LTL) of immunocastrates, probably due to the low and low/medium lysine diets being less than optimum for the growth requirement (Boler *et al.*, 2011). Although there seems to be little influence of immunocastration on the IMF, it does influence the fatty acid composition. Immunocastration decreases the saturated fatty acid content of the subcutaneous fat but does not influence the monounsaturated fatty acids compared to surgical castrates (Pauly *et al.*, 2009). However, Font-i-Furnols *et al.* (2012b) found no significant difference in saturated fatty acid composition between females, surgical castrates and immunocastrates. The diet will also influence the fatty acid composition of the fat, which is evident in the differences between the trials by Pauly *et al.* (2009) and Font-i-Furnols *et al.* (2012b). Pauly *et al.* (2009) used a diet with 2.1 % dietary crude fat, high in wheat while Font-i-Furnols *et al.* (2012b) used 3.45 % dietary crude fat which could have influenced the differences in results, since the energy contents vary and each sex has their own unique energy requirements. Font-i-Furnols *et al.* (2012b) concluded that immunocastration hardly changes the meat quality, fatty acid content and omega-6:omega-3 ratio of fresh meat and dry cured ham, which implies that the metabolic pathways of long chain omega-6 and omega-3 fatty acids are not affected.

The consumer acceptability of male pork products is not only important from a welfare aspect but also from a sensory aspect with respect to boar taint. Boar taint in entire males decreases the sensory acceptability of pork in consumers sensitive to it and is increasing in importance with the tendency to move away from surgical castration in the EU and the production of heavier male carcasses. The consumer acceptability of boar taint depends on their anosmia for androstenone and skatole. Up to 99 % of consumers are sensitive to skatole in pork (Weiler *et al.*, 1997) however the sensitivity to androstenone is ~ 45 % (Font-i-Furnols, 2012a) and is genetically determined (Wysocki & Beauchamp 1984). A world-wide study by Gilbert & Wysocki (1987) showed that women are more sensitive to androstenone than men in all countries and that the USA and UK had higher percentages of anosmic people than Africa, Asia, Australia, the Caribbean, Europe and Latin America. Thus immunocastration shows promise in terms of consumer acceptance since the levels of androstenone and skatole in immunocastrates are comparable to surgical castrates (Dunshea *et al.*, 2001) and Gispert *et al.* (2010) found that immunocastrates and surgical castrates had high consumer acceptability and were both better accepted than entire males.

Therefore immunocastration appears to have little effect on the meat quality of the result pork with very few of the instrumentally measured parameters that differed being perceived as different by

consumers. When compared to that of entire males, immunocastration improves the sensory quality of pork with regards to boar taint. Thus immunocastration improves the consumer acceptability of pork from male pigs from a sensory aspect as well as from an animal welfare point of view.

2.4.5 Reproductive system and functioning

The effect of immunocastration on the functioning of the reproductive system is especially important with regards to the testicles since it is the site of testosterone and androstenone production, which are the two most important factors involved in why producers consider vaccinating in the first place. The effects of immunocastration on the reproductive tract and functioning of male pigs seems to vary depending on the schedule used. The recommended vaccination schedule by the manufacturer specifies that the male pigs receive their first vaccination after eight to nine weeks of age, at least four weeks apart from the second and that the second be given no later than four to five weeks prior to slaughter. Thus depending on when the pig receives its second vaccination, the effect on the development of the reproductive system may vary. Various studies have shown that immunocastration decreases the size of the testicles (Dunshea *et al.*, 2001; Metz *et al.*, 2002; Zamaratskaia *et al.*, 2008a; Fuchs *et al.*, 2009b) as well as the seminal vesicles, epididymis and bulbourethral glands (Bonneau *et al.*, 1994; Dunshea *et al.*, 2001). Bulbourethral and testes sizes of immunocastrates can be up to 50 % lighter, which may indicate vaccination efficiency and monitoring on the slaughter line and Dunshea *et al.* (2001) thus suggested that a trimmed testes size of less than 400 g for Landrace x Large White immunocastrates with a mean hot carcass weight of 74.4 kg could indicate effective vaccination when the standard vaccination procedures are followed. Kubale *et al.* (2013) reported that the most differences in terms of weight were in the vesicular glands, then bulbourethral glands, testes and lastly the prostate. Even though there is an obvious effect on the testicle size, it has not been recommended to use their size as an indicator of the presence of boar taint (Fuchs *et al.*, 2009b).

In order to determine the effect of immunocastration on the testicular development and functioning, the histology of the testicles needs to be considered, since the Leydig cells are the site of steroid hormones production in the testicles, they are influenced by immunocastration. The number and nuclear area of the Leydig cells decrease in immunocastrated pigs in comparison to entire males with a smaller volume of interstitial tissue already seen from two weeks after second vaccination due the Leydig cells progressively losing shape as well as shrinkage of the cytoplasm and nucleus (Kubale *et al.*, 2013). When male pigs are vaccinated at 12 and then again at 19 weeks of age, spermatogenesis is clearly disrupted and Kubale *et al.* (2013) found that immunocastration caused mild atrophy of seminiferi with spermatocyte loss and lower germ cell numbers. At 21 weeks of age, spermatogenesis was impaired and disruption worsened until 27 weeks of age where there was almost a complete disappearance of the germ cell layer with undifferentiated germ cells, while the tubuli seminiferi were shrunken with many open spaces and almost no spermatocytes were seen. Kubale *et al.* (2013) also evaluated the histologic characteristics of the sexual accessory glands (bulbourethral gland, vesicular gland and prostate), which revealed a visible decrease in glandular

tissue of the bulbourethral gland and reduction in height of the vesicular gland epithelium. The histologic characteristics noted in terms of the regression of reproductive glands became more pronounced with increase in delay before slaughter, which is supported by Brunius *et al.* (2011) who found a more pronounced reduction in reproductive organ size in early vaccinated pigs due to arrest of development at an earlier age and prolonged time of reduced testicular function. In this situation testicle size may have a larger potential as a tool to identify non-immunised pigs than those vaccinated using the standard vaccination schedule.

2.5 Ractopamine hydrochloride: increased efficiency

In the pig industry, feed costs contribute a substantial proportion to the total cost of production. The energy and protein content of the diet are major determinants of performance and are the largest and thus most expensive component of feed. It is therefore important to make most efficient use of these sources and using feed additives such as ractopamine hydrochloride may assist this process. Ractopamine hydrochloride ($C_{18}H_{23}NO_3HCl$) is a beta-adrenergic agonist widely used as a feed additive in products such as Paylean[®] for pigs (Elanco[™] Animal Health, Eli Lilly and Company, Indianapolis). Paylean[®] is a granular premix which is mixed as part of a complete finishing ration to pigs for the last 20 to 40 kg of weight gain, receiving a diet containing at least 16 % crude protein. The mode of action is well documented with over 300 studies in 13 countries but its effect varies depending on the dosage level and duration; however, it is recommended by the manufacturer to feed Paylean[®] continuously at one of two recommended levels, 5 mg/kg or 10 mg/kg, with no withdrawal (Paylean Technical Manual, 2000). At 5 mg/kg, Paylean[®] improves weight gain rates as well as feed efficiency but at 10 mg/kg it also includes the benefits of increases carcass leanness and carcass dressing percentage (Paylean Technical Manual, 2000). When Paylean[®] is fed to pigs, no withdrawal period is needed and the acceptable daily intake is 1.25 µg/kg in the human diet according to classical and specialised toxicity tests (Freedom of Information Summary Supplemental New Animal Drug Application, 2006).

The primary action of Paylean[®] is to increase muscle protein synthesis and the amount of meat in high value cuts and thus improve production efficiency. This effect has been supported by carcass and primal cut measurements as well as carcass dissections; which all showed that Paylean[®] increases carcass lean while decreasing fat and waste and its effects are more evident when the carcass is divided into primal cuts and then boneless cuts (Paylean Technical Manual, 2000). The active ingredient of Paylean[®] is ractopamine hydrochloride (RAC) and is classified as a phenethanolamine which has beta-adrenergic activity. Ractopamine hydrochloride mimics the effects of epinephrine and is a β -1 selective adrenergic agonist which redirects available nutrients away from lipogenesis while stimulating protein synthesis thereby resulting in more lean growth (Paylean Technical Manual, 2000). Once absorbed into the blood stream, RAC binds to and stimulates beta receptors located on the surfaces of adipose tissue and skeletal muscle cells. The exact action of RAC on protein metabolism is not well understood however it is postulated that it influences cyclic AMP mediated processes such as transcription, translation and protein synthesis. In pig, α -actin

mRNA increased when fed ractopamine (Helferich *et al.*, 1990) which indicates that either transcription rate and/or stability of mRNA increases in the cytoplasm of the muscle cell thus increasing protein synthesis capacity. In terms of fat metabolism, RAC binds to beta receptors located on the surface of adipose cells thus activating adenyl cyclase which stimulates cyclic AMP production. This then activates protein kinases which have various influences on other enzymes involved in fatty acid synthesis as well as lipolysis (Paylean Technical Manual, 2000). The enzymes fatty acid synthetase, lipoprotein lipase and malic enzyme decrease in activity and the sensitivity of adipose cells to insulin is also decreased in response to RAC (Williams *et al.*, 1987; Hausman *et al.*, 1989; Mills *et al.*, 1990). Thus, RAC decreases lipogenesis while increasing protein synthesis but there is currently no evidence of RAC inhibiting protein degradation. The resultant effects include increased daily gain, decreased feed intake and improved feed efficiency.

2.5.1 Effect on performance and nutritional requirements

Ractopamine hydrochloride supplementation has various benefits in terms of growth and slaughter performance; however, the effects vary depending on the inclusion level and duration used. According to the Paylean Technical Manual (2000), an inclusion level of 5 mg/kg improves weight gain rates and feed efficiency but at 10 mg/kg the carcass leanness and carcass dressing percentage is also improved when fed for the last 20 to 40 kg of weight gain before slaughter. The recommended feeding directions for RAC stipulate that it be fed continuously for no longer than six weeks from a minimum starting weight of 70 kg to pigs receiving a diet containing at least 16 % crude protein or its equivalent by amino acid fortification with 8.5 to 9.5 g lysine/kg. The maximum growth response occurs within approximately the first two weeks of inclusion (Jacela *et al.*, 2009) and then the response declines as β -adreno receptors are down-regulated in adipose tissue or desensitized in skeletal muscle tissue (Spurlock *et al.*, 1994).

Apple *et al.* (2007) reviewed the effects of RAC at various inclusion levels from 23 published reports on live growth performed between the early 1990s to 2005 and found that regardless of the inclusion level, pigs fed RAC gained faster and more efficiently; they also produced heavier, more muscular carcasses. The results from the meta-analysis confirms the statement that the carcass leanness increases at 10 mg/kg, with those studies using 10 mg/kg and 20 mg/kg showing a greater percentages of fat-free lean than untreated controls. However little incentive is available to make use of 20 mg/kg inclusion level due to the high cost of RAC relative to the benefits. The ADG was improved by 12 % at 5 mg/kg and 11.8 % at 10 or 20 mg/kg and feed efficiency was improved by 6.3 % to 17.2 % at 5 mg/kg and 5.6 % to 25.9 % at 10 mg/kg of RAC compared to controls. There was very little effect on the feed intake, but it showed a tendency to be reduced with the inclusion of RAC in all but one trial (Apple *et al.*, 2007). Jacela *et al.* (2009) also found that feeding RAC at 5 mg/kg during last three weeks of growth improved growth performance in terms of a greater ADG and better feed to gain ratio, as well as increased carcass weights and yields. With regards to sex, the response in growth rate to RAC is less in entire males than in gilts and surgical castrates when a level of 20 mg/kg is used (Dunshea *et al.*, 1993). However, Braña *et al.* (2013) investigated the effect of RAC

supplementation at 5 mg/kg on surgical castrates, gilts and immunocastrates and found a lack of gender x RAC interaction for growth rate and feed efficiency, which meant that the improvements in these traits were consistent for gilts, surgical castrates and immunocastrates. Thus all sexes had greater ADG and gain:feed with a heavier carcass weight, but RAC had little effect on the feed intake of immune or surgical castrates while increasing the intake of gilts. These results are further supported by Rikard-Bell *et al.* (2009) who found no interaction between intact males, immunocastrates and gilts fed a step-up program of 5 mg/kg for 14 days and 10 mg/kg for 17 days.

Due to the increase in protein accretion, adequate amino acids and protein must be provided in order to realize maximum benefits of RAC. The energy content of the diet seems to have little effect on the action of RAC, which is supported by Hinson *et al.* (2011) who found no dietary energy x RAC interactions for growth performance, carcass characteristics and meat quality, which meant that the improvements were present regardless of differing dietary energy levels. Webster *et al.* (2007) found that a higher lysine level was needed to generate an increase in growth performance when RAC was included in the diet and concluded that finisher pigs of 79 – 109 kg require 12 g dietary lysine/kg for a response in growth performance at 10 mg/kg RAC, which is higher than commercially used. The influence of varying energy, amino acids and RAC at 5 mg/kg on immunocastrates was investigated by Lanferdini *et al.* (2013) and no significant differences were seen between immunocastrates and entire males for weight gain, feed intake or feed efficiency. This could indicate that the requirements of immunocastrates may not differ from entire males fed RAC at 5 mg/kg if they receive their vaccination four weeks prior to slaughter. Since information regarding feeding RAC to immunocastrates is limited, it is important to further investigate its effects on their growth performances.

2.5.2 Influence on carcass composition and meat quality

The inclusion of RAC at 5 mg/kg and 10 mg/kg increases the hot carcass weight (HCW) by approximately 2.3 % and 7.7 % respectively, and the dressing percentage by 0.2 % at 5 mg/kg and 0.6 % at 10 mg/kg (Apple *et al.*, 2007). At 10 mg/kg RAC the conclusions with regards to HCW differ, with those studies which found a significant increase in HCW (Stites *et al.*, 1991; Herr *et al.*, 2001; Merchant-Forde *et al.*, 2003; Armstrong *et al.*, 2004; Carr *et al.*, 2005a; See *et al.*, 2005;), those who found no effect (Watkins *et al.*, 1990; Crome *et al.*, 1996; Stoller *et al.*, 2003; Carr *et al.*, 2005b) and those who found a decrease in HCW (Aalhus *et al.*, 1990). However, a later meta-analysis of 29 publications from 1990 to 2007 by Andretta *et al.* (2012) agreed with Apple *et al.* (2007) that feeding RAC increased HCW. When RAC was fed to immunocastrates (step-up of 5 mg/kg for 14 days followed by 10 mg/kg for 17 days), Rikard-Bell *et al.* (2009) found that not only was the HCW increased, but the HCW of immunocastrates was greater than that of boars and gilts.

The results for the effect of RAC on backfat thickness varies since the duration of RAC supply, level of inclusion as well as the estimated RAC intake is inversely correlated to the backfat thickness (Andretta *et al.*, 2012). Again, the conclusions with regards to backfat depth vary with those who have found significant decreases in backfat depth (Watkins *et al.*, 1990; Merchant-Forde *et al.*,

2003; Apple *et al.*, 2004a; Carr *et al.*, 2005b) and those who have not (Aalhus *et al.*, 1990; Stites *et al.*, 1991; Crome *et al.*, 1996; Herr *et al.*, 2001; Stoller *et al.*, 2003; Carr *et al.*, 2005a; See *et al.*, 2005; Hinson *et al.*, 2011). Therefore, the theory that RAC repartitions nutrients away from lipid synthesis to protein accretion may be questioned. This is highlighted by the conflicting results shown in Apple *et al.*'s (2007) meta-analysis where the effects of 5 mg/kg and 10 mg/kg RAC on 10th rib backfat thickness varied from -10 to 15.3 % and -16.1 to 6.6 % respectively. However, the meta-analysis showed that on average, the backfat depth was reduced by 0.04 and 0.14 % for 5 mg/kg and 10 mg/kg RAC respectively, while the loin muscle area was increased by 0.3 and 3.5 % for 5 mg/kg and 10 mg/kg RAC respectively. When the effect of RAC on IMF content is considered, Aalhus *et al.* (1990), Stoller *et al.* (2003) and Carr *et al.* (2005a, b) reported no significant differences between pigs fed 10 mg/kg RAC and controls. However, Braña *et al.* (2013) found that the response of immunocastrates to 5 mg/kg RAC differed to that of surgical castrates in terms of IMF, since RAC decreased the IMF content in the surgical castrates but had no significant effect on the IMF of immunocastrates. Thus it appears as though RAC will have little to no effect on the IMF or marbling of pork from immunocastrated pigs. Although a number of researchers failed to show effect on midline and 10th rib fat depth, results show that RAC reduces carcass fat percentage when carcass dissections are performed (Apple *et al.* 2007). Including RAC can alter the fatty acid composition of subcutaneous fat by increasing proportions of polyunsaturated fatty acids, linoleic acid and linolenic acid with minor effects on proportions of saturated fatty acids and monounsaturated fatty acids (Apple *et al.* 2007), however Braña *et al.* (2013) showed that RAC had no effect on pork flavour which largely depends on the IMF content and fatty acid composition.

Research has suggested that due to the shift in muscle fibre type and size, RAC may influence the meat colour and increase the processing yields of pork. According to Aalhus *et al.* (1992), RAC caused an increase in the production of white muscle fibres with larger diameters and fewer intermediate fibres, while not affecting the total number of fibres or the diameter of red fibres. Rikard-Bell *et al.* (2009) showed that RAC increased the loin percentage, while Moore *et al.* (2009) who found that RAC increased lean tissue weights. Although RAC does not appear to have an influence on the CIE L* colour values (Aalhus *et al.*, 1990; Herr *et al.*, 2001; Stoller *et al.*, 2003; Armstrong *et al.*, 2004; Carr *et al.*, 2005a, b; Athayde *et al.*, 2012) RAC decreases the CIE a* and b* values and was thus less red and less yellow (Apple *et al.*, 2007). The increase in muscle fibre diameter may also be responsible for the increase in Warner Bratzler shear force values associated with RAC-fed pork (Aalhus *et al.*, 1990; Uttaro *et al.*, 1993; Carr *et al.*, 2005a, b; Athayde *et al.*, 2012). However, Apple *et al.* (2007) showed that most studies indicated little or no effect on pork eating quality. This is supported by the fact that the *post mortem* meat pH and temperature is not influenced by RAC (Aalhus *et al.*, 1990; Stites *et al.*, 1991; Dunshea *et al.*, 1993; Stoller *et al.*, 2003; Carr *et al.*, 2005a, b; Athayde *et al.*, 2012) nor the drip loss and cooking loss (Dunshea *et al.*, 1993; Uttaro *et al.*, 1993; Stoller *et al.*, 2003; Carr *et al.*, 2005a; Athayde *et al.*, 2012).

2.6 Conclusion

The banning of surgical castration in various EU countries has raised concerns about boar taint and management issues of entire males pigs and thus immunological castration appears to be an attractive solution to managing boar taint and aggression without the use of surgical castration, since if the correct vaccination protocol is followed, growth performance and meat quality does not seem to be negatively affected compared to entire males. When following standard vaccination protocol, immunocastrates grow as entire males until their second vaccination where lipid deposition and feed intake begins to increase. However, this increase can be managed should the minimum of four weeks between the booster and slaughter be used. The use of RAC is also promising in order to decrease the lipid deposition during the period between the booster and slaughter; however, it is essential that the correct dietary and protein levels be supplied in order to reap these benefits. The production of immunocastrates in this way could allow for the capitalisation of the lean and efficient growth of an entire male until the booster is administered, after which RAC may be supplied along with the correct diet in order to produce heavier male carcasses without boar taint. This may allow for a larger margin in which profit can be achieved per carcass, as well as an increase in the yields of high value cuts such as the loin. Although it has been established that approximately seven days is needed for the metabolic changes in immunocastrates, it is not clear as to how this effects their nutritional requirements and the current practise to feed immunocastrates as surgical castrates following their second vaccination may be incorrect. Since the primary focus of pig production is meat and RAC increases protein synthesis, muscle growth must be maximised and thus the dietary lysine and overall balanced protein content of the diet must not be limiting. Thus immunocastrated pigs fed RAC are likely to benefit from higher dietary protein levels than estimated for surgical castrates.

Therefore, there is currently a substantial body of literature in terms of the effects of immunocastration on the performance, behaviour, hormone levels and vaccination schedule of growing pigs; however, few of these studies have addressed how immunocastration and RAC affect their nutrient responses. It is thus necessary to understand differences in the rates of protein and lipid growth of immunocastrates in order to correctly provide nutrients for them. Therefore optimal balanced protein levels for immunocastrates fed with and without RAC need to be established in terms of their growth performance, carcass characteristics and yields.

2.7 References

- Aalhus, J., Jones, S., Schaefer, A., Tong, A., Robertson, W., Murray, A. & Merrill, J. (1990). The effect of ractopamine on performance, carcass composition and meat quality of finishing pigs. *Canadian Journal of Animal Science*, 70, 943–952.
- Andretta, I., Kipper, M., Lehnen, C. R., Demori, A. B., Remus, A. & Lovatto, P. A. (2012). Meta-analysis of the relationship between ractopamine and dietary lysine levels on carcass characteristics in pigs. *Livestock Science*, 143, 91–96.

- Apple, J., Rincker, P., McKeith, F., Carr, S., Armstrong, T. & Matzat, P. (2007). Review: Meta-analysis of the ractopamine response in finishing swine. *The Professional Animal Scientist*, 23, 179–196.
- Armstrong, T., Ivers, D., Wagner, J., Anderson, D., Weldon, W. & Berg, E. (2004). The effect of dietary ractopamine concentration and duration of feeding on growth performance, carcass characteristics, and meat quality of finishing pigs. *Journal of Animal Science*, 82, 3245–3253
- Athayde, N., Dalla Costa, O., Roça, R., Guidoni, A., Ludtke, C. & Lima, G. (2012). Meat quality of swine supplemented with ractopamine under commercial conditions in Brazil. *Journal of Animal Science*, 90, 4604–4610.
- Babol, J., Squires, E. J. & Lundström, K. (1999). Relationship between metabolism of androstenone and skatole in intact male pigs. *Journal of Animal Science*, 77, 84–92.
- Bauer, A., Lacorn, M. & Claus, R. (2009). Effects of two levels of feed allocation on IGF-I concentrations and metabolic parameters in GnRH-immunized boars. *Journal of Animal Physiology and Animal Nutrition*, 93, 744–753.
- Boler, D., Kutzler, L., Meeuwse, D., King, V., Campion, D., McKeith, F. & Killefer, J. (2011). Effects of increasing lysine on carcass composition and cutting yields of immunologically castrated male pigs. *Journal of Animal Science*, 89, 2189–2199.
- Bonneau, M. (1982). Compounds responsible for boar taint, with special emphasis on androstenone: a review. *Livestock Production Science*, 9, 687-705.
- Bonneau, M., Dufour, R., Chouvet, C., Roulet, C., Meadus, W. & Squires, E. (1994). The effects of immunization against luteinizing hormone-releasing hormone on performance, sexual development, and levels of boar taint-related compounds in intact male pigs. *Journal of Animal Science*, 72, 14–20.
- Booth, W. (1975). Changes with age in the occurrence of C₁₉ steroids in the testis and submaxillary gland of the boar. *Journal of Reproduction and Fertility*, 42, 459–472.
- Braña, D., Rojo-Gómez, G., Ellis, M. & Cuaron, J. (2013). Effect of gender (gilt and surgically and immunocastrated male) and ractopamine hydrochloride supplementation on growth performance, carcass, and pork quality characteristics of finishing pigs under commercial conditions. *Journal of Animal Science*, 91, 5894–5904.
- Brunius, C., Zamaratskaia, G., Andersson, K., Chen, G., Norrby, M., Madej, A. & Lundström, K. (2011). Early immunocastration of male pigs with Improvac[®] - Effect on boar taint, hormones and reproductive organs. *Vaccine*, 29, 9514–9520.
- Carr, S., Ivers, D. J., Anderson, D. B., Jones, D. J., Mowrey, D.H, England, M. B., Killefer, J., Rincker, P.J., & McKeith, F. (2005a). The effects of ractopamine hydrochloride on lean carcass yields and pork quality characteristics. *Journal of Animal Science*, 83, 2886–2893.
- Carr, S., Rincker, P., Killefer, J., Baker, D., Ellis, M. & McKeith, F. (2005b). Effects of different cereal grains and ractopamine hydrochloride on performance, carcass characteristics, and fat quality in late-finishing pigs. *Journal of Animal Science*, 83, 223–230.

- Claus, R., Lacorn, M., Danowski, K., Pearce, M. C. & Bauer, A. (2007). Short-term endocrine and metabolic reactions before and after second immunization against GnRH in boars. *Vaccine*, *25*, 4689–4696.
- Claus, R., Weiler, U. & Herzog, A. (1994). Physiological aspects of androstenone and skatole formation in the boar- a review with experimental data. *Meat Science*, *38*, 289–305.
- Crome, P. K., McKeith, F. K., Carr, T. R., Jones, D. J., Mowrey, D. H., & Cannon, J. E. (1996). Effect of ractopamine on growth performance, carcass composition, and cutting yields of pigs slaughtered at 107 and 125 kilograms. *Journal Animal Science*, *74*, 709-716.
- Cronin, G., Dunshea, F., Butler, K., McCauley, I., Barnett, J. & Hemsworth, P. (2003). The effects of immuno-and surgical-castration on the behaviour and consequently growth of group-housed, male finisher pigs. *Applied Animal Behaviour Science*, *81*, 111–126.
- D'Souza, D., and Mullan, B. (2002). The effect of genotype, sex and management strategy on the eating quality of pork. *Meat Science*, *60*, 95–101.
- Dunshea, F., Colantoni, C., Howard, K., McCauley, I., Jackson, P., Long, K., Lopaticki, S., Nugent, E., Simons, J., Walker, J., & Hennessey, D. P. (2001). Vaccination of boars with a GnRH vaccine (Improvac) eliminates boar taint and increases growth performance. *Journal of Animal Science*, *79*, 2524–2535.
- Dunshea, F., King, R., Campbell, R., Sainz, R. & Kim, Y. (1993). Interrelationships between sex and ractopamine on protein and lipid deposition in rapidly growing pigs. *Journal of Animal Science*, *71*, 2919–2930.
- Font i Furnols, M., Gispert, M., Guerrero, L., Velarde, A., Tibau, J., Soler, J., Hortós, M., García-Regueiro, J., Pérez, J., Suárez, P. & Oliver, M. (2008). Consumers' sensory acceptability of pork from immunocastrated male pigs. *Meat Science*, *80*, 1013–1018.
- Font i Furnols, M., González, J., Gispert, M., Oliver, M., Hortós, M., Pérez, J., Suárez, P. & Guerrero, L. (2009). Sensory characterization of meat from pigs vaccinated against gonadotropin releasing factor compared to meat from surgically castrated, entire male and female pigs. *Meat Science*, *83*, 438–442.
- Font-i-Furnols, M. (2012a). Consumer studies on sensory acceptability of boar taint: A review. *Meat Science*, *92*, 319–329.
- Font-i-Furnols, M., Gispert, M., Soler, J., Diaz, M., Garcia-Regueiro, J., Diaz, I. & Pearce, M. (2012b). Effect of vaccination against gonadotrophin-releasing factor on growth performance, carcass, meat and fat quality of male Duroc pigs for dry-cured ham production. *Meat Science*, *91*, 148–154.
- Fredriksen, B., Font-i- Furnols, M., Lundström, K., Migdal, W., Prunier, A., Tuttysens, F. A. M., Bonneau, M. (2009). Practise on castration of piglets in Europe. *Animal*, *3*, 1480-1487.
- Freedom of Information Summary Supplemental New Animal Drug Application PAYLEAN 9 and PAYLEAN 45 (Ractopamine Hydrochloride) Type A Medicated Article for Finishing Swine (NADA 140-863)*. (2006). Retrieved from: <http://www.fda.gov/downloads/AnimalVeterinary/Products/ApprovedAnimalDrugProducts/FOIA DrugSummaries/ucm115647.pdf> on 9 September 2014.

- Fuchs, T., Nathues, H., Koehrmann, A., Andrews, S., Brock, F., Sudhaus, N., Klein, G., grosse Beilage, E. (2009a). A comparison of the carcass characteristics of pigs immunized with a 'gonadotrophin-releasing factor (GnRF)' vaccine against boar taint with physically castrated pigs. *Meat science*, 83, 702–705.
- Fuchs, T., Thun, R., Parvizi, N., Nathues, H., Koehrmann, A., Andrews, S., Brock, F., Klein, G., Sudhaus, N., & grosse Beilage, E. (2009b). Effect of a gonadotropin-releasing factor vaccine on follicle-stimulating hormone and luteinizing hormone concentrations and on the development of testicles and the expression of boar taint in male pigs. *Theriogenology*, 72, 672–680.
- Gilbert, A. N., & Wysocki, C. J. (1987). The smell results: Survey. *National Geographic*, October (p 514-525).
- Gispert, M., Oliver, M. A., Velarde, A., Suarez, P., Pérez, J. & Font i Furnols, M. (2010). Carcass and meat quality characteristics of immunocastrated male, surgically castrated male, entire male and female pigs. *Meat Science*, 85, 664–670.
- Hausman, D. B., Martin, R. J., Veenhuizen E. L. & Andreson, D. B. (1989). Effect of ractopamine on insulin sensitivity and response to isolated rat adipocytes. *Journal of Animal Science*, 67, 1455-1464.
- Helferich, W. G., Jump, D. B., Anderson, D. B., Skjaerlund, D. M., Merkel, R. A., Bergen, W. G. (1990). Skeletal Muscle α -Actin Synthesis Is Increased Pretranslationally in Pigs Fed the Phenethanolamine Ractopamine. *Endocrinology*, 126, 3096-3100.
- Herr, C. T., Hankins, S. L., Schinckel, A. P., & Rickert, B. T. (2001). Evaluation of three genetic populations of pigs for response to increasing levels of ractopamine. *Journal of Animal Science*, 79 (Suppl. 2), 73.
- Hinson, R., Wiegand, B., Ritter, M., Allee, G. & Carr, S. (2011). Impact of dietary energy level and ractopamine on growth performance, carcass characteristics, and meat quality of finishing pigs. *Journal of Animal Science*, 89, 3572–3579.
- Hughes, P. E. & Varley, M. A. (1980). *Reproduction in the pig*. London, United Kingdom: Butterworth & Co. (pp 5-9.)
- Improvac: *Product Overview*. Retrieved from: <http://www.improvac.co.nz/sites/improvac/en-NZ/Pages/productoverview.aspx> online on 5 August 2014
- Jacela, J., Tokach, M. D., DeRouchey, J. M., Goodband, R. D., Nelssen, J. L. & Dritz, S. S. (2009). Effect of constant or step-up ractopamine HCl (Paylean) feeding programs on growth performance and carcass characteristics of late-finishing pigs. *Report of progress (Kansas State University. Agricultural Experiment Station and Cooperative Extension Service); 1020*. Kansas State University. Agricultural Experiment Station and Cooperative Extension Service.
- Jaros, P., Bürgi, E., Stärk, K., Claus, R., Hennessy, D. & Thun, R. (2005). Effect of active immunization against GnRH on androstenone concentration, growth performance and carcass quality in intact male pigs. *Livestock Production Science*, 92, 31–38.
- Kubale, V., Batorek, N., Škrlep, M., Prunier, A., Bonneau, M., Fazarinc G., Čandek-Potokar, M. (2013). Steroid hormones, boar taint compounds, and reproductive organs in pigs according to the delay between immunocastration and slaughter. *Theriogenology*, 79, 69-80.

- Lanferdini, E., Lovatto, P., Melchior, R., Orlando, U., Ceccantini, M. & Poleze, E. (2013). Feeding surgically castrated, entire male and immunocastrated pigs with different levels of amino acids and energy at constant protein to energy ratio with or without ractopamine. *Livestock Science*, *151*, 246–251.
- Lealiifano, A., Pluske, J. R., Nicholls, R., Dunshea, F., Campbell, R., Hennessy, D., Miller, D., Hansen, C. F., & Mullan, B. (2011). Reducing the length of time between slaughter and the secondary gonadotropin-releasing factor immunization improves growth performance and clears boar taint compounds in male finishing pigs. *Journal of Animal Science*, *89*, 2782–2792.
- Marchant-Forde, J. N., Lay, D. C., Pajor, E. A., Richert, B. T., & Schinckel, A. P. (2003). The effects of ractopamine on the behavior and physiology of finishing pigs. *Journal of Animal Science*, *81*, 416-422.
- Metz, C., Hohl, K., Waidelich, S., Drochner, W. & Claus, R. (2002). Active immunization of boars against GnRH at an early age: consequences for testicular function, boar taint accumulation and N-retention. *Livestock Production Science*, *74*, 147–157.
- Mills, S. E., Lui, C. Y., Schinckel A. P. (1990). Effects of ractopamine on adipose tissue metabolism and insulin binding in finishing hogs, interaction with genotype and slaughter weight. *Domestic animal Endocrinology*, *7*, 251-264.
- Moore, K., Dunshea, F., Mullan, B., Hennessy, D. & D'Souza, D. (2009). Ractopamine supplementation increases lean deposition in entire and immunocastrated male pigs. *Animal Production Science*, *49*, 1113–1119.
- Patterson, R. L. S. (1968a). 5 α -Androst-16-ene-3-one: Compound responsible for taint in boar fat. *Journal of Science and Food Agriculture*, *19*, 31–37.
- Patterson, R.L.S. (1968b). Acidic components of boar preputial fluid. *Journal of the Science of Food and Agriculture*, *19*, 38-40.
- Pauly, C., Spring, P., O'Doherty, J., Ampuero Kragten, S. & Bee, G. (2009). Growth performance, carcass characteristics and meat quality of group-penned surgically castrated, immunocastrated (Improvac[®]) and entire male pigs and individually penned entire male pigs. *Animal*, *3*, 1057–1066.
- Paylean[™] Technical Guide, Version 1.0. (2000). Prepared by R. D. Muller (Technical Services). Elanco Animal Health, A Division of Eli Lilly and Company, Four Parkwood, Suite 125, 500 E. 96th St., Indianapolis, Indiana, 46240, U.S.A.
- Rault, J.L., Lay, D. C. & Marchant-Forde, J. N. (2011). Castration induced pain in pigs and other livestock. *Applied Animal Behaviour Science*, *135*, 214–225.
- Rikard-Bell, C., Curtis, M., Van Barneveld, R. J., Mullan, B., Edwards, A. C., Gannon, N., Henman, D. J., Hughes, P. E., & Dunshea, F. (2009). Ractopamine hydrochloride improves growth performance and carcass composition in immunocastrated boars, intact boars, and gilts. *Journal of Animal Science*, *87*, 3536–3543.
- See, M. T., Armstrong, T. A., Matzat, P. D., Belstra, B. A., McKeith, F. K., Rincker, P. J., Xi, L., Odle, J., Culbertson, M., Herring, W., & Hansen, J. (2005). Effect of ractopamine feeding level on growth performance and carcass composition. *Journal of Animal Science*, *83* (Suppl. 2), 27.

- Spurlock, M., Cusumano, J., Ji, S., Anderson, D., Smith, C., Hancock, D. & Mills, S. (1994). The effect of ractopamine on beta-adrenoceptor density and affinity in porcine adipose and skeletal muscle tissue. *Journal of Animal Science*, *72*, 75–80.
- Stites, C., McKeith, F., Singh, S., Bechtel, P., Mowrey, D. & Jones, D. (1991). The effect of ractopamine hydrochloride on the carcass cutting yields of finishing swine. *Journal of Animal Science*, *69*, 3094–3101.
- Stoller, G., Zerby, H., Moeller, S., Baas, T., Johnson, C. & Watkins, L. (2003). The effect of feeding ractopamine (Paylean) on muscle quality and sensory characteristics in three diverse genetic lines of swine. *Journal of Animal Science*, *81*, 1508–1516.
- Tuytens, F., Vanhonacker, F., Verhille, B., De Brabander, D., Verbeke, W. (2012). Pig producer attitude towards surgical castration of piglets without anaesthesia versus alternative strategies. *Research in Veterinary Sciences*, *79*, 69-80.
- Uttaro, B., Ball, R., Dick, P., Rae, W., Vessie, G. & Jeremiah, L. (1993). Effect of ractopamine and sex on growth, carcass characteristics, processing yield, and meat quality characteristics of crossbred swine. *Journal of Animal Science*, *71*, 2439–2449.
- Watkins, L. E., Jones, D. J., Mowrey, D. H., Anderson, D. B. & Veenhuizen, E. L. (1990). The effect of various levels of ractopamine hydrochloride on the performance and carcass characteristics of finishing swine. *Journal of Animal Science*, *68*, 3588-3595.
- Webster, M. J., Goodband, R., Tokach, M., Nelssen, J., Dritz, S., Unruh, J., Brown, K., Real, D., Derouchey, J., Woodworth, J., Groesbeek, C. N., & Marsteller, T. A. (2007). Interactive effects between ractopamine hydrochloride and dietary lysine on finishing pig growth performance, carcass characteristics, pork quality, and tissue accretion. *The Professional Animal Scientist*, *23*, 597–611.
- Weiler, U., Fischer, K., Kemmer, H., Dobrowski, A., & Claus, R. (1997) Influence of androstenone sensitivity on consumer reactions to boar meat. In M. Bonneau, K. Lundström, & B. Malmfors (Eds.), *Boar taint in entire male pigs. European Association for Animal Production Working Group, Stockholm, Sweden. EAAP Publication No. 92.* (pp. 147-151): Wageningen Pers.
- Williams, A. C., Brunjes, P. M., Martin, R. J., Veenhuizen, E. L. & Anderson, D. B. (1987). Influence of feeding ractopamine to finishing swine on the in vitro response of adipose tissue to ractopamine and insulin. *Journal of Animal Science*, *65* (Supp 1): 249 (abstract nr 139).
- Wysocki, C. J., & Beauchamp, G. K. (1984). Ability to smell androstenone is genetically determined. *Proceedings of the Natural Academy of Sciences of the United States of America*, *81*, 4899-4902.
- Zamaratskaia, G., Babol, J., Andersson, H. & Lundström, K. (2004a). Plasma skatole and androstenone levels in entire male pigs and relationship between boar taint compounds, sex steroids and thyroxine at various ages. *Livestock Production Science*, *87*, 91–98.
- Zamaratskaia, G., Babol, J., Madej, A., Squires, E. & Lundström, K. (2004b). Age-related Variation of Plasma Concentrations of Skatole, Androstenone, Testosterone, Oestradiol-17 β , Oestrone Sulphate, Dehydroepiandrosterone Sulphate, Triiodothyronine and IGF-1 in Six Entire Male Pigs. *Reproduction in Domestic Animals*, *39*, 168–172.

- Zamaratskaia, G., Andersson, H., Chen, G., Andersson, K., Madej, A. & Lundström, K. (2008a). Effect of a Gonadotropin-releasing Hormone Vaccine (ImprovacTM) on Steroid Hormones, Boar Taint Compounds and Performance in Entire Male Pigs. *Reproduction in Domestic Animals*, 43, 351–359.
- Zamaratskaia, G., Rydhmer, L., Andersson, H. K., Chen, G., Lowagie, S., Andersson, K. & Lundström, K. (2008b). Long-term effect of vaccination against gonadotropin-releasing hormone, using ImprovacTM, on hormonal profile and behaviour of male pigs. *Animal Reproduction Science*, 108, 37–48.
- Zeng, X., Turkstra, J., Jongbloed, A., Van Diepen, J. T. M., Meloen, R., Oonk, H., Guo, D., & Van de Wiel, D. (2002). Performance and hormone levels of immunocastrated, surgically castrated and intact male pigs fed ad libitum high-and low-energy diets. *Livestock Production Science*, 77, 1–11.

CHAPTER 3

Growth responses of entire and immunocastrated male pigs to dietary protein with and without ractopamine hydrochloride

Abstract

The growth performance of 120 individually penned entire (E) and immunocastrated (C) male pigs (PIC[®] Large White x Landrace x White Duroc maternal line cross PIC 410 terminal sire) fed diets containing varying levels of balanced protein with or without ractopamine hydrochloride (RAC) were evaluated. The pigs entered the trial at 16 weeks of age (live weight = 57.52 ± 5.36 kg) and were assigned to 12 treatments using a 2 x 2 x 3 factorial design. Three diets were formulated with increasing lysine levels; 7.50 g/kg (low), 9.79 g/kg (medium) and 12.07 g/kg (high) and all other amino acids balanced in terms of lysine. The treatment combinations were: E Low, E Medium and E High; E Low RAC, E Medium RAC and E High RAC; C Low, C Medium and C High; and C Low RAC, C Medium RAC and C High RAC. Vaccination protocol involved two doses of Improvac[®] administered at 16 and 20 weeks of age, four weeks before slaughter. Ractopamine hydrochloride was supplemented to the applicable treatments for 28 days preslaughter. The live weight, backfat depth and feed intake were measured on a weekly basis and used to determine the growth rate (average daily gain/ADG), change in voluntary feed intake, feed conversion ratio and backfat gain. Immunocastrated pigs fed RAC had the highest body weights from 22 weeks of age, with immunocastration and RAC improving the ADG. Immunocastration increased the average daily feed intake (ADFI) and backfat deposition rate while increasing the FCR after the second vaccination. The effect of RAC on the feed conversion ratio (FCR) depended on the level of dietary balanced protein, with the medium and high protein diets providing the best FCRs. Therefore, immunocastrated pigs performed as entire males until the second vaccination, after which ADFI, FCR and leanness was sacrificed, while RAC supplementation improved feed efficiency when pigs were fed the appropriate dietary protein level.

Keywords: Swine, Paylean, GnRH, Boar taint, ADG, ADFI

3.1 Introduction

The increase in consumer awareness in terms of animal welfare and the ethical treatment of animals in meat production systems has raised various issues within the pork industry. One such issue is the surgical castration of piglets without anaesthesia; however, the production of intact boars raises different issues such as aggression, sexual behaviour and boar taint. According to a review by Font-i-Furnols (2012), the consumer acceptability of pork is decreased by boar taint and depends on the levels of both androstenone and skatole. The sensitivity of consumers also differs, with females being more sensitive than males (Font-i-Furnols, 2012) and it is likely that the consumer acceptability of pork will decrease with the current movement away from surgical castration of male pigs. Thus the banning of surgical castration in various European Union countries has led to the increased interest in alternatives to controlling the prevalence of boar taint such as chemical castration, otherwise known as immunocastration.

Currently, the only registered vaccine available for immunocastration is a product known as Improvac[®] (Improvest[®]/ Vivax[®]/ Innosure[®]) which contains an incomplete analogue of gonadotropin-

releasing hormone conjugated with a carrier protein in an aqueous adjuvant (Fuchs *et al.*, 2009) and is manufactured by ZoetisTM (Pfizer Animal Health). The standard recommended vaccination protocol involves two vaccinations given at least four weeks apart and four to six weeks prior to slaughter. The first vaccination primes the body for the second (booster) vaccination, after which a rapid increase in circulating antibodies against GnRH is initiated. These antibodies bind to GnRH and render it unable to attach to the receptors located on the pituitary gland, thus disrupting the hypothalamic-pituitary-gonadal axis. This removes the stimulus for LH and FSH production and ultimately steroid production by the testes; which is essentially the same desired effect as surgical castration. Antibody titres increase within seven days of the booster vaccination, reach a peak and then decline slightly but remain elevated up until slaughter, even up to twenty-two weeks after the booster vaccination using the standard vaccination schedule (Claus *et al.*, 2007; Zamaratskaia *et al.*, 2008; Brunius *et al.*, 2011). This causes an immediate and significant decrease in LH (Dunshea *et al.*, 2001; Claus *et al.*, 2007) followed by a decrease in testosterone levels similar to that of surgical castrates within two weeks after the booster (Brunius *et al.*, 2011) as the Leydig cells within the testes undergo involution in response to a lack of LH (Wagner & Claus, 2004). The increase in antibody titre and the immediate response in terms of decreased LH and thus testosterone indicates that the potential anabolic growth of immunocastrates may be compromised within a matter of days after the second vaccination.

Therefore, the immunocastrated male pig grows and acts as an entire male until the second vaccination, after which the animal seems to transition to perform and behave similarly to a surgically castrated male (Boler *et al.*, 2011). This allows for initial capitalisation on the improved lean growth and feed conversion efficiency of an entire male that would otherwise be lost with surgical castration. After the second vaccination, tissue deposition rates as well as voluntary feed intake appear to change as the pig transitions from an entire male to a surgical castrate in terms of its metabolic state (Dunshea *et al.*, 2001; Bauer *et al.*, 2009). Due to the fact that immunocastration decreases testosterone production, one would expect immunocastrates to grow at a rate lower than boars after the second vaccination and start to perform similar to barrows. The metabolic changes that the immunocastrates undergo should be complete after approximately seven days; however, it is not clear as to the exact effect of these changes on growth and nutritional requirements. Immunocastration does not only influence androgen synthesis but oestrogen synthesis as well, with levels reported to be as low as surgical castrates at slaughter (Brunius *et al.*, 2011). This indirectly influences protein synthesis since oestrogens up-regulate hepatic growth hormone (GH) receptors, thus increasing insulin-like growth factor-1 (IGF-1) expression through the effect of GH. Brunius *et al.* (2011) found that plasma IGF-1 levels were highest in entire males, lowest in surgical castrates and intermediate in immunocastrates and according to Claus *et al.* (2007), plasma IGF-1 levels gradually decline after the booster vaccination and then stabilise after two to three weeks. Since immunocastrates have intermediate IGF-1 levels, this implies that they could have a higher anabolic growth potential than surgical castrates since IGF-1 stimulates protein synthesis (Bauer *et al.*, 2009). Brunius *et al.* (2011) demonstrated that GH levels in immunocastrates do not change following the second vaccination and thus remain at the same level as that within an entire male pig and did not fall to that within a surgical castrate. Therefore, even though anabolic growth appears to be compromised

due to decreased androgen production, the fact that GH levels do not change and IGF-1 levels are intermediary with immunocastration indicates that growth rates of immunocastrates may not decrease to that of surgical castrates provided nutritional requirements are met. However, according to Dunshea *et al.* (2001), Improvac[®]-vaccinated boars actually performed better than entire boars most probably due to the fact that they exhibited reduced aggressive and sexual behaviour in group housing, thus spending more time eating and portioning energy towards growth rather than physical activity.

The current practise commercially is to feed immunocastrates as surgical castrates following their second vaccination, however the differences in hormonal and thus metabolic status indicates that immunocastrates may have different nutritional requirements to both entire males and surgical castrates. Since the primary focus of pig production is meat, one must ensure that muscle growth is maximised and thus the dietary lysine and overall balanced protein content of the diet is not limiting. This is supported by the fact that backfat thickness decreased and percentage lean tissue yield increased as immunocastrates were fed dietary lysine levels increasing from 7 to 10 g lysine/kg, probably due to the fact that at 7 g lysine/kg, lysine was limiting (Boler *et al.*, 2011). It is also important to note that the voluntary feed intake of immunocastrates increases rapidly after the second vaccination (Claus *et al.*, 2007) most likely due to the absence of oestrogens and androgens and thus this also needs to be taken into account when determining the nutrient requirements for immunocastrates.

Body protein accretion can be further increased by the addition of a β -adrenergic agonist such as ractopamine hydrochloride, or Paylean[®], to the pig feed. Ractopamine hydrochloride (RAC) had been shown to increase weight gain, feed efficiency and leanness by repartitioning nutrients from lipogenesis towards protein synthesis. Since RAC increases protein synthesis, pigs fed RAC are likely to benefit from higher dietary protein levels. Rikard-Bell *et al.* (2009) demonstrated that immunocastrates fed diets supplemented with RAC had a better growth performance than the entire male controls; however, limited data are available for feeding RAC to immunocastrates. Thus the adjustment of nutritional strategies, especially in terms of balanced protein, as well as the use of additives in the diet of immunocastrates can aid in better growth and slaughter performance while eliminating boar taint in male carcasses. Since information regarding feeding RAC to immunocastrates is limited, it is important to further investigate its effect on growth performance. Therefore the aims of the study were to assess whether immunocastrates maintained their growth performance after the second vaccination when feeds are supplemented with RAC; to determine whether RAC supplementation would improve gains and efficiency while decreasing fat deposition; and to measure the response of immunocastrates to various dietary protein levels with and without RAC supplementation.

3.2 Materials and methods

This study was approved by the Research Ethics Committee: Animal Care and Use of Stellenbosch University (SU-ACUM13-00022) and the animals were handled according to South African National

Standards 10386: 2008 and following the South African Pig Welfare Code (NSPCA, 2014). The experiment was conducted from August to November 2013 at the Agricultural Research Council's Boar Testing Facilities at Elsenburg (Stellenbosch, Western Cape, South Africa).

3.2.1 Feed formulation

The 120 male pigs received a commercial grower pig feed (Table 3.1) from 16 weeks of age and approximately 60 kg live weight to 20 weeks of age (approximately 88 kg live weight); designated as Time 1 (T1). The commercial grower feed was fed to the pigs before they were moved into the experimental facilities and consisted of maize as the primary energy source and soybean meal as the primary protein source. Although the Ca:P ratio represented in Table 3.1 is low, they both represent minimum values and thus would be correctly balanced by the feed manufacturer within their indepth nutrient composition tables.

Table 3.1 Nutrient composition of the commercial grower pig feed used from 16 to 20 weeks of age (T1)

Nutrient	Value	Bounds
DE pig (MJ/kg)	12.3	
Moisture	12.00	maximum
Crude protein	16.00	minimum
Crude fat	2.5	minimum
Crude fibre	8.00	maximum
Total lysine	0.85	minimum
Calcium	0.60	minimum
Phosphorous	0.50	minimum

DE pig: digestible energy for pig

Bounds representing the maximum or minimum levels of each nutrient

At 20 weeks of age the pigs were changed to the experimental diets at the time of second vaccination, which would thus represent a finisher ration. Since PIC[®] pigs were used for the current trial, the PIC[®] Nutrients Specification Manual (2011) was consulted to determine the standard ileal digestible (SID) lysine percentages. The PIC[®] Nutrients Specification Manual (2011) makes use of the NRC publication (1998), as well as published and internal research which has been validated in various commercial environments using their PIC[®] pigs.

The average standardized ileal digestible (SID) lysine for immunocastrates is estimated to be approximately 20 % greater than for surgical castrates from 60 to 104 kg, after which the difference decreases to approximately 5 % greater than surgical castrates. According to the PIC[®] growth curves, the pigs should have a mass of 88 kg at 20 weeks of age should their growth be considered good and thus the weight ranges of 80 to 104 kg were used for the estimations given by PIC[®] to calculate a predicted SID lysine (g/kg) requirement for immunocastrates taking into account a 0.20 increase (Table 3.2). These values fit within the range which Boler *et al.* (2011) used for their trial.

Table 3.2 Prediction of SID lysine requirements for immunocastrated pigs using the PIC[®] Nutrients Specification Manual (2011) for PIC barrow SID lysine requirements

	< 80 kg	> 80 kg	< 104 kg	> 104 kg	Average
SID lysine (g/kg) PIC barrows	7.9	6.5	6.9	6.2	7.0
SID lysine (g/kg) * 20 %	1.6	1.3	1.4	1.2	1.4
Final lysine (g/kg)	9.5	7.8	8.3	7.4	8.4

SID: standard ileal digestible

According to the manufacturer's feeding directions of RAC (Paylean[®], Elanco[®] Animal Health, Eli Lilly) the diet must contain at least 160 g crude protein/kg with 8.5 to 9.5 g lysine/kg since it increases protein synthesis and thus lysine requirements. PIC[®] specifications for those pigs finished using RAC suggests 8.5 g SID lysine/kg for RAC fed for longer than 21 days and 9.5 g SID lysine/kg for RAC fed for less than 21 days. The three SID lysine levels chosen were 7.5, 9.8 and 12 g lysine/kg by taking into account the estimated average SID lysine level for immunocastrates as 8.4 g lysine/kg (Table 3.2), the recommendations of Apple *et al.* (2004), Webster *et al.* (2007) and Boler *et al.* (2011) as well as the feeding directions of RAC. The current feeding directions stipulate that when RAC is included at 10 mg/kg, 8.5 to 9.5 g lysine/kg needs to be given in the diet. Apple *et al.* (2004) suggested that at least 10 g lysine/kg be used in finisher pig diets in order to optimise growth while Webster *et al.* (2007) found that finisher pigs require approximately 8 g lysine/kg without RAC and 12 g lysine/kg at 10 mg/kg RAC. Lastly, Boler *et al.* (2011) concluded that immunocastrates performed best at 9 and 10 g lysine/kg in their trial. All other amino acids were balanced in terms of lysine. All other nutrient requirements were based on those recommended for PIC[®] pigs and nutrient bounds were established using WinFeed (EFG Software) for the low and high protein diets. A commercial feed manufacturing company mixed the two basal feeds and a third that was a 1:1 mixture of the two protein basal diets in accordance with standard industry practices (Table 3.3). The resultant feeds were formulated to contain digestible lysine contents of 7.5, 9.8 and 12 g lysine/kg respectively. The three balanced protein diets were fed from 20 to 24 weeks of age; designated as Time 2 (T2).

Table 3.3 Formulated ingredient composition (g/kg) of the diets representing low and high balanced protein diets, mixed at the commercial feed manufacturer fed to the experimental pigs from 20 to 24 weeks of age (T2)

Ingredient Composition	Low	High
Maize	326.77	205.67
Wheat bran	294.67	124.33
Barley meal	150.00	150.00
Soya oil cake (470 g CP/kg)	126.67	300.17
Sunflower oil cake (360 g CP/kg)	50.00	150.00
Canola oil	25.00	30.00
Limestone	14.17	12.67
Salt	4.30	4.42
L-lysine HCL	2.00	1.80
Vitamin & mineral premix	2.00	2.00
Monocalcium phosphate	1.90	0.00
Mycotoxin binder	1.00	1.00
L-threonine	0.53	0.18
Phytase enzyme	0.50	0.50
DL-methionine	0.27	0.37
Choline chloride liquid	0.13	0.13
Xylanase & β -glucanase enzyme combination	0.10	0.10
Maize gluten meal (600 g CP/kg)	0.00	16.67

Vitamin & Mineral premix: Vitamin A: 5489.5 IU/kg, Vitamin D: 1005.3 IU/kg, Vitamin E: 27.6 IU/kg, Vitamin K: 2.8 mg/kg, Niacin: 22.0 mg/kg, Riboflavin 4.9 mg/kg, d-Pantothenate: 16.5 mg/kg, Vitamin B12: 22.0 mcg/kg, Zinc: 100 mg/kg, Iron: 66 mg/kg, Manganese: 25 mg/kg, Copper: 10 mg/kg, Iodine: 0.33 mg/kg and Selenium: 0.25 mg/kg. This is an approximate premix composition obtained for grow-finish PIC[®] pigs from 68 kg live weight to slaughter from PIC[®] Nutrients Specification Manual (2011). These are suggested specifications for the amount of vitamins/minerals per kg of a complete diet.

CP: crude protein

HCL: hydrochloride

Table 3.4 Calculated nutrient composition of the diets representing low and high balanced protein diets fed to the experimental pigs from 20 to 24 weeks of age (T2); all amino acid values shown are digestible values

Calculated Nutrient Composition	Low	High	
NE pig (MJ/kg)	9.20	9.20	
DE pig (MJ/kg)	13.29	13.83	
Crude protein (g/kg)	161.16	255.80	
Crude starch (g/kg)	359.20	271.02	
Crude fiber (g/kg)	60.76	68.07	
Crude fat (g/kg)	49.12	47.40	
Ash (g/kg)	59.31	71.02	
Amino acids (g/kg)			
	Lysine	7.50	12.07
	<i>Methionine</i>	2.47	4.09
	<i>TSAA</i>	4.74	7.48
	<i>Tryptophan</i>	1.59	2.66
	<i>Threonine</i>	4.88	7.85
	<i>Arginine</i>	9.24	16.12
	<i>Isoleucine</i>	5.08	9.09
	<i>Leucine</i>	10.33	17.24
	<i>Valine</i>	6.11	10.12
	<i>Histidine</i>	3.48	5.61
Calcium (g/kg)	7.51	7.49	
Total phosphorus (g/kg)	6.86	7.42	
Available phosphorus (g/kg)	2.50	2.53	
Sodium (g/kg)	2.00	2.00	
Potassium (g/kg)	9.86	12.94	
Chloride (g/kg)	3.79	3.76	

NE: net energy

ME: metabolizable energy

TSSA: total sulphur-containing amino acids

The moisture, crude fat, crude fibre, ash, amino acid and mineral contents were analysed according to AOAC (2002) approved methods (AOAC methods 934.01; 954.02; 962.09; 994.12; 942.05) and the crude protein contents using the DUMAS method (AOAC methods 984.13 & 976.06) by the Central Analytical Laboratories (5 Cartwright Street, Stormill, Florida) and the LCMS Laboratory, Central Analytical Facility (University of Stellenbosch, South Africa) (Table 3.5).

Table 3.5 Analysed nutrient composition (g/kg) of the low, medium and high balanced protein diets used from 20 to 24 weeks (T2) of age for the experimental pigs

Analysed Nutrient Composition	Low	Medium	High	
Moisture	94.0	93.0	86.0	
Crude protein	162.1	209.9	253.5	
Crude fat	50.0	51.2	52.6	
Crude fibre	58.2	55.7	57.6	
Ash	50.3	53.8	56.4	
Amino Acids				
	Lysine	8.4	9.2	13.7
	<i>Methionine</i>	1.6	1.6	2.8
	<i>Threonine</i>	5.5	6.2	8.9
	<i>Arginine</i>	8.7	10.2	15.0
	<i>Isoleucine</i>	5.0	6.1	9.0
	<i>Leucine</i>	10.6	12.3	17.4
	<i>Valine</i>	6.7	7.7	11.3
	<i>Histidine</i>	3.6	4.1	5.9
	<i>Serine</i>	6.4	7.4	11.1
	<i>Glycine</i>	6.4	7.4	10.9
	<i>Aspartic acid</i>	12.3	14.9	21.2
	<i>Glutamic acid</i>	26.6	30.1	41.5
	<i>Alanine</i>	6.5	7.4	10.4
	<i>Proline</i>	8.4	9.0	12.3
	<i>Cysteine</i>	0.7	0.7	1.0
	<i>Tyrosine</i>	4.8	5.7	7.7
	<i>Phenylalanine</i>	6.6	7.8	10.8
Calcium	7.5	7.6	6.7	
Phosphorous	6.3	6.4	6.4	
Sodium	2.1	1.9	1.7	
Potassium	9.6	10.5	11.8	
Magnesium	2.6	2.7	2.8	
Sodium	2.1	2.5	2.7	
Iron (mg/kg)	265.6	272.4	274.1	
Manganese (mg/kg)	122.5	127.6	117.7	
Copper (mg/kg)	37.8	42.8	42.4	
Zinc (mg/kg)	237.0	195.0	199.0	

Ractopamine hydrochloride was administered at 10 mg/kg continuously for the last 28 days before slaughter (T2) and mixed in accordance with the safety guidelines. The decision to use 10 mg/kg rather than 5 mg/kg was based on the claim that 10 mg/kg RAC increased carcass leanness and dressing percentage as well as provided an improvement in weight gain and feed efficiency over that when using 5 mg/kg RAC. The low, medium and high protein diets with or without ractopamine hydrochloride were thus fed to the respective treatments from 20 to 24 weeks of age (T2).

3.2.2 Animals, housing, feeding and experimental design

The pigs were purchased from a commercial piggery with a high-health status at 13 weeks of age and the growth trial was conducted at the Agricultural Research Council's Boar Testing Facilities at Elsenburg (Stellenbosch, Western Cape, South Africa) using PIC[®] Large White x Landrace x White

Duroc maternal line cross PIC[®] 410 terminal sire boars of two sex types, namely entire males and immunocastrated males. The feeding trial followed a 2 x 2 x 3 factorial design where each animal was randomly allocated to each of the 12 treatment combinations. The first factor represents entire (E) versus immunocastrated (C) males, the second represents whether or not ractopamine hydrochloride (RAC; Paylean[®]) was added to the feed, and the last represents three levels of balanced dietary protein (Table 3.6). Ten animals were each allocated to the 12 treatment combinations, thus 120 male pigs took part in the feeding trial and the treatment combinations were randomly distributed within the pig house. The mean and range of body weights between treatments were controlled by initially ranking the pigs according to body weight and then randomly assigning these in batches of 12 pigs to the 12 treatments. Each body weight group was considered a block, however, initial statistical analyses showed that it was unnecessary to block for both location and body weight and thus initial weight was accounted for as a covariate in the statistical analyses. No pigs were removed from the trial for any reason and all pigs were slaughtered at 24 weeks of age.

Table 3.6 The 12 treatment combinations broken up into their factors: sex, RAC and lysine level

Treatment	Sex	RAC	Protein level
1	Entire	No	High
2	Entire	No	Medium
3	Entire	No	Low
4	Immunocastrated	No	High
5	Immunocastrated	No	Medium
6	Immunocastrated	No	Low
7	Immunocastrated	Yes	High
8	Immunocastrated	Yes	Medium
9	Immunocastrated	Yes	Low
10	Entire	Yes	High
11	Entire	Yes	Medium
12	Entire	Yes	Low

RAC: ractopamine hydrochloride

Prior to using the facilities and equipment (including feeders) these were washed with a high pressure sprayer and scrubbed to remove any debris, then cleaned with One Shot (Envirochem[®], Cape Town) and disinfected with GL 100 (Envirochem[®], Cape Town) which are both SABS approved products (SANS 1853). Each pen was divided into a solid concrete sleeping area covered in fresh pine wood shavings (approximately $\frac{3}{4}$ of pen) and a sloped dunging area free from shavings separated by a concrete hump so that shavings remained in the bedding area (Figure 3.1). An adjustable stainless steel feeder was placed in the clean sleeping area and bolted to the bars of the pens and a nipple drinker was situated in the concrete dunging area. Feed and water were available *ad libitum* throughout the trial. The house was divided into 120 individual pens with metal bars as dividers so that pigs were allowed social interaction through visual and limited physical contact

without being able to display aggressive behaviour. The house was naturally ventilated and temperature was recorded using temperature loggers which measured the ambient temperature every 10 minutes so that any drastic changes could be identified. The 12 treatments (Table 3.6) were randomly assigned to pens and blocked so that the effect of location within the house could be taken into account. Initial weight was recorded at 16 weeks of age so that it could be used as a covariate in statistical analyses. The period of measurement started at 16 weeks since it coincided with the first Improvac[®] vaccination, and the experiment terminated when the pigs were 24 weeks of age at which time the pigs were slaughtered.



Figure 3.1 The experimental pens showing layout with feeder in top right of pen, bolted to pen bars

The pigs were allowed to acclimatise to the new environment and all weighing and other management and recording activities for three weeks before the trial started at 16 weeks of age, during which time they continued to be fed the commercial feed (Table 3.1). Feed intake, body weight and backfat depth were recorded weekly from 16 to 24 weeks of age. The experiment was initiated at 20 weeks when they were allocated one of three protein levels either with or without RAC and therefore there were six dietary treatment combinations (three protein levels with or without RAC; Table 3.5) all of which were fed *ad libitum* in a pelleted form. The second vaccination was also given at 20 weeks of age to the immunocastrated pigs. Feed was delivered by a commercial feed company on a weekly basis due to space constraints on the research farm, but the total amount of each batch required was mixed at the same time and analysed for chemical composition using near-infrared spectroscopy as well as by CAL Laboratories using a full proximate analysis.

The immunocastrated (C) pigs were injected with 2 mL of Improvac[®] (Zoetis[™] Animal Health, Sandton, South Africa) subcutaneously behind and below the base of the ear. The first vaccination

was given at 16 weeks using an Improvac[®] safety vaccination gun. Only one immunocastrated pig exhibited a site reaction which was seen in the form of a small abscess (Figure 3.2) that appeared two weeks after vaccination and was lanced, rinsed with an iodine solution and sprayed with Necrospray (Oxytetracycline hydrochloride 40 mg; gentian violet 4 mg; Bayer[®] Animal Health) and then Supona[™] (Chlorfenvinphos 0,48 % m/m; dichlorphos 0,74 % m/m; gentian violet 0,145 % m/m; Zoetis[™] Animal Health) for the subsequent days until healed.

The second vaccination was given at 20 weeks; four weeks before the termination of the trial. Control (E) pigs were not given a placebo.

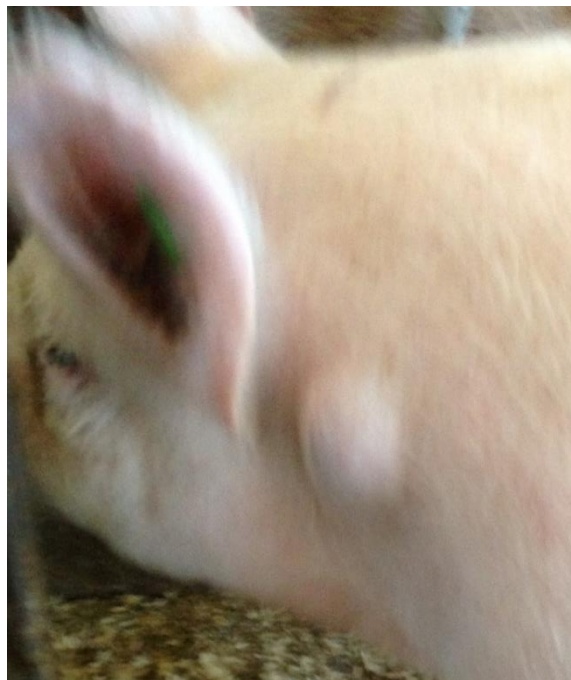


Figure 3.2 The site reaction showing abscess on the neck of an immunocastrated pig two weeks after the first vaccination with Improvac[®]

The pigs were checked daily in terms of food and water availability as well as health. Each pen was cleaned daily, shavings replaced and feeders were adjusted accordingly to ensure provision of fresh feed continuously. The pigs were weighed individually every Tuesday, during which their backfat thickness was measured 65 mm from the dorsal midline at the last rib. The pigs were weighed (kg) using a metal crate with gates at the front and back, on top of Gallagher[™] load bars connected to a Gallagher[™] livestock indicator (W310, Gallagher Animal Management Systems, New Zealand) accurate to two decimal places. Every Wednesday, the feeders were weighed (kg) individually in order to measure weekly feed intake. Each feeder was suspended from a hanging scale accurate to two decimal places (Nagata HTR, Tainan) to establish the “before” weight, filled and then re-weighed to establish the “after” weight (Figure 3.3). The weekly feed intake was calculated by subtracting the “before” weight from the previous week’s “after” weight. The ADG and FCR were calculated every week so that any animal far below average was visited to note any possible problems. For the backfat

thickness, measurements were taken while the pigs were in the weighing crate using a Renco Lean-meater digital backfat indicator (Renco Corporation, USA). The position of measurement was marked using a liquid livestock marker so that each measurement would be taken at the same position each week.

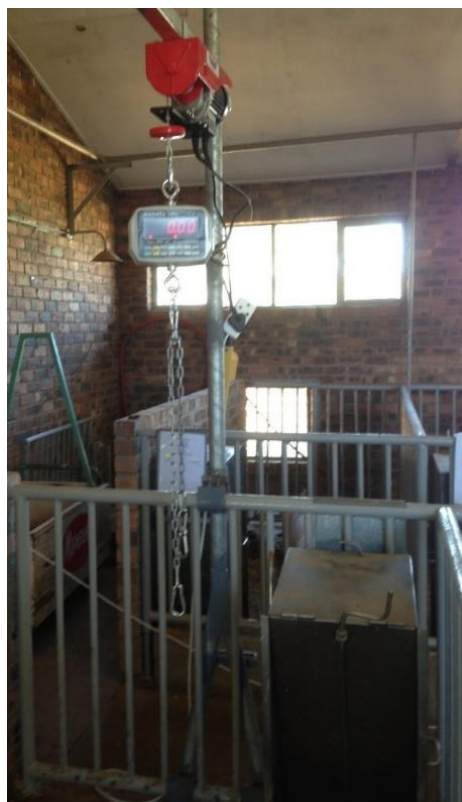


Figure 3.3 The hanging scale used to weigh the feeders (bottom right) hoisted by a mechanical winch

3.2.3 Statistical analysis

Statistical analysis was performed using the Variance Estimation, Precision and Comparison (VEPAC) procedure in STATISTICA 64 version 11 (2012). The normality of the data was tested using normal probability plots of the residuals after which linear mixed models were fitted to the variables weight (kg), backfat depth (mm) and weekly feed intake (kg) using the Restricted Maximum Likelihood (REML) method. The grouping variables specified were: pen/animal number, house, RAC, protein level, sex and age. The initial mass at 16 weeks of age was used as a covariate when the overall trial period was analysed (T1/2) and mass at 19 weeks was used when the period from 20 to 24 weeks of age was analysed (T2). The fixed effects were initial mass, house, RAC, protein, sex, age and the various second, third and fourth order interactions. Thus the random effects were pen/animal number. Linear regressions were then fitted to weight (kg), backfat depth (mm) and weekly feed intake (kg) to obtain the ADG, fat gain and ADFI respectively from the relative slopes. From these analyses, it was deemed unnecessary to block for location within the pig house. The FCR was calculated for two periods; from 16 to 20 weeks (T1) and 20 to 24 weeks (T2) and analysed using univariate analysis of variances (ANOVA's) following the general linear models (GLM) procedure which was also the

process used to analyse the slopes. Fishers LSD comparison of LSMeans was the chosen *post hoc* test and was used where interactions were significant. A significance level of 5 % was chosen and thus p-values for interactions, effects and differences below 5 % are reported as significant and those below 10 % are reported as trends. All means are reported as LSMeans \pm Standard Error of the mean (SEM).

3.3 Results

The results for the various treatments on mean body weight, backfat depth and weekly intake are reported in Table 3.7 to 3.9 and the results for ADG, fat gain, feed intake and FCR can be found in Table 3.10.

The fixed effects test for T1/2 showed that the initial vaccination had no effect on the variables and thus it was not necessary to analyse T1 separately. For T1/2, RAC and sex had an interaction over time for weight ($p = 0.001$) which showed that E and C responded differently to RAC over time (Table 3.7). The E treatment had no difference in weights between those fed RAC and those which did not receive RAC over T1/2. However, the C fed RAC were heavier from weeks 22 to 24 (Figure 3.4). This resulted in C RAC having the highest LSMean final weight of 134.1 ± 1.84 kg and E no RAC had the lowest (124.4 ± 1.50 kg).

Table 3.7 The LSMeans \pm SEM for the main effects and significant interactions for sex, RAC and protein level on the live body weight (kg) at 16, 20 and 24 weeks of age

Effect	Body Weight (kg)		
	16 weeks	20 weeks	24 weeks
Sex			
E	57.4 \pm 0.72	89.5 \pm 1.08	125.5 \pm 1.34
C	57.6 \pm 0.67	89.0 \pm 0.93	130.9 \pm 1.20
RAC mg/kg			
0	57.5 \pm 0.65	89.4 \pm 0.93	126.0 \pm 1.02
10	57.6 \pm 0.74	89.1 \pm 1.08	130.3 \pm 1.51
Protein			
Low	58.0 \pm 0.83	90.3 \pm 1.24	127.5 \pm 1.80
Med	57.1 \pm 0.90	88.3 \pm 1.28	128.0 \pm 1.54
High	57.5 \pm 0.83	89.1 \pm 1.18	129.0 \pm 1.51
Sex*RAC			
E 0	57.7 \pm 0.94	90.0 \pm 1.41	124.4 ^c \pm 1.50
E 10	57.1 \pm 1.11	89.0 \pm 1.65	126.6 ^{bc} \pm 2.24
C 0	57.3 \pm 0.90	88.8 \pm 1.22	127.6 ^b \pm 1.33
C 10	58.0 \pm 1.00	89.2 \pm 1.44	134.1 ^a \pm 1.84

^{a,b} LSMeans within columns for the main effects and interactions with different superscripts are significantly different ($p < 0.05$)

E: entire males
C: immunocastrates
0: 0 mg/kg
10: 10 mg/kg
Med: medium

RAC: ractopamine hydrochloride

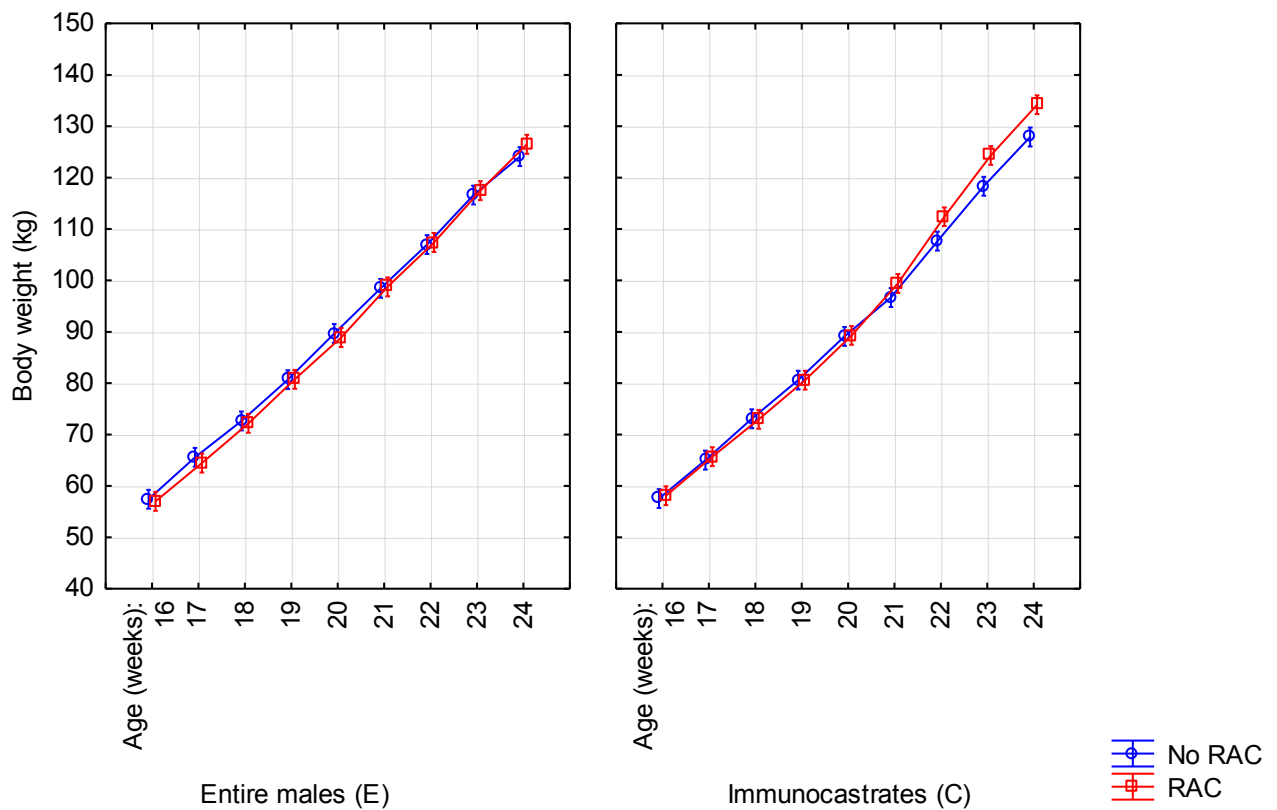


Figure 3.4 Body weight (kg) of entire males (left) and immunocastrates (right) from 16 to 24 weeks of age (T1/2) fed with and without RAC. The vertical bars indicate 95 % confidence intervals

When the data from T2 were analysed separately, the C pigs overtook the E pigs between 21 and 22 weeks of age in terms of body weight and the effect of RAC on increasing body weight also took place between 21 and 22 weeks of age, which can also be seen in T1/2 shown in Figure 3.4. Thus both treatments took approximately two weeks to have an effect on body weight. The VEPAC for T2 showed an interaction between RAC, protein and sex over time ($p = 0.012$) which indicated that when RAC is not fed, the dietary protein level did not have a large effect on body weight. However the E pigs had the lowest 24 week body weights when they were fed the low (1263.7 ± 3.06 kg) or medium protein diets (122.5 ± 2.71 kg) (Table 3.7). All the other combinations of dietary protein and sex (C Low, C Med, C High and E High) did not differ from each other, but both the C and E pigs had the highest 24 weeks body weights with the high protein diet (128.0 ± 2.52 and 127.0 ± 2.71 kg respectively) (Figure 3.5).

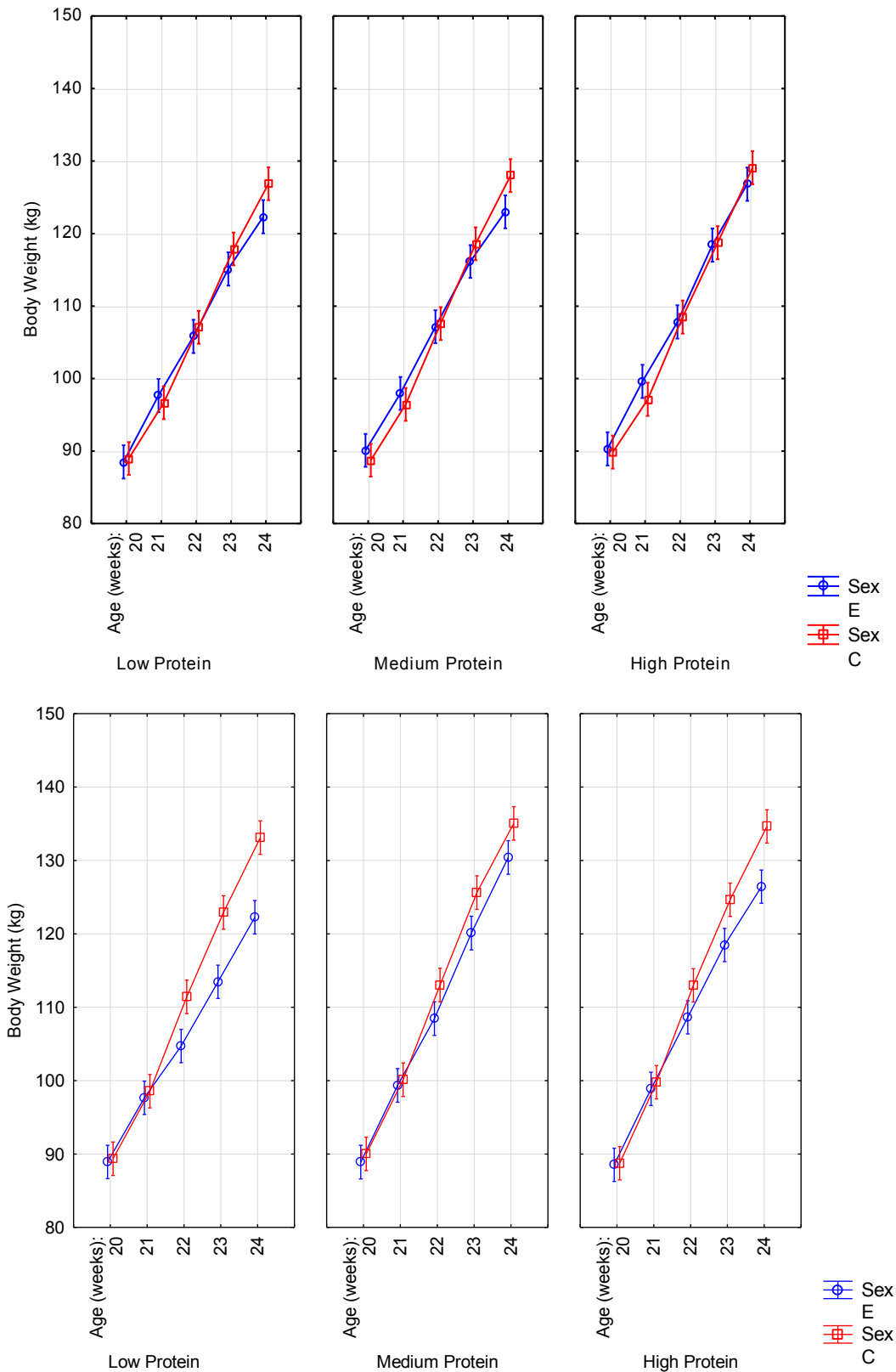


Figure 3.5 Body weight (kg) of entire males (E) and immunocastrates (C) from 20 to 24 weeks of age (T2) fed without RAC (above) and with RAC (below) for the various balanced protein diets (Low, Medium and High). The vertical bars indicate 95 % confidence intervals

When 10 mg/kg RAC was included in the diet, E Low (123.0 ± 4.00 kg) had the lowest 24 week body weight, followed by E High (126.9 ± 4.17 kg). The treatments C Low, C Med, C High and E Med did not differ from each other, with C Low having the highest 24 week body weight over all treatments with and without RAC (136.7 ± 3.45 kg) and for the E pigs, feeding medium protein showed the best body weights (129.9 ± 3.50 kg) (Figure 3.5).

In order to further evaluate the effects of the various factors on body weight, a linear regression was fitted to the body weights of each animal over time. The slope of each regression represents the change in body weight over time, or the ADG. The results of the ANOVA for ADG showed that sex and RAC both had significant effects on ADG, with C pigs having a higher ADG ($p < 0.001$), while feeding RAC improved ADG ($p < 0.001$) (Table 3.10).

The VEPAC analysis for the live backfat depths and the ANOVA for the slopes representing the change in backfat gain showed that immunocastration increased the mean backfat depth and backfat gains ($p < 0.001$) (Table 3.8 & Table 3.10) between 22 and 23 weeks of age (Figure 3.6).

Table 3.8 The LSM means \pm SEM for the main effects for sex, RAC and protein level on the live backfat depths (mm) at 16, 20 and 24 weeks of age

Effect	Backfat depth (mm)		
	16 weeks	20 weeks	24 weeks
Sex			
E	6.3 ± 0.16	9.2 ± 1.11	$11.3^b \pm 0.18$
C	6.4 ± 0.16	9.0 ± 0.10	$12.5^a \pm 0.17$
RAC mg/kg			
0	6.3 ± 0.16	9.1 ± 0.13	12.0 ± 0.18
10	6.5 ± 0.16	9.1 ± 0.12	11.8 ± 0.19
Protein			
Low	6.5 ± 0.18	9.3 ± 0.13	12.0 ± 0.26
Med	6.3 ± 0.22	9.0 ± 0.16	12.0 ± 0.26
High	6.3 ± 0.17	9.1 ± 0.16	11.7 ± 0.23

^{a,b} LSM means within columns for the main effects and interactions with different superscripts are significantly different ($p < 0.05$)

E: entire males

C: immunocastrates

0: 0 mg/kg

10: 10 mg/kg

Med: medium

RAC: ractopamine hydrochloride

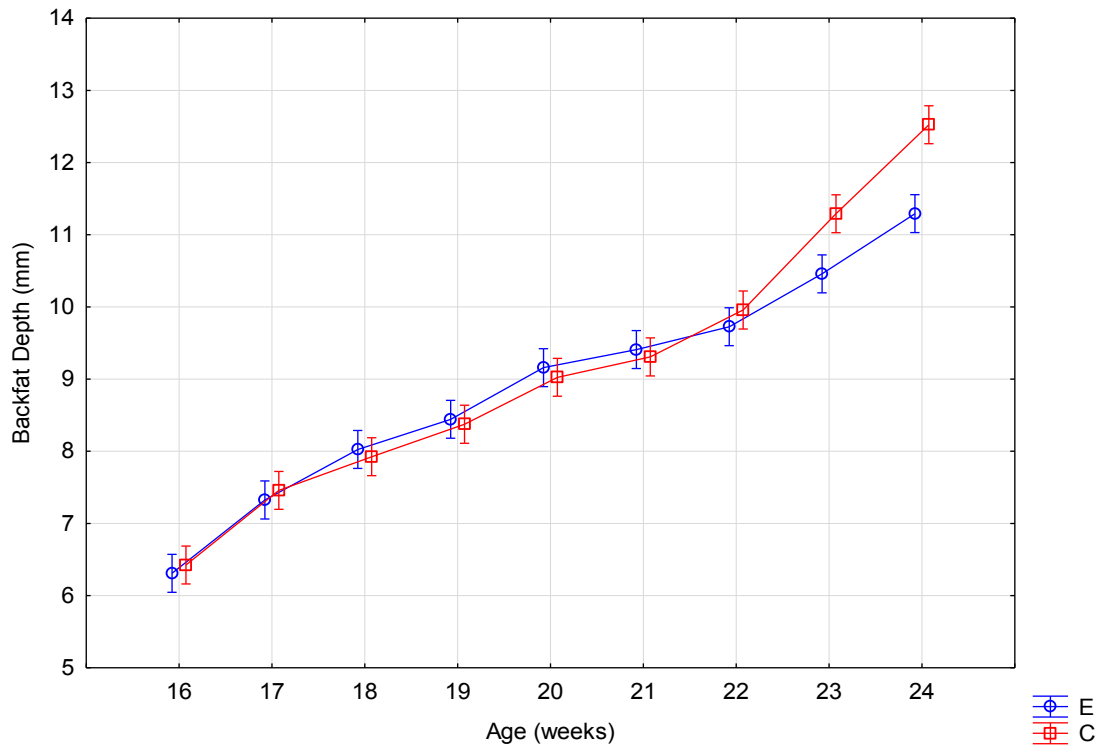


Figure 3.6 Backfat depth (mm) of entire males (E) and immunocastrates (C) from 16 to 24 weeks of age (T1/2). The vertical bars indicate 95 % confidence intervals

A sex and RAC interaction ($p = 0.009$) was evident for weekly feed intake, which revealed that C fed RAC had an increased intake from week 21 when compared to C which did not receive RAC (Figure 3.7). However, the effect on intake seems to be largely due to the effect of sex. Figure 3.8 shows that from 21 weeks of age, the weekly feed intake of C pigs increased from 19.9 ± 0.38 kg to 30.1 ± 0.56 kg per week. When C pigs are compared to E pigs at 23 weeks of age, the weekly feed intake was ~ 8.8 kg more per week (Table 3.9). The results for the change in feed intake over time, or ADFI, indicated an interaction for sex and RAC ($p = 0.040$) where feeding RAC decreased ADFI in E but increased ADFI in C pigs (Table 3.8). However, the increase in ADFI for C was not significant nor was the decrease in ADFI in E (Table 3.10).

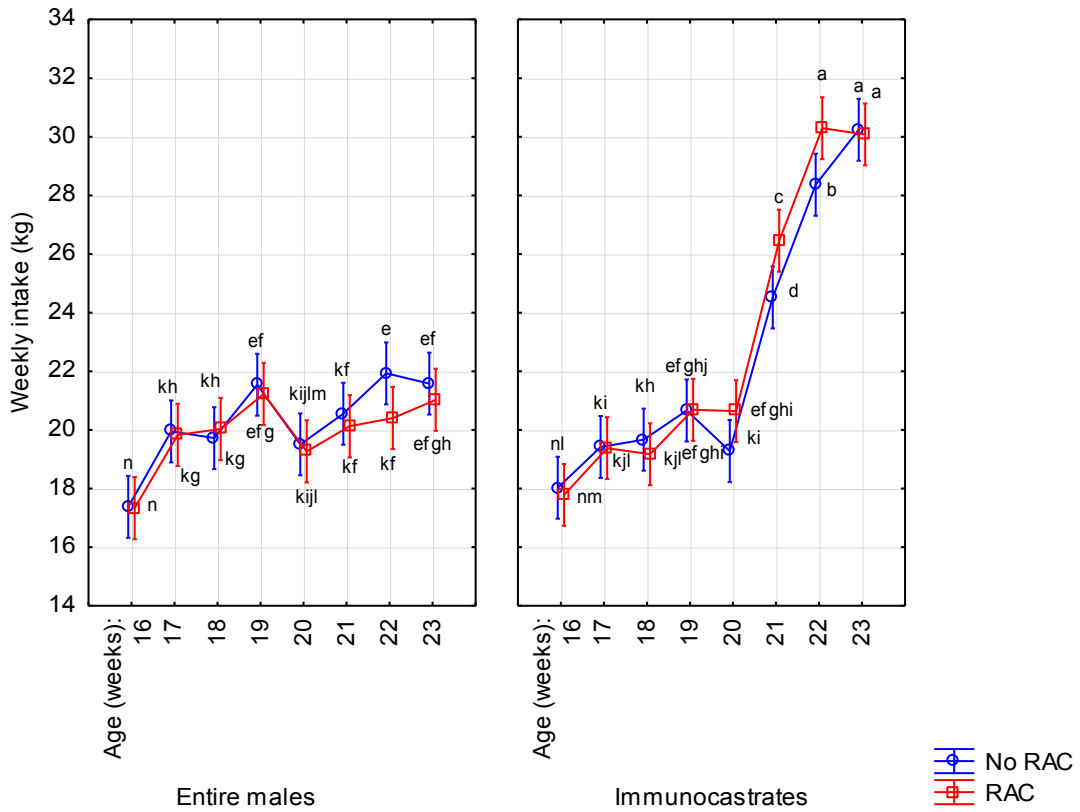


Figure 3.7 Weekly feed intake (kg) of entire males and immunocastrates from 16 to 24 weeks of age (T1/2) fed with and without RAC. The vertical bars indicate 95 % confidence intervals

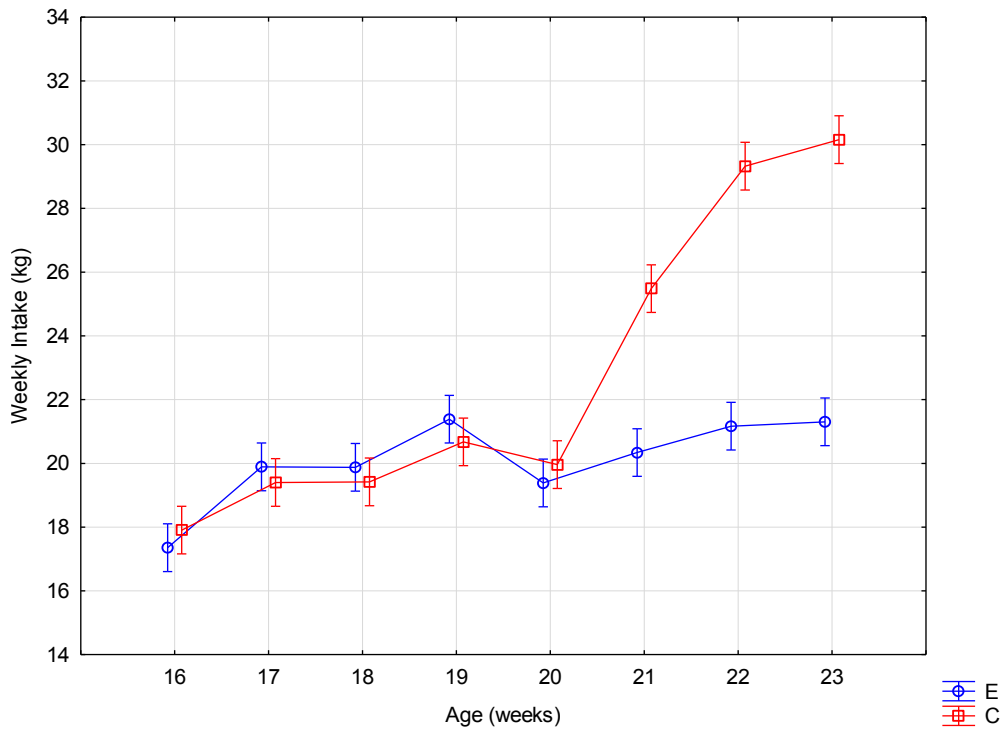


Figure 3.8 Weekly feed intake (kg) of entire males (E) and immunocastrates (C) from 16 to 24 weeks of age (T1/2). The vertical bars indicate 95 % confidence intervals

Table 3.9 The LSM means \pm SEM for the main effects and significant interactions for sex, RAC and protein level on the weekly feed intake (kg) at 16, 20 and 23 weeks of age

Effect	Weekly Feed Intake (kg)		
	16 weeks	20 weeks	23 weeks
Sex			
E	17.4 \pm 0.33	19.4 \pm 0.40	21.3 ^b \pm 0.36
C	17.9 \pm 0.42	19.9 \pm 0.38	30.1 ^a \pm 0.56
RAC mg/kg			
0	17.7 \pm 0.38	19.4 \pm 0.37	25.9 \pm 0.73
10	17.6 \pm 0.38	20.0 \pm 0.42	25.6 \pm 0.75
Protein			
Low	17.5 \pm 0.37	20.0 \pm 0.57	26.0 \pm 0.97
Med	17.4 \pm 0.56	19.6 \pm 0.44	25.7 \pm 0.88
High	18.0 \pm 0.45	19.4 \pm 0.42	25.5 \pm 0.88
Sex*RAC			
E 0	17.4 \pm 0.49	19.5 \pm 0.52	21.6 \pm 0.48
E 10	17.4 \pm 0.47	19.3 \pm 0.62	21.1 \pm 0.52
C 0	18.0 \pm 0.58	19.2 \pm 0.53	30.2 \pm 0.83
C 10	17.8 \pm 0.61	20.6 \pm 0.54	30.1 \pm 0.77

^{a,b} LSM means within columns for the main effects and interactions with different superscripts are significantly different ($p < 0.05$)

E: entire males

C: immunocastrates

0: 0 mg/kg

10: 10 mg/kg

Med: medium

RAC: ractopamine hydrochloride

The FCR for the T1 period showed no significant differences between treatments, as expected (Table 3.10). For the T2 period, an interaction between RAC and dietary protein level ($p = 0.01$) revealed that when no RAC was fed, the high dietary protein level gave the lowest (or best) FCR and when RAC was fed, both the medium and high dietary protein levels provided the lowest FCR (Figure 3.9). The main effect of sex was also significant ($p < 0.0001$) such that immunocastration increased the FCR (Table 3.10).

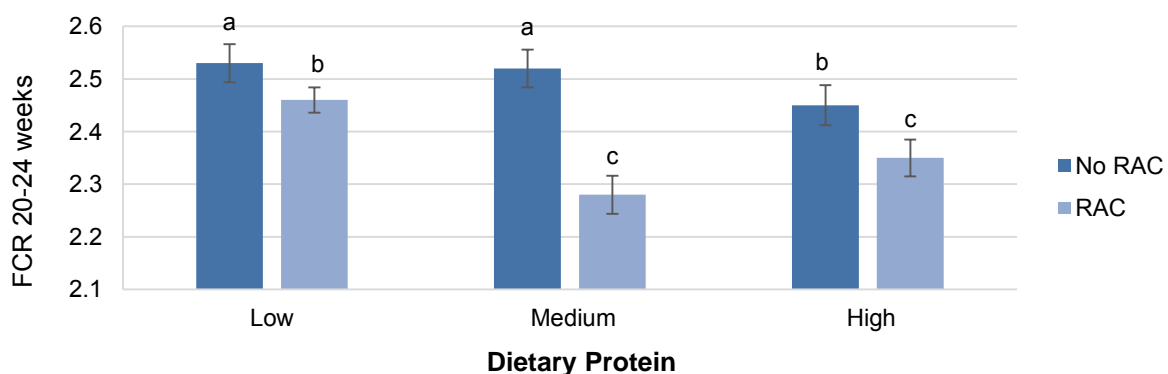


Figure 3.9 FCR (kg/kg) 20 to 24 weeks of age (T2) over the various Low, Medium and High dietary protein levels. The vertical bars indicate SEM.

Table 3.10 The LSMeans \pm SEM for the main effects and significant interactions for sex, RAC and protein level on the ADG (kg/day), ADFI (kg/day), Fat gain (mm/day) and FCR for T1, T2 (kg/kg)

Effect	ADG (kg/day)	Fat gain (mm/day)	ADFI (kg/day)	FCR T1 (kg/kg)	FCR T2 (kg/kg)
Sex					
E	1.23 ^b \pm 0.017	0.56 ^b \pm 0.019	0.40 \pm 0.064	2.48 \pm 0.032	2.37 ^b \pm 0.023
C	1.31 ^a \pm 0.017	0.68 ^a \pm 0.017	1.82 \pm 0.076	2.48 \pm 0.022	2.48 ^a \pm 0.019
RAC mg/kg					
0	1.23 ^b \pm 0.013	0.64 \pm 0.020	1.10 \pm 0.107	2.47 \pm 0.023	2.50 \pm 0.021
10	1.31 ^a \pm 0.020	0.60 \pm 0.019	1.13 \pm 0.124	2.49 \pm 0.031	2.37 \pm 0.021
Protein					
Low	1.28 \pm 0.020	0.64 \pm 0.021	1.20 \pm 0.146	2.46 \pm 0.038	2.49 \pm 0.022
Med	1.25 \pm 0.024	0.62 \pm 0.021	1.08 \pm 0.141	2.45 \pm 0.038	2.40 \pm 0.032
High	1.29 \pm 0.021	0.60 \pm 0.028	1.04 \pm 0.141	2.49 \pm 0.040	2.40 \pm 0.026
Sex*RAC					
E 0			0.47 ^b \pm 0.076		
E 10			0.32 ^b \pm 0.101		
C 0			1.71 ^a \pm 0.121		
C 10			1.93 ^a \pm 0.088		
Protein* RAC					
Low 0					2.53 ^a \pm 0.036
Low 10					2.46 ^b \pm 0.024
Med 0					2.52 ^a \pm 0.036
Med 10					2.28 ^c \pm 0.036
High 0					2.45 ^b \pm 0.038
High 10					2.35 ^c \pm 0.035

^{a,b} LSMeans within columns for the main effects and interactions with different superscripts are significantly different ($p < 0.05$)

E: entire males

C: immunocastrates

0: 0 mg/kg

10: 10 mg/kg

Med: medium

RAC: ractopamine hydrochloride

3.4 Discussion

In the current trial, Paylean[®] was the chosen β -adrenergic agonist since it is a widely used and researched product, does not require a withdrawal period, can be fed rather than implanted and it photodegrades and thus does not build up in waste water (Paylean[®] Technical Manual, 2000). The vaccination timing was selected based on the fact that peak levels of plasma steroids are reached at approximately 19 weeks of age (França *et al.*, 2000) and thus the second vaccination was to be given at 20 weeks of age so that the benefit of the male steroid hormones could be realised in terms of anabolic growth. According to personal communications, producers in the Western Cape (South Africa) are currently slaughtering at approximately 22 weeks of age but in spite of this it was decided to slaughter the trial pigs at 24 weeks of age to reap the benefits of the extra growth period, which further motivated the timing of 20 weeks of age for the second vaccination to allow a minimum of four weeks between the booster and slaughter. This schedule using vaccination at eight and then again at four weeks prior to slaughter has also been proven to be effective in terms of androstenone suppression (Jaros *et al.*, 2005).

Boler *et al.* (2011) performed a study on immunocastrated PIC[®] pig crosses to determine the effects of increasing lysine on carcass composition and cutting yields at 7, 8, 9 and 10 g lysine/kg. This was described as low, low/medium, medium/high and high for this genotype in the late finishing phase. They reported increased lean cutting yields and carcass cutting yields of immunocastrates as the levels of lysine increased from 7 to 10 g lysine/kg. The importance of dietary lysine in the lean growth responses of pigs was investigated by Apple *et al.* (2004) and Webster *et al.* (2007). Apple *et al.* (2004) showed that the level of dietary lysine influenced the response of pigs to RAC and that pigs require at least 10 g lysine/kg in combination with RAC to optimise growth. Webster *et al.* (2007) also found that increasing the dietary lysine level improved the responses of finisher pigs fed with and without RAC, but when RAC was fed that a higher lysine level was needed to generate an increase in growth performance. Webster *et al.* (2007) concluded that finisher pigs of 79 – 109 kg require approximately 8 g lysine/kg and at 10 mg/kg RAC, 12 g lysine/kg was needed for a response in growth performance, of which both levels are higher than commercially used. Thus for the current trial a range of 7.5, 9.8 and 12 g digestible dietary lysine/kg were chosen for the low, medium and high balanced protein diets respectively, assuming that an increase in balanced dietary protein and the provision of RAC would allow for an increase in ADFI of immunocastrates to be used for lean growth in terms of body weight and an improvement in feed efficiency.

The C and E males reacted differently to RAC in terms of body weight, with greater body weights being achieved in C pigs fed RAC. When the data were separated into T1 and T2, it showed that dietary protein levels could also influence the responses of the sexes to RAC. However, the ADG provides a more descriptive and economic parameter with which to compare the treatments. Following the described vaccination schedule, immunocastration increased ADG between 21 and 22 weeks of age, within two weeks after the second vaccination which is the same point at which the voluntary feed intake increased (Figure 3.8). Therefore, some of the increase in gain could possibly be attributed to increased gut fill. This is in accordance with the findings from Dunshea *et al.* (2001)

who found that immunocastrates had a higher weight gain after the booster vaccination than entire male pigs which seemed to be due to an increased feed intake rather than a change in FCR. Since the stomach has a limited capacity, an increase in ADFI may increase the feed through-flow rate in the digestive system and thus not all of the increase in ADG can be attributed to gut fill. However, in the current study, immunocastration increased the FCR by ~ 4.6 % for the T2 period and thus the increase in ADFI allowed for an increase in ADG but also a decrease in feed efficiency which further supports the conclusion that gut fill may have an influence on a portion of the increase seen in the ADG.

In most of the research done with immunocastrates fed RAC backfat has been measured at the time of slaughter using the Hennessey Grading Probe or Fat-O-Meater and not on the live pig using an ultrasound scanner. Claus *et al.* (2007) evaluated the endocrine and metabolic changes in immunocastrates receiving their second vaccination at 22 weeks, and found that the total body fat content measured using D₂O dilution did not differ significantly to the total body fat content pre-booster. In the current trial, backfat thickness was also measured at slaughter using the Hennessey Grading Probe and is discussed in Chapter 4, section 4.3.1. Differences in fat gain are also likely to depend on whether or not the nutritional requirements of immunocastrates are met, since an imbalance or deficiency may lead to an increased fat deposition. It is thus important that the timing of the second vaccination, nutritional requirements and feeding strategy be carefully considered when immunocastrates are used for the production of finisher pigs.

Immunocastration increased the live backfat depth. However, this occurred approximately three weeks after the second vaccination (Figure 3.6) and is thus likely due to the direct effect of the lack of testosterone, since testosterone levels are negatively correlated with feed intake (Weiler *et al.*, 1996). Testosterone levels drop within two weeks after the second vaccination in response to the lack of LH (Dunshea *et al.*, 2001; Claus *et al.*, 2007; Brunius *et al.*, 2011) which lifts its inhibitory effect on appetite as well as its stimulatory effect on protein synthesis. Lealiifano *et al.* (2011) demonstrated that the increase in ADFI is apparent two weeks after immunisation regardless of the timing (in terms of pig age) of the second vaccination and thus the longer the period between the booster and slaughter, the greater the opportunity for fat deposition. A change in nutrient requirements may also influence ADFI, since an attempt is made to rectify any deficiencies by increasing feed intake. Since the increase in feed intake was seen over all dietary protein levels and the dietary energy content of 9.2 MJ NE/kg is comparable to the high energy diet (9.7 MJ NE/kg) used by Zeng *et al.* (2002), the increase in ADFI is most likely due to the lack of testosterone inhibiting feed intake. In group housed pigs, a decrease in distraction from aggressive activities will also influence the differences in ADFI between immunocastrated and intact male pigs. Cronin *et al.* (2003) showed that before the second vaccination, both entire males and immunocastrates were more active than surgical castrates. However, after the second vaccination the entire males displayed more social behaviour and spent less time at the feeders than the immunocastrates and surgical castrates. Although the pigs in the current trial were individually housed, social interaction was still possible through the pen dividers and even standing activity may contribute to increased energy expenditure and distraction from feed intake, since the energy expenditure for standing is relatively high in pigs (Noblet *et al.*, 1993).

Depending on the inclusion level and duration used, RAC is said to benefit growth performance traits with 5 mg/kg improving the weight gain rates and feed efficiency, while at 10 mg/kg the additional benefits include improved carcass leanness and carcass dressing percentage (Paylean[®] Technical Manual, 2000). However, a meta-analysis of 23 published reports on live growth performed between the early 1990s to 2005 found that pigs fed RAC had a higher ADG and better feed efficiency while producing larger and more lean carcasses, regardless of the inclusion level (Apple *et al.*, 2007). At 10 mg/kg, ADG was improved by 11.8 % and feed efficiency between 5.6 % to 25.9 %, with little effect on the ADFI, however, the latter tended to be reduced (Apple *et al.*, 2007). In the current study, the inclusion of 10 mg/kg RAC in the diets increased the ADG by 80 g/day, or 6.5 %, and decreased the ADFI in entire males, which could be due to the lower energy requirements of lean growth versus fat deposition. Although RAC increased the ADFI in immunocastrates while decreasing ADFI in entire males, these differences were not significant, which agrees with Braña *et al.* (2013) who noted no significant differences in ADFI for immunocastrates fed 0 and 5 mg/kg RAC for 28 days. Thus the significant interaction for sex and RAC regarding ADFI simply indicates a change in trends for each sex when RAC is included in the diet.

The interaction between RAC and dietary protein level for the FCR for T2 showed that the effect on FCR depended on the dietary protein level with the lowest dietary protein level resulting in the poorest FCR, whether RAC was included in the diet or not. When RAC was not included, the high protein diet provided the best FCR (2.45 ± 0.038 kg/kg) which indicates that the balanced protein, and thus lysine, requirements are higher than commercially used. The medium and high protein diets provided the best FCRs with RAC and although they did not differ significantly from one another, the medium protein diet with RAC provided a lower FCR (2.28 ± 0.036 kg/kg) compared to the high protein diet (2.35 ± 0.035 kg/kg). This difference may have practical implications since the feed cost is the single most expensive factor of a pig production unit and thus over a large number of finisher pigs it could have a significant impact on the production cost per unit. Therefore, it is necessary to perform an economic comparison to establish if the improvement in FCR will justify the inclusion of RAC and a higher protein diet, also taking into account the effect of RAC on economically important carcass traits (Chapter 4). The current results thus tend to agree with those of Webster *et al.* (2007) who found that a higher dietary lysine level was needed to generate an increase in growth performance when RAC was included in the diet.

The mode of action of RAC suggests that it inhibits the repartitioning of nutrients towards lipogenesis by decreasing the sensitivity of the adipose cells to insulin, while increasing protein synthesis capacity (Williams *et al.*, 1987; Haussman *et al.*, 1989; Mills *et al.*, 1990; Paylean Technical Manual, 2000). The enzymes fatty acid synthetase, lipoprotein lipase and malic enzyme decrease in activity and the sensitivity of adipose cells to insulin is also decreased in response to RAC (Williams *et al.*, 1987; Haussman *et al.*, 1989; Mills *et al.*, 1990). However, previous results in terms of this theory vary. No differences in their live backfat thickness were seen in pigs fed RAC, but the data at 24 weeks indicated a slight decrease in backfat depth. Therefore, if RAC was fed for a longer period, significant effects could possibly be seen when the decrease in sensitivity of the adipose β -receptors is taken into account with an appropriate step-up program. Rikard-Bell *et al.* (2009) demonstrated that

when immunocastrates (vaccinated at 11 and 17 weeks old) were fed 5 mg/kg RAC for 14 days after the second vaccination, followed by 10 mg/kg for 17 days, a consistent decrease in fat mass was seen. However, should the period between the second vaccination and slaughter be optimised, differences in backfat depths should not be seen between entire males and immunocastrates. This is supported by Lealiifano *et al.* (2011) who investigated various vaccination schedules where the booster was given at two, three, four and six weeks prior to slaughter. The results indicated that vaccinating at four weeks prior to slaughter had no influence on the backfat thickness, but those vaccinated at six weeks prior to slaughter showed an increase in backfat thickness compared to the non-vaccinated control.

3.5 Conclusion

The results indicate that immunocastration, dietary protein level and feeding 10 mg/kg RAC all influenced ADG, ADFI, FCR and fat deposition. Therefore, immunocastrates perform at the same level as entire males up until the second vaccination and thus can be managed as entire males. However, after the second vaccination ADFI increases and FCR decreases, thus the period between second vaccination and slaughter is important when trying to minimise these effects. Although immunocastration increased ADG, the increase seen in ADFI negatively influenced feed efficiency (FCR). The difference in backfat depth at 24 weeks of age between the sexes was ~ 1.2 mm and it is therefore questionable whether significant differences will be seen at slaughter using the Hennessey Grading Probe. The influence of gut fill on body weight and thus ADG can also be evaluated by comparing the hot carcass masses (and dressout percentages) at slaughter. There was no response in backfat thickness seen with RAC supplementation, but this may not be the case with other carcass cuts. Thus the influence of RAC on the lean meat yields of the various carcass cuts needs to be investigated in order to ascertain a better representation of the effect of RAC on fat deposition. Supplying the growing pigs feed with a higher protein level improved feed efficiency whether RAC was fed or not, and thus an economic comparison is important to establish whether the increased cost of a high protein diet and RAC inclusion is warranted by the improvement in FCR. Therefore, the inclusion of 10 mg/kg RAC for the last 28 days of growth could improve the feed efficiency of immunocastrates, provided the correct dietary protein level is fed.

3.6 References

- AOAC International (2002). Official Method of Analysis (17th ed.) Arlington, Virginia, USA: Association of Official Analytical Chemists Inc.
- Apple, J., Maxwell, C., Brown, D., Friesen, K., Musser, R., Johnson, Z., & Armstrong, T. (2004). Effects of dietary lysine and energy density on performance and carcass characteristics of finishing pigs fed ractopamine. *Journal of Animal Science*, 82, 3277–3287.

- Apple, J., Rincker, P., McKeith, F., Carr, S., Armstrong, T., & Matzat, P. (2007). Review: Meta-analysis of the ractopamine response in finishing swine. *The Professional Animal Scientist*, *23*, 179–196.
- Bauer, A., Lacorn, M., & Claus, R. (2009). Effects of two levels of feed allocation on IGF-I concentrations and metabolic parameters in GnRH-immunized boars. *Journal of Animal Physiology and Animal Nutrition*, *93*, 744–753.
- Boler, D., Kutzler, L., Meeuwse, D., King, V., Champion, D., McKeith, F., & Killefer, J. (2011). Effects of increasing lysine on carcass composition and cutting yields of immunologically castrated male pigs. *Journal of Animal Science*, *89*, 2189–2199.
- Braña, D., Rojo-Gómez, G., Ellis, M., & Cuaron, J. (2013). Effect of gender (gilt and surgically and immunocastrated male) and ractopamine hydrochloride supplementation on growth performance, carcass, and pork quality characteristics of finishing pigs under commercial conditions. *Journal of Animal Science*, *91*, 5894–5904.
- Brunius, C., Zamaratskaia, G., Andersson, K., Chen, G., Norrby, M., Madej, A., & Lundström, K. (2011). Early immunocastration of male pigs with Improvac® - Effect on boar taint, hormones and reproductive organs. *Vaccine*, *29*, 9514–9520.
- Claus, R., Lacorn, M., Danowski, K., Pearce, M. C., & Bauer, A. (2007). Short-term endocrine and metabolic reactions before and after second immunization against GnRH in boars. *Vaccine*, *25*, 4689–4696.
- Cronin, G., Dunshea, F., Butler, K., McCauley, I., Barnett, J., & Hemsworth, P. (2003). The effects of immuno- and surgical-castration on the behaviour and consequently growth of group-housed, male finisher pigs. *Applied Animal Behaviour Science*, *81*, 111–126.
- Dunshea, F., Colantoni, C., Howard, K., McCauley, I., Jackson, P., Long, K., Lopaticki, S., Nugent, E., Simons, J., Walker, J., & Hennessey, D. P. (2001). Vaccination of boars with a GnRH vaccine (Improvac) eliminates boar taint and increases growth performance. *Journal of Animal Science*, *79*, 2524–2535.
- Font-i-Furnols, M. (2012). Consumer studies on sensory acceptability of boar taint: A review. *Meat Science*, *92*, 319–329.
- França, L. R., Silva, V. A., Chiarini-Garcia, H., Garcia, S. K., & Debeljuk, L. (2000). Cell proliferation and hormonal changes during postnatal development of the testis in the pig. *Biology of Reproduction*, *63*, 1629–1636.
- Fuchs, T., Thun, R., Parvizi, N., Nathues, H., Koehrmann, A., Andrews, S., Brock, F., Klein, G., Sudhaus, N., & grosse Beilage, E. (2009). Effect of a gonadotropin-releasing factor vaccine on follicle-stimulating hormone and luteinizing hormone concentrations and on the development of testicles and the expression of boar taint in male pigs. *Theriogenology*, *72*, 672–680.
- Jaros, P., Bürgi, E., Stärk, K., Claus, R., Hennessey, D., & Thun, R. (2005). Effect of active immunization against GnRH on androstenone concentration, growth performance and carcass quality in intact male pigs. *Livestock Production Science*, *92*, 31–38.
- Lealiifano, A., Pluske, J. R., Nicholls, R., Dunshea, F., Campbell, R., Hennessey, D., Miller, D., Hansen, C. F., & Mullan, B. (2011). Reducing the length of time between slaughter and the

- secondary gonadotropin-releasing factor immunization improves growth performance and clears boar taint compounds in male finishing pigs. *Journal of Animal Science*, 89, 2782–2792.
- Noblet, J., Shi, X. S., & Dubois S. (1993). Energy cost of standing activity in sows. *Livestock Production Science*, 34, 127–136.
- Paylean™ Technical Guide, Version 1.0. (2000). Prepared by R. D. Muller (Technical Services). Elanco Animal Health, A Division of Eli Lilly and Company, Four Parkwood, Suite 125, 500 E. 96th St., Indianapolis, Indiana, 46240, U.S.A.
- PIC® Nutrients Specification Manual (2011). *Grow-Finish Specifications* (pp 20 - 22). Retrieved from: <http://piclatam.com/Agroinformacion/MANUAL.pdf> on 17 April 2014.
- Rikard-Bell, C., Curtis, M., Van Barneveld, R. J., Mullan, B., Edwards, A. C., Gannon, N., Henman, D. J., Hughes, P. E., & Dunshea, F. (2009). Ractopamine hydrochloride improves growth performance and carcass composition in immunocastrated boars, intact boars, and gilts. *Journal of Animal Science*, 87, 3536–3543.
- South African Code for the Welfare of Pigs (2014). Retrieved from: <http://www.nspca.co.za/clientdata/10072/uploads/codes/code%20pig%20welfare.pdf> on 7 September 2014.
- Wagner, A. & Claus, R. (2004). Involvement of glucocorticoids in testicular involution after active immunization of boars against GnRH. *Reproduction*, 127, 275-283.
- Webster, M. J., Goodband, R., Tokach, M., Nelssen, J., Dritz, S., Unruh, J., Brown, K., Real, D., Derouchey, J., Woodworth, J., Groesbeek, C. N., & Marsteller, T. A. (2007). Interactive effects between ractopamine hydrochloride and dietary lysine on finishing pig growth performance, carcass characteristics, pork quality, and tissue accretion. *The Professional Animal Scientist*, 23, 597–611.
- Weiler, U., Claus, R., Dehnhard, M. & Hofäcker, S. (1996). Influence of the photoperiod and a light reverse program on metabolically active hormones and food intake in domestic pigs compared with a wild boar. *Canadian Journal of Animal Science*, 76, 531–539.
- Zamaratskaia, G., Rydhmer, L., Andersson, H. K., Chen, G., Lowagie, S., Andersson, K. & Lundström, K. (2008). Long-term effect of vaccination against gonadotropin-releasing hormone, using Improvac™, on hormonal profile and behaviour of male pigs. *Animal Reproduction Science*, 108, 37–48.
- Zeng, X., Turkstra, J., Jongbloed, A., Van Diepen, J. T. M., Meloen, R., Oonk, H., Guo, D., & Van de Wiel, D. (2002). Performance and hormone levels of immunocastrated, surgically castrated and intact male pigs fed ad libitum high-and low-energy diets. *Livestock Production Science*, 77, 1–11.

CHAPTER 4

Carcass traits and cutting yields of entire and immunocastrated pigs fed increasing protein levels with and without ractopamine hydrochloride supplementation

Abstract

Carcass traits and yields of 120 PIC[®] entire (E) and immunocastrated (C) male pigs were evaluated following the feeding of diets varying in balanced protein levels with and without ractopamine hydrochloride (RAC). Vaccination occurred at 16 and 20 weeks of age and RAC was supplemented at 0 or 10 mg/kg for 28 days prior to slaughter at 24 weeks of age. Low, medium and high protein diets were formulated and all amino acids balanced with regards to lysine (7.50, 9.79 and 12.07 g digestible lysine/kg) and pigs were allocated to one of 12 treatment combinations: E Low, E Medium and E High; E Low RAC, E Medium RAC and E High RAC; C Low, C Medium and C High; and C Low RAC, C Medium RAC and C High RAC. Variables collected at slaughter included hot carcass weight (HCW), Hennessey Grading Probe (HGP) backfat thickness and commercial classification (PORCUS). The left side of each carcass was divided into head, trotters, shanks, fillets, shoulder, hindquarter, loin and belly, weighed and expressed as a percentage of the dressed hot carcass or side weight. Each cut was trimmed into muscle, bone and subcutaneous fat with skin and weighed individually. A carcass lesion score was given 24 hours *post mortem* and at 48 hours *post mortem* backfat depth and skin thickness was measured using an engineering calliper on the right loin. Immunocastration and RAC supplementation increased the live weight at slaughter, however, no differences were found between treatments for the dressed HCW. The HGP backfat thickness showed no differences between treatments but there was a significant sex and protein interaction for calliper backfat depth while RAC supplementation significantly lowered the backfat depth. For the commercial cut weights, feeding RAC significantly increased the weights and the percentage of the trotters, fillets, shoulder, hindquarter and loin. Supplementing 10 mg/kg RAC increased the muscle weight and percentage of the shoulder, hindquarter and loin and decreased the fat weight and percentage of the hindquarter and belly fat percentage. Immunocastration increased fat weights and percentages compared to E in the loin cut as well as in the belly. Therefore, RAC supplementation increased carcass cutting and lean yields, while decreasing the backfat depth. The interaction for sex and dietary protein levels for the calliper backfat depths could indicate that the protein requirements differ between the sexes and that the negative effects due to immunocastration on backfat thickness could be avoided should the correct dietary protein be provided.

Keywords: *Paylean, Improvac, pork, swine, GnRH, boar taint, carcass yield*

4.1 Introduction

Immunological castration, or immunocastration, involves vaccinating against gonadotropin-releasing hormone (GnRH) in order to terminate the production of male steroid hormones. This technique has received increased attention internationally in the pork industry to control boar taint in response to the rise in welfare concern and implementation of legislation with regards to surgical castration. Since vaccination protocol calls for two vaccinations for the castration effect to take place, the male pigs

function as entire males until the second vaccination after which testicular hormone production ceases within two weeks (Dunshea *et al.*, 2001). It would thus be tempting to treat immunocastrates as surgical castrates after their second booster vaccination in terms of nutrient requirements; however, Boler *et al.* (2011) has shown that immunocastrates should be fed at a higher lysine level than surgical castrated pigs in order to increase carcass cutting yields without detrimental effects on meat quality. Boler *et al.* (2011) reported that immunocastrates fed medium/ high (9 g/kg) lysine and high (10 g/kg) lysine had a 2.5 % higher carcass cutting yield than surgical castrates and that feeding immunocastrates increasing levels of lysine decreased back fat thickness and increased the percentage lean of their carcasses. The importance of dietary lysine in terms of carcass characteristics is further highlighted by Webster *et al.* (2007) who showed that increased dietary lysine is associated with improved carcass characteristics.

Since the South African carcass classification system is largely based on subcutaneous fat thickness, focus is placed on increasing the lean: fat ratio of the carcass. Thus there is an interest in additives which may allow manipulation of carcass characteristics further than genetics, sex and nutrition. Ractopamine hydrochloride (RAC) is a feed additive which had been shown to increase the leanness of pig carcasses (Armstrong *et al.*, 2004) and to decrease the daily rate of fat deposition in entire males but not barrows (Dunshea *et al.*, 1993). Ractopamine hydrochloride also increases the lean tissue weight in immunocastrates which is accompanied by a decrease in fat weight (Rikard-Bell *et al.*, 2009), however, the increase in protein synthesis is not always at the expense of fat accretion (Dunshea *et al.*, 1993).

Apple *et al.* (2004b) showed that dietary energy had little influence on the carcass composition, meat quality and growth performance of RAC-fed pigs, but that the level of dietary lysine influenced the response to RAC. Webster *et al.* (2007) also found that increasing dietary lysine improved the responses of finisher pigs fed with and without RAC but when pigs were fed RAC, a higher lysine level was needed to generate an increase in growth performance, but carcass muscling increased and fat decreased in pigs fed RAC as lysine increased. They concluded that finisher pigs of 79 – 109 kg require approximately 8g lysine /kg and at 10 mg/kg RAC, 12 g lysine /kg was needed for a response in growth performance. Apple *et al.* (2004b) agreed with the conclusion that finisher pigs require additional lysine and noted that pigs require at least 10 g lysine/kg to optimise growth and carcass yields, which is higher than presently used commercially. It is thus evident that dietary lysine is important in order to optimize the carcass characteristics and yields of finisher pigs when fed with and without RAC.

Limited studies have been performed on the responses of immunocastrates to various levels of balanced dietary protein with lysine as an indicator amino acid, especially when RAC was used. Also, the vaccination schedules, lysine levels, control sex and carcass fabrication techniques used vary between studies. In Chapter 3 it was shown that C pigs fed RAC had an increased body weight compared to E pigs fed with or without RAC and C fed without RAC from approximately 22 weeks of age, two weeks into RAC supplementation. At 22 weeks of age, the C pigs also had increased backfat depth compared to E, which could affect their carcass classification and the increase in body weight could influence their hot carcass weight and carcass cutting yields. The significant increase in feed

intake seen in the C pigs within one week of second vaccination could influence the gut fill and thus limit the differences between hot carcass weights of the two sexes. In Chapter 3, little response was seen in the growth performances of the pigs fed at the different dietary protein levels, although, an effect of varying protein levels may be seen in the muscle and fat deposition of the various carcass cuts. The effect of feeding increasing levels of lysine with and without RAC on the carcass yields of E and C has not yet been quantified under South African production conditions using local genetics. Thus, the objective of this study is to determine the effects of increasing balanced protein (lysine) in finisher diets of immunocastrates on carcass cutting yields and their respective muscle, fat and bone proportions when carcasses were fabricated using commercial South African procedures. The hypotheses for this study included: 1) that RAC would increase muscle weight while decreasing fat weight thus decreasing the negative effects of immunocastration and 2) that an increase in balanced protein would allow additional lysine (amino acids) to be deposited as lean tissue rather than fat.

4.2 Materials and Methods

All experimental procedures were approved by the Research Ethics Committee: Animal Care and Use of Stellenbosch University (SU-ACUM13-00022) and conform to the South African National Standards 10386: 2008 in terms of the accepted standards for the use of animals in research and teaching. Further details regarding the experimental design of the growth trial, experimental diet formulation, husbandry of the trial and experimental housing of the pigs is discussed in detail in Chapter 3 (section 3.2).

4.2.1 Animals, housing and feeding

One hundred and twenty individually penned entire male pigs formed part of a growth performance trial at the ARC boar testing facilities at Elsenburg in the Western Cape Province, South Africa. They were randomly selected from commercial slaughter stock produced from a PIC[®] Camborough maternal line (Large White x Landrace x White Duroc) and a PIC[®] 410 terminal sire which has been selected for lean growth. The trial was arranged as a 2 x 2 x 3 factorial experiment with the main effects of sex (immunocastrates versus entire males), RAC (0 or 10 mg/kg) and balanced protein level (low, medium and high). The pigs were randomly placed at 13 weeks of age to allow for acclimatization in terms of environment and weighing procedures. Until 20 weeks of age the pigs received a commercial grower feed (Table 3.1) *ad libitum* in the pelleted form and fresh water was freely available from a nipple drinker in each pen. The pens were divided into a concrete sleeping area covered in clean pine wood shavings and a slanted dunging area free from shavings. From 16 weeks of age, each pig was weighed, back fat was measured 65 mm from the dorsal midline using a Renco Lean-meater digital backfat indicator (Renco Corporation, USA) at the last rib counted from the cranial end and feed intake was recorded. The pigs randomly allocated for immunocastration were vaccinated at 16 weeks and 20 weeks of age using an Improvac[®] safety vaccination gun at a dose of 2 mL of Improvac[®] behind and below the base of the ear. Vaccinations were thus four weeks apart

and the booster was given four weeks before slaughter. At 20 weeks of age, the diets were changed to those of varying protein content (7.50, 9.79 and 12.07 g digestible lysine/kg). Paylean® (RAC) was given continuously as part of the complete diet for the last 28 days of growth at 10 mg/kg to the respective allocated treatments and a zero day withdrawal was used.

4.2.2 Slaughter

At 24 weeks of age, all pigs were slaughtered at a commercial abattoir in the Western Cape Province, South Africa 45 minutes away from the ARC boar testing facilities at Elsenburg. Each pig was loaded individually but transported as a mixed group and held in a communal lairage for a minimum of one hour, which was chosen due to the relatively short transport period. They were electrically stunned (220 Volts, 1.4 Amps for four seconds) with electrodes at the base of each ear and exsanguinated using a thoracic stick. The head and left hand side trotters, shanks, fillets, shoulder, hindquarter, loin, belly and the right hand side loin was numbered with the pig's individual ear tag number using a carcass pencil after being dehaired and before their ear tags were removed. The carcasses were then eviscerated, the testicle pairs were collected (Chapter 6) and the carcass was health inspected according to commercial practises. Each carcass was weighed to determine the hot carcass weight and the Hennessey Grading Probe (HGP) was used to determine the backfat thickness between the 2nd and 3rd last rib from the cranial end and 45 mm from the dorsal midline. The hot carcass weight and back fat thickness was used to compute a commercial carcass classification score according to the PORCUS system (Table 4.1) with any pig carcass over 101 kg being classified as a "Sausage" carcass regardless of fat thickness or lean meat percentage.

Table 4.1 PORCUS classification system used to grade pig carcasses under the Agricultural Product Standards Act 119 of 1990 and calculated using the lean meat percentage and Hennessey Grading Probe fat thickness (mm)

Class	Meat percentage	Fat thickness (mm)
P	70 and more	More than 1 but less than 12
O	More than 68 but less than 69	More than 12 but less than 17
R	More than 66 but less than 67	More than 17 but less than 22
C	More than 64 but less than 65	More than 22 but less than 27
U	More than 62 but less than 63	More than 27 but less than 32
S	61 and less	More than 32
Sausage	*	*

The pH and temperature was taken at the 2nd and 3rd last rib and 45 mm from the dorsal midline spot on the loin from the inside of the carcass within 45 minutes *post mortem* using a calibrated portable Crison PH25 pH meter (Allela, Barcelona) after which the pigs entered a cold room at 4°C for 24 hours whilst being hung by both Achilles tendons (data discussed in section 5.3.2). Slaughtering started at 08:49 with the first testicles being collected at 09:00 and slaughtering was finished by 11:00. A summary of the slaughter data collected in the abattoir included:

- Dressed Hot Carcass Weight (HCW) (kg)
- pH at 45 minutes (pH₄₅) in the *Longissimus thoracis* (Chapter 5)
- Temperature (45 minutes) in the *Longissimus thoracis*
- Conformation class (1 to 5) according to the PORCUS classification
- Fat thickness (mm) between the 2nd and 3rd last rib and 45 mm from the dorsal midline
- Collection of testicle pairs for size measurements (Chapter 6)

4.2.3 Carcass fabrication and deboning

After approximately 24 hours, the carcasses were deboned by trained and experienced staff at the abattoir using commercial techniques. The first pH measurements taken 24 hours *post mortem* (pH₂₄) were measured at approximately 07:30, the weighing of the heads started at 08:00 and the cutting of the carcasses started at 09:00 and all carcasses were cut and weighed by 13:00. Each carcass was also given a carcass lesion score as described by McCauley *et al.* (2001) and used by Lealiifano *et al.* (2011):

- 0 = unmarked
- 1 = minimal bruising/scratching
- 2 = obvious bruising/ scratching
- 3 = severe bruising (entire carcass)

The carcasses were sectioned into commercial cuts in a similar manner described by Hoffman (1987) and Pieterse (2006). The head of each carcass was removed by cutting between the atlas and axis of the neck at a 90° angle to the ventral line and weighed individually. The two *psaos major* muscles were also removed and weighed together for each individual pig. Each carcass was then placed on a band saw and sectioned into the shoulder, belly, loin, hindquarter, shanks and trotters. Firstly, the trotters were removed at the carpal for the fore legs and just above the hock, or the tarsal joint, for the hind legs. The hind leg shank was then removed by cutting just below the knee joint where the femur meets the tibia, passing through approximately the mid-point of the tibia and fibula in the hind legs. The fore leg shank was removed by cutting just above the elbow joint where the humerus meets the radius and ulna. The shoulder was removed by cutting directly behind the fore leg (“armpit”) at approximately in the region between the 2nd thoracic vertebra and the 2nd and 3rd rib. The hindquarter was sectioned off by cutting within the sacral vertebrae region by passing through the narrow part of the hip bone also known as the shaft of the ilium. The belly was then sectioned from the loin by cutting a straight line just below, or ventrally to, the 4th thoracic vertebra and the *psaos major* and *psaos minor* muscles (tenderloin) position towards the hindquarters. The right hand side loins of 96 pigs were sampled by selecting eight of the mid-weight pigs from each treatment in terms of their live weight at slaughter. Each loin was taken from the right hand side of the carcass and cut at between the 2nd and 3rd last rib. The cranial section of each untrimmed loin (bone-in, rind-on) was wrapped in individual water impermeable plastic bags, placed in cardboard boxes and taken back under insulated conditions to Stellenbosch University for analyses (meat quality data discussed in

section 5.3). The fat thickness and skin thickness of each of these 96 loins were measured using an engineering calliper at the same position as the HGP backfat thickness measurement.

The left hand side shoulder, belly, loin, hindquarter, shanks (fore and hind together) and trotters (fore and hind together) were weighed. The shoulder, belly, loin and hindquarter were then deboned and trimmed into muscle and subcutaneous fat using commercial abattoir techniques. The hindquarter was trimmed of the skin and subcutaneous fat by cutting as close as possible to the muscle. The vertebrae, pelvic bone as well as the sections of the femur and tibio-tarsal bones were removed from the muscular tissue. Any hard connective tissues were trimmed. The hindquarter was thus divided into:

- Skin and subcutaneous fat
- Lean meat: including inter- and intramuscular fat, blood vessels, glands and connective tissue
- Bone: sacral vertebrae, portion of the pelvis, femur, patella, portion of the tibia-tarsus, remaining muscular tissue between the vertebrae and remaining attached connective tissue on the bone

The shoulder was also trimmed of all skin and fat. The meat was then sectioned as closely as possible from the cervical and thoracic vertebrae dorsal projections, as well as from the first rib bones. The scapula and humerus were removed along with the associated cartilage. The shoulder was thus divided into:

- Skin and subcutaneous fat
- Lean meat: including inter- and intramuscular fat, blood vessels, glands and connective tissue
- Bone: cervical vertebrae, approximately the first seven thoracic vertebrae, ribs, scapula, humerus, radius, ulna, cartilage, muscular tissue between the dorsal projections of the vertebrae as well as remaining attached connective tissue

The loin was trimmed of all the skin and subcutaneous fat by hand after which the vertebrae along with the attached rib bones were removed by cutting straight along the ribs as closely as possible and along the spinal processes. The loin was thus divided into:

- Skin and subcutaneous fat
- Lean meat: including inter- and intramuscular fat, blood vessels, glands and connective tissue
- Bone: remaining thoracic vertebrae, remaining ribs, lumbar vertebrae, remaining small portion of pelvis, muscular tissue between the vertebrae as well as remaining attached connective tissue and intercostal muscles between the ribs and their associated connective tissue

The belly was also trimmed of the skin and subcutaneous fat by hand and the remaining ribs removed, which were sectioned off when the belly was separated from the loin. In all cuts the meat was removed as close to the bone as possible, however the intercostal muscles of the ribs were left on the bone. The belly was thus divided into:

- Skin and subcutaneous fat
- Lean meat: including inter- and intramuscular fat, blood vessels, glands and connective tissue

- Bone: ventral portion of ribs, intercostal muscles between the ribs and their associated connective tissue, portion of sternum, remaining canal and kidney fat and remaining portions of diaphragm

For each cut, the muscle, bone and fat (with skin) were weighed and expressed either as a percentage of the dressed HCW or the dressed side HCW. The dressed HCW included the head, trotters and fillets.

4.2.4 Statistical analysis

Statistical analysis was performed using STATISTICA 64 version 11 (2012) for the 2 x 2 x3 factorial experimental design and the main effects tested were sex, RAC and protein level as well as their interactions. The data was tested for normality of the residuals and homogeneity using Levene's test. Univariate analysis of variances (ANOVA's) were then performed using the general linear models (GLM) procedure and followed up with *post hoc* tests where effects or interactions were significant using Fisher LSD comparison of LS (least squared) means. Significant interactions, effects and differences were reported at a significance level of 5 % and trends were noted at the 10 % significance level. Data values are reported as LSMeans \pm Standard Error (SEM) of the mean. The cuts were examined in terms of actual weight (kg) and then as a percentage of the hot carcass side weight (including trotters, shanks, shoulder, hindquarter, belly and loin) or whole carcass weight (including fillets and head) as well so that the carcass weight of the pigs could be included for a better representation of differences in the carcass yields.

4.3 Results

The results for carcass traits are shown in Table 4.1 and carcass yields in Table 4.2 to 4.6. Both weights and percentages of cuts are discussed, however, only the percentages are shown in the tables. Unless stated otherwise, there were no interactions between the main effects and the latter are therefore discussed.

4.3.1 Live weight, hot carcass weight, carcass scores and fat depth

The live weight at slaughter was influenced by the main effects of sex and RAC such that C pigs were ~5 kg heavier ($p = 0.003$) than E pigs and RAC-fed pigs were ~4 kg heavier ($p = 0.017$) than non-RAC fed pigs (Table 4.1). However, there were no significant differences for the dressed HCW (~101 kg) between the treatments. There were also no significant differences for HGP fat depth measured in the abattoir; however, the calliper fat thickness showed a sex and protein interaction ($p = 0.039$) and the main effect RAC influenced calliper-measured backfat depth (Figure 4.1). The C fed Medium protein carcasses had greater calliper backfat depths than E on all protein levels (E Low: $p = 0.021$, Medium: $p = 0.010$ and High: $p = 0.001$) as well as C Low protein ($p = 0.003$; Table 4.1). Also, E High

protein had lower ($p = 0.030$) backfat depths than C High protein. Pigs fed RAC had lower ($p = 0.027$) backfat depths than those who did not receive RAC in their feed. There was no effect of sex, RAC or protein level on the calliper skin thickness (Table 4.1). Due to the lack of effect on the HCW and HGP fat thickness, there are no differences seen in the lean meat percentage between treatments.

Since the average HCW was approximately 101 kg, there was no significant differences in carcass classification since most of the pigs were classified as “Sausage pigs” under the South African PORCUS system. However, the HGP fat thickness was approximately 17 mm, which was more than the calliper measurements in Table 4.1 and thus the carcasses would have been classified as “R” if they had been lighter. There were also no differences between the various factors for the carcass conformation score (visual appraisal according to South African classification system) with an average score of 4 (round carcass) across the treatments. The carcass lesion score data showed no significant differences between the sexes with an average score of 2 for E and C.

Table 4.1 The LSMMeans \pm SEM for the main effects and significant interactions for sex, RAC and protein level on the live weight, hot dressed carcass weight, Hennessey Grading Probe fat depth, calliper fat depth and calliper skin depth (mm)

Effect	Live Weight (kg)	HCW (kg)	HGP Fat Depth (mm)	Calliper Fat Depth (mm)	Calliper Skin Depth (mm)	Lean Meat (%)
Sex						
E	125.5 ^b \pm 1.34	101.4 \pm 0.89	17.4 \pm 0.40	9.2 \pm 0.31	3.8 \pm 0.14	67.5 \pm 0.16
C	130.7 ^a \pm 1.20	101.8 \pm 1.14	17.2 \pm 0.37	10.4 \pm 0.38	3.6 \pm 0.22	67.5 \pm 0.15
RAC mg/kg						
0	126.0 ^b \pm 1.02	101.95 \pm 0.96	17.4 \pm 0.37	10.3 ^a \pm 0.34	3.6 \pm 0.22	67.4 \pm 0.15
10	130.3 ^a \pm 1.51	101.31 \pm 1.09	17.1 \pm 0.40	9.3 ^b \pm 0.36	3.9 \pm 0.14	67.6 \pm 0.16
Protein						
Low	127.5 \pm 1.80	101.3 \pm 0.87	17.0 \pm 0.43	9.3 \pm 0.43	3.7 \pm 0.25	67.6 \pm 0.17
Med	128.0 \pm 1.54	102.3 \pm 1.48	17.7 \pm 0.53	10.4 \pm 0.49	3.8 \pm 0.26	67.3 \pm 0.21
High	129.0 \pm 1.51	101.3 \pm 1.35	17.1 \pm 0.45	9.6 \pm 0.38	3.7 \pm 0.17	67.6 \pm 0.18
Sex* Protein						
E Low				9.6 ^{cb} \pm 0.66		
E Med				9.4 ^{cb} \pm 0.44		
E High				8.7 ^c \pm 0.52		
C Low				9.0 ^{cb} \pm 0.55		
C Med				11.5 ^a \pm 0.79		
C High				10.5 ^{ab} \pm 0.47		

^{a,b} LSMMeans within columns for the main effects and interactions with different superscripts are significantly different ($p < 0.05$)

HCW: dressed hot carcass weight

HGP: Hennessey Grading Probe

E: entire males

C: immunocastrates

0: 0 mg/kg

10: 10 mg/kg

Med: medium

RAC: ractopamine hydrochloride

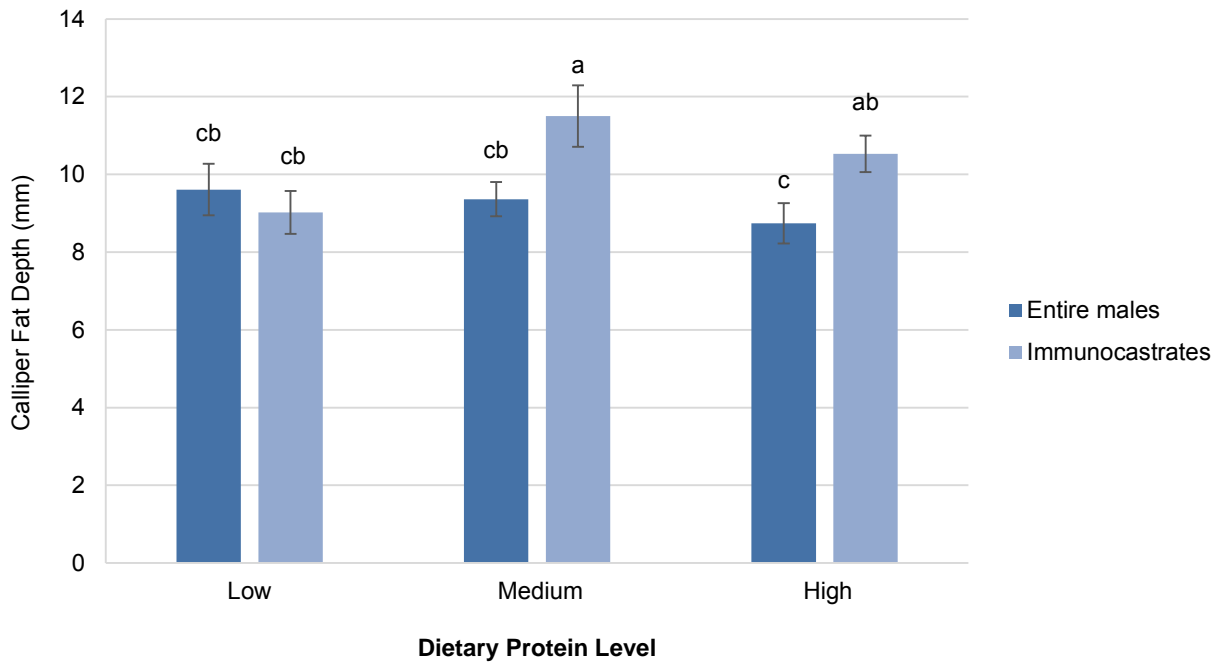


Figure 4.1 Interaction ($p = 0.039$) between sex and protein for the mean calliper backfat depth (mm) of the *Longissimus thoracis* muscle for entire males and immunocastrates fed at low, medium and high dietary protein. The different alphabetical letters denote significant differences ($p < 0.05$) between the mean values and vertical bars indicate SEM

4.3.2 Carcass cutting yields

There were no significant difference between the weights and the percentage weight of the heads in terms of the HCW or the shanks as a percentage of the half carcass weight (Table 4.2). However, RAC had an effect ($p = 0.040$) on the trotters, such that those pigs fed RAC had a lower proportion of the side being trotters than those who did not receive RAC. This was also seen in the weight of the trotters where RAC-fed pigs had lighter ($p = 0.001$) trotters than those fed without RAC. Sex influenced the weight of the shanks and trotters such that E had heavier shanks ($p = 0.014$) and trotters ($p = 0.020$) than C. When the percentage of the HCW represented by the shanks and trotters were considered, there was only a trend for increased percentages in E. The fillets were weighed in pairs and thus expressed in terms of whole carcass weight. The fillets from RAC-fed carcasses formed a higher ($p < 0.001$) proportion of the carcass and were heavier ($p < 0.001$) than the fillets from non-RAC pigs.

Table 4.2 The effects of sex, RAC and protein level on the head, trotters, shanks and fillets expressed as a percentage of the carcass weight (head and fillets) and half carcass weight (trotters and shanks) (LSMeans \pm SEM)

Effect		Head (%)	Trotters (%)	Shanks (%)	Fillets (%)
Sex					
	E	8.3 \pm 0.11	2.8 \pm 0.05	4.9 \pm 0.07	0.5 \pm 0.01
	C	8.4 \pm 0.14	2.7 \pm 0.04	4.7 \pm 0.09	0.5 \pm 0.01
RAC mg/kg					
	0	8.3 \pm 0.11	2.8 ^a \pm 0.04	4.8 \pm 0.08	0.46 ^b \pm 0.009
	10	8.5 \pm 0.13	2.6 ^b \pm 0.04	4.9 \pm 0.07	0.54 ^a \pm 0.012
Protein					
	Low	8.4 \pm 0.12	2.7 \pm 0.04	4.9 \pm 0.07	0.5 \pm 0.01
	Med	8.3 \pm 0.16	2.7 \pm 0.05	4.8 \pm 0.12	0.5 \pm 0.01
	High	8.4 \pm 0.17	2.7 \pm 0.06	4.8 \pm 0.09	0.5 \pm 0.02

^{a,b} LSMeans within columns for the main effects and interactions with different superscripts are significantly different ($p < 0.05$)

RAC mg/kg fillet data shown to two decimal places for LSMeans and three for SEM to illustrate significant differences

E: entire males

C: immunocastrates

0: 0 mg/kg

10: 10 mg/kg

RAC: ractopamine hydrochloride

Med: medium

Ractopamine hydrochloride had an effect on the weight of the green shoulder cut, with those pigs that received RAC (13.9 \pm 0.18 kg) having heavier ($p = 0.002$) shoulders than those who did not (13.1 \pm 0.14 kg; Table 4.3). Since there was no significant difference between the treatments for the bone and fat weight of the shoulder, this weight increase should be in the muscle weight. This was confirmed by RAC-fed treatments having heavier ($p < 0.001$) muscle weights in the shoulder than the no-RAC control treatments. The RAC-fed pigs had heavier shoulder muscle weights (8.6 \pm 0.12 kg) than those pigs who did not receive RAC (8.0 \pm 0.08 kg). When the shoulder was expressed as a percentage of the half carcass weight, RAC still had an effect by increasing the proportion of the carcass represented by the shoulder ($p = 0.010$) and the shoulder muscle ($p = 0.001$).

Table 4.3 The effects of sex, RAC and protein level on the shoulder, shoulder muscle, fat and bone expressed as a percentage of the half carcass weight (LSMeans \pm SEM)

Effect	Shoulder (%)	Shoulder Muscle (%)	Shoulder Fat (%)	Shoulder Bone (%)
Sex				
E	26.7 \pm 0.38	16.4 \pm 0.26	4.6 \pm 0.09	5.0 \pm 0.09
C	26.9 \pm 0.53	16.5 \pm 0.34	4.7 \pm 0.10	5.0 \pm 0.12
RAC mg/kg				
0	25.9 ^b \pm 0.39	15.7 ^b \pm 0.25	4.6 \pm 0.09	4.9 \pm 0.09
10	27.6 ^a \pm 0.50 ^b	17.2 ^a \pm 0.32	4.6 \pm 0.10	5.1 \pm 0.12
Protein				
Low	26.7 \pm 0.47	16.2 \pm 0.32	4.7 \pm 0.11	5.0 \pm 0.12
Med	26.7 \pm 0.52	16.5 \pm 0.35	4.6 \pm 0.13	5.0 \pm 0.11
High	26.9 \pm 0.69	16.6 \pm 0.43	4.6 \pm 0.11	5.1 \pm 0.15

^{a,b} LSMean within each column for the main effects and interactions with different superscripts are significantly different ($p < 0.05$)

E: entire males

C: immunocastrates

0: 0 mg/kg

10: 10 mg/kg

RAC: ractopamine hydrochloride

Med: medium

There was a significant effect of RAC and sex on the actual weight of the hindquarter cut (Table 4.4). The C had heavier ($p = 0.023$) hindquarters than E and those pigs fed RAC had heavier ($p = 0.009$) hindquarters than those not fed RAC, regardless of sex. The supplementation of RAC also had an effect on the muscle and fat weights of the hindquarter, but not the bone weight. Those pigs who were fed RAC had heavier ($p = 0.001$) hindquarter muscle weights and lighter ($p = 0.012$) hindquarter fat weights than those fed 0 mg/kg RAC. When the hindquarter was expressed as a percentage of the half carcass weight, RAC had an effect on the hindquarter percentage ($p = 0.026$), muscle percentage ($p = 0.0007$) and fat percentage ($p = 0.032$) but sex no longer had an effect. Feeding RAC increased the hindquarter percentage and hindquarter muscle percentage while decreasing the hindquarter fat percentage. The bone percentage showed significant interaction between sex, RAC and protein levels (Figure 4.2). Due to the small differences for the bone percentages, the LSMean and standard errors are shown to two and three decimal places respectively in Table 4.4.

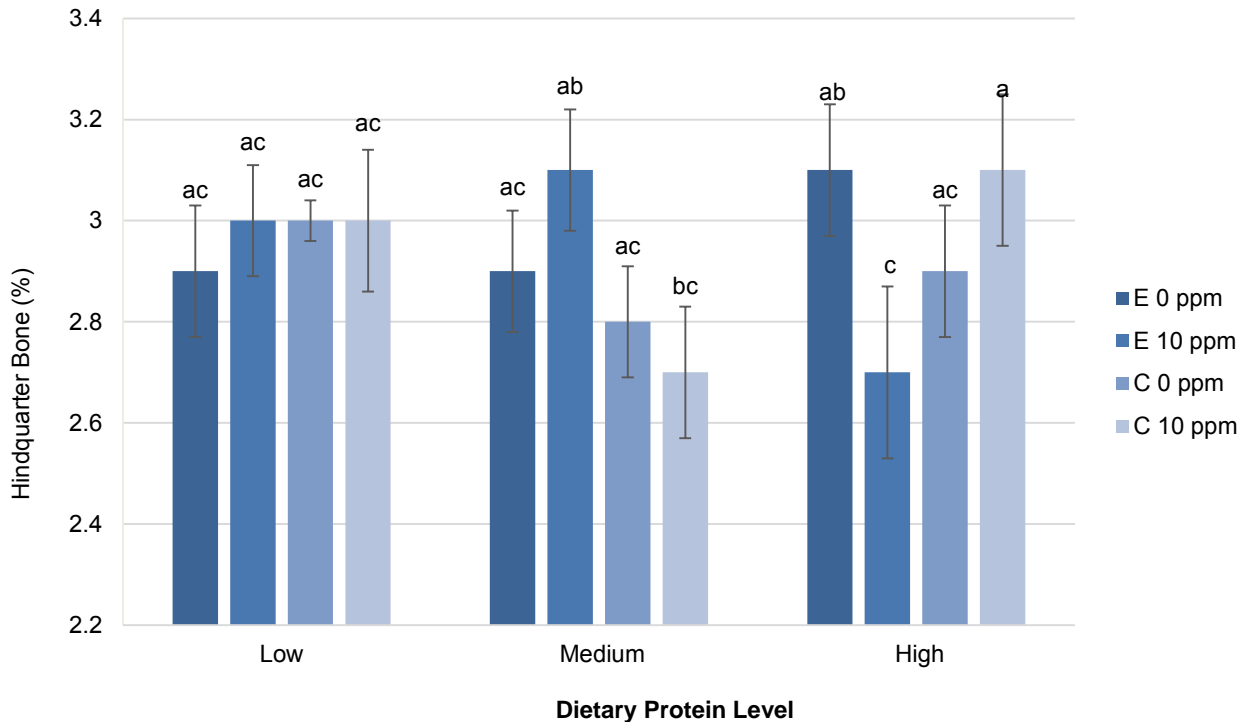


Figure 4.2 Interaction ($p = 0.049$) between sex, ractopamine hydrochloride and protein for the hindquarter bone percentage for entire males (E) and immunocastrates (C) fed at low, medium and high protein with (10 mg/kg) and without (0 mg/kg) RAC. The different alphabetical letters denote significant differences ($p < 0.05$) between the mean values and vertical bars indicate SEM

The RAC had a significant effect on the weight of the green loin cut, with those fed RAC having heavier loins than those fed without RAC (Table 4.5). Sex influenced the loin weight and C had heavier ($p = 0.030$) loins than the E. Ractopamine hydrochloride also influenced the muscle weight of the loin so that those fed 10 mg/kg RAC had heavier ($p = 0.005$) loin muscle weights than those fed 0 mg/kg RAC. Sex had a significant effect on loin fat weight and C had heavier ($p < 0.001$) loin fat weights than E. When one considers the proportion of the carcass represented by the loin, RAC still had a significant effect by increasing the loin percentage ($p = 0.028$) and loin muscle percentage ($p = 0.013$). Sex still had an effect on fat percentage with C having a higher ($p < 0.001$) proportion of the carcass represented by the loin fat. Neither sex, RAC nor lysine influenced loin bone weight or percentage.

Table 4.4 The effects of sex, RAC and protein level on the hindquarter, hindquarter muscle, fat and bone expressed as a percentage of the half carcass weight (LSMeans \pm SEM)

Effect	Hindquarter (%)	Hindquarter Muscle (%)	Hindquarter Fat (%)	Hindquarter Bone (%)
Sex				
E	26.2 \pm 0.41	19.2 \pm 0.31	4.3 \pm 0.14	2.9 \pm 0.05
C	27.2 \pm 0.48	19.6 \pm 0.38	4.6 \pm 0.12	2.9 \pm 0.05
RAC mg/kg				
0	26.0 ^b \pm 0.36	18.5 ^b \pm 0.26	4.6 ^a \pm 0.14	2.9 \pm 0.05
10	27.3 ^a \pm 0.51	20.2 ^a \pm 0.39	4.2 ^b \pm 0.11	2.9 \pm 0.06
Protein				
Low	26.6 \pm 0.52	19.2 \pm 0.39	4.5 \pm 0.14	3.0 \pm 0.05
Med	26.6 \pm 0.52	19.4 \pm 0.40	4.3 \pm 0.15	2.9 \pm 0.06
High	26.7 \pm 0.60	19.5 \pm 0.48	4.5 \pm 0.17	2.9 \pm 0.07
Sex*Protein*RAC				
E Low 0				2.92 ^{ac} \pm 0.126
E Med 0				2.93 ^{ac} \pm 0.118
E High 0				3.08 ^{ab} \pm 0.129
E Low 10				2.95 ^{ac} \pm 0.111
E Med 10				3.06 ^{ab} \pm 0.120
E High 10				2.70 ^c \pm 0.167
C Low 0				3.00 ^{ac} \pm 0.039
C Med 0				2.80 ^{ac} \pm 0.112
C High 0				2.85 ^{ac} \pm 0.131
C Low 10				2.98 ^{ac} \pm 0.141
C Med 10				2.74 ^{bc} \pm 0.130
C High 10				3.12 ^a \pm 0.149

^{a,b} LSMMeans within columns for the main effects and interactions with different superscripts are significantly different ($p < 0.05$)

E: entire males

C: immunocastrates

0: 0 mg/kg

10: 10 mg/kg

Med: medium

RAC: ractopamine hydrochloride

Table 4.5 The effects of sex, RAC and protein level on the loin, loin muscle, fat and bone expressed as a percentage of the half carcass weight (LSMeans \pm SEM)

Effect		Loin (%)	Loin muscle (%)	Loin fat (%)	Loin bone (%)
Sex					
	E	14.4 \pm 0.28	8.3 \pm 0.20	2.7 ^b \pm 0.08	3.0 \pm 0.10
	C	15.1 \pm 0.30	8.5 \pm 0.20	3.3 ^a \pm 0.10	2.9 \pm 0.07
RAC mg/kg					
	0	14.3 ^b \pm 0.23	8.1 ^b \pm 0.15	3.0 \pm 0.08	2.8 \pm 0.06
	10	15.2 ^a \pm 0.34	8.8 ^a \pm 0.23	3.0 \pm 0.11	3.1 \pm 0.11
Protein					
	Low	14.8 \pm 0.38	8.4 \pm 0.23	3.1 \pm 0.13	2.9 \pm 0.08
	Med	14.7 \pm 0.31	8.4 \pm 0.20	3.0 \pm 0.12	2.9 \pm 0.08
	High	14.8 \pm 0.39	8.5 \pm 0.31	3.0 \pm 0.12	3.0 \pm 0.15

^{a,b} LSMMeans within each column for the main effects and interactions with different superscripts are significantly different ($p < 0.05$)

E: entire males

C: immunocastrates

0: 0 mg/kg

10: 10 mg/kg

RAC: ractopamine hydrochloride

For belly weight, there was a sex and RAC interaction ($p = 0.045$) with C fed RAC having heavier bellies than E with ($p < 0.001$) and without RAC ($p < 0.001$) as well as C fed without RAC ($p = 0.003$; Table 4.6). There was also a sex and RAC interaction for belly muscle weight so that C fed RAC had heavier belly muscle weights than E with ($p < 0.001$) and without RAC ($p = 0.002$) as well as C fed without RAC ($p = 0.013$). For belly fat weight, the main effect of sex was significant and E had less ($p = 0.034$) belly fat than C. There was a significant interaction between sex and lysine for the bone weight of the belly ($p = 0.007$) with E Low protein having lower belly bone weights than E Medium ($p = 0.001$) and E High ($p = 0.018$). The C High treatments also had lower belly bone weights than E Medium ($p = 0.001$) and E High ($p = 0.015$). When the belly was evaluated as a proportion of the carcass, the main effects of sex and RAC were significant. Immunocastrates had a larger ($p = 0.006$) proportion of the carcass being bellies and RAC increased ($p = 0.044$) the belly percentage. For the belly muscle, the sex and RAC interaction was still significant ($p = 0.026$) with C fed RAC having larger belly muscle percentages than E with ($p = 0.005$) and without RAC ($p = 0.003$) as well as the C fed without RAC ($p = 0.009$) (Figure 4.3). There was also an interaction between sex and dietary protein for the bone percentage of the belly ($p = 0.014$) with E Low having lower belly bone percentages than E Medium ($p = 0.003$) and E High ($p = 0.041$) and E Medium having higher belly bone percentages than C Medium ($p = 0.037$) and C High (0.006) (Figure 4.4). A log transformation of the belly fat percentage data was done since this better represented the data. It was then found that sex influenced belly fat percentage ($p < 0.001$), such that C had higher belly fat percentages than E.

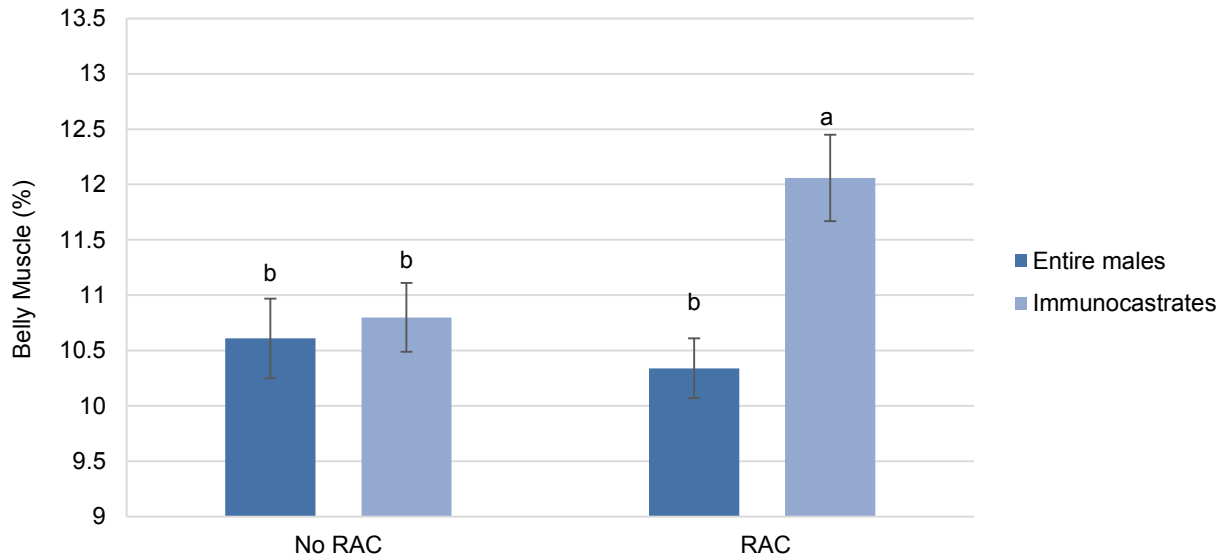


Figure 4.3 Interaction ($p = 0.026$) between sex and ractopamine for the belly muscle percentage for entire males (E) and immunocastrates (C). The different alphabetical letters denote significant differences ($p < 0.05$) between the mean values and vertical bars indicate SEM

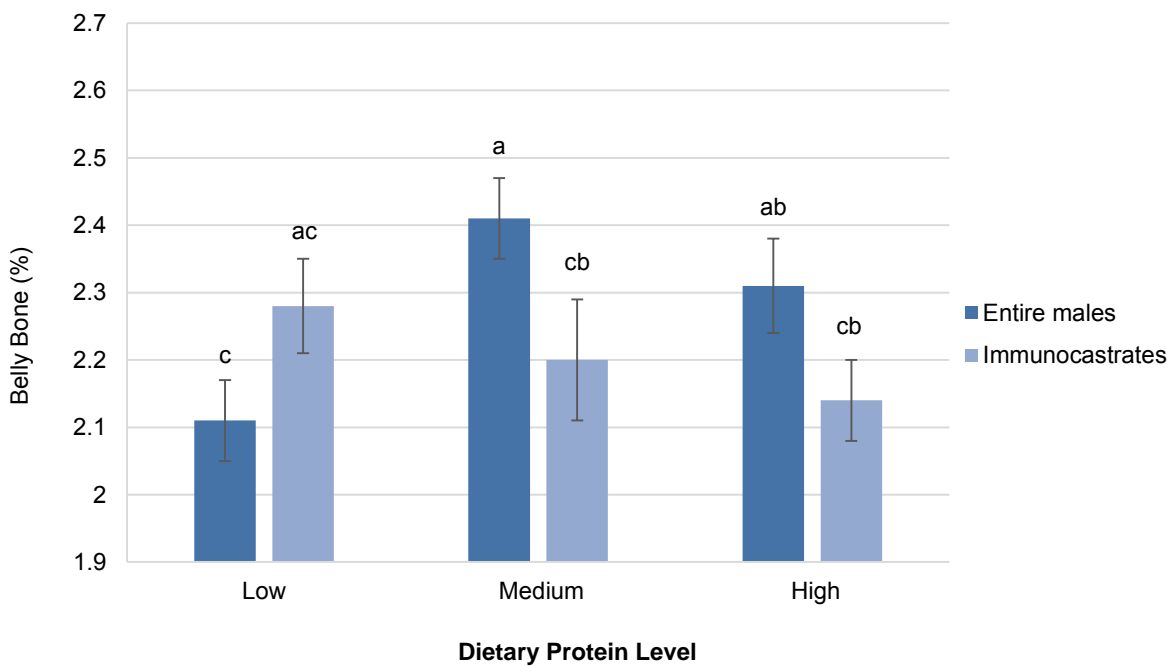


Figure 4.4 Interaction ($p = 0.014$) between sex and protein level for the belly bone percentage for entire males (E) and immunocastrates (C) fed at low, medium and high dietary protein. The different alphabetical letters denote significant differences ($p < 0.05$) between the mean values and vertical bars indicate SEM

Table 4.6 The effects of sex, RAC and lysine level on the belly, belly muscle, fat and bone expressed as a percentage of the half carcass weight (LSMeans \pm SEM)

Effect	Belly (%)	Belly Muscle (%)	Belly Fat (%)	Belly Bone (%)
Sex				
E	13.8 ^b \pm 0.27	10.5 \pm 0.22	0.9 ^b \pm 0.03	2.3 \pm 0.04
C	14.9 ^a \pm 0.31	11.4 \pm 0.26	1.3 ^a \pm 0.03	2.2 \pm 0.04
RAC mg/kg				
0	14.0 ^b \pm 0.25	10.7 \pm 0.23	1.0 \pm 0.03	2.2 \pm 0.04
10	14.8 ^a \pm 0.33	11.2 \pm 0.26	1.0 \pm 0.03	2.3 \pm 0.04
Protein				
Low	14.2 \pm 0.41	10.9 \pm 0.35	1.0 \pm 0.03	2.2 \pm 0.05
Med	14.5 \pm 0.35	11.0 \pm 0.31	1.1 \pm 0.04	2.3 \pm 0.06
High	14.4 \pm 0.34	10.9 \pm 0.25	1.0 \pm 0.04	2.2 \pm 0.05
Sex*RAC				
E 0		10.6 ^b \pm 0.36		
E 10		10.3 ^b \pm 0.27		
C 0		10.8 ^b \pm 0.31		
C 10		12.1 ^a \pm 0.39		
Sex*Protein				
E Low				2.1 ^c \pm 0.06
E Med				2.4 ^a \pm 0.06
E High				2.3 ^{ab} \pm 0.07
C Low				2.3 ^{ac} \pm 0.07
C Med				2.2 ^{cb} \pm 0.09
C High				2.1 ^{cb} \pm 0.06

^{a,b} LSMeans within each column for the main effects and interactions with different superscripts are significantly different ($p < 0.05$)

E: entire males

C: immunocastrates

0: 0 mg/kg

10: 10 mg/kg

Med: medium

RAC: ractopamine hydrochloride

4.4 Discussion

4.4.1 Live weight, hot carcass weight, carcass scores and fat thickness

Immunocastration increased the live weight at slaughter by approximately 5 kg compared to entire males; however, no differences were seen in dressed HCW. This is most likely due to the significant increase in feed intake seen from the growth performance results in Chapter 3 and thus increased gut fill. Also, bladder fill and organ weight could have influenced the fact that no differences were seen in the HCW. The absence of male steroid hormones allows the liver to function more efficiently with regards to the metabolism of compounds such as skatole (Babol *et al.*, 1999) and therefore increasing liver activity which could have an influence on the liver weight. The effect of immunocastration on liver functioning is unclear and thus investigation into this could be beneficial. Numerous factors may influence the differences between sexes (males and immunocastrates) in terms of live weight at slaughter and dressed carcass mass, including gut fill, the amount of time between the second vaccination and slaughter and by whether or not weights were distributed evenly across treatment groups. Therefore, the conclusion varies between studies as to whether live weight and HCW differ between sexes (Fàbrega *et al.*, 2010, Gispert *et al.*, 2010; Weiler *et al.*, 2013) or not (Bonneau *et al.*, 1994; Zamaratskaia *et al.*, 2008; Lealiifano *et al.*, 2011). The backfat thickness is also influenced by various factors such as diet, the physiological age at which the booster vaccination is given and its timing relative to slaughter and thus results also vary. The current results indicated no differences in HGP backfat thickness between the two sexes which is in agreement with Dunshea *et al.* (2001) and Lealiifano *et al.* (2011) who both also allowed for four weeks between the booster and slaughter. Results from backfat measurements can also vary between instruments used as well as between users; for instance should the carcass be particularly long or the user is short in comparison, the HGP may not be inserted perpendicularly to the carcass. It was for this reason that the calliper fat thickness of the loins was measured at 48 hours *post mortem*. In Chapter 3, no response to protein level was seen in backfat gain, however, the results from the calliper backfat depths showed significant interaction for sex and protein levels. From the interaction, one could say that the level of lysine affected the backfat thickness and the response of which depended on the sex of the pig and that the low protein diet resulted in the numerically lowest backfat depth for immunocastrates and the high protein diet resulted in the numerically lowest backfat depth for entire males. Although the differences were not significant, the calliper backfat depth of E decreased as the protein level increased (Figure 4.1). A similar trend was evident in the trial by Boler *et al.* (2011) who found that increased lysine levels numerically decreased backfat thickness in immunocastrates but only the C fed high lysine had significantly higher backfat depths than E fed high lysine.

Feeding RAC also influenced the live weight at slaughter and calliper backfat thickness without affecting the hot dressed carcass weight or HGP backfat thickness. Including RAC in the diets increased final body weight by approximately 4 kg and decreased calliper backfat thickness by approximately 1 mm. Results with regards to the effects of 10 mg/kg on carcass traits vary since the duration of inclusion, dietary protein levels and genotype of the pig could influence the response. Carr

et al. (2005b) fed 10 mg/kg RAC for 28 days to PIC[®] pigs and found a significant difference for final body weight between 0 and 10 mg/kg treatments as well as no difference in dressed HCW. It can also be seen by their results that whether the differences in backfat thickness were significant or not depended on the site measured, such that there was a significant difference between treatments for 10th and last rib measurements and no differences for the 1st rib and last lumbar measurements. However, a meta-analysis of 29 publications from 1990 to 2007 by Andretta *et al.* (2012) showed that feeding RAC increased HCW and an earlier meta-analysis by Apple *et al.* (2007) showed that feeding 10 mg/kg RAC increased HCW by 3.1 %. It was highlighted by Apple *et al.* (2007) that conclusions regarding HCW and 10 mg/kg RAC differed, with some researchers finding a significant increase (Stites *et al.*, 1991; Herr *et al.*, 2001; Merchant-Forde *et al.*, 2003; Armstrong *et al.*, 2004; Carr *et al.*, 2005a; See *et al.*, 2005), those who found no effect (Watkins *et al.*, 1990; Crome *et al.*, 1996; Stoller *et al.*, 2003; Carr *et al.*, 2005b) and those who found a negative effect (Aalhus *et al.*, 1990; Stoller *et al.*, 2003). The data regarding feeding immunocastrates is limited; however, one study performed by Rikard-Bell *et al.* (2009) found that HCW increased by feeding RAC to immunocastrates and that HCW were greater for immunocastrates than boars and gilts. Although this agrees with Apple *et al.* (2007) and Andretta *et al.* (2012), it cannot be compared with the current trial's results since a step-up in inclusion level of RAC (5 mg/kg for 14 days followed by 10 mg/kg for 17 days) was used by Rikard-Bell *et al.* (2009).

Backfat thickness is inversely correlated to the duration of RAC supply, level of inclusion as well as the estimated RAC intake (Andretta *et al.*, 2012) and thus it is expected for results to vary between those who found differences (Watkins *et al.*, 1990; Merchant-Forde *et al.*, 2003; Apple *et al.*, 2004a; Carr *et al.*, 2005b;) and those who did not (Aalhus *et al.*, 1990; Stites *et al.*, 1991; Crome *et al.*, 1996; Herr *et al.*, 2001; Stoller *et al.*, 2003; Carr *et al.*, 2005a; See *et al.*, 2005; Hinson *et al.*, 2011). Therefore the theory that RAC repartitions nutrients away from lipid synthesis to protein accretion does not always hold true. However, Apple *et al.* (2007) showed that there is a numerical decrease in backfat thickness when RAC is fed but it is not always statistically significant which is true for the current backfat depth data, since the HGP values did not differ but the calliper measurements showed a significant decrease. The calliper backfat measurements and linear carcass results thus agree to a certain extent with Webster *et al.* (2007) who found that increased dietary lysine decreased the average backfat thickness but not the carcass characteristics of pigs when fed 10 mg/kg RAC, except in the current research sex affected the response to protein levels. Lysine levels have been shown to increase live slaughter weight and HCW of immunocastrated pigs (Boler *et al.*, 2011), however no effect was seen in the current results for protein levels. This could be due to the fact that Boler *et al.* (2011) increased only the dietary lysine levels and not the other amino acids as well as followed a step-down lysine program from grower to first vaccination and then first vaccination to the booster. This was not done in the current trial where all pigs received the same lysine level up until the booster when they were changed to the various increased lysine levels with all amino acid ratios maintained. Thus at the low protein level, all other amino acids were not limiting in the current trial due to being unbalanced, which could have occurred at the low lysine level of Boler *et al.* (2011), which would affect factors such as lipid versus protein deposition.

When the HGP fat thickness and calliper fat thicknesses are considered, a large variation in measurements is seen and thus could be a possible reason for a lack in significant differences for HGP fat thickness. The average fat thickness across the various main effects was 17 mm for the HGP and between 8.7 and 11.5 mm for the calliper measurements, thus a difference of approximately 6 to 9 mm exists. The HGP includes the skin thickness in its measurements; however, skin thickness was ~ 3.8 mm which leaves 3 to 6 mm unaccounted for. However, some of this difference seen between instruments could be attributed to shrinkage of the adipose tissue since the calliper measurements were taken on the cold loin cut. This reiterates the effect of measurement techniques on the results for backfat thickness and suggests that calliper measurements be taken in order to avoid incorrect measurement using the HGP due to factors such as the insertion not being perpendicular to the skin or calibration issues. Also, it could be necessary to compare calliper measurements taken on a hot carcass to those measurements taken after the carcass has been cooled in order to establish if shrinkage of the fat could influence the measurements.

Lealiifano *et al.* (2011) found that entire male pigs had a greater carcass lesion score than immunocastrates; however their animals were group housed during the trial and thus allowed to interact with each other. The current carcass lesion score data collected at the abattoir could not conclude anything about fighting in lairage most probably due to the short period in lairage of one hour and thus it was not possible to identify if immunocastrates were bullied by entire males. Thus in future, if the experimental objective is to evaluate the effect of castration on lesion scores, the pigs should be kept longer in lairage and the group numbers and a chosen sex ratio be kept constant if they are to be split into groups. Thus the short lairage time provided minimal opportunity for fighting and the fact that the *post mortem* pH and drip loss were normal (Chapter 5, section 5.3) indicates that a longer lairage time was not necessary in terms of recovery from transport stress. The carcass classification data was also of little use since the average carcass weight was approximately 101 kg which meant that they were immediately classified as sausage pigs regardless of their fat thickness using the current South African PORCUS system. However, should just the backfat thickness be considered, most of the carcasses would have been considered “R” since the average HGP fat thickness across the sexes, diets and RAC inclusion level was 17 mm.

4.4.2 Carcass cutting yields

Since the way in which the carcass has been fabricated varies between studies, carcass cutting yield data differs which may limit ability to compare them. As a result, very few studies have trimmed the green cuts into muscle, fat and bone as has been done in the current study and therefore this data is important from a practical perspective as it determines the value of the carcass with a higher value been placed on muscle (for example the loin muscle used in the making of back bacon) than on fat (and skin) as the latter is typically used in the production of cheaper emulsions.

The only green cut percentage influenced by immunocastration was the belly, which increased by 1 %. Immunocastration also increased the loin fat percentage by 0.6 % and the belly fat percentage by 0.4 %, which is in agreement with Gispert *et al.* (2010) who found a higher proportion

of subcutaneous loin fat in immunocastrates. The lack of effect of immunocastration on the head, trotters, shoulder, hindquarter and loin percentages is supported by Bonneau *et al.* (1994) as well as Gispert *et al.* (2010), except for the loin percentage where Gispert *et al.* (2010) found immunocastration increased loin percentage which was not observed in the current research. The current results further agree and disagree with Gispert *et al.* (2010) in various ways. The results are in agreement in terms of the fact that immunocastration increased the proportion of the carcass represented by the belly cut and did not influence tenderloin (fillet) percentage.

They disagree with the conclusion that immunocastrates had more muscle, more subcutaneous fat and less bone in the ham than entire males in terms of percentage of the carcass and that immunocastrates had lighter heads than entire males. Since the ham cut was used, the trotter and shank were included in this measurement by Gispert *et al.* (2010) but in the current study the trotters and shanks were evaluated separately which could influence the findings. Both the current results and Gispert *et al.* (2010) do not agree with Bonneau *et al.* (1994), who found no difference in the belly cut between entire males and immunocastrates in terms of the carcass percentage.

Interactions existed between sex and protein for the belly bone percentage as well as between sex, RAC and protein for the hindquarter bone percentage. The effect of sex, RAC and lysine on bone can be seen in research by Moore *et al.* (2009) who found that immunocastration and RAC decreased the percentage of bone when the side of the carcass was analysed using dual energy X-ray absorptiometry and Boler *et al.* (2011) who found bone weight to be heavier in entire males than immunocastrates and surgical castrates, with the exception of immunocastrates fed 9 g lysine/kg. Most of the differences for the hindquarter bone in the current results between the treatments appear to be negligible biologically except for that fed RAC at medium lysine and high lysine. For example, the highest hindquarter bone percentage was 3.12 ± 0.15 % for C RAC High protein and the lowest was 2.70 ± 0.17 % for E RAC High protein. This may become important in cuts for cured meat production, such as the hindquarter, where the price of the cut is determined by the bone: meat ratio. The fact that there was no effect of protein on any of the green cuts, muscle or fat percentage agrees with Boler *et al.* (2011) who did not find differences in whole ham percentages from immunocastrates, entire males and surgical castrates regardless of lysine percentage fed.

Including 10 mg/kg RAC increased the percentage of the HCW represented by the shoulder (1.7 %), hindquarter (1 %), loin (0.9 %) and belly (0.8 %) as well as the shoulder muscle (1.4 %), hindquarter muscle (1.5 %) and loin muscle (0.7 %) while decreasing the hindquarter fat percentage (0.4 %). Thus the increase in cut percentages seem to be largely due to increased lean muscle growth rather than decreased fat deposition, with the exception of the hindquarter. A decreased fat percentage in the hindquarter may also negatively affect the desirability of the cut for dried cured meat production and thus the value if it is sold for this particular purpose. Those pigs fed RAC also had higher fillet percentages and lower trotter and shank percentages, however, the statistical differences are negligible from an industry point of view since the trotters have a low re-sale value and the fillets comprise such a small proportion of the carcass. The increase in loin percentage agrees with Rikard-Bell *et al.* (2009) and the influence of RAC on the muscle and fat of the shoulder agrees with Moore *et al.* (2009) who found that RAC increased lean tissue weights but did not alter fat tissue

weights of the half carcass. However, they differ from Carr *et al.* (2005a) who found no difference in the loin, shoulder and belly as a percentage of the carcass when RAC was fed, but did find differences in their respective weights. This could be due to the fact that RAC was fed until the pigs reached slaughter weight and thus the feeding period varied from 25 to 41 days. It has been noted that the effect of RAC decreases as the duration of supply increases, as the β -adrenoceptors are down-regulated within the fat tissue and/or desensitized in skeletal muscle tissue (Spurlock *et al.*, 1994). Although the inclusion of RAC does not affect the total number of muscle fibres, the diameters of the white muscle fibres increase in response to RAC supplementation (Aalhus *et al.*, 1992). Thus the increase in the proportion of the HCW represented by the muscle tissue of the various cuts is likely due to the effect of RAC on the muscle fibre diameters.

4.5 Conclusion

Immunocastration and RAC inclusion resulted in heavier live body weights at slaughter but not HCW most likely due to differences in genital weight (Chapter 6) and gut fill in the live animal. An interaction for sex and protein levels for the calliper backfat depths indicates that the protein requirements differ between the sexes and an oversupply or under supply of balanced protein can result in increased fat deposition. Since the PORCUS system and the price of the carcass relies on the backfat thickness, as well as weight, it could prove beneficial to further evaluate the effect of protein levels on immunocastrates using a larger sample size and wider protein level range. The HGP backfat thickness measurements did not show the differences seen with the calliper measurements and thus immunocastration did not influence the classification in terms of backfat thickness. Therefore, calliper measurements are preferred above the HGP when backfat is measured *post mortem* due to the differences in the results between the two methods. Including 10 mg/kg RAC decreased calliper backfat thickness and increased the percentages of all cuts evaluated as well as the lean tissue content of these cuts, thus increasing the value of the carcasses. Immunocastration increased the percentage of the half carcass weight represented by the belly cut, loin fat and belly fat while the significant interaction between sex and RAC for belly muscle indicated that by including RAC at 10 mg/kg in the diet of immunocastrates, belly muscle percentage increased by approximately 2 % of the half carcass weight. Further research should also be done in terms of the bone percentage of the ham weight since the bone: muscle ratio is an important factor in the cost of cured ham products. Therefore, one can conclude that RAC increased the muscle percentages of all the cuts in terms of the HWC but only decreased the fat percentage of the hindquarter when expressed as a proportion of the HCW. The current results indicate that the carcass traits of immunocastrates are close to entire males but the negative effects on backfat thickness due to immunocastration could be avoided should the correct dietary lysine level be provided and including RAC in the diet provided improved carcass cutting yields and lean yields of the shoulder, hindquarter and loin.

A question that should be debated further regarding the South African carcass classification is whether the cut-off point should not be raised so that heavier pigs will not be penalised. When the present carcass classification system was developed, heavier pigs were invariably fatter and intact

males had a higher incidence of and stronger boar taint. However, with new genetics, more efficient diet compositions and interventions such as the use of immunocastration and feeding of RAC, it could be argued that the meat quality and lean meat yield of these heavier pigs is not compromised. Research would be required to evaluate the meat quality of the heavier pigs. Another factor that could influence whether or not the carcass classification should change would be the market; for example, heavier carcasses would lead to larger back bacon portions which would change the packaging criteria and thus display space usage in the refrigeration units. The production of heavier carcasses could thus provide incentive to make use of immunocastration as a means of controlling boar taint while allowing for a larger profit margin on heavier carcasses without being penalised for being a male.

4.6 References

- Aalhus, J. L., Jones, S. D. M., Schaefer, A. L., Tong, A. K. W., Robertson, W. M., Merrill, J. K., & Murray, A. C. (1990). The effect of ractopamine on performance, carcass composition and meat quality of finishing pigs. *Canadian Journal of Animal Science*, 70, 943-952.
- Andretta, I., Kipper, M., Lehnen, C. R., Demori, A. B., Remus, A. & Lovatto, P. A. (2012). Meta-analysis of the relationship between ractopamine and dietary lysine levels on carcass characteristics in pigs. *Livestock Science*, 143, 91–96.
- Apple, J. K., Kutz, B. R., Maxwell, C. V., Davis, M. E., Rakes, L. K., Johnson, Z. B., & Armstrong, T. A. (2004a). Effects of ractopamine and dietary fat source on performance and carcass characteristics of growing-finishing swine. *Journal of Animal Science*, 82 (Suppl. 1), 135.
- Apple, J., Maxwell, C., Brown, D., Friesen, K., Musser, R., Johnson, Z. & Armstrong, T. (2004b). Effects of dietary lysine and energy density on performance and carcass characteristics of finishing pigs fed ractopamine. *Journal of Animal Science*, 82, 3277–3287.
- Apple, J., Rincker, P., McKeith, F., Carr, S., Armstrong, T. & Matzat, P. (2007). Review: Meta-analysis of the ractopamine response in finishing swine. *The Professional Animal Scientist*, 23, 179–196.
- Armstrong, T., Ivers, D., Wagner, J., Anderson, D., Weldon, W. & Berg, E. (2004). The effect of dietary ractopamine concentration and duration of feeding on growth performance, carcass characteristics, and meat quality of finishing pigs. *Journal of Animal Science*, 82, 3245–3253.
- Babol, J., Squires, E. J. & Lundström, K. (1999). Relationship between metabolism of androstenone and skatole in intact male pigs. *Journal of Animal Science*, 77, 84–92.
- Boler, D., Kutzler, L., Meeuwse, D., King, V., Champion, D., McKeith, F. & Killefer, J. (2011). Effects of increasing lysine on carcass composition and cutting yields of immunologically castrated male pigs. *Journal of Animal Science*, 89, 2189–2199.
- Bonneau, M., Dufour, R., Chouvet, C., Roulet, C., Meadus, W. & Squires, E. (1994). The effects of immunization against luteinizing hormone-releasing hormone on performance, sexual development, and levels of boar taint-related compounds in intact male pigs. *Journal of Animal Science*, 72, 14–20.

- Carr, S., Ivers, D. J., Anderson, D. B., Jones, D. J., Mowrey, D.H, England, M. B., Killefer, J., Rincker, P.J., & McKeith, F. (2005a). The effects of ractopamine hydrochloride on lean carcass yields and pork quality characteristics. *Journal of Animal Science*, *83*, 2886–2893.
- Carr, S., Rincker, P., Killefer, J., Baker, D., Ellis, M. & McKeith, F. (2005b). Effects of different cereal grains and ractopamine hydrochloride on performance, carcass characteristics, and fat quality in late-finishing pigs. *Journal of Animal Science*, *83*, 223–230.
- Crome, P. K., McKeith, F. K., Carr, T. R., Jones, D. J., Mowrey, D. H., & Cannon, J. E. (1996). Effect of ractopamine on growth performance, carcass composition, and cutting yields of pigs slaughtered at 107 and 125 kilograms. *Journal Animal Science*, *74*, 709-716.
- Dunshea, F., Colantoni, C., Howard, K., McCauley, I., Jackson, P., Long, K., Lopaticki, S., Nugent, E., Simons, J., Walker, J., & Hennessey, D. P. (2001). Vaccination of boars with a GnRH vaccine (Improvac) eliminates boar taint and increases growth performance. *Journal of Animal Science*, *79*, 2524–2535.
- Dunshea, F., King, R., Campbell, R., Sainz, R. & Kim, Y. (1993). Interrelationships between sex and ractopamine on protein and lipid deposition in rapidly growing pigs. *Journal of Animal Science*, *71*, 2919–2930.
- Fàbrega, E., Velarde, A., Cros, J., Gispert, M., Suárez, P., Tibau, J. & Soler, J. (2010). Effect of vaccination against gonadotrophin-releasing hormone, using Improvac[®], on growth performance, body composition, behaviour and acute phase proteins. *Livestock Science*, *132*, 53–59.
- Gispert, M., Àngels Oliver, M., Velarde, A., Suarez, P., Pérez, J. & Font i Furnols, M. (2010). Carcass and meat quality characteristics of immunocastrated male, surgically castrated male, entire male and female pigs. *Meat Science*, *85*, 664–670.
- Herr, C. T., Hankins, S. L., Schinckel, A. P., & Rickert, B. T. (2001). Evaluation of three genetic populations of pigs for response to increasing levels of ractopamine. *Journal of Animal Science*, *79* (Suppl. 2), 73.
- Hinson, R., Wiegand, B., Ritter, M., Allee, G. & Carr, S. (2011). Impact of dietary energy level and ractopamine on growth performance, carcass characteristics, and meat quality of finishing pigs. *Journal of Animal Science*, *89*, 3572–3579.
- Hoffman, L. C. 1987. Die doeltreffendheid van sekere parameters ter bepaling van die weefselamestelling van varkkarkasse. M.Sc thesis, University of Stellenbosch, Western Cape, South Africa.
- Lealiifano, A., Pluske, J. R., Nicholls, R., Dunshea, F., Campbell, R., Hennessey, D., Miller, D., Hansen, C. F., & Mullan, B. (2011). Reducing the length of time between slaughter and the secondary gonadotropin-releasing factor immunization improves growth performance and clears boar taint compounds in male finishing pigs. *Journal of Animal Science*, *89*, 2782–2792.
- Marchant-Forde, J. N., Lay, D. C. Jr., Pajor, E. A., Richert, B. T., & Schinckel, A. P. (2003). The effects of ractopamine on the behavior and physiology of finishing pigs. *Journal of Animal Science*, *81*, 416-422.

- McCauley, I., Cronin, G. M., Hennessy, D. P., Smits, R. J. & Dunshea, F. R. (2001). Carcass damage at slaughter is reduced equally by surgical and immunological castration. In Cranwell, P. D. (ed.), *Manipulating Pig Production VIII*. Australasian Pig Science Association, Adelaide, South Australia. (pp 82)
- Moore, K., Dunshea, F., Mullan, B., Hennessy, D. & D'Souza, D. (2009). Ractopamine supplementation increases lean deposition in entire and immunocastrated male pigs. *Animal Production Science*, *49*, 1113–1119.
- Pieterse, E. 2006. Effects of increased slaughter weight of pigs on pork production. Ph.D thesis, University of Stellenbosch, Western Cape, South Africa.
- Republic of South Africa. (1990). *Agricultural Product Standards Act 119 of 1990*. Retrieved from www.nda.agric.za/docs/NPPOZA/APS%20Act.pdf
- Rikard-Bell, C., Curtis, M., Van Barneveld, R. J., Mullan, B., Edwards, A. C., Gannon, N., Henman, D. J., Hughes, P. E., & Dunshea, F. (2009). Ractopamine hydrochloride improves growth performance and carcass composition in immunocastrated boars, intact boars, and gilts. *Journal of Animal Science*, *87*, 3536–3543.
- See, M. T., Armstrong, T. A., Matzat, P. D., Belstra, B. A., McKeith, F. K., Rincker, P. J., Xi, L., Odle, J., Culbertson, M., Herring, W., & Hansen, J. (2005). Effect of ractopamine feeding level on growth performance and carcass composition. *Journal of Animal Science*, *83* (Suppl. 2), 27.
- Spurlock, M., Cusumano, J., Ji, S., Anderson, D., Smith, C., Hancock, D. & Mills, S. (1994). The effect of ractopamine on beta-adrenoceptor density and affinity in porcine adipose and skeletal muscle tissue. *Journal of Animal Science*, *72*, 75–80.
- Stites, C. R., McKeith, F. K., Singh, S. D., Bechtel, P. J., Mowrey, D. H., & Jones, D. J. (1991). The effect of ractopamine hydrochloride on the carcass cutting yields of finishing swine. *Journal of Animal Science*, *69*, 3094-3101.
- Stoller, G. M., Zerby, H. N., Moeller, S. J., Baas, T. J., Johnson, C., & Watkins, L. E. (2003). The effect of feeding ractopamine (Paylean) on muscle quality and sensory characteristics in three diverse genetic lines of swine. *Journal of Animal Science*, *81*, 1508-1516.
- Watkins, L. E., Jones, D. J., Mowrey, D. H., Anderson, D. B. & Veenhuizen, E. L. (1990). The effect of various levels of ractopamine hydrochloride on the performance and carcass characteristics of finishing swine. *Journal of Animal Science*, *68*, 3588-3595.
- Webster, M. J., Goodband, R., Tokach, M., Nelssen, J., Dritz, S., Unruh, J., Brown, K., Real, D., Derouchey, J., Woodworth, J., Groesbeek, C. N., & Marsteller, T. A. (2007). Interactive effects between ractopamine hydrochloride and dietary lysine on finishing pig growth performance, carcass characteristics, pork quality, and tissue accretion. *The Professional Animal Scientist*, *23*, 597–611.
- Weiler, U., Götz, M., Schmidt, A., Otto, M. & Müller, S. (2013). Influence of sex and immunocastration on feed intake behavior, skatole and indole concentrations in adipose tissue of pigs. *Animal*, *7*, 300–308.
- Zamaratskaia, G., Rydhmer, L., Andersson, H. K., Chen, G., Lowagie, S., Andersson, K. & Lundström, K. (2008). Long-term effect of vaccination against gonadotropin-releasing hormone,

using Improvac™, on hormonal profile and behaviour of male pigs. *Animal Reproduction Science*, 108, 37–48.

CHAPTER 5

Physical meat quality and chemical composition of the *Longissimus thoracis* of entire and immunocastrated pigs fed varying dietary protein with and without ractopamine hydrochloride supplementation

Abstract

The physical and chemical attributes of the *Longissimus thoracis* (LT) of 96 PIC[®] entire (E) and immunocastrated (C) pigs was evaluated to determine the effects of varied dietary protein levels fed with or without ractopamine hydrochloride (RAC). Three diets of low, medium and high protein were balanced with regards to three chosen digestible lysine levels; 7.50, 9.79 and 12.07 g lysine/kg respectively. Vaccination occurred at 16 and 20 weeks of age and RAC was included at 0 or 10 mg/kg to the respective treatments for the last 28 days before slaughter at 24 weeks of age. The trial was a 2 x 2 x 3 factorial design with the following treatment combinations: E Low, E Medium and E High; E Low RAC, E Medium RAC and E High RAC; C Low, C Medium and C High; and C Low RAC, C Medium RAC and C High RAC. The LT muscles were chemically analysed in terms of moisture, protein, fat and ash content and physically analysed for surface colour (CIE L*a*b*), drip loss, cooking loss and Warner-Bratzler shear force (WBSF). There were no differences between treatments for moisture and ash contents. The E High and C Medium treatments had greater protein contents in the LT than the C High pigs, while C Low had the highest numerical IMF content and E High RAC had the lowest. There were also sex and protein interactions for LT protein content, CIE L* values and Warner-Bratzler shear force (WBSF) values. These indicated that E Low had lower L* values than C Low and E Medium and that E High had the greatest WBSF values although it did not differ from E Low, while C Low had the lowest WBSF values which only differed from E low and C High. Feeding RAC increased WBSF values, while decreasing CIE a* and b* values thus resulting in less tender, less red and less yellow pork. The pigs fed medium protein had lower cooking losses than those fed low protein or high protein and immunocastrated pigs had lower cooking losses than E, however, there were no significant differences between the various main effects for drip loss. Thus the effect of immunocastration on meat quality depends on dietary protein levels and RAC; although the differences may be negligible from a sensory aspect and may not influence consumer acceptability.

Keywords: *Paylean, Improvac, GnRH, boar taint, colour, tenderness*

5.1 Introduction

The South African pork industry involves the production and sale of both fresh pork and processed pork products in almost equal quantities (DAFF, 2012). The quality of fresh meat products can be defined in various ways; where factors such as *post mortem* pH_u, surface colour, tenderness, drip and cooking loss are especially important for the consumer from a sensory and shelf-life perspective. Of these, meat colour is extremely important when evaluating the quality of meat, especially since it is often the first, if not the only characteristic which the consumer uses when purchasing meat (Brewer & McKeith, 1999). Processing characteristics also depend on various physical attributes of pork such as

water holding capacity and drip loss, since they determine the processing yields of value-added products. Thus pork meat quality is important in terms of the consumer acceptability of fresh meat as well as from a processing perspective.

Meat quality is not only measured by its physical attributes, but also by its sensory attributes. A current and long-standing problem in the pork industry is boar taint, an unpleasant characteristic smell or taste in the meat caused by compounds such as androstenone which is produced by the testes of entire boars and decreases the sensory eating quality of pork (Font-i-Furnols, 2012). Since the production of entire male slaughter pigs is preferable over surgical castrated pigs due to growth performance and welfare reasons, alternative means to controlling the prevalence of boar taint in entire male carcasses are receiving increasing attention (Pauly *et al.*, 2009). Immunological or chemical castration can be used to clear the compounds associated with boar taint from storage in the adipose tissue, thus providing an ethical and simple answer to controlling boar taint and allowing for the production of heavier male carcasses.

Immunocastration involves the vaccination of entire male pigs with a GnRH analogue so that antibodies are produced against GnRH and thus the hypothalamic-pituitary-gonadal axis is blocked, decreasing testicular steroid hormone production. Currently there is only one product registered for commercial use, namely Improvac[®] (Improvast[®]/Vivax[®]/Innosure[®]), which has been available in Australia and New Zealand since 1998 and in South Africa since 2006. However, the South African market has not embraced this technology fully yet. Vaccination protocol involves the administration of two subcutaneous doses into the neck with at least four weeks between them and the last being four to six weeks prior to slaughter. Following the standard vaccination schedule, the second, or booster, vaccination initiates the production of GnRH antibodies within three to five days (Claus *et al.* 2007), thus immediately inhibiting LH and FSH secretion causing testosterone and androstenone levels to drop to levels seen in surgical castrates within two weeks (Brunius *et al.* 2011) without a significant increase until slaughter (Claus *et al.*, 2007). However, Brunius *et al.* (2011) found that GH levels are not affected by standard immunization but IGF-1 levels were intermediate in immunocastrates, with surgical castrates having the lowest levels and entire males having the highest. Thus immunocastrates may have a higher potential for anabolic growth than surgical castrates due to the unique changes in their hormone production.

Immunocastrates thus grow similarly to entire males until their booster vaccination after which their lean growth seems to be sacrificed in lieu of fat deposition and immunocastrates are fatter than entire males when backfat thickness and carcass dissection are investigated (Metz *et al.*, 2002; Gispert *et al.*, 2010; Boler *et al.*, 2011). Pauly *et al.* (2009) further demonstrated that the lean meat percentage of immunocastrates was intermediate to that of entire males and surgical castrates. However, when intramuscular fat is analysed, immunocastrates do not differ significantly from entire males and surgical castrates. In Chapter 3, it was shown that immunocastrates have an ADG and fat gain comparable to entire males when they are vaccinated four weeks prior to slaughter, however, in the calliper backfat thickness measurements *post mortem* showed that immunocastration increased subcutaneous fat thickness (Chapter 4). This was also supported by the carcass yield results which showed that immunocastrates had higher loin subcutaneous fat percentages. Therefore, in order to

counteract such an increase in fat gain, products such as Paylean® can be used in order to promote protein synthesis through the action of a β -adrenergic agonist known as ractopamine hydrochloride (RAC). Ractopamine is said to redirect the available nutrients from lipogenesis to protein synthesis, thus increasing muscle fibre diameter. This is supported by Aalhus *et al.* (1992) who reported that RAC caused an increase in large diameter white muscle fibres and to a lesser extent, intermediate fibres but did not affect the total number of fibres. An increase in muscle fibre diameter can be associated with decreased tenderness however, Braña *et al.*, (2013) showed that feeding RAC had no effect on pork tenderness or flavour but improved juiciness scores.

Since the performance of immunocastrates seems to be intermediate to that of entire males and surgical castrates, their nutrient requirements are likely to differ. Since testosterone production is affected by immunocastration, protein synthesis is likely to decrease as well. Lysine is the first limiting amino acid in pigs due to their primary focus being on muscle growth and thus the lysine requirements of immunocastrates should decrease but not to that of surgical castrates. This is in agreement with Boler *et al.* (2011) who found that immunocastrates should be fed at a higher lysine level than surgical castrates in order to improve their cutting yields. However, when adding RAC to the diets of immunocastrates, protein synthesis should increase and thus so too should the lysine requirements. Andretta *et al.* (2012) performed a meta-analysis of the relationship between lysine and RAC and found that from the 29 articles analysed, pigs fed higher lysine levels had a higher loin area and lower backfat depth than those untreated. Although RAC has been thoroughly researched over the past twenty years, few studies have focused on the lysine requirements of immunocastrates and the effects on the chemical composition and quality of the resultant meat. Likewise, research into the effects of varied lysine levels fed with RAC on immunocastrates is lacking; this includes investigations into how chemical composition of the meat, as well as the meat quality is affected.

The aim of this research is thus to quantify the impact of varied lysine levels fed to intact boars and immunocastrates with and without RAC on the chemical composition and meat quality of the *Longissimus thoracis* muscle (LT). The objectives are firstly to establish whether immunocastrates have more intramuscular fat than entire males. Secondly, to determine if feeding RAC decreases intramuscular fat content. Thirdly, to determine which protein level is best for immunocastrates in terms of fat deposition when they are fed either with or without RAC, and lastly, to confirm that immunocastration and RAC do not adversely affect meat quality.

5.2 Materials and Methods

Details regarding the experimental design of the growth trial, experimental diet formulation, husbandry of the trial and experimental housing of the pigs are discussed in detail in Chapter 3 (section 3.2). Further details regarding carcass sectioning and sampling of the LT is discussed in Chapter 4 (section 4.2.3).

5.2.1 Animals, housing and feeding

One hundred and twenty entire male pigs were randomly selected from a commercial grower herd and placed into individual pens at the ARC boar testing facilities at Elsenburg in the Western Cape Province, South Africa. The pigs were slaughter stock produced from a PIC[®] Camborough maternal line (Large White x Landrace x White Duroc) and a PIC[®] 410 terminal sire selected for lean growth. The feeding trial followed a 2 x 2 x 3 factorial design where each animal was randomly allocated to each of the 12 treatment combinations and thus there were 10 animals per treatment. Three diets of varying balanced protein were formulated with regards to three chosen digestible lysine levels and are considered low, medium and high (7.50, 9.79 and 12.07 g digestible lysine/kg respectively) (Table 3.5). Immunocastrated animals received their Improvac[®] vaccinations at 16 and 20 weeks of age and Paylean[®] was added to the applicable diets at 10 mg/kg for the last 28 days of growth after which the pigs were slaughtered at 24 weeks of age without feed or RAC withdrawal. At the time of second vaccination and the addition of RAC (20 weeks of age), the diets were changed to the low, medium or high protein diets. Prior to this, all pigs received a commercial grower diet (Table 3.1).

5.2.2 Slaughter and sampling

All of the pigs were slaughtered at 24 weeks of age at a commercial abattoir in the Western Cape Province, South Africa 45 minutes away from the research facility. The pigs were maintained in lairage for an hour in one group after which they were slaughtered and inspected according to commercial practises using electrical stunning followed by exsanguination (thoracic stick). The pH and temperature were recorded 45 minutes *post mortem* (pH₄₅ and temp₄₅) between the 2nd and 3rd last rib, 45 mm from the dorsal midline and into the loin from the inside of the carcass using a calibrated portable Crison PH25 pH meter, (Allela, Barcelona) consisting of a glass electrode exposed within a blade to allow insertion into meat, which measures both pH and temperature. The carcasses then entered a cold room at 4°C for 24 hours whilst being hung by both Achilles tendons. After 24 hours, the pH and temperature (pH₂₄ and temp₂₄) was measured again in the same manner as before, and the carcasses were deboned by trained and experienced staff at the abattoir using commercial techniques as described in Chapter 4 (4.2.3). At this point, 96 loins (LT) were sampled by selecting eight of the mid-weight pigs from each treatment in terms of their live mass at slaughter. Each deboned loin was taken from the right hand side of the carcass and cut just above the point of pH measurement (between the 2nd and 3rd last rib 45 mm from the dorsal midline). The cranial section (LT) of each untrimmed loin was wrapped in individual water impermeable plastic bags, placed in cardboard boxes and taken back to Stellenbosch University for further analyses.

At 48 hours post mortem, the pH and temperature (pH₄₈ and temp₄₈) was taken within the loin on the side cut at the same location as that measured at 24 hours *post mortem* and described in 5.2.2. Four 2 cm thick steaks were then cut perpendicular to the long axis of the muscle fibres from each loin from the cut side (between the 2nd and 3rd last rib 45 mm from the dorsal midline and counted from the cranial end) towards the cranial side (A, B, C and D) and trimmed of all fat and

excess tissue until the steak only consisted of the LT muscle. The first steak, A, was used for colour measurements on the freshly cut surface, steak B was used for cooking and then shear force measurements, steak C was used for drip loss and steak D was used for proximate analyses.

5.2.3 Physical meat quality

The pH and temperature were measured in the loin at the same location as previously described (5.2.2) at 48 hours *post mortem*. Meat surface colour tests were performed instrumentally on the LT after it reached pH_u at 48 hours *post mortem*, using a Color-guide 45°/0° colorimeter (BYK-Gardner GmbH, Gerestried, Germany) in accordance to the CIE L*a*b* colour system described by Honikel (1998). Steak A for each loin was used for colour measurements, which was approximately 2 cm thick, cut perpendicular to the long axis of the muscle fibres. The steaks were allowed to bloom for 45 minutes in order to allow the surface myoglobin to be fully oxygenated. Three measurements were taken on the freshly cut side of the bloomed steak at the top, middle and bottom of the steak in order to compensate for the fact that pork meat tends to have a colour gradient from top to bottom. Average L*, a*, b* measurements were used for statistical analyses, where L* represents brightness, a* represents the red-green range and b* represents the blue-yellow range. The a* and b* values were used to calculate the chroma value, which represents the saturation/intensity of colour, and the hue-angle, which represents the definition of the colour, as follows:

$$\text{Chroma } (C^*) = (a^{*2} + b^{*2})^{-0.5}$$

$$\text{Hue - angle } (^{\circ}) = \tan^{-1} \left(\frac{b^*}{a^*} \right)$$

Cooking loss and drip loss was determined according to the methods described by Honikel (1998). For cooking loss, steak B from each loin was weighed and placed in a thin-walled water-impermeable plastic bag in a water-bath at 80 °C with the opening above the surface. Samples were cooked to an internal temperature of 75 °C (approximately 60 minutes) and then removed from the water-bath and the cooking loss removed. The meat was cooled in ice slurry inside their plastic bags. The meat was then blotted, weighed and the cooking loss expressed as a percentage of the initial sample as follows:

$$\text{Cooking loss \%} = \frac{\text{weight}_{\text{before}} - \text{weight}_{\text{after}}}{\text{weight}_{\text{before}}} \times 100$$

Steak C from each loin was weighed individually and suspended from a corner of the slice by a piece of wire within an impermeable plastic bag, ensuring that the meat was not touching the sides of the bag, at 4°C for 24 hours. The meat was then removed, blotted, weighed and then the drip loss expressed as a percentage of the initial meat mass as follows:

$$Drip\ loss\ \% = \frac{weight_{before} - weight_{after}}{weight_{before}} \times 100$$

The samples from the cooking loss (steak B) were used for shear force measurements using an Instron Universal Testing Machine (model 4444, Apollo Scientific cc, South Africa) fitted with a Warner Bratzler blade. The load cell was 2 kN and the crosshead speed was 200 mm/ minute. The Warner Bratzler blade fitting was 1.2 mm thick, with a triangular opening which has a base length of 13 mm and a perpendicular height of 15 mm. A cylindrical core borer was used to cut 6 samples from the cooked meat steaks with a diameter of 1.27 cm and at least 20 mm long, such that the longitudinal axis of the core was parallel to the direction of the muscle fibres to ensure the sample cores were sheared at right angles to the fibre axis (Honikel, 1998). The shear force was measured as the peak force in Newton required to shear each core and the average was calculated.

5.2.4 Chemical analyses

Each LT steak (D) was homogenised individually and the moisture (method 934.01; AOAC, 2002a), protein (Dumas combustion method 992.15; AOAC, 2002) and ash (method 942.05; AOAC 2002b) content were determined for each sample in duplicate. Moisture content ($\text{g}\cdot 100\ \text{g}^{-1}$) was determined by weighing 2.5 g of each homogenized sample into desiccated crucibles, dried at $\pm 100\ ^\circ\text{C}$ for 24 hours and weighed again after cooling in a desiccator for 30 minutes. These moisture-free samples were then analysed for ash content ($\text{g}/100\text{g}$) by placing them in a furnace at 500°C for 6 hours, cooled for 2 hours, desiccated and then weighed again. All weighing performed for the chemical analyses was done using a RADWAG AS 220/C/2 balance (max: 220 g; min: 10 mg, T = - 220 g; e = 1 mg) accurate to 0.0001 g except for the 5 g fat samples which were weighed using a RADWAG PS750/C/2 (max: 750 g) accurate to 0.001g (Wagi Elektroniczne, Poland). Crude fat analyses were performed using chloroform/methanol (2:1 v/v) extraction according to the rapid solvent extraction method described by Lee *et al.* (1996) to determine the intramuscular fat content ($\text{g}\cdot 100\ \text{g}^{-1}$). The filtrate from the fat extraction was dried, finely ground and used for protein analysis in a Leco Nitrogen/Protein Analyser (Leco Fp-528, Leco Corporation) by weighing off approximately 0.15 g into Leco™ foil. Since protein contains 16 % nitrogen, the nitrogen percentage is multiplied by a conversion factor of 6.25 to yield the crude protein content ($\text{g}\cdot 100\ \text{g}^{-1}$) (McDonald *et al.*, 2002). An EDTA calibration sample (Leco Corporation, USA) was analysed in the Leco Nitrogen/Protein Analyser prior to each batch of protein samples, with the intention of ensuring the accuracy and recovery rate of each sample.

Bi-monthly tests are performed for the above proximate analyses as part of a National Inter-laboratory Scheme (AgriLASA: Agricultural Laboratory Association of South Africa) to ensure accuracy and repeatability of the chemical composition analysis methods using blind sample analyses.

5.2.5 Statistical analysis

Statistical analysis was performed using STATISTICA 64 version 11 (2012). Levene's test of homogeneity of variances was performed to determine whether the data was homoscedastic or not. Following this, normality plots were drawn to establish possible outliers and ensure normality of the data. Univariate ANOVA's were conducted and Fisher LSD was chosen for *post hoc* testing to compare the various LS Means. The chosen level of significance was 5% to determine significant differences of effects and trends were noted at the 10 % significance level. The values for the data are reported as LSMeans and SEM.

5.3 Results

The results for chemical composition are reported in Table 5.1 and physical meat quality in Table 5.3 and 5.4. The results for pH and temperature over the *post mortem* period are summarised in Table 5.2. Unless stated otherwise, there were no interactions between the main effects and the later are therefore discussed.

5.3.1 Chemical composition

When comparing the LSMeans for moisture content and ash content of the LT, none of the main effects were statistically or biologically significant (Table 5.1). The interaction between sex, RAC and protein level was significant ($p = 0.053$) for intramuscular fat (IMF) content (Figure 5.1). The post-hoc Fisher's LSD test indicated that E High RAC had less IMF than E Medium ($p = 0.042$), C Low ($p = 0.004$) and C High RAC ($p = 0.028$). Also, E Medium RAC had lower IMF than C Low ($p = 0.018$) and C Medium RAC had lower IMF than C Low ($p = 0.040$) (Figure 5.1). The differences between the treatments for IMF are biologically insignificant except that of C Low and E High. The C Low had the highest numerical LS Mean IMF content (4.37 ± 1.68 g/100g) and E High RAC had the lowest numerical LS Mean (2.81 ± 0.64 g/100g), which indicates that the C Low has almost double the amount of IMF than the E High.

The LT protein content was the only chemical composition parameter which showed an interaction (Figure 5.2) between sex and protein level ($p = 0.010$) with E High having a greater LT protein content than C High ($p = 0.017$). C High also had a significantly lower ($p = 0.017$) LT protein content than C Medium. However, it is debatable whether the differences between the LSMeans for the E High (23.6 ± 0.21 g/100g) and C High (22.7 ± 0.26 g/100g) as well as the C High (22.7 ± 0.26 g/100g) and C Medium (23.6 ± 0.18 g/100g) are of any biological significance.

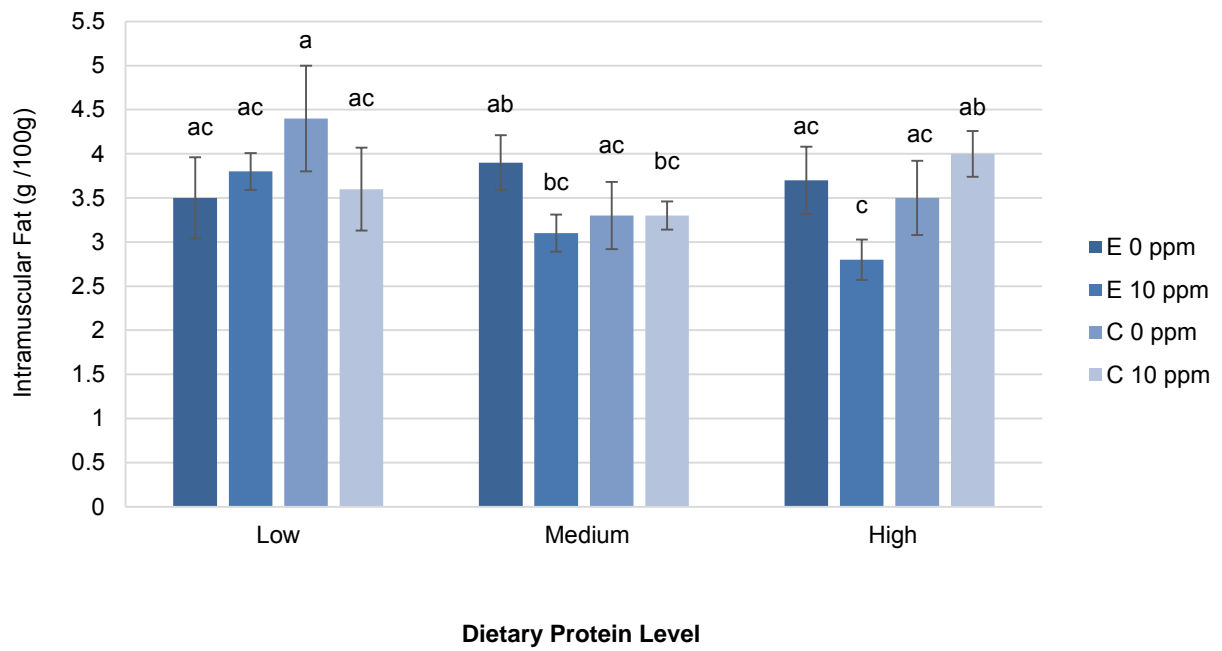


Figure 5.1 Interaction between sex, ractopamine and protein for the mean intramuscular fat content (g/100g) of the *Longissimus thoracis* muscles for entire males (E) and immunocastrates (C) fed at low, medium and high digestible protein with 0 mg/kg or 10 mg/kg RAC. The different alphabetical letters denote significant differences ($p < 0.05$) between the mean values and vertical bars indicate SEM

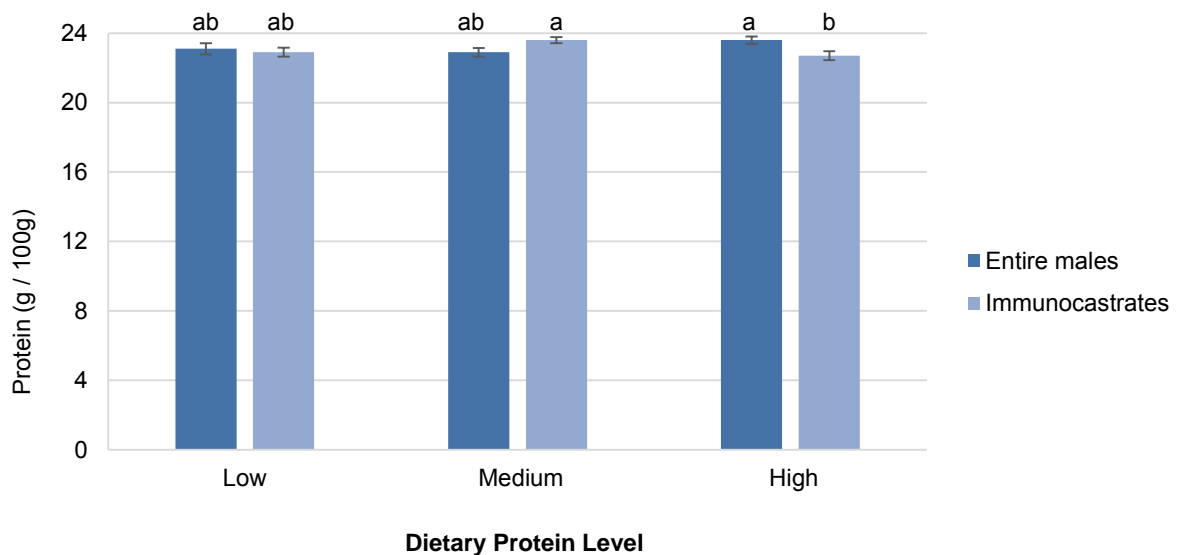


Figure 5.2 Interaction ($p = 0.010$) between sex and protein for the mean protein content (g/100g) of the *Longissimus thoracis* muscles for entire males and immunocastrates fed at low, medium and high protein. The different alphabetical letters denote significant differences ($p < 0.05$) between the mean values and vertical bars indicate SEM

Table 5.1 LSMeans \pm SEM for the chemical composition of the *Longissimus thoracis* for each of the main effects and significant interactions

Effect	Moisture (g/100g)	Protein (g/100g)	Fat (g/100g)	Ash (g/100g)
Sex				
E	72.9 \pm 0.13	23.2 \pm 0.16	3.5 \pm 0.14	1.3 \pm 0.04
C	72.8 \pm 0.16	23.1 \pm 0.14	3.7 \pm 0.17	1.4 \pm 0.04
RAC mg/kg				
0	72.8 \pm 0.14	23.1 \pm 0.16	3.7 \pm 0.17	1.3 \pm 0.03
10	72.9 \pm 0.15	23.2 \pm 0.14	3.4 \pm 0.13	1.4 \pm 0.04
Protein				
Low	72.7 \pm 0.22	23.0 \pm 0.21	3.8 \pm 0.24	1.3 \pm 0.04
Med	72.9 \pm 0.13	23.2 \pm 0.16	3.4 \pm 0.14	1.4 \pm 0.04
High	72.9 \pm 0.17	23.2 \pm 0.18	3.5 \pm 0.18	1.3 \pm 0.05
Sex*Protein				
E Low		23.1 ^{ab} \pm 0.33		
E Med		22.9 ^{ab} \pm 0.25		
E High		23.6 ^a \pm 0.20		
C Low		22.9 ^{ab} \pm 0.26		
C Med		23.6 ^a \pm 0.18		
C High		22.7 ^b \pm 0.26		
Sex*Protein*RAC				
E Low 0			3.5 ^{ac} \pm 0.46	
E Med 0			3.9 ^{ab} \pm 0.31	
E High 0			3.7 ^{ac} \pm 0.38	
E Low 10			3.8 ^{ac} \pm 0.39	
E Med 10			3.1 ^{bc} \pm 0.21	
E High 10			2.8 ^c \pm 0.23	
C Low 0			4.4 ^a \pm 0.60	
C Med 0			3.3 ^{ac} \pm 0.38	
C High 0			3.5 ^{ac} \pm 0.42	
C Low 10			3.6 ^{ac} \pm 0.47	
C Med 10			3.3 ^{bc} \pm 0.16	
C High 10			4.0 ^{ab} \pm 0.26	

^{a,b} LSMeans within main effects and interactions with different superscripts are significantly different ($p < 0.05$)

E: entire males

C: immunocastrates

0: 0 mg/kg

10: 10 mg/kg

Med: medium protein

RAC: ractopamine hydrochloride

5.3.2 pH and temperature 45 minutes, 24 hours and 48 hours post-mortem

The pH at 45 minutes, 24 hours and 48 hours *post mortem* did not differ significantly for sex, protein level and RAC (Table 5.2). This is an indication that the pigs across all treatments had similar glycogen levels in their muscles at slaughter and as none of the pH₄₅ values were < 6.0, thus all indications are that the pigs were not exposed to excessive *ante mortem* stress which would have resulted in pale soft exudative (PSE) meat. There was no significant difference for LT temp₄₅, however, entire males had a lower ($p = 0.007$) LT temp₂₄ than immunocastrates and the temp₄₈ of the LT of those pigs fed with RAC was significantly higher ($p = 0.025$) than the LT of those not fed RAC (Table 5.2). The differences in temperature 24 hours and 48 hours *post mortem* were approximately 1 °C and thus the probability that these differences in temperature were not large enough to affect the *post mortem* pH.

Table 5.2 The influence of sex, protein level and RAC on *Longissimus thoracis* pH and temperature at 45 minutes, 24 hours and 48 hours *post mortem* (LSMean ± SEM)

Effect	pH 45 min	Temp 45 min °C	pH 24 hrs	Temp 24 hrs °C	pH 48 hrs	Temp 48 hrs °C
Sex						
E	6.33 ± 0.02	38.22 ± 0.06	5.34 ^a ± 0.01	8.88 ^b ± 0.14	5.46 ± 0.01	7.02 ± 0.09
C	6.37 ± 0.03	38.23 ± 0.05	5.33 ^b ± 0.01	9.46 ^a ± 0.16	5.45 ± 0.01	7.17 ± 0.10
RAC mg/kg						
0	6.36 ± 0.03	38.18 ± 0.04	5.33 ± 0.01	9.16 ± 0.15	5.453 ^a ± 0.01	6.94 ^b ± 0.08
10	6.34 ± 0.03	38.26 ± 0.06	5.35 ± 0.02	9.18 ± 0.16	5.448 ^b ± 0.01	7.25 ^a ± 0.10
Protein						
Low	6.32 ± 0.04	38.17 ± 0.06	5.31 ± 0.01	9.04 ± 0.17	5.43 ± 0.01	7.11 ± 0.12
Med	6.34 ± 0.04	38.26 ± 0.08	5.37 ± 0.02	9.35 ± 0.19	5.45 ± 0.01	7.16 ± 0.10
High	6.38 ± 0.03	38.24 ± 0.06	5.34 ± 0.01	9.11 ± 0.19	5.46 ± 0.01	7.02 ± 0.13

^{a,b} LSMeans within main effects and interactions with different superscripts are significantly different ($p < 0.05$)

E: entire males

C: immunocastrates

min: minutes

hrs: hours

Temp: temperature

Note that for pH and temperature 48 hours, N = 96 and that pH 48 hours for RAC not rounded to two decimals in order to express differences

0: 0 mg/kg

10: 10 mg/kg

Med: medium protein

RAC: ractopamine hydrochloride

5.3.3 Surface colour

For the L* value (lightness), there was an interaction between sex and protein levels ($p = 0.045$), with E Low having a lower L* value than E Medium ($p = 0.044$) and C Low ($p = 0.019$) (Figure 5.3). For both a* (redness) and b* (yellowness), RAC had a significant effect; pigs fed RAC had lower a* values ($p = 0.004$) as well as lower b* values ($p = 0.005$) than those who did not receive RAC (Table 5.3). When hue-angle and chroma value are calculated, RAC also had an effect. Pork from pigs who received RAC had a higher hue-angles ($p = 0.003$) and lower chroma values ($p = 0.005$) than those who did not receive RAC.

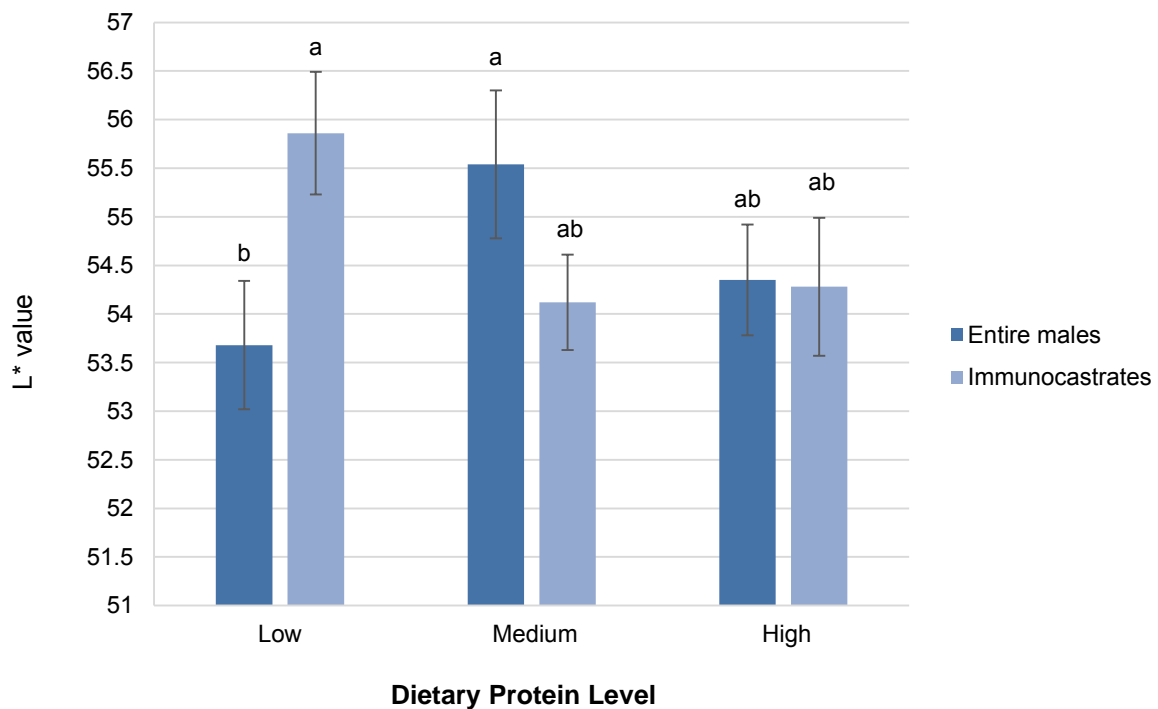


Figure 5.3 Interaction ($p = 0.022$) between sex and dietary protein for the mean surface CIE L* value of the *Longissimus thoracis* muscles for entire males and immunocastrates fed at low, medium and high protein. The different alphabetical letters denote significant differences ($p < 0.05$) between the mean values. Vertical bars indicate SEM

Table 5.3 CIE L*a*b* values, hue-angle and chroma values of the *Longissimus thoracis* for the various main effects and significant interactions (LSMean \pm SEM)

Effect	L*	a*	b*	Hue	Chroma
Sex					
E	54.52 \pm 0.39	0.66 \pm 0.15	9.90 \pm 0.17	86.75 \pm 0.80	9.97 \pm 0.18
C	54.75 \pm 0.37	0.61 \pm 0.17	9.80 \pm 0.16	87.04 \pm 0.90	9.88 \pm 0.17
RAC mg/kg					
0	54.48 \pm 0.35	0.95 ^a \pm 0.15	10.18 ^a \pm 0.15	95.15 ^a \pm 0.78	10.26 ^a \pm 0.16
10	54.80 \pm 0.41	0.32 ^b \pm 0.15	9.52 ^b \pm 0.16	88.64 ^b \pm 0.85	9.58 ^b \pm 0.17
Protein					
Low	54.77 \pm 0.49	0.88 \pm 0.19	10.06 \pm 0.19	85.55 \pm 1.02	10.14 \pm 0.20
Med	54.83 \pm 0.46	0.71 \pm 0.21	10.00 \pm 0.22	86.65 \pm 1.10	10.08 \pm 0.24
High	54.31 \pm 0.45	0.31 \pm 0.17	9.50 \pm 0.17	88.49 \pm 0.95	9.54 \pm 0.18
Sex*Protein					
E Low	53.68 ^b \pm 0.66				
E Med	55.54 ^a \pm 0.76				
E High	54.35 ^{ab} \pm 0.57				
C Low	55.86 ^a \pm 0.63				
C Med	54.12 ^{ab} \pm 0.49				
C High	54.28 ^{ab} \pm 0.71				

^{a,b} LSMeans within main effects and interactions with different superscripts are significantly different ($p < 0.05$)

E: entire males

C: immunocastrates

0: 0 mg/kg

10: 10 mg/kg

Med: medium protein

RAC: ractopamine hydrochloride

5.3.4 Drip loss, cooking loss and Warner-Bratzler shear force

There was no significant difference for drip loss between the various treatments. This indicates that the *ante mortem* stress and cooling rates were similar across all treatments and reiterates the fact that all treatments experienced a normal decline in muscle pH *post mortem*. There were no significant interactions for cooking loss and thus the main effects of sex, protein and RAC could be investigated (Table 5.4). For the main effect of protein ($p = 0.009$), those pigs fed at a low protein level (31.21 ± 0.239 %) had a higher ($p = 0.012$) cooking loss than those fed at a medium protein level (30.34 ± 0.239 %). Those pigs fed medium dietary protein had cooking losses which were also different ($p = 0.004$) from those fed high dietary protein. The effect of sex was also significant ($p = 0.009$) with the cooking loss of the LT of entire males (31.33 ± 0.195 %) being higher than immunocastrates (30.59 ± 0.195 %).

With regards to WBSF, there was an interaction ($p = 0.006$) between sex and protein levels for tenderness described using shear force values (Figure 5.4); E Low had a higher WBSF values than C Low ($p = 0.030$). Similarly, C High had greater WBSF values than E Medium ($p = 0.000$) and C Low ($p = 0.000$) although C High had lower WBSF values than E High ($p = 0.04$) and C Medium ($p = 0.024$). The main effect of RAC was significant ($p = 0.013$) with those pigs receiving RAC having higher WBSF values than those who did not.

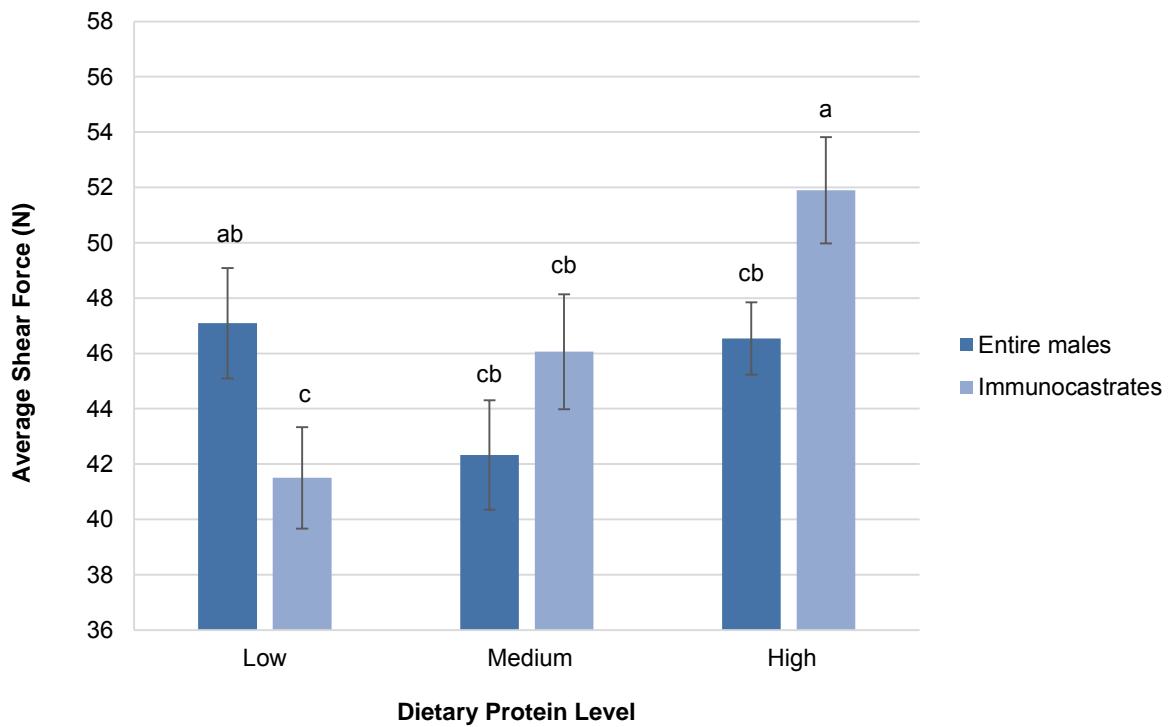


Figure 5.4 Interaction ($p = 0.006$) between sex and protein for the mean Warner-Bratzler shear force value of the *Longissimus thoracis* muscles for entire males and immunocastrates fed at low, medium and high protein. The different alphabetical letters denote significant differences ($p < 0.05$) between the mean values and the vertical bars indicate standard errors of the LSMeans

Table 5.4 The LSMeans \pm SEM for drip loss, cooking loss and Warner-Bratzler shear force for the *Longissimus thoracis* from the various treatment combinations

Effect	Drip loss (%)	Cooking loss (%)	Shear Force (N)
Sex			
E	3.72 \pm 0.21	31.33 ^a \pm 0.20	45.32 \pm 1.06
C	3.70 \pm 0.18	30.59 ^b \pm 0.21	46.49 \pm 1.26
RAC mg/kg			
0	3.94 \pm 0.23	30.76 \pm 0.23	44.05 ^a \pm 1.06
10	3.48 \pm 0.15	31.16 \pm 0.19	47.76 ^b \pm 1.21
Protein			
Low	3.92 \pm 0.26	31.21 ^a \pm 0.24	44.30 \pm 1.43
Med	3.59 \pm 0.20	30.34 ^b \pm 0.24	44.20 \pm 1.45
High	3.62 \pm 0.26	31.33 ^a \pm 0.27	49.22 \pm 1.24
Sex*Protein			
E Low			47.09 ^a \pm 2.00
E Med			42.33 ^b \pm 1.98
E High			46.54 ^{ab} \pm 1.31
C Low			41.50 ^b \pm 1.83
C Med			46.06 ^{ab} \pm 2.08
C High			51.90 ^{ab} \pm 1.92

^{a,b} LSMeans within main effects and interactions with different superscripts are significantly different ($p < 0.05$)

E: entire males

C: immunocastrates

0: 0 mg/kg

10: 10 mg/kg

Med: medium protein

RAC: ractopamine hydrochloride

5.4 Discussion

The study demonstrated that immunocastration had no effect on moisture and ash content of the LT, however, numerous interactions with sex (entire and immunocastrated males) existed for the chemical and physical meat quality attributes. The interaction between sex, protein and RAC for IMF content was significant of the 10 % and boarded significance on the 5 % level. From the interaction, the medium protein diet provided the lowest numerical IMF content for C pigs for both RAC levels, while the high protein level resulted in the numerically lowest IMF for E pigs fed RAC and the low protein level for E pigs fed 0 mg/kg RAC, however, it is difficult to establish any trends from the interaction. Based on previous research from earlier studies, immunocastration had no effect on the IMF of the *semimembranosus* muscle compared to surgical castrates, entire males as well as female pigs (Gispert *et al.*, 2010). Boler *et al.* (2011) found that feeding increasing levels of dietary lysine decreased the extractable lipid content of the loin of immunocastrates and concluded that this was

probably due to the low and low/medium lysine diets they fed being less than optimal for growth requirements. Therefore, it is likely that the lysine intake was below the requirements for the C pigs fed low protein with no RAC and thus they had the highest IMF. Fernandez *et al.* (1999a) compared the effect of varying IMF content on the sensory qualities of pork and concluded that flavour and juiciness were significantly enhanced when IMF content was above 2.5 % and that there were favourable effects of high IMF (> 3.5 %) on the tenderness of pork. They further noted that the acceptability of pork was improved by increasing IMF levels up to approximately 3 % IMF but this was associated with a decrease in willingness to purchase meat with high visible fat. However, a further experiment suggested that when the consumers evaluated an entire chop, their perception of visible fat was dependent on intermuscular fat rather than intramuscular fat (Fernandez *et al.*, 1999b). Thus the numerical difference in IMF content between the E High RAC (2.81 ± 0.64 g/100g) and C Low (4.37 ± 1.68 g/100g) treatments could also be significant in terms of the sensory acceptability of the meat. All treatments except E High RAC had IMF contents higher than 3 % and thus could be preferable in terms of flavour, tenderness and juiciness but could also be discriminated against visually should the intermuscular fat and subcutaneous fat content be unfavourable. Depending on whether the aim is to decrease LT IMF content or increase marbling; the optimal dietary protein level will differ for each sex and whether the pig receives RAC or not. Also, it is important to recognise that the degree of differences in IMF is also likely to differ between E and C depending on the timing of the second vaccination in terms of the physiological maturity of the pig as well as time prior to slaughter. In order to establish the possible effects of the differences in IMF content, it would be beneficial to follow up this study with a sensory evaluation using a tasting panel. Although Pauly *et al.* (2009) found that immunocastration decreased the degree of saturation with regards to the fatty acid composition when compared to entire males, the effects of the combination of immunocastration, RAC and dietary protein levels on the fatty acid composition need to be investigated to further describe the effect on meat quality.

The effect of sex on the protein content of the LT differed depending on the dietary protein content. The result that the E high had the highest LT protein content could be attributed to lysine not being as limiting as within the medium and low protein diets. Thus the entire male pigs were able to synthesise more protein using the higher levels of balanced protein along with their anabolic growth potential. From the results, it appears as if the immunocastrates were limited in terms of lysine at the low protein level and thus IMF deposition increased and protein deposition decreased when compared to the medium protein level. At the medium protein level, the protein deposition increased and IMF deposition decreased, indicating that the lysine and balanced protein requirements were probably closer to the actual requirements of the immunocastrates. However, if one increases the protein level further (High), the protein deposition decreases and the IMF deposition increased again, regardless of whether they were fed RAC or not. This indicates that lysine within the balanced protein is no longer limiting but most likely over supplied. This is not reflected in the voluntary feed intake since there was not a significant interaction between sex and dietary protein level for ADFI (Table 3.8) but rather a significant interaction for sex and RAC.

Immunocastration increased the LT temp₂₄ and decreased the pH₂₄, however no differences were found at 48 hours *post mortem* between the sexes which supports the results from Zamaratskaia *et al.* (2008), Pauly *et al.* (2009) and Gispert *et al.* (2010). The LT temp₄₈ was influenced by RAC, however, this difference was not large enough to affect the pH measurement. The protein level did not influence the pH which was expected since varying lysine level in the feed has been shown to not influence pH_u (Witte *et al.*, 2000). This is further supported by Boler *et al.* (2011) who found that feeding increasing levels of lysine to immunocastrates had no influence on pH_u. The results are thus in favour of RAC and sex not influencing *post mortem* meat pH and temperature which is in accordance with previous research using RAC (Aalhus *et al.*, 1990; Stites *et al.*, 1991; Dunshea *et al.*, 1993; Stoller *et al.*, 2003; Carr *et al.*, 2005a, b; Athayde *et al.*, 2012)). Therefore the differences in the current trial could possibly be due to the insulating effect of fat, placement within the cold rooms and precision of pH probe in terms of measuring temperature. If one considers the calliper fat thickness of the LT (Table 4.1), there was a significant interaction between sex and protein, however, RAC also had a significant effect on fat thickness. Those pigs fed RAC had less fat (9.27 ± 0.36 mm) than those who did not receive RAC (10.32 ± 0.34 mm) and if one had to ignore the interaction, entire males had less fat (9.24 ± 0.31 mm) than immunocastrates (10.35 ± 0.38 mm). The effect of RAC on LT temp₄₈ likely to be a random effect of placement within the fridge and/or differences in subcutaneous fat depth since pH_u and drip loss were not affected.

Previous research on the meat colour of immunocastrates tends to favour the trend that immunocastrates have lighter (L*), redder (a*) and more yellow (b*) meat than entire male pigs (Moore *et al.*, 2009; Gispert *et al.*, 2010). The current results for the a* values are particularly low; however the results for pH₄₈ and WBSF tenderness indicate that PSE was not evident. Another sex and protein interaction existed for the L* value, where the E pigs on the low protein diet had significantly lower L* values than C Low and C Medium. Webster *et al.* (2007) found that when no RAC is included in the diet, an increase in dietary lysine from 0.8 to 1.4 % decreased lightness (L*), b* and the saturation index. In the current trial, the effect of protein on L* values depended on the sex but it did not follow a simple increase or decrease pattern as observed by Webster *et al.* (2007). For the entire males, the L* value increased between Low to Medium protein and then decreased again, where the opposite was true for immunocastrates. Earlier research showed that entire males had lower L* values than immunocastrates; but the differences were small, as with the current L* results, and were within the range considered acceptable by consumers (Boler *et al.*, 2011). Therefore it is unlikely that these differences will affect consumer acceptability of immunocastrate pork. In accordance to results reported by Aalhus *et al.* (1990), Herr *et al.* (2001), Stoller *et al.* (2003), Armstrong *et al.* (2004), Carr *et al.* (2005a, b) and Athayde *et al.* (2012) the current results showed no effect of 10 mg/kg RAC on CIE L* values. A meta-analysis of research performed between 1990 to 2005 concluded that RAC appeared to have little or no effect on the lightness (L*) and lowered a* and b* values and thus RAC-fed pork was less red and less yellow than controls (Apple *et al.*, 2007) which is in agreement with the current research. These differences in CIE a* and b* values could be as a result of the changes in muscle fibres since RAC increases the proportion and diameter of intermediate and white muscle fibres (Aalhus *et al.*, 1992). Thus the increase in white and

intermediate muscle fibre diameters could dilute the red muscle fibres, causing pork from RAC fed animals to appear less red than controls. As mentioned in section 5.2.4, the hue and chroma values are calculated using the a^* and b^* values and represent the definition of the colour and the saturation/intensity of colour respectively. Although significant research has been done into the effects of RAC on meat colour, very few report the effect on hue and chroma values.

Feeding 10 mg/kg RAC had little effect on the chemical composition of the LT, except for the IMF of E fed high protein with RAC which had the lowest IMF content (Figure 5.1). The majority of the articles which formed part of Apple *et al.*'s (2007) meta-analysis did not report significant differences between pigs fed RAC and controls for IMF content, as well as marbling. Sex x RAC interactions for immunocastrates and surgical castrates have been reported at 5 mg/kg (Braña *et al.*, 2013). It has also been shown that IMF content decreased and crude protein content increased in the loin of gilts and boars fed 10 mg/kg for 28 days (Webster *et al.*, 2007). Therefore, it is possible that the trend for a significant interaction between sex, RAC and protein for IMF could become clearer should a wider range of protein levels be used. Although the results from Chapter 4 indicated that RAC increased the proportion of the carcass represented by the shoulder muscle, hindquarter muscle and loin muscle, the LT protein content was not affected. Thus RAC would have increased the total crude protein content of the entire carcass but does not functionally change an individual muscle such as the LT.

The drip loss and cooking loss was not affected by including RAC, which is in agreement with current literature (Dunshea *et al.*, 1993; Uttaro *et al.*, 1993; Stoller *et al.*, 2003; Carr *et al.*, 2005a; Athayde *et al.*, 2012). The results that immunocastration did not influence drip loss is in agreement with results reported by D'Souza & Mullan (2002) and Pauly *et al.* (2009). This opposes the results found by Boler *et al.* (2011) that immunocastrates fed on a medium/ high (9 g/kg) lysine diet had a higher drip loss than immunocastrates fed a high lysine (10 g/kg) diet; however, sex and dietary protein influenced the current results for cooking loss. Although there was no significant interaction between sex and protein, immunocastration decreased cooking losses and pork from pigs fed medium protein had lower cooking losses than the low and high protein diets. The influence of dietary protein on cooking losses is not in agreement with Boler *et al.* (2011), however, the difference of ~ 1 % does not necessarily warrant further investigation. A sex x protein interaction was evident in the WBSF results which highlighted that WBSF values increased as dietary protein levels increased in immunocastrates, such that C Low had the lowest numerical WBSF values and C High had the highest, however, dietary protein had no effect on the WBSF values for E. The increase in the WBSF of C with increased dietary protein level is similar to the increase Apple *et al.* (2004) observed when the dietary lysine:ME ratio increased. Boler *et al.* (2011) did not find significant differences in WBSF between immunocastrates fed various lysine levels which is not in agreement with the current findings although this could be due to the difference in cooking methods used between the two studies; fresh meat was used for the current trial 48 hours *post mortem* and cooked in a water bath whereas Boler *et al.* (2011) aged the pork for 14 days *post mortem* before freezing, thawing and cooking on a Farberware Open Hearth grill (455N, Walter Kidde, Bronx, NY). The effect of RAC increasing WBSF is in accordance with the results from Aalhus *et al.* (1990), Uttaro *et al.* (1993), Carr *et al.* (2005a, b) and Athayde *et al.* (2012). The increase in WBSF values could be due to the increase in muscle fibre

diameter in response to RAC, which was observed by Aalhus *et al.* (1992) and Dunshea *et al.* (1993). An increase in dietary protein may influence muscle growth to a certain extent and thus the increase in WBSF values seen with C High could also be due to an increase in muscle fibre diameter in response to increased protein synthesis. Although a significant increase in muscle percentage was not seen the various commercial cuts in Chapter 4, a slight increase in muscle fibre diameter may have an influence on the tenderness measured by WBSF.

5.5 Conclusion

Immunocastration, RAC and various protein levels did not negatively affect the LT moisture and ash content as well as pH₄₈ and drip loss. While interactions existed for fat and protein content, the effect of these biologically are questionable and only the difference between IMF contents of E High and C Low may influence the sensory qualities of the resultant pork. The pork from immunocastrates also tends to decrease in lightness (L*) as protein levels increase which does not hold true for the entire males. Thus further research is required into the sensory properties of pork resulting from feeding immunocastrates at various protein levels with and without RAC. The difference between the various LSMeans for cooking losses for protein levels as well as sexes is approximately 1 % which questions its sensory significance since drip loss was not significantly affected. Another sex x protein interaction was evident for WBSF, which further motivates the use of a sensory trial on these treatments in order to establish whether such interactions can be perceived by a sensory panel and will in fact influence the eating quality of the pork. The WBSF values were also increased by RAC, thus decreasing the tenderness of resultant pork.

It can therefore be concluded that the effect of immunocastration on IMF content depends on the dietary protein level provided and inclusion of RAC in the diet, with the medium protein diet providing the lowest IMF for immunocastrates with and without RAC and the high protein diet with RAC providing the lowest IMF for entire males. Immunocastration decreased cooking losses but the effect on meat colour and WBSF depends on the dietary protein provided, although the differences appear to be negligible from a sensory aspect and thus consumer acceptance will most likely be unaffected. Pork from RAC-fed pigs was less red (a*) and less yellow (b*) than those not fed RAC most likely due to a shift in muscle fibre types. However, the in CIE L*a*b* colour values are unlikely to be picked up subjectively and thus will most likely not influence consumer acceptability, a postulation that should also be validated using a sensory trial since meat colour is often the first, if not the only, characteristic which the consumer uses when purchasing meat.

5.6 References

A Profile of the South African Pork Market Value Chain (2012). *Market Structure* (pp 6). Retrieved from: <http://www.nda.agric.za/docs/AMCP/Pork2012.pdf> on 16 August 2014.

- Aalhus, J., Jones, S., Schaefer, A., Tong, A., Robertson, W., Murray, A. & Merrill, J. (1990). The effect of ractopamine on performance, carcass composition and meat quality of finishing pigs. *Canadian Journal of Animal Science*, *70*, 943–952.
- Aalhus, J., Schaefer, A., Murray, A. & Jones, S. (1992). The effect of ractopamine on myofibre distribution and morphology and their relation to meat quality in swine. *Meat Science*, *31*, 397–409.
- Andretta, I., Kipper, M., Lehnen, C. R., Demori, A. B., Remus, A. & Lovatto, P. A. (2012). Meta-analysis of the relationship between ractopamine and dietary lysine levels on carcass characteristics in pigs. *Livestock Science*, *143*, 91–96.
- Apple, J., Maxwell, C., Brown, D., Friesen, K., Musser, R., Johnson, Z. & Armstrong, T. (2004). Effects of dietary lysine and energy density on performance and carcass characteristics of finishing pigs fed ractopamine. *Journal of Animal Science*, *82*, 3277–3287.
- Apple, J., Rincker, P., McKeith, F., Carr, S., Armstrong, T. & Matzat, P. (2007). Review: Meta-analysis of the ractopamine response in finishing swine. *The Professional Animal Scientist*, *23*, 179–196.
- Athayde, N., Dalla Costa, O., Roça, R., Guidoni, A., Ludtke, C. & Lima, G. (2012). Meat quality of swine supplemented with ractopamine under commercial conditions in Brazil. *Journal of Animal Science*, *90*, 4604–4610.
- Boler, D., Kutzler, L., Meeuwse, D., King, V., Champion, D., McKeith, F. & Killefer, J. (2011). Effects of increasing lysine on carcass composition and cutting yields of immunologically castrated male pigs. *Journal of Animal Science*, *89*, 2189–2199.
- Braña, D., Rojo-Gómez, G., Ellis, M. & Cuaron, J. (2013). Effect of gender (gilt and surgically and immunocastrated male) and ractopamine hydrochloride supplementation on growth performance, carcass, and pork quality characteristics of finishing pigs under commercial conditions. *Journal of Animal Science*, *91*, 5894–5904.
- Brewer, M., and F. McKeith. (1999). Consumer-rated Quality Characteristics as Related to Purchase Intent of Fresh Pork. *Journal of Food Science*, *64*, 171–174.
- Brunius, C., Zamaratskaia, G., Andersson, K., Chen, G., Norrby, M., Madej, A. & Lundström, K. (2011). Early immunocastration of male pigs with Improvac[®] - Effect on boar taint, hormones and reproductive organs. *Vaccine*, *29*, 9514–9520.
- Carr, S., Ivers, D. J., Anderson, D. B., Jones, D. J., Mowrey, D.H, England, M. B., Killefer, J., Rincker, P.J., & McKeith, F. (2005a). The effects of ractopamine hydrochloride on lean carcass yields and pork quality characteristics. *Journal of Animal Science*, *83*, 2886–2893.
- Carr, S., Rincker, P., Killefer, J., Baker, D., Ellis, M. & McKeith, F. (2005b). Effects of different cereal grains and ractopamine hydrochloride on performance, carcass characteristics, and fat quality in late-finishing pigs. *Journal of Animal Science*, *83*, 223–230.
- Claus, R., Lacorn, M., Danowski, K., Pearce, M. C. & Bauer, A. (2007). Short-term endocrine and metabolic reactions before and after second immunization against GnRH in boars. *Vaccine*, *25*, 4689–4696.

- Dunshea, F., King, R., Campbell, R., Sainz, R. & Kim, Y. (1993). Interrelationships between sex and ractopamine on protein and lipid deposition in rapidly growing pigs. *Journal of Animal Science*, *71*, 2919–2930.
- D'Souza, D., and Mullan, B. (2002). The effect of genotype, sex and management strategy on the eating quality of pork. *Meat Science*, *60*, 95–101.
- Fernandez, X., Monin, G., Talmant, A., Mourot, J. & Lebret, B. (1999a). Influence of intramuscular fat content on the quality of pig meat—1. Composition of the lipid fraction and sensory characteristics of *m. longissimus lumborum*. *Meat Science*, *53*, 59–65.
- Fernandez, X., Monin, G., Talmant, A., Mourot, J. & Lebret, B. (1999b). Influence of intramuscular fat content on the quality of pig meat—2. Consumer acceptability of *m. longissimus lumborum*. *Meat Science*, *53*, 67–72.
- Font-i-Furnols, M. (2012). Consumer studies on sensory acceptability of boar taint: A review. *Meat Science*, *92*, 319–329.
- Gispert, M., Àngels Oliver, M., Velarde, A., Suarez, P., Pérez, J. & Font i Furnols, M. (2010). Carcass and meat quality characteristics of immunocastrated male, surgically castrated male, entire male and female pigs. *Meat Science*, *85*, 664–670.
- Metz, C., Hohl, K., Waidelich, S., Drochner, W. & Claus, R. (2002). Active immunization of boars against GnRH at an early age: consequences for testicular function, boar taint accumulation and N-retention. *Livestock Production Science*, *74*, 147–157.
- Moore, K., Dunshea, F., Mullan, B., Hennessy, D. & D'Souza, D. (2009). Ractopamine supplementation increases lean deposition in entire and immunocastrated male pigs. *Animal Production Science*, *49*, 1113–1119.
- Pauly, C., Spring, P., O'Doherty, J., Ampuero Kragten, S. & Bee, G. (2009). Growth performance, carcass characteristics and meat quality of group-penned surgically castrated, immunocastrated (Improvac[®]) and entire male pigs and individually penned entire male pigs. *Animal*, *3*, 1057–1066.
- Stites, C., McKeith, F., Singh, S., Bechtel, P., Mowrey, D. & Jones, D. (1991). The effect of ractopamine hydrochloride on the carcass cutting yields of finishing swine. *Journal of Animal Science*, *69*, 3094–3101.
- Stoller, G., Zerby, H., Moeller, S., Baas, T., Johnson, C. & Watkins, L. (2003). The effect of feeding ractopamine (Paylean) on muscle quality and sensory characteristics in three diverse genetic lines of swine. *Journal of Animal Science*, *81*, 1508–1516.
- Uttaro, B., Ball, R., Dick, P., Rae, W., Vessie, G. & Jeremiah, L. (1993). Effect of ractopamine and sex on growth, carcass characteristics, processing yield, and meat quality characteristics of crossbred swine. *Journal of Animal Science*, *71*, 2439–2449.
- Webster, M. J., Goodband, R., Tokach, M., Nelssen, J., Dritz, S., Unruh, J., Brown, K., Real, D., Derouchey, J., Woodworth, J., Groesbeek, C. N., & Marsteller, T. A. (2007). Interactive effects between ractopamine hydrochloride and dietary lysine on finishing pig growth performance, carcass characteristics, pork quality, and tissue accretion. *The Professional Animal Scientist*, *23*, 597–611.

- Witte, D., Ellis, M., McKeith, F. & Wilson, E. (2000). Effect of dietary lysine level and environmental temperature during the finishing phase on the intramuscular fat content of pork. *Journal of Animal Science*, 78, 1272–1276.
- Zamaratskaia, G., Andersson, H., Chen, G., Andersson, K., Madej, A. & Lundström, K. (2008). Effect of a Gonadotropin-releasing Hormone Vaccine (Improvac™) on Steroid Hormones, Boar Taint Compounds and Performance in Entire Male Pigs. *Reproduction in Domestic Animals*, 43, 351–359.

CHAPTER 6

Effects of immunocastration on boar taint compounds and testicle size, colour and histology on boars fed with or without ractopamine hydrochloride at varying balanced protein levels

Abstract

Backfat samples from 96 PIC[®] pigs was analysed for androstenone (5 α -androst-16-en-3-one), skatole (3-methylindole) and indole following a simultaneous extraction and analyses using mass spectrometry and fluorescence. The adipose samples were taken from pigs which formed part of a growth trial following a 2 x 2 x 3 factorial design where half of the pigs were immunocastrated at 16 and 20 weeks of age; RAC was supplemented at 0 or 10 mg/kg for 28 days prior to slaughter and each pig was allocated to either a low, medium or high protein diet balanced with regards to lysine (7.50, 9.79 and 12.07 g digestible lysine/kg) from 20 weeks of age until slaughter at 24 weeks. For androstenone, 83.3 % of immunocastrates (C) had levels under the detection limit (0.02 μ g/g fat), with 100 % being under the sensory threshold of 0.5 μ g/g fat while 77.1 % of entire males (E) had levels above the detection limit with none over 0.5 μ g/g. The C pigs had skatole and indole levels significantly lower than the E pigs. The testes size was decreased with immunocastration; however this was not significant and the changes in testicular morphology and activity can be seen by the histology and colour results. Testicular histology showed a visual decrease in seminiferous tubule diameter, deformation, increased lumen and decreased spermatogenesis. The CIE L*a*b* cut surface colour measurements indicated a decrease in testicular activity due to increased L*, b* and decreased a* values. Therefore the current vaccination schedule was successful in inhibiting testicular functioning and thus androstenone production. Also, testicle size does not provide an indication of vaccination success and alternatives such as the CIE L*a*b* cut surface colour should be considered.

Keywords: *Improvac, GnRH, testes, boar taint, androstenone, skatole*

6.1 Introduction

The production of entire male pigs is preferable over that of surgical castrates due to their superior growth performance and carcass characteristics. This can be attributed to the favourable effects of androgens produced by the testes which stimulate protein synthesis and thus lean growth. In addition to this, the testes of entire male pigs produce androstenone (5 α -androst-16-en-3-one), which is a pheromone. The primary function of androstenone is to stimulate the standing reflex in sows such that the boar may mount her; but, androstenone is lipophilic and thus accumulates in adipose tissue (Bonneau, 1982). This results in an unpleasant flavour and aroma of the meat of entire male pigs, commonly referred to as boar taint. Thus surgical castration has traditionally been implemented in order to prevent the development of boar taint, which is caused by androstenone, skatole (3-methylindole) (Babol *et al.*, 1995) and to a lesser extent indole. Skatole is a product of the bacterial

breakdown of tryptophan in the large intestine, which is reabsorbed and is associated with a faecal smell or taste in the meat to those who are sensitive to it while androstenone is described as an unpleasant urine or sweat smell or taste. Since the contribution of indole to boar taint is often seen as negligible it is frequently ignored in studies on boar taint. The sensory threshold for the detection of androstenone in pork is 0.5 to 1 µg/g fat and 0.20 to 0.25 µg/g fat for skatole (Zamaratskaia *et al.*, 2004) with approximately 45 % of consumers being sensitive to boar taint (Font-i-Furnols, 2012). Furthermore, when skatole and androstenone are present together in pork, the risk of the consumer detecting boar taint increases (Font-i-Furnols, 2012). Although under 50 % of consumers are able to perceive boar taint with more people being anosmic to androstenone than skatole, a frequent exposure to androstenone can induce the ability to perceive androstenone in those people considered anosmic to androstenone which could further decrease the consumer acceptability of pork containing boar taint (Andresen, 2006).

Surgical castration has raised welfare issues since castrated piglets are prone to infection, morbidity and mortality (Rault *et al.*, 2011). In Europe, approximately 79.3 % of male pigs are surgically castrated (Fredriksen *et al.*, 2009), but the European Union (EU) aims to voluntarily ban surgical castration without anaesthesia by 2017 (Font-i-Furnols, 2012). In order to prevent boar taint, male pigs are either surgically castrated shortly after birth or entire male pigs are slaughtered after puberty but before sexual maturity. Surgical castration results in the loss of the effects of anabolic male hormones and thus less efficient growth and fatter carcasses, while the alternative results in small carcasses and thus small profit margins for producers. The production of small entire males also produces lean carcasses which do not have the thick layer of fat or large enough cuts needed for the production of dry-cured products (Fuchs *et al.*, 2009a). Surgical castration has also been receiving attention from a welfare perspective as it has raised various welfare concerns to such an extent that countries have started to implement legislation either limiting or completely banning its application. Thus alternative solutions such as immunocastration have also been receiving increased attention. Immunocastration involves a GnRH vaccine which blocks the hypothalamic-pituitary-gonadal axis through the production of GnRH-specific antibodies. GnRH is a peptide produced in the hypothalamus which acts on the anterior pituitary gland to stimulate LH and FSH secretion. The gonadotropins LH and FSH stimulate steroidogenesis in the testicles, such as testosterone, which provide a feedback regulation for GnRH, LH and FSH (Hughes & Varley, 1980). Vaccination using products such as Improvac[®] disrupt the hypothalamic-pituitary-gonad axis, thus inhibiting testes functioning and steroidogenesis with the intention of reducing the accumulation of androstenone in the adipose tissue. This process is known as immunocastration or chemical castration and has been successful in inhibiting fertility in various livestock species using various methods of vaccine preparation.

In male pigs, the second vaccination is administered four to six weeks prior to slaughter. This enables the capitalisation of improved feed efficiency of entire males until the second vaccination which then allows for the elimination of boar taint compounds. After the second vaccination is given, antibody titres increase within seven days followed by an immediate decrease in LH and then testosterone (Dunshea *et al.*, 2001; Claus *et al.*, 2007; Brunius *et al.*, 2011). Thus within two weeks after the booster vaccination, testosterone levels are reduced to those comparable to surgical

castrates (Brunius *et al.*, 2011) due to Leydig cells involution (Wagner & Claus, 2004). Testicle size reduces as a result of the absence of LH and FSH and thus testicle functioning is affected (Metz *et al.*, 2002; Zamaratskaia *et al.*, 2008; Fuchs *et al.*, 2009b). Immunocastration also decreases the size of the seminal vesicles, epididymis and bulbourethral glands by up to 50 % (Bonneau *et al.*, 1994; Dunshea *et al.*, 2001) Although immunocastration influences the size of the various components of the male reproductive tract, their sizes cannot be used as an efficient monitoring strategy at slaughter to determine if vaccination was successful or not or as an indication of the presence of boar taint (Fuchs *et al.*, 2009b).

The effect of immunocastration on testicular functioning depends on the vaccination schedule used, thus the objectives of the study was to evaluate the effect of vaccinating at 16 and 20 weeks of age, four weeks prior to slaughter, on the testicle size, weight, volume and histology as well as analysing the presence of androstenone, skatole and indole in the subcutaneous backfat simultaneously.

6.2 Materials and methods

The Research Ethics Committee: Animal Care and Use of Stellenbosch University approved all the experimental procedures used in the study (SU-ACUM13-00022). The procedures used conform to the accepted standards for the use of animals in research and teaching described by the South African National Standards 10386: 2008. The experimental design of the growth trial, experimental diet formulation, husbandry of the trial and experimental housing of the pigs is discussed further in Chapter 3 (section 3.2).

6.2.1 Animals, housing and feeding

A growth performance trial involving 120 entire male pigs was conducted at the ARC boar testing facilities at Elsenburg (Western Cape Province, South Africa) using individual housing. At 13 weeks of age, the pigs were randomly selected from commercial slaughter stock (PIC[®] Camborough maternal line: Large White x Landrace x White Duroc crossed with PIC[®] 410 terminal sire) and allocated to one of 12 treatment combinations following a 2 x 2 x 3 factorial experimental design. The main effects used in the factorial design are: sex (immunocastrates versus entire males), RAC supplementation (0 or 10 mg/kg) and three balanced protein levels (low, medium and high). From 13 to 20 weeks of age, they were allowed to acclimatise to the environment, weighing procedures and other management activities during which time they were fed a commercial grower feed (Table 3.1) *ad libitum*. Each pen consisted of a concrete sleeping area with clean pine wood shavings and a separate slanted dunging area free from shavings with a nipple drinker. Those pigs randomly allocated for immunocastration received their vaccinations (2 mL Improvac[®] below ear) at 16 and again at 20 weeks of age. At 20 weeks of age, the diets were changed to one of three balanced protein diets (7.50, 9.79 and 12.07 g digestible lysine/kg) (Table 3.3 - 3.5) with or without RAC supplementation at 0 or 10 mg/kg for the last 28 days of growth, summarised in Table 3.6.

6.2.2 Slaughtering and testicle measurements

All of the 120 pigs were slaughtered at 24 weeks of age; four weeks after the immunocastrates received their second vaccination. They were transported to a commercial abattoir in the Western Cape Province, South Africa 45 minutes away from the ARC boar testing facilities (Eisenburg). The pigs were electrically stunned with electrodes at the base of each ear (220 Volts, 1.4 Amps for four seconds) and exsanguinated using a thoracic stick, after being held in communal lairage for an hour. Testes were collected in the abattoir just prior to evisceration, placed in numbered plastic bags and placed in cooler boxes on ice. Slaughtering started at 08:49 with the first testicles were collected at 09:00 and slaughtering was finished by 11:00. The testicles were transported to Stellenbosch University approximately 45 minutes away and analysed in a similar method to that used by Lealiifano *et al.* (2011). The epididymis and any connective tissue were removed using scalpel blades before each individual testicle was weighed using a RADWAG PS750/C/2 scale (Wagi Elektroniczne, Poland) accurate to 0.001g. The length and width of each testicle was measured using a calibrated engineering calliper and the volume measured using water displacement by placing the testicle into a beaker filled to 1000 mL of water and measuring the amount of water displaced by recording the difference between the initial and final volumes. Of the 12 treatment combinations, it was decided that eight mid-weight pigs would be selected from four treatment combinations for testicle histology and surface colour measurements. The treatments chosen were all on the medium balanced protein diet, with and without RAC supplementation for both entire and immunocastrated males (Table 6.1).

Table 6.1 Treatment combinations chosen showing the sex and if RAC was supplemented at the medium balanced protein diet or not

Treatment	Sex	RAC	Protein level
2	Entire	No	Medium
5	Immunocastrated	No	Medium
8	Immunocastrated	Yes	Medium
11	Entire	Yes	Medium

RAC: ractopamine hydrochloride

The 32 chosen testicle pairs were cut in half (Figure 6.1) and measured for CIE L*a*b* colour using a Color-guide 45°/0° colorimeter (BYK-Gardner GmbH, Gerestried, Germany) without bloom time as described by Lealiifano *et al.* (2011). For each testicle pair, three colour measurements were taken over the four cut surfaces and an average was calculated for L*, a* and b* values.



Figure 6.1 The cut surfaces of two testicles taken from an entire male (left) and an immunocastrate (right)

After the colour measurements were taken, a 5 to 10 mm slice was taken from each testicle and then cut in half and then into quarters. A quarter was then taken from each of the two testicle pairs and preserved in buffered formalin (50 mL 40 % formalin buffered with 450 mL 0.9 % NaCl). The histology slides were then prepared using haematoxylin and eosin staining.

6.2.3 Chemical analyses

Approximately 24 hours *post mortem*, the carcasses were deboned at the abattoir using commercial techniques and 96 right hand side loins were sampled by selecting eight of the mid-weight (live weight at slaughter) pigs from each treatment. Subcutaneous fat samples were taken from the loins at approximately the 3rd last rib and frozen at - 20°C until analysis. The method was based on the liquid chromatographic multiple mass spectrometric (LC-MS) method used for the simultaneous analysis of androstenone (5 α -androst-16-en-3-one), skatole (3-methylindole) and indole (2, 3-benzopyrrole) described by Verheyden *et al.* (2007) and Bekaert *et al.* (2012). The samples were thawed and 5 g was weighed off in duplicate, cut into thin flakes and inserted into a stomacher bag with 5 mL methanol (MeOH) containing the internal standard, 2-methylindole (2-MID), at a concentration of 200 μ g/kg. They were then homogenized in a stomacher BagMixer® 400W (Interscience, France) for two minutes. The supernatant was transferred to a sterile test tube and cooled by submersing the tube in liquid nitrogen. The samples were then centrifuged for six minutes at 5000 rpm and then frozen again to clear the upper phase. They were then filtered into another sterile tube using a syringe filter (0.22 μ m). The extract was diluted by placing 300 μ L of the extract into a sterile glass vial with 200 μ L of 1 % acetic acid prior to analysis. The samples were then analysed using a Waters Xevo TQ triple quadrupole mass spectrometer and a Waters Acquity UPLC and fluorescence detector with a Phenomenex Kinetix C18, 2.6 μ m, 150x2.1 mm column and using two solvent gradient: 7.5 % formic acid and 49:49:2 methanol: acetonitrile: isopropanol plus 7.5 % formic acid. The column temperature

was 40 °C and 10 µL of each sample was injected. The 5 α -androst-16-en-3-one was quantified using tandem mass spectrometry whereas 3-methylindole and indole were quantified using fluorescence. The calibration range and limit of quantification for 5 α -androst-16-en-3-one was 0.01 to 13 µg/g and 0.02 µg/g respectively whereas the calibration range and limit of detection for both 3-methylindole and indole was 0.008 to 0.08 µg/g and 0.004 µg/g respectively.

6.3 Statistical analyses

In order to establish whether sex, RAC supplementation or dietary balanced protein level had an effect on the concentration of skatole and indole in the subcutaneous fat, statistical analysis were performed using STATISTICA 64 version 11 (2012). Before univariate analysis of variances (ANOVA's) were performed using the general linear models (GLM) procedure, the normality of the residuals and homogeneity (Levene's test) were tested. This was followed by the Fisher LSD comparison of LSMeans (least squared means). The same statistical procedure was used to analyse the testicle measurements; for all measurements excluding the colour the body weight was taken into account as a covariate. Colour result values are reported as LSMeans and Standard Error of the Mean (SEM) and a significance level of 5 % was used.

6.4 Results

6.4.1 Androstenone, skatole and indole

The results for the androstenone levels in the adipose tissue samples showed that 40 out of 48 (83.3 %) of the immunocastrates (C) had androstenone levels under the detection limit (0.02 µg/g fat), while none of the immunocastrates had androstenone levels over the sensory threshold of 0.5 µg/g fat (Zamaratskaia *et al.*, 2004). For the eight samples which contained androstenone, the average androstenone concentration was 0.08 µg/g fat with a range of 0.03 to 0.17 µg/g fat. The entire males (E) had 37 out of 48 (77.1 %) fat samples which had androstenone levels above the detection limit but none of them had concentrations above 0.5 µg/g. The average androstenone concentration for E was 0.10 µg/g fat with a range of 0.02 to 0.22 µg/g fat for the 37 samples which were above the detection limit. For skatole, 41 out of 48 (85.4 %) of the C pigs and 46 out of 48 (93.8 %) of the E pigs had levels over the detection limit (0.004 µg/g fat). For the C pigs that had levels over the detection limit, the range was 0.004 to 0.034 µg/g fat with an average of 0.013 µg/g fat. For the E pigs over the detection limit, the average skatole concentration was 0.04 µg/g with a range of 0.004 to 0.04 µg/g fat. Of the C pigs, 6 out of 48 (12.5 %) were over the sensory detection limit of 0.02 µg/g fat (Zamaratskaia *et al.*, 2004) and 30 out of 48 (62.5 %) of the E pigs were above the sensory detection limit. From the statistical analyses, E had higher levels of skatole than C ($p < 0.001$) but there was no effect of RAC supplementation or dietary balanced protein level on the levels of skatole. Although the results were not significant, from Figure 6.2 it can be seen that the skatole levels increased slightly with an increase in dietary protein level.

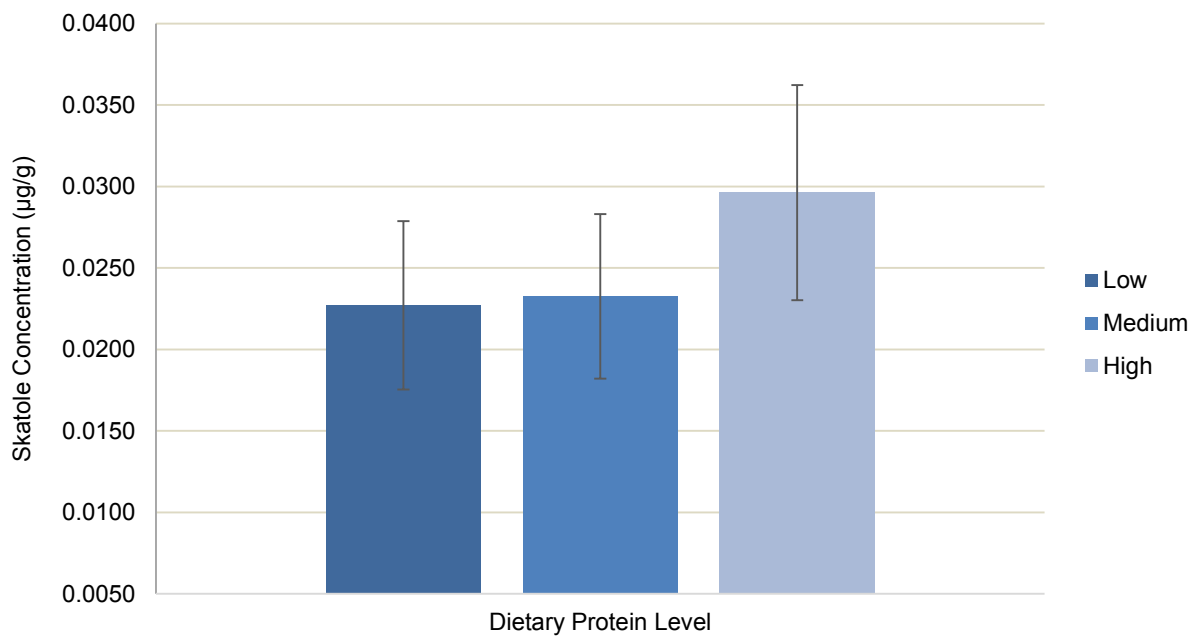


Figure 6.2 The skatole concentration ($\mu\text{g/g}$ fat) for the low, medium and high balanced protein diets

The C pigs had 27 out of 48 (56.3 %) who had indole levels above the detection limit ($0.004 \mu\text{g/g}$ fat) with an average of $0.006 \mu\text{g/g}$ fat with a range of 0.004 to $0.012 \mu\text{g/g}$ fat. The E pigs had higher indole levels in their adipose tissue ($p = 0.030$) with 27 out of 48 pigs having levels above the detection limit with an average of $0.012 \mu\text{g/g}$ fat and a range of 0.004 to $0.092 \mu\text{g/g}$ fat.

6.4.2 Testicle measurements

Immunocastration decreased the weight, volume, length and width of the individual testicles, but these differences were not significant. The results for the CIE $L^*a^*b^*$ colour values indicated that immunocastration increased the L^* values ($p < 0.001$) and b^* values ($p < 0.001$) while decreasing the a^* values ($p < 0.001$). Thus the cut surface colour of the testicles from the immunocastrates was lighter, more yellow and less red than entire males.

Table 6.2 CIE L*a*b* values for the main effects of sex and RAC for the cut surface colour of the 32 testicles sampled from pigs fed the medium balanced protein diet (LSMean \pm SEM)

Effect		L*	a*	b*
Sex				
	E	52.57 ^a \pm 0.66	9.98 ^a \pm 0.26	11.01 ^a \pm 0.14
	C	59.13 ^b \pm 0.72	6.95 ^b \pm 0.42	12.44 ^b \pm 0.21
RAC mg/kg				
	0	56.48 \pm 1.22	8.07 \pm 0.54	11.75 \pm 0.22
	10	55.67 \pm 0.97	8.63 \pm 0.51	11.79 \pm 0.29

^{a,b} LSMeans within main effects and interactions with different superscripts are significantly different ($p < 0.05$)

E: entire males

C: immunocastrates

0: 0 mg/kg

10: 10 mg/kg

RAC: ractopamine hydrochloride

The visual assessment results on the testicular histology show a visual decrease in the seminiferous tubule diameters and atrophy of the seminiferous tubules with spermatocyte loss (Figure 6.3) compared to the non-vaccinated males (Figure 6.4).

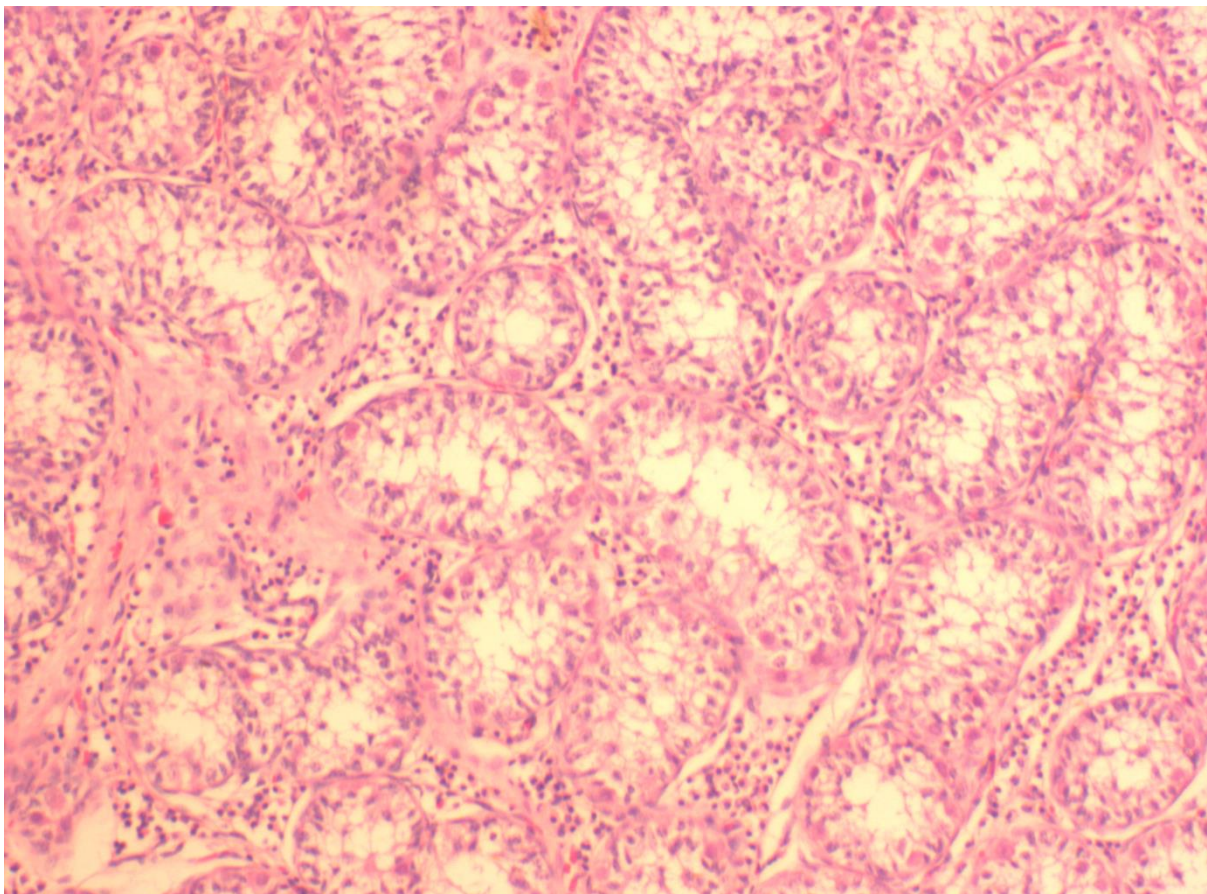


Figure 6.3 The testicle morphology of an immunocastrate showing a decreased in seminiferous tubule diameters and atrophy of the seminiferous tubules, as well as spermatocyte loss and thus an increase in lumen (4X magnification; photograph has not been resized)

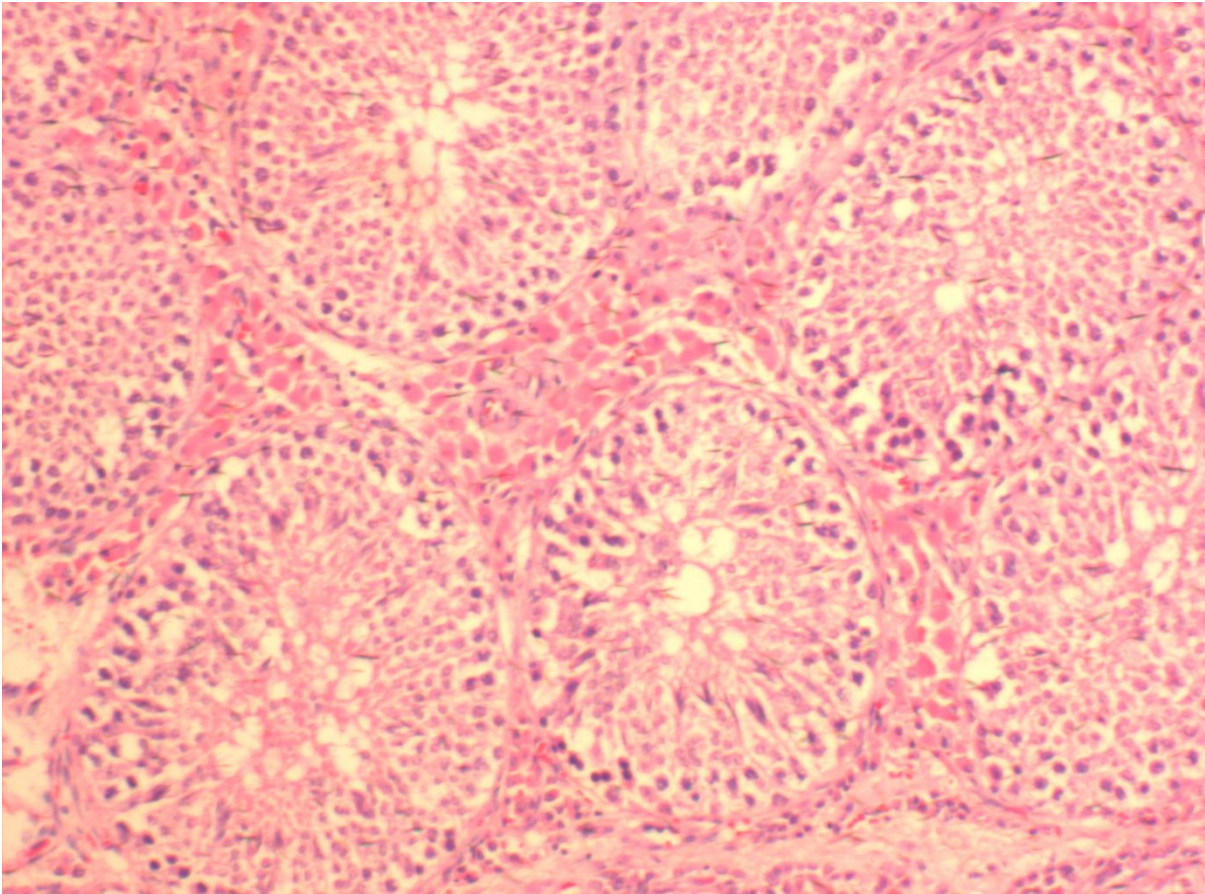


Figure 6.4 The testicle morphology of an entire male (4X magnification; photograph has not been resized)

The decrease in seminiferous tubule diameter and decreased spermatogenesis resulted in an increase in lumen and deformation of the seminiferous tubules (Figure 6.5). The seminiferous tubules were tightly packed and thus the effects on the Leydig cells were difficult to observe at 10X magnification. In some cases, the effects on the seminiferous tubule morphology and size were more drastic (Figure 6.6).

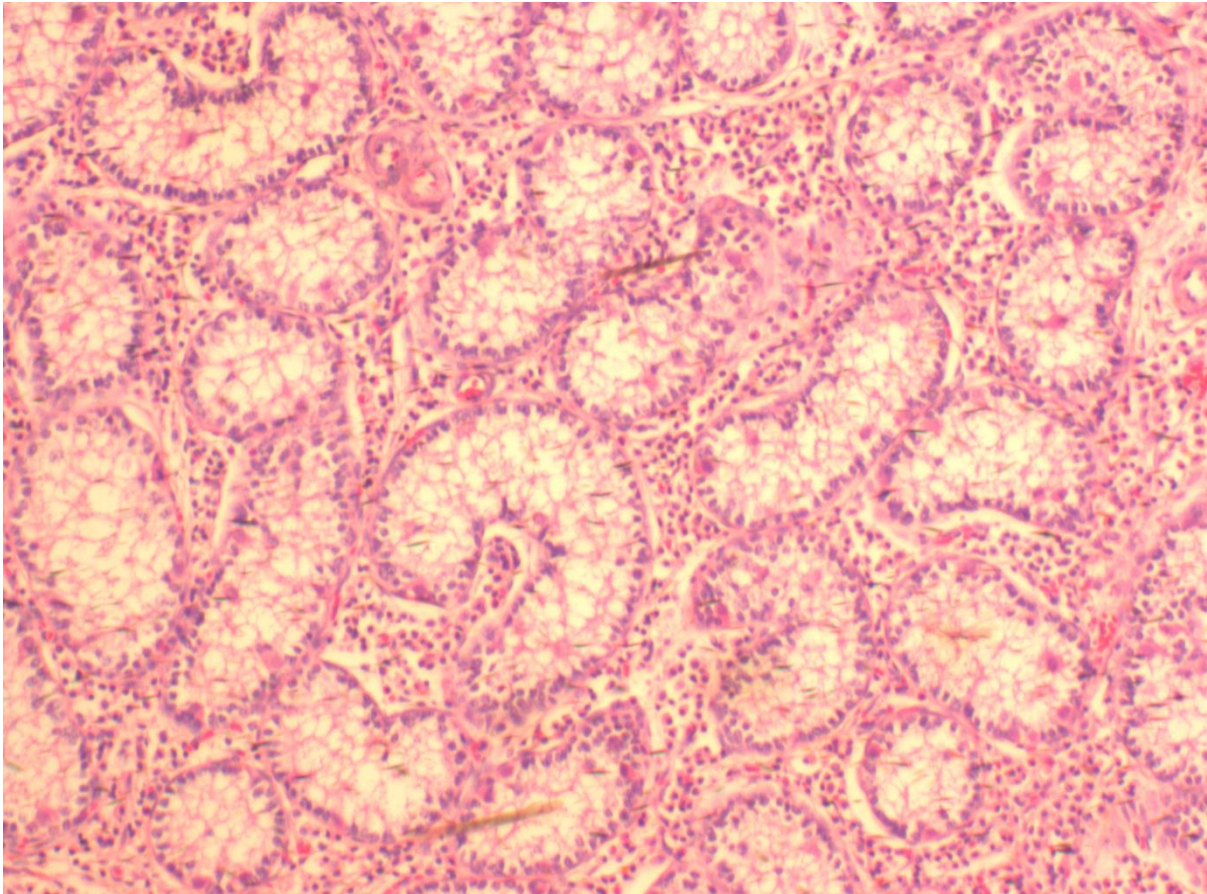


Figure 6.5 The testicle morphology of an immunocastrate showing an increase in lumen and deformation of the seminiferous tubules (4X magnification; photograph has not been resized)

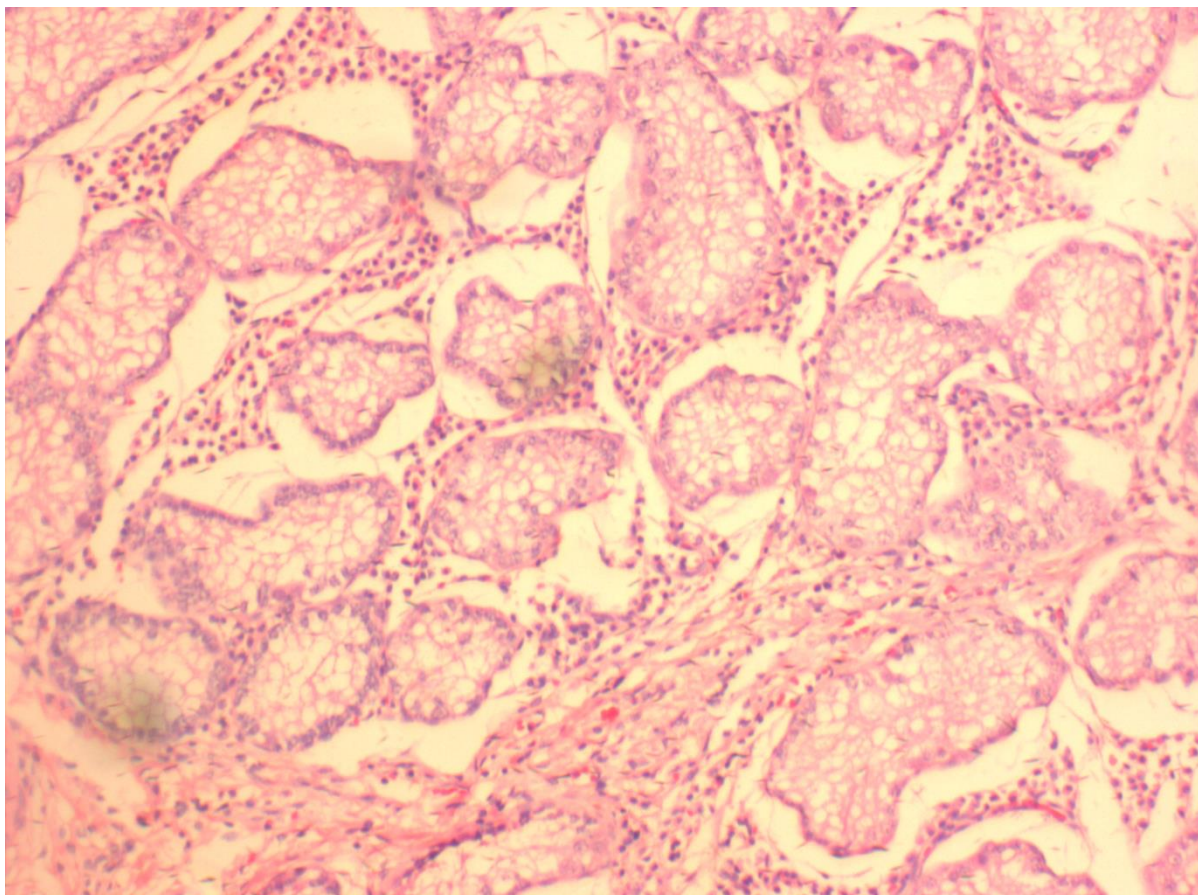


Figure 6.6 The testicle morphology of an immunocastrate showing a severe increase in lumen and deformation of the seminiferous tubules with no spermatogenesis evident (4X magnification; photograph has not been resized)

6.5 Discussion

Immunocastration resulted in 83.3 % of the treated pigs having androstenone levels below the detection limit of the methodology used for analysis and although the highest androstenone level seen was 0.08 $\mu\text{g/g}$, this is not close to the sensory threshold of 0.5 to 1.0 $\mu\text{g/g}$ of adipose tissue (Zamaratskaia *et al.*, 2004). The fact that there is still androstenone present in the adipose tissue samples of the immunocastrates is not unexpected, since Weiler *et al.* (2013) reported an average value of 0.10 $\mu\text{g/g}$ fat for immunocastrates who received their second vaccination at approximately 21 weeks of age, four weeks prior to slaughter. The same result was found by Lealiifano *et al.* (2011) who investigated various timings of the second vaccination and when administered at four weeks prior to slaughter, the androstenone concentration in the backfat at slaughter was $0.10 \pm 0.05 \mu\text{g/g}$ fat, which did not differ significantly from the androstenone concentrations from those pigs vaccinated at six weeks prior to slaughter ($0.13 \pm 0.05 \mu\text{g/g}$). However, the androstenone concentrations in the entire male pigs used for the current trial do not compare to those of Lealiifano *et al.* (2011) and Weiler *et al.* (2013) which could be due to numerous reasons. Firstly, the methodologies for the determination of the various boar taint compounds vary and the methodology used by Verheyden *et*

al. (2007) was developed for large scale analysis of samples with quick sample preparation and simultaneous analysis of the three boar taint compounds. Although equipment with a high sensitivity was used, the extraction method requires more investigation since matrix interferences were found for the androstenone determination (Verheyden *et al.*, 2007). Androstenone has a relatively high heritability (Robic *et al.*, 2008) and thus the breed or genotype can influence the androstenone fat concentrations. Weiler *et al.* (2013) made use of a Large White x Landrace maternal line and a Pietrain terminal sire to produce the progeny used for their study and found an average androstenone level of 1.75 µg/g which is above the sensory threshold. Lealiifano *et al.* (2011) used Large White x Landrace boars with an average androstenone value of 0.91 µg/g. In the current study a maternal line of Large White x Landrace x White Duroc was crossed with a PIC[®] 410 terminal sire and therefore the genotype could have influenced the amount of androstenone in the adipose tissue since the slaughter age was similar in all three studies. Aluwé *et al.* (2011) found an interaction between breed and slaughter weight for androstenone levels, with Large White pigs having a higher androstenone concentration than Pietrain pigs at 110 kg slaughter weight. Pieterse (2006) showed that the androstenone levels of five different genotypes of boars increased when slaughter weights of 102 to 113 kg are compared to those above 133 kg, however, none where above 1.0 µg/g. Since the pigs in the current trial were slaughtered at an average weight of ~ 130 kg (Chapter 4; section 4.3.1), the low values could thus be attributed to the use of modern genotypes. Also, many of the studies involving the analysis of boar taint compounds in immunocastrates and entire males have made use of group-housing which could allow for the exhibition of sexual behaviour such as mounting which could have a stimulatory effect on the production of testicle steroids. Some have also used females in their experimental design in order to compare the growth performances and thus the presence of females may also have a stimulatory effect even if they are not housed within the same pen.

Immunocastration lowered the skatole levels in the fat, which agrees with Weiler *et al.* (2013); it also decreased indole levels whereas Weiler *et al.* (2013) observed the opposite. Since both skatole and indole levels are highly dependent on environmental factors such as management and feed composition (Bonneau, 1982), differences in results are to be expected. The possible reason for lowered skatole levels in immunocastrates without a significant dietary effect could be due to decreased testicular steroid production; however the mechanism of this is still unclear in current literature. Various theories with regards to the decrease in skatole in the absence of testicular steroids exist, including the involvement of steroids in intestinal cell apoptosis (Claus *et al.*, 1994) or the increase in hepatic clearance of skatole (Babol *et al.*, 1999) and the inhibitory effect of androstenone on the skatole-induced expression of the CYP2E1 enzyme which is involved in skatole metabolism (Doran *et al.*, 2002). Skatole and androstenone levels in adipose tissue are highly correlated for pigs between 20 and 24 weeks of age (Zamaratskaia *et al.*, 2004) and since androstenone and skatole may have a synergistic effect on the perception of boar taint (Font-i-Furnols, 2012a), the fact that 83.3 % of the immunocastrated carcasses had androstenone levels below the detection limit (0.02 µg/g) could mean that the two carcasses with skatole levels just above 0.025 µg/g may not be rejected as readily by consumers than entire male carcasses with both androstenone and skatole present. The reason for the two immunocastrated carcasses having skatole levels just above 0.025 µg/g is not

clear since the pens were cleaned daily in order to minimise the effect of environment; however, factors such as the level of skatole prior to second vaccination can also have an influence on the degree of skatole clearance. When the meat from immunocastrates was evaluated by a sensory panel, Gispert *et al.* (2010) showed that meat from immunocastrates was accepted better than that from entire males when both androstenone and skatole odours and taste were analysed.

Immunocastration decreases the testes weight as demonstrated by Dunshea *et al.* (2001), Jaros *et al.* (2005), Zamaratskaia *et al.* (2008) and Pauly *et al.* (2009). However, the testicle sizes vary between the results; for example when Lealiifano *et al.* (2011) vaccinated at four weeks prior to slaughter the average testes weight was 98 g for the immunocastrates and 209 g for the entire males whereas Dunshea *et al.* (2001) reported mean testes weights of 182.6 g for immunocastrates and 421.6 g for entire males also vaccinated four weeks prior to slaughter. However, testes weights/size varies depending on body weight at slaughter as well as the genotype used. Although it was not significant, immunocastration decreased the individual testicle weight (196.1 ± 10.23 g) compared to entire males (207.6 ± 10.23 g); furthermore, the effects of immunocastration on the testicle function could be seen by its effect on the morphology and colour of the testicles. The visual assessment of the testicular morphology was in accordance with the effects observed by Kubale *et al.* (2013), who also found decreased seminiferous tubule diameters, seminiferous tubule atrophy and loss of spermatocyte. Additionally, Kubale *et al.* (2013) noted that the Leydig cells become deformed as the cytoplasm shrunk, with the effects worsening with time. Therefore, although the testicle sizes did not differ significantly, the vaccination affected testicular morphology of which the effects could probably be seen in terms of size if the delay between vaccination and slaughter was longer.

The results from the CIE L*a*b* colour measurements on the cut surfaces of the testicles showed that immunocastration caused lighter, less red and more yellow testicles which agrees with the findings of Lealiifano *et al.* (2011) and show that immunocastration not only had an effect on the morphology but also the functioning of the testes. Since the number and nuclear area of the Leydig cells decreased in immunocastrated pigs in comparison to entire males (Kubale *et al.*, 2013), an increase in L* (lightness) and a decrease in a* (redness) could be as a result of decreased activity in the testes. The current results thus agree with Fuchs *et al.* (2009b) that testicle size measurements are not a good indication of the efficacy of vaccination and therefore the possibility of boar taint. However, the testicle colour measurements showed more promise in determining whether testicular functioning was terminated with vaccination and could pose a more reliable method for slaughter-line application (Lealiifano *et al.*, 2011).

6.6 Conclusion

Immunocastration resulted in 100 % of the treated animals having androstenone concentrations below the sensory threshold of 0.5 µg/g fat and decreased skatole and indole concentrations in comparison to the entire males. Two out of 48 immunocastrates had skatole levels above the sensory threshold of 0.025 µg/g fat and thus it would be beneficial to further investigate the sensory properties of pork from immunocastrates with various skatole levels without the presence of androstenone using

a trained sensory panel. Although the methodologies used for the analysis of the boar taint compounds confirmed the expected outcomes with regards to androstenone levels in immunocastrates, the extraction process needs to be further optimised for androstenone. The methodology allowed for the quick and simultaneous analysis of 5 α -androst-16-en-3-one, skatole (3-methylindole) and indole. Immunocastration decreased the testicle weight by ~ 5 % compared to entire males but the effects on the testicular functioning were better represented by the changes in the morphology and colour of the testes. The effects of immunocastration on the testicles indicates a decrease in functioning and activity and thus supports the conclusion that vaccinating at 16 and 20 weeks of age, four weeks prior to slaughter, is successful in inhibiting testicular steroid production. Therefore, the findings agree with that found in current literature that the testicle size measurements should not be used as an indication of the success of immunocastration or presence of boar taint but the CIE L*a*b* cut surface colour measurements shows promise.

6.7 References

- Aluwé, M., Millet, S., Bekaert, K., Tuytens, F., Vanhaecke, L., De Smet, S. & De Brabander, D. (2011). Influence of breed and slaughter weight on boar taint prevalence in entire male pigs. *Animal*, 5, 1283–1289.
- Andresen, Ø. (2006). Boar taint related compounds: Androstenone/skatole/other substances. *Acta Veterinaria Scandinavica*, 48 (Suppl 1).
- Babol, J., Squires, E. & Gullett, E. (1995). Investigation of factors responsible for the development of boar taint. *Food Research International*, 28, 573–581.
- Babol, J., Squires, E. J. & Lundström, K. (1999). Relationship between metabolism of androstenone and skatole in intact male pigs. *Journal of Animal Science*, 77, 84–92.
- Bekaert, K., Vanden Bussche, J., François, S., Tuytens, F., De Brabander, H., Vandendriessche, F. & Vanhaecke, L. (2012). A validated ultra-high performance liquid chromatography coupled to high resolution mass spectrometry analysis for the simultaneous quantification of the three known boar taint compounds. *Journal of Chromatography A*, 1239, 49–55.
- Bonneau, M. (1982). Compounds responsible for boar taint, with special emphasis on androstenone: a review. *Livestock Production Science*, 9, 687-705.
- Bonneau, M., Dufour, R., Chouvet, C., Roulet, C., Meadus, W. & Squires, E. (1994). The effects of immunization against luteinizing hormone-releasing hormone on performance, sexual development, and levels of boar taint-related compounds in intact male pigs. *Journal of Animal Science*, 72, 14–20.
- Brunius, C., Zamaratskaia, G., Andersson, K., Chen, G., Norrby, M., Madej, A. & Lundström, K. (2011). Early immunocastration of male pigs with Improvac[®] - Effect on boar taint, hormones and reproductive organs. *Vaccine*, 29, 9514–9520.
- Claus, R., Weiler, U. & Herzog, A. (1994). Physiological aspects of androstenone and skatole formation in the boar- a review with experimental data. *Meat Science*, 38, 289–305.

- Doran, E., Whittington, F. W., Wood, J. D., McGivan, J. D. (2002). Cytochrome P450IIE1 (CYP2E1) is induced by skatole and this induction is blocked by androstenone in isolated pig hepatocytes. *Chemico-Biological Interactions*, 140, 81–92.
- Dunshea, F., Colantoni, C., Howard, K., McCauley, I., Jackson, P., Long, K., Lopaticki, S., et al. (2001). Vaccination of boars with a GnRH vaccine (Improvac) eliminates boar taint and increases growth performance. *Journal of Animal Science*, 79, 2524–2535.
- Font-i-Furnols, M. (2012). Consumer studies on sensory acceptability of boar taint: A review. *Meat Science*, 92, 319–329.
- Fredriksen, B., Font-i-Furnols, M., Lundström, K., Migdal, W., Prunier, A., Tuttysens, F. A. M., Bonneau, M. (2009). Practise on castration of piglets in Europe. *Animal*, 3, 1480-1487.
- Fuchs, T., Nathues, H., Koehrmann, A., Andrews, S., Brock, F., Sudhaus, N., Klein, G., et al. (2009a). A comparison of the carcass characteristics of pigs immunized with a 'gonadotrophin-releasing factor (GnRF)' vaccine against boar taint with physically castrated pigs. *Meat Science*, 83, 702–705.
- Fuchs, T., Thun, R., Parvizi, N., Nathues, H., Koehrmann, A., Andrews, S., Brock, F., et al. (2009b). Effect of a gonadotropin-releasing factor vaccine on follicle-stimulating hormone and luteinizing hormone concentrations and on the development of testicles and the expression of boar taint in male pigs. *Theriogenology*, 72, 672–680.
- Gispert, M., Àngels Oliver, M., Velarde, A., Suarez, P., Pérez, J. & Font i Furnols, M. (2010). Carcass and meat quality characteristics of immunocastrated male, surgically castrated male, entire male and female pigs. *Meat Science*, 85, 664–670.
- Hughes, P. E. & Varley, M. A. (1980). *Reproduction in the pig*. London, United Kingdom: Butterworth & Co. pp 5-9.
- Jaros, P., Bürgi, E., Stärk, K., Claus, R., Hennessy, D. & Thun, R. (2005). Effect of active immunization against GnRH on androstenone concentration, growth performance and carcass quality in intact male pigs. *Livestock Production Science*, 92, 31–38.
- Kubale, V., Batorek, N., Škrlep, M., Prunier, A., Bonneau, M., Fazarinc G., Čandek-Potokar, M. (2013). Steroid hormones, boar taint compounds, and reproductive organs in pigs according to the delay between immunocastration and slaughter. *Theriogenology*, 79, 69-80.
- Lealiifano, A., Pluske, J. R., Nicholls, R., Dunshea, F., Campbell, R., Hennessy, D., Miller, D., Hansen, C. F., & Mullan, B. (2011). Reducing the length of time between slaughter and the secondary gonadotropin-releasing factor immunization improves growth performance and clears boar taint compounds in male finishing pigs. *Journal of Animal Science*, 89, 2782–2792.
- Metz, C., Hohl, K., Waidelich, S., Drochner, W. & Claus, R. (2002). Active immunization of boars against GnRH at an early age: consequences for testicular function, boar taint accumulation and N-retention. *Livestock Production Science*, 74, 147–157.
- Pauly, C., Spring, P., O'Doherty, J., Ampuero Kragten, S. & Bee, G. (2009). Growth performance, carcass characteristics and meat quality of group-penned surgically castrated, immunocastrated (Improvac®) and entire male pigs and individually penned entire male pigs. *Animal*, 3, 1057–1066.

- Pieterse, E. 2006. Effects of increased slaughter weight of pigs on pork production. Ph.D thesis, University of Stellenbosch, Western Cape, South Africa.
- Rault, J.-L., Lay Jr, D. C. & Marchant-Forde, J. N. (2011). Castration induced pain in pigs and other livestock. *Applied Animal Behaviour Science*, 135, 214–225.
- Robic, A., Larzul, C. & Bonneau, M. (2008). Genetic and metabolic aspects of androstenone and skatole deposition in pig adipose tissue: a review. *Genetics Selection Evolution*, 40, 581.
- Verheyden, K., Noppe, H., Aluwé, M., Millet, S., Vanden Bussche, J. & De Brabander, H. (2007). Development and validation of a method for simultaneous analysis of the boar taint compounds indole, skatole and androstenone in pig fat using liquid chromatography-multiple mass spectrometry. *Journal of Chromatography A*, 1174, 132–137.
- Weiler, U., Götz, M., Schmidt, A., Otto, M. & Müller, S. (2013). Influence of sex and immunocastration on feed intake behavior, skatole and indole concentrations in adipose tissue of pigs. *Animal*, 7(02), 300–308.
- Zamaratskaia, G., Andersson, H., Chen, G., Andersson, K., Madej, A. & Lundström, K. (2008). Effect of a Gonadotropin-releasing Hormone Vaccine (Improvac™) on Steroid Hormones, Boar Taint Compounds and Performance in Entire Male Pigs. *Reproduction in Domestic Animals*, 43, 351–359.
- Zamaratskaia, G., Babol, J., Andersson, H. & Lundström, K. (2004). Plasma skatole and androstenone levels in entire male pigs and relationship between boar taint compounds, sex steroids and thyroxine at various ages. *Livestock Production Science*, 87, 91–98.

CHAPTER 7

General conclusions and recommendations

Increased consumer awareness is placing pressure on various aspects of pork production, especially with regards to animal welfare. Therefore pig producers are facing new challenges in terms of management practices, one of which is controlling the prevalence of boar taint without the use of surgical castration in response to the banning of surgical castration without anaesthesia in numerous countries. Although the production of intact male pigs is favourable in terms of feed efficiency and lean growth, the production of heavy male carcasses increases the risk of boar taint and thus entire male carcasses over 100 kg are currently downgraded in South Africa. This resulted in the slaughtering of male pigs before puberty and sexual maturity and thus small carcasses with narrow profit margins. In order to produce larger male carcasses while controlling boar taint and without the use of surgical castration, immunological castration can be used and has received increased attention in the pig industry and pig research. Since feed (dietary protein in particular) contributes to a large proportion of pig production expenses, the provision of correct nutrition for favourable growth and carcass characteristics is of utmost importance. In order to fully exploit the nutrients provided, feed additives such as ractopamine hydrochloride (RAC) have been shown to improve growth performance parameters, carcass traits and carcass cutting yields. Thus the need to determine the nutrient requirements of immunocastrated male pigs with and without feed additives such as RAC was identified.

The results of this study indicated that immunocastrates perform as entire males until their second vaccination and thus should be fed as such, after which the weight gain (ADG) and feed intake (ADFI) increases while the feed efficiency decreases as a result of the castration. In light of this result and current literature findings, the time period between the second vaccination and slaughter should not be extended in order to minimise this effect. The current vaccination schedule was favourable with regards to the fact that although the backfat deposition rate was increased, there were no differences seen in backfat thickness at slaughter when using the Hennessey Grading Probe. The supplementation of RAC did not influence the backfat deposition rate during growth or the HGP backfat measurement; however it decreased the calliper backfat thickness and thus its effect on fat deposition remains questionable. The supplementation of RAC at 10 mg/kg for the last 28 days of growth improved the ADG while its effect on feed efficiency depends on the level of dietary balanced protein such that the medium and high diets provided the best results. Therefore, the provision of a higher dietary protein level than what is commercially used, improved feed efficiency both with, and without RAC supplementation and thus an economic comparison is needed for the individual pig producer to determine whether this improvement justifies the cost input of both the RAC as well as the higher protein diet.

The increase in ADG due to immunocastration and RAC supplementation resulted in heavier live weights at slaughter, although this was not reflected in the dressed hot carcass weights. Since immunocastration increased ADFI, gut fill could be partially responsible for this, but RAC did not influence ADFI. Thus the HCW measurement could have been influenced by various factors in the

abattoir such as the movement of the carcass over the scale. The lack of differences between the backfat measurements using the HGP could also be attributed to user error as well as the fact that the differences in calliper backfat thicknesses were small. The latter is unlikely since the variation in results from the two instruments used to measure backfat depth was large (11.5 mm versus 17 mm for HGP). Therefore, it would be recommended that calliper measurements be taken when backfat measurements are being compared during research projects. The calliper backfat depths provided an indication that the dietary protein requirements differ between the sexes, since an oversupply or under supply can influence fat deposition. These results indicated that entire males fed the high balanced protein diet will have the least subcutaneous backfat deposition, whereas immunocastrates should be fed the low balanced protein diet to minimise backfat deposition. Since the PORCUS system and carcass price relies on the backfat thickness and since the HGP results showed no significant differences the carcasses would not have been penalised for backfat thickness on the slaughter line. Nonetheless, it could be beneficial to further investigate the effect of a wider range of balanced protein levels on the carcass traits and growth performance of immunocastrates using a larger sample size.

Supplementing RAC improved the carcass cutting yields of all the commercial cuts measured and improved the lean yield of these cuts either by increasing the muscle or decreasing the fat. However, the lack of effect on the fat deposition on all the cuts except the belly and hindquarter further questions the direct effect of RAC on fat deposition. Immunocastration increased the belly yield but also increased the belly fat and loin fat but by supplementing RAC, the belly muscle percentage increases (~ 2 % of side weight). Thus the increase in fat deposition due to immunocastration could be altered by RAC supplementation, while increasing the value of the carcass through improved carcass cutting yields. Various interactions were evident for the bone percentages of the various cuts which appear to be negligible but further research could be beneficial when a market that produces cured ham products is considered since the cost is largely determined by the bone: muscle ratio. Further research is also needed into the mechanism of action of RAC with regards to lipogenesis since the effects are unclear and contradictory in this and previous research.

Immunocastration, RAC and dietary protein level had little effect on the chemical composition of the *Longissimus thoracis* (LT) muscle, with the statistically significant effect on crude protein content of the LT being biologically negligible. The pH and temperature for 45 minutes, 24 hours and 48 hours *post mortem* showed no significant effects or any indication of PSE pork, which implies that excessive stress was not experienced at slaughter. Both immunocastration and RAC influenced the CIE L*a*b* colour values and although they are unlikely to be picked up subjectively when compared to current literature, this should be validated by a sensory/consumer trial since the combination of immunocastration and RAC may have an influence and colour is considered one of the most important factors considered when purchasing meat. The decrease in cooking loss experienced with immunocastration and feeding the medium protein diet is questionable since the drip loss was not significantly affected; however, the WBSF tenderness showed that feeding a high protein diet to immunocastrates and RAC supplementation increased the WBSF values, thus decreasing tenderness. This further motivates the need to quantify the effect of these differences seen in the

physical quality of the meat from a sensory perspective in order to determine their effects on consumer acceptability.

The absence of androstenone in the adipose tissue of the immunocastrates supports the use of immunocastration as a means of controlling boar taint in heavy entire male carcasses. Although the effects of immunocastration on the testicular function could not be seen using size measurements, the CIE L*a*b* colour measurements and testicle histology provided an indication of decreased activity and functioning. The current results are thus in agreement that testicular size should not be used as an indication of vaccination efficacy or boar taint. The testicle surface colour measurements could prove more useful in achieving this and thus further research is needed into the correlation between these measurements and the endocrinological activity of the testes. Therefore, the vaccination schedule used was successful in eliminating androstenone while providing minimal delay between the second vaccination and slaughter so as to minimise the negative effects of immunocastration on fat deposition. Again, this is in agreement with current literature that the period between the second vaccination and slaughter not be extended past four weeks when using a modern, lean and fast-growing genotype such as the current genotype (PIC®).

Taking the above into account, it can be concluded that immunocastration was effective in eliminating androstenone from the adipose tissue while providing a carcass similar in quality to that of entire males. Although feed efficiency is sacrificed when immunocastration is practised, this can be rectified and improved by the supplementation of 10 mg/kg RAC for the last 28 days of growth along with the correct provision of dietary balanced protein. This indicates that the dietary requirements for immunocastrates differ with regards to dietary balanced protein, with and without RAC supplementation. By supplementing RAC at 10 mg/kg 28 days prior to slaughter, producers can improve the cutting yield and lean yield of carcasses which increases the value of expensive cuts, thus increasing the return per carcass. Therefore, with the use of immunocastration, RAC and correct dietary balanced protein, pig producers have the option to produce heavier male carcasses efficiently without sacrificing the meat quality or the lean meat yield, while providing a product which consumers will be acceptable of in terms of both the sensory meat quality traits and animal welfare. However, the movement towards the production of heavier immunocastrated male carcasses will rely on the processor not penalising them and thus a change in the current classification system could be required, possibly treating immunocastrated carcasses as a separate sex within the abattoir.