

**Improvement of Energy and Nitrogen
Utilisation in Pork production –Genetics and
Growth Models-**

Thesis committee**Thesis supervisors**

Prof. dr. R. Roehe
Professor of Animal Breeding and Genetics
Scotland's Rural College (SRUC)

Prof. dr. ir. J.A.M. van Arendonk
Professor of Animal Breeding and Genetics
Wageningen University

Thesis co-supervisors

Dr. E. Kanis
Assistant professor, Animal Breeding and Genetics Group
Wageningen University

Other members

Prof. dr. R.G.F. Visser - Professor of Plant Breeding, Wageningen University
Dr. J.P. Bidanel - Deputy Head of Animal Genetics division INRA, France
Prof. dr. ir M.W.A. Verstegen, Wageningen University
Dr. ir E.F. Knol - Research manager TOPIGS Research Center IPG

This research was conducted under the auspices of the Graduate School of Wageningen Institute of Animal Sciences (WIAS).

**Improvement of Energy and Nitrogen
Utilisation in Pork production –Genetics and
Growth Models-**

Mahmoud Shirali

Thesis

submitted in fulfillment of the requirements for the degree of doctor at
Wageningen University
by the authority of the Rector Magnificus
Prof. Dr. M.J. Kropff,
in the presence of the
Thesis Committee appointed by the Academic Board
to be defended in public
on Thursday March 13, 2014
at 11.00 a.m. in the Aula.

M. Shirali

Improvement of Energy and Nitrogen Utilisation in Pork production –Genetics and Growth Models-

PhD thesis, Wageningen University, the Netherlands (2014)

With references, with summaries in English and Dutch

ISBN 978-94-6173-847-9

Abstract

M. Shirali (2013). "Improvement of Energy and Nitrogen Utilisation in Pork production –Genetics and Growth Models-". PhD thesis, Wageningen University, the Netherlands

Expansion of demand for pork is expected to meet the nutritional requirements of an increasing world population. However, higher levels of pig production are using limited feed resources and are often associated with higher levels of environmental pollution, which provide substantial challenges for pork producers. Therefore, strategies that simultaneously improve feed efficiency and increase production with reduction of environmental pollution of pork production (e.g. per kg product) is necessary. The general aim of the current project was to investigate opportunities for improvement of energy usage and nitrogen excretion at different stages of growth in pigs, and to determine their phenotypic and genetic background in association with other performance traits as well as to provide the basis for developing strategies for improvement of these traits using biological growth models. Feed efficiency was characterised by residual energy intake (REI) as the surplus of energy intake which is not used for protein and lipid deposition along with maintenance throughout growth to 140 kg BW whereas nitrogen excretion was estimated as the difference between nitrogen intake and retention. The results of phenotypic analyses indicate that nitrogen excretion increases substantially during growth of pigs and can be reduced most effectively by improving feed efficiency and to a lesser extent through the improvement of weight gain and/or body composition. Results of genetic analyses indicate that REI as a measure of feed efficiency is highly heritable ($h^2 = 0.44$), suggesting great potential for genetic improvement. REI has different genetic background at different stages of growth, suggesting that genetic improvement of REI should consider the stage of growth. In addition, REI explains a large portion of variance in nitrogen excretion, suggesting that selection for lower REI is expected to reduce nitrogen excretion of pork production as well as improve feed efficiency. Genomic analysis showed that different genes are responsible for efficiency of feed utilisation at different stages of growth. The results further suggest that only a small proportion of the variance in REI was explained by variation in feed intake, whereas underlying factors of feed utilisation, such as metabolism and protein turnover, are likely to have great influence on REI. A biological growth model was used to characterize a crossbred population regarding feed energy and nitrogen efficiency in comparison to two purebred population selected for different performances. The results of the biological growth model can be used to developed optimal genetic, nutritional and

production strategies, e.g. the impact of reduction in slaughter weight on marginal energy efficiency and nitrogen excretion was estimated. Furthermore, based on the results of the biological growth model, the influence of changes of production traits during growth on energy and nitrogen efficiency can be estimated to optimise genetic strategies. Furthermore, opportunities for further improvement of energy and nitrogen utilisation have been outlined.

Contents

Chapter 1	General introduction	9
Chapter 2	Nitrogen excretion at different stages of growth and its association with production traits in growing pigs	19
Chapter 3	Novel insight into the genomic architecture of feed and nitrogen efficiency measured by residual energy intake and nitrogen excretion in growing pigs	45
Chapter 4	Estimation of residual energy intake and its genetic background during the growing period in pigs	73
Chapter 5	Predicting energy efficiency and nitrogen excretion in growing pigs with different growth potential using a mechanistic biological growth model	93
Chapter 6	General discussion	123
	Summary	157
	Samenvatting	163
	Publications	169
	Curriculum vitae	173
	Training and supervision plan	177
	Acknowledgements	181
	Colophon	185

1

General introduction

1.1 Introduction

Commercial breeding programs aim to produce highly productive animals with high efficiency to convert feed into meat. Increased animal productivity and efficiency of quality lean meat production lead the future of competitiveness of pork in the food market (Clutter and Brascamp, 1998). Feed intake is of high economical importance in growing pigs, as feed is the highest cost input associated with pig production. To reduce production costs and the environmental footprint of pig production, feed efficiency needs to be extensively subjected to research. In the future, with an increase in human world population size and purchasing power, demand for meat will increase substantially (Neeteson-van Nieuwenhoven et al., 2013). Pig and poultry are major sources for animal protein in human diets (OECD-FAO, 2013). Therefore, commercial pig breeding programs must ensure an increase in production without neglecting concerns about the environmental footprint of pork production (Neeteson-van Nieuwenhoven et al., 2013). Environmental impacts of pork production are widespread, affecting soil, water, air and fauna. Manure surplus causes major problems in highly intensive pig production areas, especially for nitrogen, because of water pollution by nitrates and air pollution by gaseous ammonia emissions (Dourmad et al., 1999b). The primary nutrient of concern in excreta is nitrogen. The global nitrogen excretion from livestock sectors was estimated to be 137 million tonnes per year in 2006 (Steinfeld et al., 2006). Legislation such as the Integrated Pollution Prevention and Control (IPPC) Directive (OJ, 2008) which restricts the amount of manure that can be applied to land as fertilizer creates additional challenges for pig producers. Reduction in environmental pollution by pig production combined with an improvement in feed efficiency is one of the main research priorities in the UK (Kleanthous, 2009). Therefore, methods to reduce the amount of nitrogen in pig excreta are required. Several methods for reducing the environmental pollution by animals have been studied. Proposed mitigation strategies include improvement of animal nutrition, manure management, as well as genetic improvement in production, as a complementary approach to the first two methods (Dourmad et al., 1999a; Crocker and Robison, 2002; Steinfeld et al., 2006). As suggested by Kanis et al. (2005), the quantity of minerals (in particular nitrogen and phosphorous) and heavy metals excreted per kilogram of meat produced, largely depends on production and reproduction efficiencies. In particular, improving growth rate and feed efficiency have a favourable environmental impact. Kanis et al. (2005) also suggested putting more emphasis on these traits in the breeding objective.

1.2 Feed efficiency and nitrogen excretion at different stages of growth

Feed efficiency is not only of high importance from the economic point of view but also to reduce environmental pollution per kg product. In order to minimize environmental pollution and improve feed efficiency, both the dietary supply and the nutrient requirements of the pigs at different growth stages should be known as precise as possible. The changes of feed energy usage and nitrogenous losses during the growing-finishing period have been under limited investigation in large populations of pigs. In addition, energy usage and nitrogen excretion are related to nitrogen retention of which limited information is available during the growth of pigs. Supplying feed protein to animals without considering their specific requirements at different stages of growth will result in more nitrogen loss than necessary and consequently environmental pollution, whereas in case of undersupply is expected to reduce growth rate and therefore a longer growth period will result in an increased nitrogen loss per kg product. Furthermore, the use of nitrogen in metabolism is associated with food energy (Susenbeth et al., 1999).

It is widely known that the physiological requirements of pigs change during growth as well as their dietary requirements. To optimise production efficiency and minimize nitrogen excretion, the diet should match the physiological requirements. Therefore, growth, feed efficiency and nitrogen excretion of pigs should be studied at different stages of their growth. Currently, there is limited knowledge of energy efficiency and nitrogen excretion rates at different stages of growth and their associations with production traits. These associations will contribute to identify the effects of improvements in production traits on energy efficiency and nitrogen excretion and to reveal the biological explanation underlying the variation in these traits. Accurate measurements of energy efficiency and nitrogen excretion from pigs are of great interest for genetic selection to reduce energy usage and nitrogen excretion and for the understanding of their genetic background. Optimisation of food energy supply with respect to protein and fat deposition and maintenance requirements will save limited energy resources. Therefore, optimising pig production with respect to energy use and protein requirement, depending on the potential performance of specific genotypes, is essential to reduce the environmental impact, energy resources and costs of pork production.

Improving feed energy efficiency is already one of the major objectives of current animal breeding programs to reduce feed costs and improve lean growth. In growing-finishing pigs, energy usage is divided into energy used for production (protein and lipid deposition) and energy needed for maintenance. Direct selection

for higher production has generally resulted in an increase in nutrient intake relative to maintenance requirements (Tolkamp et al., 2010). Tolkamp et al. (2010) reported substantial variation within and between breeds in efficiency of production and maintenance requirements.

Variation in maintenance requirements contributes directly to variation in production efficiency. Most of the focus in animal breeding has been on traditional measures of feed efficiency that are based on the ratio of feed intake and growth. Boggess et al. (2009) claimed that around 500 million dollars could be saved annually by pig producers in the US by reducing the average feed conversion ratio (FCR) from 2.75 to 2.45. There are different ways of improving feed efficiency such as decreasing maintenance requirements and improving efficiency of utilisation of energy and protein (Tolkamp et al., 2010). Selection for feed conversion ratio has been shown to be suboptimal because it is a ratio trait of feed intake over body weight gain, which showed disproportionate selection pressure on the component traits (Gunsett et al., 1984; Kieter and Presuhn, 1997). This may result in a reduction of feed intake, which in turn may limit further improvement of growth. In particular in Pietrain, selection for leanness has resulted in reduced feed intake capacity, which may limit future genetic improvement of growth rate (Roehe et al. 2003). Therefore, residual feed intake (RFI) has been introduced as measure of feed efficiency (De Haer et al., 1993) which captures variation in maintenance requirements, digestion efficiency, and tissue turnover rate among others. Some studies have shown that a significant proportion of feed intake is not explained by production and maintenance requirements, therefore suggesting that selection on RFI can be carried out without altering growth in order to increase feed efficiency and reduce the production costs. Although intense selection for lean growth has significantly improved feed efficiency in pork production, further improvements require direct selection on components of feed intake such as RFI that are independent of lean growth.

1.3 Genomic association study

Genomics is the study of the structure, function and interactions within the genome. The potential of genomics in animal breeding relies on the ability to identify the causative gene differences giving rise to a difference in phenotype, or to identify a linked genetic marker, and to use the polymorphism in a breeding program. Knowledge of the “ideal genotype” and the genotype of every animal can facilitate increased genetic gain through a combination of improving accuracy of estimated breeding values (EBVs) and shortening the generation interval

(Meuwissen et al., 2001). The executed genomic study in this thesis will give insight into the causes of genetic variation in production, feed efficiency and nitrogen excretion traits and will identify the genetic relationships of nitrogen excretion and energy usage traits with other performance traits. The use of genomic tools for improvement is most cost-effective for traits which are difficult and costly to measure such as feed energy efficiency and nitrogen efficiency. Consequently, the use of genomic breeding for these traits may have a high impact on environmental sustainability, energy resources, food security and economics of pork production. There have been very few genomic studies for residual feed intake (Fan et al., 2010; Gilbert et al., 2010). Likewise genomic studies to reduce nitrogen loss and energy usage per kg meat over the entire growing period are scarce. Therefore, this study aims to identify quantitative trait loci (QTL), which are associated with energy usage and nitrogen loss, to be potentially used for marker assisted selection and to obtain more insight into the biological regulation of those traits.

Substantial progress has been made in the selection of animals for specific traits using traditional quantitative genetics approaches. In particular, feed efficiency has been substantially improved by increase in lean tissue growth. Nevertheless considerable variation in phenotypes remains unexplained, and therefore represents potential additional gain for animal production. In addition, the paradigm shift in new technologies now being applied to animal breeding represents a powerful opportunity to open the “black box” underlying the response to selection and to better understand the genetic architecture controlling the traits of interest. These arguments particularly apply to complex traits such as energy partitioning and feed efficiency. The use of genomic information to understand the genetic regulation of complex and/or novel traits provides a great opportunity to optimise animal breeding programs.

1.4 Growth models

Pig growth models that integrate current knowledge about the influence of various genetic and environmental factors underlying pig growth may represent a powerful alternative to conventional statistical approaches to improve efficiency and reduce nitrogen excretion in pork production systems during the entire growth period and at different target weights. Biological growth models have been used to optimise feed energy usage with minimum nitrogenous losses (Knap, 2012). Growth models have been developed for identifying methods to improve the efficiency of mineral retention in growing-finishing pigs and thus to minimize the potential environmental pollution of pig production (Jongbloed and Lenis, 1991). Moreover,

the biological growth model can be used to compare production estimates from the optimal growth model that can fit and predict best, based on the available observed data, within the literature (Moughan et al., 1995; Whittemore et al., 2001; Knap et al., 2003). Knap (2000) developed a growth model to analyze the relationship between protein turnover or thermoregulation with body composition. Future selection strategies may utilize biological growth models using protein accretion and minimum lipid to protein deposition ratio to optimise feed intake capacity (De Vries and Kanis, 1992). There is need for more advanced and precise growth models, e.g. capable of expressing feed intake and nitrogen loss on amino acid level according to the genetic potential of each individual on a daily bases during the growing-finishing period. This study aims to develop a more advanced growth model. This has the advantage that efficiency can be modelled as a function of either age or weight, reflecting that efficiency can be represented by different biological traits in different growth phases.

1.5 Aims of the thesis

The objectives of this study were to reveal the change in nitrogen excretion and energy usage at different stages of growth and to determine their genetic and biological background in association with performance traits using phenotypic, genetic, genomic and growth modelling tools. For this study, a unique dataset containing phenotypes with longitudinal information for growth, chemical body composition and feed intake information along with accurate pedigree and genomic information, was available for each individual animal during growth. As the study was set up as a full-sib design population, which is a powerful design for QTL mapping, it provided a valuable and informative dataset to perform genomic scanning for traits of interest. In addition, unique measurements of protein and lipid body content using deuterium dilution technique were available longitudinally on live animals which are expected to result in higher accuracy of feed efficiency and nitrogen excretion estimates than using proxy traits. The objectives of this thesis that follow their overarching aim of study are therefore:

- 1) To investigate nitrogen loss traits in a commercial pig population and to determine the effects of gender, halothane gene and housing type on nitrogen loss at different stages of growth as well as during the entire growing period.
- 2) To determine associations between nitrogen loss and production traits at different stages of growth and for the entire growing period.

1 General introduction

- 3) To compare residual energy intake (REI) estimating models based on deuterium dilution technique measurements of lean and fat tissue growth in live animals with common models using their proxy (average daily gain and backfat thickness) traits.
- 4) To determine genetic characteristics of REI at different growth stages.
- 5) To examine the genetic and phenotypic relationships of REI with production traits.
- 6) To detect QTL for REI and nitrogen excretion as measures reflecting feed efficiency and environmental impact, at different stages of growth and over the entire growing period.
- 7) To determine the genomic architecture of feed efficiency measured by REI in association with growth, feed intake and nitrogen excretion traits in a commercial population
- 8) To extend an existing pig growth model (Knap, 2000) to estimate REI and nitrogen excretion for each individual animal per day during growth.
- 9) To obtain biological estimates for feed efficiency and nitrogen excretion and underlying growth traits for pigs from genetically different commercial lines by fitting the above mechanistic model to available data.
- 10) To determine how improvement in growth is likely to affect feed efficiency and nitrogen excretion using the above growth model, adapted to the different modern pig populations.

The general discussion will further address the biological background of feed efficiency and nitrogen excretion in pigs and suggest possible future areas that can further improve nutrient efficiency and reduces environmental pollution.

References

- Boggess, M. 2009. A pork industry prospective. Pig Genome III Conference. November 2-4, 2009, Hinxton, UK .
- Clutter, A.C. and E.W. Brascamp. 1998. Genetics of performance traits. In: Rothschild, M.F., and A. Ruvinsky (Eds.). The Genetics of the Pig. CAB International, pp. 427-461.
- Crocker, A.W. and O.W. Robison. 2002. Genetic and nutritional effects on swine excreta. *J. Anim Sci.* 80:2809-2816.
- De Haer, L.C.M., P. Luiting and H.L.M. Aarts. 1993. Relations among individual (residual) feed intake, growth performance and feed intake pattern of growing pigs in group housing. *Livest. Prod. Sci.* 36:233-253.
- De Vries, A.G. and E. Kanis. 1992. A growth model to estimate economic values for food intake capacity in pigs. *Anim. Prod.* 55:241-246.
- Dourmad, J.Y., N. Guingand, P. Latimier and B. Seve. 1999a. Nitrogen and phosphorus consumption, utilisation and losses in pig production: France. *Livest. Prod. Sci.* 58:199-211.
- Dourmad, J.Y., B. Seve, P. Latimier, S. Boisen, J. Fernandez, C. van der Peet-Schwering and A.W. Jongbloed. 1999b. Nitrogen consumption, utilisation and losses in pig production in France, The Netherlands and Denmark. *Livest. Prod. Sci.* 58:261-264.
- Fan, B., S. Lkhagvadorj, W. Cai, J. Young, R.M. Smith, J.C.M. Dekkers, E. Huff-Lonergan, S.M. Lonergan and M.F. Rothschild. 2010. Identification of genetic markers associated with residual feed intake and meat quality traits in the pig. *Meat Sci.* 84:645-650.
- Gilbert, H., J. Riquet, J. Gruand, Y. Billon, K. Feve, P. Sellier, J. Noblet and J.P. Bidanel. 2010. Detecting QTL for feed intake traits and other performance traits in growing pigs in a Pietrain-Large White backcross. *Anim.* 4:1308-1318.
- Gunsett, F. C. 1984. Linear Index Selection to Improve Traits Defined as Ratios. *J. Anim. Sci.* 59:1185-1193.
- Jongbloed, A.W. and N.P. Lenis. 1991. Nutrition as a means to reduce environmental pollution by pigs. Proceedings of 42nd Annual Meeting of EAAP. Berlin, Germany.
- Kanis, E., K.H. De Greef, A. Hiemstra and J.A.M. van Arendonk. 2005. Breeding for societally important traits in pigs. *J. Anim. Sci.* 83:948-957.
- Kleanthous, A. 2009. Pigs and the environment. A report to BPEX, Warwickshire, UK. HERETOMORROW Publishing.

- Knap, P.W. 2000. Stochastic simulation of growth in pigs: relations between body composition and maintenance requirements as mediated through protein turnover and thermoregulation. *Anim. Sci.* 71:11-30.
- Knap, P.W. 2012. Pig breeding for increased sustainability. In *Encyclopedia of sustainability science and technology* (ed. R.A. Meyers), Springer Science+Business Media, New York NY, USA. Volume 11, pp. 7972-8012.
- Knap, P.W., R. Roehe, K. Kolstad, C. Pomar and P. Luiting. 2003. Characterization of pig genotypes for growth modeling. *J. Anim. Sci.* 81:E187-E195.
- Krieter, J. and U. Presuhn. 1997. The use of traits defined as product or ratios in pig breeding. *Zuchtungskunde.* 69:130-141.
- Moughan, P.J., M.W.A. Verstegen and M.I. Visser-Reyneveld. 1995. *Modelling growth in the pig.* Wageningen Press, Wageningen.
- Meuwissen, T.H.E., B.J. Hayes and M.E. Goddard. 2001. Prediction of Total Genetic Value Using Genome-Wide Dense Marker Maps. *Genetics* 157:1819-1829.
- Neeteson-van Nieuwenhoven, A.M., P.W. Knap and S. Avendano. 2013. The role of sustainable commercial pig and poultry breeding for food security. *Anim. Frontiers* 3:52-57.
- OECD-FAO. 2013. *OECD-FAO Agricultural Outlook 2013-2022.* OECD Publishing. doi: 10.1787/agr_outlook-2013-en.
- OJ. 2008. Council Directive 2008/1/EC of the European Parliament and of the Council of 15 January 2008 concerning integrated pollution prevention and control. *Official Journal of the European Union.* OJL 24 (2008). pp. 8-29.
- Roehe, R., G.S. Plastow and P.W. Knap. 2003. Quantitative and molecular genetic determination of protein and fat deposition. *HOMO* 54:119-131.
- Steinfeld, H., P. Gerber, T. Wassenaar, V. Castel, M. Rosales and C. de Haan. *Livestock's long shadow: environmental issues and options.* 2006. Rome, Italy, Food and Agriculture Organization of the United Nations.
- Susenbeth, A., T. Dickel, A. Diekenhorst and D. Hohler. 1999. The effect of energy intake, genotype, and body weight on protein retention in pigs when dietary lysine is the first-limiting factor. *J. Anim. Sci.* 77:2985-2989.
- Tolkamp, B., E. Wall, R. Roehe, J. Newbold and K. Zaralis. 2010. Review of nutrient efficiency in different breeds of farm livestock. Defra report. IFO183.
- Whittemore, C.T., D.M. Green and P.W. Knap. 2001. Technical review of the energy and protein requirements of growing pigs: protein. *Anim. Sci.* 73:363-373.

2

Nitrogen excretion at different stages of growth and its association with production traits in growing pigs

M. Shirali^{1&5}, A. Doeschl-Wilson², P.W. Knap³, C. Duthie⁴, E. Kanis⁵, J.A.M. van Arendonk⁵ and R. Roehe¹

¹Animal and Veterinary Sciences, SRUC, West Mains Road, Edinburgh, EH9 3JG, UK;

²Division of Genetics and Genomics, The Roslin Institute, R(D)SVS, University of Edinburgh, Easter Bush, Midlothian, EH25 9RG, UK; ³PIC International Group, Ratsteich 31, 24837 Schleswig, Germany; ⁴Future Farming Systems Group, SRUC, West Mains Road, Edinburgh, EH9 3JG, UK; and ⁵Animal Breeding and Genomics Centre, Wageningen Institute of Animal Sciences, Wageningen University, P.O. Box 338, 6700 AH Wageningen, The Netherlands.

Journal of Animal Science (2012) 90:1756-1765

Abstract

The objectives of this study were to determine nitrogen loss at different stages of growth and during the entire growing period, and to investigate the associations between nitrogen excretion and production traits in growing pigs. Data from 315 pigs of an F_2 population which originated from crossing Pietrain sires with a commercial dam line were used. Nitrogen retention was derived from protein retention as measured using the deuterium dilution technique during different stages of growth (60 to 90 kg, 90 to 120 kg and 120 to 140 kg). Pigs were fed *ad libitum* with two pelleted diets containing 17% (60 to 90 kg) and 16.5% (90 to 120 and 120 to 140 kg) CP. Average daily nitrogen excretion (ADNE) within each stage of growth was calculated based on the accumulated difference between average daily nitrogen intake (ADNI) and average daily nitrogen retention (ADNR). Lowest ADNE, nitrogen excretion per weight gain (NEWG) and total nitrogen excretion (TNE) were observed during growth from 60 to 90 kg. In contrast, the greatest ADNE, NEWG and TNE were found during growth from 120 to 140 kg. Statistical analyses indicated that gender, housing type, the *ryanodine receptor 1* (*RYR1*) gene and batch influenced nitrogen excretion ($P < 0.05$), but the degree and direction of influences differed between growth stages. Gender differences showed that gilts excreted less nitrogen than barrows ($P < 0.05$), which was associated with lower feed conversion ratio (FCR; Feed:gain) and lipid:protein gain ratio. Single-housed pigs showed lower nitrogen excretion compared to group-housed pigs ($P < 0.05$). In comparison to other genotypes, pigs carrying genotype *NN* (homozygous normal) at the *RYR1* locus had the lowest nitrogen excretion ($P < 0.05$) at all stages of growth except from 60 to 90 kg. The residual correlations indicated that NEWG and TNE have large positive correlations with FCR ($r = 0.99$ and 0.91 , respectively) and moderate negative correlations with ADG ($r = -0.53$ and -0.48 , respectively), for the entire growing period. Improvement in FCR, increase in ADG and reduction in lipid:protein gain ratio by one phenotypic standard deviation reduced TNE per pig by 709 g, 307 g and 211 g, respectively, over the entire growing period. The results indicate that nitrogen excretion changes substantially during growth and it can be reduced most effectively by improvement of feed efficiency and to a lesser extent through the improvement of weight gain and/or body composition.

Key words: Body composition, Feed efficiency, Growth, Nitrogen excretion, Pigs

2.1 Introduction

Environmental impacts of pig production are widespread, affecting soil, water, air and fauna. The global nitrogen excretion from livestock sectors was estimated to be 137 million tonnes per year in 2006 (Steinfeld et al., 2006). The pollutant of primary concern in pig excreta is nitrogen, which forms harmful components such as nitrate, nitrous oxide and ammonia. Legislation such as Integrated Pollution Prevention and Control (IPPC) Directive (OJ, 2008) which restricts the amount of manure that can be applied to land as fertilizer have created challenges for pig producers. Therefore, methods to reduce the amount of nitrogen in swine excreta are required.

Nitrogen excretion depends on many factors such as genetics and gender (Crocker and Robison, 2002), nutrition (Dourmad et al., 1999), housing system, weight and age (Murrells et al., 2010). The *ryanodine receptor 1* (*RYR1*) gene influences feed efficiency (Leach et al., 1996) and carcass composition (Salmi et al., 2010), but its effect on nitrogen excretion is not currently known. Accurate measurements of nitrogen excretion from pigs are of great interest for genetic selection on reduction of nitrogen excretion and for understanding its biological background. Currently, there is limited knowledge of nitrogen excretion rates at different stages of growth and their associations with production traits. These associations will contribute to identify the effects of improvements in production traits on nitrogen excretion and to reveal the biological explanation underlying the variation in nitrogen excretion in pigs.

The aims of this study were to investigate different nitrogen loss traits in a commercial pig population and to determine the effects of gender, *RYR1* gene and housing type on nitrogen loss at different stages of growth as well as during the entire growing period. Moreover, associations between nitrogen loss and production traits were examined at different stages of growth and for the entire growing period.

2.2 Materials and Methods

All animal care and handling procedures in the federal testing station were reviewed and approved by the Landwirtschaftskammer Schleswig-Holstein, Rendsburg, Germany.

Animals

The animals were from a 3-generation full-sib design. The founder generation (F_0) consisted of seven Pietrain grand-sires and 16 grand-dams bred from a three-way cross of Leicoma boars with Landrace $\times$ Large White dams. All grand-sires were heterozygous (Nn) at the *RYS1* locus. Of the F_1 generation, 8 boars and 40 sows were selected to develop the F_2 population. The F_2 generation consisted of 315 pigs from the first two parities of the F_1 sows. Detailed information about these animals of full-sib design and their use in a genomic study is presented in Duthie et al. (2010).

Data

The present study is based on measurements obtained on animals from the F_2 population. Forty eight gilts and 46 barrows from the F_2 generation were single-housed in straw-bedded pens and fed manually, with feed disappearance recorded on a weekly basis. The remaining 117 gilts and 104 barrows were housed in mixed-sexed groups of up to 15 pigs in straw-bedded pens. Animals housed in groups were fed using electronic feeders (ACEMA 48, ACEMO, Pontivy, France), which recorded feed disappearance at each visit. Pigs started the performance test at about 30 kg BW and were weighed on a weekly basis. For this study, only the testing period from 60 kg onwards were considered because at this stage pigs were entirely adapted to the electronic feeders. Pigs were weighed at target weights 60, 90, 120 and 140 kg BW. Average BW (SD) at target weights were 61 kg (2.58), 91 kg (2.60), 120 kg (2.69) and 140 kg (2.80), respectively. During growth from 60 to 90, and 90 to 140 kg of BW, pigs were fed ad libitum with a diet containing 17% CP, 13.8 MJ of ME/kg and 1.1% lysine, and a diet containing 16.5% CP, 13.4 MJ of ME/kg and 1.0% lysine, respectively. The diets consisted of adequate nutrient supplies to permit maximum protein deposition. For a more detailed description of the data see Landgraf et al. (2006), and Mohrmann et al. (2006).

The deuterium dilution technique was used to determine chemical body composition at the target weights of 60, 90, 120 and 140 kg. This technique is an *in vivo* method based on the empty body water content of the pigs. Using this method, the percentage of fat-free substance of pigs was estimated from the empty body water content. Protein and ash content of the empty body were estimated based on the percentage of the fat-free substance. Percentage of lipid content was the deviation of the percentage of fat-free substance from 100%. The accuracy of the deuterium dilution technique to determine body composition has been verified using magnetic resonance imaging on live animals and chemical

analysis of serially slaughtered animals using data of the F₁ population of the present experiment in previous studies (Landgraf et al., 2006; Mohrmann et al., 2006). A detailed description of the use and analysis of the deuterium dilution technique is presented by Landgraf et al. (2006). Average daily protein (APD) and lipid deposition (ALD) rates were calculated as the difference between protein or lipid composition at the two adjacent target weights divided by the number of days between the target weights. The ALD to APD gain ratio (ALD:APD) was calculated as well. Average daily gain was calculated within each stage of growth and the entire growing period. Average daily feed intake was calculated as the sum of feed disappearance (kg) divided by number of days for each stage of growth and over the entire growing period. Average daily energy intake (ADEI) was based on ME content of the diet and daily feed intake. Feed conversion ratio (FCR) was the sum of feed disappearance (kg) divided by body weight gain (kg) in each stage of growth and the entire growing period. The neck, shoulder, ham and loin of the right carcass side were dissected into fat and lean. Saleable meat was determined on the right carcass side as the sum of trimmed neck, shoulder, ham and loin weight. The fat to saleable meat ratio was obtained as the sum of external fat over the saleable meat for the above four carcass cuts.

Nitrogen Excretion

Average daily nitrogen excretion (ADNE) was calculated using the mass balance equation (Whittemore et al., 2003):

$$\text{ADNE} = \text{ADNI} - \text{ADNR}$$

where average daily nitrogen intake (ADNI) is the average daily feed intake multiplied by CP of feed intake divided by 6.25 (g/d), and the average daily nitrogen retention (ADNR) is equal to APD divided by 6.25 (g/d). Average daily nitrogen excretion was calculated for each stage of growth and for the entire growing period.

Total nitrogen excretion (TNE; kg/pig) was calculated for each pig during each stage of growth and during the entire growing period using equation:

$$\text{TNE} = \text{ADNE} \times \text{days}$$

where days is the length of growing period for each stage of growth and the entire growing period.

The nitrogen excretion per kg weight gain (NEWG) was calculated as the ratio between ADNE and ADG in each stage of growth as well as the entire growing period. Nitrogen excretion per saleable meat (NESM) was calculated as the ratio between TNE (kg/pig) and saleable meat (kg/pig) for each pig during the entire growing period (60 to 140 kg) only.

Statistical Analysis

The GLM procedure (SAS Inst. Inc., Cary, NC) was used to estimate least squares means for nitrogen excretion and production traits at different stages of growth and over the entire growing period using model:

$$y_{ijklmn} = \mu + S_i + G_j + B_k + HT_l + BF_m + b_1(SW) + b_2(EW) + e_{ijklmn} \quad [1]$$

where y_{ijklmn} is the observed trait, μ is the intercept, S_i is the fixed effects of gender with 2 levels (gilts or barrows), G_j is the fixed effect of *RYS1*-genotype with 3 levels (*NN*, *Nn*, *nn*), B_k is the fixed effect of batch with 9 levels (animals entering the test station), HT_l is the fixed effect of housing type with 2 levels (single or group-housed), BF_m is the fixed effect of birth farm with 2 levels, b_1 is the linear regression coefficient of start weight (*SW*) at each growth period, b_2 is the linear regression coefficient of end weight (*EW*) at each growth period and e_{ijklmn} is the random residual. The significance of interactions among fixed effects was tested and all interactions were non-significant ($P > 0.05$). The GLM procedure (SAS Inst. Inc., Cary, NC) was used to perform multivariate analysis of variance to predict residual correlations between nitrogen excretion and production traits based on model [1].

2.3 Results

Table 2.1 shows the means, coefficients of variation and standard errors of nitrogen excretion and production traits. The results indicate that nitrogen excretion increases as the growing period increases; where the lowest and greatest ADNE were achieved during growth from 60 to 90 kg and 120 to 140 kg, respectively. The most nitrogen efficient stage of growth was from 60 to 90 kg, where NEWG and ADNE were at their lowest level and ADNR was at its greatest level. This stage of growth was also associated with lowest FCR and ALD:APD, as well as greatest ADG.

2 Nitrogen excretion during growth

Table 2.1 Nitrogen excretion and production traits, and their standard errors (SE) and coefficients of variation (CV), at different stages of growth and during the entire growing period.

Trait ¹	Stages of growth											
	60-90 kg			90-120 kg			120-140 kg			60-140 kg		
	Mean	SE	CV	Mean	SE	CV	Mean	SE	CV	Mean	SE	CV
ADNI, g/d	67.4 ^a	± 0.60	14.9	74.7 ^b	± 0.58	13.0	75.6 ^b	± 0.69	15.2	72.8	± 0.52	11.5
ADEI, MJ/d	34.1 ^a	± 0.29	14.8	37.8 ^b	± 0.29	13.3	38.1 ^b	± 0.35	15.6	36.7	± 0.25	11.8
ADNR, g/d	21.6 ^a	± 0.21	16.5	20.0 ^b	± 0.21	17.5	18.7 ^c	± 0.26	23.5	20.1	± 0.16	12.5
ADNE, g/d	45.8 ^a	± 0.47	17.3	54.7 ^b	± 0.47	14.4	56.9 ^c	± 0.53	15.5	52.7	± 0.41	12.7
NEWG, g/kg	55.6 ^a	± 0.62	18.9	71.5 ^b	± 0.82	19.1	81.0 ^c	± 1.07	21.7	69.6	± 0.55	12.8
TNE, kg/pig	1.68 ^a	± 0.03	26.2	2.04 ^b	± 0.03	23.1	1.54 ^c	± 0.03	31.6	5.35	± 0.04	13.3
FCR, kg/kg	2.98 ^a	± 0.02	12.8	3.66 ^b	± 0.03	14.0	4.02 ^c	± 0.05	19.6	3.58	± 0.02	11.4
ADG, g/d	837 ^a	± 7.99	16.4	783 ^b	± 7.99	17.5	726 ^c	± 10.2	24.2	781	± 8.24	12.6
APD, g/d	135 ^a	± 1.32	16.5	125 ^b	± 1.30	17.4	117 ^c	± 1.65	23.5	126	± 1.38	12.5
ALD, g/d	271 ^a	± 3.55	22.0	273 ^a	± 3.89	23.9	269 ^a	± 5.54	34.1	272	± 2.72	16.2
ALD:APD	2.01 ^a	± 0.02	12.8	2.18 ^b	± 0.02	15.2	2.31 ^c	± 0.03	24.7	2.17	± 0.01	9.95
DAYS, d	37.1 ^a	± 0.53	24.1	37.9 ^a	± 0.55	24.3	27.5 ^b	± 0.55	33.2	102	± 0.88	13.8

^{a-c} Within a row, means without a common superscript differ ($P < 0.05$)

¹ ADNI = average daily nitrogen intake; ADEI = average daily energy intake; ADNR = average daily nitrogen retention; ADNE = average daily nitrogen excretion; NEWG = nitrogen excretion per weight gain; TNE = total nitrogen excretion; FCR = feed conversion ratio; APD = average daily protein deposition; ALD = average daily lipid deposition; ALD:APD = lipid to protein gain ratio; DAYS = length of growing period.

2 Nitrogen excretion during growth

To determine the strength of the associations between TNE and FCR, ADG, ALD:APD and ADNI from 60 to 140 kg, linear regression coefficients were estimated based on the residuals (including the mean) of these traits obtained using model [1] (Figure 2.1a-d). The association between TNE and FCR was very strong ($R^2 = 0.80$ and $RMSE = 0.24$) during the entire growing period (Figure 2.1a), whereby a decrease in FCR of 100 g lower feed intake per 1 kg weight gain is associated with a reduction in TNE of 173 g per pig, which corresponds to 709 g reduction in TNE per phenotypic standard deviation of FCR. The association between TNE and ADG was substantially lower ($R^2 = 0.22$ and $RMSE = 0.48$) and the linear regression indicated that with 100 g/d faster growth, pigs were on average excreting 310 g less nitrogen over the entire growing period (Figure 2.1b). This indicates that an increase in ADG by one phenotypic standard deviation reduced TNE by 307 g. A slightly greater coefficient of determination ($R^2 = 0.27$ and $RMSE = 0.46$) was obtained between TNE and DAYS from 60 to 140 kg compared to that between TNE and ADG, whereby a decrease by 1 day was associated with a reduction in TNE by 25 g (data not shown). Thus, a reduction in DAYS by one phenotypic standard deviation resulted in 350 g decrease in TNE. The association between TNE and ALD:APD ($R^2 = 0.12$ and $RMSE = 0.50$) was slightly lower, whereby a reduction of ALD:APD by 0.1 is associated with a reduction in TNE by 96 g over the entire growing period (Figure 2.1c). In terms of phenotypic standard deviation, reducing ALD:APD by one standard deviation resulted in 211 g reduction in TNE. The lowest association ($R^2 = 0.05$ and $RMSE = 0.52$) was found between TNE and ADNI, whereby a reduction of ADNI by 10 g/d is associated with a reduction in TNE by 179 g over the entire growing period (Figure 2.1d). This showed that reducing ADNI by one phenotypic standard deviation reduced TNE by 149 g.

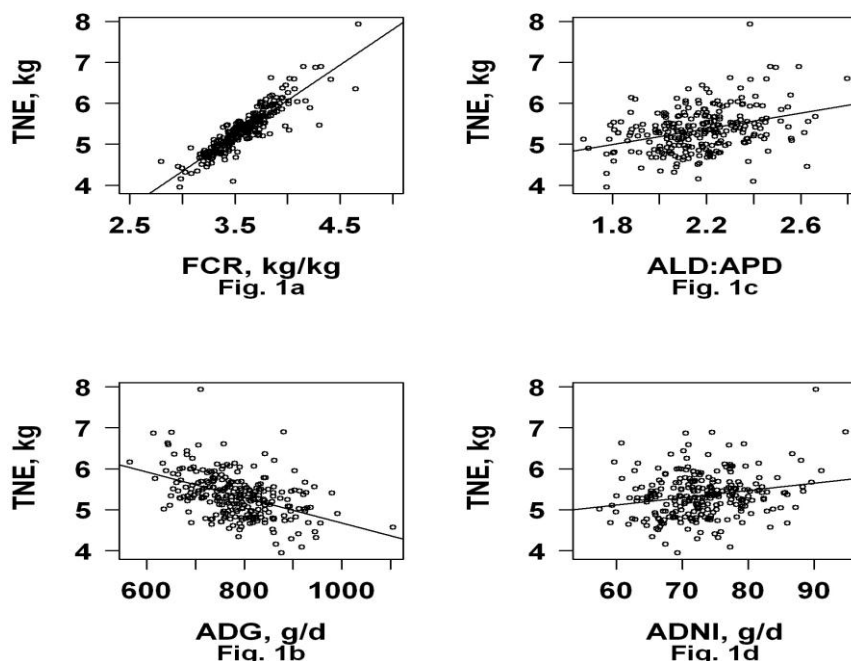


Figure 2.1a-d. Associations between total nitrogen excretion (TNE) and 1a) feed conversion ratio (FCR), 1b) ADG, 1c) lipid to protein gain ratio (ALD:APD), and 1d) average daily nitrogen intake (ADNI) during the entire growing period from 60 to 140 kg.

Differentiations in Nitrogen Excretion and Production Traits

The results of the GLM analysis indicate that gender, housing type and batch significantly influenced ADNE, NEWG and TNE ($P < 0.05$), but the degree and direction of the influences differed between growth stages. Pigs were born on two different birth farms, which significantly affected ADNE ($P < 0.05$) but showed no effect on NEWG and TNE. To determine the changes in nitrogen excretion and production traits over different stages of growth and during the entire growing period, least squares means of the effects gender, housing type and *RYR1* genotypes at different stages of growth were estimated for nitrogen excretion and production traits (Tables 2.2 and 2.3).

The analysis revealed that ADNE, NEWG and TNE were significantly less in gilts than barrows from 60 to 140 kg. This coincides with significantly lower FCR and less

2 Nitrogen excretion during growth

ADG, APD, ALD and ALD:APD in gilts than barrows. Throughout the growing period the differences between genders in these production traits declined, and no significant differences were found at the last growth stage (120 to 140 kg). From 120 to 140 kg, the gender differences were significant for ADNE and TNE but not for NEWG.

Nitrogen excretion per weight gain and TNE were significantly lower ($P < 0.01$) in single-housed pigs during the entire growing period and for all growth stages except from 60 to 90 kg. For single-housed pigs, lower NEWG and TNE were generally associated with lower FCR, greater ADG and APD during the entire growing period and different stages of growth except for growth from 60 to 90 kg, when ADG was greater for group-housed pigs ($P < 0.05$).

The differences between *RYR1* genotypes indicated that pigs with genotype *NN* (homozygous normal) showed the lowest NEWG and FCR of all genotypes and also had lower TNE compared to *Nn* (heterozygous carrier) genotype during growth from 60 to 140 kg. Within stages of growth, the *NN* genotype compared to *Nn* had consistently lower TNE and FCR in all stages of growth except for 60 to 90 kg.

For NESM, the least squares means were only available for 60 to 140 kg period. Gender, batch, birth farm and *RYR1* genotype influenced ($P < 0.01$) NESM. Housing type did not have a significant effect on NESM. Gilts excrete significantly ($P < 0.001$) less NESM than barrows (0.18 ± 0.003 and 0.20 ± 0.003 , respectively). The least squares mean of NESM for the *RYR1* genotypes showed that all three genotypes differed ($P < 0.05$), with the *Nn* genotype being highest and the *NN* genotype being lowest.

2 Nitrogen excretion during growth

Table 2.2 Least squares means and standard errors of nitrogen excretion traits, nitrogen intake, and energy intake for the effects of gender, housing type and *ryanodine receptor 1* (*RYR1*) genotype at different stages of growth and during the entire growing period

Growth period	Effect	Level	Trait ¹									
			ADNE, g/d		NEWG, g/kg		TNE, kg/pig		ADNI, g/d		ADEI, MJ/d	
60-90 kg	Gender	Barrow	47.52 ^a	± 0.72	58.8 ^a	± 1.18	1.76 ^a	± 0.04	69.00 ^a	± 0.89	34.97 ^a	± 0.45
		Gilt	42.01 ^b	± 0.72	54.3 ^b	± 1.17	1.64 ^b	± 0.04	62.63 ^b	± 0.89	31.72 ^b	± 0.45
	Housing type	Single	43.49 ^a	± 1.13	57.9 ^a	± 1.84	1.73 ^a	± 0.06	63.62 ^a	± 1.40	32.20 ^a	± 0.71
		Grouped	46.04 ^a	± 0.58	55.2 ^a	± 0.96	1.67 ^b	± 0.03	68.01 ^b	± 0.73	34.49 ^b	± 0.37
		<i>NN</i>	43.59 ^a	± 1.24	56.8 ^a	± 2.03	1.70 ^{a,b}	± 0.06	64.38 ^a	± 1.54	32.60 ^a	± 0.78
	<i>RYR1</i>	<i>Nn</i>	46.22 ^b	± 0.66	57.7 ^a	± 1.08	1.75 ^a	± 0.03	67.19 ^a	± 0.82	34.07 ^a	± 0.42
		<i>nn</i>	44.49 ^a	± 0.74	55.2 ^a	± 1.21	1.66 ^b	± 0.04	65.87 ^a	± 0.92	33.37 ^a	± 0.47
90-120 kg	Gender	Barrow	57.79 ^a	± 0.75	71.0 ^a	± 1.38	2.04 ^a	± 0.04	79.08 ^a	± 0.93	40.11 ^a	± 0.47
		Gilt	52.45 ^b	± 0.75	66.4 ^b	± 1.40	1.92 ^b	± 0.04	73.14 ^b	± 0.94	37.11 ^b	± 0.48
	Housing type	Single	56.07 ^a	± 1.19	64.9 ^a	± 2.19	1.90 ^a	± 0.06	78.40 ^a	± 1.47	39.76 ^a	± 0.75
		Grouped	54.17 ^a	± 0.61	72.4 ^b	± 1.13	2.06 ^b	± 0.03	73.83 ^b	± 0.76	37.46 ^b	± 0.39
		<i>NN</i>	54.28 ^a	± 1.29	65.4 ^a	± 2.38	1.88 ^a	± 0.06	75.88 ^a	± 1.60	38.48 ^a	± 0.81
	<i>RYR1</i>	<i>Nn</i>	56.09 ^a	± 0.70	70.9 ^b	± 1.29	2.05 ^b	± 0.03	76.73 ^a	± 0.87	38.95 ^a	± 0.44
		<i>nn</i>	54.99 ^a	± 0.76	69.7 ^{a,b}	± 1.40	2.00 ^{a,b}	± 0.04	75.73 ^a	± 0.94	38.41 ^a	± 0.48
120-140 kg	Gender	Barrow	58.38 ^a	± 0.94	78.8 ^a	± 1.97	1.48 ^a	± 0.03	78.26 ^a	± 1.21	39.72 ^a	± 0.62
		Gilt	56.48 ^b	± 0.92	75.1 ^a	± 1.92	1.39 ^b	± 0.03	76.61 ^a	± 1.18	38.89 ^a	± 0.60
	Housing type	Single	57.22 ^a	± 1.46	71.2 ^a	± 3.07	1.31 ^a	± 0.05	78.76 ^a	± 1.89	39.98 ^a	± 0.96
		Grouped	57.64 ^a	± 0.75	82.6 ^b	± 1.58	1.56 ^b	± 0.03	76.11 ^a	± 0.97	38.63 ^a	± 0.49
		<i>NN</i>	57.77 ^a	± 1.60	72.5 ^a	± 3.36	1.34 ^a	± 0.06	79.20 ^a	± 2.07	40.20 ^a	± 1.05
	<i>RYR1</i>	<i>Nn</i>	57.55 ^a	± 0.86	78.6 ^{a,b}	± 1.80	1.48 ^b	± 0.03	77.12 ^a	± 1.11	39.14 ^a	± 0.56
		<i>nn</i>	56.96 ^a	± 0.94	79.6 ^b	± 1.97	1.49 ^b	± 0.03	75.99 ^a	± 1.22	38.57 ^a	± 0.62

2 Nitrogen excretion during growth

Table 2.2 Continued

Growth period	Effect	Level	Trait ¹									
			ADNE, g/d		NEWG, g/kg		TNE, kg/pig		ADNI, g/d		ADEI, MJ/d	
60-140 kg	Gender	Barrow	55.08 ^a	± 0.68	69.5 ^a	± 0.95	5.38 ^a	± 0.07	75.97 ^a	± 0.85	38.59 ^a	± 0.43
		Gilt	50.09 ^b	± 0.67	65.1 ^b	± 0.94	5.08 ^b	± 0.07	70.30 ^b	± 0.85	35.72 ^b	± 0.43
	Housing type	Single	52.30 ^a	± 1.06	64.7 ^a	± 1.49	5.08 ^a	± 0.11	73.56 ^a	± 1.33	37.39 ^a	± 0.68
		Grouped	52.87 ^a	± 0.54	69.9 ^b	± 0.76	5.38 ^b	± 0.06	72.71 ^a	± 0.68	36.92 ^a	± 0.35
	RYR1	NN	52.37 ^a	± 1.16	64.6 ^a	± 1.64	5.08 ^a	± 0.12	73.46 ^a	± 1.52	37.33 ^a	± 0.77
		Nn	53.23 ^a	± 0.61	69.1 ^b	± 0.86	5.36 ^b	± 0.06	73.63 ^a	± 0.76	37.40 ^a	± 0.38
		nn	52.15 ^a	± 0.69	68.4 ^b	± 0.97	5.24 ^{a,b}	± 0.07	72.32 ^a	± 0.86	36.73 ^a	± 0.44

^{a,b} Within each effect and trait, means without a common superscripts differ ($P < 0.05$)

¹ ADNE = average daily nitrogen excretion; NEWG = nitrogen excretion per weight gain; TNE = total nitrogen excretion; ADNI = average daily nitrogen intake; ADEI = average daily energy intake.

2 Nitrogen excretion during growth

Table 2.3 Least squares means and standard errors of production traits for the effects of gender, housing type and ryanodine receptor 1 (*RYR1*) genotype at different stages of growth and during the entire growing period.

Growth period	Effect	Level	Trait ¹											
			FCR, kg/kg		ADG, g/d		APD, g/d		ALD, g/d		ALD:APD		DAYS, d	
60-90 kg	Gender	Barrow	3.11 ^a	± 0.04	832 ^a	± 14	134 ^a	± 2	279 ^a	± 6	2.08 ^a	± 0.03	38 ^a	± 1
		Gilt	2.94 ^b	± 0.04	794 ^b	± 14	128 ^b	± 2	248 ^b	± 6	1.94 ^b	± 0.03	39 ^a	± 1
	Housing type	Single	3.08 ^a	± 0.07	776 ^a	± 22	125 ^a	± 4	252 ^a	± 9	2.03 ^a	± 0.04	40 ^a	± 1
		Grouped	2.98 ^a	± 0.04	850 ^b	± 11	137 ^b	± 2	275 ^b	± 5	2.00 ^a	± 0.02	37 ^b	± 1
	RYR1	NN	3.04 ^a	± 0.07	801 ^a	± 24	129 ^a	± 4	258 ^a	± 10	2.01 ^a	± 0.04	39 ^a	± 1
		Nn	3.07 ^a	± 0.04	812 ^a	± 13	131 ^a	± 2	265 ^a	± 5	2.02 ^a	± 0.02	38 ^a	± 1
		nn	2.97 ^a	± 0.04	826 ^a	± 14	133 ^a	± 2	267 ^a	± 6	2.01 ^a	± 0.03	38 ^a	± 1
90-120 kg	Gender	Barrow	3.66 ^a	± 0.05	829 ^a	± 14	133 ^a	± 2	303 ^a	± 7	2.30 ^a	± 0.03	36 ^a	± 1
		Gilt	3.49 ^b	± 0.05	805 ^a	± 14	129 ^a	± 2	272 ^b	± 7	2.10 ^b	± 0.04	37 ^a	± 1
	Housing type	Single	3.43 ^a	± 0.08	870 ^a	± 22	139 ^a	± 3	313 ^a	± 11	2.28 ^a	± 0.06	34 ^a	± 1
		Grouped	3.72 ^b	± 0.04	763 ^b	± 11	123 ^b	± 2	263 ^b	± 5	2.12 ^b	± 0.03	39 ^b	± 1
	RYR1	NN	3.45 ^a	± 0.09	838 ^a	± 24	135 ^a	± 4	286 ^a	± 11	2.12 ^a	± 0.06	35 ^a	± 1
		Nn	3.66 ^b	± 0.05	807 ^a	± 13	129 ^a	± 2	293 ^a	± 6	2.27 ^b	± 0.03	37 ^a	± 1
		nn	3.61 ^{a,b}	± 0.05	805 ^a	± 14	129 ^a	± 2	284 ^a	± 7	2.20 ^{a,b}	± 0.04	37 ^a	± 1
120-140 kg	Gender	Barrow	3.95 ^a	± 0.07	775 ^a	± 19	124 ^a	± 3	276 ^a	± 11	2.22 ^a	± 0.07	26 ^a	± 1
		Gilt	3.81 ^a	± 0.07	785 ^a	± 18	126 ^a	± 3	278 ^a	± 11	2.21 ^a	± 0.07	25 ^a	± 1
	Housing type	Single	3.67 ^a	± 0.12	837 ^a	± 29	135 ^a	± 5	279 ^a	± 17	2.07 ^a	± 0.11	24 ^a	± 1
		Grouped	4.10 ^b	± 0.06	723 ^b	± 15	115 ^b	± 2	275 ^a	± 9	2.36 ^b	± 0.05	28 ^b	± 1
	RYR1	NN	3.72 ^a	± 0.13	836 ^a	± 32	134 ^a	± 5	300 ^a	± 19	2.22 ^a	± 0.12	24 ^a	± 1
		Nn	3.95 ^b	± 0.07	763 ^b	± 17	122 ^b	± 3	273 ^{a,b}	± 10	2.24 ^a	± 0.06	26 ^b	± 1
		nn	3.99 ^b	± 0.07	741 ^b	± 19	119 ^b	± 3	258 ^b	± 11	2.18 ^a	± 0.07	27 ^b	± 1

2 Nitrogen excretion during growth

Table 2.3 Continued

Growth period	Effect	Level	Trait ¹											
			FCR, kg/kg		ADG, g/d		APD, g/d		ALD, g/d		ALD:APD		DAYS, d	
60-140 kg	Gender	Barrow	3.58 ^a	± 0.04	817 ^a	± 11	131 ^a	± 2	289 ^a	± 5	2.21 ^a	± 0.02	99 ^a	± 2
		Gilt	3.40 ^b	± 0.04	791 ^b	± 11	127 ^b	± 2	264 ^b	± 5	2.08 ^b	± 0.02	102 ^b	± 2
	Housing type	Single	3.40 ^a	± 0.06	826 ^a	± 17	133 ^a	± 3	283 ^a	± 8	2.13 ^a	± 0.04	99 ^a	± 2
		Grouped	3.58 ^a	± 0.03	782 ^b	± 9	125 ^b	± 1	270 ^a	± 4	2.16 ^a	± 0.02	103 ^a	± 1
	<i>RYR1</i>	<i>NN</i>	3.37 ^a	± 0.06	832 ^a	± 19	134 ^a	± 3	282 ^a	± 9	2.11 ^a	± 0.04	98 ^a	± 3
		<i>Nn</i>	3.56 ^b	± 0.03	794 ^a	± 9	127 ^b	± 2	278 ^a	± 4	2.18 ^a	± 0.02	102 ^a	± 1
		<i>nn</i>	3.54 ^b	± 0.04	787 ^a	± 11	126 ^b	± 2	270 ^a	± 5	2.14 ^a	± 0.03	102 ^a	± 2

^{a,b} Within each effect and trait, means without a common superscripts differ ($P < 0.05$)

¹ FCR = feed conversion ratio; APD = average daily protein deposition; ALD = average daily lipid deposition; ALD:APD = lipid to protein gain ratio; DAYS = length of growing period.

Residual Correlations

To provide a better understanding of underlying causes, or means for improvements in nitrogen excretion traits, the residual correlations between nitrogen excretion and various production traits were estimated after adjustment for the effects described in model [1] (Tables 2.4 and 2.5).

The results of the multivariate analysis of variance indicate a large positive residual correlation between NEWG and TNE during the entire growing period and in all the stages of growth. In contrast, ADNE was only lowly to moderately correlated with NEWG and TNE, which indicates that ADNE cannot be used as a good predictor for TNE and NEWG. The correlations between ADNE and ADNI, or ADEI were consistently positive and large. However, NEWG and TNE were lowly correlated with intake traits ADNI and ADEI. In contrast, NEWG and TNE showed large positive correlation with FCR during the entire growing period and in all stages of growth.

Average daily nitrogen excretion was positive and moderately correlated with the production traits ADG, APD, ALD and ALD:APD in all the stages of growth, except for correlations with ALD:APD during growth from 90 to 120 kg and 120 to 140 kg, which were small. In contrast to ADNE, NEWG and TNE showed moderate negative correlations with the growth traits ADG, APD and ALD in all stages of growth. The correlations were strongest for NEWG and TNE with ADG and APD from 120 to 140 kg. For NESM (data not shown), large positive correlations with NEWG, TNE, and FCR ($r = 0.79, 0.86$, and 0.79 , respectively) were observed during the entire growing period. The correlations of fat to saleable meat ratio with nitrogen excretion and production traits were low to moderate (data not shown). The correlation of fat to saleable meat ratio with ADNE was moderate ($r = 0.50$; $P < 0.001$), and with NEWG and TNE were low (0.21 and 0.19 , respectively; $P < 0.05$). The correlation of fat to saleable meat ratio with NESM was the greatest between nitrogen excretion traits ($r = 0.58$; $P < 0.001$).

2 Nitrogen excretion during growth

Table 2.4 Correlations between the residuals of nitrogen excretion and production traits at growth from 60 to 90 kg (above diagonal) and 90 to 120 kg (below diagonal).

Trait ¹	ADNE	NEWG	TNE	ADEI	ADNI	FCR	ADG	APD	ALD	ALD:APD	DAYS
ADNE		0.49 ***	0.51 ***	0.92 ***	0.92 ***	0.48 ***	0.30 ***	0.28 ***	0.42 ***	0.34 ***	-0.30 ***
NEWG	0.39 ***		0.98 ***	0.12 *	0.12 *	0.99 ***	-0.66 ***	-0.66 ***	-0.41 ***	0.17 **	0.66 ***
TNE	0.39 ***	0.96 ***		0.15 *	0.15 *	0.98 ***	-0.63 ***	-0.64 ***	-0.39 ***	0.20 **	0.64 ***
ADEI	0.93 ***	0.05	0.06		1.00 ***	0.12 *	0.64 ***	0.63 ***	0.67 ***	0.29 **	-0.62 ***
ADNI	0.93 ***	0.05	0.06	1.00 ***		0.12 *	0.64 ***	0.63 ***	0.67 ***	0.29 **	-0.62 ***
FCR	0.38 ***	0.99 ***	0.96 ***	0.05	0.05		-0.66 ***	-0.67 ***	-0.42 ***	0.15 *	0.66 ***
ADG	0.35 ***	-0.69 ***	-0.68 ***	0.66 ***	0.66 ***	-0.69 ***		1.00 ***	0.83 ***	0.08	-0.94 ***
APD	0.34 ***	-0.69 ***	-0.68 ***	0.65 ***	0.65 ***	-0.70 ***	1.00 ***		0.81 ***	0.05	-0.94 ***
ALD	0.39 ***	-0.48 ***	-0.47 ***	0.60 ***	0.60 ***	-0.49 ***	0.78 ***	0.75 ***		0.61 ***	-0.78 ***
ALD:APD	0.21 **	0.005	-0.001	0.20 **	0.20 **	-0.01	0.12 *	0.07	0.70 ***		-0.08
DAYS	-0.33 ***	0.69 ***	0.71 ***	-0.63 ***	-0.63 ***	0.69 ***	-0.95 ***	-0.95 ***	-0.77 ***	-0.15 *	

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

¹ADNE = average daily nitrogen excretion; NEWG = nitrogen excretion per weight gain; TNE = total nitrogen excretion; ADEI = average daily energy intake; ADNI = average daily nitrogen intake; FCR = feed conversion ratio; APD = average daily protein deposition; ALD = average daily lipid deposition; ALD:APD = lipid to protein gain ratio; DAYS = length of growing period.

2 Nitrogen excretion during growth

Table 2.5 Correlations between the residuals of nitrogen excretion and production traits at growth from 120 to 140 kg (above diagonal) and during the entire growing period from 60 to 140 kg (below diagonal)

Trait ¹	ADNE	NEWG	TNE	ADEI	ADNI	FCR	ADG	APD	ALD	ALD:APD	DAYS
ADNE		0.21 ***	0.24 ***	0.93 ***	0.93 ***	0.21 ***	0.42 ***	0.41 ***	0.41 ***	0.20 *	-0.43 ***
NEWG	0.37 ***		0.94 ***	-0.13 *	-0.13 *	0.99 ***	-0.74 ***	-0.75 ***	-0.47 ***	-0.01	0.72 ***
TNE	0.41 ***	0.91 ***		-0.10	-0.10	0.94 ***	-0.70 ***	-0.71 ***	-0.44 ***	-0.01	0.74 ***
ADEI	0.96 ***	0.12 *	0.17 **		1.00 ***	-0.13 *	0.72 ***	0.71 ***	0.58 ***	0.18 **	-0.68 ***
ADNI	0.96 ***	0.12 *	0.17 **	1.00 ***		-0.13 *	0.72 ***	0.71 ***	0.58 ***	0.18 **	-0.68 ***
FCR	0.36 ***	0.99 ***	0.91 ***	0.11	0.11		-0.75 ***	-0.75 ***	-0.48 ***	-0.03	0.72 ***
ADG	0.54 ***	-0.53 ***	-0.48 ***	0.75 ***	0.75 ***	-0.54 ***		1.00 ***	0.72 ***	0.13 *	-0.90 ***
APD	0.52 ***	-0.55 ***	-0.49 ***	0.73 ***	0.73 ***	-0.55 ***	1.00 ***		0.67 ***	0.06	-0.90 ***
ALD	0.64 ***	-0.25 ***	-0.17 *	0.75 ***	0.75 ***	-0.26 ***	0.80 ***	0.77 ***		0.76 ***	-0.65 ***
ALD:APD	0.40 ***	0.25 ***	0.31 ***	0.33 ***	0.33 ***	0.23 ***	0.09	0.05	0.67 ***		-0.13 *
DAYS	-0.53 ***	0.51 ***	0.54 ***	-0.71 ***	-0.71 ***	0.51 ***	-0.92 ***	-0.92 ***	-0.73 ***	-0.08	

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

¹ ADNE = average daily nitrogen excretion; NEWG = nitrogen excretion per weight gain; TNE = total nitrogen excretion; ADEI = average daily energy intake; ADNI = average daily nitrogen intake; FCR = feed conversion ratio; APD = average daily protein deposition; ALD = average daily lipid deposition; ALD:APD = lipid to protein gain ratio; DAYS = length of growing period.

2.4 Discussion

Nitrogen Excretion, Retention and Intake Change during the growing period

This study revealed that nitrogen excretion is changing throughout the entire growing period. Greatest nitrogen efficiency (32%) (nitrogen retention/nitrogen intake), lowest nitrogen excretion and greatest retention were found during early growth from 60 to 90 kg. Nitrogen efficiency gradually decreased with increasing growth stages. The lowest nitrogen efficiency (25%) was during growth from 120 to 140 kg, which was associated with lowest nitrogen retention and greatest nitrogen intake in comparison with corresponding traits in other stages of growth.

Dourmad et al. (2008) suggested that when animal growth rate is low, the relative maintenance contribution is high, which results in more feed required per kg product and consequently higher emissions. Our research indicates that this effect is more pronounced at the later stages of growth because of increased maintenance requirement with decreasing growth rate. According to this analysis, the best strategy to improve nitrogen efficiency would be to improve feed efficiency, as its coefficient of determination to predict TNE was 0.80. In comparison, ADG and ALD:APD showed substantially lower coefficient of determination of 0.22 and 0.12, respectively.

Influencing Factors of Nitrogen Efficiency

The significant effects associated with ADNE, NEWG and TNE in this study were gender, housing type, RYR1 genotype and batch. The batch effect reflects environmental changes for successive groups of animals tested within the testing station and were reported in the literature (Bermejo et al., 2003; Schulze et al., 2003; Otto et al., 2007) to significantly influence production traits. The results of the present study indicate that this is also the case for nitrogen excretion traits.

In the current study, barrows had consistently greater nitrogen excretion than gilts, which agrees with the results of Crocker and Robison (2002). Over the entire growing period, greater nitrogen excretion in barrows was associated with greater FCR (5%), ADG (3%) and ALD:APD (6%). A greater FCR of barrows has been reported in several studies (Kanis, 1988; Latorre et al., 2003). Crocker and Robison (2002) concluded that faster growth of barrows results in higher turnover of nutrients, and thus higher amount of all nutrient excretion per kg of pig weight. Our study showed an association between nitrogen excretion with ALD:APD and fat to saleable meat ratio, because the amount of feed energy (and thus ADFI) required for producing fat tissue is much greater than that for lean tissue. In a simulation study, Morel and

Wood (2005) showed that nitrogen excretion was less in leaner pigs, which agrees with results of our study. From the results of the present study, it can be concluded that different pig genders have different quantity of nitrogen in their slurry, and improvement in FCR of barrows by using different strategies of feeding and husbandry (e.g. adjusting diet according to genders) is expected to result in a reduction of nitrogen excretion from these pigs. Recently, due to the issues related to surgical castration of male pigs, the EU has planned to stop castration of male piglets by 2018 (European Commission, 2010). This will result in growing entire males, which are expected to have better feed efficiency than barrows and consequently result in less nitrogen excretion. In addition, entire males will likely be slaughtered at lower weight to avoid boar taint, which will further reduce nitrogen excretion.

Individually housed pigs had significantly lower TNE which was associated with lower FCR (5%), ALD:APD (1.4%) and significantly greater ADG (5%) compared to group-housed pigs during the entire growing period. This indicates a higher efficiency of feed energy conversion among single-housed pigs, likely due to lower activity and absence of competition for food, in particular at later stage of growth. In contrast, for the early stage of growth from 60 to 90 kg, TNE of single-housed pigs was 3.6% greater than that of group-housed pigs, which was associated with lower ADG and a longer growing period. This could be due to a favourable competition i.e. peer pressure to eat among group-housed pigs which resulted in significantly greater ADEI as well as ADG and lower nitrogen excretion in this stage. In later growth stages, group-housed pigs showed 8% and 19% greater TNE during growth from 90 to 120 kg and 120 to 140 kg, respectively, which may indicate that in these stages of growth the larger activity, competition and maintenance resulted in lower efficiency. Therefore, strategies in feeding, husbandry and genetics to reduce TNE are expected to be most efficient under group-housed conditions at later stages of growth, in particular when pigs are grown to a heavy finishing weight.

The homozygotes for the *n*-allele at the *RYS1* gene (*nn*-stress susceptible) are known to have a greater risk of the malignant hyperthermia syndrome and reduced meat quality such as pale, soft, exudative meat and low water holding capacity (Zhang et al., 1992). In the present study, the *nn* and *Nn* genotypes showed significantly greater NEWG than *NN* genotypes and the *Nn* genotypes also showed greater TNE than *NN* genotypes over the entire growing period. This suggests that stress susceptibility has an unfavourable influence on nitrogen excretion, in

particular if animals are grown to a large finishing weight, because the difference between *NN* genotypes with others in TNE were particularly large during growth from 120 to 140 kg. Especially the *Nn* and *nn* genotypes showed a greater NEWG compared to *NN* genotype during the entire growing period. This was associated with a greater FCR and lesser APD. Leach et al. (1996) using pigs grown from 40 kg BW and slaughtered at 110, 125 and 140 kg BW reported that *Nn* genotype had better feed efficiency than *NN* genotypes. Similar results were not obtained in our study, despite growing pigs to similar BW. Furthermore, Zhang et al. (1992) using Pietrain breed grown from 24.5 kg to 100 kg of BW found that *Nn* genotypes have higher weight gain, better feed efficiency and leaner body composition than *nn* genotypes. The disagreement between those studies and our study may be due to different crosses of breeds, resulting in different genetic effects depending on the *RYR1* genotype.

Relationship between Nitrogen Emission and Production Traits

The current study showed that there are very close correlations ($r > 0.90$) among FCR, TNE and NEWG. Therefore, reduction of nitrogen excretion by improvement of FCR using strategies of feeding, optimisation of diets, husbandry, genetics, etc. is the primary choice to reduce its environmental impact. According to our study, the improvement in FCR by one phenotypic standard deviation would reduce TNE by 709 g over the entire growing period. Moderate negative correlations between ADG or APD and TNE or NEWG indicate that improvements of these growth traits are a secondary choice to reduce nitrogen loss and thus the environmental impact of pig production. An increase in ADG by one phenotypic standard deviation would reduce TNE by 307 g over the entire growing period. The ALD and ALD:APD showed only low correlations to TNE and NEWG over the entire growing period and their improvement may be the third choice of indirectly mitigating nitrogen emissions. Over the entire growing period, an improvement of ALD:APD by one phenotypic standard deviation would reduce TNE by 211 g. Of all previously discussed associations, ADNI showed the lowest correlations with TNE and NEWG, so that a decrease in ADNI by one phenotypic standard deviation reduced TNE by 149 g during the entire growing period. The reason for the stronger association of TNE with FCR may be that the regressions of TNE on ADG and TNE on ADNI are of opposite directions so that only the ratio of ADNI and ADG (similar to FCR) resulted in the best association with TNE. Generally, this study suggested that a substantial reduction in nitrogen excretion can be obtained by improving production traits.

Estimates for the effect of changes in production traits on environmental pollution of pig production have been derived in several studies. Jones et al. (2008) estimated 0.8% annual reduction in global warming potential of methane and nitrogen emissions as a result of genetic trends for growth rate (+8.5, g/d per year), FCR (-0.02, kg/kg per year) and litter size (0.16, pigs/litter per year) in the UK pig sector from 1988-2007. They found that genetic increase in ADG is responsible for 70% reduction in ammonia and genetic improvement in FCR is responsible for 70% reduction of nitrous oxide. Furthermore, they concluded that this rate of genetic improvement will continue over the next few decades and may increase due to the use of molecular genetic tools. Moreover, the results of Jones et al. (2008) may be underestimated as the genetic trend of lean content (+ 0.5 %, lean meat per year) has not been taken into account (P.W. Knap, PIC, Schleswig, Germany, personal communication). In the current study, a 10% improvement of FCR, increase of ADG and decrease of ADNI in their corresponding means resulted in 12%, 5% and 2% reduction in TNE, respectively, during the entire growing period. Fernandez et al. (1999) in national study in Denmark on growing pigs grown from 30 to 100 kg estimated a reduction in nitrogen emission of 13%, 13% and 15% from 10% improvement of the mean of FCR, growth rate and dietary nitrogen, respectively. The improvement of FCR resulted in similar mitigation of nitrogen emission with our study, whereas the deviations between studies for the other traits may likely be due to differences in growth period, population means, breeds, etc.

In the current study, 28% nitrogen retention (ADNR as percentage of ADNI) and 72% nitrogen excretion (ADNE as percentage of ADNI) were achieved on average during the entire growth period from 60 to 140 kg, with greatest nitrogen retention of 32% and lowest nitrogen excretion of 68%, achieved during growth from 60 to 90 kg. Dourmad et al. (1999) reported average nitrogen retention of 33% and nitrogen excretion of 67% during growth from 28 to 108 kg. The slightly greater nitrogen excretion in the current study was mainly due to a greater mean of FCR, which is partly associated with a greater length of the growing period at a substantially greater slaughter weight, and differences in breeds. These results indicate the relatively low efficiency of nutrient utilization in pigs. Oenema and Tamminga (2005) reported that nitrogen efficiency is greater in poultry and pigs than in dairy cattle, beef cattle and sheep. The current study shows that there are large coefficients of variation for TNE, NEWG and ADNE; therefore improvement of these traits within populations has great potential. Dourmad et al. (2008) estimated, based on results of French farms, that greenhouse gas emissions will be reduced or increased by about 7% each in the 30% best- and 30% worst-performing

farms, respectively, in comparison to average-performing farms. P.W. Knap (PIC, Schleswig, Germany, personal communication) modelled nitrogen retention and excretion for six pig sire lines grown from 20 to 120 kg BW on nucleus level during the years from 1969-2004. The analysis showed that the 2004 genotype had 19% greater nitrogen retention or, alternatively, a 20% lower nitrogen excretion than the 1969 genotype. This indicates that genetic improvement of body composition in these years resulted in a substantial reduction of nitrogen excretion per pig produced. In summary, all practices, including genetic, nutrition and management, that result in the improvement of feed efficiency are potential strategies to reduce greenhouse gas emissions per unit of product.

Future Research

A future research area that could accelerate the reduction in nitrogen emission due to breeding may be based on the genomic background of nitrogen losses i.e. by identifying quantitative trait loci and their genomic associations with production traits. In particular the genetic association of FCR with nitrogen excretion may be used to efficiently reduce nitrogen due to genetic selection. Jones et al. (2008) proposed different methods for increasing the production efficiency such as increasing the digestion efficiency and an improvement in post-absorptive nutrient utilization. Further research on genetic variation of maintenance requirements and efficiency of utilization of energy and protein to increase the production efficiency and reduce the environmental pollution of production is necessary. In addition, selecting for low residual feed intake has been discussed as an alternative measure to improve feed efficiency (Kennedy et al., 1993) and to reduce the environmental pollution of pig production. Variation in residual feed intake captures differences in efficiency of digestion, efficiency of metabolic utilization of feed energy, maintenance requirements and tissue turnover rates, among others (Dekkers and Gilbert, 2010). Furthermore, reduction in nitrogen emission of pigs requires knowledge about the amino acid availability in feedstuffs, and the changes in amino acid requirements according to growth stage or physiological state. These may be approachable by using biological growth models such as the mechanistic dynamic model of Knap (2000) to optimally match nutrients to the animal's requirements and to consequently reduce the environmental pollution of pig production.

References

- Bermejo, J. L., R. Roehe, V. Schulze, G. Rave, H. Looft, and E. Kalm. 2003. Random regression to model genetically the longitudinal data of daily feed intake in growing pigs. *Livest. Prod. Sci.* 82:189-199.
- Crocker, A. W. and O. W. Robison. 2002. Genetic and nutritional effects on swine excreta. *J. Anim Sci.* 80:2809-2816.
- Dekkers, J. C. M. and H. Gilbert. 2010. Genetic and Biological Aspect of Residual Feed Intake in Pigs. No. 287 in *Proc. 9th World Congr. Genet. Appl. Livest. Prod.*, Leipzig, Germany.
- Dourmad, J. Y., N. Guingand, P. Latimier, and B. Seve. 1999. Nitrogen and phosphorus consumption, utilisation and losses in pig production: France. *Livest. Prod. Sci.* 58:199-211.
- Dourmad, J. Y., C. Rigolot, and H. van der Werf. 2008. Emission of greenhouse gas, developing management and animal farming systems to assist mitigation. Pages 36-39 in *Proc. Livest. Glob. Clim. Change.*, Hammamet, Tunisia.
- Duthie, C., G. Simm, A. Doeschl-Wilson, E. Kalm, P. W. Knap, and R. Roehe. 2010. Epistatic analysis of carcass characteristics in pigs reveals genomic interactions between quantitative trait loci attributable to additive and dominance genetic effects. *J. Anim Sci.* 88:2219-2234.
- European Commission. 2010. Animal welfare: voluntary end to the surgical castration of piglets by 2018. Accessed Jul. 7, 2011. http://ec.europa.eu/food/animal/welfare/farm/docs/castration_pigs_press_release_en.pdf.
- Fernandez, J. A., H. D. Poulsen, S. Boisen, and H. B. Rom. 1999. Nitrogen and phosphorus consumption, utilisation and losses in pig production: Denmark. *Livest. Prod. Sci.* 58:225-242.
- Jones, H. E., C. C. Warkup, A. Williams, and E. Audsley. 2008. The effect of genetic improvement on emissions from livestock systems. Page 6 in *Proc. 59th EAAP.*, Vilnius, Lithuania.
- Kanis, E. 1988. Food intake capacity in relation to breeding and feeding of growing pigs. PhD Diss. Wageningen Univ., The Netherlands.
- Kennedy, B. W., J. H. J. Vanderwerf, and T. H. E. Meuwissen. 1993. Genetic and Statistical Properties of Residual Feed-Intake. *J. Anim Sci.* 71:3239-3250.
- Knap, P. W. 2000. Stochastic simulation of growth in pigs: relations between body composition and maintenance requirements as mediated through protein turnover and thermoregulation. *Anim. Sci.* 71:11-30.

- Landgraf, S., A. Susenbeth, P. W. Knap, H. Looft, G. S. Plastow, E. Kalm, and R. Roehe. 2006. Allometric association between in vivo estimation of body composition during growth using deuterium dilution technique and chemical analysis of serial slaughtered pigs. *Anim. Sci.* 82:223-231.
- Latorre, M. A., P. Medel, A. Fuentetaja, R. Lazaro, and G. G. Mateos. 2003. Effect of gender, terminal sire line and age at slaughter on performance, carcass characteristics and meat quality of heavy pigs. *Anim. Sci.* 77:33-45.
- Leach, L. M., M. Ellis, D. S. Sutton, F. K. McKeith, and E. R. Wilson. 1996. The growth performance, carcass characteristics, and meat quality of halothane carrier and negative pigs. *J. Anim. Sci.* 74:934-943.
- Mohrmann, M., R. Roehe, A. Susenbeth, U. Baulain, P. W. Knap, H. Looft, G. S. Plastow, and E. Kalm. 2006. Association between body composition of growing pigs determined by magnetic resonance imaging, deuterium dilution technique and chemical analysis. *Meat Sci.* 72:518-531.
- Morel, P. C. H. and G. R. Wood. 2005. Optimisation of Nutrient Use to Maximize Profitability and Minimise Nitrogen Excretion in Pig Meat Production Systems. *Acta Hortic. (ISHS)* 674:269-275.
- Murrells, T., N. R. Passant, G. Thistlethwaite, A. Wagner, Y. Li, T. Bush, J. Norris, P.J. Coleman, C. Walker, R.A. Stewart, I. Tsagatakis, C. Conolly, N. Brophy, and S. Okamura. 2010. UK Emissions of Air Pollutants 1970 to 2008. Accessed Nov.7,2010.http://ukair.defra.gov.uk/reports/cat07/1009030925_2008_Report_final270805.pdf.
- Oenema, O. and S. Tamminga. 2005. Nitrogen in global animal production and management options for improving nitrogen use efficiency. *Sci. China Ser. C: Life Sci.* 48:871-887.
- OJ. 2008. Council Directive 2008/1/EC of the European Parliament and of the Council of 15 January 2008 concerning integrated pollution prevention and control. *Off. J. Eur. Union. OJL* 24. pp. 8-29.
- Otto, G., R. Roehe, H. Looft, L. Thoelking, P. W. Knap, M. F. Rothschild, G. S. Plastow, and E. Kalm. 2007. Associations of DNA markers with meat quality traits in pigs with emphasis on drip loss. *Meat Sci.* 75:185-195.
- Salmi, B., L. Trefan, J. Bloom-Hansen, J. P. Bidanel, A. B. Doeschl-Wilson, and C. Larzul. 2010. Meta-analysis of the effect of the halothane gene on 6 variables of pig meat quality and on carcass leanness. *J. Anim. Sci.* 88:2841-2855.
- Schulze, V., R. Roehe, J. L. Bermejo, H. Looft, and E. Kalm. 2003. The influence of feeding behaviour on feed intake curve parameters and performance traits of station-tested boars. *Livest. Prod. Sci.* 82:105-116.

- Steinfeld, H., P. Gerber, T. Wassenaar, V. Castel, M. Rosales, and C. de Haan. 2006. Livestock's long shadow: environmental issues and options. FAO, Rome, Italy.
- Whittemore, C. T., M. J. Hazzledine, and W. H. Close. 2003. Nutrient Requirement Standards for Pigs. Br. Soc. Anim. Sci., Penicuik, Scotland, UK.
- Zhang, W., D. L. Kuhlers, and W. E. Rempel. 1992. Halothane Gene and Swine Performance. J. Anim Sci. 70:1307-1313.

3

Novel insight into the genomic architecture of feed and nitrogen efficiency measured by residual energy intake and nitrogen excretion in growing pigs

M. Shirali^{1&5}, C. Duthie⁴, A. Doeschl-Wilson², P.W. Knap³, E. Kanis⁵, J.A.M. van Arendonk⁵ and R. Roehe¹

¹Animal and Veterinary Sciences, SRUC, West Mains Road, Edinburgh, EH9 3JG, UK;

²Division of Genetics and Genomics, The Roslin Institute, R(D)SVS, University of Edinburgh, Easter Bush, Midlothian, EH25 9RG, UK; ³PIC International Group, Ratsteich 31, 24837 Schleswig, Germany; ⁴Future Farming Systems Group, SRUC, West Mains Road, Edinburgh, EH9 3JG, UK; and ⁵Animal Breeding and Genomics Centre, Wageningen Institute of Animal Sciences, Wageningen University, P.O. Box 338, 6700 AH Wageningen, The Netherlands.

Abstract

Improvement of feed efficiency in pigs is of great economical and environmental interest and contributes to use limited resources efficiently to feed the world population. Genome scans for feed efficiency traits are of importance to reveal the underlying biological causes and increase the rate of genetic gain. The aim of this study was to determine the genomic architecture of feed efficiency measured by residual energy intake (REI), in association with production, feed conversion ratio (FCR) and nitrogen excretion traits through the identification of quantitative trait loci (QTL) at different stages of growth using a three generation full-sib design population which originated from a cross between Pietrain and a commercial dam line. Six novel QTL for REI were detected explaining 2.7-6.1% of the phenotypic variance in REI. At growth from 60-90 kg body weight (BW), a QTL with a significant dominance effect was identified for REI on SSC14, at a similar location to the QTL for feed intake and nitrogen excretion traits. At growth from 90-120 kg BW, three QTL for REI were detected on SSC2, SSC4 and SSC7 with significant additive, imprinting and additive effects, respectively. These QTL (except for the imprinted QTL) were positionally overlapping with QTL for FCR and nitrogen excretion traits. During final growth (120-140 kg BW), a further QTL for REI was identified on SSC8 with significant additive effect, which overlapped with QTL for nitrogen excretion. During entire analysed growth (60-140 kg BW), a novel additive QTL for REI on SSC4 was observed, with no overlapping with QTL for any other traits considered. The occurrence of only one overlapping QTL of REI with feed intake suggests that only a small proportion of the variance in REI was explained by change in feed intake, whereas four overlapping QTL of REI with those of nitrogen excretion traits suggests that mostly underlying factors of feed utilisation such as metabolism and protein turnover were the reason for change in REI. Different QTL for REI were identified at different growth stages, indicating that different genes are responsible for efficiency in feed utilisation at different stages of growth.

Key words: Feed efficiency, Growth, Nitrogen excretion, Pigs, Quantitative trait loci, Residual energy intake

3.1 Introduction

Improving feed efficiency, in light of high production costs and environmental impact, is one of the main aims in pig breeding, which contributes to the efficient use of limited resources to feed the world population. Genome scans are of great importance for the identification of genomic regions, which are associated with economically important traits (Roehe et al., 2003). Additionally, genome scans can reveal the biological background of important traits. Feed conversion ratio (FCR), traditionally used to determine feed efficiency, is inter-related with growth, body composition and feed intake (Johnson et al., 1999). Residual feed intake has become increasingly of interest as an alternative measurement of feed efficiency, which is phenotypically independent from production (De Haer et al., 1993), and has been shown to improve feed efficiency in experimental selection lines (Gilbert et al., 2007; Cai et al., 2008). Interestingly, among the numerous quantitative trait loci (QTL) mapping studies carried out in pigs, to the best of our knowledge only two studies have performed a genome scan for residual feed intake (Fan et al., 2010; Gilbert et al., 2010) but no study analysed QTL for such trait at different stages of growth. To obtain more accurate estimates of feed efficiency, in the present study, residual energy intake (REI) was calculated from regressing metabolizable energy intake on estimates of protein and lipid deposition as component traits in successive growth stages. Using the same experimental population as the current study, a large number of QTL for production and feed intake traits have been reported (Mohrmann et al., 2006a; Duthie et al., 2008 and 2010), suggesting the potential to detect possible QTL for REI in the current population. Shirali et al. (2012) identified a substantial favourable phenotypic association between FCR and nitrogen excretions. Therefore, the genomic association between REI and nitrogen excretions can be explored to determine possible methods of mitigating the environmental impact of pig production.

The aims of this study were to detect QTL for REI and nitrogen excretion as measures reflecting feed efficiency and environmental impact, at different stages of growth and over the entire analysed growing period, and to determine the genomic architecture of feed efficiency measured by REI in association with growth, feed intake and nitrogen excretion traits in a commercial population originating from a cross of Pietrain and a commercial dam line.

3.2 Materials and Methods

All animal care and handling procedures in the federal testing station were reviewed and approved by the Landwirtschaftskammer Schleswig-Holstein, Rendsburg, Germany.

Design and data

The QTL mapping analyses was based on animals from a three-generation full-sib design population. The founder generation (F_0) consisted of 7 unrelated Pietrain grand-sires and 16 unrelated grand-dams bred from a 3-way cross of Leicoma boars with Landrace $\times$ Large White dams. All grand-sires were heterozygous (Nn) at the *ryanodine receptor 1* (*RYR1*) locus. Of the F_1 generation, 8 boars and 40 sows were selected to develop the F_2 generation, which consisted of 315 pigs from the first two parities of the F_1 sows.

From the F_2 generation, 48 gilts and 46 barrows were single-housed in straw-bedded pens and fed manually, with feed disappearance recorded on a weekly basis. The remaining 117 gilts and 104 barrows were housed in mixed-sexed groups of up to 15 pigs in straw-bedded pens. Animals housed in groups were fed using electronic feeders (ACEMA 48, ACEMO, Pontivy, France), which recorded feed disappearance at each visit. Pigs started the performance test at about 30 kg body weight and were weighed on a weekly basis. For this study, only the testing period from 60 kg onwards was considered because at this stage pigs were entirely adapted to the electronic feeders. Pigs were weighed at target live weights of 60, 90, 120 and 140 kg, where the average live weight (SD) at these target weights were 61 kg (2.58), 91 kg (2.60), 120 kg (2.69) and 140 kg (2.80), respectively. During growth from 60 to 90 and 90 to 140 kg of body weight, pigs were fed *ad libitum* with a diet containing 13.8 MJ of ME/kg, 17% CP and 1.1% lysine and a diet containing 13.4 MJ of ME/kg, 16.5% CP and 1.0% lysine, respectively. The diets consisted of adequate nutrient supplies to permit maximum protein deposition. For a more detailed description of the data see (Landgarf et al., 2006; Mohrmann et al., 2006b).

The deuterium dilution technique was used to determine chemical body composition at each target weight. This technique is an *in vivo* method based on the empty body water content of the pigs. Using this method, the percentage of fat-free substance of pigs was estimated from the empty body water content. Protein and ash content of the empty body were estimated based on the percentage of the fat-free substance. Percentage of lipid content was the deviation of the percentage of fat-free substance from 100%. The accuracy of the deuterium

dilution technique to determine body composition has been verified using magnetic resonance imaging on live animals and chemical analysis of serially slaughtered animals using data of the F_1 population of the present experiment (Landgarf et al., 2006; Mohrmann et al., 2006b). Average daily protein (APD) and lipid deposition (ALD) rates were calculated as the difference between protein or lipid content at the two adjacent target weights divided by the number of days between the target weight measurements. Average daily gain was calculated within each growth stage and for the entire analysed growing period (60 to 140 kg). Backfat thickness (BF) was measured on the cold left carcass side. Average daily feed intake was calculated as the sum of feed disappearance (kg) divided by number of days for each stage of growth and over the growing period. Average daily energy intake (ADEI) was based on ME content of the diet and daily feed intake. The REI was estimated using a regression model for ADEI that included, besides a number of systematic effects, pre-adjusted APD and ALD. The pre-adjustment for APD and ALD was obtained accounting for the same systematic effects as ADEI as described in detail in section statistical analysis. The FCR was calculated as the sum of feed disappearance (kg) divided by body weight gain (kg) in each stage of growth and the entire analysed growing period. Three nitrogen excretion traits were estimated at each stage of growth and during the entire analysed growing period: average daily nitrogen excretion (ADNE), nitrogen excretion per body weight gain (NEWG) and total nitrogen excretion (TNE), as an indication of environmental nitrogen pollution by pigs. The ADNE was estimated as the difference between average daily nitrogen intake and nitrogen retention, NEWG was the ratio between ADNE and ADG and the TNE was calculated as ADNE multiplied by days of growth. More detailed information about the nitrogen excretion traits outlined above is presented in a previous study (Shirali et al., 2012).

Genotypic data

Blood samples (9 ml) were collected from the F_0 , F_1 and F_2 animals by puncture of the vena jugularis and genomic DNA was extracted using the silicagel method following Myakishev et al. (1995). Chromosomes SSC1, SSC2, SSC4, SSC6, SSC7, SSC8, SSC9, SSC10, SSC13 and SSC14 were chosen for genotyping because of their likely associations with lean and fat tissue. All pigs were genotyped for 88 informative microsatellite markers, of which 10, 9, 9, 9, 10, 8, 9, 9, 8 and 7 genomic markers were located on SSC1, SSC2, SSC4, SSC6, SSC7, SSC8, SSC9, SSC10, SSC13 and SSC14, respectively. The position of markers on the genome and their distance from each other, and allele information were obtained using the published USDA linkage map (Rohrer et al., 1996). The average distances between markers were

16.0, 16.5, 16.3, 20.6, 17.3, 18.4, 17.3, 16.0, 18.0 and 17.4 cM and the largest gaps between markers were 27.7, 25.2, 26.5, 28.7, 26.2, 23.1, 21.7, 20.8, 24.0 and 23.6 cM on SSC1, SSC2, SSC4, SSC6, SSC7, SSC8, SSC9, SSC10, SSC13 and SSC14, respectively.

Statistical analysis

The QTL analysis was performed using GridQTL software (Seaton et al., 2006), which adopts a least squares regression method of QTL mapping (Haley et al., 1994), and genomic parent-of-origin (imprinting) effect analysis developed by Knott et al. (1998). In this analysis, the estimate of the additive effect is defined as half of the difference between the effects corresponding to pigs homozygous for alleles from the grandpaternal sire line and pigs homozygous for alleles from the grandmaternal dam line. A positive additive genetic value indicates that the allele originating from the grandpaternal sire line (Pietrain) showed a higher effect than the allele from the grandmaternal dam line and vice versa. The dominance effect is defined as deviation of heterozygous animals from the mean of both types of homozygous animals. A positive dominance value indicates an increase in the trait of interest as a result of a heterozygous genotype and vice versa. In this study, combined additive and dominance effect analysis was performed for all traits; and in the absence of a significant dominance effect an additive only model was used. Furthermore, all significant QTL were tested for genomic imprinting. In this analysis, imprinting is defined as the phenotypic difference between the two heterozygous states of 2 alleles caused by inheritance of the Pietrain allele from paternal or maternal side. A detectable difference between the two alternative heterozygous states has been used to define the parent of origin effect. A QTL with paternally expressed effect (maternal imprinting) is defined if the effect of parent of origin is in the same direction as the additive effect; whereas, a QTL with maternally expressed effect (paternal imprinting) is defined if the parent of origin and the additive effects are in different directions. Individual QTL analysis was performed for ADFI, ADEI, FCR, APD, ALD, ADG, BF, ADNE, NEWG and TNE using a model that accounted for systematic effects of gender, *RYR1*-genotype, batch, housing type, birth farm, as well as start and end body weight. QTL analysis for REI was performed using a model for ADEI that accounted for, besides the above systematic effects, the pre-adjusted values of APD and ALD. The pre-adjusted measures of APD and ALD were obtained after adjustment for the same systematic effect as described above for the traits using the GLM procedure (SAS Inst. Inc., Cary, NC). The statistical significance threshold level in the QTL analysis was the chromosome-wide significance level obtained by permutation test with 1000

iterations using the GridQTL (Seaton et al., 2006). Along with investigating each individual growth stage, QTL analyses were performed based on data over the entire analysed growing period from 60 to 140 kg body weight.

3.3 Results

The genome scan identified 47 QTL above the 95% chromosome-wide significance level and 22 QTL at the suggestive level (90% chromosome-wide significance level) at different stages of growth and during the entire analysed growing period for production, feed intake and nitrogen excretion traits (Table 3.1). The QTL analysis detected 6 QTL for REI, 7 QTL for TNE, 7 QTL for NEWG, 9 QTL for ADNE, 7 QTL for FCR, 6 QTL for ADEI, 10 QTL for ADFI, 6 QTL for APD, 6 QTL for ALD, 4 QTL for average daily gain (ADG) and 1 QTL for BF. Based on the aim of this study, only the QTL associated with feed efficiency and environmental impact due to nitrogen excretion are presented in detail.

QTL for REI during growth from 60 to 90 kg

On SSC14, a QTL with significant dominance effects was identified for REI at position 19 centimorgan (cM) close to SW245 with a QTL effect of -1.75 ± 0.43 MJ/d ME, explaining 6.1% of the phenotypic variation in REI. This QTL was located in a position close to QTL for ADNE (-4.18 ± 1.06 , g/d), ADEI (-2.15 ± 0.57 , MJ/d ME) and ADFI (-0.18 ± 0.05 , kg/d) (Figure 3.1). No further QTL for production traits (e.g. ADG, APD and ALD) were identified in this chromosomal region, indicating that the QTL for REI in this region is independent to production traits.

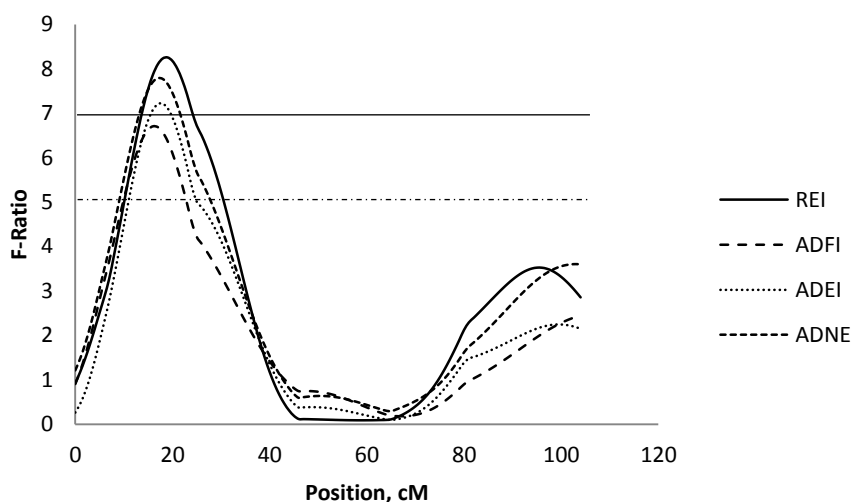


Figure 3.1 Test-statistic along SSC14 for evidence of QTL for residual energy intake (REI), average daily feed intake (ADFI), average daily energy intake (ADEI) and average daily nitrogen excretion (ADNE) at the growth period of 60 to 90 kg body weight. The solid and dashed horizontal lines denote the 99% and 95% chromosome-wide significance level, respectively.

QTL for REI during growth from 90 to 120 kg

Three QTL for REI were detected at 90 to 120 kg body weight. Firstly, on SSC2 at 16 cM close to SWR783, an additive QTL was detected with a significant QTL effect of -0.96 ± 0.32 MJ/d ME, explaining 3.5% of phenotypic variance in REI. This QTL was identified at the similar location as the QTL for FCR (-0.18 ± 0.05), TNE (-0.13 ± 0.03 , kg/pig) and NEWG (-4.16 ± 1.15 , g/kg) (Figure 3.2). Secondly, on SSC4 a unique QTL for REI was captured which showed paternal genomic imprinting (i.e. only the maternally inherited allele is expressed). This QTL was identified at position 130 cM close to SW856 with a QTL effect of 0.60 ± 0.26 MJ/d ME, explaining 5.6% of the phenotypic variance in REI (Figure 3.3). No further QTL were identified in this chromosomal region for any other traits in this study. Thirdly, on SSC7, an additive QTL was identified at position 117 cM close to SWR773 with a significant QTL effect of 0.75 ± 0.27 MJ/d ME, explaining 2.9% of the phenotypic variance in REI. This QTL was identified at the similar location as a dominant QTL for FCR (-0.22 ± 0.08) and NEWG (-5.27 ± 1.93 , g/kg) (Figure 3.4).

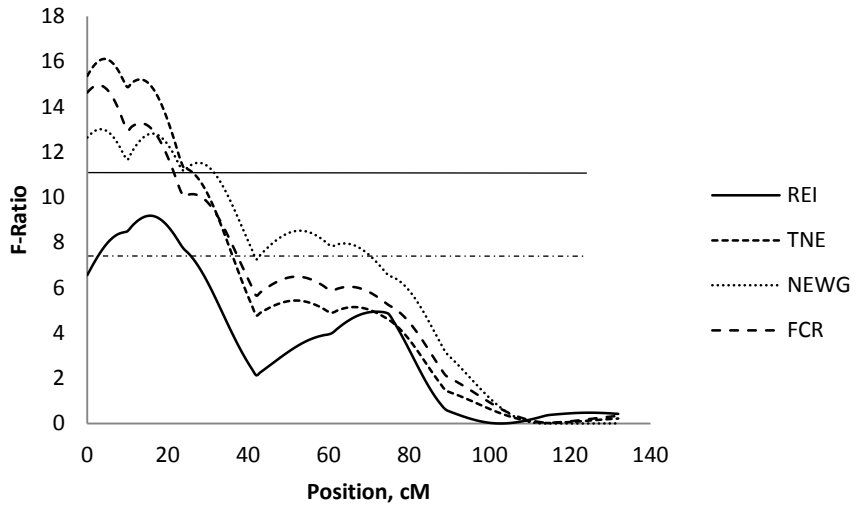


Figure 3.2 Test-statistic along SSC2 for evidence of QTL for residual energy intake (REI), total nitrogen excretion (TNE), nitrogen excretion per weight gain (NEWG) and feed conversion ratio (FCR) at the growth from 90 to 120 kg body weight. The solid and dashed horizontal lines denote the 99% and 95% chromosome-wide significance level, respectively.

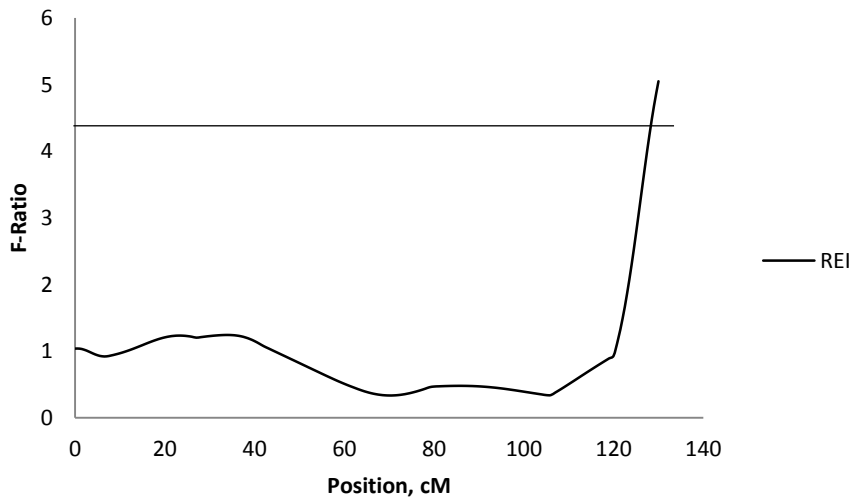


Figure 3.3 Test-statistic along SSC4 for evidence of QTL for residual energy intake (REI) at the growth period of 90 to 120 kg body weight. The solid horizontal line denotes the 95% chromosome-wide significance level.

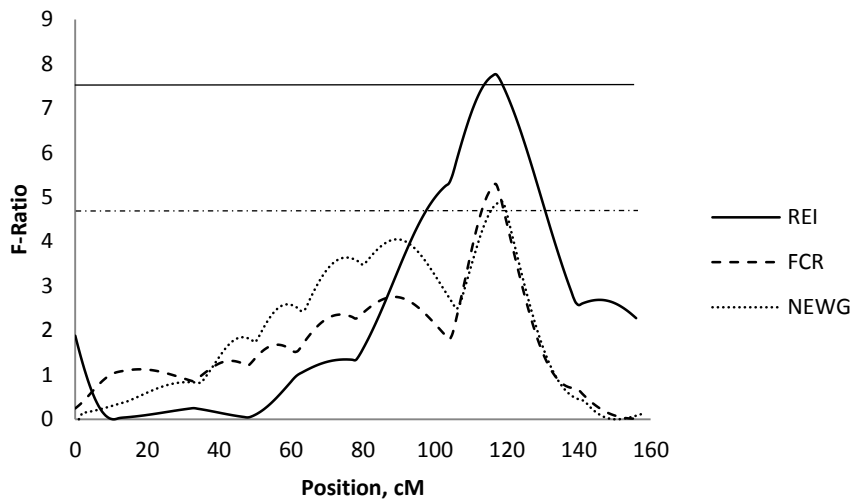


Figure 3.4 Test-statistic along SSC7 for evidence of QTL for residual energy intake (REI), feed conversion ratio (FCR) and nitrogen excretion per weight gain (NEWG) at the growth period of 90 to 120 kg body weight. The solid horizontal line denotes the 95% chromosome-wide significance level for additive QTL for REI, and the dashed horizontal line denotes the 90% chromosome-wide significance level for dominance QTL for FCR and NEWG.

QTL for REI during growth from 120 to 140 kg

On SSC8, an additive QTL for REI at 120 to 140 kg body weight was detected at position 0 cM close to SW2410 with a QTL effect of -1.26 ± 0.33 MJ/d ME, explaining 5.5% of the phenotypic variance in REI. Within this chromosomal region, a QTL for ADNE was also located, with an additive mode of inheritance associated with a reduction in ADNE (-2.56 ± 0.77 , g/d) (Figure 3.5).

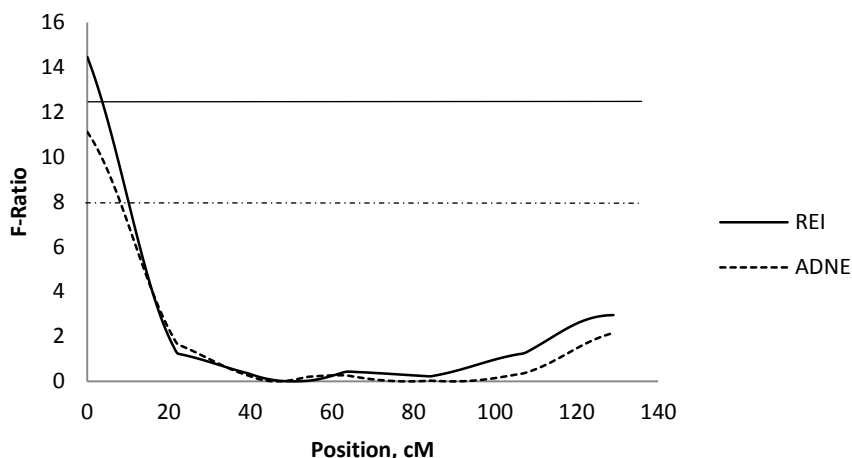


Figure 3.5 Test-statistic along SSC8 for evidence of QTL for residual energy intake (REI) and average daily nitrogen excretion (ADNE) at the growth period of 120 to 140 kg body weight. The solid and dashed horizontal lines denote the 99% and 95% chromosome-wide significance level, respectively.

QTL for REI during the entire analysed growth period (60 to 140 kg)

When investigating the entire analysed growing period (60 to 140 kg) none of the above QTL were identified. An additional QTL was however identified on SSC4 at 130 cM for REI in 90% significance level with additive effects accounting for 2.7% of the phenotypic variance in REI. This chromosomal region did not harbour any QTL for the other traits analysed in this study (Figure 3.6).

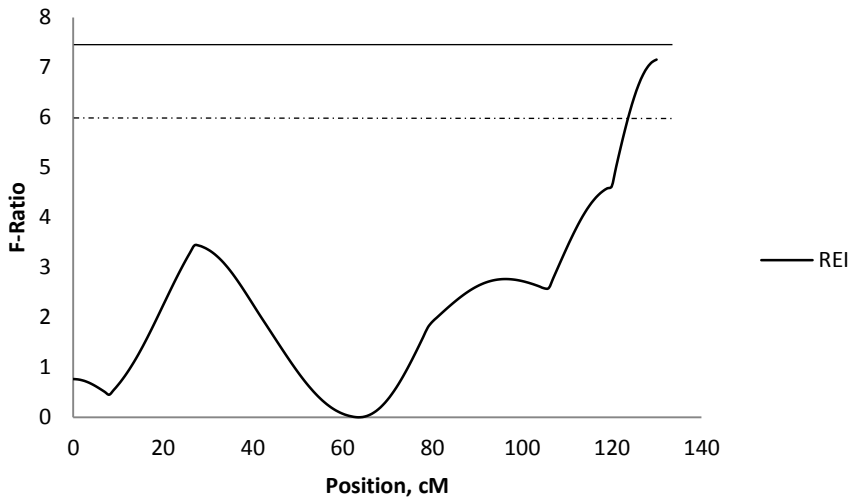


Figure 3.6 Test-statistic along SSC4 for evidence of QTL for residual energy intake (REI) for the entire analysed growth period (60 to 140 kg body weight). The solid and dashed horizontal lines denote the 95% and 90% chromosome-wide significance level, respectively.

QTL for nitrogen excretions during growth from 60 to 90 kg

In total 23 QTL were identified throughout the genome for traits associated with nitrogen excretions. Of these QTL, 15 were identified at specific stages of growth and 8 were only identified when considering the entire analysed growing period. On SSC6, a dominance QTL for ADNE (3.83 ± 1.16 , g/d) was identified at positions 134 cM, between markers SW1881 and SW322, which was in a similar region to a QTL for ADFI with significant dominance genetic effects (0.18 ± 0.05 , kg/d). On SSC10, at position 44 cM a significant QTL was identified for ADNE with an additive genetic effect indicating that the grandpaternal Pietrain allele is favourably associated with a reduction in ADNE by -2.17 ± 0.64 g/d. In addition, QTL were identified within this region of SSC10 for ADEI and ADFI with significant additive effects of -1.12 ± 0.34 MJ/d ME and -0.10 ± 0.03 kg/d, respectively.

QTL for nitrogen excretions during growth from 90 to 120 kg

On SSC4, at 14 to 24 cM, between SW2404 and SW489, QTL were identified for ADG, NEWG, TNE and FCR with significant additive genetic effects. This genomic region was associated with an unfavourable effect of the Pietrain allele on reduction of ADG (-37.54 ± 11.65 , g/d) combined with an unfavourable increase in NEWG (3.33 ± 1.14 , g/kg), TNE (0.10 ± 0.03 , kg/pig) and FCR (0.16 ± 0.04). On SSC6,

three QTL were identified at three different positions for ADNE, FCR and ADFI at 70, 105 and 128 cM, respectively. On SSC7, QTL were identified at positions 88, 58 and 117 cM for TNE, ADNE and NEWG, respectively, indicating the association of different regions of this chromosome with possible environmental pollution of pig production. On SSC13, a unique additive QTL for NEWG was identified at position 119 cM, between markers SW2440 and S0291, which explained 3.4% of the phenotypic variation. This QTL showed that the additive genetic effect of the allele originating from the Pietrain grandpaternal breed was associated with an increase in nitrogen excretions by 3.43 ± 1.14 g/kg of body weight.

QTL for nitrogen excretions during growth from 120 to 140 kg

On SSC2, QTL at positions 115 to 116 cM, close to SWR345, identified with the Pietrain allele showing an unfavourable additive genetic association with TNE (0.08 ± 0.03 , kg/pig) and NEWG (4.53 ± 1.45 , g/kg). On SSC6, at positions 148 and 150 cM, close to SW322, 2 QTL with a significant dominance effect were identified for ADFI and ADEI, respectively. This region also showed QTL association with ADFI at other stages of growth.

QTL for nitrogen excretions during the entire growing period (60 to 140 kg)

On SSC2, at positions 0 to 4 cM, between SWR2516 and SW2623, favourable additive genetic effects of the Pietrain allele with APD, FCR and TNE were identified, where the grandpaternal Pietrain breed was associated with an increase in APD (3.37 ± 1.33 , g/d), a reduction in FCR (-0.09 ± 0.03) and TNE (-0.18 ± 0.06 , kg/pig). In addition, at a slightly different location on SSC2 (13 cM), close to marker SW2623, a favourable additive genetic effect of the Pietrain allele for NEWG (-2.22 ± 0.75 , g/kg) was identified, indicating that these regions on the same chromosome are highly associated with both feed efficiency and potentially environmental pollution. On SSC4, at positions 15 to 24 cM, between SW489 and S0301, positive additive genetic associations with FCR (0.07 ± 0.02), NEWG (1.97 ± 0.69 , g/kg) and TNE (0.16 ± 0.06 , kg/pig) were identified. On SSC6 from 131 to 135 cM, between SW1881 and SW322, 5 QTL with significant dominance effects were identified suggesting that the heterozygous genotype is associated with an unfavourable increase in ADNE (3.60 ± 0.95 , g/d) and ALD (20.15 ± 6.45 , g/d) as well as a favourable increase in APD (7.43 ± 2.41 , g/d) related to an increased ADEI (2.22 ± 0.57 , MJ/d ME) and ADFI (0.18 ± 0.05 , g/kg). In addition, a favourable additive QTL effect of the Pietrain allele was identified for BF (-0.12 ± 0.04) around this region. On SSC7, a QTL with significant dominance effects for TNE was identified at 111 cM, between SW632 and SWR773, with a favourable effect of -0.31 ± 0.10 , kg/pig. This

3 Genomic architecture of energy and nitrogen efficiency

region did not show any association with any other traits in this study. On SSC9, position 82 cM had a significant dominance association with ADNE (2.55 ± 0.79 , g/d) and ADEI (1.59 ± 0.48 , MJ/d ME) without any influence on production traits, indicating that a heterozygous genotype was associated with an unfavourable increase in these traits. On SSC14, three QTL with significant dominance effects were identified between positions 14 and 15 cM, between S0089 and SW245, with favourable decrease in ADNE (-2.65 ± 0.89 , g/d) and ALD (-13.34 ± 6.25 , g/d) related to a decrease in ADFI (-0.14 ± 0.04 , kg/d).

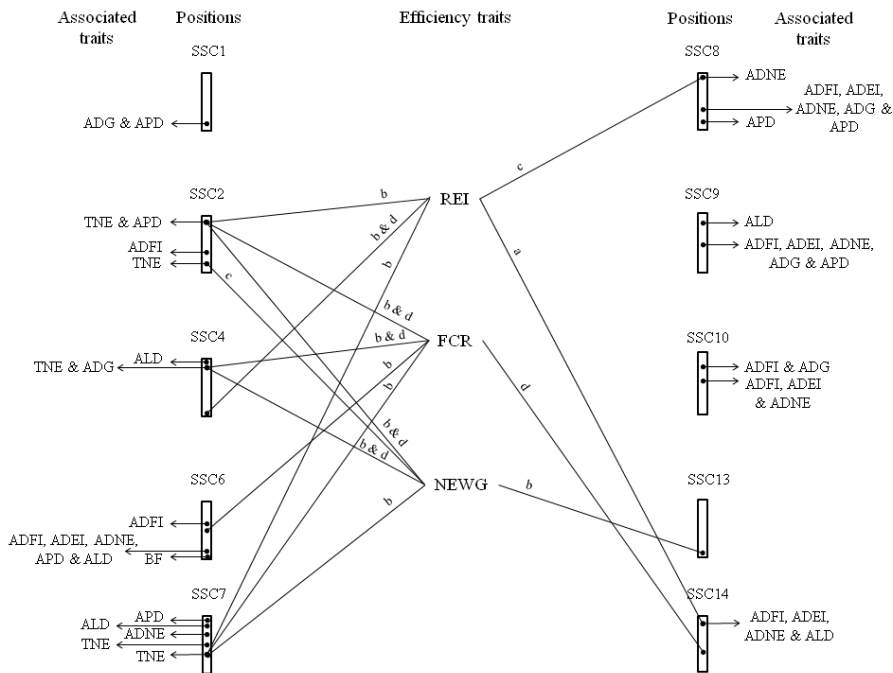


Figure 3.7 The genomic architecture of feed and nitrogen efficiency in association with growth, feed intake and nitrogen excretion traits are presented using QTL information for residual energy intake (REI), feed conversion ratio (FCR), nitrogen excretion per weight gain (NEWG), total nitrogen excretion (TNE), average daily nitrogen excretion (ADNE), average daily feed intake (ADFI), average daily energy intake (ADEI), average daily gain (ADG), average daily protein deposition (APD) and average daily lipid deposition (ALD). The a, b, c and d represent 60 to 90 kg, 90 to 120 kg, 120 to 140 kg growth stages and the entire analysed growth period (60 to 140 kg), respectively.

3 Genomic architecture of energy and nitrogen efficiency

Table 3.1 Evidence of QTL for REI, production and nitrogen excretion traits throughout growth.

Growth stage	SSC	Trait ¹	F-Ratio	Position, cM	Marker interval	% Var ²	Mode of inheritance	QTL effect ³
60 to 90 kg	2	ALD, g/d	4.98†	59	SW240-SW1026	3.66	Dominance	-0.02 ± 0.01
	6	ADNE, g/d	5.53†	134	SW1881-SW322	3.99	Dominance	3.83 ± 1.16
	6	ADFI, kg/d	5.87*	135	SW1881-SW322	4.07	Dominance	0.18 ± 0.05
	10	ADEI, MJ/d ME	10.61**	44	SW2195	3.79	Additive	-1.12 ± 0.34
	10	ADFI, kg/d	12.88**	44	SW2195	4.44	Additive	-0.10 ± 0.03
	10	ADNE, g/d	11.64**	44	SW2195	4.18	Additive	-2.17 ± 0.64
	14	ADFI, kg/d	6.71**	16	S0089-SW245	4.64	Dominance	-0.18 ± 0.05
	14	ADNE, g/d	7.79**	17	S0089-SW245	5.53	Dominance	-4.18 ± 1.06
	14	ADEI, MJ/d ME	7.22*	18	S0089-SW245	5.11	Dominance	-2.15 ± 0.57
	14	REI, MJ/d ME	8.26**	19	S0089-SW245	6.10	Dominance	-1.75 ± 0.43
90 to 120 kg	1	APD, g/d	7.61*	113	SW1311-SW1828	2.82	Additive	-5.13 ± 1.86
	2	FCR	14.95**	3	SWR2516-SW2623	5.17	Additive	-0.18 ± 0.05
	2	NEWG, g/kg	13.00**	3	SWR2516-SW2623	4.78	Additive	-4.16 ± 1.15
	2	TNE, kg/pig	16.11**	4	SWR2516-SW2623	4.02	Additive	-0.13 ± 0.03
	2	REI, MJ/d ME	9.18*	16	SW2623-SWR783	3.46	Additive	-0.96 ± 0.32
	4	TNE, kg/pig	10.39*	14	SW489-S0301	3.81	Additive	0.10 ± 0.03
	4	FCR	12.67**	17	SW489-S0301	4.42	Additive	0.16 ± 0.04
	4	NEWG, g/kg	8.57*	19	SW489-S0301	3.20	Additive	3.33 ± 1.14
	4	ADG, g/d	10.39*	24	SW489-S0301	3.67	Additive	-37.54 ± 11.65
	4	REI, MJ/d ME	5.05*	130	MP77-SW856	5.63	Imprinting	0.60 ± 0.26
	6	ADNE, g/d	8.51*	70	S0087-SW122	3.15	Additive	-2.00 ± 0.68
	6	FCR, MJ/d ME	8.32*	105	S0228	2.95	Additive	-0.13 ± 0.04
	6	ADFI, kg/d	4.76†	128	SW1881-SW322	3.37	Dominance	0.15 ± 0.05

3 Genomic architecture of energy and nitrogen efficiency

Table 3.1 Continued

Growth stage	SSC	Trait1	F-Ratio	Position, cM	Marker interval	% Var2	Mode of inheritance	QTL effect3
90 to 120 kg	7	ADNE, g/d	5.40*	58	SW1841-S0087	3.97	Dominance	-3.50 ± 1.07
	7	TNE, kg/pig	4.83†	88	SW122-S0228	3.57	Dominance	-0.17 ± 0.06
	7	NEWG, g/kg	4.88†	116	SWR773	3.65	Dominance	-5.27 ± 1.93
	7	REI, MJ/d ME	7.77*	117	SWR773	2.94	Additive	0.75 ± 0.27
	7	FCR	5.29†	117	SWR773	3.73	Dominance	-0.22 ± 0.08
	10	ADG, g/d	9.09*	3	SW830-SWR136	3.22	Additive	-34.19 ± 11.34
	10	ADFI, kg/d	7.51*	6	SW830-SWR136	2.67	Additive	-0.08 ± 0.030
	13	NEWG, g/kg	9.06*	119	SW2440-S0291	3.38	Additive	3.43 ± 1.14
120 to 140 kg	1	ADG, g/d	4.5†	116	SW1311-SW1828	3.20	Dominance	60.73 ± 20.32
	2	ADFI, kg/d	5.63*	82	SW1370-SWR2157	3.96	Dominance	0.20 ± 0.06
	2	TNE, kg/pig	8.45*	115	SWR345	3.19	Additive	0.08 ± 0.03
	2	NEWG, g/kg	9.84*	116	SWR345	3.73	Additive	4.53 ± 1.45
	4	ALD, g/d	7.43**	5	SW2404-SW489	5.57	Dominance	-38.44 ± 12.34
	6	ADFI, kg/d	5.00†	148	SW322	3.53	Dominance	0.19 ± 0.06
	6	ADEI, MJ/d ME	4.61†	150	SW322	3.32	Dominance	2.10 ± 0.69
	7	ALD, g/d	4.81†	28	SWR1343-SW2155	3.68	Dominance	49.33 ± 15.93
	8	REI, MJ/d ME	14.46**	0	SW2410	5.53	Additive	-1.26 ± 0.33
	8	ADNE, g/d	11.12*	0	SW2410	4.16	Additive	-2.56 ± 0.77
	9	ADG, g/d	5.11†	81	S0019-SW2093	3.62	Dominance	70.29 ± 22.74
	9	ADEI, MJ/d ME	6.88*	82	S0019-SW2093	4.88	Dominance	2.56 ± 0.73
	9	ADFI, kg/d	5.48*	82	S0019-SW2093	3.86	Dominance	0.20 ± 0.06
	9	APD, g/d	4.47†	86	S0019-SW2093	3.43	Dominance	10.76 ± 3.67

3 Genomic architecture of energy and nitrogen efficiency

Table 3.1 Continued

Growth stage	SSC	Trait1	F-Ratio	Position, cM	Marker interval	% Var2	Mode of inheritance	QTL effect3
60 to 140 kg	2	APD, g/d	6.44†	0	SWR2516-SW2623	2.51	Additive	3.37 ± 1.33
	2	FCR	12.08**	4	SWR2516-SW2623	4.09	Additive	-0.09 ± 0.03
	2	TNE, kg/pig	9.91*	4	SWR2516-SW2623	3.57	Additive	-0.18 ± 0.06
	2	NEWG, g/kg	8.81*	13	SW2623-SWR783	3.50	Additive	-2.22 ± 0.75
	4	NEWG, g/kg	8.08*	15	SW489-S0301	3.22	Additive	1.97 ± 0.69
	4	TNE, kg/pig	8.77*	22	SW489-S0301	3.17	Additive	0.16 ± 0.06
	4	FCR	7.54†	24	SW489-S0301	2.60	Additive	0.07 ± 0.02
	4	REI, MJ/d ME	7.16†	130	MP77-SW856	2.70	Additive	-0.45 ± 0.17
	6	ALD, g/d	4.91†	131	SW1881-SW322	3.58	Dominance	20.15 ± 6.45
	6	ADEI, MJ/d ME	7.56**	133	SW1881-SW322	5.51	Dominance	2.22 ± 0.57
	6	APD, g/d	4.91†	134	SW1881-SW322	3.64	Dominance	7.43 ± 2.41
	6	ADNE, g/d	7.28*	134	SW1881-SW322	5.17	Dominance	3.60 ± 0.95
	6	ADFI, kg/d	7.50*	135	SW1881-SW322	4.92	Dominance	0.18 ± 0.05
	6	BF, cm	11.42*	163	SW322-SW2052	3.75	Additive	-0.12 ± 0.04
	7	APD, g/d	4.69†	5	SW2564-SWR1343	3.42	Dominance	4.81 ± 2.13
	7	TNE, kg/pig	6.53*	111	SW632-SWR773	4.66	Dominance	-0.31 ± 0.10
	8	APD, g/d	6.90*	101	SW374-SW1551	2.51	Additive	3.54 ± 1.35
	9	ALD, g/d	4.58†	18	SW21-SW911	3.32	Dominance	20.64 ± 6.96
	9	ADEI, KJ/d ME	5.66*	82	S0019	4.19	Dominance	1.59 ± 0.48
	9	ADNE, g/d	5.23*	82	S0019	3.77	Dominance	2.55 ± 0.79
	14	ALD, g/d	4.31†	14	S0089-SW245	3.14	Dominance	-13.34 ± 6.25
	14	ADNE, g/d	4.56†	14	S0089-SW245	3.30	Dominance	-2.65 ± 0.89
	14	ADFI, kg/d	5.13*	15	S0089-SW245	3.41	Dominance	-0.14 ± 0.04
	14	FCR	4.55†	88	SW1557-SWC27	3.13	Dominance	0.15 ± 0.05

† = 0.1, * = 0.05 and ** = 0.01 chromosome-wide significance level

¹ REI = residual energy intake; ADEI = average daily energy intake; APD = average protein deposition; ALD = average lipid deposition; FCR = feed conversion ratio; TNE = total nitrogen excretion; ADNE = average daily nitrogen excretion; NEWG = nitrogen excretion per weight gain; BF = backfat thickness

3 Genomic architecture of energy and nitrogen efficiency

² Percentage of variance explained by the QTL calculated as the proportion of residual sum of square from the full model divided by the residual sum of square from null model (excluding QTL effect)

³ Estimated additive, dominance or imprinting effects and their standard error

3.4 Discussion

QTL for REI

In the current study, six novel QTL were detected for REI at different stages of growth, bringing new insight into the genomic architecture of efficiency of feed utilisation. Based on QTL information, the genomic architecture of feed and nitrogen efficiency in growing pigs is broadly illustrated in Figure 3.7. The identification of QTL for REI in this study in different genomic locations for different stages of growth, suggests that different genes are switched on and off throughout growth.

On SSC2, a QTL for REI showed associations with QTL for TNE, NEWG and FCR at 90 to 120 kg of growth. Additionally, this region was found to have an association with protein deposition during the entire analysed growing period, suggesting that the allele originating from the Pietrain breed is associated with an increase in protein deposition and an improvement in feed efficiency and reduced environmental pollution of pig production. The same QTL for FCR using the same experimental population as in the current study was reported in a previous study where the authors also suggested the segregation of the IGF2 allele as a candidate gene for this QTL, which is associated with fatness and growth (Duthie et al., 2008). In addition, this region has been shown in the literature to be associated with ADG, ADFI, body weight, ultrasonic backfat and FCR (Knott et al., 1998; De Koning et al., 2001; De Koning et al., 2003; Houston et al., 2005; Duthie et al., 2008; Gilbert et al., 2010). The QTL for REI, FCR, NEWG, TNE and APD reported in the current study had an additive mode of inheritance compared to the QTL for IGF2, which showed genomic imprinting, indicating that, besides the QTL for IGF2, there might be an additional QTL for APD on SSC2 around this location causing the improvement in efficiency of protein deposition. The results described in this study, indicate that this chromosomal region may play a role in improving the efficiency of feed utilisation through an increase in leanness, decrease in feed conversion ratio and consequently a reduction in the environmental impact of pig production.

On SSC4, the QTL for REI (MP77-SW856) showed a change in mode of inheritance depending on the considered growth period. During growth from 90 to 120 kg body weight, this QTL showed paternal imprinting so that the maternally inherited Pietrain allele expressed an undesirable increase in REI. In contrast, at growth from 60 to 140 kg body weight, an additive QTL effect with a desirable reduction in REI was identified. This may indicate that the responsible gene (or genes) in this region

have changed in function during the growing period. It has to be considered that the estimated parent of origin effects may not be caused by imprinting but maternal effects as this effect can be confounded with imprinting effects as estimated in this study. However, a maternal effect would most likely be expected at earlier (60 to 90 kg) and not at later stage of growth (90 to 120 kg). There are reports of QTL around this region for ADG, loin muscle area and ultrasonic backfat (Malek et al., 2001; Knott et al., 2002). This chromosome has been shown to harbour a QTL for FCR with an additive mode of inheritance in a region different to the QTL for REI, as reported by Duthie et al. (2008), using the same experimental population as in the current study. In addition, Sahana et al. (2013) reported two significant SNPs associated with FCR on SSC4 but at different positions (63.9 cM and 64.0 cM, respectively) to the QTL identified in the present study for FCR and REI. The lack of association with feed intake traits suggest that the REI QTL may be associated with underlying causes of variation in REI such as metabolism, protein turnover, etc.

On SSC6, the identified additive QTL for FCR, at 90 to 120 kg body weight, was the only QTL associated with feed efficiency on this chromosome in current study. In agreement, Yue et al. (2003b) reported a QTL for FCR around the FCR QTL region identified in the current study. Fan et al. (2010) suggested an association of fat mass and obesity related (FTO) p.Ala198Ala SNP on SSC6, at 28.28 cM to 28.33 cM. In the current study, in this chromosomal region, no QTL for REI was detected, which could be expected as REI is adjusted for fat growth, assuming the QTL for fatness is the underlying biological reason for the FCR QTL.

On SSC7, at 90 to 120 kg, the identified additive QTL for REI (SW632-SWR773) was overlapping with the dominant QTL for FCR and NEWG. In addition, the QTL of this genomic region were associated with a dominance QTL effect for TNE during the entire analysed growing period. The results suggest that heterozygous genotypes at this QTL are associated with efficient use of energy and nitrogen intake during growth. In the present population, when the allele originating from the Pietrain grandpaternal breed is present, an increase in REI was obtained. This may be caused by one QTL with pleiotropic effects or by multiple QTL with different mode of inheritance in this region. In contrast to our study, this region has been shown to be associated with ADG, average feeding rate and backfat thickness (Knott et al., 1998; Houston et al., 2005; Kim et al., 2005; Edwards et al., 2008). Furthermore, Zhang et al. (2009) reported a FCR QTL on this chromosome at 64.8 cM, from a cross of White Duroc and Chinese Erhualian breeds, whereas this QTL was not detected in the current study.

On SSC8, at the latest growth period considered in this study (120 to 140 kg), the QTL for REI (SW2410-SW905) was only found to have an association with nitrogen excretions. These results suggest that the QTL for REI may be associated with underlying variation in efficiency of digestion, feed utilisation, protein turnover, etc, due to independence of REI from production, and no positional association with QTL for feed intake traits in this region. However, other studies have found QTL for ADG in this region (e.g. De Koning et al., 2001; Zhang et al., 2009).

On SSC14, the identified QTL for REI at 60 to 90 kg of growth expressed dominance effects and was overlapping with QTL for both ADNE and ADEI. Furthermore, the animals with heterozygous genotype had reduced lipid deposition, feed intake and nitrogen excretion during the entire analysed growing period. In the current study, this QTL region (S0089-SW245) did not show any association with protein growth indicating that this chromosomal region of SSC14 harbours a QTL for REI which is caused by reduced feed intake. The reduced feed intake may again be the reason for the identified QTL for ALD. However, it cannot be distinguished from this study whether low feed intake is the driver for low lipid deposition or vice versa. Moreover, it is difficult to determine whether this region carries one or multiple QTL for those traits. De Koning et al. (2001) reported a maternally imprinted QTL for backfat (measured by ultrasound) in this region (SW857-SW295) for a cross between Chinese Meishan and Dutch commercial pigs. Additionally, Rohrer and Keele (1998) reported a suggestive additive QTL for average backfat thickness in this region (SW510-SW2439) for a three generation reciprocal backcross of Meishan and Large White composite pigs. Furthermore, a QTL for daily feed intake has been reported in the same region (SW857-S0007) by Liu et al. (2007). The result suggests that the QTL for REI in this region of SSC14 may be associated with improved production efficiency and reduced nitrogen excretions through reduction in energy usage and fatness. Furthermore, Sahana et al. (2013) reported eight SNPs to be associated with FCR on SSC14 in different genomic regions (120 to 124 cM) to the QTL obtained in current study for FCR and REI. Fan et al. (2010) suggested an association of TCF7L2 c.646+514A>G SNP on SSC14, at 134.5 cM to 134.78 cM position, with residual feed intake as possible genetic markers for this trait. Fan et al. (2010) concluded that the involvement of these SNPs with variation in residual feed intake suggests that there is a common pathway or network regulating fatness, energy balance and feed intake.

Following analyses for epistatic QTL in the current study, no epistatic QTL was detected for REI at any of the growth stages considered in this study.

QTL for nitrogen excretions

On SSC2, positions 115 and 116 cM, close to SWR345, were associated with an increase in NEWG and TNE at 120 to 140 kg body weight. This region was not associated with any other traits in the current study. Although, QTL for ADG have been reported around this region (Bidanel et al., 2001), no QTL for feed intake or feed efficiency have been reported. This suggests that this region may be associated with underlying causes of variation in nitrogen excretion such as metabolism, protein utilisation, or maintenance requirements, etc.

On SSC4, at 90 to 120 kg body weight, the region between 14 and 24 cM (SW489-S0301) had an additive effect of the Pietrain allele associated with a reduction in ADG, and an increase in NEWG, TNE and FCR. In addition, this region showed the same unfavourable QTL effects of the Pietrain allele on FCR, NEWG and TNE during the entire analysed growing period. Duthie et al. (2008) reported this QTL for FCR using the same experimental data as in the current study. These results indicate that the additive allele from the grandpaternal Pietrain breed is associated with a reduction in production, and consequently an increase in FCR and nitrogen excretion. This is surprising as the Pietrain breed has been greatly selected for improved productivity.

On SSC6, the region between 128 to 150 cM (SW1881-SW322) was found to have a dominance effect associated with increase in ADFI, ADEI and ADNE at different stages of growth. Furthermore, this region was also associated with an increase in APD and ALD during the entire analysed growing period from 60 to 140 kg body weight. This suggests that there might be a pleiotropic QTL or QTL in high linkage disequilibrium that increases production such as APD and to a higher extent ALD, therefore, resulting in increased feed intake, energy usage and consequently nitrogen excretions. These QTL for ADFI at 60 to 90 kg, and 90 to 120 kg growth stages has been reported by Mohrmann et al. (2006a) using the same experimental population as in the current study. Gilbert et al. (2010) reported a QTL for FCR in this region for Pietrain-Large White backcross. In addition, on SSC6 at position 70 cM, the additive effect QTL associated with reduction of ADNE, indicates that the allele originating from the Pietrain grandpaternal breed is associated with a reduction in nitrogen excretions. This region was not associated with any other trait analysed in this study suggesting that this is a unique QTL for nitrogen efficiency. However, Gilbert et al. (2010) reported a QTL for ADFI around this region (83 cM), which would suggest that the increase in ADNE may be due to an increase in feed intake as these traits are highly correlated (Shirali et al., 2012).

The QTL on SSC7, at 58 and 88 cM, showed favourable dominance associations with ADNE and TNE, respectively, suggesting that heterozygous animals have less nitrogen excretions. These regions were not associated with any other traits in the current study, suggesting a unique QTL for nitrogen efficiency. However, Gilbert et al. (2010) reported a suggestive ($P < 0.1$) QTL for ADFI at the same position as the QTL for ADNE and additionally a QTL for FCR (74 cM) in nearby region to the QTL for TNE.

On SSC9, the QTL in the region from 81 to 86 cM (S0019-SW2093), which was associated with nitrogen excretions during the entire analysed growing period, had also associations with an increase in ADEI, ADFI, APD and ADG from 120 to 140 kg body weight. This suggests the presence of a QTL for production, and in particular lean production, which increases feed intake and energy usage, and consequently results in an increased nitrogen excretion. Furthermore, the QTL associated with production shows a positive correlation with the QTL for feed intake which is in agreement with the genetic correlation between these traits (Johnson et al., 1999). Duthie et al. (2008) reported QTL for ADG, APD, and ALD in this region using the same experimental data as in the current study.

On SSC10, a QTL at position 44 cM was associated with ADNE, ADFI and ADEI at the stage of growth from 60 to 90 kg body weight. The result indicates that the allele originating from the Pietrain grandsire is associated with reduced feed intake, energy usage, and consequently may results in a reduction in the environmental impact of pig production. The QTL for ADFI has been previously reported in a study which utilised the same experimental data (Duthie et al., 2008). A QTL for ADG in nearby region has been previously reported for a cross of outbred Wild boar and Large White pigs (Knott et al., 1998). Furthermore, in the current study, the Pietrain allele at the QTL on SSC10, between markers SW830 and SWR136 (3 and 6 cM) had an unfavourable additive genetic association with ADFI (-0.08 ± 0.03 , kg/d) and ADG (-34.19 ± 11.34 , g/d) during growth from 90 to 120 kg. Duthie et al. (2008) reported a QTL with unfavourable additive effects of the Pietrain on APD in this region at the same growth period, using the same experimental data as in the current study. This suggests that the allele originating from Pietrain grandsire breed is associated with a reduction in protein deposition, and consequently growth and the feed intake required for growth.

On SSC13, an additive QTL at 119 cM was associated with an increase in NEWG. In this region no QTL were detected which were associated with any growth and feed

efficiency traits. This indicates that the allele originating from the Pietrain grandpaternal breed is associated with an increase in nitrogen excretion through influencing the underlying causes of variation in nitrogen excretion. In addition, no QTL for feed efficiency has been reported in this region of the genome; however, a QTL for ADG was reported in this region with an additive effect which is associated with growth (Knott et al., 1998). Yue et al. (2003a) also reported a QTL for feed intake around this region.

3.5 Conclusions

This study revealed six novel QTL for REI revealing the genomic architecture of efficiency in feed utilisation and indicating that the regulation of feed efficiency is partly independent from that of production traits. As expected no QTL for REI were overlapping with QTL for APD and ALD within the considered growth period, but between growth periods some overlapping occurred, suggesting change genomic regulations of the these traits during growth. One of the six novel QTL for REI had positional association with QTL for feed intake suggesting that some of the variation in REI can be explained by variation in feed intake. However, four of the six novel QTL for REI had positional association with QTL related to nitrogen excretions suggests the change in efficiency of feed utilisation due to underlying causes of variation in REI such as metabolism, digestion, protein turnover, etc. Different QTL for REI were identified at different growth stages, suggesting different genes are responsible for efficiency in feed utilisation at different stages of growth. This also suggests that selection for REI is most efficient if carried out within stages of growth.

References

- Beeckmann, P., G. Moser, H. Bartenschlager, G. Reiner, and H. Geldermann. 2003. Linkage and QTL mapping for *Sus scrofa* chromosome 8. *Journal of Animal Breeding and Genetics* 120:66-73.
- Bidanel, J. P., D. Milan, N. Iannuccelli, Y. Amigues, M. Y. Boscher, F. Bourgeois, J. C. Caritez, J. Gruand, P. Le Roy, H. Lagant, R. Quintanilla, C. Renard, J. Gellin, L. Ollivier, and C. Chevalet. 2001. Detection of quantitative trait loci for growth and fatness in pigs. *Genetics Selection Evolution* 33:289-309.
- Cai, W., D. S. Casey, and J. C. M. Dekkers. 2008. Selection response and genetic parameters for residual feed intake in Yorkshire swine. *J. Anim Sci.* 86:287-298.

- De Haer, L. C. M., P. Luiting, and H. L. M. Aarts. 1993. Relations among individual (residual) feed intake, growth performance and feed intake pattern of growing pigs in group housing. *Livestock Production Science* 36:233-253.
- De Koning, D. J., R. Pong-Wong, L. Varona, G. J. Evans, E. Giuffra, A. Sanchez, G. Plastow, J. L. Noguera, L. Andersson, and C. S. Haley. 2003. Full pedigree quantitative trait locus analysis in commercial pigs using variance components. *J. Anim Sci.* 81:2155-2163.
- De Koning, D. J., A. P. Rattink, B. Harlizius, M. A. M. Groenen, E. W. Brascamp, and J. A. M. van Arendonk. 2001. Detection and characterization of quantitative trait loci for growth and reproduction traits in pigs. *Livestock Production Science* 72:185-198.
- Duthie, C., G. Simm, A. Doeschl-Wilson, E. Kalm, P. W. Knap, and R. Roehe. 2008. Quantitative trait loci for chemical body composition traits in pigs and their positional associations with body tissues, growth and feed intake. *Animal Genetics* 39:130-140.
- Duthie, C., G. Simm, A. Doeschl-Wilson, E. Kalm, P. W. Knap, and R. Roehe. 2010. Epistatic analysis of carcass characteristics in pigs reveals genomic interactions between quantitative trait loci attributable to additive and dominance genetic effects. *J. Anim Sci.* 88:2219-2234.
- Edwards, D. B., C. W. Ernst, N. E. Raney, M. E. Doumit, M. D. Hoge, and R. O. Bates. 2008. Quantitative trait locus mapping in an F2 Duroc × Pietrain resource population: II. Carcass and meat quality traits. *J. Anim Sci.* 86:254-266.
- Fan, B., S. Lkhagvadorj, W. Cai, J. Young, R. M. Smith, J. C. M. Dekkers, E. Huff-Lonergan, S. M. Lonergan, and M. F. Rothschild. 2010. Identification of genetic markers associated with residual feed intake and meat quality traits in the pig. *Meat Science* 84:645-650.
- Gilbert, H., J. P. Bidanel, J. Gruand, J. C. Caritez, Y. Billon, P. Guillouet, H. Lagant, J. Noblet, and P. Sellier. 2007. Genetic parameters for residual feed intake in growing pigs, with emphasis on genetic relationships with carcass and meat quality traits. *J. Anim Sci.* 85:3182-3188.
- Gilbert, H., J. Riquet, J. Gruand, Y. Billon, K. Fève, P. Sellier, J. Noblet, and J. P. Bidanel. 2010. Detecting QTL for feed intake traits and other performance traits in growing pigs in a Pietrain-Large White backcross. *Animal* 4:1308-1318.
- Haley, C. S., S. A. Knott, and J. M. Elsen. 1994. Mapping quantitative trait loci in crosses between outbred lines using least-squares. *Genetics* 136 (3):1195-1207.
- Houston, R. D., C. S. Haley, A. L. Archibald, and K. A. Rance. 2005. A QTL affecting daily feed intake maps to Chromosome 2 in pigs. *Mamm. Genome*. 16 (6): 464-70.

- Johnson, Z. B., J. J. Chewning, and R. A. Nugent, III. 1999. Genetic parameters for production traits and measures of residual feed intake in large white swine. *J. Anim Sci.* 77:1679-1685.
- Kim, J. J., H. H. Zhao, H. Thomsen, M. F. Rothschild, and J. C. M. Dekkers. 2005. Combined line-cross and half-sib QTL analysis of crosses between outbred lines. *Genetical Research* 85:235-248.
- Knott, S. A., L. Marklund, C. S. Haley, K. Andersson, W. Davies, H. Ilegren, M. Fredholm, I. Hansson, B. Hoyheim, K. Lundstrom, M. Moller, and L. Andersson. 1998. Multiple Marker Mapping of Quantitative Trait Loci in a Cross Between Outbred Wild Boar and Large White Pigs. *Genetics* 149:1069-1080.
- Knott, S. A., P. E. Nystrom, L. ndersson-Eklund, S. Stern, L. Marklund, L. Andersson, and C. S. Haley. 2002. Approaches to interval mapping of QTL in a multigeneration pedigree: the example of porcine chromosome 4. *Animal Genetics* 33:26-32.
- Landgraf, S., A. Susenbeth, P. W. Knap, H. Looft, G. S. Plastow, E. Kalm, and R. Roehe. 2006. Allometric association between in vivo estimation of body composition during growth using deuterium dilution technique and chemical analysis of serial slaughtered pigs. *Anim. Sci.* 82:223-231.
- Liu, G., D. G. J. Jennen, E. Tholen, H. Juengst, T. Kleinwachter, M. Holker, D. Tesfaye, G. Un, H. J. Schreinemachers, E. Murani, S. Ponsuksili, J. J. Kim, K. Schellander, and K. Wimmers. 2007. A genome scan reveals QTL for growth, fatness, leanness and meat quality in a Duroc-Pietrain resource population. *Animal Genetics* 38:241-252.
- Malek, M., J. C. M. Dekkers, H. K. Lee, T. J. Baas, and M. F. Rothschild. 2001. A molecular genome scan analysis to identify chromosomal regions influencing economic traits in the pig. I. Growth and body composition. *Mamm. Genome.* 12 (8): 630-6.
- Mohrmann, M., R. Roehe, P. W. Knap, H. Looft, G. S. Plastow, and E. Kalm. 2006a. Quantitative trait loci associated with AutoFOM grading characteristics, carcass cuts and chemical body composition during growth of *Sus scrofa*. *Animal Genetics* 37:435-443.
- Mohrmann, M., R. Roehe, A. Susenbeth, U. Baulain, P. W. Knap, H. Looft, G. S. Plastow, and E. Kalm. 2006b. Association between body composition of growing pigs determined by magnetic resonance imaging, deuterium dilution technique and chemical analysis. *Meat Sci.* 72:518-531.
- Myakishev, M. V., G. I. Kapanadze, G. O. Shaikhayev, G. P. Georgiev, and D. R. Beritashvili. 1995. Extraction of DNA from the whole blood by silica gel. *Inst. Gene Biol. , Moscow, Russia.*

- Roehe, R., G. S. Plastow, and P. W. Knap. 2003. Quantitative and molecular genetic determination of protein and fat deposition. *Homo-Journal of Comparative Human Biology* 54:119-131.
- Rohrer, G. A., L. J. Alexander, Z. L. Hu, T. P. L. Smith, J. W. Keele, and C. W. Beattie. 1996. A comprehensive map of the porcine genome. *Genome Res.* 6:371:-391.
- Rohrer, G. A. and J. W. Keele. 1998. Identification of quantitative trait loci affecting carcass composition in swine: I. Fat deposition traits. *J. Anim Sci.* 76:2247-2254.
- Sahana, G., V. Kadlecova, H. Hornshoj, B. Nielsen, and O. F. Christensen. 2013. A genome-wide association scan in pig identifies novel regions associated with feed efficiency trait. *J. Anim Sci.* 91:1041-1050.
- Seaton, G., J. Hernandez, J. A. Grunchec, I. White, J. Allen, D. J. De Koning, W. Wei, D. Berry, C. S. Haley, and S. A. Knott. 2006. A Grid Portal for QTL Mapping of Compute Intensive Datasets. *Proceedings of the 8th World Congress on Genetics Applied to Livestock Production*. August 13-18. Belo Horizonte, Brazil.
- Shirali, M., A. Doeschl-Wilson, P. W. Knap, C. Duthie, E. Kanis, J. A. M. van Arendonk, and R. Roehe. 2012. Nitrogen excretion at different stages of growth and its association with production traits in growing pigs. *J. Anim Sci.* 90:1756-1765.
- Yue, G., V. Russo, R. Davoli, I. Sternstein, C. Brunsch, D. Schroffellova, A. Stratil, G. Moser, H. Bartenschlager, G. Reiner, and H. Geldermann. 2003a. Linkage and QTL mapping for *Sus scrofa* chromosome 13. *Journal of Animal Breeding and Genetics* 120:103-110.
- Yue, G., A. Stratil, M. Kopecny, D. Schroffellova, J. Schroffel, J. Hojny, S. Cepica, R. Davoli, P. Zambonelli, C. Brunsch, I. Sternstein, G. Moser, H. Bartenschlager, G. Reiner, and H. Geldermann. 2003b. Linkage and QTL mapping for *Sus scrofa* chromosome 6. *Journal of Animal Breeding and Genetics* 120:45-55.
- Zhang, Z. Y., J. Ren, D. R. Ren, J. W. Ma, Y. M. Guo, and L. S. Huang. 2009. Mapping quantitative trait loci for feed consumption and feeding behaviors in a White Duroc x Chinese Erhualian resource population. *J. Anim Sci.* 87:3458-3463.

4

Estimation of residual energy intake and its genetic background during the growing period in pigs

M. Shirali^{1&5}, A. Doeschl-Wilson², C. Duthie⁴, P.W. Knap³, E. Kanis⁵, J.A.M. van Arendonk⁵ and R. Roehe¹

¹Animal and Veterinary Sciences, SRUC, West Mains Road, Edinburgh, EH9 3JG, UK;

²Division of Genetics and Genomics, The Roslin Institute, R(D)SVS, University of Edinburgh, Easter Bush, Midlothian, EH25 9RG, UK; ³PIC International Group, Ratsteich 31, 24837 Schleswig, Germany; ⁴Future Farming Systems Group, SRUC, West Mains Road, Edinburgh, EH9 3JG, UK; and ⁵Animal Breeding and Genomics Centre, Wageningen Institute of Animal Sciences, Wageningen University, P.O. Box 338, 6700 AH Wageningen, The Netherlands.

Accepted for publication in Livestock Science 2013

Abstract

The aims of this study were to (I) compare models estimating residual energy intake (REI) using lean and fat tissue growth or their proxy traits (average daily gain (ADG) and backfat thickness (BF)); (II) determine genetic characteristics of REI at different growth stages and during the growing period; and (III) examine the genetic and phenotypic relationships of REI with other production traits. Data from 315 pigs of an F₂ generation were used, originated from crossing of Pietrain sires with a commercial dam line. Average daily protein (APD) and lipid deposition (ALD), as measurements of lean and fat tissue growth, were obtained using deuterium dilution technique on live animals. The REI was estimated using 4 different models for energy intake that included, besides other systematic effects, 1) ADG and BF; 2) APD and ALD; 3) and 4) incorporated the same covariables as the first two models, respectively, but pre-adjusted for systematic effects. Genetic parameters and EBV were estimated based on univariate animal models using REML analysis. Over the growing period, heritabilities of different REI, obtained from different models, were all estimated at 0.44 and their genetic correlations were at unity. At different stages of growth, heritabilities for REI were greater ranging from 0.47 to 0.50. Genetic correlations between REI estimates at different stages of growth, obtained using genetic model 4, indicated that REI at 60 to 90 kg was non-significantly ($P > 0.05$) associated with REI at 90 to 120 kg (0.32 ± 0.29) and 120 to 140 kg (0.28 ± 0.28), but REI of the latter stages showed a significant ($P < 0.05$) moderate genetic correlation (0.58 ± 0.21). There were substantial favourable genetic correlations of REI with feed conversion ratio (FCR, 0.84 ± 0.13) and total nitrogen excretion (TNE, 0.85 ± 0.11). The results indicate that REI estimated based on models using proxy traits for lean and fat tissue deposition resulted in only slightly lower accuracies compared to models fitting APD and ALD, which explained a greater variation of energy intake. There is great potential for improvement of REI due to its large heritability. Genetic selection for REI should consider the stages of growth, because of their differences in genetic background. REI explained a large portion of variance in FCR and TNE, therefore selection for REI is expected to result, beside improvement of feed efficiency, in a substantial reduction in environmental pollution of pig production.

Key words: Feed efficiency, Genetic parameters, Growth, Nitrogen excretion, Pigs, Residual energy intake

4.1 Introduction

Improvement of feed efficiency is of great economical interest and therefore one of the main goals within pig breeding programs. Residual feed intake (RFI) has been discussed as an alternative trait to improve feed efficiency (De Haer et al., 1993). Differences in models used to estimate RFI have been shown to result in different relationships between RFI and production traits (Mrode and Kennedy, 1993; Johnson et al., 1999), which can influence genetic gain. In pigs, RFI has been mostly estimated using models adjusting feed intake for average daily gain (ADG) and backfat thickness (BF), often together with metabolic body weight (Gilbert et al., 2007; Cai et al., 2008). Changes in the energy content of diets during the growing period most often occurs, which may have an effect on the accuracy of RFI estimation. Therefore, in the present study, metabolizable energy intake was used to estimate residual energy intake (REI).

Traits used to estimate RFI in the above referenced studies are proxies for lean and fat tissue growth, which are mostly available in practical pig breeding programs. In the present study, however, measures of protein and lipid deposition at different stages of growth were available, which may improve the accuracy of REI estimation. Furthermore, there is a lack of information about genetic characteristics of REI at different growth stages. Shirali et al. (2012) showed the large impact of improving feed conversion ratio (FCR) on nitrogen emissions. This may be due to interrelationships of growth and feed efficiency traits, therefore, the association between REI and nitrogen excretions is of interest to mitigate the environmental pollution of pig production.

The aims were to compare REI-estimating models based on unique measurements of lean and fat tissue growth on live animals with common models using their proxy traits; determine genetic characteristics of REI at different growth stages; examine the genetic and phenotypic relationships of REI with production traits, and to investigate the genetic background of residual energy intake.

4.2 Materials and methods

Animals

The animals, which provided the data used in this study were from a three-generation full-sib design. The founder generation (F_0) consisted of 7 unrelated Pietrain grandsires and 16 unrelated grand-dams bred from a 3-way cross of Leicoma boars with Landrace $\times$ Large White dams. All grand-sires were

heterozygous (*Nn*) at the *ryanodine receptor 1* (*RYR1*) locus. Of the F_1 generation, 8 boars and 40 sows were selected to produce the F_2 generation. The F_2 generation consisted of 315 pigs from the first two parities of the F_1 sows. The pedigree contained 386 individuals in total. All animal care and handling procedures in the federal testing station were reviewed and approved by the Landwirtschaftskammer Schleswig-Holstein, Rendsburg, Germany.

Data

The REI estimates are based on 315 animals from the F_2 generation. Forty eight gilts and 46 barrows from the F_2 generation were single-housed in straw-bedded pens and fed manually, with feed disappearance recorded on a weekly basis. The remaining 117 gilts and 104 barrows were housed in mixed-sexed groups of up to 15 pigs in straw-bedded pens. Animals housed in groups were fed using electronic feeders (ACEMA 48, ACEMO, Pontivy, France), which recorded feed disappearance at each visit. Pigs started the performance test at about 30 kg body weight (BW) and were weighed on a weekly basis. For this study, only the testing period from 60 kg onwards was considered because at this stage pigs were entirely adapted to the electronic feeders. Pigs were weighed at target weights 60, 90, 120 and 140 kg BW. Average BW (SD) at target weights were 61 kg (2.58), 91 kg (2.60), 120 kg (2.69) and 140 kg (2.80), respectively. During growth from 60 to 90, and 90 to 140 kg of BW, pigs were fed *ad libitum* with a diet containing 13.8 MJ of ME/kg, 17% CP and 1.1% lysine, and a diet containing 13.4 MJ of ME/kg, 16.5% CP and 1.0% lysine, respectively. The diets consisted of adequate nutrient supplies to permit maximum protein deposition. For a more detailed description of the data see Landgraf et al. (2006), and Mohrmann et al. (2006).

The deuterium dilution technique was used to determine chemical body composition at the target weights of 60, 90, 120 and 140 kg. This technique is an *in vivo* method predicting empty body water content of the pigs. Using this method, the percentage of fat-free substance of pigs was estimated from the empty body water content. Protein and ash content of the empty body were estimated based on the percentage of the fat-free substance. Percentage of lipid content was the deviation of the percentage of fat-free substance from 100%. The accuracy of this technique to determine body composition has been verified using magnetic resonance imaging on live animals and chemical analysis of serially slaughtered animals using data of the F_1 population of the present experiment in previous studies (Landgraf et al., 2006; Mohrmann et al., 2006). Mohrmann et al. (2006) reported the correlations between the estimates for empty body water, fat free

substances, and protein in fat-free substances obtained from deuterium dilution technique and chemically analysed methods to be 0.92, 0.90, and 0.85, respectively. A detailed description of the use and analysis of the deuterium dilution technique is presented by Landgraf et al. (2006). Average daily protein (APD) and lipid deposition (ALD) rates were calculated as the difference between protein or lipid weight at the two adjacent target weights divided by the number of days between those target weights. Average daily gain was calculated within each growth stage and for the growing period (60 to 140 kg). The BF was measured on the cold left carcass side at the 13th/14th rib interface. Average daily feed intake was calculated as the sum of feed disappearance (kg) divided by number of days for each stage of growth and over the growing period. Average daily energy intake (ADEI) was calculated using ME content of the diet and ADFI. Feed conversion ratio was calculated as the sum of feed disappearance (kg) divided by body weight gain (kg) in each stage of growth and the growing period. Total nitrogen excretion (TNE) was calculated as the average daily nitrogen excretion multiplied by days of growth within each stage of growth as well as the entire growing period. More detailed information about nitrogen excretion is presented in Shirali et al. (2012).

Estimating Residual Energy Intake

Residual metabolizable energy intake estimates were obtained as the residuals of 4 different models for ADEI, as specified in Table 4.1. The analysis was carried out using the GLM procedure of SAS software (SAS Inst. Inc., Cary, NC) and those models will be referred to as basic models.

Models [1] and [3] are based on proxy estimates for protein and lipid deposition whereas models [2] and [4] are based on direct measures of protein and lipid deposition. As BF was only measured on slaughtered pigs, REI models [1] and [3] were only applied to the entire growing period. In preliminary analyses, metabolic mid-BW ($\text{kg BW}^{0.75}$), calculated as the average of start and end $\text{BW}^{0.75}$, was additionally included in the models to estimate REI in order to account for maintenance requirements. However, the effect of metabolic mid-BW was not significant ($P > 0.05$) in those models and the EBV correlation between REI models with and without metabolic mid-BW was 0.99. This indicates that maintenance requirements were already accounted for in the models by other effects fitted such as start and end BW and body composition.

4 Genetics of energy efficiency

Table 4.1 Basic models used to obtain different residual energy intake estimates.

Model	Trait ^a
[1]	REI1 $ADEI = FIXED + COV + b_3 \times ADG + b_4 \times BF + REI1$
[2]	REI2 $ADEI = FIXED + COV + b_3 \times APD + b_4 \times ALD + REI2$
[3]	REI3 $ADEI = FIXED + COV + b_3 \times ADGA + b_4 \times BFA + REI3$
[4]	REI4 $ADEI = FIXED + COV + b_3 \times APDA + b_4 \times ALPDA + REI4$

^a Where in Table 4.1, FIXED = fixed effects of $\mu + SEX + HT + BT + RYR1 + HR$ where μ = the population mean; SEX = sex (gilts vs. barrows); HT = housing type (Single vs. Group housed); BT = batch (9 levels); RYR1 = ryanodine receptor 1 (NN, Nn and nn); HR = herd (2 levels); COV = covariates of $b_1 \times SW + b_2 \times EW$ where SW = body weight at start of the growth period, kg; EW = body weight at the end of the growth period, kg; APD = average daily protein deposition, g/d; ALD = average daily lipid deposition, g/d; BF = backfat thickness, cm; APDA = adjusted average daily protein deposition estimated as residual of model $APD = FIXED + COV + APDA$; ALDA = adjusted average daily lipid deposition estimated as the residual of model $ALD = FIXED + COV + ALDA$; ADGA = adjusted average daily gain estimated as the residual of model $ADG = FIXED + COV + ADGA$; BFA = adjusted backfat thickness estimated as the residual of model $BF = FIXED + COV + BFA$; REI1 to REI4 = residual energy intake using models [1] to [4].

Genetic parameter estimation

Estimates for genetic parameters and EBV for REI traits at different stages of growth and during the growing period were obtained in REML analyses, using the program ASReml (Gilmour et al., 2009), and adding to the basic models [1] to [4] the random animal effect. These models will be referred to as genetic models [1] to [4]. The genetic parameters and EBV for production traits were only calculated for the growing period. The mixed model included sex, housing type, batch, RYR1, herd, start and end BW as systematic effects and the animal as random effect. The effects of litter and permanent environment were not included in the model as the proportions of variance explained due to these effects were not significantly different from zero when tested separately in the model. The mixed model is represented as $\mathbf{y} = \mathbf{Xb} + \mathbf{Za} + \mathbf{e}$, where $\mathbf{y}$ is the vector of observations; $\mathbf{X}$ and $\mathbf{Z}$ are incidence matrices relating records to effects $\mathbf{b}$ and $\mathbf{a}$; $\mathbf{b}$ is the vector of solution for fixed effects; $\mathbf{a}$ is the vector of solution for additive genetic effects [$\sim N(0, \mathbf{A}\sigma_a^2)$]; and $\mathbf{e}$ is the vector of residuals [$\sim N(0, \mathbf{I}\sigma_e^2)$]. The matrix $\mathbf{A}$ describes the additive genetic relationships between animals and $\mathbf{I}$ is the identity matrix. Because the multi-trait analysis did not converge, likely due to the small data size and high number of parameters to be estimated, genetic correlations of REI with production

traits during the growing period were approximated by the correlations between the univariate EBV of these traits, following Calo et al (1973):

$$r_{A_{XY}} = \frac{\sqrt{\sum r_{Xi}^2 \sum r_{Yi}^2}}{\sum r_{Xi}^2 \sum r_{Yi}^2} r_{\hat{A}_X \hat{A}_Y}$$

where $r_{A_{XY}}$ is the genetic correlation between traits X and Y, $r_{\hat{A}_X \hat{A}_Y}$ is the correlation between EBV of the two traits, and r_{Xi}^2 and r_{Yi}^2 are the corresponding reliabilities of the trait EBV for ith individual respectively. Reliability (i.e. square of accuracy) was estimated as $1 - \frac{s_i^2}{(1 + f_i)\sigma_A^2}$ where s_i is the standard error for the ith individual, f_i is the inbreeding coefficient, $1 + f_i$ is the diagonal element of relationship matrix, and σ_A^2 is the genetic variance.

The standard error of r_A was calculated using the following formula (Falconer, 1981):

$$\sigma(r_{A_{XY}}) = \frac{1 - r_A^2}{\sqrt{2}} \sqrt{\left[\frac{\sigma(h_X^2) \sigma(h_Y^2)}{h_X^2 h_Y^2} \right]}$$

where σ denotes standard error, and h_X^2 and h_Y^2 are the heritabilities of traits X and Y, respectively.

The phenotypic correlations (r_p) were calculated by performing multivariate analysis of variance to predict residual correlations between REI and production traits after adjusting production traits for sex, housing type, batch, *RYS1*, herd, start and end BW as systematic effects using GLM procedure in SAS software (SAS Institute, 2006). Moreover, EBV of the analysed traits were examined in a principal component analysis (Statistical graphics Corp., Rockville, MD, USA) to get insight into the biological background of different trait complexes, the weight of REI in those trait complexes and the variance explained by each of the traits complexes, which gives an indication of their potential genetic improvement in breeding programmes.

4.3 Results

Model Comparison of REI

Table 4.2 presents the characteristics of REI using different models during the growing period. The coefficient of determination (R^2) associated with the four basic REI models indicated that models [2] and [4], which included APD and ALD or their adjusted estimates, respectively, explained 2.6% more variance in ADEI than

4 Genetics of energy efficiency

models [1] and [3], in which their proxy measurements, (ADG and BF) were fitted. Genetic models [2] and [4] resulted in 8.6% less phenotypic variation in REI than genetic models [1] and [3], indicating that more variation is explained by fitting APD and ALD in the model compared with fitting ADG and BF. Interestingly, no differences in heritability estimates were obtained using these different models. Although, basic models [2] and [4] resulted in equal goodness of fit, basic and genetic models [4], fitting pre-adjusted values of APD and ALD, was used in further analyses to avoid any influence of systematic effects on the results. Genetic and phenotypic correlations among all REI estimates were unity.

Table 4.2 Estimates of root mean square error (RMSE, MJ/d ME), coefficient of determination (R^2), phenotypic variance (σ_p^2), heritability (h^2) and its SE for residual energy intake measurements during the growing period.

Model ^a	RMSE	R^2	σ_p^2	h^2	SE
[1]	1.99	0.77	4.43	0.44	0.20
[2]	1.89	0.79	4.05	0.44	0.21
[3]	1.99	0.77	4.43	0.44	0.20
[4]	1.89	0.79	4.05	0.44	0.21

^a RMSE and R^2 estimated using the basic models; σ_p^2 , h^2 and its SE estimated using the genetic models.

Table 4.3 shows the RMSE and R^2 of REI using basic model [4] and the genetic parameters using the genetic model [4] at different growth stages. The greatest coefficient of determination was obtained during 60 to 90 kg body weight. The phenotypic variance of REI increased with increase in stage of growth. Phenotypic variance of REI from 60 to 90 kg was 16% and 38% lower than from 90 to 120 and 120 to 140 kg, respectively; and phenotypic variance of REI from 90 to 120 kg was 26% lower than during growth from 120 to 140 kg. The REI heritabilities were large and significantly different from zero ($P < 0.05$) and showed small differences between stages of growth. The magnitude of heritabilities was not influenced by the lower R^2 at later stages. The genetic correlations between REI estimates at 60 to 90 kg and those at later stages of growth were low and non-significantly different from zero ($P > 0.05$). Only the REI at 90 to 120 kg resulted in a significant moderate genetic correlation (0.58 ± 0.21) with that at 120 to 140 kg. The genetic correlations between REI during the total analysed growing period (60 to 140 kg) with those at different stages of growth showed an increase from 0.60 ± 0.21 at 60 to 90 kg, to 0.82 ± 0.11 at 90 to 120 kg, and 0.84 ± 0.09 at 120 to 140 kg (data not shown).

Characteristics of production traits

Characteristics of the production traits during the growing period are shown in Table 4.4, along with univariate estimates of heritabilities. The heritabilities for ADG, APD, ALD, BF, and ADEI were significantly different from zero ($P < 0.05$) and of moderate to large magnitude in the range from 0.39 to 0.66, whereas those of FCR and TNE were lower and non-significantly different from zero. The proxy traits used in the REI models ADG (0.64 ± 0.19) and BF (0.44 ± 0.17) showed larger heritabilities than the corresponding protein and lipid deposition traits APD (0.46 ± 0.19) and ALD (0.39 ± 0.18). Furthermore, the CVs of ADG and APD were similar, indicating similar variation in these traits, whereas the CV of ALD was greater than BF, indicating that BF did not fully account for the relative variation in fat deposition.

Correlations between REI and production traits

Estimates of genetic and phenotypic correlations between REI and production traits for the growing period (60 to 140 kg) are given in Table 4.4. As expected, REI was phenotypically uncorrelated with APD and ALD, because these traits were fitted in the model to predict REI. In addition, REI showed no phenotypic correlation with ADG and BF. The genetic correlations of REI with ADG (0.27 ± 0.25), APD (0.30 ± 0.29), ALD (0.36 ± 0.29) and BF (0.16 ± 0.30) deviated from zero, although these estimates had large standard errors and were thus statistically not significantly different from zero ($P > 0.05$). The REI and ADEI had large phenotypic (0.55) and genetic (0.80 ± 0.10) correlations, as expected from part-whole traits. The REI showed substantial favourable phenotypic (0.69) and genetic (0.84 ± 0.13) correlations with FCR, indicating that REI explained a great proportion of the variance in FCR. The REI showed a favourable phenotypic (0.69) and genetic (0.85 ± 0.11) correlation with TNE, highlighting that REI could be used to reduce the environmental impact of pig production.

4 Genetics of energy efficiency

Table 4.3 Estimates of root mean square error (RMSE, MJ/d ME), coefficient of determination (R^2), phenotypic variance (σ_p^2), heritability (h^2), its SE for residual energy intake (REI) at different stages of growth, and their genetic and phenotypic correlations

Stages of growth	REI ^a				Genetic and phenotypic correlations ^b		
	RMSE	R^2	σ_p^2	h^2 (SE)	60-90 kg	90-120 kg	120-140 kg
60-90 kg	2.55	0.74	7.52	0.47 (0.21)		0.17*	0.24**
90-120 kg	2.86	0.66	8.95	0.47 (0.22)	0.32 ±0.29		0.31**
120-140 kg	3.30	0.68	12.10	0.50 (0.20)	0.28 ±0.28	0.58 ±0.21	

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

^a RMSE and R^2 estimated using the basic model [4]; the σ_p^2 , h^2 and its SE estimated using the genetic model [4]

^b Estimated based on genetic model [4] and below and above the diagonal are genetic and phenotypic correlations with their SE, respectively.

Table 4.4 Estimates of mean, coefficient of variation (CV), minimum (MIN), maximum (MAX), phenotypic variance (σ_p^2), heritabilities (h^2), their SE for production traits during the growing period, and their phenotypic (r_p), and genetic (r_g) correlations with residual energy intake

Traits ^a	Mean	CV	MIN	MAX	σ_p^2	h^2 (SE)	r_g (SE) ^b	r_p
ADG, g/d	775	12.82	398	1071	8061	0.64 (0.19)	0.27 (0.25)	- 0.03
APD, g/d	125	12.37	82	172	185	0.46 (0.19)	0.30 (0.29)	- 0.03
ALD, g/d	268	15.55	131	390	1411	0.39 (0.18)	0.36 (0.29)	- 0.07
BF, cm	3.16	14.72	2.00	4.70	0.19	0.44 (0.17)	0.16 (0.30)	0.12
ADEI, MJ/d ME	36.60	11.02	25.58	47.85	10.81	0.66 (0.19)	0.80 (0.10)	0.55 **
FCR	3.47	8.45	2.70	4.39	0.07	0.26 (0.20)	0.84 (0.13)	0.69 **
TNE, kg/pig	5.31	13.45	3.56	7.31	0.33	0.32 (0.21)	0.85 (0.11)	0.69 **

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

^a APD = average daily protein deposition; ALD = average daily lipid deposition; BF = backfat thickness; ADEI = average daily energy intake; FCR = feed conversion ratio; TNE = total nitrogen excretion

^b The genetic correlations are approximations based on correlations between the univariate EBV following Calo et al. (1973).

Principal component analysis

Principal component analysis was performed on EBV estimates of REI and production traits to determine the weights of REI in biological different trait complexes. The results showed that principal component (PC) 1 explained about half of the variance in the data (49%), followed by PC 2 explaining 34% (Table 4.5). Thereafter, the amount of variance explained by PCs dropped significantly, with PC 3 and PC 4 explaining only 11.3% and 3.0%, respectively.

Table 4.5 Characteristic of principal component analysis for residual energy intake (REI), ADG, average daily protein deposition (APD), average daily lipid deposition (ALD), backfat thickness (BF), average daily energy intake (ADEI), feed conversion ratio (FCR), and total nitrogen excretion (TNE)

Component	Eigenvalue	Percentage of variance	Accumulative variance
1	3.91	48.9	48.9
2	2.73	34.2	83.1
3	0.91	11.3	94.4
4	0.24	3.00	97.4
5	0.11	1.32	98.7
6	0.06	0.72	99.4
7	0.04	0.44	99.9
8	0.01	0.15	100

Figure 4.1a shows that PC 1 was associated with growth traits such as ADG, APD, ALD, and their required energy intake (ADEI), which had large weights within this principal component. Moreover, REI shows a small weight in the first PC suggesting that REI is not entirely genetically independent of growth. The PC 2 was associated with efficiency in feed utilization as it was mostly influenced by FCR, TNE, and REI. The PC 3 was associated with body composition as BF showed the largest weight (Figure 4.1b). The 4th PC suggests that an increase in REI is almost equivalent to a loss in body energy content due to decreased ALD, after adjustment for PC 1 to PC 3. In addition, the results also indicated that ADG and APD, as well as TNE and FCR are largely genetically correlated as their weights in different PC are very similar.

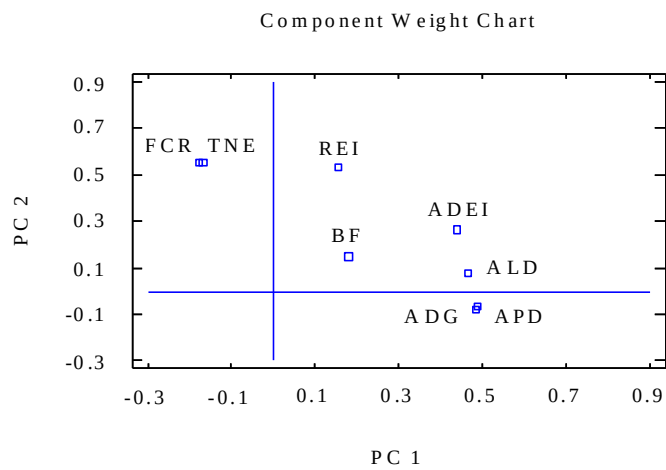


Fig. 1a

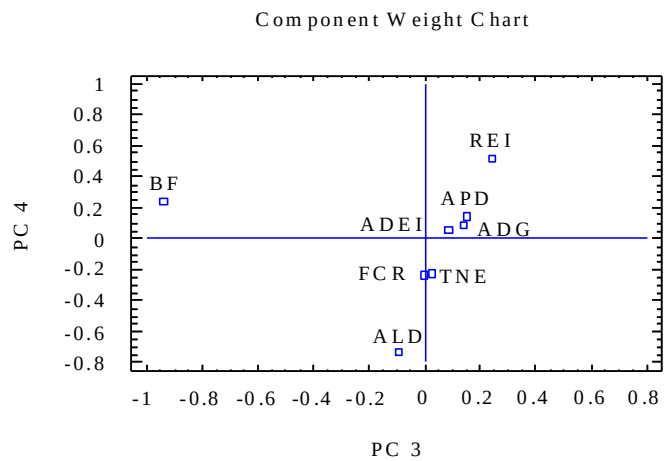


Fig. 1b

Figure 4.1a-b Component weights chart from principal component (PC) analysis of EBV for residual energy intake (REI), ADG, average daily protein deposition (APD), average daily lipid deposition (ALD), average daily energy intake (ADEI), backfat thickness (BF), feed conversion ratio (FCR), and total nitrogen excretion (TNE).

4.4 Discussion

REI model comparison

Accurate measurement of feed efficiency such as residual energy intake, in the light of increasing cost of animal production and shrinking resources, is essential for genetic improvement of efficiency in feed utilization of animals. Comparison of the models to predict REI showed that fitting, besides other systematic effects, the covariables APD and ALD or their pre-adjusted values, explained a larger proportion of the phenotypic variance (79%) in ADEI. This variance was only slightly lower (77%) when using the proxy measures (ADG and BF) in addition to all other systematic effects. Cai et al. (2008) estimated that RFI explained a lower phenotypic proportion on the feed intake variance at 66% when fitting, besides systematic effects, the adjusted values of ADG and ultrasonic backfat (UBF). Bunter et al. (2010) reported that 60% of the phenotypic variance in feed intake was accounted for by using a model that included adjusted values of ADG, UBF and metabolic BW in addition to systematic effects. Gilbert et al. (2007) found that 66% to 76% of variance in feed intake was accounted for, using alternative models for feed intake containing ADG and UBF; or ADG, lean meat content and average metabolic BW. Possible reasons for the larger variance explained by models in the current study may be due to the use of ADEI, which incorporate the changes in energy of diets during the growth period, instead of ADFI; the use of BF, which was measured on the carcass and is expected to be more accurately measured than UBF, which is widely used in RFI studies; and the different genetic make-up of those experimental populations, which is in the present study comprised of a F_2 population founded from crosses of Pietrain sires and commercial crossbred dams, whereas Cai et al., 2008 used Yorkshire pigs selected for reduced RFI and Gilbert et al., 2007 used Large White growing pigs selected upward and downward for RFI. The heritabilities of different REI estimates were similar and the estimated genetic correlations between different REI estimates were unity. This indicates that fitting proxy measures for lean and fat deposition (ADG and BF) to predict REI provide similar genetic results compared with the use of the measures of lean and fat deposition themselves. This also means that the proportion of the phenotypic variance explained by the model did not influence the estimates of the genetic parameters for REI. However, the 8.6% greater phenotypic variance for REI using ADG and BF as proxies for lean and fat tissue growth indicates that not all variance in energy intake associated with growth is accounted for, so that less accurate estimates of REI may be obtained. Ignoring in the present case, parts of the systematic effects by using proxy measurements to estimate REI can result in

biased EBV and result in greater mean square errors of those EBV as analytically shown by Henderson (1975). The biological reason is that ADG and BF explain approximately protein and lipid deposition.

Heritabilities

The estimated heritability of 0.44 for REI in the present study was in the upper range of heritabilities estimated in the literature, which ranged from 0.10 to 0.40 (Hoffmann et al., 1992; De Haer et al., 1993; Mrode and Kennedy, 1993; De Vries et al., 1994; Von Felde et al., 1996; Johnson et al., 1999; Labroue et al., 1999; Gilbert et al., 2007; Cai et al., 2008; Bunter et al., 2010; Saintilan et al., 2011). Kennedy et al. (1993) discussed that the heritability of RFI can be variable depending on the genetic and phenotypic parameters among its component traits, and depends considerably on the environmental correlation between feed intake and production. Mrode and Kennedy (1993) obtained heritabilities for RFI of 0.33, 0.30, and 0.38 using models in which feed intake was adjusted for ADG; ADG and UBF; or lean growth rate, respectively, for pen averages of Yorkshire, Landrace and Duroc boars growing from 30 to 90 kg BW. Johnson et al. (1999) reported heritabilities for RFI of 0.17, 0.11, 0.15, and 0.10 using models in which feed intake was adjusted for ADG; ADG, and UBF; ADG, and loin eye area; and ADG, UBF, and loin eye area, respectively, in Large White boars grown from 100 to 176 days. Cai et al. (2008) estimated a heritability of 0.29 for RFI using a model fitting adjusted values of ADG and UBF in Yorkshire pigs with on test age of 90 days and end weight of 115 kg. Saintilan et al. (2011) reported a heritability of 0.40 for RFI using a model including ADG, BF, dressing percentage, lean meat content, and maintenance requirements based on data of Pietrain raised from 35 to 107 kg BW. Some possible underlying reasons for differences between heritability estimates include breed differences, population structure, model differences, and length of growth. In the current study a crossbred F_2 population has been used, which may inflate the variance component and the heritability estimates through partitioning some of the dominance variance as additive variance, as dominance effects could not be fitted in the models. Johansson et al. (1993) showed that the including a litter effect in the model accounts for most of the dominance variance. However, the litter effect was not significant for the analysed traits suggesting that the effect of dominance may be negligible for the analysed traits.

To our knowledge very few heritability estimates exist for nitrogen excretion traits. The heritability of 0.32 for total nitrogen excretion trait was in agreement with Saintilan et al. (2013) reporting heritabilities in the range of 0.31 and 0.40 for nitrogen excretion in growing pigs for four pig breeds: two dam breeds Landrace

and Large White, as well as two sire breeds Large White and Pietrain for a growth period from 30 to 110 kg BW.

In the current study, heritabilities of ADG, ADEI and BF were 0.64, 0.66 and 0.46, respectively. Mrode and Kennedy (1993) reported lower heritability estimates of 0.43 and 0.45 for ADG and ADFI, respectively, but a larger heritability of 0.59 for BF. Johnson et al. (1999) obtained consistently lower heritabilities for ADG (0.24), ADFI (0.23) and UBF (0.36) than those obtained in the present study. Cai et al. (2008) estimated lower heritabilities for ADG (0.42) and ADFI (0.51) but a larger heritability for UBF (0.68). Dufrasne et al. (2011) estimated for ADG a heritability of 0.41, which was lower than the result of our study, for a crossbred population based on Pietrain boars and Landrace sows performance tested between 100 to 210 days. Consistent with heritability of BF in current study, Solanes et al. 2004 reported heritability of 0.37 to 0.43 for UBF at 100 kg BW for Landrace, Yorkshire, and Hampshire breeds.

In the present study, the heritability of APD and ALD was 28% and 15% lower than their proxy traits: ADG and BF, respectively. For ALD the lower heritability can be explained by the observation that growth rate of tissue showed lower genetic determination than body composition. The lower heritability of APD compared to ADG can be explained by the fact that the former trait resembling lean tissue growth only and the latter trait a combination of largely correlated lean and fat tissue growth. Mrode and Kennedy (1993) reported a heritability of 0.39 for lean growth rate, which is smaller than the heritability for APD identified in the present study. Saintilan et al. (2011) estimated the heritability for lean meat content (0.48), which is in accordance with the heritability for APD obtained in the current study. Greater heritabilities of body composition traits are shown by Edwards et al. (2006), who reported heritabilities of 0.55 and 0.46 for empty body protein and empty body lipid at 26 weeks of age, respectively, for crosses of Duroc and Pietrain-sired pigs with Yorkshire or Yorkshire-Landrace dams.

REI at different stages of growth

Although heritabilities of REI were similar at different stages of growth, phenotypic and genetic variance of REI increased with increasing growth. Greater phenotypic and genetic variance in REI at later growth stages indicated that these stages are associated with lower efficiency in feed utilization. Shirali et al. (2012) reported that nitrogen efficiency gradually decreased with increasing growth stages. This study estimated during early growth (from 60 to 90 kg) the greatest nitrogen efficiency (32%) (nitrogen retention/nitrogen intake), the lowest nitrogen excretion

and greatest retention; however found during later growth (from 120 to 140 kg) the lowest nitrogen efficiency (25%), which was associated with lowest nitrogen retention and greatest nitrogen intake. The present results indicated that selecting for REI in particular at later growth stages is expected to improve production efficiency and reduce nitrogen excretion (greenhouse gas emission) from pig production. In addition, variation in REI may be influenced by metabolism, efficiency in digestion, feed energy utilization, tissue turnover rates and activity levels (Herd and Arthur, 2009; Dekkers and Gilbert, 2010). The low to moderate genetic correlation among REI estimates obtained at different stages of growth revealed that REI had a different genetic background at each stage of growth. Duthie et al. (2008) reported that different quantitative trait loci of growth as well as feed intake traits are switched on and off at different growth stages. As the genetic correlations suggest that this may also be the case for REI, it should be treated as different traits at different stage of growth to obtain the greatest selection response. In addition, the genetic variance at later stages of growth was almost two times larger compared with earlier growth stages, implying that greater selection response could be achieved under selection for REI at later growth stages.

Genetic and phenotypic correlations

Feed efficiency, growth and feed intake are all interrelated (Johnson et al., 1999; Ruten et al., 1999), where better understanding of their relationship is required to accurately predict the outcome of selection. As expected, the phenotypic correlations of REI with APD and ALD were zero. This was due to the fact that REI was estimated by adjustment for those traits at the phenotypic level. The genetic correlations of REI with APD, ADG, ALD and BF showed low deviations from zero, which were not significant, suggesting that adjustment on the phenotypic level resulted also in independence of REI from lean and fat tissue growth on the genetic level. In the current study, REI did not show any phenotypic or genetic correlation with ADG, which is expected because ADG is the sum of lean and fat tissue growth. In addition, a non-significant genetic and phenotypic correlations were obtained between REI and BF, which indicates that BF (measured on the carcass) is a good proxy measurement for fat deposition. Bunter et al. (2010) obtained genetic correlations of RFI with ADG (0.24 ± 0.12) and UBF (0.20 ± 0.11) in purebred Yorkshire pigs, when RFI accounted for the adjusted values of ADG, UBF and metabolic mid-BW along with other fixed effects. Johnson et al. (1999) found non-significant genetic correlations of RFI with ADG (0.17) and UBF (0.22) in Large White boars tested in individual pens, when RFI accounted for initial test age and BW as well as ADG and UBF on test. Johnson et al. (1999) reported a large genetic

correlation (0.67) between RFI and BF when RFI was not adjusted for UBF. Cai et al. (2008) reported a genetic correlation of RFI with ADG (0.17 ± 0.18), which was smaller than in the current study, and a slightly negative genetic correlation of RFI with UBF (-0.14 ± 0.16) in purebred Yorkshire pigs grown from 40 to 115 kg BW, when RFI was obtained by adjustment for ADG and UBF besides other fixed effects. Although ADEI was phenotypically and genetically correlated with both growth and REI, selecting for REI is expected to influence the proportion of ADEI, which is not associated with lean and fat tissue growth. The strong favourable phenotypic and genetic correlations between REI and FCR suggested that selection for REI will substantially improve FCR. The genetic correlations of REI with ADEI (0.80), and FCR (0.84) are at the upper level compared with the literature estimates of RFI with ADFI, which ranged from 0.52 to 0.77 and RFI with FCR, which ranged from 0.71 to 0.85 (Gilbert et al., 2007; Cai et al., 2008; Bunter et al., 2010; Saintilan et al., 2011; Saintilan et al., 2013). The knowledge of genetic and phenotypic associations of RFI with nitrogen excretion traits is scarce. The current study suggested a large genetic (0.85) and phenotypic (0.69) correlations between REI and TNE which is in agreement with results of Saintilan et al. (2013) reporting large genetic (0.84) and phenotypic (0.79) correlations for RFI and nitrogen excretion. Kennedy et al. (1993) showed that RFI is phenotypically independent from the component traits, except for feed intake, but it is not genetically independent, and the sign and magnitude of the genetic correlations are influenced by the genetic and environmental correlations of component traits with feed intake. Aside from sampling errors, the differences in genetic correlation estimates between studies may be due to population differences in phenotypic and genetic correlations among traits (Cai et al., 2008). The genetic correlations of RFI with component traits are a direct result of the phenotypic and genetic parameters of its component traits in the population (Kennedy et al., 1993). The first PC indicated that most of the variation of EBV in the analysed traits is associated with growth and its required energy intake, suggesting a great potential for genetic improvement of those traits. Only slightly less variation of EBV is explained by the second PC reflecting traits associated with feed efficiency, which also suggests their great potential for genetic improvement. In this PC, growth and body composition traits showed only minor influence, implying that REI as a measure of feed efficiency can improve production efficiency, reduce environmental pollution, and reduce the use of energy resources independent from growth and body composition. Relatively small variation is explained by the third PC, which reflects body composition determined by BF. BF is shown to be greatly independent from all other analysed traits and demonstrated the greatest weight of all traits for this PC.

4.5 Conclusions

The results indicate that the use of proxy traits for lean and fat tissue growth, such as ADG and BF, to estimate REI, resulted in similar estimates than their direct measurements (ALD and APD). However, the lower variance of energy intake explained by models, which fitted proxy traits (ADG and BF), indicates less accuracy in estimation of REI, which may result in lower selection response compared with using the direct measurements of lean and fat tissue growth. The large heritability identified for REI suggests great selection potential. The REI demonstrated different genetic background at different growth stages, implying that selection for REI will be more efficient if each stage of growth is considered separately. Most importantly, the later stages of growth, which are expected to result in larger selection response due to large genetic variance of REI. The principal components of breeding values indicate that there are two main components, firstly growth and secondly feed efficiency, which explains most of the genetic variation in the analysed traits. This indicated that these components can be improved independently and is expected to result in substantial genetic improvement. The REI demonstrated favourable large genetic correlations with TNE and FCR, implying that selection for REI will result in a reduction of TNE and consequently a reduction in the environmental pollution of pig production, as well as a reduction in the use of energy resources.

References

- Bunter, K. L., Cai, W., Johnston, D.J., Dekkers, J.C.M., 2010. Selection to reduce residual feed intake in pigs produces a correlated response in juvenile insulin-like growth factor-I concentration. *J. Anim. Sci.* 88:1973-1981.
- Cai, W., Casey, D.S., Dekkers, J.C.M., 2008. Selection response and genetic parameters for residual feed intake in Yorkshire swine. *J. Anim. Sci.* 86:287-298.
- Calo, L.L., Mcdowell, R.E., Van Vleck, L.D., Miller, P.D., 1973. Genetic aspects of beef production among Holstein-Friesians pedigree selected for milk-production. *J. Anim. Sci.* 37:676-682.
- De Haer, L.C.M., Luiting, P., Aarts, H.L.M., 1993. Relations among individual (residual) feed-intake, growth-performance and feed-intake pattern of growing pigs in-group housing. *Livest. Prod. Sci.* 36:233-253.
- De Vries, A.G., van der Wal, P.G., Long, T., Eikelenboom, G., Merks, J.W.M., 1994. Genetic-parameters of pork quality and production traits in Yorkshire populations. *Livest. Prod. Sci.* 40:277-289.

- Dekkers, J.C.M. Gilbert, H., 2010. Genetic and biological aspect of residual feed intake in pigs. No. 287 in Proc. 9th World Congr. Genet. Appl. Livest. Prod. , Leipzig, Germany.
- Dufresne, M., Rustin, M., Jaspard, V., Wavreile, J., Gengler, N., 2011. Using test station and on-farm data for the genetic evaluation of Pietrain boars used on Landrace sows for growth performance. *J. Anim. Sci.* 89:3872-3880.
- Duthie, C., Simm, G., Doeschl-Wilson, A., Kalm, E., Knap, P.W., Roehe, R., 2008. Quantitative trait loci for chemical body composition traits in pigs and their positional associations with body tissues, growth and feed intake. *Anim. Genet.* 39:130-140.
- Edwards, D.B., Tempelman, R.J., Bates, R.O., 2006. Evaluation of Duroc- vs. Pietrain-sired pigs for growth and composition. *J. Anim. Sci.* 84:266-275.
- Falconer, D. S., 1981. Introduction to quantitative genetics. Second ed. Longman Group Ltd, UK.
- Gilbert, H., Bidanel, J.P., Gruand, J., Caritez, J.C., Billon, Y., Guillouet, P., Lagant, H., Noblet, J., Sellier, P., 2007. Genetic parameters for residual feed intake in growing pigs, with emphasis on genetic relationships with carcass and meat quality traits. *J. Anim. Sci.* 85:3182-3188.
- Gilmour, A.R., Gogel, B.J., Cullis, B.R., Thompson, R., 2009. ASReml user guide. Release 3.0. VSN Int. Ltd., Hemel Hempstead, UK.
- Henderson, C. R., 1975. Comparison of alternative sire evaluation method. *J. Anim. Sci.* 41:760.
- Herd, R. M., Arthur, P. F., 2009. Physiological basis for residual feed intake. *J. Anim. Sci.* 87:E64-E71.
- Hoffmann, L., Jentsch, W., Beyer, M., 1992. Untersuchungen zum Energieumsatz wachsender Schweine im Lebendmasseabschnitt von 10-50 kg. 3: Energieerhaltungsbedarf wachsender Schweine. *Arch. Anim. Nutr.* 42:235-248.
- Johansson, K., Kennedy, B.W., Quinton, M., 1993. Prediction of breeding values and dominance effects from mixed models with approximations of the dominance relationship matrix. *Livest. Prod. Sci.* 34:213-223.
- Johnson, Z. B., Chewning, J.J., Nugent, III, R.A., 1999. Genetic parameters for production traits and measures of residual feed intake in large white swine. *J. Anim. Sci.* 77:1679-1685.
- Kennedy, B.W., van der Werf, J.H., Meuwissen, T. H., 1993. Genetic and statistical properties of residual feed intake. *J. Anim. Sci.* 71:3239-3250.
- Labroue, F., Maignel, L., Sellier, P., Noblet, J., 1999. Consommation résiduelle chez le porc en croissance alimenté à volonté. Méthode de calcul et variabilité génétique. *Journ. Rech. Porcine Fr.* 31:167-174.

- Landgraf, S., Susenbeth, A., Knap, P.W., Looft, H., Plastow, G.S., Kalm, E., Roehe, R., 2006. Allometric association between in vivo estimation of body composition during growth using deuterium dilution technique and chemical analysis of serial slaughtered pigs. *Anim. Sci.* 82:223-231.
- Mohrmann, M., Roehe, R., Susenbeth, A., Baulain, U., Knap, P.W., Looft, H., Plastow, G. S., Kalm, E., 2006. Association between body composition of growing pigs determined by magnetic resonance imaging, deuterium dilution technique and chemical analysis. *Meat Sci.* 72:518-531.
- Mrode, R. A., Kennedy, B.W., 1993. Genetic-variation in measures of food efficiency in pigs and their genetic-relationships with growth-rate and backfat. *Anim. Prod.* 56:225-232.
- Ruten, W., Tholen, E., Rodehutsord, M., Trappmann, W., 1999: Evaluation of the efficiency of protein utilization as an additional selection criterion in pigs. *Zuchtungskunde* 71(4): 277-292
- Saintilan, R., Merour, I., Schwob, S., Sellier, P., Bidanel, J.P., Gilbert, H., 2011. Genetic parameters and halothane genotype effect for residual feed intake in Pietrain growing pigs. *Livest. Sci.* 142(1):203-209. (Abstr.)
- Saintilan, R., Merour, I., Brossard, L., Tribout, T., Dourmad, J.Y., Sellier, P., Bidanel, J.P., van Milgen, J., Gilbert, H., 2013. Genetics of residual feed intake in growing pigs: relationships with production traits, and nitrogen and phosphorus excretion traits. *J. Anim. Sci.* 91:2542-2554.
- Shirali, M., Doeschl-Wilson, A., Knap, P.W., Duthie, C., Kanis, E., van Arendonk, J.A.M., Roehe, R., 2012. Nitrogen excretion at different stages of growth and its association with production traits in growing pigs. *J. Anim. Sci.* 90:1756-1765.
- Solanes X.F., Kihlberg, M., Andersson, K., Lundeheim, N., 2004. Maternal effects on daily weight gain and ultrasonically recorded backfat in Swedish Landrace, Swedish Yorkshire, and Swedish Hampshire purebred pigs. *Acta Agric. Scand. A Anim. Sci.* 54(2):67-74.
- Von Felde, A., Roehe, R., Looft, H., Kalm, E., 1996. Genetic association between feed intake and feed intake behaviour at different stages of growth of group-housed boars. *Livest. Prod. Sci.* 47:11-22.

5

Predicting energy efficiency and nitrogen excretion in growing pigs with different growth potential using a mechanistic biological growth model

M. Shirali^{1&5}, A. Doeschl-Wilson², P.W. Knap³, E. Kanis⁵, C. Duthie⁴, J.A.M. van Arendonk⁵ and R. Roehe¹

¹Animal and Veterinary Sciences, SRUC, West Mains Road, Edinburgh, EH9 3JG, UK;

²Division of Genetics and Genomics, The Roslin Institute, R(D)SVS, University of Edinburgh, Easter Bush, Midlothian, EH25 9RG, UK; ³PIC International Group, Ratsteich 31, 24837 Schleswig, Germany; ⁴Future Farming Systems Group, SRUC, West Mains Road, Edinburgh, EH9 3JG, UK; and ⁵Animal Breeding and Genomics Centre, Wageningen Institute of Animal Sciences, Wageningen University, P.O. Box 338, 6700 AH Wageningen, The Netherlands.

Submitted to Animal 2013

Abstract

In this study a mechanistic biological growth model was used to obtain estimates for feed efficiency and nitrogen excretion traits throughout the growth period, and to investigate how selection for growth impacts these traits. The model was fitted to longitudinal body protein and lipid measurements of 315 crossbred pigs to obtain input parameters which were used to generate an *in-silico* population of 1000 pigs to investigate feed efficiency and nitrogen excretion and other performance traits. In comparison, input model parameters presented in the literature from two genetically different purebred commercial lines (dam Line A and sire Line B) were used to estimate feed efficiency and pollution traits over growth. The effect of selection for ADG (using three categories – lower, average and upper defined by 1 SD deviation from the average ADG from 30 to 100 kg BW) was explored by comparing model predictions for body composition, REI and TNE of pigs. During growth from 30 to 100 kg, the comparison of the lower and upper ADG category of the crossbred population showed 20% lower TNE (3.06 ± 0.04 vs. 3.82 ± 0.04 , kg/pig) and 26% lower REI (473 ± 3.12 vs. 635 ± 3.15 , MJ ME/pig) corresponding to faster lean growth represented by 30% to 32% larger growth rate and 32% shorter growth period. The largest differences in TNE between upper and lower growth categories were achieved at 150 days of age which coincide with time of maximum protein and lipid deposition. Comparing Line A, highly selection for fast growth, with the average category of crossbred pigs showed 21% and 33% lower REI and TNE, respectively. The Line B, highly selection for feed efficiency and leanness, had 10% lower REI and 26% lower TNE compared to the average category of crossbred pigs. This indicated the effect of selection for fast growth, feed efficiency and leanness on energy and nitrogen efficiency. The results of the biological growth model can be used to develop optimal genetic and production strategies, e.g. reduction in slaughter weight from 130 to 100 kg BW resulted in a substantial decrease in marginal REI of 302 MJ ME or (£9.96 Cost saving from REI) and marginal nitrogen excretion of 1.60 kg per pig space and year in the finishing unit. Moreover, based on the results of the biological growth model, the influence of changes of production traits during growth on energy and nitrogen efficiency can be estimated to optimise genetic strategies.

Key words: Feed efficiency, Growth models, Nitrogen excretion, Pigs

5.1 Introduction

Expansion of pork production to meet the nutritional requirements of an increasing world population has caused challenges associated with limited feed resources for animals and environmental impact of production. To overcome these challenges improving feed efficiency and reducing nitrogen pollution has gained great importance. Previous studies by Fernandez et al. (1999); Jones et al. (2008); Knap, (2012) and Shirali et al. (2012) indicate a substantial favourable association between growth and feed efficiency and nitrogen excretion in growing pigs. For genetic improvement programmes, residual feed intake (RFI) has been discussed as a measure of feed efficiency (De Haer et al., 1993) and has been shown to improve feed efficiency in experimental selection lines (Cai et al., 2008; Gilbert et al., 2007). Accurate estimates of RFI and nitrogen excretion based on measures of individual lean and fat tissue growth in large numbers of animals is however difficult and expensive. Therefore, these traits have been estimated using approximate models, e.g. RFI has been mostly estimated using models adjusting feed intake for proxy traits of lean and fat tissue growth such as ADG and backfat thickness and often together with metabolic BW (Gilbert et al., 2007; Cai et al., 2008). Measurements of protein and lipid growth are expected to yield more accurate estimates of feed efficiency and nitrogen excretion as they are more closely linked to the underlying biological processes, but are usually not available in practical breeding programs. In addition, residual metabolizable energy intake (REI) would be more appropriate for modelling of feed efficiency than RFI, because it allows generalisation of the results since the diets used can vary in energy content during growth within and between populations. Also, differences in the models used to estimate RFI have been shown to result in different relationships between RFI and production traits (Johnson et al., 1999; Mrode and Kennedy, 1993), which can influence genetic gain.

Thus, statistical methods with readily available proxy traits may result in inaccurate estimates of feed efficiency and nitrogen excretion, as they provide a poor representation of the underlying biological processes for these traits. Also, nitrogen excretion and feed efficiency are likely to vary throughout the growth of pigs, but statistical models are not well equipped to describe the dynamics of these traits.

Mechanistic biological pig growth models, on the other hand, integrate current knowledge about the dynamic influence of various genetic and environmental factors underlying pig growth. Such growth models have been used to determine and parameterize biological traits of pigs that by definition are a more accurate representation of the animal's intrinsic growth potential and thus less dependent

on environmental and dietary conditions than readily observable production traits (Doeschl-Wilson et al., 2007), and to evaluate their effect on production traits (e.g. De Lange et al., 2001). There are several types of mechanistic models that simulate the growth of pigs (Black et al., 1986; De Lange, 1995; Green and Whittemore, 2003; van Milgen et al., 2000; Wellock et al., 2003). Despite of differences in individual model assumptions, scope and resolution, all of these models however essentially simulate the allocation of dietary protein and energy into different biological processes associated with basic maintenance and growth processes over time (De Lange et al., 2001). The nutritional framework upon which these models are built presents a valuable opportunity for estimating nitrogen excretion and residual energy intake, i.e. the metabolizable energy that has not been used for protein and lipid retention as well as satisfy maintenance requirements, from a biological rather than a pure statistical perspective and thus to explore novel ways of minimizing these.

To our knowledge no pig growth model has been applied to estimate feed efficiency by estimating REI or alternatively RFI. However, some studies have shown estimations of nitrogen excretion through growth modelling (Knap, 2012; Morel and Wood, 2005). In the current study a semi-stochastic, dynamic growth model to estimate performance for a population of pigs (Knap, 1999; Knap, 2000b; Knap, 2000c) was extended to estimate residual energy intake and nitrogen excretion. The model takes genetic differences in growth potentials and maintenance requirements into account and describes the allocation of nutrients towards maintenance and growth processes on a daily basis depending on the physiological status of the individual, the environmental and dietary conditions and the genetic requirements of each individual animal.

The aims of this study were to i) adapt the existing mechanistic pig growth model to represent the growth dynamics of modern pig populations. This is achieved by fitting the model to longitudinal measurements of energy and protein intake as well as protein and lipid retention from a crossbred population, and by using literature estimates for the model input parameters corresponding to two highly selected commercial pig lines; ii) extend the model to estimate residual energy intake and nitrogen excretion for these pig populations over the growth period between 30 to 130 kg BW; iii) use the model, adapted to the different modern pig populations, to determine how improvement in growth is likely to affect feed efficiency and nitrogen excretion.

5.2 Material and Methods

All animal care and handling procedures in the federal testing station were reviewed and approved by the Landwirtschaftskammer Schleswig-Holstein, Rendsburg, Germany.

Data

i) Experimental crossbred population

The mechanistic biological growth model used in this study was fitted to data of a crossbred F_2 population, for which longitudinal data of energy and protein intake as well as protein and lipid deposition were available. The founder generation (F_0) consisted of 7 unrelated Pietrain grandsires and 16 unrelated grand-dams bred from a 3-way cross of Leicoma boars with Landrace $\times$ Large White dams. All grandsires were heterozygous (Nn) at the *ryanodine receptor 1* (*RYR1*) locus. Of the F_1 generation, 8 boars and 40 sows were selected to produce the F_2 generation. The F_2 generation consisted of 315 pigs from the first two parities of the F_1 sows.

Pigs started the performance test at about 30 kg BW and were weighed on a weekly basis. Pigs are weighed at target weights 30, 60, 90, 120 and 140 kg BW. Average BW (SD) at target weights were 30 (2.55), 61 (2.58), 91 (2.60), 120 (2.69) and 140 (2.80) kg, respectively. During growth from 30 to 60, 60 to 90, and 90 to 140 kg of BW, pigs were fed *ad libitum* with a diet containing 13.8 MJ of ME/kg, 18.5% CP and 1.2% lysine, a diet containing 13.8 MJ of ME/kg, 17% CP and 1.1% lysine, and a diet containing 13.4 MJ of ME/kg, 16.5% CP and 1.0% lysine, respectively. The diets consisted of adequate nutrient supplies to permit maximum protein deposition. For a more detailed description of the data see Landgraf et al. (2006), and Mohrmann et al. (2006).

Chemical body composition at the target weights of 30, 60, 90, 120 and 140 kg were determined using the deuterium dilution technique. This technique is an *in vivo* method predicting empty body water content of the pigs. Using this method, the percentage of fat-free substance of pigs was estimated from the empty body water content. Based on the percentage of the fat-free substance the protein and ash content of the empty body were estimated. Percentage of lipid content was the deviation of the percentage of fat-free substance from 100%. The accuracy of this technique to determine body composition has been verified using magnetic resonance imaging on live animals and chemical analysis of serially slaughtered animals using data of the F_1 population of the present experiment in previous studies (Landgraf et al., 2006; Mohrmann et al., 2006). The correlations between

the estimates for empty body water, fat free substances, and protein in fat-free substances obtained from deuterium dilution technique and chemically analysed methods reported to be 0.92, 0.90, and 0.85, respectively (Mohrmann et al., 2006). A detailed description of the use and analysis of the deuterium dilution technique is presented by Landgraf et al. (2006).

ii) Purebred commercial lines

In addition to the crossbred pig population described above, the mechanistic biological model was applied to two genetically different PIC pig lines. The model input parameters for these lines had been obtained previously by Doeschl-Wilson et al. (2007). Line A is a dam line mainly selected for fast growth, high reproductive performance and high robustness; line B is a sire line mainly selected for high feed efficiency and leanness.

Description of the mechanistic biological growth model

The semi-stochastic mechanistic biological model of Knap (1999) for simulating growth of a genetically heterogeneous population of pigs was extended to simulate feed efficiency and nitrogen excretion traits during the growth phase. The stochastic nature of the mechanistic biological model implies that its predictions are influenced by the random sampling used to generate the simulated animal populations. The interactions between the traits representing the genetic potentials, and prevailing physiological, nutritional, environmental constraints are described by a system of mathematical equations that integrate the present knowledge about the metabolic and physiological processes involved in pig growth. More specifically, the model describes the allocation of dietary nutrients into processes associated with maintenance, protein and lipid deposition. In accordance with potential growth rules presented by Emmans' (1988), the model represents the genetic potentials of animals for growth as the animal's biological ability to deposit protein and lipid and cope with various kinds of stressors. The genetic growth potentials of pigs are described by three parameters: mature protein and lipid mass (P_{∞} and L_{∞} , respectively) and a Gompertz rate parameter (B_{Gomp}) to regulate the growth of both portions; specifying the animal's potential for protein (P , kg) and lipid (L , kg) mass growth in optimal environmental conditions according to:

$$dP/dt = P \times B_{Gomp} \times \ln(P_{\infty}/P) \quad [1]$$

$$dL/dt = L \times B_{Gomp} \times \ln(L_{\infty}/L) \quad [2]$$

where the same rate parameter B_{Gomp} is used for protein and lipid deposition, assuming full allometry between body protein and lipid. The asymptotes P_{∞} and L_{∞}

correspond to protein and lipid mass at maturity. Protein and lipid growth constitute two of the resource demanding processes; all others are characterised as maintenance processes, which are also considered as genotype dependent. Maintenance processes include basic metabolic processes related to survival, such as thermoregulation, activity, as well as energetic work for protein and lipid deposition. As the Gompertz parameters are expected to be correlated, the parameter B_{Gomp} was scaled to $B^* = B_{Gomp} \times P_{\infty}^{0.27}$ to ensure independence between the model parameters (Taylor, 1985). For convenience, L_{∞} is expressed as its ratio to P_{∞} (LP_{∞} , in kg/kg), which is assumed to be uncorrelated to both P_{∞} and B^* . This leads to three presumed-independent model parameters P_{∞} and LP_{∞} and B^* , representing three of the four underlying biological traits describing the animal genotype (Doeschl-Wilson et al., 2007). The fourth genotype specific model parameter, ME_m relates to the maintenance requirements of maintenance processes. These maintenance energy requirements (ME_{maint}) are calculated according to Knap and Schrama (1996) as a function of the metabolic body weight ($BW^{0.75}$):

$$ME_{maint} = (1 + ME_m) \times f(BW) \times BW^{0.75}$$

where

$$f(BW) = 325 - 0.5 \times BW$$

ME_{maint} thus depends on metabolic BW, and thus on P_{∞} , LP_{∞} and B^* as BW is calculated as the composition of protein, lipid, ash and water. But the genotype specific parameter ME_m is assumed uncorrelated to all three growth parameters. From now on, we will refer to P_{∞} , LP_{∞} , B^* and ME_m as the four underlying biological traits.

In addition to the four underlying biological traits mentioned above the model uses as inputs the pigs' initial body weight, as well as a description of the diet composition. Based on the provided initial body weight the model first calculates the chemical composition of the pig in terms of protein, lipid, ash, and water mass at the start of the simulation according to the rules of Emmans and Kyriazakis (1995) and Emmans and Fisher (1996).

The model predicts *ad libitum* feed intake as the intake required to satisfy both the protein and energy needs of potential growth, as defined by equations [1] and [2], plus maintenance processes, subject to the nutrient composition of the diet and physiological capacity constraints to feed intake volume. After decomposition of consumed feed into its nutrient components, the nutrients are partitioned into growth and maintenance processes outlined by Knap and Schrama (1996). The model iteratively calculates the actual protein and lipid mass growth on a daily bases until 500 days of maturation, subject to the physical, nutritional and

environmental constraints that are captured by the model. The model outputs are simultaneous daily predictions for various observable phenotypic performance traits (e.g. body weight, feed efficiency, body composition, etc).

A more detailed description of the model concepts and the mathematical equations, including pseudo code, is provided in Knap (1999; 2000c).

Model extension

As the purpose of this study was to examine feed efficiency and nitrogen excretion traits during the pigs' growth period, the simulation model was extended to estimate residual energy intake (REI) and total nitrogen excretion (TNE). Residual energy intake was calculated at every time step as the surplus of metabolizable energy intake which is not used for protein and lipid deposition, and maintenance requirements. Total nitrogen excretion was estimated as the accumulation of daily nitrogen excretion estimated as the difference between daily nitrogen intake and nitrogen retention in the body. The daily nitrogen intake was estimated as the average daily feed intake multiplied by crude protein of feed intake divided by 6.25 (g/d), and the average daily nitrogen retention is the average daily protein deposition (APD) divided by 6.25 (g/d) (Whittemore et al., 2003). In addition to production traits and feed intake traits, the extended simulation model provides as output daily estimates of REI and TNE as measurements for feed efficiency and environmental pollution.

Model adaptation

Step 1: Curve fitting and data selection

The mechanistic biological growth model described above assumes that protein and lipid accretion of pigs can be described by a Gompertz curve (Knap, 2000c). To test whether this assumption holds for the pigs of the present study, a preliminary curve fitting procedure was applied using the individual protein and lipid measures obtained at 30 kg, 60 kg, 90 kg, 120 kg and 140 kg of BW. Thus, using Gompertz functions according to models [3] and [4], the body protein and lipid mass (P and L, respectively) in relation to age (days) for each individual was predicted until 500 days of age for the crossbred population. This model fitting process was carried out using the non-linear procedure, proc NLIN, of the SAS software (SAS Inst. Inc., Cary, NC), applied to each individual pig.

$$P(t) = P_{\infty} \times e^{-e^{-B_P \times (t - t_P^*)}} [3]$$

$$L(t) = L_{\infty} \times e^{-e^{-B_L \times (t - t_L^*)}} [4]$$

where P_{∞} and L_{∞} are the asymptotic values that represent mature protein and lipid mass in kg, t_p^* and t_L^* denote the x-coordinates of age (d) at points of inflection of the estimated P and L curves, and B_p , and B_L are the Gompertz rate parameters for growth of P and L.

Using this model, Gompertz curves for both protein and lipid accretion could be adequately fitted to a subset of 195 out of the 315 individuals. Only these individuals were used for the subsequent analysis. More specifically, models [3] and [4] were used to predict P and L for these individuals from 70 until 500 days of age. Then, the predicted values for P and L for each individual in 10 days interval were used for the subsequent growth model Gompertz parameter estimation.

Step 2: Estimation of input parameter values for the mechanistic biological model

To obtain realistic model predictions for production, efficiency and pollution traits for modern pig populations, appropriate values for the four biological model input traits (P_{∞} , LP_{∞} , B^* and ME_m) specifying the individuals' intrinsic growth potentials were required. For this purpose the longitudinal estimates for protein and lipid mass derived by the above curve fitting procedure for the individuals from 70 to 500 days of age with 10 days interval, were used to adapt the mechanistic biological growth model to the pig population of the present study.

More specifically, the differential evolutionary computational algorithm (Storn and Price, 1997) was used to infer for each individual animal estimates for the four biological model input traits specifying the individual's genetic growth potential that, under the given dietary and environmental constraints, best correspond to the estimated protein and lipid masses. This algorithm had been previously applied to the same (non-extended) simulation model to derive genetic parameter estimates for the same four underlying biological growth traits (Doeschl-Wilson et al., 2007). However, in contrast to the previous study, the algorithm was now applied to every individual animal rather than the population as a whole.

Details of the algorithm are outlined in Storn and Price (1997). Briefly, the differential evolutionary algorithm is a modified genetic algorithm that adapts evolutionary concepts to search efficiently through the multi-dimensional (4D in the present study) parameter space to find the optimum solution. In the present study, the latter is defined as the set of parameter values for which the corresponding model predictions for protein and lipid mass provide the closest fit to the data as specified by the criterion outlined below. The algorithm is an iterative process consisting of many iterations. In each iteration a number of solutions (12 in the present study) are simultaneously produced, of which the best (according to tournament selection) contribute to the initial estimates of the next

iteration. Each solution corresponds to a set of estimates for the 4 model parameters. Convergence towards a final solution was assumed if the best solution of an iteration did not change over 10,000 successive iterations.

Five replicates of the differential evolutionary algorithm were generated for the population of 195 individuals. Each replicate comprised 100,000 iterations and started with different initial values for the four biological input traits and different seeds for the random number generators used in the model and the optimisation process.

The criteria in optimisation included protein and lipid mass for each individual from 70 to 500 days of age with 10 days interval. The goodness of fit corresponding to a set of parameter values was evaluated according to the relative prediction error sum of squared (RPESS):

$$RPESS = \sum_{j=1}^{44} \left(\frac{\hat{P}_j - P_j}{P_j} \right)^2 + \sum_{j=1}^{44} \left(\frac{\hat{L}_j - L_j}{L_j} \right)^2$$

where $\hat{P}_j$ and $\hat{L}_j$ refer to the protein and lipid mass of the j -th time point (i.e. $70 + j \times 10$ days of age), respectively, predicted by the mechanistic biological model, and P_j and L_j refer to the corresponding protein and lipid mass obtained by the Gompertz curve fitting procedure described above. Thus, the lower RPESS, the better the model fit.

The differential evolutionary computational algorithm thus identifies adequate input parameter values for the current population, and thus predict values for traits of importance (e.g. the Gompertz parameters describe the genetically controlled growth potential of pigs, and maintenance requirements), that are difficult to measure in real situations.

Model application

After validating the mechanistic biological model as an adequate representation of a modern pig population, a population of pigs comprising 1000 individuals was generated with the same means and coefficients of variations for the four underlying biological growth traits as obtained from the model adaptation process described above, and assuming pigs with similar initial body weight as the mean of the experimental population in this study (30 kg). Predicted growth, energy efficiency, and nitrogen excretion traits for all individuals were recorded in 10 days interval until 500 days of age. Pigs were then categorised according to their predicted ADG at 100 kg BW to investigate the effects of improvement in observed growth on growth potential, energy efficiency and nitrogen excretion. For this purpose, three categories (Lower, Average, Upper) were created referring to

individuals whose ADG was at least 1 standard deviation (SD) below, within 1 SD, and more than 1 SD above the average ADG at 100 kg body weight, respectively. At 100 kg, average daily protein and lipid deposition (APD and ALD) rates were calculated as the difference between protein or lipid weight at the begin weight and 100 kg target weight divided by the number of days between those target weights. Average daily gain was also calculated between the target weights. In the current study, the lower category crossbred pigs can be considered as a population with negligible selection for growth in comparison to the upper category, which may represent a population selected for fast growth. The comparison between categories for the estimates of traits of interest, such as REI and TNE, indicates the effect of selection for ADG on economically and environmentally important traits. In addition, comparing the results from crossbred categories with purebred PIC lines, can give insight into the effect of different genetic selection on growth, feed efficiency and reproduction traits on the REI and nitrogen excretion traits. Note furthermore that in this study, the obtained REI value based on the growth model is a biological estimation of REI in comparison literature studies that estimate this trait based on a statistical model with zero mean. Similarly, the absolute values of TNE from the biological growth model and those estimated based on statistical models are not comparable. Considering that the estimates from the used growth model can be biologically explained, these estimates are better suited for developing selection and mitigation strategies.

5.3 Results

Curve fitting

Fitting the non-linear models [3] and [4] to the available point measurements of protein and lipid mass showed that 62% of the individuals of the crossbred population closely followed a Gompertz curve trend for these traits. For the remaining 38%, the curve fitting procedure could not provide realistic estimates for the Gompertz parameters and resulted in a poor fit of their individual curves. This resulted in selecting 195 individuals for subsequent analysis in this study. The close fit of the predicted curves to the data (see Figure 5.1 for one representative individual) may be indicative for a high accuracy in predicting protein and lipid mass for the used subset of pigs throughout the growth period until about 300 days of age.

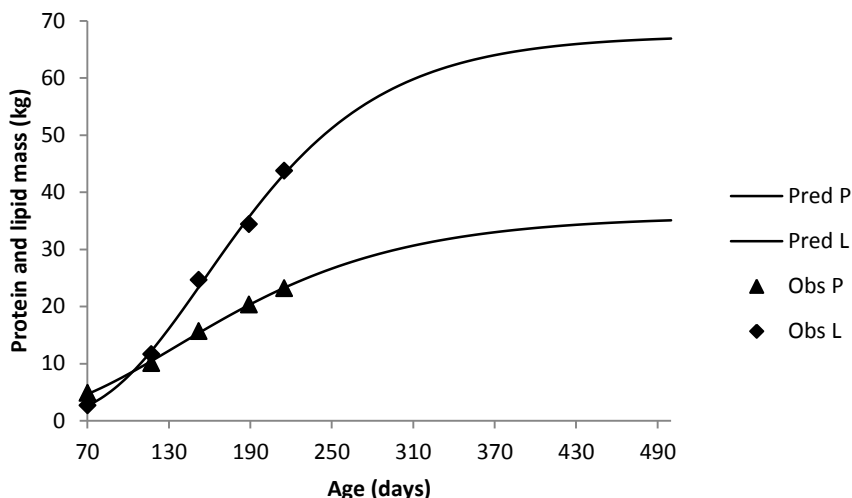


Figure 5.1 Predicted protein (Pred P) and lipid (Pred L) mass by fitting Gompertz curves to observed average protein (Obs P) and lipid (Obs L) mass for one individual pig of the 195 pigs analysed.

The means and standard errors for P_{∞} , L_{∞} , t_p^* , t_L^* , B_p , and B_L for the 195 individuals that followed Gompertz curve trends are presented in Table 5.1.

Table 5.1 Means and standard errors of Gompertz parameters obtained for protein and lipid mass using individual observations for 195 analysed pigs of the crossbred population

Variable ¹	Protein		Variable ¹	Lipid	
	Mean	SE		Mean	SE
P_{∞}	35.32	0.05	L_{∞}	62.97	0.15
t_p^*	136.95	0.20	t_L^*	159.90	0.26
B_p	0.011	0.00002	B_L	0.013	0.00003

¹ P_{∞} and L_{∞} = mature protein and lipid mass in kg; t_p^* and t_L^* = age at point of inflection for protein and lipid mass in days; B_p and B_L = the rate parameter regulating the growth of protein and lipid mass

Differential evolutionary algorithm parameter estimation

The differential evolutionary computational algorithm converged for all of the 195 pigs and produced realistic input parameter values. The different optimisation runs corresponding to different starting values and random seeds resulted in the same parameter estimates for almost all of the pigs, confirming that the global rather than local optima were identified. For the minority of pigs (n=20) with different solutions, the absolute optimum value was chosen by selecting the solution corresponding to the lowest RPRESS value. The mean, standard errors, and CV of four parameter estimates obtained from the differential evolutionary computational algorithm are presented in Table 5.2.

Table 5.2 Parameter estimates obtained from differential evolutionary computational algorithm for 195 analysed pigs of the crossbred population

	Parameter estimates ¹				
	P_{∞} (kg)	LP_{∞} (kg/kg)	B^* (kg/(day×day))	B_{Gomp} (kg/(day×day))	ME_m (KJ/(day×kg ^{0.75}))
Mean	32.73	1.97	0.0308	0.012	-0.373
SE	0.34	0.02	0.0004	0.0002	0.04
CV	12.50	13.40	15.24	17.46	-130

¹ P_{∞} = mature protein mass in kg; LP_{∞} = mature lipid to protein ratio; B^* = potential rate at which mature mass is retained; B_{Gomp} = Gompertz growth rate parameter; ME_m = energy requirements for the maintenance process

The goodness of fit of the mechanistic biological model to the crossbred pig population of the present study was further evaluated by performing correlation analysis for protein and lipid mass between 80 to 500 days of age obtained from either curve fitting or predicted by the mechanistic biological model, with the input parameters estimated as described above (Figure 5.2). The results show that for all growth stages a high correlation between the respective predicted protein and lipid mass were obtained. For protein mass, the largest correlation was at the beginning of growth with 0.99, and the lowest achieved at 190 days of age with a value of 0.82. For lipid mass, the largest correlation was achieved at the beginning of growth with a value of 0.97, and lowest was at 230 days of age with a value of 0.75. The difference between predicted values of protein and lipid mass obtained through using differential evolutionary algorithm and curve fitting, respectively, is presented in the residual plot of Figure 5.3. This residual plot shows that discrepancies between both types of models are generally less than 2 kg, demonstrating a high concordance between predictions. The mechanistic biological growth model however has a tendency to over-predict protein mass and to under-

predict lipid mass at the early growth stages (i.e. before 300 days of age). These differences may arise because the mechanistic biological growth model assumes full allometry between P and L depositions, which may not be fully justified, and because it may over-simplify some bio-chemical processes. However, the general high concordance between the models indicates that the mechanistic biological growth model is a valid representation of growth and change in body composition during the growth phase for the analysed 195 pigs in this study.

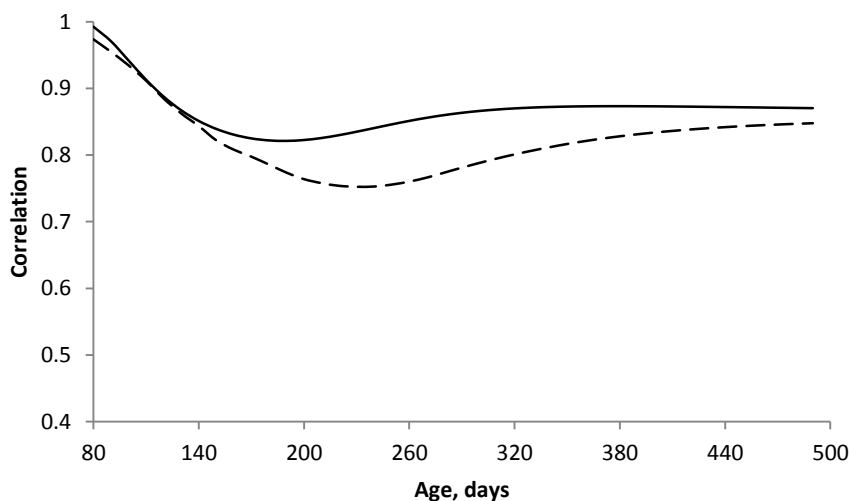


Figure 5.2 Correlations between protein (solid line) and lipid (dash line) mass during growth of pigs from 80 to 500 days of age predicted from the mechanistic biological growth model with the optimal values for the model input parameters and the corresponding estimated values obtained from fitting Gompertz curves through available data (n=195)

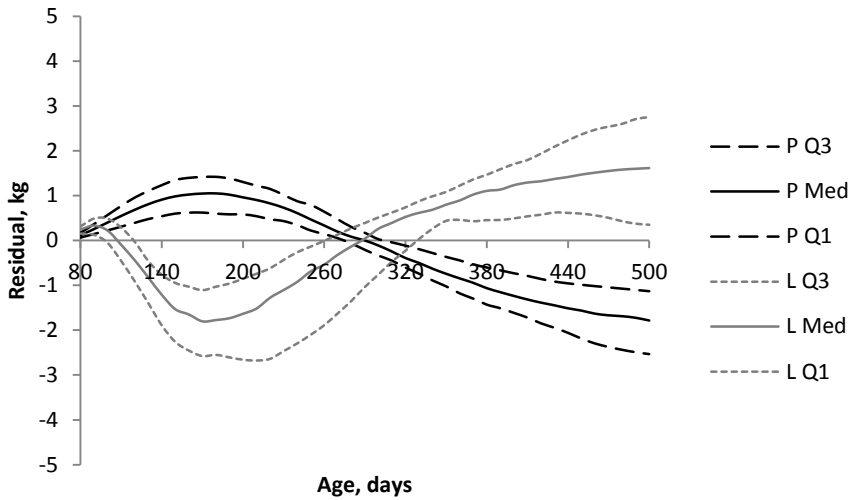


Figure 5.3 Residual estimates for protein and lipid mass during growth of pigs from 80 to 500 days of age predicted from the mechanistic biological growth model with the optimal values for the model input parameters and the corresponding estimated values obtained from fitting Gompertz curves to available data. The median residual for protein and lipid mass (P Med and L Med, respectively) along with third quartile (P Q3 and L Q3, respectively) and first quartile (P Q1 and L Q1, respectively) are presented. Positive values correspond to over-prediction of the mechanistic biological growth model.

Simulation analysis

Table 5.3 summarises the predicted values for growth, energy efficiency, nitrogen excretion and the Gompertz parameters associated with the lower, average and upper ADG categories of the simulated pig population comprising 1000 pigs with the same mean and CV for the underlying biological parameters as estimated above. At 100 kg BW point, upper category pigs not only showed significantly ($P < 0.05$) greater ADG (30% to 32%) and shorter growth period (32%), but also, a favourably lower REI (26%) and TNE (20%) compared to the lower category. Furthermore, in conjunction with better growth, feed efficiency and nitrogen pollution, the upper category pigs also showed to have greater underlying biological traits with 30% higher B^* , and B_{Gomp} , which consequent in 31% greater PD_{max} compared to lower category pigs.

5 Growth modelling for energy and nitrogen efficiency

Table 5.3 Characteristics of growth, energy efficiency, nitrogen excretion, and Gompertz parameters presented as mean and their standard errors (SE) for the entire population (Average) and for selected populations which were at least 1 standard deviation below (Lower) or above (Upper) the average ADG from 30 to 100 kg body weight using a simulation study based on the mechanistic biological growth model developed based on the crossbred population

Traits ¹	Lower		Average		Upper	
	Mean	SE	Mean	SE	Mean	SE
P _∞ (kg)	32.89 ^a	0.32	32.48 ^a	0.15	33.11 ^a	0.32
LP _∞ (kg/kg)	1.99 ^a	0.02	1.96 ^a	0.01	1.94 ^a	0.02
B* (kg/(day×kg))	0.0250 ^c	0.0003	0.0309 ^b	0.0001	0.0358 ^a	0.0003
B _{Gomp} (kg/(day×kg))	0.0098 ^c	0.0001	0.0121 ^b	0.0001	0.0140 ^a	0.0001
PD _{max} (g/day)	116 ^c	0.87	143 ^b	0.41	169 ^a	0.86
ADG (g/day)	672 ^c	4.35	832 ^b	2.02	990 ^a	4.30
APD (g/day)	109 ^c	0.80	135 ^b	0.37	159 ^a	0.79
ALD (g/day)	169 ^c	2.12	209 ^b	0.99	244 ^a	2.10
DAY (day)	104.5 ^c	0.48	84.4 ^b	0.22	70.9 ^a	0.47
REI (MJ ME/pig)	635 ^c	3.15	538 ^b	1.47	473 ^a	3.12
TNE (kg/pig)	3.82 ^c	0.04	3.37 ^b	0.02	3.06 ^a	0.04

^{a-c} Within each trait, means without a common superscripts differ ($P < 0.05$)

¹ P_∞ = mature protein; LP_∞ = mature lipid to protein ratio; B* = potential rate at which mature mass is retained; B_{Gomp} = Gompertz growth rate parameter; PD_{max} = maximum protein deposition; ADG = average daily gain; APD = average protein deposition; ALD = average lipid deposition; DAY = days during growth from 30 to 100 kg; REI = residual energy intake; TNE = total nitrogen excretion. All the traits are obtained during growth from 30 to 100 kg BW.

Trends for simulated growth, energy efficiency and nitrogen excretion

Predicted growth trends of protein weight, lipid weight, residual energy intake, and nitrogen excretion in the crossbred population of 195 pigs presented in Figure 5.4a-d. It shows that pigs in the upper ADG category have consistently greater protein and lipid deposition than pigs in the lower ADG category throughout the entire growth period. The largest difference was achieved at 150 days of age with 23% for protein weight and 35% for lipid weight, where upper category pigs had 17.96 kg protein weight and 22.68 kg lipid weight, and lower category pigs had 13.78 kg protein weight and 14.84 kg lipid weight.

The growth pattern of residual energy intake and total nitrogen excretion show that at each time point upper category pigs have considerably larger REI and TNE compared to lower category pigs. These differences were at their greatest at 170 days of age for REI and 150 days of age for TNE with 21% and 25% differences, respectively. The upper category pigs had 756 MJ ME of REI and 3.59 kg of TNE compared to the lower category pigs with 600 MJ ME of REI and 2.70 kg of TNE. But

the higher REI and TNE of the upper category of pigs at a given age was overcompensated by a shorter growth period so that REI and TNE over the entire test period were less compared to the lower category.

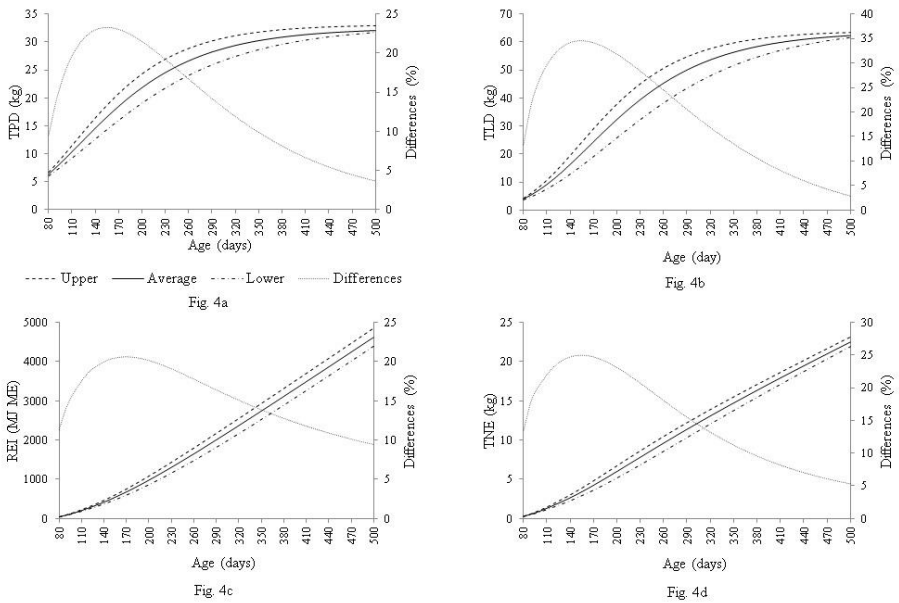


Figure 5.4a-d Growth patterns of total protein weight (TPD), lipid weight (TLD), residual energy intake (REI) and nitrogen excretion (TNE) from 80 to 500 days of age for the entire population (Average) and for selected populations which were at least 1 standard deviation below (Lower) or above (Upper) the average ADG from 30 to 100 kg body weight. The proportional differences between upper and lower category pigs in each 10 day interval are presented as percentages.

PIC lines

Estimates for REI, TNE along with growth traits and Gompertz parameters at 100 kg BW point for the PIC pig lines undergoing selection mainly for fast growth and reproduction (Line A) and feed efficiency and leanness (Line B) are presented in Table 5.4. The results indicate that pigs from line A have significantly larger B^* (13%) and PD_{max} (15%) associated with larger ADG (14%), APD (13%), and ALD (21%) and less days (14%) needed to grow from 30 to 100 kg BW compared to line

B. Therefore, this resulted in better efficiency as indicated by lower REI (13%) and TNE (10%) in line A.

Comparison between line A (Table 5.4) with the average of the crossbred population (Table 5.3), showed 21% lower REI and 33% lower TNE in the line A pigs, which was at least partly due to 26% larger APD and 37% lower ALD; in addition to 19% higher ADG and 19% lower duration of growth (DAY). Line B was also compared to the average of the crossbred pigs. The results indicate that line B has a 10% lower REI and a 26% lower TNE which could be explained by 16% higher APD, 50% lower ALD, 5% higher ADG, and 6% shorter duration of growth to reach 100 kg BW.

Table 5.4 Characteristics of growth, energy efficiency, nitrogen excretion, and Gompertz parameters presented as mean and their standard errors (SE) from 30 to 100 kg body weight for the commercial Lines A and B.

Traits ¹	Line A		Line B	
	Mean	SE	Mean	SE
P _∞ (kg)	59.3 ^a	0.09	57.4 ^b	0.09
LP _∞ (kg/kg)	1.27 ^a	0.01	1.13 ^b	0.01
B* (kg/(day×kg))	0.032 ^a	0.0001	0.028 ^b	0.0001
B _{Gomp} (kg/(day×kg))	0.011 ^a	0.0001	0.009 ^b	0.0001
PD _{max} (g/day)	232 ^a	0.42	198 ^b	0.42
ADG (g/day)	1024 ^a	1.65	877 ^b	1.65
APD (g/day)	184 ^a	0.28	161 ^b	0.28
ALD (g/day)	131 ^a	0.41	103 ^b	0.41
DAY (day)	68.3 ^a	0.15	79.6 ^b	0.15
REI (MJ ME/pig)	424 ^a	0.99	485 ^b	0.99
TNE (kg/pig)	2.25 ^a	0.01	2.50 ^b	0.01

^{a,b} Within each trait, means without a common superscripts differ ($P < 0.05$)

¹ P_∞ = mature protein; LP_∞ = mature lipid to protein ratio; B* = potential rate at which mature mass is retained; B_{Gomp} = Gompertz growth rate parameter; PD_{max} = maximum protein deposition; ADG = average daily gain; APD = average protein deposition; ALD = average lipid deposition; DAY = length of growth; REI = residual energy intake; TNE = total nitrogen excretion. All the traits are representing the 30 to 100 kg growing period.

5.4 Discussion

The ability to define genetically controlled characteristics of pigs with a few parameters through growth modelling is appealing for exploring potential consequences of genetic selection on a variety of traits simultaneously. Furthermore, individual estimates for biological traits that are difficult to observe in

Reliability of the parameter estimates

The predictions for production, feed efficiency, and nitrogen excretion traits depend strongly on the model input traits, i.e. the Gompertz parameters describing the genetic potential of pigs. Therefore, by fitting the model to data from three different pig lines, a crossbred and two purebreds, the Gompertz parameters should reflect the expected line differences. The P_{∞} and B^* estimates obtained from the differential evolutionary algorithm for the crossbred population (32.73 ± 0.34 and 0.0308 ± 0.0004 , respectively) was within the range of literature estimates (24.5 to 40.7, kg and 0.0218 to 0.0445 kg/(day×kg), respectively) reported previously (e.g. Ferguson and Gous, 1993a; Ferguson and Gous, 1993b; Knap, 2000a; Knap, 2000c; Knap et al., 2003); but the LP_{∞} (1.97 ± 0.02) was in the lower range of the reported values (0.97 to 5.16). Regarding the PD_{max} , the upper ADG category of the crossbred population (169 ± 0.86) was in the middle and the lower category (116 ± 0.87) was at lower level of the range of PD_{max} values (110 to 193 g/day) reported by Knap (2000a and c). For the purebred lines A and B, the P_{∞} values were substantially larger than the values obtained for the different categories obtained for the crossbred population, and the ones reported by (Knap 2000a and c). Consequently, LP_{∞} for lines A and B were also substantially lower than the values for the crossbred population and they were at the lower level of the values reported by (Knap 2000a and c). Only the B^* estimates for both purebred lines were in the range of values obtained for crossbred populations. Knap (2000a) indicated that both Gompertz parameters B_{Gomp} and LP_{∞} have shown a response to genetic selection over time. This also can be concluded from the current analysis, as selection for faster growing animals (ADG at least 1 standard deviation over the average of the population) was predicted to result in larger B_{Gomp} and lower LP_{∞} . Knap (2000a) also pointed out that the progress towards larger and leaner animals due to breeding strategies is expected to be reflected in increasing values for P_{∞} and decreasing values of LP_{∞} . Our study supports this finding that lines with higher growth rate (Line A) and leanness (Line B) resulted in higher mature protein and lower mature lipid to protein ratio in comparison with the crossbred population.

Comparison between upper and lower ADG categories mimics response to selection on ADG. Emmans and Kyriazakis (1998) stated that the trait selection has to change the mean values for the Gompertz parameters over time; and Whittemore (1994) reported that selection for higher growth rate, feed efficiency and leanness would result in higher mature body weight. In the current study, the different categories of crossbred pigs had no significant differences in the P_{∞} and

the LP_{∞} . This is in agreement with results of Ferguson and Kyriazis (2003) who reported no significant differences for P_{∞} and LP_{∞} in six different commercial crossbred pig genotypes. Pigs were categorised according to performance at 100 kg BW, long before maturity; therefore the differences are mirrored by differences in Gompertz parameters for B^* , B_{Gomp} and PD_{max} , and not by differences in P_{∞} and L_{∞} . Although pig genotypes have become leaner, the mature body size and thus the mature protein have remained unchanged for commercial crossbred pigs. This can be due to selection for lean growth and feed efficiency at a substantially lower weight than that at maturity. Using the 1969-2004 trend of PD_{max} in pig sire lines reported by Doeschl-Wilson et al. (2007), the PD_{max} obtained for different categories in the current study (upper, average, and lower) can be located in time at years 1990, 1985 and 1970, respectively; indicating that the upper and lower category pigs are 20 years of sire line genetic improvement apart. The LP_{∞} was not different between categories in the current study, and it was located in time at 1985 of pig sire lines from Doeschl-Wilson et al. (2007). In summary, the predicted estimates for the model input parameters fall within the range of expected values based on previous estimates and available information about the pig line characteristics. Therefore, this provides confidence for the reliability of predictions of output parameters such as REI and TNE.

Predicted impact of selection for growth on REI and TNE

The upper ADG category animals had 30% to 32% larger growth compared to lower category pigs which is associated with 30% larger B^* and B_{Gomp} . Nitrogen excretion is 20% lower and REI is 26% lower for upper category crossbred pigs. This can be attributed to lean growth as reflected in greater B^* and B_{Gomp} , and 32% shorter duration to reach the target BW of 100 kg. The larger PD_{max} in the upper category pigs is responsible for the prediction of greater nitrogen retention efficiency and also better energy efficiency in these pigs compared to other categories as shown by lower nitrogen excretion and REI. Furthermore, comparing line A with the upper category crossbred pigs indicates that improvement in Gompertz parameters such as B_{Gomp} (21%) and LP_{∞} (34%) not only improves growth (14% to 36%), but simultaneously reduces nitrogen excretion (27%) and REI (10%). In addition, improvement in ALD (36%) and APD (14%) are also reflected in lower nitrogen excretion and REI. Comparison of PIC line B with the upper category pigs emphasises the substantial association of B_{Gomp} and LP_{∞} with nitrogen excretion and REI. The B_{Gomp} was lower (38%) in line B, but there was no significant differences in ADG and duration of growth, and consequently also neither in REI compared to the upper category pigs. However, the LP_{∞} was substantially lower

(42%) in line B with lower ALD (58%) and 18% lower TNE. The better nitrogen and energy efficiency in line A compared to line B could be due to effectively larger and faster growth, despite the fact that line B is expected to be more efficient due to lower feed intake and backfat depth at a given body weight, as reported by Doeschl-Wilson et al. (2007). Shirali et al. (2012) showed substantial phenotypic associations of ADG (-0.48), APD (-0.49), and ALD (-0.17) with TNE for growing pigs from 60 to 140 kg BW. Furthermore, Saintilan et al. (2013) reported large genetic (0.48 to 0.84) and phenotypic (0.67 to 0.70) association between RFI and TNE for four pig breeds. As the results of the current study suggest, at a given point in time, fast growing pigs have larger REI and TNE. However, as fast growing pigs reach the target BW faster, they have therefore lower TNE and REI at a given body weight or over the entire growth period. Morel and Wood (2005) quantified the nitrogen flux in growing pigs with low (120 g/d), medium (160 g/d) and high (200 g/d) PD_{max} genotypes using a simulation model and reported 15.8, 12.4 and 10 kg nitrogen excretion per pig place per year for these genotypes, respectively. They concluded that improved genotypes with greater PD_{max} have less nitrogen excretion. The low (120 g/d) and medium (160 g/d) PD_{max} genotypes reported by Morel and Wood (2005) can be compared to lower and upper categories in the current study with 116 ± 0.87 and 169 ± 0.86 g/d PD_{max} , respectively. In the above study, a computational genetic algorithm was used to find a feeding strategy that optimises the objective function of weighted gross margin and nitrogen excretion cost, and the simulation aim was to determine the effect of different pig genotypes, economic gross margin, and nitrogen excretion on profitability and nitrogen excretion under different feeding practices. This is different from the aims of the simulations in the current study. Nevertheless, the results are comparable and indicate the effectiveness of genetic improvement for faster growth with greater protein deposition rate on environmental pollution of production. Shirali et al. (2012), using the same crossbred experimental population of pigs as in the current study, reported 5.35 ± 0.04 kg total nitrogen excretion in 102 ± 0.88 days of growth from 60 to 140 kg of BW calculated as the difference between nitrogen intake and nitrogen retention in the body obtained using deuterium dilution technique which estimated the protein and lipid content of body. This is in agreement with 5.09 kg TNE estimated in the current study for the mean of crossbred population for 60 to 140 kg BW. Dourmad et al. (1993) reported 3.9 kg nitrogen excretion for growing pigs between 30 to 102 kg of live weight, which is only slightly higher than the estimated 3.4 kg TNE of the crossbred population.

Furthermore, the time trends for the estimated traits indicate that nitrogen excretion and residual energy intake increase non-linearly throughout the growth

practice - such as APD, ALD, REI or TNE examined here - can be estimated by fitting these models to available measurements of observable traits. In this study, such a growth model revealed characteristics of feed efficiency and nitrogen excretion during growth in pigs. Furthermore, the model shed light on the association between observable growth rate and underlying potentials for protein and lipid deposition, as well as feed efficiency and nitrogen excretion.

Interpretation of the results from the mechanistic biological growth model

The mechanistic model provided estimates for TNE and REI that were based on knowledge of biochemical processes associated with energy utilization rather than using statistical methods based on observable proxy traits for growth. This has several advantages: firstly, as a consequence of the explicit description of the interactions between genetically controlled growth potential and physiological and environmental constraints in the growth models, the mechanistic models are expected to be capable of providing reliable extrapolation of the predictions outside the data range. Second, the dynamic nature of the model allows for estimating economically and environmentally important traits on a daily basis rather than as average over a long period of time, thus revealing the changes in these traits at different stages of growth. Lastly, the mechanistic biological growth model has been built upon a description of the pig phenotype in terms of traits that are considered more closely related to the underlying biology and more likely to be stable across a range of environments, in addition to taking into account the environmental factors such as diet and feeding characteristic during the growth trajectory (Doeschl-Wilson et al., 2007). Thus, the model would be able to predict nitrogen excretion and feed efficiency of the same population of pigs under different environmental and nutritional constraints.

However, the mechanistic model also has several intrinsic assumptions that may generate bias in the results. One important assumption made in the growth model, which can affect the results, is that feed intake is restricted by assuming that the pigs will not exceed their desired feed intake to meet their maintenance requirements and growth potential. In reality pigs may have more feed intake than their requirements due to different environmental and/or genetic factors such as variation in feeding behaviour and competition (Von Felde et al., 1996), thus resulting in larger REI and TNE. In addition, the diet composition in the model matched the real diet as much as possible, but it was assumed that the amino acid composition was ideal. The model is therefore more likely to under-estimate rather than over-estimate REI and TNE traits in practice.

period. The results are in agreement with our previous results based on statistical analysis of protein and lipid mass measurements of the same population (Shirali et al., 2012) that showed substantial differences in nitrogen excretion at different stages of growth. In addition, greatest differences in nitrogen excretion between upper and lower categories were found at 150 days of age which coincide with time of maximum protein and lipid deposition which would lead to greater feed intake. However, the higher excretion of the upper category at given age are more than offset by the higher growth rate as obtained by a substantially lower TNE during growth from 30 to 100 kg.

Predictions for time trends in REI and TNE

Knap (2012) estimated nitrogen retention and excretion for six pig sire lines from 20 to 120 kg BW during the years from 1969 to 2004 using the same growth model as in the current study. The analysis showed that the 2004 genotype had on average 19% greater nitrogen retention or, alternatively, a 20% less nitrogen excretion than the 1969 genotype. This indicates that genetic improvement of body growth and composition in these years resulted in a substantial reduction of nitrogen excretion per pig produced. However, Knap (2012) only had crude estimates for the model input parameters that were based on summary statistics rather than individual measurements as in this study. The current study complement the Knap (2012) study by i) providing more accurate parameter estimates based on more sophisticated fitting process, ii) expanding the model to estimate REI in addition to nitrogen excretion, iii) examining in depth the relationship between traits of interest over growth period. Jones et al. (2008) estimated 0.8% annual reduction in global warming potential of methane and nitrogen emissions as a result of genetic trends for growth rate [$+8.5$, g/(d/yr)], FCR [-0.02 , kg/(kg/yr)] and litter size [0.16 , pigs/(litter/yr)] in the United Kingdom pig sector from 1988 to 2007. They found that genetic increase in ADG is responsible for 70% reduction in ammonia and genetic improvement in FCR is responsible for 70% reduction of nitrous oxide. Furthermore, they concluded that this rate of genetic improvement will continue over the next few decades and may increase because of the use of molecular genetic tools. Moreover, the results of Jones et al. (2008) may be underestimated as the genetic trend of lean content ($+0.5$ %, lean meat per year) has not been taken into account (Knap, 2012). The favourable association of REI and TNE with the Gompertz parameters B^* and LP_{∞} in the upper ADG category animals indicate a possible genetic improvement in feed and nitrogen utilization efficiency through genetic selection on B^* and LP_{∞} . Additionally, it indicates the effect of genetic selection for growth and leanness on

TNE and REI, which is in accordance with the finding of Jones et al. (2008) and Knap et al. (2012). Furthermore, based on differences in the Gompertz parameters of the different ADG performance categories in the current study and comparison with PIC lines, it can be concluded that the genetic improvement practices that has been carried out in pig breeding through the years has resulted in improvement of feed efficiency and reduction of nitrogen excretion. The results of the current studies would further suggest that the underlying biological traits such as P_{∞} , LP_{∞} and B^* that specify the growth characteristic of pigs and will respond stronger to genetic selection (Doeschl-Wilson et al., 2007). These underlying traits also have a strong positive influence on the energy efficiency and nitrogen excretion in pigs, therefore, more rapid improvement in energy efficiency and nitrogen excretion could be achieved by selection for these underlying traits instead.

The results of this study can be used in numerous ways. For example the IPCC (2006) developed a prediction equation to determine nitrogen excretion per pig. This prediction equation can be improved using the results of nitrogen excretion of the studied pig lines. In this prediction equation, the IPCC (2006) suggested 30% nitrogen retention efficiency (NRE; nitrogen retention per nitrogen intake) in pigs, which can be much more accurate reflected by using specific NRE as presented in Figure 5.5a using the data of the crossbred population with the potential to consider different slaughter weight. The NRE of the studied purebred populations showed even greater NRE of 48% and 47% for line A and B, respectively, compared to the crossbred line with 35% considering the growth period from 30 to 100 kg BW. The results of the growth models can also be used to develop new or optimise genetic, nutritional and management strategies to improve feed energy and nitrogen efficiency. One plausible management strategy in reducing the amount of nitrogen excretion could be optimising (reducing) the slaughter weight within an economical sustainable range. Comparisons between different slaughter weights showed that animals tend to excrete between 600 to 790 g more nitrogen by successively increasing slaughter weight by 10 kg in the range from 90 to 130 kg (Figure 5.5b). This is also associated with an increase in REI used with 100 to 136 MJ ME between each target slaughter weights (Figure 5.5c). To investigate the effect of optimising slaughter weight in a growing system, the marginal increase in REI and TNE are estimated per pig space and year in the finishing unit, considering a starting weight at 30 kg and assuming that the unit is occupied by pigs for 330 days per year. The marginal energy saving per pig space and year was 74 MJ ME, 126 MJ ME and 102 MJ ME when reducing finishing weights from 110 to 100 kg BW, from 120 to 110 kg BW and from 130 to 120 kg BW, respectively. These slaughter weight

reductions were associated with the marginal nitrogen saving of 437 g, 674 g and 486 g per pig space and year, respectively. When reducing slaughter weight from 130 to 100 kg BW, the total marginal energy saving per pig space and year was 302 MJ ME. To obtain the energy cost per MJ ME, which is associated with the marginal increase in energy due to increase in slaughter weight, the cost of each feed ingredient (www.indexmundi.com) of a typical pig feed diet was estimated by obtaining the values of each feed ingredient (www.indexmundi.com) multiplied by its amount of MJ ME in the diet. The energy and cost saving involved in the marginal decrease in REI, when slaughter weight was decreased from 130 to 100 kg, were 302 MJ ME that equivalents to £9.96 per pig space and year. This slaughter weight reduction was associated with a substantial decrease in nitrogen excretion of 1.60 kg of nitrogen per pig space and year in the finishing unit. Moreover, reducing slaughter weight is a feasible strategy due to issues related to surgical castration of male pigs, the EU has planned to stop castration of male piglets by 2018 (European Commission, 2010). This will result in growing entire males, which are expected to have better feed efficiency than barrows and consequently result in less nitrogen excretion. In addition, entire males will likely be slaughtered at lower weight to avoid boar taint, which will further reduce nitrogen excretion. Growing gilts and boars under same housing conditions is suggested by Dammgen et al. (2012) therefore, this may result in lower slaughter weight for gilts as well. Furthermore, selection strategies for example for improvement of ADG at early stage of growth could be developed based on the estimates of the biological growth model regarding energy and nitrogen efficiency. In conclusion, economical and environmental benefits can be achieved in pork production systems through change in slaughter weight, improvement in growth rate, feed utilization efficiency and body composition along with underlying biological traits presented in this study. Furthermore, these results can provide guidelines for pork producers and policy makers to enhance management strategies by identifying the amount of nitrogen excretion and energy usage throughout the growth and identifying the underlying factors influencing these traits.

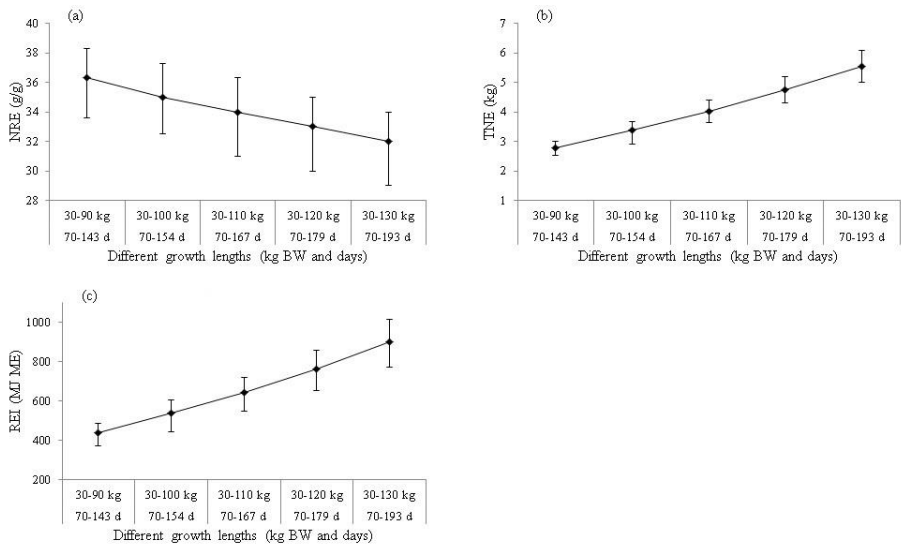


Figure 5.5a-c Average nitrogen retention efficiency (NRE), total nitrogen excretion (TNE) and residual energy intake (REI) at different growth lengths from 30 to 90, 100, 110, 120 and 130 kg slaughter weight, and their respective days of growth for the crossbred population. The upper and lower error bars in NRE represent the estimated trait value for pigs with more than 1 standard deviation above the average ADG at the respective body weight and at least 1 standard deviation below the average ADG at respective body weight, respectively, and vice versa for TNE and REI traits.

Implications

The study provides input parameters for biological growth model based on a crossbred population to examine the impact of change in growth on improvement feed energy efficiency and reduction of nitrogen excretion. Using the biological growth model allows to study the change of energy and nitrogen efficiency during growth, which can be used for developing optimal selection and production strategies, e.g. the biological growth model was used to analyse the impact of reducing slaughter weight on energy intake and nitrogen excretion and showed that this is a feasible strategy to improve energy efficiency and reduce environmental pollution of pork production.

References

- Black, J.L., R.G. Campbell, I.H. Williams, K.J. James and G.T. Davies. 1986. Simulation of energy and amino acid utilisation in the pig. *Research and Development in Agriculture* 3, 121-145.
- Cai, W., D.S. Casey and J.C.M. Dekkers. 2008. Selection response and genetic parameters for residual feed intake in Yorkshire swine. *J. Anim. Sci.* 86:287-298.
- De Haer, L.C.M., P. Luiting and H.L.M. Aarts. 1993. Relations among individual (residual) feed-intake, growth-performance and feed-intake pattern of growing pigs in-group housing. *Livest. Prod. Sci.* 36:233-253.
- De Lange, C.F.M. 1995. Framework for a simplified model to demonstrate principles of nutrient partitioning for growth in the pig. In *Modelling Growth in the Pig*, pp. 71-85 [P.J. Moughan, M.W.A. Verstegen and M.I. Visser-Reyneveld, editors]. Wageningen: Wageningen Press.
- De Lange, C.F.M., B.J. Marty, S. Birkett, P. Morel and B. Szkotnicki. 2001. Application of pig growth models in commercial pork production. *Can. J. Anim. Sci.* 81:1-8.
- Doeschl-Wilson, A.B., P.W. Knap, B.P. Kinghorn and H.A. Van der Steen. 2007. Using mechanistic animal growth models to estimate genetic parameters of biological traits. *Animal: an international journal of animal bioscience* 1:489-499.
- Dourmad J.Y., Y. Henry, D. Bourdon, N. Quiniou and D. Guillou. 1993. Effect of growth potential and dietary protein input on growth performance, carcass characteristics and nitrogen output in growing-finishing pigs. In: *Nitrogen Flow in Pig Production and Environmental Consequences*. Edited by M.W.A. Verstegen, L. A. den Hartog, G.J.M. van Kempen and J.H.M. Metz. P. 206-211.
- Emmans, G.C. 1988. Genetic component of potential and actual growth. In *Animal breeding opportunities* (Eds. R.B. Land, G. Bulfield and W.G. Hill). BSAP occasional publication 12. Pp. 153-181.
- Emmans, G.C. and C. Fisher. 1996. Problems in nutritional theory. In *Nutrient Requirements of poultry and nutritional research* (ed. C. Fisher and K. N. Boorman). pp. 9-39, Butterworths, London.
- Emmans, G.C. and I. Kyriazakis. 1995. A general method for predicting the weight of water in the empty bodies of pigs. *Anim. Sci.* 61:103-108.
- Emmans, G.C. and I. Kyriazakis. 1998. Growth and body composition. In: *A Quantitative biology of the pig*. Ed. Kyriazakis, I., CABI-Publishing, Willingford. pp. 181-197.
- Ferguson, N.S. and R.M. Gous. 1993a. Evaluation of pig genotypes. 1. Theoretical aspects of measuring genetic parameters. *Anim. Prod.* 56:243.

- Ferguson, N.S. and R.M. Gous. 1993b. Evaluation of pig genotypes. 2. Testing experimental procedure. *Anim. Prod.* 56:249.
- Ferguson, N.S., R.M. Gous and G.C. Emmans. 1997. Predicting the effects of animal variation on growth and food intake in growing pigs using simulation modelling. *Anim. Sci.* 64: 513-522.
- Ferguson, N.S. and S.T. Kyriazis. 2003. Evaluation of the growth parameters of six commercial crossbred pig genotypes 1. Under commercial housing conditions in individual pens. *S. Afr. J. Anim. Sci.* 33:11-20.
- Fernandez, J.A., H.D. Poulsen, S. Boisen and H.B. Rom. 1999. Nitrogen and phosphorus consumption, utilisation and losses in pig production: Denmark. *Livest. Prod. Sci.* 58:225-242.
- Gilbert, H., J.P. Bidanel, J. Gruand, J.C. Caritez, Y. Billon, P. Guillouet, H. Lagant, J. Noblet and P. Sellier. 2007. Genetic parameters for residual feed intake in growing pigs, with emphasis on genetic relationships with carcass and meat quality traits. *J. Anim. Sci.* 85:3182-3188.
- Green D.M. and C.T. Whittemore. 2003. Architecture of a harmonized model of the growing pig for the determination of dietary net energy and protein requirements and of excretions into the environment (IMS Pig). *Anim. Sci.* 77, 113-130.
- International Panel for Climate Change (IPCC). 2006. Chapter 10: emissions from livestock and manure management. In 2006 IPCC guidelines for national greenhouse gas inventories (ed. HS Eggleston, L Buendia, K Miwa, T Ngara and K Tanabe), chapter 10, pp. 10.1-10.87. Institute for Global Environmental Strategies (IGES), Hayama, Japan.
- Johnson, Z.B., J.J. Chewning and R.A. Nugent III. 1999. Genetic parameters for production traits and measures of residual feed intake in large white swine. *J. Anim. Sci.* 77:1679-1685.
- Jones, H.E., C.C. Warkup, A. Williams and E. Audsley. 2008. The effect of genetic improvement on emissions from livestock systems. Page 6 in Proceedings of 59th EAAP, Vilnius, Lithuania.
- Knap, P.W. 1999. Simulation of growth in pigs: evaluation of a model to relate thermoregulation to body protein and lipid content and deposition. *Anim. Sci.* (Penicuik, Scotland) 68:655-679.
- Knap, P.W. 2000a. Time trends of Gompertz growth parameters in 'meat-type' pigs. *Anim. Sci.* 70:39-49.
- Knap, P.W. 2000b. Stochastic simulation of growth in pigs: relations between body composition and maintenance requirements as mediated through protein turnover and thermoregulation. *Anim. Sci.* 71, 11-30.

- Knap, P.W. 2000c. Variation in maintenance requirements of growing pigs in relation to body composition. A simulation study. Landbouwniversiteit Wageningen (Wageningen Agricultural University).
- Knap, P.W. 2012. Pig breeding for increased sustainability. In Encyclopedia of sustainability science and technology (ed. R. A. Meyers), Springer Science+Business Media, New York NY, USA. Volume 11, pp. 7972-8012.
- Knap, P.W., R. Roehe, K. Kolstad, C. Pomar and P. Luiting. 2003. Characterization of pig genotypes for growth modeling. *J. Anim. Sci.* 81:E187-E195.
- Knap PW and Schrama JW 1996. Simulation of growth in pigs: approximation of protein turn-over parameters. *Anim. Sci.* 63:533-547.
- Landgraf, S., A. Susenbeth, P.W. Knap, H. Looft, G.S. Plastow, E. Kalm and R. Roehe. 2006. Allometric association between in vivo estimation of body composition during growth using deuterium dilution technique and chemical analysis of serial slaughtered pigs. *Anim. Sci.* 82:223-231.
- Mohrmann, M., R. Roehe, A. Susenbeth, U. Baulain, P.W. Knap, H. Looft, G.S. Plastow and E. Kalm. 2006. Association between body composition of growing pigs determined by magnetic resonance imaging, deuterium dilution technique and chemical analysis. *Meat Sci.* 72:518-531.
- Morel, P.C.H. and G.R. Wood. 2005. Optimisation of nutrient use to maximise profitability and minimise nitrogen excretion in pig meat production systems. *Acta Hort.* (ISHS) 674:269-275.
- Mrode, R.A. and B.W. Kennedy. 1993. Genetic-variation in measures of food efficiency in pigs and their genetic-relationships with growth-rate and backfat. *Anim. Prod.* 56:225-232.
- Saintilan, R., I. Merour, L. Brossard, T. Tribout, J.Y. Dourmad, P. Sellier, J. Bidanel, J. van Milgen and H. Gilbert. 2013. Genetics of residual feed intake in growing pigs: relationships with production traits, and nitrogen and phosphorus excretion traits. *J. Anim. Sci.* 2012-5687.
- Shirali, M., A. Doeschl-Wilson, P.W. Knap, C. Duthie, E. Kanis, J.A.M. van Arendonk and R. Roehe. 2012. Nitrogen excretion at different stages of growth and its association with production traits in growing pigs. *J. Anim. Sci.* 90:1756-1765.
- Storn, R. and K. Price. 1997. Differential evolution: A simple and efficient heuristic for global optimization over continuous spaces. *J. Global Optim.* 11, 341-359.
- Taylor, C.S. 1985. Use of genetic size-scaling in evaluation of animal growth. *J. Anim. Sci.* 61:118-143.
- van Milgen, J., N. Quiniou and J. Noblet. 2000. Modelling the relation between energy intake and protein and lipid deposition in growing pigs. *Anim. Sci.* 71, 119-130.

- Von Felde, A., R. Roehe, H. Looft and E. Kalm. 1996. Genetic association between feed intake and feed intake behaviour at different stages of growth of group-housed boars. *Livest. Prod. Sci.* 47:11-22.
- Wellock, I.J., G.C. Emmans and I. Kyriazakis. 2003. Modelling the effects of thermal environment and dietary composition on pig performance: model logic and concepts. *Anim. Sci.* 77, 255-266.
- Whittemore, C.T. 1994. Causes and consequences of change in the mature size of the domestic pig. *Outlook Agr.* 23(1):55-59.
- Whittemore, C.T., M.J. Hazzledine and W.H. Close. 2003. Nutrient Requirement Standards for Pigs. British Society of Animal Science, Penicuik, Scotland, UK.

6

General discussion

6.1 Introduction

The aim of this thesis was to investigate improvements in feed efficiency and reduction of the environmental pollution by pig production using genetics, genomics and growth modelling tools. In particular, the thesis investigated possible methods to improve energy and nitrogen utilisation by analysing individual pig performance on the phenotypic, genetic and genomic level. Moreover, a biological growth model was used to provide the fundamental information to develop strategies for improvement of energy and nitrogen efficiency. This general discussion will explore the findings in the four main chapters of this thesis, and put these into context with other published studies. Finally, it provides opportunities for future studies.

6.2 Nitrogen excretion

Several mitigation methods for reducing the environmental pollution by animals have been studied. One method is optimization of nutrition. Dourmad et al. (1999) state that; both (i) adequate daily supplies of energy and protein (amino acids) according to pigs' stages of growth and pigs' potentials, and (ii) a balanced supply of dietary amino acids, and consequent reduction in total crude protein of the diet, can improve the efficiency of nitrogen utilisation and therefore reducing the nitrogen excretion in pigs. However, even when good nutrition has been applied to reduce nitrogen excretion, large quantities of nitrogen still remain in the manure which is applied to the farm lands as fertilizers. Another method to minimize the nitrogen pollution is improved methods of handling and applying pig manure. A wide range of manure management practices to reduce environmental pollution caused by nitrogen excretion are described by (Rodhe et al., 2006; Prapasongsa et al., 2010). Another complementary method, studied in the second chapter, would be improvement of feed conversion ratio (FCR), by tailoring physiological requirements, which would substantially reduce nitrogen excretion. However, this requires accurate estimates of protein and lipid deposition during growth along with accurate feed consumption recording.

In the current study, the technique of deuterium dilution was used for the measurement of chemical body composition on live animals throughout growth (Landgraf et al., 2006; Mohrmann et al., 2006). The result of these earlier research projects were a unique dataset containing protein and lipid contents of live pigs along with performance traits available for each individual at 30, 60, 90, 120 and

140 kg target body weights (BW). The dataset allowed estimation of nitrogen excretion and feed efficiency traits at different stages of growth and during the entire growing-finishing period for live animals. In addition, this provides a baseline estimate for nitrogen excretion and energy efficiency for modern pig populations.

In chapter two, the total nitrogen excretion (TNE) was estimated to be 5.35 ± 0.04 kg per pig and the ratio of nitrogen excreted over intake was 72% during the entire growth period from 60 to 140 kg BW for pigs of current study. Saintilan et al. (2013), however, reported 2.61 to 3.36 kg TNE and 58% to 65% for the ratio of nitrogen excreted over intake for different breeds growing from 30 to 110 kg BW. The differences between these two studies could be explained by differences in the phase and length of growth period studied. In the current study animals grew to higher BW (140 kg); the later stages are known to be associated with lower production efficiency. The latest growth stage from 120 to 140 kg had 75% ratio for nitrogen excretion to nitrogen intake compared to 68% for the first stage of growth from 60 to 90 kg. In addition, feed composition could explain some of the variation in nitrogen excretion as the average crude protein of the diet in the current study was relatively speaking 6% higher than that of the diet in the study by Saintilan et al. (2013) (15.6% vs. 16.7% in our study). In the current study growth rate (ADG: 781 ± 8.24 , g/d) and feed efficiency (FCR: 3.58 ± 0.02 , kg/kg) were significantly poorer than those reported by Saintilan et al. (2013) (839 to 978, g/d and 2.49 to 2.79 kg/kg, respectively). These results indicate that the length of the growth period and association of this with growth rate and feed efficiency have substantial effect on nitrogen excretion. Increase in growth rate, improvement in feed efficiency and obtaining optimum slaughter weight have substantial effect on improving the efficiency of pork production system.

6.2.1 Influencing factors on nitrogen efficiency

Different factors have been shown to be associated with nitrogen excretion by pigs such as genetics and gender (Crocker and Robison, 2002), housing system, weight and age (Murrells et al., 2010). The statistical analyses in the second chapter indicated that gender, housing type, the *ryanodine receptor 1* (*RYR1*) gene and batch influenced nitrogen excretion ($P < 0.05$), but the degree and direction of influences differed between growth stages. These show the importance to analyse those factors at different stage of growth for optimising strategies to minimise nitrogen excretion.

Breed effect on nitrogen excretion

Saintilan et al. (2013) reported that purebred pigs have lower nitrogen excretion than crossbred pigs. Their conclusion was based on comparison of four pig breeds: Landrace dam breed, Large White dam breed, Large White sire breed, and Pietrain sire breed, with crossbred pigs used by Dourmad et al. (1999), Fernandez et al. (1999), Van der Peet-Schwering et al. (1999) and chapter 2 of this thesis (Shirali et al., 2012). However this cannot be entirely justifiable by the breed effect because there are differences in nutrition, husbandry, length of growth period and other factors.

It is evident from a review of literature conducted by Tolkamp et al. (2010) that there are considerable breed/genotype effects on energy and nutrient efficiency. These differences are often accompanied with differences in productivity, first of all, productivity in terms of growth rate. A higher efficiency is largely the result of utilising consumed energy and protein to a larger extent for energy and protein retention and to a lesser extent for maintenance. Tolkamp et al. (2010) showed that for many production systems, not only breed/genotype effects on growth rate but also on fertility, mortality, longevity and disease resistance can have considerable effects on energy and nutrient efficiency of production systems as a whole. In addition, Tolkamp et al. (2010) concluded that there is evidence for breed/genotype effects on digestive efficiency in pigs which would lead to differences in nutrient efficiencies in different breeds of pigs.

Effect of gender on nitrogen excretion

In chapter two it was shown that gilts excreted less nitrogen than barrows ($P < 0.05$), which was associated with lower FCR and average daily lipid to protein deposition ratio (ALD:APD) in gilts. Crocker and Robison (2002) concluded that faster growth of barrows (when fed *ad libitum*) resulted in higher turnover of nutrients, and thus higher excretion of all nutrients per kg pig weight. Chapter two showed an association of nitrogen excretion with ALD:APD and fat to saleable meat ratio. This is due to the fact that the amount of energy (and thus average daily feed intake (ADFI)) required for producing fat tissue is at least four times more than lean tissue growth due to differences in water content and metabolism (Kyriazakis and Whittemore, 2006). From the results of the present study, it can be concluded that different pig genders excrete different quantities of nitrogen. Consequently an improvement in FCR can be realised by using gender-adjusted strategies of feeding. This is expected to result in a reduction of nitrogen excretion. Recently, due to the issues related to surgical castration of male pigs, the EU has planned to stop castration of male piglets by 2018 (European Commission, 2010). Entire males are

expected to have better feed efficiency than barrows and consequently results in less nitrogen excretion per pig. In addition, entire males will likely be slaughtered at lower weight to avoid boar taint, which will further reduce nitrogen excretion per pig. In addition, Dammgen et al. (2013) showed that carcass lean meat content is higher in boars than barrow indicating lower nitrogen excretion per kg products in boars. Dammgen et al. (2013) studied the changes in emission of greenhouse gases (GHG) and ammonia due to a shift from growing barrows to entire males, and concluded that growing boars would result in substantial reduction in ammonia emission and to a lesser extent in nitric and nitrous oxide, and methane emissions, due to better FCR as a result of especially lower feed intake relative to growth of boars.

Effect of housing type on nitrogen excretion

In chapter two, single-housed pigs showed lower nitrogen excretion than group-housed pigs ($P < 0.05$). This indicates a better feed efficiency among single-housed pigs, likely due to lower activity and absence of competition for food, in particular at later stages of the growing-finishing period. In later growth stages, group-housed pigs showed 8% and 19% greater TNE during growth from 90 to 120 kg and 120 to 140 kg, respectively, which may indicate that the higher activity, competition and maintenance resulted in lower efficiency at these stages. Strategies (feeding and genetics) to reduce TNE are therefore expected to be most efficient under group-housed conditions at later stages of growth, in particular when pigs are grown to a heavy finishing weight. The comparison of single-housed pigs and group-housed pigs has scientific value to identify differences of extreme conditions (e.g. absence competition and high competition), but under practical condition only group-housing is economically viable.

In future evaluation of housing systems, impact on feed efficiency needs to be included along with consequences on health and welfare of animals. Dammgen et al. (2013) suggested that group-housing of boars and gilts can be an option as their differences in body weight gain is lower compared to gilts and barrows. In addition, Andersson et al. (2005) suggested that rearing boars and gilts in mixed-sex housing results in higher body weight gain than those pigs reared under single-sex housing. However, gilts and boars have different GHG emissions (calculated from methane and nitrous oxide emissions) and different amounts of nutrient in their slurry.

Effect of halothane genotype on nitrogen excretion

In the second chapter, pigs with the *NN* halothane genotype (homozygous normal) at the *RYR1* locus had the lowest nitrogen excretion ($P < 0.05$) at all stages of growth except from 60 to 90 kg BW in comparison to other halothane genotypes. The homozygotes for the *n*-allele at the *RYR1* gene (*nn*-stress susceptible) are known to respond positively to the halothane challenge and have a greater risk of malignant hyperthermia syndrome (MH) and reduced meat quality such as pale, soft, exudative (PSE) meat and low water holding capacity (Zhang et al., 1992). Zhang et al., 1992 have shown that the *nn* genotype seems to increase the water content in lean muscle and suppress fat deposition in lean tissue while reducing meat quality. The heterozygous carrier (*Nn*) pigs grow faster and have better meat quality scores with less muscling than the *nn* genotype (Zhang et al., 1992). Our study showed dominance effects of the *n* allele over the *N* allele with *NN* genotypes having lowest FCR and nitrogen excretion compared to other genotypes.

Effect of maintenance requirements on nitrogen excretion

Reviews of Tolkamp et al. (2010) and Kyriazakis (2011) suggested that maintenance requirements has influence on feed efficiency and consequently on nitrogen excretion. When growth rate is low, maintenance contributes largely on the variation in feed intake required per kg product and consequently in more excretion/emission. This thesis shows that the effect of maintenance on nutrient excretion and efficiency is more pronounced at the later stages of growth because of the increased maintenance requirement with decreasing growth rate, which consequently results in larger nitrogen excretion and lower nutrient efficiency. Studies have suggested the presence of variation in metabolisable energy requirements for maintenance which is due to differences between genotypes in number of behavioural or physiological characteristics. However, the present evidence is not entirely conclusive; reviews of Tolkamp et al. (2010) and Kyriazakis (2011) suggested that variation in energy requirements for maintenance is also associated with differences between genotypes in, especially, level of activity, ability to regulate body temperature and also level of disease resistance.

6.2.2 Effect of improving performance traits on nitrogen excretion**Feed conversion efficiency**

Chapter two showed that improvement in FCR reduces nitrogen excretion substantially. The residual correlations shown in chapter two indicated that nitrogen excretion per weight gain (NEWG) and TNE have large positive correlations with FCR ($r = 0.99$ and 0.91 , respectively). Furthermore, an

improvement in FCR by one phenotypic standard deviation would reduce TNE per pig by 709 g during the entire growing period from 60 to 140 kg BW (Figure 6.1). Based on a live cycle analysis of the pig production system, Jones et al. (2008) estimated an 0.8% annual reduction in global warming potential of methane and nitrogen emissions as a result of genetic trends for growth rate (+8.5 g/d per year), FCR (-0.02 kg/kg per year) and litter size (+0.16 piglets/litter per year) in the UK pig sector from 1988-2007. They suggested that this genetic improvement in pigs has resulted in reduction of 17% in methane, 18% in ammonia and 14% in nitrous oxide emissions from 1988-2007. In addition, they suggested that improvement in each of the traits results in reduction of environmental pollution, however, the genetic improvement in FCR is responsible for 70% reduction of nitrous oxide, 58% of reduction in global warming potential, and more than 5% of ammonia and methane emissions. Furthermore, they concluded that this rate of genetic improvement will continue over the next few decades and may increase due to the use of molecular genetic tools. In the current study, a 10% improvement of FCR from 60 to 140 kg BW resulted in a 12% reduction of TNE in the same period. Fernandez et al. (1999), in a national study in Denmark on pigs grown from 30 to 100 kg, estimated a reduction in nitrogen emission of 13% as a result of 10% improvement of FCR. The present study suggests that reduction of nitrogen excretion by improvement of FCR is the primary choice to reduce the environmental impact of pork production and can be achieved by using better feeding strategies, optimisation of diets, husbandry, genetics, etc.

Growth rate

The improvement of animal productivity was suggested by FAO (2006) as an efficient way to increase world production of animal products and meet the increasing world demand, without increasing the use of land or the emission of GHG. The results in chapter two indicates that NEWG and TNE have moderate negative correlations with ADG ($r=-0.53$ and -0.48 , respectively), for the entire growing period (60 to 140 kg BW). Furthermore, an increase in ADG by one phenotypic standard deviation would reduce TNE per pig by 307g over the entire growing period (Figure 6.1). These results indicate that increasing growth rate is the second choice for reducing the environmental pollution of pig production via nitrogen excretion. Generally, chapter two suggested that a substantial reduction in nitrogen excretion can be obtained by improving production traits such as growth rate. For most production systems, breeding programmes already include one or more production traits in their selection indices. These strategies have had a beneficial effect on energy and nutrient efficiencies of pig production. Jones et al.

(2008) showed a substantial effect of genetic trends for growth rate (+8.5 g/d per year) on annual reduction in global warming potential of methane and nitrogen emissions in the UK pig sector from 1988-2007. They found based on a life cycle analysis, considering the whole production system that the proportion of total change in emissions due to genetic improvement of ADG can be 70% reduction in ammonia and 70% reduction in methane as well as 20% reduction in the global warming potential by the pig sector. Chapter two shows that a 10% increase of ADG resulted in a 5% reduction in TNE, during the entire growing period. Fernandez et al. (1999) in a national study in Denmark on growing pigs grown from 30 to 100 kg estimated a reduction in nitrogen emission of 13% per pig, from a 10% improvement of the mean of growth rate. A review of Tolkamp et al. (2010) showed that improvement in growth rate will substantially improve nutrient efficiency due to reduction in the length of the growing period and thus reduction in maintenance requirements due to a reduction in the time required to achieve (the fixed) slaughter weight. Growth rate has a moderate heritability (e.g. von Felde et al., 1996; Dufrasne et al., 2011). In chapter four, a large heritability (0.64) for ADG was estimated in the current population indicating a substantial genetic variation for this trait for genetic improvement and reduction in TNE.

Other traits closely related to growth rate and association with nitrogen excretion, are length of the growing period and average daily nitrogen intake. A reduction in length of the growing period to a desired (thus fixed) slaughter weight, and average daily nitrogen intake by one phenotypic standard deviation resulted in 350 g and 149 g decrease in TNE, respectively (Figure 6.1).

Body composition

In meat-producing animals, feed efficiency is also affected by body composition, as in pigs fat tissue growth requires at least four times more energy than lean tissue growth due to differences in water content and metabolism (Kyriazakis and Whittemore, 2006; van Milgen and Noblet, 2003). Many selection programmes also include traits in relation to product composition, most importantly the fat to lean ratio in the product. Knap (2012) indicated that annual reduction in global warming potential of methane and nitrogen emissions will be further reduced as a result of genetic trend in lean content (+ 0.5 %, lean meat per year). In a simulation study, Morel and Wood (2005) showed that nitrogen excretion was 37% less in a group of leaner (high maximum protein deposition, 200 g/d) than in fatter (low maximum protein deposition, 120 g/d) pigs, which agrees with results of this thesis. In chapter two, the lipid to protein ratio showed moderate positive correlations with

NEWG (0.25) and TNE (0.31). Furthermore, reduction of lipid to protein gain ratio by one phenotypic standard deviation reduced TNE per pig by 211 g over the entire growing period (Figure 6.1). The results in chapter two indicate that improving body composition, in particular lean growth, can reduce nitrogen excretion and improve nutrient efficiency.

Selection for a reduction in fat tissue and increase in lean tissue has been very successful in pigs, with decreasing backfat thickness from 3.2 to 1.9 mm and increase in loin muscle area from 40 to 60 cm² in Pietrain pigs after 30 years of selection (Roehe et al., 2003). In chapter four, backfat thickness was shown to have a large heritability (0.44) indicating that this trait can still be improved by genetic selection. This further indicates that genetic improvement of backfat thickness and body composition can be carried out with consecutive result in reduction of nitrogen excretion and improvement in nutrient efficiency. However, in many pig lines the optimal fatness has been achieved and further reduction may result in reduction in fertility (e.g. Karsten et al., 2000), longevity (e.g. Lopes-Serrano et al., 2000) and meat quality (e.g. Sellier 1998).

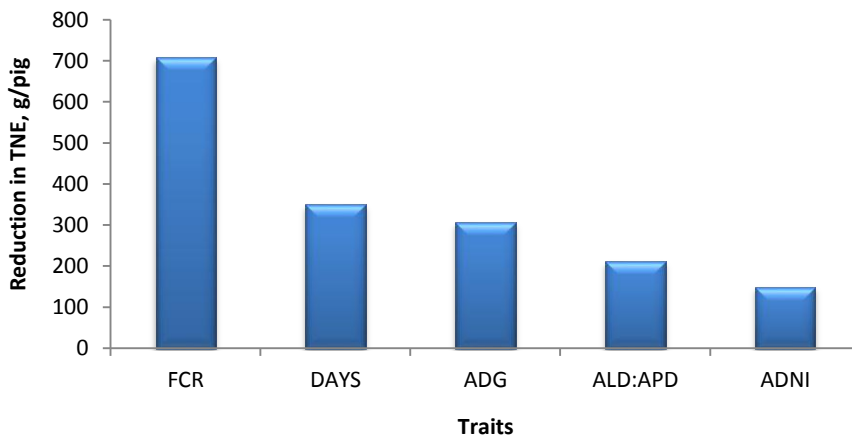


Figure 6.1 Effect of improving feed conversion ratio (FCR), length of growing period (DAYS), average daily gain (ADG), lipid to protein gain ratio (ALD:APD) and average daily nitrogen intake (ADNI) by one standard deviation on total nitrogen excretion (TNE) per pig during the entire growing period from 60 to 140 kg BW

Although, FCR has been used to evaluate feed efficiency for many years, it is not widely accepted in animal breeding programs because FCR has genetic-statistical properties (being the ratio of ADFI and ADG) with difficult prediction of genetic gain in the component traits in future generations (Sainz and Paulino, 2004; Hoque and Suzuki, 2009). Gunsett (1984 and 1986) already found that direct selection on FCR may result in disproportional or unpredictable genetic gain for ADFI or ADG. Therefore, most of the improvement in feed efficiency has been due to a reduction in backfat thickness and improvement in growth rate (Knap and Luiting, 1999; Tolkamp et al., 2010).

6.3 Efficiency of feed utilisation, various aspects

Residual feed intake (RFI) has been discussed as an alternative trait to improve feed efficiency, independent to production traits (de Haer et al., 1993). Therefore, there is considerable interest in using this efficiency measure as a useful new trait in selection programmes for a variety of production systems. Variation in RFI captures differences in efficiency of digestion, efficiency of metabolic utilisation of feed energy, maintenance requirements, tissue turnover rates, activity, and stress, among others (e.g. Herd and Arthur, 2009; Dekkers and Gilbert, 2010). In poultry, selection directly for low RFI (higher efficiency) was suggested because it could lead to reduction of maintenance requirements and improvement of feed efficiency (e.g. Luiting and Urff, 1991). In pigs, a reduction in RFI has been shown to improve feed efficiency in experimental selection lines (Gilbert et al., 2007; Cai et al., 2008).

6.3.1 RFI estimation

Differences in models used to estimate RFI have been shown to result in different relationships between RFI and production traits (Mrode and Kennedy, 1993; Johnson et al., 1999), which can influence genetic gain when selecting for RFI. In pigs, RFI has been mostly estimated using models adjusting feed intake for ADG and backfat thickness, often together with metabolic body weight (Gilbert et al., 2007; Cai et al., 2008). In these studies, changes in the energy content of diets during the growing period have been neglected, which may have an effect on the accuracy of RFI estimation. Therefore, in the present study, metabolisable energy intake was used to estimate residual energy intake (REI). Tissue deposition traits used to estimate RFI in the above referenced studies are proxies for lean and fat tissue growth, which are mostly available in practical pig breeding programs. In the present study, however, measures of protein and lipid deposition at different stages of growth were available, which may improve the accuracy of REI estimation. Feed efficiency, growth and feed intake are all interrelated, and a

better understanding of their relationship is required to accurately predict the outcome of selection. Comparison of the models to predict REI in chapter four showed that fitting various systematic effects together with the covariables average protein deposition (APD) and average lipid deposition (ALD) or their pre-adjusted values, explained a slightly larger proportion of the phenotypic variance (79%) in average daily energy intake (ADEI) than models using the proxy measures (ADG and backfat thickness) (77%) in addition to all other systematic effects. This indicates that there is a substantial proportion of variation in feed consumption (at least 21%) in growing pigs is unrelated to lean and fat growth. The results indicate that the use of proxy traits for lean and fat tissue growth, such as ADG and backfat thickness, to estimate REI, results in estimates similar to their direct measurements (ALD and APD). However, when estimating genetic parameters in chapter four, the 8.6% lower phenotypic variance of energy intake explained by prediction models fitted to proxy traits (ADG and backfat thickness) indicates lower accuracy in estimation of REI, which may result in lower selection response compared with using the direct measurements of lean and fat tissue growth. Including REI (alternatively RFI) in selection programmes could lead to further improvement of feed efficiency especially in later stages of growth and consequently reduction in nutrient excretion. This size of improvement depends on the genetic variance in REI. The estimated heritability of 0.44 for REI in chapter four was in the upper range of heritabilities estimated in the literature, which ranged from 0.10 to 0.40 (Hoffmann et al., 1992; de Haer et al., 1993; Mrode and Kennedy, 1993; de Vries et al., 1994; Von Felde et al., 1996; Johnson et al., 1999; Labroue et al., 1999; Gilbert et al., 2007; Cai et al., 2008; Bunter et al., 2010; Saintilan et al., 2011). The large heritability and genetic variance identified for REI suggests a great selection potential. In particular selection experiments showed that selection for low RFI can decrease the feed required for a given rate of production as shown in growing pigs of experimental lines (Gilbert et al., 2007; Cai et al., 2008).

6.3.3 REI at different stages of growth

Greater phenotypic and genetic variance in REI at later growth stages, as found in chapter 4, indicated that these stages show more variation in feed utilization. This was in agreement with the results in chapter two which also revealed that nitrogen excretion is changing throughout the growing period. Greatest nitrogen efficiency (32%) (nitrogen retention/nitrogen intake), lowest average daily nitrogen excretion (ADNE), NEWG and TNE were observed during the earlier growth stage from 60 to 90 kg BW. Nitrogen efficiency gradually decreased with progressing growth stages. The lowest nitrogen efficiency (25%) was during the latest growth period from 120

to 140 kg BW, which was associated with lowest nitrogen retention and greatest nitrogen intake in comparison with the corresponding traits in the other stages of growth. The change in level of nitrogen efficiency during growth indicates the necessity of selection for nitrogen efficiency at different stages of growth in order to maximise animal performance. Similarly, chapter four showed changes in the variation of REI at different stages of growth, with REI explaining 26% to 34% of the variation in average daily feed energy intake during growth, and the REI demonstrated a different genetic background at different growth stages, with no to moderate genetic correlation between REI estimates during growth, implying that selection for REI will also be more efficient if each stage of growth is considered separately (Figure 6.2).

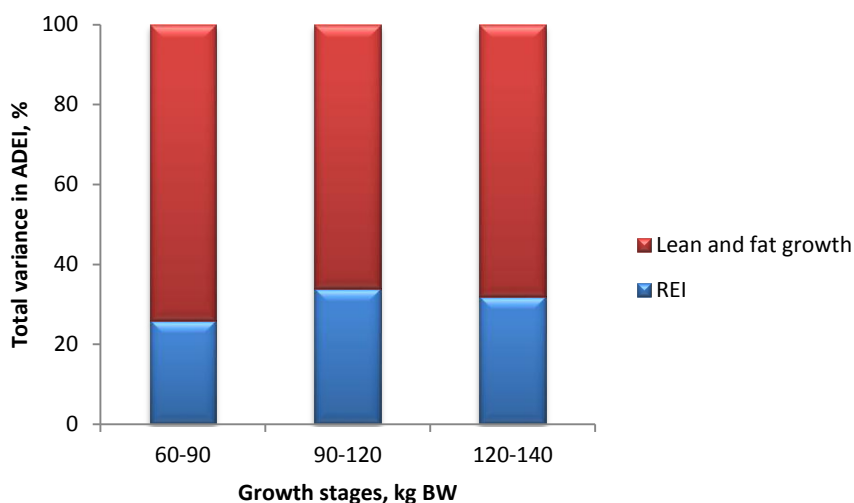


Figure 6.2 Proportion of phenotypic variance in average daily feed intake (ADFI) contributed by residual energy intake (REI) and component traits of REI model (lean and fat growth) at different stages of growth.

Most importantly, selection at the later stages of growth is expected to result in larger selection response due to larger genetic variance of REI. Webb (1998) suggested changing the shape of the feed intake curve by increasing feed intake at an early age but decreasing it at a later age. Cai et al. (2011) performed genetic analysis of longitudinal measurements of feed intake and BW, along with other performance traits in selection lines for RFI in Yorkshire pigs and concluded that selection for lower RFI has resulted in a lower feed intake curve and lower BW

curve especially towards the end of test period (210 days of age, in this case). However, to change the curves of performance traits (e.g. feed intake, growth, and backfat) significantly towards desired directions, methodologies should be studied with attention to the high inter-relationship between performance traits. The principal components of breeding values indicate that there are two main components, firstly growth and secondly feed efficiency, which explain most of the genetic variance in the analysed traits. This indicates that these components can be improved independently and are expected to result in substantial genetic improvement.

6.3.4 Underlying factors influencing REI

Association of REI with feed intake traits

In chapter four, the strong favourable phenotypic and genetic correlations between REI and FCR suggest that selection for lower REI will substantially improve FCR and nutrient efficiency. The genetic correlations of REI with ADEI (0.80), and FCR (0.84) are at the upper level compared with the literature estimates of RFI with ADFI, which range from 0.52 to 0.77, and RFI with FCR, which range from 0.71 to 0.85 (Gilbert et al., 2007; Cai et al., 2008; Bunter et al., 2010; Saintilan et al., 2011; Saintilan et al., 2013). Although ADEI was phenotypically and genetically correlated with both growth and REI, selecting for lower REI is expected to reduce the proportion of ADEI, which is not associated with lean and fat tissue growth.

Association of REI with nitrogen excretion

Knowledge of genetic and phenotypic associations between RFI and nitrogen excretion traits is scarce. In Chapter three, large favourable genetic (0.85) and phenotypic (0.69) correlations between REI and TNE were estimated, which is in agreement with results of Saintilan et al. (2013) who reported large genetic (0.84) and phenotypic (0.79) correlations between RFI and nitrogen excretion. Furthermore, Saintilan et al. (2013) showed large phenotypic (0.71 to 0.80) and genetic (0.51 to 0.85) correlations between RFI and phosphorous excretion as another important excreted nutrient of concern. In addition, they showed a favourable association between RFI and nitrogen and phosphorous efficiency, indicating the importance of this trait for reducing nutrient excretion and improving nutrient retention efficiency. In the current study, phosphorous excretion was not aimed at because it could not be estimated due to lack of measures needed for accurate estimation of phosphorous retention. The loadings of the first principle component of estimated breeding values (EBV) of all analysed performance traits (REI, FCR, TNE, ADEI, backfat thickness, ADG, ALD and APD)

indicated that most of the variation is associated with growth and its required energy intake, suggesting a great potential for genetic improvement in these traits. Only slightly less variation of EBV of all analysed performance traits is explained by the second principle component reflecting traits associated with feed efficiency, which also suggests their great potential for genetic improvement. Growth and body composition traits showed only minor influence on second principle component, implying that REI as a measure of feed efficiency can improve production efficiency, reduce environmental pollution, and reduce the use of energy resources independent from growth and body composition.

Association of REI with body composition traits

Selection for feed efficiency based on RFI led to leaner pigs in experimental populations (Gilbert et al., 2007; Cai et al., 2008; Saintilan et al., 2013) mostly through a decrease of backfat and an increase in lean meat content. This is due to partitioning nutrients towards lean tissue instead of fat tissue production as lean growth is more energy efficient than fat growth. Furthermore, Johnson et al. (1999) reported that selection for lower RFI would have a positive effect on loin eye area. Selection for lower RFI has widely shown to improve carcass composition by greater muscle to fat ratio, which suggests that carcass dressing percentage is expected to be enhanced. Saintilan et al. (2013) reported that traits related to nitrogen and phosphorous excretion are genetically more closely related to RFI in leaner sire breeds than in fatter dam breeds, and associated with higher genetic correlations between FCR and RFI in sire than in dam breeds. Chapter two of the current study also indicated the favourable positive effect of lean growth on nitrogen excretion at different stages of growth and during the entire growth.

Association of REI with meat quality traits

RFI and meat quality traits have been shown to be unfavourably associated, as low RFI pigs have a lower meat quality index, with a paler and more acid meat, as shown for Large White dam and Pietrain sire breeds (Saintilan et al., 2013). Lefaucheur et al. (2011) reported that the leaner carcasses with greater muscle content, thinner backfat and less intramuscular fat content of low RFI pigs showed more hypertrophy of muscle fibers of the type IIb. This muscular hypertrophy was associated with higher muscle glycogen content, resulting in reduced technological quality of meat such as lower pH at 30 min postmortem, paler colour and higher drip loss, suggesting that selection for lower RFI may impair some meat quality traits. In addition, Gilbert et al. (2007) reported significant correlated responses of meat quality with several carcass traits for Large White pigs; low RFI lines had

significantly lower pH, paler meat colour, heavier carcass weight (increased dressing percentage) and lean cut (weight of loin). In contrast, Cai et al. (2008) reported no significant difference between the low and high RFI lines for these traits; however showing slightly lower pH, darker meat colour, lower carcass weights, and larger loin muscle area in Yorkshire pigs. The differences between the two studies could be due to the breed differences and gender differences as Cai et al. (2008) used only gilts for carcass traits vs. gilts and castrated males reported by Gilbert et al. (2007).

Biological basis of RFI

The variation in RFI reflects differences in digestion processes, metabolic utilization of feed intake, or both, in animals of similar BW and production levels (Nguyen Hong et al., 2005). In cattle, RFI was found to be positively correlated with methane emissions and heat production or retained energy, but not with maintenance energy requirements or efficiency of utilization of ME for growth (Nkrumah et al., 2006; Castro Bulle et al., 2007). In growing pigs, Saintilan et al. (2013) showed that RFI has a large positive correlation with nitrogen and phosphorous excretion. In addition, chapter four showed large genetic and phenotypic association of REI with nitrogen excretion in growing pigs. The association of RFI with excretion traits may indicate the association of RFI with underlying biological pathways associated with these traits such as digestion and metabolism. Luiting et al. (1991) suggested that up to 85% of the difference in heat production between lines of laying hens differing in RFI can be related to differences in physical activity. Barea et al. (2010) investigated the presence of variation for energy utilisation between high and low RFI lines and reported that high and low RFI pigs have no differences in digestibility coefficients or nitrogen retention. However, high RFI lines are energetically less efficient due to greater heat production related to physical activity and basal metabolic rate. Chapter four showed that variation in ADEI explained by REI depends on the components of the prediction model. In addition, variation in REI increases over the different stages of growth suggesting different pathways to be involved in the variation in REI. Comparing nutrient efficiency in pigs with poultry, Kyriazakis (2011) in a review suggested that pigs systematically have a greater nutrient efficiency than chickens due to differences in the digestive tract and digestive processes. Furthermore, the above study suggested the presence of genetic variation in maintenance requirements in pigs, with digestive tract efficiency explaining a proportion of it. Therefore selection to decrease maintenance requirement can contribute to lower energy expenditure and consequently lower nitrogen excretion. The results of chapter two also indicate the

effect of maintenance requirement on nitrogen excretion especially in the later stages of growth. The variation in efficiency of energy and nutrient utilisation has been of interest for improvement of feed efficiency and decreasing environmental pollution; however, it has been a difficult question to tackle due to various pathways of nutrient metabolism.

RFI is expected to be associated with energy utilization and metabolism. Barea et al. (2010) indicated a substantial genetic change in the dynamics of energy partitioning in growing pigs by selecting for RFI. They showed lower efficiency in high RFI lines due to higher heat production related to physical activity and basal metabolic rate. However, no differences in digestibility coefficient and nitrogen and energy retention between low and high RFI lines were obtained. In addition, Renaudeau et al. (2013) reported no differences between high and low RFI lines of pigs for nutrient and energy digestibility. Furthermore, they reported higher urine excretion in high RFI lines which can cause more slurry and consequently higher environmental pollution by these pigs. They also showed that despite lower heat production in low RFI pigs, these pigs have not changed their ability of losing heat, and suggested no advantage of low RFI pigs on heat tolerance. Le Naou et al. (2012) investigated the metabolic changes and tissue responses in selection lines of RFI in growing pigs and suggested that greater catabolic activities in glucose and fatty acid metabolism in the liver and muscle of pigs selected for high RFI may considerably generate ATP to sustain daily BW gain and maintenance requirements in those pigs. This suggests that high RFI pigs have higher maintenance requirements compared to low RFI pigs. This is in agreement with results of Boddicker et al. (2011) who reported lower maintenance energy requirements for low RFI pigs, and concluded that maintenance requirements may be one of the main biological factors that contribute to the differences in RFI. Lkhagvadorj et al. (2010) reported a switch to short-term energy storage in low RFI pigs compared to control line pigs, which was associated with a down-regulation of expression of genes involved in lipogenesis in both liver and adipose tissues. The lack of long-term energy storage could be a problem for coping with stresses such as temperature or disease. However, Richardson et al. (2002) demonstrated that low RFI ruminants have better resistance to stress, as shown by blood cell parameters in divergently selected steers for RFI.

Dekkers and Gilbert (2010) reported the effect of genetic selection for RFI on feeding behaviour in experimental pig lines in the USA (ISU) and France (INRA). They showed that pigs from both experimental lines with low RFI had higher

consumption rate when in the feeders. The ISU low RFI line spent 10 minutes less in the feeders than the control line. The number of meals was also reduced in the INRA low RFI line, although number of visits was similar for both lines. Number of visits to the feeder tended to be lower in the ISU low RFI line but the impact on number of meals was not evaluated. These findings are consistent with the positive correlation estimated by de Haer et al. (1993) between RFI and daily feeding time and total number of visits in pigs, and the generally greater levels of activity in high RFI chickens (Luiting et al., 1991). Several studies have shown that animal behaviour is highly heritable (e.g. Von Felde et al., 1996; Schulze et al., 2003 using feed intake behaviour; Turner et al., 2008 and 2009; D'Eath et al., 2009 using aggression of pigs) and therefore behavioural traits may be used as indirect selection traits to reduce maintenance requirements. In particular, animal behaviour may be directly associated with energy requirements for maintenance. So far, selection on RFI has been shown to be associated with animal characteristics that are related to energy cost. For instance low RFI pigs are less active for feed consumption and social interactions (Dekkers and Gilbert, 2010), have a lower heat production (Barea et al., 2010; Renaudeau et al., 2013), reduced activity of enzymes involved in oxidative and glycolytic metabolisms (Le Naou et al., 2012), reduced viscera size (Dekkers and Gilbert, 2010), and lower maintenance requirements (Boddicker et al., 2011). In addition, Brossard et al. (2012) reported that low RFI pigs have higher requirements for amino acids, expressed in grams of digestible lysine per MJ of net energy. This suggests that an improvement in RFI should be carried out in-line with reformulation of diet for these new genotype pigs to cover their nutritional requirements and to capture the benefit on improved feed utilized efficiency.

6.3.5 Shortcomings in the current study and ways to overcome these

The current study was based on a limited population size, due to high costs of measurements of protein and lipid deposition on live animals during growth. This resulted in a limited number of animals to perform variance component estimations and genetic correlations. A large number of animals are necessary to conduct a more comprehensive and conclusive study. However, such a study would be very expensive due to gathering such difficult to measure phenotypes. Based on the analysis of the current data it can be decided to extend the data. In addition, the genetic correlations were approximately estimated by using correlations between EBV, which do not exactly represent the true genetic correlations, but rather the direction and the strength of the correlations. Moreover, the used population comprises of crossbred animals so that not only additive genetic effects

but also dominance genetic effects may influenced the estimates of the genetic parameters.

Direct selection on feed intake is prohibited due to the difficulties and expense of recording feed intake on a large number of animals, but possible if the genes responsible for feed intake and efficiency of feed utilization are known. A comprehensive understanding of mechanisms that control feed intake and energy metabolism helps to discover such genes and to utilize genetic information on feed intake in a manner that will enhance production efficiency. The population in the current study was designed for QTL mapping and allowed to detect potential QTL associated with REI to effectively understand the physiology and genomic basis of feed efficiency in addition to nitrogen excretion as a predictor for environmental pollution. In addition the mode of inheritance of those QTL gave insight into the importance of additive, dominance and imprinting effects on energy efficiency and additive and dominance effects on nitrogen efficiency.

6.4 Genomics of efficiency of feed utilization

Use of genomic information could improve RFI, by improving its genetic response due to more accurate selection and reduction in generation interval and also revealing the genomic architecture of feed efficiency along with excretion traits. However, only a small number of significant associations between this trait and genetic polymorphisms at individual loci have been reported so far (Fan et al. 2010; Gilbert et al. 2010). In chapter three, six novel QTL for REI were identified at different stages of growth.

Bunter et al. (2010) showed large to moderate genetic correlations of insulin-like growth factor one concentration (IGF-I) with RFI (0.63 ± 0.15), FCR (0.78 ± 0.14), feed intake (0.26 ± 0.17), and backfat (0.52 ± 0.11). They concluded that reduction in juvenile IGF-I is associated with leaner, more efficient animals. Therefore, IGF-I could be a valuable physiological indicator of genetic merit for economically important efficiency traits, particularly because it is measured early in the life of animals. However, Lefaucheur et al. (2011) reported that plasma concentrations of IGF-I were not affected by selection for the RFI.

6.4.1 Genomic architecture of REI

Genome scans for feed efficiency traits are of importance to reveal the underlying biological causes and its results can be used in selection to increase the rate of genetic gain. In chapter three, six novel QTL for REI were identified revealing the genomic architecture of efficiency in feed utilisation and indicating that the

regulation of feed efficiency is partly independent from that of production traits. In addition, 23 QTL for different nitrogen excretion traits were observed illustrating the underlying complex genetic basis of such traits. To our knowledge few QTL for RFI have been previously identified (Fan et al., 2010; Gilbert et al., 2010). Lkhagvadorj et al. (2010) studied the genes and pathways that underlie RFI through gene expression profiling, and suggested that 311 genes in subcutaneous adipose tissue and 147 genes in liver were differentially expressed due to RFI differences. They concluded that the lipid metabolic pathway was over-represented by down-regulated genes due to low RFI suggesting that low RFI pigs shifted to energy conservation and efficient utilization. The authors further proposed an energy conservation mechanism which may be controlled by metabolic pathways and transcriptional factors. The findings of Lkhagvadorj et al. (2010) are in agreement with the result of the current study. In chapter three, four of the QTL for REI (or RFI) were positionally overlapping with nitrogen excretion traits suggesting the change in efficiency of feed utilisation due to underlying causes of variation in REI such as metabolism, digestion, and protein turnover. Fan et al. (2010) reported an association of the fat mass and obesity (*FTO*) related single nucleotide polymorphism (SNP) marker p.Ala198Ala on SSC6 with RFI. In addition, this SNP was found to be associated with feed intake. Furthermore, they reported another SNP marker, c.646 + 514A > G, associated with transcription factor 7-like-2 (*TCF7L2*) gene as a suggestive marker ($P < 0.1$) for RFI. However this marker was significantly associated with ADG, feed intake, and meat colour. *FTO* and *TCF7L2* have been identified as the most promising candidate genes associated with type 2 diabetes in humans (Dina et al., 2007; Grant et al., 2006). In pigs, the mutations within *FTO* were found to be associated with growth rate and fatness (Du et al., 2009; Fan et al., 2010; Fontanesi et al., 2009), and *TCF7L2* mutations were associated with backfat (Du et al., 2009). The study of Fan et al. (2010) suggested that the involvement of *FTO* and *TCF7L2* genes with variation in RFI indicates that there is a common pathway or network regulating fatness, energy balance and feed intake. In addition, the *TCF7L2* gene was associated with meat colour traits and the individuals with lower RFI tended to have darker meat, which is different from the report of Gilbert et al. (2007). Possible reasons for the discrepancies could be lines with different genetic background; because Yorkshire was used in the study of Fan et al. (2010) while Large White was used in the other study (Gilbert et al., 2007). Fan et al. (2010) and Gilbert et al. (2007) also concluded that no common genetic markers influenced both RFI and meat quality traits, indicating that for this limited sample of candidate genes the genetic relationship between these traits is low, and therefore, meat quality of pigs might not be affected by selection for low RFI. In the

genomic regions of these markers no QTLs for REI were detected in our study. This could be due to lack of power or difference in populations used in the studies, Fan et al. (2010) used purebred Yorkshire pigs and the current study used a crossbred population of Pietrain mated with a crossbred PIC dam line.

Gilbert et al. (2010) reported two QTL for RFI on chromosomes 5 and 9. The QTL for RFI on SSC5 was at a suggestive level and showed no positional overlap with other traits in that study. The QTL for RFI on SSC9 was positionally overlapping with QTL for feed intake and ADG. These results indicate that some of the variation in RFI is explained by growth traits, and some other remaining variation by underlying biological pathways associated with RFI. This is in line with the conclusions of chapter three of the current study which identified different QTL revealing different biological backgrounds underlying feed efficiency. The QTL for RFI reported in the above study could not be detected in the current project because no genotype information of SSC5 was available.

6.5 Modelling feed efficiency and nitrogen excretion

Chapters two to four show that feed efficiency varies with different stages of growth and has different genetic and genomic background in the various growth stages. Therefore, modelling feed efficiency as a function of age or weight reflects that feed efficiency can be a different biological trait at different stages of growth. The ability to define the genetic characteristics of pigs in terms of few parameters through growth modelling is appealing for exploring potential consequences of genetic selection on a variety of traits simultaneously. Furthermore, individual estimates for biological traits that are difficult to observe in practice (such as APD, ALD, REI or TNE examined here) can be estimated by fitting these models to available measurements of observable traits. In this study, the biological growth model gave insight into the association between observable growth rate and underlying genetic potentials for protein and lipid deposition, as well as feed efficiency and nitrogen excretion.

Chapter five showed that growth models can be used as basis to identify the effect of change in growth on energy and nitrogen efficiency. Current study showed that fast growing pigs have 20% lower nitrogen excretion and 26% better energy efficiency due to better lean growth and shorter growing period compared to slow growing pigs. Growth models could be used to alter BW and feed intake curves (De Vries and Kanis, 1992)) as it provides longitudinal predictions and also to look at consequences of altered curves. The growth model also provides estimates for economically and environmentally important traits on a daily basis rather than over

a long period of time, thus revealing the continuous changes in these traits at different stages of growth. Furthermore, growth models can be used to compare different breeds and the commercial crossbreds with respect to energy and nitrogen efficiency. The results of chapter five indicate that selection for fast growth in dam lines, feed efficiency and leanness in sire lines are likely to improve energy and nitrogen efficiency. As dam and sire lines pass their genetic merits to their offspring selection would result in reduction of nitrogen excretion and improvement in feed efficiency in growing pigs. Moreover, growth models can be used to determine the improvement in nutrient efficiency by selecting for REI, nitrogen excretion traits and other production traits. Growth models can be a tool to predict REI and nitrogen excretion for use of International Panel for Climate Change (IPCC). In addition, the results of chapter five can be used to improve the prediction equations for nitrogen excretion in IPCC (2006). Growth models can be used to optimize slaughter weight with respect to energy and nitrogen efficiency. Chapter five suggested decreasing slaughter weight as a strategy to further reduce nitrogen excretion and improve profitability. The energy and cost saving involved in the decrease of REI loss when slaughter weight was decreased from 130 to 100 kg were 362 MJ ME and £11.94 per pig. This was followed by a substantial decrease in nitrogen excretion of 2.16 kg per pig. Furthermore, reduction in slaughter weight would result in reduction of maintenance requirement due to shorter growing period and thus improvement in nutrient efficiency. Growth models can be a tool for evaluating the consequences of such a strategy. These results might help for improving efficiency, and to define optimal strategies for commercial breeding programmes.

6.5.1 Shortcomings of the current growth model

An important assumption made in the current growth model, is that feed intake is restricted by assuming that pigs will not exceed their desired feed intake to meet their maintenance requirements and growth potential. In reality, pigs may consume more feed than their requirements due to different factors such as variation in feeding behaviour and competition (Von Felde et al., 1996), thus resulting in larger REI and TNE (less energy and nutrient efficiency). In addition, the diet composition in the model matched the real diet as much as possible, but it was assumed that the amino acid composition was ideal. The current model is therefore more likely to under-estimate rather than over-estimate the REI and TNE traits. However the current growth model could be extended to reflect competition among animals and investigate the deviation from the ideal amino acid composition.

6.6 Future research

Rapid technological advances allow us to collect high density SNP panel or even whole genome sequences and complex trait phenotypes from a large number of individuals. This had led to the development of selection based on high density SNP panel's information, called genomic selection. Genomic selection, as we have come to know it was first described by Meuwissen et al. (2001) and has been described as "the most promising application of molecular genetics in livestock populations since work began almost 20 years ago" (Sellner et al., 2007). It is based on the simultaneous selection for many thousands of genetic markers that densely cover the entire genome, made possible by the development of arrays with many thousands of SNPs for fast genotyping throughput. Genomic selection is particularly cost-effective to improve selection on traits related to energy efficiency, where there is limited number of animals with detailed phenotypes.

The availability of the pig SNP chip covering the pig genome, enable genome-wide association studies to be carried out in breeding stocks that are independent from the set up of experimental crossbreed populations and that potentially permit more accurate localisation of genes responsible for phenotypic variation. From results of this thesis and Gorbach et al. (2010) it can be concluded that genome-assisted selection to predict feed efficiency and genomic selection to predict breeding values for feed efficiency are feasible to genetically improve the conversion of nutrients to desired animal products. However, the detection of markers associated with trait of interest and/or the estimation of marker effects will essentially not completely contribute to the explanation of the complex biology of the trait. Meuwissen and Goddard (2010) reported that using whole-genome sequence data improves the accuracy of predicting genetic values compared to using SNP chips. Moreover, whole-genome sequence data gives the opportunity to discover causative mutations and to use this in gene assisted selection or a combination of genotype-assisted selection and genomic selection.

In order to address the biological networks responsible for variation in feed efficiency under three levels of (i) feed consumption (such as feeding behaviour and intake), (ii) nutrient digestibility and (iii) metabolism (anabolic and catabolic utilisation of nutrients) transcriptomic, proteomic and metabolomic genomic approaches are promising and have the potential to generate new hypotheses and knowledge to develop tools for genotype-assisted selection and management as well as the deduction of novel biomarkers. The genomic contribution to organismal phenotype occurs via the transcriptome, the proteome and the metabolome.

Large number of genome-wide molecular profiling experiments provide molecular phenotypes (e.g. levels of RNA, protein, metabolites, phosphorylation, glycosylation, or methylation) that are associated with the trait of interest. Genome-wide molecular interaction maps (e.g. protein-protein, protein-DNA or protein-metabolite interactions) provide insight into the structural and functional organisation of the genome. Application of appropriate statistical and bioinformatic modeling frameworks for exploiting and integrating the data resulting from these technologies, will potentially lead to improved understanding of the genetic architecture and better predictive models of complex traits. Using these new advanced technologies allow for development of a genome partitioning approach for identifying genetic variants that influence complex traits such as feed efficiency.

Transcriptomics investigates the expression levels of messenger RNA (mRNA) in a given tissue at a certain time under different conditions (Sanchez-Pla et al., 2012). Once a candidate gene (or genome region) is found to have a significant effect on a given phenotype then expression studies on the gene in the population can be undertaken to better understand the role that gene plays in the biological process affecting that trait. Understanding the biological process underpinning the expression of a phenotype can give greater insight into other potential candidate genes and molecular mechanisms regulating the trait of interest. This type of methodology could prove extremely useful for complex traits, likely to be influenced by a number of biological pathways, working differently at different life stages (e.g., growing animals vs. mature animal nutrient partitioning rules) (Tolkamp et al., 2010). This may also be of particular relevance in understanding how trait expression is influenced in different environments.

Proteomics investigates the structure and function of proteins and their interaction with the genome while metabolomics investigates the relationship between the genome and metabolic processes. The advantage of investigative research on proteins and metabolites, over that on the genome, is that proteins and metabolites in themselves are inherently biological end-products. Information contained in the nucleic acid sequence of the DNA determines, via transcription to mRNA, the amino acid sequence of a protein which in turn determines its structure and function. All biological processes and observed phenotypes are affected by this process and while the basic nucleic acid sequence does not change, factors such as environmental conditions can affect the transcription of DNA to mRNA and eventually to protein. Further steps to utilise these techniques/technologies and apply them to relevant traits in livestock, might help to understand the complexity

of traits and how they are expressed in different animals, in different environments and at different stages of life (Tolkamp et al., 2010). Also, it may help to understand the interaction between traits at a genomic, genetic and environmental level.

The metabolomics approach allows performing large scale studies with large number of individuals and on a longitudinal basis. In addition, metabolomics can be integrated with genomics or transcriptomic approaches to help revealing the molecular routes relevant for the efficient transition of nutrients into animal tissues. Furthermore, signature of metabolites can be indicative for the effect of various physiological and molecular routes along with the effect of various diets or environmental conditions on utilisation of nutrients.

As genetic improvement techniques are refined and become more powerful using modern DNA based methods, it is important that the information of change in production traits are link with nutritional and management advice. The biological growth model used in this thesis can be the basis for this advice. As new insight into genomic regulation of REI becomes available, integration into existing growth models may provide more accurate predictions. Models to quantify expected improvement from implementation of results and methods developed here will be predicted using models at different levels. Expected effects of more efficient genetic selection for improved efficiency can be quantified using stochastic or deterministic models. Improvement may be due to both more accurate evaluation (genomic selection and models of genetic effects) and improved measures of efficiency and its interaction with diets and the gut microbial meta-genome. In addition, the improvement of feed efficiency by optimization of feed intake capacity with respect to the protein deposition curve (considering a minimum lipid deposition) is expected to improve nutrient efficiency (Roehe et al., 2002 and 2004).

Future research areas that can be explored are development of biomarkers that are diagnostic for the contemporary utilisation and partitioning of nutrients, indicative towards the animal's reactivity to nutritional and management interventions towards improved feed efficiency and informative regarding the genetic potential of the animal to breed animals with inheritable beneficial feed conversion properties. Performing genome wide association studies and undertaking genomic selection approaches provide links between genomic variation and variation in feed efficiency and related growth, carcass and meat quality traits. Further research on genomics of digestive efficiency is necessary to obtain more insight into the genetics of digestive efficiency in pigs. In addition, integrative genetics, which integrates knowledge from phenotypes, gene expression, metabolomics and

genomic data, can be applied to further improve energy and nutrient efficiency so that the use of energy and nutrient resources as well as the environmental impact of pig production is minimised.

6.7 Implications

From this study it can be concluded that gender and housing type had significant influences on nitrogen losses. This suggests that farms that house only one sex may have different amounts of nutrients in manure due to differences in feed intake, body weight gain and feed efficiency between genders. Therefore, farms with different sex ratios may require different land application rates. Furthermore, nitrogen excretion showed to have favourable phenotypic and genetic associations with production traits, especially with REI. This indicates that the current selection programs have resulted in reduction of nitrogen excretion. However, the large heritability identified for REI suggests that there still is great selection potential. Therefore, genetic improvement in REI is expected to, beside improvement in energy efficiency, further reduce nitrogen excretion in pork production. Moreover, REI demonstrated different genetic background at different growth stages, implying that selection for REI will be more efficient if each stage of growth is considered separately. Most importantly, the later stages of growth are expected to result in larger selection response due to large genetic variance of REI. This study revealed six novel QTL for REI revealing the genomic architecture of efficiency in feed utilisation and indicating that the regulation of feed efficiency is partly independent from that of production traits. Different QTL for REI were identified at different growth stages, suggesting different genes are responsible for efficiency in feed utilisation at different stages of growth. This also suggests that selection for REI is most efficient if carried out within stages of growth. Moreover, using a biological growth model allows studying the change of energy and nitrogen efficiency during growth, which can be used for developing optimal selection and production strategies. The biological growth model study suggested that the reduction in slaughter weight is a feasible strategy to improve energy efficiency and reduce environmental pollution of pork production. Furthermore, the study provides input parameters for biological growth models based on a crossbred population (commercial pig population) to examine the impact of change in growth on improvement of feed energy efficiency and reduction of nitrogen excretion.

References

- Andersson, K.H., K. Andersson, G. Zamaratskaia, L. Rydhmer, G. Chen and K. Lundstrom. 2005. Effect of single-sex or mixed rearing and live weight on performance, technological meat quality and sexual maturity in entire male and female pigs fed raw potato starch. *Acta Agr. Scand. A* 55(2-1), 80-90.
- Barea, R., S. Dubois, H. Gilbert, P. Sellier, J. van Milgen and J. Noblet. 2010. Energy utilization in pigs selected for high and low residual feed intake. *J. Anim. Sci.*
- Boddicker, N., N.K. Gabler, M.E. Spurlock, D. Nettleton and J.C.M. Dekkers. 2011. Effects of ad libitum and restricted feeding on early production performance and body composition of Yorkshire pigs selected for reduced residual feed intake. *Animal* 5:1344-1353.
- Bunter, K.L., W. Cai, D.J. Johnston and J.C.M. Dekkers. 2010. Selection to reduce residual feed intake in pigs produces a correlated response in juvenile insulin-like growth factor-I concentration. *J. Anim. Sci.* 88:1973-1981.
- Cai, W., D.S. Casey and J.C.M. Dekkers. 2008. Selection response and genetic parameters for residual feed intake in Yorkshire swine. *J. Anim. Sci.* 86:287-298.
- Cai, W., M.S. Kaiser and J.C.M. Dekkers. 2011. Genetic analysis of longitudinal measurements of performance traits in selection lines for residual feed intake in Yorkshire swine. *J. Anim. Sci.* 89:1270-1280.
- Castro Bulle, F.C.P., P.V. Paulino, A.C. Sanches and R.D. Sainz. 2007. Growth, carcass quality, and protein and energy metabolism in beef cattle with different growth potentials and residual feed intakes. *J. Anim. Sci.* 85:928-936.
- Crocker, A.W. and O.W. Robison. 2002. Genetic and nutritional effects on swine excreta. *J. Anim. Sci.* 80:2809-2816.
- D'Eath, R.B., R. Roehe, S.P. Turner, S.H. Ison, M. Farish, M.C. Jack and A.B. Lawrence. 2009. Genetics of animal temperament: aggressive behaviour at mixing is genetically associated with the response to handling in pigs. *Animal* 3:1544-1554.
- Dammgen, U., A. Berk, C. Otten, W. Brade, N.J. Hutchings, H.D. Haenel, C. Rosemann, S. Danicke and M. Schwerin. 2013. Anticipated changes in the emissions of greenhouse gases and ammonia from pork production due to shifts from fattening of barrows towards fattening of boars. *Appl. Agric. Forestry Res.* 1 2013 (63) 47-60.
- De Haer, L.C.M., P. Luiting and H.L.M. Aarts. 1993. Relations among individual (residual) feed intake, growth performance and feed intake pattern of growing pigs in group housing. *Livest. Prod. Sci.* 36:233-253.

- De Vries, A.G. and E. Kanis. 1992. A growth model to estimate economic values for food intake capacity in pigs. *Anim. Prod.* 55:241-246.
- De Vries, A.G., P.G. van der Wal, T. Long, G. Eikelenboom and J.W.M. Merks. 1994. Genetic-Parameters of Pork Quality and Production Traits in Yorkshire Populations. *Livest. Prod. Sci.* 40:277-289.
- Dekkers, J.C.M. and H. Gilbert. 2010. Genetic and Biological Aspects of Residual Feed Intake in Pigs. No. 287 in *Proc. 9th World Congr. Genet. Appl. Livest. Prod.*, Leipzig, Germany.
- Dina, C., D. Meyre, S. Gallina, E. Durand, A. Korner, P. Jacobson, L.M.S. Carlsson, W. Kiess, V. Vatin, C. Lecoeur, J. Delplanque, E. Vaillant, F. Pattou, J. Ruiz, J. Weill, C. Levy-Marchal, F. Horber, N. Potoczna, S. Hercberg, C. Le Stunff, P. Bougneres, P. Kovacs, M. Marre, B. Balkau, S. Cauchi, J.C. Chevre and P. Froguel. 2007. Variation in FTO contributes to childhood obesity and severe adult obesity. *Nat. Genet.* 39:724-726.
- Dourmad, J.Y., N. Guingand, P. Latimier and B. Seve. 1999. Nitrogen and phosphorus consumption, utilisation and losses in pig production: France. *Livest. Prod. Sci.* 58:199-211.
- Dufresne, M., M. Rustin, V. Jaspard, J. Wavreile and N. Gengler. 2011. Using test station and on-farm data for the genetic evaluation of Pietrain boars used on Landrace sows for growth performance. *J. Anim. Sci.* 89:3872-3880.
- European Commission. 2010. Animal welfare: voluntary end to the surgical castration of piglets by 2018. Accessed Jul. 7, 2011. http://ec.europa.eu/food/animal/welfare/farm/docs/castration_pigs_press_release_en.pdf.
- Fan, B., S. Lkhagvadorj, W. Cai, J. Young, R.M. Smith, J.C.M. Dekkers, E. Huff-Loneragan, S.M. Lonergan and M.F. Rothschild. 2010. Identification of genetic markers associated with residual feed intake and meat quality traits in the pig. *Meat Science* 84:645-650.
- FAO. 2006. World agriculture: towards 2030/2050. Interim report. Food and Agriculture Organisation of the United Nation, Rome, Italy.
- Fernandez, J.A., H.D. Poulsen, S. Boisen and H.B. Rom. 1999. Nitrogen and phosphorus consumption, utilisation and losses in pig production: Denmark. *Livest. Prod. Sci.* 58:225-242.
- Gilbert, H., J.P. Bidanel, J. Gruand, J.C. Caritez, Y. Billon, P. Guillouet, H. Lagant, J. Noblet and P. Sellier. 2007. Genetic parameters for residual feed intake in growing pigs, with emphasis on genetic relationships with carcass and meat quality traits. *J. Anim. Sci.* 85:3182-3188.

- Gilbert, H., J. Riquet, J. Gruand, Y. Billon, K. Feve, P. Sellier, J. Noblet and J. P. Bidanel. 2010. Detecting QTL for feed intake traits and other performance traits in growing pigs in a Pietrain-Large White backcross. *Animal* 4:1308-1318.
- Grant, S.F.A., G. Thorleifsson, I. Reynisdottir, R. Benediktsson, A. Manolescu, J. Sainz, A. Helgason, H. Stefansson, V. Emilsson, A. Helgadottir, U. Styrkarsdottir, K.P. Magnusson, G.B. Walters, E. Palsdottir, T. Jonsdottir, T. Gudmundsdottir, A. Gylfason, J. Saemundsdottir, R.L. Wilensky, M.P. Reilly, D.J. Rader, Y. Bagger, C. Christiansen, V. Gudnason, G. Sigurdsson, U. Thorsteinsdottir, J.R. Gulcher, A. Kong and K. Stefansson. 2006. Variant of transcription factor 7-like 2 (TCF7L2) gene confers risk of type 2 diabetes. *Nat. Genet.* 38:320-323.
- Gunsett, F.C. 1984. Linear index selection to improve traits defiend as ratios. *J. Anim. Sci.* 59: 1185-1193.
- Gunsett, F.C. 1986. Problems associated with selection for traits defined as a ratio of two component traits. *Proc. 3rd World Congr. Genet. Appl. Livest. Prod. Uni. Nebraska, Lincoln, Nebraska, USA.* 11, 437-442.
- Herd, R.M. and P.F. Arthur. 2009. Physiological basis for residual feed intake. *J. Anim. Sci.* 87:E64-E71.
- Hoffmann, L., W. Jentsch and M. Beyer. 1992. Untersuchungen Zum Energieumsatz Wachsender Schweine Im Lebendmasseabschnitt Von 10-50 Kg. 3: Energieerhaltungsbedarf wachsender Schweine. *Archives Anim. Nutr.* 42:235-248.
- Hoque, M.A. and K. Suzuki. 2009. Genetics of residual feed intake in cattle and pigs: a review. *Asian Austral. J. Anim. Sci.* 22, 747-755.
- International Panel for Climate Change (IPCC). 2006. Chapter 10: emissions from livestock and manure management. In 2006 IPCC guidelines for national greenhouse gas inventories (ed. HS Eggleston, L Buendia, K Miwa, T Ngara and K Tanabe), chapter 10, pp. 10.1–10.87. Institute for Global Environmental Strategies (IGES), Hayama, Japan.
- Johnson, Z.B., J.J. Chewning and R.A. Nugent, III. 1999. Genetic parameters for production traits and measures of residual feed intake in large white swine. *J. Anim. Sci.* 77:1679-1685.
- Jones, H.E., C.C. Warkup, A. Williams and E. Audsley. 2008. The effect of genetic improvement on emissions from livestock systems. Page 6 in *Proc. 59th EAAP*, Vilnius, Lithuania.
- Karsten, S., R. Roehe, V. Schulze, H. Looft and E. Kalm. 2003. Genetische Beziehungen zwischen individueller Futteraufnahme wahrend der Eigenleistungsprufung und Fruchtbarkeitsmerkmalen beim Schwein. *Arch Tierz*, 43 (2000), pp. 451–461.

- Knap, P.W. 2012. Pig breeding for increased sustainability. In Encyclopedia of sustainability science and technology (ed. R. A. Meyers), Springer Science+Business Media, New York NY, USA. Volume 11, pp. 7972-8012.
- Knap, P.W. and P. Luiting. 1999. Selection limits and fitness constraints in pigs. Proc. 50th EAAP, Zurich, Switzerland.
- Kyriazakis, I. and C.T. Whittemore. 2006. Whittemore's Science and Practice of Pig Production. 3rd ed. Blackwell Publishing Ltd, Oxford, UK.
- Kyriazakis, I. 2011. Opportunities to improve nutrient efficiency in pigs and poultry through breeding. *Animal*. 5:6, pp 821-832.
- Labroue, F., L. Maignel, P. Sellier and J. Noblet. 1999. Consommation résiduelle chez le porc en croissance alimenté à volonté. Méthode de calcul et variabilité génétique. *Journées de Recherche Porcine en France* 31:167-174.
- Landgraf, S., A. Susenbeth, P.W. Knap, H. Looft, G.S. Plastow, E. Kalm and R. Roehe. 2006. Allometric association between in vivo estimation of body composition during growth using deuterium dilution technique and chemical analysis of serial slaughtered pigs. *Anim. Sci.* 82:223-231.
- Le Naou, T., N. Le Floch, I. Louveau, H. Gilbert and F. Gondret. 2012. Metabolic changes and tissue responses to selection on residual feed intake in growing pigs. *J. Anim. Sci.* 90:4771-4780.
- Lefaucheur, L., B. Lebret, P. Ecolan, I. Louveau, M. Damon, A. Prunier, Y. Billon, P. Sellier and H. Gilbert. 2011. Muscle characteristics and meat quality traits are affected by divergent selection on residual feed intake in pigs. *J. Anim. Sci.* 89:996-1010.
- Lkhagvadorj, S., L. Qu, W. Cai, O.P. Couture, C.R. Barb, G.J. Hausman, D. Nettleton, L.L. Anderson, J.C.M. Dekkers and C.K. Tuggle. 2010. Gene expression profiling of the short-term adaptive response to acute caloric restriction in liver and adipose tissues of pigs differing in feed efficiency. *Am. J. Physiol. - Regulatory, Integrative and Comparative Physiology* 298:R494-R507.
- Lopez-Serrano, M., N. Reinsch, H. Looft and E. Kalm. Genetic correlations of growth, backfat thickness and exterior with stayability in Large White and Landrace sows. *Livest. Prod. Sci.*, 64 (2000), pp. 121–131.
- Luiting, P., J.W. Schrama, W. van der Hel and E.M. Urff. 1991. Metabolic differences between White Leghorns selected for high and low residual food consumption. *Brit. Poultry Sci.* 32, 763-782.
- Luiting, P. and E.M. Urff. 1991. Optimization of a model to estimate residual feed consumption in the laying hens. *Livest. Prod. Sci.* 27, 321-338.
- Meuwissen, T.H.E., B.J. Hayes and M.E. Goddard. 2001. Prediction of Total Genetic Value Using Genome-Wide Dense Marker Maps. *Genetics* 157:1819-1829.

- Meuwissen, T. and M. Goddard. 2010. Accurate Prediction of Genetic Values for Complex Traits by Whole-Genome Resequencing. *Genetics* 185:623-631.
- Mohrmann, M., R. Roehe, A. Susenbeth, U. Baulain, P.W. Knap, H. Looft, G.S. Plastow and E. Kalm. 2006. Association between body composition of growing pigs determined by magnetic resonance imaging, deuterium dilution technique and chemical analysis. *Meat Sci.* 72:518-531.
- Morel, P.C.H. and G.R. Wood. 2005. Optimisation of nutrient use to maximise profitability and minimise nitrogen excretion in pig meat production systems. *Acta Hort. (ISHS)* 674:269-275.
- Mrode, R.A. and B.W. Kennedy. 1993. Genetic-Variation in Measures of Food Efficiency in Pigs and their Genetic-Relationships with Growth-Rate and Backfat. *Anim. Prod.* 56:225-232.
- Murrells, T., N.R. Passant, G. Thistlethwaite, A. Wagner, Y. Li, T. Bush, J. Norris, P.J. Coleman, C. Walker, R.A. Stewart, I. Tsagatakis, C. Conolly, N. Brophy and S. Okamura. 2010. UK Emissions of Air Pollutants 1970 to 2008. Accessed Nov. 7, 2010.http://ukair.defra.gov.uk/reports/cat07/1009030925_2008_Report_final270805.pdf.
- Nguyen Hong, N., C.P. McPhee and C.M. Wade. 2005. Responses in residual feed intake in lines of Large White pigs selected for growth rate on restricted feeding (measured on ad libitum individual feeding). *J. Anim. Breed. Genet.* 122:264-270.
- Prapasongsa, T., P. Christensen, J. H. Schmidt, and M. Thrane. 2010. LCA of comprehensive pig manure management incorporating integrated technology systems. *J. Clean. Prod.* 18:1413-1422.
- Renaudeau, D., G. Frances, S. Dubois, H. Gilbert and J. Noblet. 2013. Effect of thermal heat stress on energy utilization in two lines of pigs divergently selected for residual feed intake. *J. Anim. Sci.* 91:1162-1175.
- Richardson, E.C., R.M. Herd, I.G. Colditz, J.A. Archer and P.F. Arthur. 2002. Blood cell profiles of steer progeny from parents selected for and against residual feed intake. *Aust. J. Exp. Agric.* 142:203-209.
- Rodhe, L., M. Pell, and S. Yamulki. 2006. Nitrous oxide, methane and ammonia emissions following slurry spreading on grassland. *Soil. Use. Manage.* 22:229-237.
- Roehe R, S. Landgraf, M. Mohrmann., P.W. Knap, H. Looft and E. Kalm. 2004. The use of allometric and nonlinear function for longitudinal data analysis of physical and chemical body composition in pigs. *Proc. 55th Annual Meeting EAAP, Bled, Slovenia, paper PG6.7, 1-10.*
- Roehe, R., J. Lorenzo Bermejo, S. Landgraf, P.W. Knap and E. Kalm. 2002. Estimation of growth curves using linear random regression and nonlinear two-stage models

- for experimental data of protein deposition in swine. Page 40 in Proc. 53rd Annu. Mtg. Euro. Assoc. Anim. Prod., Cairo, Egypt.
- Roehe, R., G.S. Plastow and P.W. Knap. 2003. Quantitative and molecular genetic determination of protein and fat deposition. *HOMO*. 54:119-131.
- Saintilan, R., I. Merour, L. Brossard, T. Tribout, J.Y. Dourmad, P. Sellier, J. Bidanel, J. van Milgen and H. Gilbert. 2013. Genetics of residual feed intake in growing pigs: relationships with production traits, and nitrogen and phosphorus excretion traits. *J. Anim. Sci.* 91(6):2542-54.
- Saintilan, R., I. Merour, S. Schwob, P. Sellier, J. Bidanel and H. Gilbert. 2011. Genetic parameters and halothane genotype effect for residual feed intake in Pietrain growing pigs. *Livest. Sci.* 142(1), 203-209.
- Sainz, R.D. and P.V. Paulino. 2004. Residual feed intake. Proceedings UC Davis: Sierra Foothill Research and Extension Center.
- Sanchez-Pla, A., F. Reverter, M.C. Ruz de Villa and M. Comabella. 2012. Transcriptomics: mRNA and alternative splicing. *J. Neuroimmunol.* 248:23-31.
- Schulze, V., R. Roehe, J. Lorenzo Bermejo, H. Looft and E. Kalm. 2003. The influence of feeding behaviour on feed intake curve parameters and performance traits of station-tested boars. *Livest. Prod. Sci.* 82:105-116.
- Sellier, P. 1998. Genetics of meat and carcass traits. M.F. Rothschild, A. Ruvinsky (Eds.), *The Genetics of the Pig*, CABI, Wallingford, UK (1998), pp. 463–510.
- Shirali, M., A. Doeschl-Wilson, P.W. Knap, C. Duthie, E. Kanis, J.A.M. van Arendonk and R. Roehe. 2012. Nitrogen excretion at different stages of growth and its association with production traits in growing pigs. *J. Anim. Sci.* 90:1756-1765.
- Tolkamp, B., E. Wall, R. Roehe, J. Newbold and K. Zaralis. 2010. Review of nutrient efficiency in different breeds of farm livestock. Defra report. IFO183.
- Turner, S.P., R. Roehe, R.B. D'Eath, S.H. Ison, M. Farish, M.C. Jack, N. Lundeheim, L. Rydhmer and A.B. Lawrence. 2009. Genetic validation of postmixing skin injuries in pigs as an indicator of aggressiveness and the relationship with injuries under more stable social conditions. *J. Anim. Sci.* 87:3076-3082.
- Turner, S.P., R. Roehe, W. Mekkawy, M.J. Farnworth, P.W. Knap and A.B. Lawrence. 2008. Bayesian Analysis of Genetic Associations of Skin Lesions and Behavioural Traits to Identify Genetic Components of Individual Aggressiveness in Pigs. *Behav. Genet.* 38:67-75.
- van der Peet-Schwering, C.M.C., A.W. Jongbloed and A.J.A. Aarnink. 1999. Nitrogen and phosphorus consumption, utilisation and losses in pig production: The Netherlands. *Livest. Prod. Sci.* 58:213-224.
- van Milgen, J. and J. Noblet. 2003. Partitioning of energy intake to heat, protein, and fat in growing pigs. *J. Anim. Sci.* 81:E86-E93.

- Von Felde, A., R. Roehe, H. Looft and E. Kalm. 1996. Genetic association between feed intake and feed intake behaviour at different stages of growth of group-housed boars. *Livestock Production Science* 47:11-22.
- Webb, A.J. 1998. Objectives and Strategies in Pig Improvement: An Applied Perspective. *J. Dairy Sci.* 81, Supplement 2:36-46.
- Zhang, Z.Y., J. Ren, D.R. Ren, J.W. Ma, Y.M. Guo and L.S. Huang. 2009. Mapping quantitative trait loci for feed consumption and feeding behaviors in a White Duroc x Chinese Erhualian resource population. *J. Anim. Sci.* 87:3458-3463.

S

Summary

Expansion of pork production to meet the nutritional requirements of an increasing world population has faced challenges associated with limited feed resources for animals and environmental impact of production. To overcome these challenges improving feed efficiency and reducing nitrogen pollution are of increasing importance. Improvement of feed efficiency, apart from its substantial economical benefits, is expected to reduce nitrogen excretion. The association between these traits and other production traits throughout the growth of pigs is of great scientific interest to understand the biology of these traits. This knowledge will contribute to develop improvement strategies for both environmental mitigation and feed efficiency on breeding as well as commercial farm level.

The general aim of this project was to examine the phenotypic, genetic and genomic background of energy and nitrogen efficiency at different stages of growth and during the entire growth period in growing pigs and to provide the base information for developing strategies for improvement of these traits using growth modeling.

In **Chapter 2** of this thesis, the objectives were:

- To obtain accurate estimates of nitrogen excretion and nitrogen efficiency traits in growing pigs throughout the growth until 140 kg BW.
- To investigate the influencing factors, such as gender and housing type, on nitrogen excretion.
- To determine the phenotypic association of nitrogen excretion with feed efficiency and production traits at different stages of growth and during the entire growth period.

A number of factors such as gender, housing type and halothane gene status showed to be associated with nitrogen excretion. Nitrogen excretion in the first stage of growth (60 to 90 kg BW) was significantly lower than growth stages of 90 to 120 kg BW (22%) and 120 to 140 kg BW (31%) which was associated with higher lean growth (8% and 13%, respectively) and better feed efficiency (19% and 26%, respectively). Furthermore, an improvement in feed conversion ratio, average daily body weight gain or lipid to protein deposition ratio by one phenotypic standard deviation would reduce total nitrogen excretion per pig by 709 g, 307 g and 211 g, respectively, during the entire growing period from 60 to 140 kg BW.

It was concluded that nitrogen excretion increases substantially during growth and that it can be reduced most effectively by improvement of feed efficiency and to a lesser extent through improvement of weight gain and/or body composition.

In **Chapter 3**, the general objective was to determine the genomic architecture of nitrogen excretion and residual energy intake (REI), as a direct measurement of feed efficiency, throughout the growth period using quantitative trait loci (QTL) analysis. This study identified six novel QTL for REI and 23 QTL for nitrogen excretion traits at different stages of growth. The QTL for REI explained 2.7 to 6.1% of the phenotypic variance in REI. At first stage of growth from 60 to 90 kg BW, a dominance QTL for REI was detected on Sus scrofa chromosome 14 (SSC14) which was overlapping with the QTL for feed intake and nitrogen excretion traits. During the next stage of growth (90 to 120 kg BW), three QTL for REI were found on SSC2, SSC4 and SSC7 with significant additive, imprinting and additive effects, respectively. The QTL on SSC2 and SSC7 were positionally overlapping with QTL for feed conversion ratio and nitrogen excretion. During the final stage of growth (120 to 140 kg BW), an additive QTL for REI was obtained on SSC8 which was overlapping with QTL for nitrogen excretion traits. Because different QTL for REI were identified at different growth stages it can be concluded that different genes are responsible for efficiency in feed utilisation during growth. The overlapping of only one QTL for REI and feed intake suggests that only a small proportion of the variance in REI is explained by the variance in feed intake, and thus selection for REI will have only small influence on feed intake, which should not be reduced to be the limiting factor for improvement of growth. In addition, the QTL for REI overlapping with those of nitrogen excretion traits suggest that mostly underlying factors for efficiency in feed utilisation, such as metabolism and protein turnover, are responsible for variation in REI.

In **Chapter 4**, the objectives were to determine the genetic parameters for REI throughout the growth period and to identify its genetic association with growth, feed intake and nitrogen excretion traits. The results showed heritabilities of large magnitude (0.44 to 0.50) for REI at different stages of growth and during the entire growth. The REI at the first stage of growth showed no genetic correlation with REI at other stages of growth. There was only a moderate genetic correlation (0.5 ± 0.21) between REI during second (90 to 120 kg BW) and third (120 to 140 kg BW) stage of growth. The REI at this last stage of growth had the highest phenotypic variance. Furthermore, there were favourable genetic correlations of REI with feed conversion ratio (0.84 ± 0.13) and nitrogen excretion (0.85 ± 0.11).

The study concluded that there is great potential for improving feed efficiency due to a substantial genetic determination of REI. In addition, selection for feed efficiency should consider different stages of growth, due to their different genetic backgrounds. REI explained a large proportion of the variance in feed conversion

ratio and nitrogen excretion, indicating that selection for lower REI, apart from improving production efficiency, may contribute to mitigation of environmental pollution by pork production.

In **Chapter 5**, the objectives were to obtain estimates of REI and nitrogen excretion traits on a daily basis for pigs using growth modelling and to identify the effect of growth rate on energy efficiency and nitrogen excretion. The results, based on a biological growth model, showed the large decrease in nitrogen excretion with increase in growth rate which can be used for developing improvement strategies. In particular, the increase in nitrogen excretion and variation of REI during growth can be used as basis for developing strategies for environmental mitigation and improvement of energy efficiency. Furthermore, performance traits were obtained for two genetically different purebred pig lines, one line selected for high reproduction and fast growth, and the other for production including leanness. Comparing these lines with a crossbred line suggested that genetic selection for fast growth, leanness and production efficiency has resulted in reduction of nitrogen excretion and improvement in efficiency of feed utilization. Moreover, this study suggested that decreasing the slaughter weight would result in substantial reduction of nitrogen excretion and REI on a population basis. The energy and cost saving involved in the marginal decrease of REI when slaughter weight was decreased from 130 to 100 kg BW were 302 MJ ME and £9.96 per pig space and year. This was followed by a substantial decrease in nitrogen excretion of 1.60 kg per pig space and year in the finishing unit.

This chapter showed that growth models can be used to suggest possible mitigation strategies to reduce environmental pollution of pork production in addition to improving efficiency of feed utilization.

In the general discussion (**Chapter 6**), opportunities for further improvement of feed efficiency and reduction of nitrogen excretion have been discussed in depth. Various methodologies and approaches have been suggested to improve the energy and nitrogen efficiency and reduce environmental pollution in pork production. In addition, different traits and factors with influence on nitrogen excretion and feed efficiency have been discussed to provide in depth insight into nitrogen and energy efficiency area.

Main conclusions of this thesis are:

- Nitrogen excretion of growing pigs is increasing during the growth period.
- Nitrogen excretion is most effectively reduced by improvement of feed efficiency and to a lesser extent through improvement of growth rate and change of body composition.
- Efficiency of feed utilisation is influenced by several genes at various stages of growth.
- Genetic improvement of feed utilisation has substantial influence on reduction of nitrogen excretion of pigs.
- Underlying factors for efficiency in feed utilisation, such as metabolism, digestion and protein turnover, are mostly responsible for variation in residual energy intake.
- Residual energy intake has great selection potential due to large heritability identified.
- Genetic selection for energy and nitrogen efficiency should consider the stages of growth due to low genetic correlations between growth periods as well as different QTL in different growth periods for these traits.
- Genetic selection on residual energy intake is expected to result, besides improvement of feed efficiency, in a substantial reduction of nitrogen excretion by pork production.
- Growth modelling can be used to develop strategies for improvement in energy and feed efficiency of pigs; the strategies can be such as reduction of slaughter weight, or improvement of growth rate, production efficiency and body composition.

S

Samenvatting

De uitbreiding van de productie van varkensvlees om aan de voedingsbehoeften van een groeiende wereldbevolking te voldoen, heeft geleid tot problemen die verband houden met de beperkte beschikbaarheid van diervoer en met de invloed van varkensvleesproductie op het milieu. Om deze problemen op te kunnen lossen zijn verbetering van de voerefficiëntie en vermindering van de milieuverontreiniging door stikstof van toenemend belang. Verbetering van de voerefficiëntie zal, afgezien van aanzienlijke economische voordelen, naar verwachting resulteren in vermindering van de stikstofuitscheiding. De samenhang tussen deze kenmerken en andere productie eigenschappen gedurende de groei van varkens is van groot wetenschappelijk belang om de biologie van deze kenmerken te begrijpen. Deze kennis zal bijdragen aan verbetering van strategieën om de milieubelasting te verminderen en de voerefficiëntie te verbeteren op zowel het niveau van de biggenproductie als op dat van de varkensmesterij.

De algemene doelstelling van dit project was om de fenotypische, genetische en genomische achtergrond van energie- en stikstofefficiëntie te onderzoeken in verschillende stadia van de groei en gedurende de gehele groeiperiode van vleesvarkens, en om de basisinformatie te verschaffen voor het ontwikkelen van strategieën voor verbetering van deze eigenschappen met behulp groeimodellering.

Hoofdstuk 2 van dit proefschrift had de volgende doelstellingen:

- Het verkrijgen van nauwkeurige schattingen van stikstofexcretie en stikstofefficiëntie voor vleesvarkens gedurende de groei tot 140 kg lichaamsgewicht (BW).
- Te onderzoeken welke factoren, zoals geslacht en huisvestingstype, van invloed zijn op stikstofexcretie.
- Het fenotypisch verband vast stellen van stikstofexcretie met voerefficiëntie en productiekenmerken in verschillende stadia van de groei en gedurende de gehele groeiperiode.

Een aantal factoren, waaronder geslacht, huisvestingstype en status van het halothaangen, bleek geassocieerd te zijn met stikstofuitscheiding. De stikstofuitscheiding in de eerste groeifase (60 tot 90 kg BW) was significant lager dan in de groeifasen 90 tot 120 kg BW (22%) en 120 tot 140 kg BW (31%) hetgeen samenhangt met een hogere mager vleesgroei (respectievelijk 8% en 13%) en een betere voederefficiëntie (respectievelijk 19% en 26%). Verder zou een verbetering met één standaarddeviatie van de voederconversie, de gemiddelde groei per dag of de vet-/eiwitaanzet verhouding, leiden tot een vermindering van de totale

stikstofuitscheiding met respectievelijk 709, 307 en 211 gram per varken gedurende de gehele groeiperiode van 60 tot 140 kg BW.

Geconcludeerd werd dat de stikstofuitscheiding aanzienlijk toeneemt tijdens de groeiperiode en dat deze het meest effectief kan worden verlaagd door verbetering van de voerefficiëntie en in mindere mate door verbetering van de lichaamsgroei en/of de lichaamssamenstelling.

Het algemene doel in **Hoofdstuk 3** was het vaststellen van de genomische architectuur van stikstofexcretie en van residuele energieopname (REI, een directe meting van voerefficiëntie) gedurende de groeiperiode, door gebruik te maken van 'quantitative trait loci' (QTL) analyse. In deze studie werden zes nieuwe QTL voor REI en 23 QTL voor stikstofexcretie kenmerken geïdentificeerd in verschillende stadia van de groei. De QTL voor REI verklaarden 2,7 tot 6,1% van de fenotypische variantie van REI. In de eerste groeifase van 60 tot 90 kg BW werd een QTL met een dominantie effect voor REI gevonden op *Sus scrofa* chromosoom 14 (SSC14) dat overlapte met het QTL voor voeropname en stikstofexcretie. In de volgende groeifase (90 tot 120 kg BW), werden drie QTL voor REI gevonden op SSC2, SSC4 en SSC7 met respectievelijk significante additieve, imprintings en additieve effecten. De QTL op SSC2 en SSC7 overlaptten positioneel met QTL voor voederconversie en stikstofexcretie. In de laatste groeifase (120 tot 140 kg BW) werd een additief QTL voor REI gevonden op SSC8 dat overlapte met QTL voor stikstofexcretie kenmerken. Omdat verschillende QTL voor REI werden geïdentificeerd in verschillende groeifasen, kan worden geconcludeerd dat verschillende genen verantwoordelijk zijn voor de efficiëntie van voerbenutting tijdens de groei. Aangezien slechts één QTL voor REI overlapte met een QTL voor voeropname, suggereert dit dat slechts een klein deel van de variantie in REI wordt verklaard door de variantie in voeropname, en dat selectie op REI dus maar een kleine invloed zal hebben op voeropname. Voeropname moet echter niet zodanig verminderen dat het een beperkende factor wordt voor verbetering van de groei. Verder suggereren de QTL voor REI die overlappen met die voor stikstofexcretie dat vooral onderliggende factoren voor efficiëntie van voerbenutting, zoals metabolisme en eiwit turnover, verantwoordelijk zijn voor variatie in REI.

Het doel in **Hoofdstuk 4** was het vaststellen van de genetische parameters voor REI gedurende de groeiperiode en van zijn genetische samenhang met groei, voeropname en uitscheiding van stikstof. De erfelijkheidsgraden voor REI in de verschillende stadia van de groei en tijdens de gehele groeiperiode bleken hoog te zijn (0,44 tot 0,50). De REI in de eerste groeifase was genetisch niet gecorreleerd

met REI in de andere stadia van de groei. Er was slechts een matige genetische correlatie ($0,5 \pm 0,21$) tussen REI tijdens de tweede (90 tot 120 kg BW) en derde (120 tot 140 kg BW) groeifase. De REI in deze laatste groeifase vertoonde de hoogste fenotypische variantie. Verder waren er gunstige genetische correlaties van REI met voederconversie ($0,84 \pm 0,13$) en stikstofuitscheiding ($0,85 \pm 0,11$).

Uit deze studie werd geconcludeerd dat er goede mogelijkheden zijn voor het verbeteren van de voerefficiëntie omdat REI sterk genetisch bepaald is. Bij selectie op voerefficiëntie moet wel rekening gehouden worden met verschillende stadia van de groei, vanwege hun verschillende genetische achtergrond. REI verklaarde een groot deel van de variantie in voederconversie en uitscheiding van stikstof, wat aangeeft dat selectie op een lagere REI kan bijdragen aan vermindering van de milieubelasting door de productie van varkensvlees, nog afgezien van verbetering van de productie-efficiëntie.

In **Hoofdstuk 5** waren de doelstellingen om schattingen te verkrijgen van REI en stikstofexcretie op een dagelijkse basis voor varkens door middel van groeimodellering, en om het effect van groeisnelheid op de energie-efficiëntie en stikstofuitscheiding vast te stellen. De resultaten, op basis van een biologisch groeiemodel, toonden de grote afname van stikstofexcretie met de toename van de groeisnelheid. Dit verband kan worden gebruikt voor het ontwikkelen van verbeteringsstrategieën. In het bijzonder kan de toename van de stikstofuitscheiding en van de variatie in REI tijdens de groeiperiode gebruikt worden als basis voor het ontwikkelen van strategieën ter vermindering van de milieubelasting en verbetering van de energie-efficiëntie. Ook werden prestatiekenmerken verkregen van twee genetisch verschillende raszuivere varkenslijnen, een lijn geselecteerd op hoge reproductie en snelle groei, en de andere op hoge voerefficiëntie en beveelsheid. Vergelijking van deze lijnen met een hybride lijn suggereerde dat genetische selectie op snelle groei, beveelsheid en productie-efficiëntie heeft geleid tot vermindering van stikstofexcretie en verbetering van de efficiëntie van voergebruik. Deze studie suggereerde verder dat vermindering van het slachtgewicht zal leiden tot een aanzienlijke vermindering van de stikstofuitscheiding en van REI op populatieniveau. De besparing op voerenergie en voerkosten die gepaard gaat met de afname in REI bij een verlaging van het slachtgewicht van 130 naar 100 kg BW, bedroeg 302 MJ ME en £9,96 per varken ruimte en jaar. Het gevolg was een substantiële daling van de stikstofuitscheiding met 1,60 kg per varken ruimte en jaar.

Dit hoofdstuk liet zien dat groei modellen kunnen worden gebruikt om mogelijke strategieën voor te stellen die de verontreiniging van het milieu door

varkensvleesproductie verlagen naast verbetering van de efficiëntie van het voergebruik.

In de algemene discussie (**Hoofdstuk 6**) zijn de mogelijkheden voor verdere verbetering van voerefficiëntie en vermindering van stikstofexcretie uitgebreid besproken. Verschillende methoden en benaderingen zijn voorgesteld om de energie- en stikstofefficiëntie te verbeteren en de milieuverontreiniging door de productie van varkensvlees te verminderen. Bovendien zijn verschillende kenmerken en factoren die invloed hebben op de stikstofuitscheiding en voerefficiëntie besproken, om diepgaand inzicht te verkrijgen op het gebied van stikstof- en energie-efficiëntie.

De belangrijkste conclusies van dit proefschrift zijn:

- De stikstofexcretie van vleesvarkens neemt toe tijdens de groeiperiode.
- De stikstofexcretie kan het meest effectief worden verminderd door verbetering van de voerefficiëntie en in mindere mate door verbetering van de groei en toename van de beveelsheid.
- De efficiëntie van voerbenutting wordt beïnvloed door meerdere genen in verschillende groeifasen.
- Genetische verbetering van voerbenutting heeft een aanzienlijke invloed op de vermindering van de stikstofexcretie van varkens.
- Onderliggende factoren betrokken bij de voerefficiëntie, zoals stofwisseling, vertering van voer en eiwitturnover, zijn grotendeels verantwoordelijk voor de variatie in residuele energieopname.
- Residuele energieopname is een kenmerk met goede selectie mogelijkheden vanwege zijn hoge erfelijkheidsgraad.
- Bij genetische selectie op energie- en stikstofefficiëntie moet rekening gehouden worden met de groeifasen vanwege lage genetische correlaties en verschillende QTL voor deze kenmerken tussen groeiperioden.
- Naar verwachting resulteert genetische selectie op residuele energieopname, naast verbetering van de voederefficiëntie, in een aanzienlijke vermindering van de stikstofexcretie door de productie van varkensvlees.
- Groeimodellering kan worden gebruikt om strategieën te ontwikkelen ter verbetering van de energie- en voerefficiëntie van varkens. Mogelijke strategieën zijn verlaging van het slachtgewicht of verbetering van de groei, de productie-efficiëntie en de lichaamssamenstelling.

P

Publications

Peer-reviewed publications

- Shirali, M.,** A. Doeschl-Wilson, P.W. Knap, C. Duthie, E. Kanis, J.A.M. van Arendonk and R. Roehe. 2012. Nitrogen excretion at different stages of growth and its association with production traits in growing pigs. *J. Anim. Sci.* 90:1756-1765.
- Shirali, M.,** C. Duthie, A. Doeschl-Wilson, P.W. Knap, E. Kanis, J.A.M. van Arendonk and R. Roehe. 2013. Novel insight into the genomic architecture of feed and nitrogen efficiency measured by residual energy intake and nitrogen excretion in growing pigs. *BMC Genet.* 14:121.
- Shirali, M.,** A. Doeschl-Wilson, C. Duthie, P.W. Knap, E. Kanis, J.A.M. van Arendonk and R. Roehe. 2013. Estimation of residual energy intake and its genetic background during the growing period in pigs. *Livest. Sci.* Accepted for publication.
- Shirali, M.,** A. Doeschl-Wilson, P.W. Knap, E. Kanis, C. Duthie, J.A.M. van Arendonk and R. Roehe. 2013. Predicting energy efficiency and nitrogen excretion in growing pigs with different growth potential using a mechanistic biological growth model. *Animal.* Submitted.

Conference Proceedings

- Shirali, M.,** A. Doeschl-Wilson, P.W. Knap, C. Duthie, E. Kanis, J.A.M. van Arendonk, and R. Roehe. 2013. Using a mechanistic growth model to investigate energy efficiency and nitrogen excretion in pigs. *In: Book of abstracts of 64th Annual Meeting of the European Federation of Animal Production (EAAP), Nantes, France.* Page 593.
- Shirali, M.,** C. Duthie, A. Doeschl-Wilson, P.W. Knap, E. Kanis, J.A.M. van Arendonk, and R. Roehe. 2012. Novel Insight into the Genomic Architecture of Feed Efficiency in Growing Pigs. *In: Book of abstracts of 4th International Conference of Quantitative Genetics (ICQG), Edinburgh, UK.* Pages 233-234.
- Shirali, M.,** A. Doeschl-Wilson, C. Duthie, P.W. Knap, E. Kanis, J.A.M. van Arendonk, and R. Roehe. 2012. Estimation of residual energy intake and its genetic background during the growing period in commercial pigs. *In: Book of abstracts of Annual Conference of British Society of Animal Science (BSAS), Nottingham, UK.* Abstract 055.
- Shirali, M.,** A. Doeschl-Wilson, P. W. Knap, C. Duthie, E. Kanis, J. A. M. van Arendonk, and R. Roehe. 2011. Novel estimation of residual energy intake and their quantitative trait loci in growing-finishing pigs. *In: Book of abstracts of 62nd*

Annual Meeting of the European Federation of Animal Production (EAAP), Stavanger, Norway. Page 87.

Shirali, M., A. Doeschl-Wilson, P. W. Knap, C. Duthie, E. Kanis, J. A. M. van Arendonk, and R. Roehe. 2011. Nitrogen excretion during the growing-finishing period and its relationships with daily feed intake, feed conversion ratio and body composition in commercial pigs. *In*: Book of abstracts of Annual Conference of British Society of Animal Science (BSAS), Nottingham, UK. Abstract 0155.

Curriculum vitae

Mahmoud Shirali was born on 22 September 1984 in Ahvaz, Iran, where he lived till the end of his elementary school. Mahmoud completed his middle and high schools in Tehran, Iran. His adventure to become an animal breeding scientist started in 2002 by doing Animal Sciences in the Islamic Azad University, Iran. Adventurous personality and high interest in animal breeding and genetics led Mahmoud to move to Edinburgh, UK to obtain his Master's degree in Quantitative Genetics and Genome Analysis from Edinburgh University, UK during 2008 to 2009. For his M.Sc. degree, Mahmoud worked on QTL mapping for production traits in broiler chickens in the Roslin Institute, and genome wide association studies for colorectal cancer in human at the Medical Research Council. In 2009, Mahmoud enrolled as a PhD student in Wageningen University, the Netherlands in a joint project with SRUC (formerly SAC), Edinburgh, UK to further advance his interest in feed efficiency and pig breeding. This PhD was a multinational project that gave Mahmoud the possibility to expand his network and also get the best of each institution. During this PhD project, Mahmoud became an expert in feed efficiency of pigs. After his PhD, Mahmoud's interest in feed efficiency and travelling led him to take a position as post doctoral researcher in Aarhus University, Denmark on feed efficiency in pigs and minks, which he is currently involved in.

Training and Supervision Plan



Training and supervision plan

The Basic Package	year
WIAS Introduction Course	2011
Course on philosophy of science and/or ethics	2011
Introduction interview with WIAS scientific director and secretary	2011
Scientific Exposure	
<i>International conferences</i>	
Annual Conference of BSAS, Nottingham, UK	2011
Annual Conference of BSAS, Nottingham, UK	2012
4 th ICQG, Edinburgh, UK	2012
62 nd Annual Meeting of the EAAP, Stavanger, Norway	2011
64 th Annual Meeting of the EAAP, Nantes, France	2013
<i>Seminars and workshops</i>	
Scotland's Rural College (SRUC) Seminar, Edinburgh, UK	2010-2012
British Pig Executive (BPEX) Seminar, Birmingham, UK	2009-2012
Genomic Selection Workshop, SRUC, Edinburgh, UK	2010
The Roslin Institute and University of Edinburgh Seminar Program	2009-2012
Easter Bush Research Consortium Conference, Edinburgh, UK	2009-2012
Advanced Breeding Programs for Sustainable Crop and Livestock Production, Edinburgh, UK	2012
Feed Efficiency in livestock Workshop, SRUC, Edinburgh, UK	2012
<i>Presentations</i>	
BPEX Seminar, Birmingham, UK (oral)	2009
BPEX Seminar, Birmingham, UK (oral)	2010
BPEX Seminar, Birmingham, UK (oral)	2011
BPEX Seminar, Birmingham, UK (oral)	2012
SRUC Seminar, Edinburgh, UK (oral)	2010
SRUC Seminar, Edinburgh, UK (poster)	2011
SRUC Seminar, Edinburgh, UK (oral)	2012
Annual Conference BSAS, Nottingham, UK (oral)	2011
Annual Conference BSAS, Nottingham, UK (oral)	2012
Annual Meeting of the EAAP, Stavanger, Norway (oral)	2011

Training and Supervision Plan

In depth studies

Disciplinary and interdisciplinary courses

Genomic Selection	2011
-------------------	------

Advanced statistics courses

Statistics for the Life Sciences	2011
----------------------------------	------

PhD students' discussion groups

Quantitative Genetics Journal Club, UofEdinburgh (approx. 28 h/y)	2009-2012
---	-----------

SRUC Students discussion group (approx 28 h/y)	2010-2012
--	-----------

Professional Skills Support Courses

The Techniques for Writing and Presenting a Scientific Paper	2011
--	------

How to be an Effective Researcher	2010
-----------------------------------	------

Academic Writing event for international PGR students	2010
---	------

Getting Started with your PhD	2010
-------------------------------	------

SRUC Training Workshop	2010
------------------------	------

SRUC Training Workshop	2011
------------------------	------

SRUC Training Workshop	2012
------------------------	------

Reference Manager	2010
-------------------	------

Fast Reading	2010
--------------	------

Voice in Action	2010
-----------------	------

Research Skills Training

Preparing own PhD research proposal	2010
-------------------------------------	------

Didactic Skills Training

Lecturing

Teaching Assistant, SRUC, Animal Breeding	2010-2012
---	-----------

Preparing course material

Animal Breeding at SRUC	2010-2012
-------------------------	-----------

Management Skills Training

Organisation of seminars and courses

Quantitative Genetics Journal Club, University of Edinburgh, UK	2012
---	------

Education and Training Total Credit 44.1

Acknowledgement

This thesis is the result of a collaborative project between Scotland's Rural College (SRUC), Edinburgh, UK and the Animal Breeding and Genomics Centre of Wageningen UR, the Netherlands. I am very grateful to the British Pig Executive (BPEX), Scotland's Rural College (SRUC), Scottish Government and Wageningen University for funding my PhD project. The PIC, Deutsche Forschungsgemeinschaft (DFG) and University of Kiel are gratefully acknowledged for funding a previous experiment that provided the data for my PhD thesis. First of all, I would like to thank Rainer Roehe for appointing me as PhD student on this PhD project. It has been a great pleasure to work with him. Rainer is a great supervisor and group manager with excellent ideas to guide the students and the team through a project. Andrea Wilson, apart from being a great person, she is also a great supervisor. Andrea with her energetic personality was always there to help and guide me. Carol-Anne Duthie is a very collaborative and friendly supervisor, which made working with her pleasant. Pieter Knap has been of a great help to me with his extensive expertise in feed efficiency and pig breeding. I would like to thank Egbert Kanis, especially for his comments and suggestions on this thesis. Egbert and his wife Loes were very nice and supportive during my stay in Wageningen, this is really appreciated. Johan van Arendonk has always been a great help to me during this PhD project, his kind personality made working with him a great experience for me. All the supports from my supervisors are greatly appreciated as my knowledge and consequently this thesis would not have been at this level without them. I would like to give a shout out to my friends and colleagues in the Roslin Institute, SRUC, MRC and Wageningen University for all the scientific discussions during my PhD trajectory that expanded my knowledge of animal breeding, and above all for all the good times we had together which made my PhD a nice and memorable part of my life. Finally, I would like to express my gratitude to my family, Habib (my father), Fakhri (my mother), Masoud (my brother), Azadeh (my sister-in-law) and Mahsa (my sister) for their massive support during my life and especially during my PhD. It has been great to have Masoud as brother and also as an experienced colleague in quantitative genetics; he has been of great help to me. I would like to especially thank Mahsa for designing the cover page of this thesis.

All the best!
Mahmoud

Colophon

The printing of this thesis was funded by the Animal Breeding and Genomics Centre, Wageningen University, the Netherlands.

The cover photo of this thesis is designed by Mahsa Shirali.

This thesis was printed by GVO drukkers & vormgevers B.V. | Ponsen & Looijen, Ede, The Netherlands.

