The Effect of the *RN*$^{-}$ allele and Production System on Meat Quality and the Formation of Heterocyclic Amines in Pork

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Abstract

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This thesis summarises and discusses results of four separate studies on the combined effects of *RN* genotype and production system on technological and nutritional pig meat quality. That *RN* genotype considerably affects technological meat quality is well known but little attention has been directed towards potential effects on the nutritional quality of the meat. Mutagenic/carcinogenic heterocyclic amines (HCAs) in fried meat are an important nutritional safety issue and the high muscle glycogen content in *RN⁻* carriers could affect HCA formation. Further, despite an increased interest in alternative production systems for pig meat there are few and inconsistent reports on meat quality implications.

Three animal materials, representing different aspects of alternative production systems, formed the basis for the experimental work. Besides production conditions, *RN* genotype and sex of the animals were known. The water-holding capacity, colour, texture and chemical composition of the meat were analysed. In two studies the meat was fried and the level of heterocyclic amines formed was determined and in one study the fatty acid composition and the level of the antioxidant α-tocopherol were analysed. In the last study, a questionnaire complemented with colour photographs was used to obtain information on dietary practices and preferences regarding home-prepared pork chops of different *RN* genotypes.

The results stress the importance of combinatory effects of different production factors on final meat quality. Alternative production systems did not have a large impact on final pig meat quality, but in non-carriers of the *RN⁻* allele organic production with increased physical activity lead to impaired water-holding and texture of the meat. The high glycogen content of meat from *RN* carriers prevented negative effects of pre-slaughter treatment on meat quality. Free-range reared pigs with access to green feed resulted in more polyunsaturated fatty acids and α-tocopherol in the meat compared to indoor reared pigs. The high glycogen content in carriers of the *RN⁻* allele led to a browner crust and considerably lower levels of HCAs in fried meat compared to that of non-carriers. It was indicated that pork chops only marginally contribute to the daily intake of HCAs; nonetheless, by choosing meat of carriers of the *RN⁻* allele and by using lower cooking temperatures, this intake can be further reduced.

Keywords: pig meat, RN genotype, sex, organic, free-range, water-holding capacity, precursors, frying

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Svensk populärvetenskaplig sammanfattning

Marknaden för ekologiskt producerat griskött växer men det finns kunskapsluckor i hur alternativa uppfödningssystem påverkar köttets teknologiska kvalitet, till exempel dess färg, vattenhållande förmåga och textur. De flesta svenska slaktsvin är korsningsgrisar med Hampshire som faderras. Man länge känt till att en dominant mutation, den så kallade *RN*$^{-}$-allelen, har stor effekt på köttets teknologiska kvalitet hos dessa djur.

Grisköttet skall inte bara vara funktionellt, det skall även vara säkert och hälsosamt att konsumera. Näst efter tobaks-rökning är kosten en av de allra viktigaste orsakerna till uppkomsten av cancer hos människor. Förekomsten av heterocykliska aminer, så kallade stekytemutagener, som kan bildas när man tillagar kött är därför en viktig säkerhetsaspekt. Flera av de heterocykliska aminerna har klassificerats som möjliga eller troliga humancarcinogener av den internationella cancerkommittén IARC. Trots allmänt kända råd som att undvika hårdstekning och grillning över öppen eld krävs mer omfattande kunskaper om vilka betingelser som gynnar uppkomsten av heterocykliska aminer. Griskött innehåller alla de ämnen som i modellförsök visat sig vara nödvändiga för bildningen av heterocykliska aminer. Man vet dock lite om hur mycket halterna av dessa ämnen varierar inom ett djurmaterial. Exempelvis skulle *RN*$^{-}$-allelens stora inverkan på köttests glykogeninnehåll kunna ha en stor effekt på bildningen av stekytemutagener. Vidare är människors preferenser och vanor rörande stekning, i samverkan med köttets egenskaper, och hur dessa faktorer påverkar bildningen av heterocykliska aminer, inte så väl kända.

Grisköttets fettsyrasammansättning kan manipuleras genom utfodring. Genom att ge grisar mer fleromättat fett i fodret kan köttets nutritionella värde förbättras till en viss gräns. Om man däremot utfodrar allt förmycket fleromättat fett kan späck och kött få en lös konsistens och köttet riskerar att härskna snabbare. I viss mån kan dessa negativa konsekvenser avseende härskning förhindras genom att ge grisarna antioxidanter, till exempel vitamin E.

I den här avhandlingen har vi velat studera de sammanvägda konsekvenserna av *RN*-genotyp, uppfödningsform och kön för några av grisköttets teknologiska och nutritionella egenskaper.

Vi fann att uppfödningsformen har en relativt liten inverkan på grisköttets kvalitet. I kombination kan dock grisarnas *RN*-genotyp, uppfödning och kön ha både positiva och negativa effekter på köttet. Den vattenhållande förmågan var sämre i ekologiskt producerat kött från icke-bärare av *RN*$^{-}$-allelen men köttets fettsyrasammansättning förbättrades något utan att köttets hållbarhet försämrades av att grisarna fått möjlighet till utevistelse. Vidare kunde vi se att det bildas lägre halter mutagena heterocykliska aminer i kött från grisar som är bärare av *RN*$^{-}$-allelen. Våra resultat indikerar också att, trots att fläskkotlett är en populär rätt på de svenska matborden, så bidrar den i liten grad till det dagliga intaget av heterocykliska aminer. Genom att välja kött från icke-bärare av RN-genen och genom att steka kotletterna vid lägre temperaturer skulle det dagliga intaget ytterligare kunna reduceras.

Contents

Appendix

Papers I-IV

The present thesis is a synthesis of the following papers, referred to by their Roman numerals.

I. Nilzén V., Babol J., Dutta P.C., Lundeheim N., Enfält A-C., and Lundström, K. 2001. Free-range rearing of pigs with access to pasture grazing – effect on fatty acid composition and lipid oxidation products. *Meat Science*, 58, 267-275.

II. Olsson, V., Solyakov, A., Skog, K, Lundström, K. and Jägerstad, M. 2002. Natural variations of precursors in pig meat affect the yield of heterocyclic amines - Effects of *RN* genotype, feeding regime and sex. *Journal of Agricultural and Food Chemistry,* 50: 2962-2969.

III. Olsson, V., Andersson, K., Hansson, I. and Lundström, K. 2003. Differences in meat quality between organically and conventionally produced pigs. *Meat Science*, 64, 287-297.

IV. Olsson, V., Skog, K, Lundström, K. and Jägerstad, M. Colour photographs for estimation of heterocyclic amine intake from fried pork chops of different *RN* genotypes indicate large variations. *Accepted for publication in Food Quality and Preference.*

Viktoria Olsson's maiden name was Nilzén.

List of abbreviations

ATP	adenosine triphosphate
ANOVA	analysis of variance
BF	*M. biceps femoris*
DFD	dark firm and dry
4,8-DiMeIQx	2-amino-3,4,8-trimethylimidazo[4,5-f]quinoxaline
FAA	free amino acids
FAME	fatty acid methyl esters
G-6-P	glucose-6-phosphate
GLM	general linear model
Harman	1 -methyl-9H-pyrido[3,4-b]indole
HCAs	heterocyclic amines
HPLC	high performance liquid chromatography
IARC	International Agency of Research on Cancer
IMF	intramuscular fat
IQx	2-amino-3-methylimidazo[4,5-f]quinoxaline
LD	*M. longissimus dorsi*
MDA	malondialdehyde
MeIQx	2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline
Norharman	9H-pyrido[3,4-b]indole
NPN	non-protein nitrogen
pH_u	ultimate pH
pH_{45}	pH value measured 45 min *post mortem*
PhIP	2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine
PSE	pale soft and exudative
PUFA	polyunsaturated fatty acid
RFN	red, firm and nonexudative
RSE	red, soft and exudative
RN	Rendement Napole
rn^+ allele	the wild type on the PRKAG3 (RN) locus
*rn** allele	second mutant of the PRKAG3 (RN) locus
RN^- carrier	RN^-/rn^+ (with Hampshire as terminal sire)
TBA	thiobarbituric acid
WB	Warner-Bratzler shear force
WHC	water-holding capacity

Introduction

Modern pig meat production faces new challenges. High product quality is taken for granted by the consumers but other aspects, such as animal welfare (production quality) and wholesomeness of the cooked meat attract attention. Alternative pig meat production systems, managing new solutions for animal welfare and environmental problems are emerging. Presently, the production of organic pork accounts for less than 1% of the pig meat production in Sweden and about 100 Swedish farms specialise in organic pig production (Benfalk, 2003). The effect of these new systems on meat quality is yet not fully elucidated. One objective of the present thesis was to study if and how organic production of pig meat affects overall meat quality.

The RN^- allele, a mutation found in Hampshire-breed pigs, is a factor known to influence the quality of fresh and processed meat. In the Swedish slaughter-pig population, the frequency of RN^- carriers is approximately 60% (Josell *et al.*, 2000). This mutation considerably affects the chemical composition of the meat. A second objective of the thesis was to study how the *RN* genotype, in combination with altered production systems, influences the level of precursors for the formation of heterocyclic amines (HCAs) in cooked pig meat. Mutagenic/carcinogenic HCAs are formed in low concentrations (ng/g cooked meat) during heat treatment of meat and fish. Better understanding of the importance of the level of precursors and cooking conditions for their formation will help reduce exposure. Thus, a third objective of the thesis was to deepen the knowledge of the consumer cooking practices and preferences regarding pig meat from carriers and non-carriers of the RN^- allele.

Next, a brief account of the concept of meat quality and related factors, relevant for this thesis, will follow. Thereafter the objectives of the thesis are presented and the material and methods used are described. The results of the four investigations are summarised and discussed after which concluding remarks are presented. The last part of the thesis contains the four original papers, comprising the experimental work, *i.e*. the new knowledge gained during this project.

Meat Quality

To cover the complex series of processes from breeding to the fried pork chop on the plate, the concept meat quality can be divided into production quality and product quality (Hofmann, 1994). Product quality can be further divided into technological, (*i.e.* the functional properties of the meat), sensory (eating experience), nutritional (wholesomeness) and toxicological/hygienic (absence of *e.g.* contaminants, harmful micro-organisms) quality. This thesis focuses on how production system and *RN* genotype influence technological, and to some extent, nutritional meat quality.

Technological meat quality

The technological quality describes the suitability of raw material for further processing as well as the yield during processing. The technological quality attributes of pig meat include its water-holding capacity (WHC), colour, texture and chemical composition. These parameters are influenced by multiple interacting factors including breed, genetics, feeding, pre-slaughter treatment and stunning, slaughter method, chilling and storage conditions as reviewed by Rosenvold and Andersen (2003). The occurrence of a few known major genes, especially the halothane (a point mutation in the ryanodine receptor; Fujii *et al.*, 1991) and *RN*$^{-}$ genes, considerably affects the quality characteristics of fresh and processed pork. However, when major gene effects are excluded, genetics explains less of the variation in meat quality traits, which are only slightly, to moderately heritable (Sellier, 1998). There is a large individual variation in meat quality both within and between animals of the same breed, sex and environment, which is not well understood. This variation is likely to be caused by differences in various known and unknown intrinsic (genetic) and extrinsic (environmental) factors, which interact and determine the outcome of metabolic processes in the *petri-* and *post mortem* period (Klont *et al.*, 1998).

At slaughter of the animal the supply of oxygen, glucose and free fatty acids to muscle ceases when the blood circulatory system stops. Any subsequent metabolism must be anaerobic and adenosine triphosphate (ATP) can only be regenerated through breakdown of glycogen through glycolysis since oxidative decarboxylation and phosphorylation no longer operate. Lactic acid accumulates and the muscle gradually acidifies. The decline in pH depends on the initial concentration of creatine phosphate and glycogen (Bendall, 1951) and the characteristics of the *post mortem* pH decline are determined by the physiological condition of the animal at stunning (Bendall, 1973; Warriss, *et al.*, 1989). In a well-fed and unstressed pig, the pH value typically falls from about 7.2 to an ultimate pH (pH_u) of about 5.4, reaching a plateau when enzymes participating in the glycolysis are inactivated (Lawrie, 1992). The biochemical and physical processes taking place during the *post mortem* conversion of muscle to meat are crucial for the final product; more specifically, *post mortem* pH and temperature development are very important causes of variation in pork quality (Sellier & Monin, 1994; Schäfer *et al.*, 2002). Both the rate and extent of pH decline are of importance (Briskey, 1964). The rate of pH decline is often indicated by values measured 45 minutes post mortem (denoted pH_{45}) whereas the extent (pH_u) is normally measured 24 h *post mortem* but may decline further. In a study by Josell *et al.* (2003a) pH_u values were not reached until 48h *post mortem*.

Water-holding capacity (WHC)

WHC can be defined as the ability of meat to retain inherent water during storage, processing and cooking. Water loss, and subsequent inferior technological quality, causes financial losses for the industry and results in a less attractive product appearance for the consumer. WHC may also influence the sensory quality of meat. The rate and progress of the biochemical and physical processes *post mortem* determines the structural arrangement and state of the proteins in the meat, thereby

affecting its water-holding properties (Bendall & Wismer-Pedersen, 1962; Penny, 1969). pH and temperature development early *post mortem* are especially critical for the WHC of pork in populations where major gene effects are excluded (Schäfer *et al.*, 2002).

Colour

Meat colour is an important quality attribute for the consumer (Risvik, 1994). As for WHC, the temperature and pH history of the muscle *post mortem* are of importance for the meat colour through its effect on the physical structure and light scattering properties of the meat proteins (MacDougall, 1982). Further, meat colour is affected by the concentration and properties of the meat pigments myoglobin and, to lesser extent, haemoglobin (Lindahl *et al.*, 2001). Muscle myoglobin (80-90% of total pigments) concentration varies between species, breed, sex, age, type of muscle and level of training (Ledward, 1992). In fresh meat myoglobin can exist in three different forms: the reduced form of myoglobin (deoxymyoglobin) is purplish, and the oxygenated form (oxymyoglobin) is bright red whereas the oxidized form (metmyoglobin) is brown. Fresh meat colour is affected by the relative abundance of these three forms (Govindarajan, 1973).

Both the light scattering and WHC phenomena can be illustrated by two quality extremes; pale soft and exudative (PSE) and dark firm and dry (DFD) meat. PSE is associated with pale meat with low water-holding capacity. It is caused by extensive protein denaturation and lateral shrinkage of the myofibrils due to an early *post mortem* pH decline in muscles with a still relatively high temperature (Bendall & Wismer-Pedersen, 1962; Briskey, 1964; Offer & Knight, 1988a). In PSE meat, light does not penetrate far into the meat but is scattered, which makes the meat appear pale (Offer *et al.*, 1989). PSE develops for several reasons; the most investigated is pre-slaughter stress, often in stress-sensitive carriers of the Halothane gene, increasing the *post mortem* glycolytic rate and muscle temperature (Gariepy *et al.*, 1989).

DFD meat, on the other hand, is characterised by high pH_u, dark appearance and high WHC. The pH (>6.0) is much higher than the isoelectric point of actomyosin, resulting in that more water is kept between the myofilaments and less is exuded out of the meat matrix (Offer *et al.*, 1989). The DFD muscle has a compact structure and appears darker because its surface only slightly scatters incident light (Govindarajan, 1973). DFD is caused by low muscle glycogen levels at slaughter resulting in restricted formation of lactate *post mortem* and a rapid onset of rigor mortis. Low glycogen levels in turn, may be due to long-term stress, lack of food for several days or strenuous exercise (Bendall & Swatland, 1988; Warriss *et al.*, 1989).

Besides, DFD and PSE, two other pork qualities exist: red, soft and exudative (RSE), and red, firm and nonexudative (RFN). RSE pork has an acceptable colour but excessive exudation (Kauffman *et al.*, 1993; Warner *et al.*, 1993). Normal pork, sometimes referred to as RFN pork, is red, firm and nonexudative (van Laack *et al.*, 1994).

Texture

Tenderness is one of the most important factors for the perceived sensory quality of pig meat (Wood, *et al.*, 1992; Van Oeckel *et al.*, 1999). Since evaluation of tenderness by a taste panel is time consuming and costly, instrumental Warner-Bratzler shear force is often used as a measure for meat tenderness (Boccard *et al.*, 1981). Warner-Bratzler devices and sample configuration vary considerably; however, the general principle is to measure the force needed to cut through a standardized meat sample. Using the recommended equipment, a force deformation curve is obtained from which the parameters peak force and total work are measured (Honikel, 1998). Two major phenomena are known to influence final tenderness in meat: (1) shortening of muscle fibres (contraction during rigor) affects meat toughness and (2) ageing affects the gradual tenderisation. *Post mortem* degradation of myofibrillar proteins is the main reason for the improvement in meat tenderness during ageing (Quali, 1992). Also for the processes of tenderisation, a combination of pH and temperature decline has been found important, here through a possible effect on proteolytic enzymes during rigor development (O'Halloran *et al.*, 1997). Further, the level of intramuscular fat (IMF; Bejerholm & Barton-Gade, 1986; van Laack *et al.*, 2001) as well as the live animal growth rate and protein turnover (Kristensen *et al.*, 2002) may affect meat tenderness and shear force values. The properties of intramuscular collagen may also influence shear force values. However, as pigs are slaughtered young the immature connective tissue does not significantly influence pork tenderness (Avery *et al.*, 1996). These suggested factors affecting tenderness are, as reviewed by Wood *et al.* (1992), in turn affected by several processing factors, including pre-slaughter handling, stunning method, carcass chilling rate, carcass suspension system, and aging time. Processing factors are generally more important for pork tenderness than production factors including breed, carcass weight, fat level and feeding regime (Wood *et al.,* 1996).

Chemical composition, nutritional and food safety aspects

Lean pig meat contains approximately 75% water, 21% proteins, 1-3% intramuscular fat, 1-2% carbohydrates, mainly glycogen, and 1% inorganic constituents (Hedrick, 1994). Meat is a concentrated source of protein with high biological value, containing all amino acids essential for human health. It is also an important source of B vitamins, as well as iron, copper, zinc and selenium. However, this thesis has addressed the nutritional and safety aspects of pork by studying the amount and quality of intramuscular lipids, as well as the formation and level of HCAs in fried meat.

Water

Water is the principal constituent of intra- and extramyofibrillar fluid in which numerous chemical constituents are dissolved or suspended. Because of this it serves as the medium for transport of substances between the vascular bed and muscle fibres (Hedrick, 1994). Little water (4-7%) is chemically bound to muscle proteins. Instead it is the structure of the myofibrils that physically trap water in tertiary and/or quaternary protein structures and spatial domains, including the actin and myosin filament structures (Offer & Knight, 1988b; Wismer-Pedersen,

1988; Bertram *et al.*, 2001). Nuclear magnetic resonance (NMR) relaxation provides further information about the state of water in muscle tissue and relaxation data indicate the existence of three distinct water populations in muscle throughout the conversion to meat (Bertram, *et al.*, 2004b). Muscle glycogen can also bind water, and variations in water content of muscle can be attributed to those in protein and glycogen content (Sellier & Monin, 1994; Lundström *et al.*, 1996). In meat, water can be lost through evaporation or drip or during cooking (Offer & Knight, 1988b). Factors governing the water content in meat are thus related to those governing WHC.

Proteins and non-protein nitrogen

As the principal component of the dry matter, protein content can vary between 16-22% of the muscle mass. Proteins have a wide range of functions in the body. They may be structural or contractile, they may be enzymes, hormones or antibodies or they may have transport (*e.g.* haemoglobin and myoglobin) or osmotic functions (*e.g.* blood plasma albumin). The relationship between protein and water is rather constant at 0.3 in the muscle (Wismer-Pedersen, 1988). Nonetheless, the protein level may be affected by *e.g.* the occurrence of the RN^{-} allele (Sellier & Monin, 1994). Of total nitrogen content in muscle, approximately 95% is protein and 5% non-protein nitrogen (NPN), *i.e.* nitrogenous compounds that are not proteins. Free amino acids, simple (di)peptides, creatine, creatine phosphate, creatinine, some vitamins, nucleosides and nucleotides belong to NPN. Free amino acids and dipeptides (*e.g.* anserine and carnosine) are small molecules occuring in low concentrations in muscle. Metabolic type of muscle, physical activity and nutrition may influence free amino acid and dipeptide content (Cornet & Bousset, 1999; Essén-Gustavsson & Blomstrand, 2002). The concentrations of free amino acids and peptides in meat may also be affected by *post mortem* handling of meat including refrigerated storage, curing or fermentation when micro-organisms and their enzymes hydrolyse proteins. In addition, heating and cooking might induce hydrolysis of proteins (Felton, *et al.*, 2000). The physiological role of creatine is to serve, in its phosphorylated form, as a reservoir of high-energy phosphate used for ATP generation during muscle contraction. This explains the relatively high (0.3-0.6%) content of creatine in muscle in comparison with other tissues (Dvorák, 1981). In muscle cells, almost all creatine (>90%) is in a free state a few hours after slaughter (Sulser, 1978). The creatinine content is low, (about 6% of that of creatine) but when meat is cooked the creatine content decreases as creatine is converted to creatinine through water elimination and ring closure (Cambero *et al.*, 1992; del Campo *et al.*, 1998; Persson *et al.*, 2003). During cooking, creatine is transported to the surface and concentrated in the crust, where the temperature is high enough to transform creatine to creatinine (Laser-Reuterswärd *et al.*, 1987)

Intramuscular fat and fatty acid composition

Intramuscular fat or more correctly, lipid content, of pig meat can vary substantially in *M. longissimus dorsi* (LD) and *M. biceps femoris* (BF) typically in the range of 0.5-4% (Essén-Gustavsson *et al.*, 1994; Fernandez *et al.*, 1999). Traditionally, lipids have been divided into structural lipids and depot lipids. Intramuscular lipids consist mainly of neutral lipids (triacylglycerols) and

phospholipids and they function as vehicles for energy storage (depot) as well as essential parts of cell membranes (structural). In addition, lipids form the basis of steroid hormones as precursors in eicosanoid metabolism. They are a highly concentrated storage form for energy and their energy value is almost double that of carbohydrate and protein. Polyunsaturated fatty acids (PUFAs) in the diet of pigs affect the fatty acid composition in both structural and depot lipids, and changes occur earlier in the depot fat, *e.g.* back fat (Warnants *et al.*, 1996).

In recent years, there has been an increased interest in producing meat with improved fatty acid composition, designed to meet the dietary recommendations for humans. According to Swedish recommendations, essential (polyunsaturated n-6 and n-3) fatty acids should provide at least 3 % of the total energy intake. A minimum of 0.5 energy percent of n-3 is recommended. In the diet of pregnant and nursing women, essential fatty acids should contribute at least 5 energy percent (NFA, 2003). More specifically, British dietary recommendations on the fatty acid composition suggest a ratio of polyunsaturated to saturated fatty acids (P:S ratio) more than 0.4 and a ratio of n–6 to n-3 fatty acid less than 4 (DH, 1994). The reason for these recommendations is that both the P:S ratio and the n-6:n-3 PUFA ration are risk factors in various cancers and especially coronary heart disease (Staessen *et al.*, 1997; Riccardi & Rivellese, 2000). The P:S ratio of pork muscle and adipose tissue usually falls well above the minimum recommended, at approximately 0.6, due to the rateher high level of linoleic acid (C 18:2) in European cereal-based diets (Enser *et al.*, 1996; Enser, 2000). Instead, the driving force has been to increase the n-3, and thus improve the n-6:n-3 ratio of pig meat which can be achieved by feeding sources such as linseed (as reviewed by Wood *et al.*, 2004) or green feed with high n-3 content (Jakobsen, 1995). However, increasing the unsaturation of the lipids may result in "soft" meat and meat products and may cause undesirable colour (yellowness) of the depot fat (as reviewed by Wood *et al.*, 2004). Further, because lipids in pig meat are relatively unsaturated, attempts to further increase the concentration of PUFA may lead to a risk for the generation of lipid oxidation products, leading to off-odours and flavours and colour changes reducing the shelf life of the product (*e.g.* Allen & Foegeding, 1981). To some extent these adverse effects could be prevented through administration of dietary antioxidants. For example, α-tocopherol (vitamin E) can be used to delay lipid and colour oxidation and to extend shelf life (reviewed by Wood *et al.*, 2004).

Several studies (*e.g.* Enser *et al.*, 2000; Sheard *et al.*, 2000), show that it is possible to improve the fatty acid composition of pork through correct feeding strategies without adversely affecting lipid oxidation. However, the relationship between the content of PUFA, pro- and antioxidants and the formation of lipid oxidation products in pig meat calls for careful consideration.

Carbohydrates

Glycogen is the most abundant carbohydrate in the muscle, comprising approximately 0.5-2%, of muscle weight, higher values often attributable the RN^- allele. Other carbohydrates are glycosaminoglycans (associated with connective tissues), glucose and some mono- and disaccharides, as well as the intermediates of glycolytic metabolism (*e.g.* glucose-6-phosphate (G-6-P)) (Hedrick, 1994).

Determination of the glycolytic potential in meat according to Monin and Sellier (1985) enables an estimate of the glycogen present in muscle at slaughter. The glycolytic potential includes glycogen as well as lactate, glucose and G-6-P, *i.e.* products and intermediates of *post mortem* glycolysis. For some analytical purposes the determination of lactate can be omitted, and the residual glycogen (glycogen + glucose + G-6-P) is adequate (Lundström *et al.*, 1996). Glycogen serves as an important source of energy for contracting muscle under both aerobic and anaerobic conditions (Lawrie, 1992). Breed, genotype within breed, muscle type and stress, exercise as well as the amount and type of feed affect the muscle glycogen level both short and long term. As described later, the *RN*$^{-}$ allele particularly affects glycogen metabolism. *In vivo* muscle glycogen levels can, for example, be manipulated through strategic feeding schemes (Rosenvold *et al.*, 2001a; Rosenvold *et al.*, 2001b). The reason for wanting to manipulate the muscle glycogen stores at the time of slaughter is the decisive role it plays for meat quality through its effect on pH progress and thereby WHC and colour (Briskey, 1964; Rosenvold *et al.*, 2001a).

Inorganic constituents

Muscle contains numerous inorganic constituents, notably cations and anions of physiological significance such as potassium, phosphorus, sodium, magnesium, calcium, zinc and iron (Hedrick, 1994). Ash is a crude measurement of all inorganic residues obtained after removal of moisture and dry matter after heat treatment. Because pigs are monogastric animals, many dietary components, including minerals, are partly transferred from the feed to the muscle (reviewed by Rosenvold & Andersen, 2003).

Heterocyclic amines in fried pig meat

Uncooked meat has little or no aroma and only a metallic, blood-like taste. Meat flavour develops during cooking through complex interactions of precursors derived from both lipids and lean meat. The Maillard reaction is important in the development of meat flavour and an appetizing brown crust colour during cooking (Pearson *et al.*, 1966; Mottram, 1998). Further, proper cooking ensures the microbiological safety of meat and may inactivate endogenous enzymes potentially causing deteriorative changes (Hedrick, 1994). Frying is a common method of preparing meat because it is fast and easy and it gives all benefits of cooking.

After tobacco, diet is probably the second most important factor in the etiology of human cancer, responsible for about one third of all cases (Ferguson, 2002). Heterocyclic amines (HCAs) are genotoxic carcinogens produced during cooking of meat. Since the late 1970s, a series of highly mutagenic/carcinogenic HCAs has been isolated from cooked meat and fish products (Felton & Knize, 1991; Wakabayashi *et al.*, 1992; Sugimura, 2000)

Some of the most common HCAs in cooked meat, are 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP), 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx), 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline (4,8-DiMeIQx), and 2-amino-3-methylimidazo[4,5-f]quinoxaline (IQx; Figure 1). These substances are more extensively studied concerning reaction pathways, precursors and conditions than other HCAs (for references see Jägerstad *et al.*, 1998; Skog *et al.*, 1998; Felton *et al.*, 2000).

Naturally occurring water-soluble substances in meat, creatine and creatinine, free amino acids and carbohydrates (mainly glucose) function as precursors in the formation of HCAs (Sugimura *et al.*, 1982; Jägerstad *et al.*, 1983; Felton *et al.*, 1986; Nyhammar, 1986; Pais *et al.*, 1999). During cooking of meat, the Maillard reaction between amino compounds and reducing sugars is important for both crust colour and flavour formation (Pearson *et al.*, 1966; Mottram, 1998). Unfortunately, the imidazoquinoline and imidazoquinoxaline type (IQ-type) of HCAs are probably formed via the Maillard reaction (Jägerstad *et al.*, 1983; Nyhammar, 1986). Reaction mechanisms have been proposed for several but not all HCAs (Jägerstad *et al.*, 1983; Nyhammar, 1986; Yaylayan & Lachambre, 1990; Felton, & Knize, 1991; Pearson *et al.*, 1992; Murkovic *et al.*, 1999). The amino-imidazo part of the

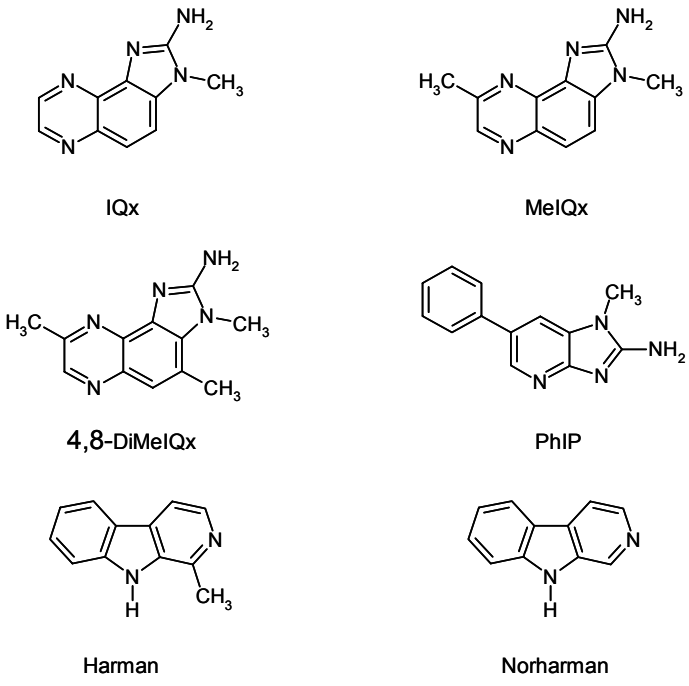

Figure 1. The HCAs detected in fried pig meat.

molecule is thought to originate from creatine (probably via creatinine) and the pyridine or pyrazine part from Strecker degradation products formed in the Maillard reaction. Aldol condensations were suggested to link the two parts together via a Strecker aldehyde (or related Shiff base). A controversial hypothesis about the reaction mechanism behind the formation of IQ type HCAs, involving free radical reactions has also been presented (Pearson *et al.*, 1992) and partly supported (Johansson & Jägerstad, 1996).

Cooking conditions, *i.e.* cooking methods, temperature and time, play a crucial role for the type and level of HCAs formed (Knize *et al.*, 1985; Knize *et al.*, 1994; Skog *et al.*, 1995; Persson *et al.*, 2003). Other important factors are the presence and relative amounts of the precursors, creatine and creatinine, free amino acids and carbohydrates. Furthermore, the properties of meat carry a range of more or less studied factors; for example the amount and fatty acid composition of lipids, occurrence of pro- and antioxidants, water content and WHC, which may serve as enhancers or inhibitors for the formation of HCAs (Jägerstad *et al.*, 1998; Felton *et al.*, 2000; Persson *et al.*, 2003). For domestic cooking practices, pan frying and grilling/broiling give much higher concentrations of HCAs than roasting because the meat is in direct contact with the hot surface (Felton *et al.*, 2000; Keating & Bogen, 2001; Zimmerli *et al.,* 2001). Water-soluble precursors of HCA, *e.g.* creatine and free amino acids, migrate with water towards the surface and into the meat juice formed. During crust formation, the water content in surface decreases and the temperature rise. This may explain why the concentration of HCA is high in the crust whereas almost negligible amounts are reported in the interior of the cooked meat products (Felton *et al.*, 2000). The level of HCAs in pan residues after frying is generally the same as in the food product fried, but sometimes the amounts are considerably higher (Skog *et al.,* 1998). Thus, the pan residues, if used for preparing *e.g.* gravy, are a source of considerable additional exposure to HCAs.

Eight HCAs have been classified as possible human carcinogens and 2-aminio-3-methylimidazo[4,5-*f*]quinoline (IQ) as a probable human carcinogen by the International Agency of Research on Cancer (IARC, 1993). Classification is based on long-term animal studies that have shown that these HCAs are carcinogenic at multiple sites (Adamson *et al.*, 1990; Ohgaki *et al.*, 1991; Sugimura, 2000). Further, compiled data indicate that PhIP and MeIQx form DNA adducts in human tissue in a dose-dependent manner (Nagao, 2000). Like many other carcinogens, HCAs require metabolic activation before binding to DNA and potentially inducing mutations and cancer (Felton *et al.*, 1997). The β-carbolines, harman and norharman, are in themselves not mutagenic but may modify the mutagenic potential of several different mutagens in various test systems, thereby acting as co-mutagens (de Meester, 1995). After bioactivation in the brain, β-carboline-derived neurotoxins may be involved in chronic neurodegenerative processes, such as Parkinson's disease reviewed by Herderich and Gutsche (1997).

PhIP, MeIQx and DiMeIQx are commonly found in well-done pork. The highest concentrations are found in cooked bacon where extreme levels of PhIP up to 53 ng/g have been reported in samples cooked at 170ºC for as long as 16 min (Gross *et al.*, 1993). In pork chops cooked under well-controlled conditions, the level of

PhIP and MeIQx would typically range from undetectable to about 5 ng/g, most often somewhat lower levels than in other meats (Table 1).

The human exposure to HCAs may be estimated using dietary assessment in combination with analytical data on HCA levels in various foods (Skog, 2002). Dietary assessment is often based on food frequency questionnaires, but it is a great challenge to cover all important factors regarding eating habits of the individual. The exposure of HCAs is related to the type of food, preference for doneness, cooking method, and quantity consumed (Felton *et al.*, 1997). These differ between different populations and conclusions from one population may not be applicable to another; *e.g.* the use of pan residues to make gravy may be common in Sweden but not in other countries (Skog, 2002). In addition, compared to literature data from the past century, identification and quantification of HCAs in normally cooked foods have become more accurate because of the improvement of current analytical methods. Literature data clearly show that HCAs are ingested by human beings in many populations. Individual exposure assessments vary between investigations, ranging from 160 to 1820 ng total HCAs/day and person (Augustsson & Steineck, 2000).

Table 1. *The concentrations of major HCAs (ng/g cooked meat) in intact and ground pork, beef and chicken breasts as reported in recent studies*

Sample	Cooking method	Initial cooking temp. (°C)	Total cooking time (min)	Amounts (ng/g cooked meat)				reference
				PhIP	MeIQx	DiMeIQx	IQ	
Pork chop	fried	150-225	8-9.5	nd-4.8	nd-2.6	nd-1.1	nd	Skog *et al.*, 1995
Pork chop	fried	175	5-15	nd	nd-3.8	nd	nd	Sinha *et al.*, 1998
Pork chop	heated	275	30	4.7	3.5	0.4		Pais *et al.*, 1999
Pork chop	unspecified	unspecified	unspecified	2.4	0.4	nd		Pais *et al.*, 2000
Pork chop	Fried/cooked	unspecified	unspecified	nd-2.5	nd-2.0	nd-0.6		Zimmerli, 2001
Beef steak[a)]	fried	150-225	7	0.02-1.8	0.02-1.6	0.02-0.6		Skog *et al.*, 1995
Beef steak[b)]	heated	275	30	1.2	1.4	0.2		Pais *et al.*, 1999
Beef patty	fried	150-225	5-7	nd-1.1	nd-2.2	nd-0.8		Skog *et al.*, 1995
Beef patty	fried	180/220	3.5/5.5	0.8-8.1	0.6-2.8	0.4-1.7		Persson *et al.*, 2003
Chicken breast	fried	140-220	12-34	nd-38.2	0.1-1.8	0.1-0.6		Solyakov & Skog, 2002
Chicken breast	heated	275	30	37.5	0.5	0.2		Pais *et al.*, 1999
Chicken breast	fried	175-225	30	0.5-10.0	0.4-0.5	0.2-0.5		Skog *et al.*, 1997

a) Sirloin steak
b) Top round steak.

The effects of *RN* genotype on pig meat quality

The effect of the RN^- allele on technological meat quality

The Hampshire breed was introduced as terminal sire in Swedish slaughter pigs in the 1970s, mainly to reduce stress susceptibility and subsequent meat quality problems such as PSE (Lundström *et al.*, 1996). In the late 1980s, researchers were able to deduce the specific characteristics of meat from the Hampshire breed of pigs to the dominant RN^- allele (Naveau, 1986; Le Roy *et al.*, 1990; Fernandez *et al.*, 1992). The allele was named after its influence on the Napole yield ("Rendement Napole" in French), a laboratory method used to evaluate the technological yield in cured and cooked hams. The most obvious effects of the RN^- allele is a high glycogen content in glycolytic muscles, followed by low ultimate pH (pH_u), pale meat and reduced technological yield and WHC (Lundström *et al.*, 1996; Le Roy *et al.*, 2000). Meat from carriers of the RN^- allele has lower protein content (Estrade *et al.*, 1993; Enfält *et al.*, 1997a) compared to that of non-carriers. Further, in almost all studies meat from RN^- carriers has lower shear force values (Lundström *et al.*, 1996; van Laack *et al.*, 2001; Josell *et al.*, 2003b) and is considered more tender in sensory evaluations (Jonsäll *et al.*, 2000; Miller *et al.*, 2000; Jonsäll *et al.*, 2001; Josell *et al.*, 2003b) compared to meat of non-carriers.

Recently, the identification of a second mutant allele (V199I or rn*) at the *RN* locus (Milan *et al.*, 2000; Ciobanu *et al.*, 2001) made it possible for Lindahl *et al.* (2004) to conclude that all three alleles at the *RN* locus, altogether six genotypes (RN^-/RN^-, RN^-/rn^+, RN^-/rn^*, rn^+/rn^+, rn^+/rn^* and rn^*/rn^*), affect important technological meat quality traits. The RN^- allele was found to be dominant over the other two alleles, rn^+ and rn*. The rn* allele affected pH_u in LD of non-carriers of the RN^- allele, giving higher pH_u. Further, a glycogen lowering effect of the rn* allele on the RN^-/- genotypes in female, but not entire male pigs, was observed (Lindahl *et al.*, 2004). In contrast to the RN^- mutation, which is found only in pure and cross-bred Hampshire pigs, the rn* allele is found in breeds other than Hampshire. Ciobanu et al. (2001) found that it contributes to lower levels of glycogen, lactate and glycolytic potential and higher pH_u in the pig breeds Landrace, Large White, Berkshire, Duroc and Duroc Synthetic.

Obviously, the effects of the RN^- allele in terms of technological and sensory quality of the meat have been well investigated. Overall, less focus has been directed towards how the RN^- allele affects the nutritional meat quality. This thesis presents new knowledge on the potential effects of the RN^- allele on fatty acid composition as well as its role in the formation of HCAs in pig meat.

The effect of the RN^- *allele on intramuscular fat content and fatty acid composition*

No effect of *RN* genotype on the total IMF content is generally reported (Lundström *et al.*, 1998b; Lebret *et al.*, 1999a) and to my knowledge few studies have been concerned with the effect of this mutation on the quality of IMF, *i.e.* its fatty acid composition. Using the same animal material as us in Study III, Högberg

et al. (2001a) found differences in fatty acid composition between the *RN* genotypes in the polar lipid fraction. Both the polar n-3 and n-6 PUFAs were affected, and the effect differed between castrates and gilts. Based on a hypothesis by Clarke (2000) on the involvement of PUFA n-3 fatty acids in the maintenance of energy and glucose metabolism, Högberg *et al.* (2001a) presented two hypotheses: (1) the difference in membrane fatty acid composition affects the glycogen metabolism in pigs and (2) the *RN* genotype affects the incorporation of fatty acids in membrane lipids.

Another theory about how *RN* genotype affects the intramuscular fatty acid composition could be attributable to the muscle fibre composition. According to Leseigneur-Meynier & Gandemer (1991), total phospholipids and phospholipid PUFAs are influenced by muscle fibre type. Based on enzyme activity, the LD was shown to be more oxidative in carriers of the RN^- allele compared to non- carriers (Lebret *et al.*, 1999a). Moreover, muscle fibre characteristics imply more oxidative muscles with increased type IIA fibres in carriers compared to non-carriers (Lundström *et al.*, 1998a; Lebret *et al.*, 1999a). Thus, the small alterations in fatty acid composition between the *RN* genotypes may be due to this change in the relative proportions of different muscle fibre types.

The effect of the RN^- *allele on the precursors and formation of HCAs*

Model systems as well as experiments with meat have revealed that the concentrations and relative amount of precursors influence which and how much of the heterocyclic amines are formed (for a review see Skog *et al.,* 1998). It is well known that glycolytic muscles of carriers of the RN^- allele may contain up to 70% more glycogen than non-carriers of the same or other breeds (Estrade *et al.*, 1993). A dominant negative mutation inhibiting AMP (adenosine monophosphate) activation and glycogen degradation, or a gain-of-function mutation leading to an increased glucose transport and/or glycogen synthesis have been put forward as possible explanations for the hyperaccumulation of glycogen in RN^- carriers (Milan *et al*., 2000). The different levels of monosaccharides derived from this glycogen pool were in a preliminary study, based on one animal of each *RN* genotype, shown to affect both the level of HCAs and the surface browning of cooked meat of different *RN* genotypes. A browner surface but less HCAs formation was found in cooked meat of carriers compared to non-carriers of the RN^- allele (Skog, 2002). This agrees well with findings in model studies, showing an optimal formation of HCAs with a relationship of mono- or disaccharides at about half the molar amount of creatine and free amino acids. When the concentrations of sugar increase above half the molar amount of creatine and free amino acids, the formation of mutagens decrease (Skog & Jägerstad, 1990, 1991; Skog *et al.*, 1992).

Surface browning is sometimes used as an indicator of HCA content, assuming that the degree of crust colour correlates positively with the HCA level. In pig meat from populations with the RN^- allele, this assumption may lead to false values when estimating the intake of HCAs. Moreover, it is common that consumers use the surface browning as an indicator of doneness when frying meat (Warren *et al.*, 1996; Lien *et al.*, 2002). Cooking temperature and time influence the level of

HCAs formed, and because meat from carriers of the RN^- allele is more readily browned compared to that of non-carriers, shorter cooking times may lead to lower HCA levels in the *RN* meat.

The effects of production system on pig meat quality

Organic pork production

Social concerns about animal welfare, environmental impact, and sometimes also product quality in conventional systems are the main reasons for developing alternative, more acceptable, types of production methods for pig meat. However, only small quantities of this type of meat are produced today and it is a challenge to develop the production, distribution and market according to political goals. Two recent studies illustrate the complexity of these issues. One study show that the perceived quality advantages of organic pork among consumers is a cognitive phenomenon (Scholderer *et al.*, 2004). The other show that the behaviour of Swedish consumers purchasing pork is only slightly affected by a negative image of industrial meat production methods (Ngapo *et al.*, 2004).

There are variations in the definition and standards of organic production among European countries (Barton-Gade, 2002) and in the scientific literature the same problems occur. Intensive or conventional production systems are compared to organic, outdoor, extensive, ecological, sustainable, alternative or free-range systems, all with different implications regarding feeding, exercise and overall management. This sometimes makes comparisons of the effects of different systems difficult; consequently emphasis must be put on precise description of the production conditions of the specific trial.

The most well-defined alternative system for production of pig meat in Sweden today is the KRAV (The Certification Organization for Organic Production) certified production of organic meat. KRAV is a member organization of the International Federation of Organic Agriculture Movement (IFOAM) and elaborates standards and monitoring programmes for organic food producers. The KRAV standards follow the European Council Regulation (European Council Regulation no 2092/91 & no 1804/99) of organic farming but may be stricter. For organic pig meat production this implies a range of commitments. To produce organic meat, feed must be grown according to KRAV regulations. However, when necessary, to cover the nutritional needs of animals, 15% of the annual feed intake can be conventionally produced. Most of the feed should ideally be produced on the farm where the animals are raised and pigs should have free access to roughage. Synthetic amino acids are not allowed in KRAV-certified pig feed. Moreover, pigs raised according to KRAV standards should be able to exercise their natural behaviour, such as rooting and searching for food, and the animals should have access to mud or a water bath during the warm season (KRAV, 2004). There are further KRAV regulations on how the pigs should be kept and treated; for example, concerning integrated production (sow and suckling pigs in the same production unit) and medication. Provided an adequate nutritional status, factors of potential importance for meat quality in this system would be increased spontaneous activity, exposure to climatic conditions, a changed diet including

access to green feed, and an enriched environment with many stimuli. If the production system results in slower growth rate and in older pigs sent to slaughter, the age at slaughter may further affect *e.g.* meat colour (pigment content; Mayoral *et al.*, 1999) and texture (the properties of collagen; Avery *et al.*, 1996).

Factors of importance for meat quality

Increased spontaneous activity

Compared to organic pig meat production in many other European countries, the Swedish system provides large outdoor areas with ample opportunities for spontaneous physical activity. The willingness to exploit these opportunities varies between individual pigs but all of the animals move more than conventionally penned pigs which are almost totally physically inactive (Essén-Gustavsson & Jensen-Waern, 1993). The effect of physical training (*e.g.* regular exercise training on a treadmill) has been investigated in several species. However, data obtained from these types of studies may be unsuitable for prediction of the effects of spontaneous activity like that pigs get in alternative production systems (Petersen *et al.*, 1998b).

Regular spontaneous activity may impose endurance type training on muscles, leading to an improved capacity of the muscle for aerobic rather than anaerobic ATP generation (Petersen *et al.*, 1998a). These adaptations could theoretically result in a relative increase in the proportion of more endurant (red oxidative) fibres, better capillarisation of the muscle, an increased activity of enzymes involved in aerobic energy metabolism as well as changes of the quantitative and/or qualitative properties of connective tissue. Physical activity influences primarily those muscles directly involved in locomotion; in muscles of the hind limb (*e.g.* BF), spontaneous activity has been shown to increase the ratio of FTa- to FTb-firbres (Petersen *et al.*, 1998a). Moreover, unrestricted activity patterns may impose strength- and to some extent endurance-type training on back muscles (*e.g* LD; Petersen *et al.*, 1998a). Essén-Gustavsson & Jensen-Waern (1993) found no effect of treatment on fibre type composition but concluded that outdoor reared pigs have a higher oxidative capacity in LD. Further, compared to pigs kept indoors, outdoor reared animals had lower blood lactate and potassium levels at slaughter, indicating a better tolerance to pre-slaughter stress. However, the effects of increased space allowance on muscle metabolic traits are not consistent (Petersen *et al.*, 1997b; Gentry *et al.*, 2002b) and presumably depend to a large extent on the degree of activity, muscle and sex of the animals.

It appears that prolonged and repeated muscular exercise during growth may increase muscle glycogen content in pigs. Essén-Gustavsson *et al.* (1988) found that moderate treadmill exercise during growth may increase the muscle glycogen levels pre slaughter , and some studies suggest higher energy levels at slaughter in LD of outdoor raised pigs (Barton-Gade & Blaabjerg, 1989; Enfält *et al.*, 1997b). Nonetheless, other studies found no (Essén-Gustavsson & Jensen-Waern, 1993) or the opposite (Petersen *et al.*, 1997b) effect on glycogen content at slaughter in LD and BF respectively, when comparing spontaneously exercised pigs to controls. Metabolic adaptations to spontaneous activity, in combination with better tolerance for physical and mental stress (Warriss *et al.*, 1983; Barton-Gade & Blaabjerg,

1989; Essén-Gustavsson & Jensen-Waern, 1993; Sather *et al.*, 1997) may lead to a better capacity to metabolise substrates other than glycogen during transport to the slaughterhouse and in the lairage, providing more glycogen for *post mortem* glycolysis (Barton-Gade & Blaabjerg, 1989; Enfält *et al.*, 1997b) in alternatively produced pigs compared to conventional pigs. Petersen et al. (1997b) found that the amount of glycogen catabolized prior to slaughter and during the initial *post mortem* phase was lower in pigs housed so that spontaneous activity was obtained compared to confined or exercise-trained pigs. Lambooij *et al.* (2004) showed that meat from free-range animals had higher glycogen and glucose stores 1 hour *post mortem* compared to conventionally raised pigs. Variations in muscle metabolic state and glycogen stores at slaughter caused by regular spontaneous activity may affect the rate and extent of pH decrease *post mortem* and thereby important meat quality traits, such as WHC, and colour, as discussed earlier. However, different experiments have obtained various results regarding the effect of regular spontaneous activity on pH-decline and pH_u (Petersen *et al.*, 1997b).

Extensive production systems with increased possibilities for exercise for the pigs could theoretically lead to either darker/more red meat through increased muscle pigmentation as a result of muscle adaptations (Gentry *et al.*, 2002c; Millet *et al.*, in press) or lighter meat through pH effects on sarcoplasmic and myofibrillar protein denaturation and the subsequent light scattering of the meat (Barton-Gade & Blaabjerg, 1989; Enfält *et al.*, 1997b). In many cases however, none of the effects are pronounced or they might even counterbalance each other.

Exposure to climatic conditions

From a producer (and pig) point of view, temperature may be an important limiting factor for outdoor production (Lebret *et al.*, 2002). The average temperature in southern Sweden ranges from ±0° to -4°C in January and 16 to 18°C in July. Environmental temperature per se, as well as variations in temperature during daytime and from day to day, has been shown to influence carcass, muscle and adipose tissue traits (Lefaucheur *et al.*, 1991; Lebret *et al.*, 1999b; Lebret *et al.*, 2002). For pigs reared outdoor during winter in France, a tendency towards higher oxidative capacity and an increase of monounsaturated fatty acids and softness of the back fat was found, compared to controls (Lebret *et al.*, 2002). Sather et al. (1997) found limited seasonal effects on the overall quality of LD, but suggested metabolic adaptations to low and variable temperatures during the Canadian winter may lead to increased muscle glycogen levels and incidence of PSE.

A changed feeding strategy including access to green feed

Organic feed differs from conventional feed mixtures in several aspects. An important difference is a high overall protein level to compensate for restrictions in adding synthetic amino acids. Further, in organic production systems all animals must have access to roughage, primarily for better welfare and health of the animals. In season, organically produced pigs in Sweden have access to pasture. Palatable roughage in the form of *e.g.* silage or pasture can cover some of the energy requirements of pigs (Andersen 2000; Danielsen *et al.*, 2000; Muriel *et al.*, 2002). This may potentially effect the technological and sensory quality of the pork. Texture and oxidative stability, manifested in the development of off-

flavours after frozen storage, may be impaired in meat of pigs fed silage (Danielsen *et al.*, 2000; Jonsäll *et al.*, 2000). On the other hand, extensive production systems may result in much appreciated products, such as the Iberian ham whose particular and intense flavour is a consequence of pigs consuming grass and acorn (García *et al.*, 1996).

An enriched environment

Transport and handling prior to slaughter imposes both physical and emotional stress on pigs. Environmental enrichment and better stimulation for pigs during the husbandry phase may lead to differences in behaviour that might influence the physiological and behavioural responses of pigs before slaughter (Klont *et al.*, 2001). Long-term exposure to external stimuli and muscle adaptations to spontaneous activity may result in calmer behaviour in the abattoir (Warriss, *et al.*, 1983; Barton-Gade & Blaabjerg, 1989) and greater resistance to stress-induced glycogen depletion. However, it is also conceivable that alternatively raised animals are less used to handling and confinement and therefore become more stressed during transportation and lairage (Gandemer *et al.*, 1990; Benfalk, *personal communication*). Long-term stress, including poor handling at the farm, during loading and transportation is often associated with DFD meat. However, it may also increase the susceptibility to pre-slaughter stress and lead to lower glycogen stores early post mortem and lower pH_u, as well as higher incidence of PSE compared with correctly handled animals (D´Souza *et al.*, 1998). Short-term stress immediately prior to stunning results in lower pH values and higher temperature early post mortem which in turn often leads to inferior WHC (Henckel *et al.*, 2000; Rosenvold *et al.*, 2001a; Stoier *et al.*, 2001).

Objectives

Despite an increased interest in alternative production systems for pig meat there are, to date, few and often inconsistent reports on how these changes in production systems affect meat quality. With a holistic approach, the overall objectives of the thesis were to study the effects of production factors in combination with RN genotype and sex, and to evaluate their impact on technological and nutritional meat quality.

Specific objectives for the present work were:

- To increase the understanding of how different rearing systems, RN genotype and sex affect overall pig meat quality.
- To monitor the natural variation of precursors of HCAs in a defined (rearing regime, RN genotype and sex) animal material.
- To investigate the formation and exposure of HCAs in relation to pig meat composition as well as consumer cooking practices and preferences.

Materials and methods

Animal materials

The thesis is based on three separate animal materials (Figure 2). The design of the experiments was elaborated in collaboration with other departments at the Swedish University of Agricultural Sciences and aimed to study different aspects of alternative production systems on, not only, production, but also meat quality parameters. The meat quality assessments were performed on subsamples of larger materials, selected with different techniques depending on the objective of the specific study. In all studies the animals were Hampshire cross-breeds ([Swedish Landrace x Swedish Yorkshire] x Hampshire), a three-way combination commonly used for slaughter pig production in Sweden.

Study I was based on a split litter design where half the litters were raised indoors in large boxes and the other half were raised outdoors with access to green feed from July until slaughter in the autumn. Animals originated from a research herd at Lövsta Research Station, SLU. All pigs were fed a conventional slaughter-pig diet.

In Study II all pigs were supplied from commercial herds and kept indoors in conventional pens. Half the animals were fed a conventional diet while for time the other half were fed an organic diet. The experiment was performed at Bjertorp Research Station, SLU.

In Study III the entire organic production system was compared with a conventional system. The animals in the organic system were progenies of organically produced sows and fed and kept according to the rules of KRAV, a Swedish organisation certifying organic food production. The conventionally produced pigs were supplied from commercial herds, fed a conventional diet and kept indoors in conventional pens. The experiment was performed at Bjertorp Research Station, SLU.

In Study I-III, all animals were sent to slaughter at two different commercial abattoirs at an average live weight of 102-108 kg. The animals within each study were slaughtered at the same abattoir and kept in lairage for approximately 2 hours before being stunned using CO_2, following the routines at the abattoir.

In Study IV production parameters were unknown. Meat originating from commercials herds was collected at the local abattoir in Uppsala, Sweden.

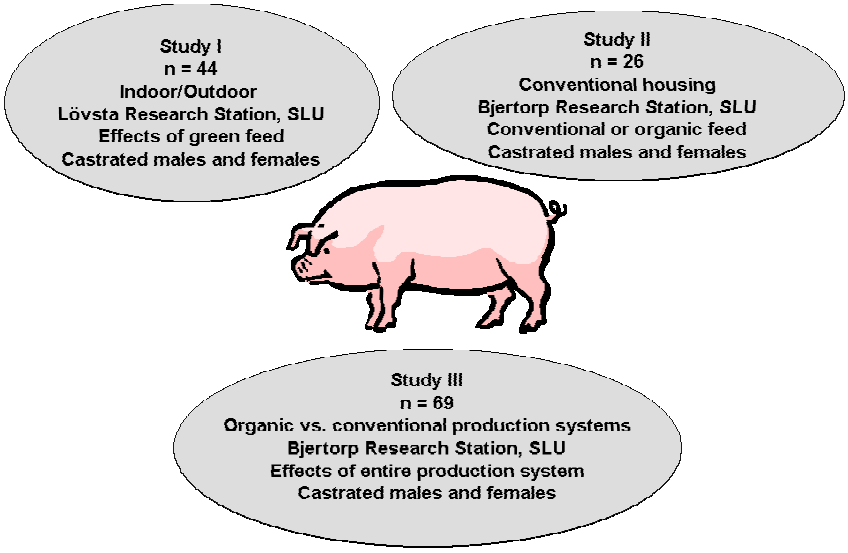

Figure 2. The three animal materials on which the thesis is based.

Methods

Carcass assessments

In Study III all carcasses were assessed for lean meat percentage by partial dissection. On ham and back, all subcutaneous fat was carefully trimmed off (Andersson, 1980). Calculation of lean meat percentage was performed with the equation:

Lean meat percentage = -45.91 + 0.634 * % of ham + 0.713 * % of back + 0.588 * (% of lean + bone in ham) + 0.298 * (% of lean + bone in back);

where the percentages of ham and back, respectively, are expressed as percentage of the carcass side without head. The equation is based on dissections performed in carcasses of the same type performed in experiments conducted at the same time as this study.

Technological meat quality

For all studies, except Study I, analyses of the technological and chemical meat quality were performed in *M. longissimus dorsi* (LD). In Study I, the analyses were performed in the ham muscle *M. biceps femoris* (BF).

pH

Ultimate pH (pH_u) was registered at the abattoir using a portable pH meter (Knick Portamess digital pH meter 651-2, Berlin, Germany) equipped with a combination gel electrode (SE 104, Knick, Berlin, Germany) as well as in muscle samples

homogenised in 0.15 M KCl using a Radiometer combination electrode (pHC2451) and pH meter PHM92 (Radiometer Analytical A/S, Copenhagen, Denmark). In most cases results from one of the two methods were presented.

Water-holding capacity

Water-holding capacity was measured as filter paper wetness (Kauffman *et al.*, 1986) and as drip loss in slices stored at +4ºC, either horizontally for 4 days (Study I, II and III) or suspended in an inflated plastic bag for 48 h (Study IV, Barton-Gade *et al*., 1994; Honikel, 1998).

Colour

In Study III, meat colour of samples was measured about 5 h after cutting using a Minolta Chroma Meter CR-300 (Osaka, Japan) with a D65 light source calibrated against a white tile. The tristimulus parameters L*, a* and b*, representing lightness, redness and yellowness were recorded at the middle of each sample. The Minolta Chroma Meter was also used for colour recordings of the crust (II & IV) and interior (IV) of cooked meat. These measurements were complemented with subjective scorings of crust colour.

Shear force measurements to assess tenderness

Samples intended for Warner-Bratzler shear force measurements in Study I and III were aged for four days and stored at -20ºC until analysis.

In Study I shear force determinations were made using the Warner-Bratzler apparatus as described by (Lundström *et al.*, 1996). The muscle samples were thawed at +4ºC overnight and cooked without cover in boiling water until an internal temperature of +72ºC was reached. Three 13-mm cores cut along the fibre axis were taken from each sample, and three recordings were made on each core. The mean value of the nine recordings was used in the calculations of shear force. In Study III the Warner-Bratzler shear force was measured using a Stable Micro Systems Texture Analyser TA-HDi (Godalming, U.K.) equipped with a Warner-Bratzler blade with a rectangular hole, 11 mm wide and 15 mm high, with a blade thickness of 1.2 mm (Honikel, 1998). Before shear force assessment, the samples (weighing about 300 grams) were thawed at +4ºC overnight (to approximately -2ºC), cut into a standardised shape (6 x 4 x 8 cm), weighed and vacuum packed once again. The samples were then kept for 90 min in a water bath at 70ºC, a time at which previous experiments had been shown to result in an internal temperature of 69-70ºC for that sample size. The samples were cooled in the vacuum bag under running water, the surface rinsed and wiped with paper and the samples were reweighed to calculate the cooking loss. For each cooked sample, on average 9 rectangular strips with a cross-section of 100 mm^2 (10x10mm) and with the fibre direction parallel to a long dimension of 30 mm were measured at a test speed of 50 mm/min (Honikel, 1998). The maximum shear force (N) was recorded.

Frying experiments to determine HCA occurrence

In Study II the meat was thawed until semi-soft and minced twice through a powered mincing machine (Electrolux Assistent, Sweden), the final plate with

holes 4 mm in diameter. The minced meat was formed into patties weighing 70 g each with a diameter of 8.5 cm and a height of 1.5 cm. The patties were fried for 3 min per side on a temperature-controlled frying device at 200°C. Patties (2 or 3) originating from the same animal were fried simultaneously in 10 g of commercial Swedish frying fat for each patty. The final internal temperature of the patties was measured using thermocouples. The temperature of the surface of the frying device was recorded once before the patties were applied and three times during frying using a temperature probe. After the patties had cooled, the patties were reweighed and the cooking loss calculated.

In Study IV, the fresh meat was fried as intact 2-cm-thick chops. Before frying, the edges of the chops were slightly cut to avoid an uneven curving during frying and the meat was tempered until the mean internal temperature was 18.7 ± 1ºC. Three RN^{-}/rn^{+} chops at a time, followed by three rn^{+}/rn^{+} chops were fried at low, medium and high temperatures in a cast iron pan on an ordinary household stove with a ceramic top (Figure 3). The temperature in the fat layer on the bottom of the pan was allowed to reach 160 (low), 180 (medium) or 200ºC (high temperature) before the chops were put into the pan. The chops were fried in 10 g of a commercial vegetable frying fat for 3 min per side. The temperature was monitored in the middle of the pan four times during the course of frying after 1, 2, 4 and 5 min using a probe coupled to a recorder (Pentronic TC1100, Line Seiki, Gunnebo, Sweden). After frying, the internal temperature of the chops was recorded using a penetration needle probe made of T/C K kapton insulated wire (Pentronic, Gunnebo, Sweden). The chops were left to cool to room temperature, weighed and photographed. Cooking loss was calculated as the percentage change in chop weight before and after frying.

Chemical analysis

Solvents and chemicals were of analytical or HPLC grade and purchased from well-recognised companies. Standards were obtained from Toronto Research Chemicals (Toronto, Canada), Larodan Fine Chemicals AB (Malmö, Sweden), Nu-

Figure 3. Cooking of chops, in Study IV.

Check-Prep INC. (Elysian, USA), Merck and Sigma. When appropriate, water was passed through Milli-Q water purification systems (Millipore, Bedford, MA). All determinations were made in duplicate or triplicate.

In the analyses of dry matter, ash, and crude protein, a standard meat reference material (SMRD 2000, LGC Promchem AB, Borås, Sweden) was used to control the method applications and the continuous accuracy and precision of the determinations. For the analysis of residual glycogen and creatine in-house standards were run for continuous quality assurance.

Dry matter and ash contents of the meat were determined gravimetrically after drying at 105ºC for 16-18 h and heat treatment in an ash oven at 550ºC for 4 h (NMKL, 1991).

Crude protein was analysed by the Kjeldahl method using the Kjeltec apparatus (Foss-Tecator AB, Höganäs, Sweden) applying the conversion factor 6.25. The contents of free amino acids (FAA) and dipeptides in the meat were analysed at the Department of Biochemistry, Uppsala University, Uppsala, Sweden using both a Biotronik LC-5001 analyzer and an Alpha-Plus analyzer.

In the Studies II and III the intramuscular fat (IMF) content of the meat was analysed after hydrolysis, using petroleum ether for extraction (Soxtec System H+ equipment, Foss-Tecator AB, Höganäs, Sweden). In Study I, IMF was determined after cold extraction using hexan:isopropanol (Hara & Radin, 1978) whereupon lipid oxidation products, fatty acid composition and tocopherol contents were analysed in the lipid fraction as follows:

The main initial products of auto-oxidation were measured in the form of hydroperoxides using a standard method (IDF, 1991). Approximately 0.2 g of lipid was dissolved in a mixture of chloroform and methanol (70:30 v/v). An ammonium thiocyanate solution as well as an iron (II) chloride solution was added and the reaction was left to proceed for five minutes. The absorbance of the red iron (III) complex formed was determined spectrophotometrically at 500 nm. The peroxide value was defined as the number of milli-equivalents of oxygen per kilogram lipid from a standard curve of known concentrations of iron (III) ions as described in the method.

The content of malondialdehyde (MDA) in muscle samples was assessed using a modification of the method described by (Draper *et al.*, 1993, method 4). In this method the TBA (thiobarbituric acid) reactive substances were extracted with 2 ml of 1-butanol. The high performance liquid chromatographic (HPLC) analysis was performed by applying 0.1 ml of the extract to a 5-ml LiChrospher 60 RP-18 column, and the effluent was monitored at 532 nm with a Merck-Hitachi L-4250 UV-VIS Detector (Hitachi Ltd., Tokyo, Japan). As mobile phase two buffers were used: Buffer A - 10% acetonitrile, 0.6 % tetrahydrofuran in 5 mM phosphate buffer, pH 7.0; Buffer B - 15% acetonitrile, 0.6 % tetrahydrofuran in 5 mM phosphate buffer, pH 7.0, with the following gradient: 0 min - A, 15%; 7.5 min - A, 100%; 12.5 min - A, 100%; 12.6 min - A, 15%, 17 min - A, 15%. The flow rate was 1 ml/min. Two peaks of TBA reactive substances were detected, one eluting at 6.2 min and another at 8.5 min and they were both used to calculate the MDA concentration in the samples utilising external standards.

In order to analyse fatty acid composition, lipids were transformed to fatty acid methyl esters (FAME) using a method described by Dutta *et al.* (1994). The composition of FAME was analysed using GLC operating conditions as described by Pickova *et al.* (1997). FAME were identified by comparing retention times with those of the external standard mixtures, FO 7 (Larodan Fine Chemicals AB, Malmö, Sweden) and 68 A (Nu-Check-Prep INC., Elysian, USA).

The tocopherol content was determined by HPLC according to the method described by (Dutta *et al.*, 1994). For detection, a fluorimeter detector was used at an excitation wavelength of 295 nm and emission at 320 nm. Quantification was done using external standard methods.

Determination of *RN* phenotype/genotype and residual glycogen content

The animal material consisted of three-way crossbreeds and the RN^- allele was transferred only from the Hampshire breed. Therefore the studied pigs were either carriers (RN^-/rn^+) or non-carriers (rn^+/rn^+) of the RN^- allele. The prediction of *RN* phenotype was based on two different methods. 1) The concentration of residual glycogen (glycogen, glucose and glucose-6-phoshate (G-6-P)) determined enzymatically in homogenized muscle tissue (Dalrymple & Hamm, 1973). In short, muscle samples were homogenised in cold perchloric acid, whereupon tissue glycogen was hydrolysed to glucose using amyloglucosidase. The concentrations of glucose and G-6-P were determined with a combined enzymatic assay and a spectrophotometric method (SIGMA, 1995). 2) The concentration of glucose + G-6-P in meat juice was also determined using the SIGMA assay (Lundström & Enfält, 1997). Both methods gave rise to bimodal distributions and the valley point was used as an empirical threshold between non-carriers and carriers of the RN^-allele (Fernandez *et al.*, 1992; Lundström *et al.*, 1996). For both methods, animals with a concentration of glucose and G-6-P ≥35 μmol/g meat or μmol/ml meat juice, respectively, were classified as carriers of the RN^- allele.

In Study II, an additional, rapid method for glucose measurement in meat juice was used for screening *RN* phenotype already in the abattoir (Lundström & Enfält, 1997; Nilzén *et al.*, 1999). At cutting, a 5-g sample was taken from the anterior section surface of LD, placed in a test tube and centrifuged for 5-10 min (Simplex, Hereaus Christ GmbH, Osterode, Harz, Tyskland). In approximately 80 % of the cases this rendered enough meat juice to measure the glucose concentration using a Glukometer Elite™ device, more commonly used for blood glucose measurements. An experimental value for division between carriers and non-carriers of the RN^- allele was set at a glucose concentration of 8 μmol/ml meat juice.

In Study II, III and IV all, or some samples with intermittent residual glycogen values were genotyped using a relatively new DNA test based on a PCR method developed by (Milan *et al.*, 2000).

Creatine and creatinine

Creatine and creatinine were quantified enzymatically by a Boehringer-Mannheim end-point method applied for food samples (Wahlefeld *et al.*, 1974). Approximately 1.4 g of the freshly minced meat was homogenised (Ultra-Turrax T25, IKA Labortechnik, Staufen, Germany) with 2 ml of hot (57-63 °) re-distilled water. The extraction was repeated twice, and the pooled homogenate was centrifuged. The supernatants were diluted with re-distilled water up to 10 ml and 10 ml 1 M perchloric acid was added. After centrifugation, 5 ml of the supernatant was neutralised using KOH. The solution was cooled at + 4°C and filtered (filter paper No. 0B, Munktell Filter AB, Grycksbo, Sweden). Then, 100 µl of the clear filtrate was taken to enzymatic analysis following a Boehringer-Mannheim end-point method (Wahlefeld *et al.*, 1974). Absorbance was measured at 340 nm (UV-2101PC, Shimadzu Scientific Instruments, Inc. or Jasco V-530 UV/VIS spectrophotometer). The mean concentration of creatinine was low, < 1 µmol/g meat, and therefore only the level of creatine is reported.

Heterocyclic amines

During the five-year time span of producing this thesis, the methods for determining the concentration of HCAs in cooked meat was developed from solid-phase extraction and reversed phase HPLC separation in Study II to blue chitin extraction and LC/MS detection in Study VI. The low amounts of HCAs present in cooked pig meat (ng/g), the complex food matrix, and the need for several isolation steps make accurate quantification challenging.

In both studies, the crust, *i.e.* the brown surface of the fried patties/chops, was removed using a scalpel, stored at -20°C and thereafter used for extraction of HCAs. The separation of crust and crumb was done as a first concentration step because HCAs are mainly found in outer layer of cooked foods (Bjeldanes *et al.*, 1983; Skog *et al.*, 1995).

In Study II, HCAs were extracted and purified according to the solid-phase extraction method of Gross *et al.* (1992) with some modifications (Fay *et al.*, 1997). Briefly, the freeze-dried crust was homogenised in NaOH, mixed with diatomaceous earth and packed into a cartridge. The HCAs were extracted using ethyl acetate. HCAs were separated using reverse-phase HPLC and the column (ODS 80™ TosoHaas, 250 x 4.6 mm i.d., 5µm) was eluted with acetonitrile and 0.01 M triethyl amine (pH adjusted to 3.6 with acetic acid). Chromatograms and spectra were obtained using a photodiode array UV detector (Varian 9065, Polychrome). HCAs were identified and quantified using retention times and the spectra from reference samples of known concentrations were run under the same conditions. The following HCAs were quantified: MeIQx, PhIP, 2-amino-3,4,8-trimethylimidazo[4,5-f]quinoxaline (4,8-DiMeIQx), 9H-pyrido[3,4-b]indole (norharman) and 1-methyl-9H-pyrido[3,4-b]indole (harman), all obtained from Toronto Research Chemicals (Toronto, Canada). Almost 70% of the samples were run as triplicates; of the triplicates one sample was spiked with a known amount of reference compounds to correct for incomplete extraction recovery. The rest of the samples were analysed as in duplicates.

In Study IV, the concentration of HCAs in the crust was analysed according to Bång *et al.* (2002). In short, the freeze-dried crust was homogenised in NaOH, mixed with diatomaceous earth and packed into a cartridge. The HCAs were extracted with ethyl acetate. The eluate was evaporated to dryness, dissolved in NaOH and applied on a Blue Chitin column that was then washed with water. The HCAs were eluted with MeOH/ NH4OH (9:1 v/v). The eluate was evaporated to dryness, dissolved in MeOH, and then analysed using LC/MS. The HCAs were separated by reversed-phase LC using a Zorbax SB-C8 StableBond Analytical HPLC column (150 x 4.6 mm i.d., 5 µm) and a Zorbax SB-C8 Analytical guard column (12.5 x 4.6 mm i.d., 5 µm) from Agilent Technologies (Palo Alto, CA, USA). The column was eluted with acetonitrile and water (pH adjusted to 3.6 with acetic acid). The effluent was connected to an LCQDECA ion-trap mass spectrometer, with Xcalibur software (Thermo Finnigan, San José, CA, USA), using the electrospray as ion source. The recoveries ranged between 30-80% depending of type of HCA and recovery corrections were made before presenting the results.

Data analysis

Data were analysed using the ANOVA GLM procedure in the software programme Minitab (Minitab® Statistical Software, Release 12). Principal component analysis (PCA) was carried out using the software programme Unscrambler version 7.7 (Camo, Oslo, Norway).

Summary of presented investigations

Study I

The influence of free-range rearing, RN genotype and sex on different pig meat quality traits, including intramuscular fatty acid composition and levels of lipid oxidation products, were studied. A total of 60 Hampshire cross-bred pigs were reared outdoors for two months with access to green feed, while 60 others were kept indoors, in a 120-m^2-large pen, throughout the rearing period. From these 120 animals a subsample of 44 animals were chosen for meat quality analysis. Of the three factors studied, the RN genotype had the largest influence on basic technological meat quality traits, whereas the rearing conditions and sex had limited effects. However, outdoor rearing resulted in higher levels of polyunsaturated fatty acids in the intramuscular fat ($p = 0.026$) and in an increased level of vitamin E ($p = 0.030$) compared with the pigs that had been reared indoors. The sex and *RN* genotype of the animals also had an effect on the fatty acid profile: females had higher levels of unsaturated fatty acids ($p = 0.003$) as well as lower levels of saturated fatty acids ($p = 0.011$) than castrated males. Carriers of the RN^- allele expressed a higher sum of omega-3 fatty acids ($p = 0.047$) and C22:5 ($p = 0.012$) than did the non-carriers. In a storage study where meat from free-range and indoor reared pigs was stored for 3 months at -20 °C, it was shown that the lipid oxidation product malondialdehyde was formed at increased levels in animals that had a higher lean meat percentage than others, *i.e.* females that were carriers of the RN^- allele and that were reared outdoors.

Study II

Pig meat shows natural variations in the concentrations of precursors of heterocyclic amines, which may affect their formation in cooked pig meat. To study this, 26 pigs with an inherent genetic variation (carriers and non-carriers of the RN^-allele) were subjected to different diets (a conventional diet compared with a diet composed according to organic standards). In addition, the effect of sex (castrated males or females) was considered when assessing technological meat quality parameters. Concentrations of precursors of heterocyclic amines (HCAs), *i.e.* creatine, residual glycogen, dipeptides, and free amino acids, were analysed in the raw meat, and the level of some HCAs (4,8-DiMeIQx, MeIQx, PhIP, harman and norharman) was then determined in fried meat patties prepared from these pigs. The *RN* genotype had the largest impact on technological meat quality parameters and the level of precursors of HCAs, especially the level of residual glycogen where carriers of the RN^- allele showed four times as high levels as non-carriers (17.2 ± 2.4 compared with 75.3 ± 2.6 μmol/g meat, least-squares means ± SE). The increased level of residual glycogen resulted in about 50 % lower amounts of total mutagenic HCAs in cooked meat compared with cooked meat from normal pigs. Fried meat from carriers of the RN^- allele obtained darker crust colour than meat from non-carriers. Diet and sex did not significantly affect the chemical composition of the meat or the formation of HCAs.

Study III

This study was set up to compare organic pig meat production with conventional production on a system level regarding carcass- and meat quality traits. The study consisted of 80 cross-bred female and castrated male pigs ([Swedish Landrace x Swedish Yorkshire] x Hampshire) of which 40 were raised under organic conditions and the other 40 were raised in a conventional production system. The organic pigs were raised outdoors in one large group following the regulations of the organic standards. The conventionally raised animals were kept indoors in groups of eight, and they were given a conventional diet. When the carcass traits and meat quality characteristics were investigated it was found that meat of organically raised non-carriers of the RN^- allele was of poorer quality (higher drip loss and increased shear force values) compared to the meat of other animals. The *RN* genotype had a relatively small effect on carcass and technological traits in this study whereas sex of the animals affected carcass traits.

Study IV

A questionnaire complemented with colour photographs was used to obtain information on dietary practices and preferences regarding home-prepared pork chops in a small (n=151) sample of Swedish consumers. The results from the questionnaire were combined with analytical results from meat of different *RN* genotypes, and showed that fried chops from a pig that was carrying the RN^- allele (high glycogen content) had a darker crust and contained lower levels of mutagenic heterocyclic amines (HCAs) than chops from a non-carrier (low glycogen content). In this study population, the intake of fried pork chops only contributed slightly to the total HCA exposure; the total *monthly* intake of mutagenic HCAs was on average 256 ng, ranging from 0 to 1982 ng/month. However, using lower frying temperatures and meat from pigs carrying the RN^- allele can further reduce the intake. From the photographs, most of the respondents chose fried chops from the non-carrier, which would result in an average contribution to the monthly HCA intake of 359 ± 402 ng (mean ± SD) compared to 35 ± 60 ng/month for consumers who preferred the RN^-/rn^+ chops. More than 20 times the amount of mutagenic HCAs was formed when frying chops of the non-carrier of the RN^- allele at an initial pan temperature of 200ºC instead of 160ºC; 4.13 compared to 0.18 ng/g cooked meat.

Results and general discussion

The results are presented and discussed in relation to the objectives of the thesis.

The effects of different production systems on overall pig meat quality (Studies I and III)

Production performance and carcass composition

The effect of production system on production results and carcass composition were not in focus in the present study and only briefly accounted for in study III. In this study organically produced pigs had a higher daily weight gain than the conventionally produced animals, 829 compared to 764 g/day ($p=0.003$). Most studies on the daily weight gain of pigs kept outdoor report lower growth intensity for these animals than for conventionally/indoor raised pigs (Enfält *et al.*, 1997b; Sather *et al.*, 1997; Danielsen *et al.*, 2000). However, Bridi et al. (1998) could not record any differences in average daily weight gain for indoor and outdoor finishing and Stern *et al.* (2003) and Gentry *et al.*, (2002c), similarly to us, found a higher growth rate for outdoor pigs compared to indoor pigs in the first part of the growing period. As stated by Gentry *et al.* (2002a) the differing results reported on the effects of outdoor finishing environments on pig growth rates are due to the many factors influencing pig performance, including genetics, physical environment, climatic conditions and management level. The feed conversion ratio for both the organically and conventionally raised pigs in the present study was close to 3.0. The conventionally raised pigs had 2.2 percentage points higher carcass lean meat content than the organically raised animals. This was true both for estimated as well as commercially graded lean meat content and was associated with thinner side fat and more meat in the back of the conventionally produced carcasses compared to those produced organically. The average lean meat percentage of commercially slaughtered organic pigs in Sweden during the year 2002 was 56.7%, somewhat lower that that of conventional pigs, 57.4% (Alarik, 2003). This agrees with van der Wal *et al.* (1993) who found lower lean meat percentage and thicker back fat in "scharrel" (free range) pigs and Bridi *et al.* (1998) and Gentry *et al*. (2002) who also found thicker back fat in outdoor raised pigs. However, it is also common that free-range raising increases carcass lean meat content (Jones, 1993; Enfält *et al.*, 1997b; Sather *et al.*, 1997; Stern *et al.*, 2003) and decreases back fat thickness in pigs (Warriss *et al.*, 1983) reflecting higher energy requirements to maintain body temperature and for exercise.

Technological quality

Most of the technological quality traits measured in Studies I, II and III are presented in Table 2. The results from Study II are based on the 26 animals described in the article, although present results on the effects of organic versus conventional diet were not published before. The technological quality was investigated in the muscles BF (Study I) and LD (Studies II and III). These two muscles are known to differ in their fibre composition and metabolic characteristics (Lundström *et al.*, 1989; Karlsson *et al.*, 1993).

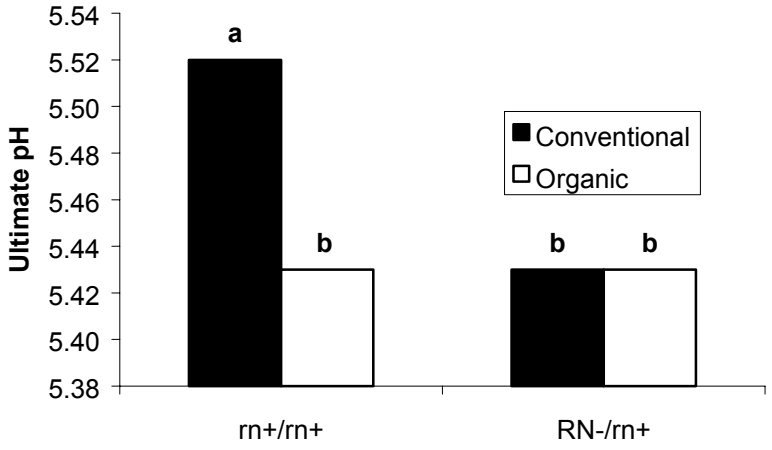

Figure 4. The combined effect of production system and RN genotype on pH_u, bars with different superscripts (a and b) differ significantly, $p < 0.05$.

BF represents a more red oxidative type than the more glycolytic LD, which should be considered when comparing results.

pH

The alternative production forms studied in this thesis did not dramatically affect pH_u (Table 2). However, when the combined effects of production system and *RN* genotype were examined in Study III, organically produced non-carriers had the same pH_u as carriers of the RN^- allele whereas conventionally produced non-carriers had significantly higher pH_u-values (Figure 4). This indicates that production conditions may be more important for technological meat quality in meat with normal or low levels of glycogen than in meat of carriers of the RN^- allele with high glycogen content. This was supported by findings of Andersson *et al.* (2003) where pre-slaughter routines affected technological yield in meat of non-carriers but not carriers of the RN^- allele and was further exemplified by the lack of any pH_u increasing effects of lairage duration and mixing of unfamiliar animals when studying Hampshire cross-bred pigs with high muscle glycogen content (Fernandez *et al.*, 1992). Thus, high initial levels of glycogen may prevent effects of pre-slaughter treatments and stress on meat quality. Muscle glycolytic potential correlates with pH_u better at low muscle glycogen levels at slaughter than at high glycogen levels at slaughter (Lundström *et al.*, 1996; Henckel *et al.*, 2002, Study III). With the possible future removal of the RN^- allele from the genetic pool in Sweden, these data emphasise the need for a holistic approach to pre-slaughter factors influencing meat quality.

Diverging results from different studies make it difficult to draw consistent conclusions about the effects of alternative production systems on pH_u in different muscles, (predominantly measured in LD). Many studies find no effect of outdoor rearing and spontaneous activity on muscle pH_u whereas others report lower pH_u in

meat of outdoor reared animals compared to conventional production (Table 3). No obvious general difference (*e.g.* in space allowance) between the studies reporting pH_u effects and those reporting no pH_u effects was however, noted.

The rate of pH decline, especially important for WHC and measured as pH_{45}, has in some studies been found to be faster in LD of free-range reared pigs (Sather *et al.*, 1997; Millet *et al.*, in press) whereas other studies found no effect (Barton-Gade & Blaabjerg, 1989; Bridi *et al.*, 1998; Gentry *et al.*, 2002a; Gentry *et al.*, 2002c) and one study found a slower pH fall in free-range pigs (Lambooij *et al.*, 2004). The WHC was not recorded in all these studies, but in the studies of Sather *et al.* (1997) and Lambooij *et al.* (2004) a faster pH decline clearly led to increased drip loss.

Concerning more isolated effects of training, Essén-Gustavsson *et al.* (1988) found a lower pH_u in BF in trained pigs compared to untrained pigs and Petersen *et al*. (1997b) observed that pH_{45} in LD was affected both by training (decreased compared to control) and spontaneous activity (increased compared to control). No training effects on muscle pH were seen in studies by Lewis *et al.* (1989) and by Enfalt *et al.* (1993). The latter authors suggested that this absence was partly due to the use of Hampshire crossbreds in the study.

An interesting observation, relevant for Swedish climatic conditions, was made by Lefaucheur *et al.* (1991) who found a faster *post mortem* pH decline, higher glycolytic potential and lower pH_u in pigs reared in cold (12ºC compared to28ºC) environments. In a more recent study, with less extreme temperature differences, 17ºC compared to 24ºC, these effects were not seen (Lebret *et al.*, 2002).

To summarise, organic production systems can be expected to have either no or a lowering effect on ultimate pH and thereby affect meat quality characteristics dependent on pH. More attention should be focused on the combinatory effects of e.g. rearing systems, genotype, sex and preslaughter treatments.

Water holding capacity

In this thesis, the most serious drawback of alternative production systems on technological meat quality was the inferior water-holding capacity in meat from animals with increased opportunities for exercise. In both Study I and III meat of the outdoor reared animals lost more than one percentage point more as drip when compared to conventionally reared counterparts (Table 2). However, in Study III there was a combined effect of production system and *RN* genotype and in analogy to the effect on pH_u, organically produced non-carriers of the RN^- allele had higher drip loss than conventionally produced non-carriers. Some studies have reported a decreased water-holding capacity of meat from extensive production systems (Andersson, *et al.*, 1990; Gandemer *et al.*, 1990; Enfält *et al.*, 1997b; Sather *et al.*, 1997; Claudi-Magnussen, 1999; Gentry *et al.*, 2002a) whereas others reported no effect of outdoor rearing on water holding capacity. (Millet *et al*., in press; Warriss *et al*., 1983; van der Wal *et al.,* 1993; Gentry *et al.,* 2002c). Two recent studies differ somewhat from the mainstream. First, Lambooij *et al.* (2004) found a considerably slower *post mortem* temperature and pH decline in free-range reared pigs compared to meat from conventionally reared pigs. This led to less

Table 3. *An overview of different studies investigating the effect of alternative production systems with increased spontaneous activity on muscle pH_u*

Study	***Muscle***	***No effect on pH_u***	***Lower pH_u***
Warriss *et al.*, 1983	LD	X	
Gandemer *et al.*, 1990	LD	X	
Stecchini *et al.*, 1990	SM	X	
Jones, 1993	LD	X	
van der Wal *et al.*, 1993	LD	X	
Dufey, 1995	LD	X	
Sather *et al.*, 1997	LD	X	
Bridi *et al.*, 1998	LD	X	
Hansen *et al.*, 2001	LD	X	
Gentry *et al.*, 2002a	LD	X	
Gentry *et al.*, 2002b	LD	X	
Lebret *et al.*, 2002,	SM	X	
Stern *et al.*, 2003	BF	X	
Barton-Gade & Blaabjerg, 1989	LD		X
Gandemer *et al.*, 1990	AD		X
Enfält *et al.*, 1997	LD		X
Sather *et al.*, 1997	SM		X
Study I, 2001	BF		X
Stern *et al.*, 2003	LD		X
Millet *et al.*, in press	LD		X
Study III, 2003	LD		X
Lambooij *et al.*, 2004,	LD		X

LD) *M. Longissimus dorsi*
SM) *M. Semimembranosus*
BF) *M. Biceps femoris*
AD) *M. Adductor.*

water exuded from the muscles at portioning but no difference in drip loss. Second, comparing meat of pigs raised under barren and enriched (added straw) housing conditions Klont *et al.* (2001) found an improved water-holding capacity in the meat of the pigs raised in the enriched environment.

In Study III, somewhat unexpectedly, the meat of organically produced pigs lost less water during cooking than that of conventionally produced pigs (Table 2). The higher protein content of the organic meat may explain the better water-holding properties of this meat during cooking.

To summarise, the effects of alternative production systems and exercise on the water-holding properties of meat may vary, probably due to the intensity of the activity to which the animal is subjected. About half of the above-mentioned studies indicate reduced water-holding capacities in alternatively produced meat. The possible muscle adaptations and increased muscle glycogen contents of spontaneously exercised, alternatively produced pigs may be followed by

differences in the regulatory mechanisms of *post mortem* glycolysis resulting in impaired water-holding capacity compared to conventional animals.

Colour

The L*a*b* recordings of the fresh meat in Study III were not affected by production system. Our notion is that Swedish consumers often associate organically produced pig meat with darker /redder meat colour but the evidence for this is ambiguous. There may be several theoretical backgrounds to the perceived darker meat of outdoor reared pigs. First, in some cases, a use of more traditional breeds with higher muscle haem pigment concentration than more selected pig breeds (Estevez *et al.*, 2003) may be the reason. Second, adaptations to spontaneous activity and colder temperatures leading to muscles with increased oxidative capacity and improved capillarization (Essén-Gustavsson & Jensen-Waern, 1993; Petersen *et al.*, 1998a; Lebret *et al.*, 2002) has been put forward. Last, if alternatively produced pigs are slaughtered later than conventional pigs, their age may lead to higher muscle myoglobin content (Mayoral *et al.*, 1999) and a more red meat. Comparing the effects in another species, meat of extensively produced young bulls had relatively more slow contracting fibres, better vascularization, higher oxidative metabolic potential, darker meat (lower L* values) and higher pigmentation than intensively produced meat (Vestergaard *et al.*, 2000). Comparing animals of the same age and breed, a direct effect of outdoor rearing and/or increased spontaneous activity on porcine muscle fibre composition has been reported by Petersen *et al.* (1998a) and Lebret *et al.* (2002) but refuted in other studies (Essén-Gustavsson & Jensen-Waern, 1993; Gentry *et al.*, 2002b). Nonetheless, a darker/redder colour and higher pigmentation of extensively produced pork is sometimes reported (Millet *et al.*, in press; Warriss *et al.*, 1983; Bridi *et al.*, 1998; Claudi-Magnussen, 1999; Gentry *et al.*, 2002c).

When paler meat and/or a higher PSE incidence of alternatively produced pigs has been reported (Barton-Gade & Blaabjerg, 1989; Jones, 1993; Enfält *et al.*, 1997b; Sather *et al.*, 1997) this has often been explained by more muscle glycogen at slaughter due to greater resistance to stress-induced glycogen depletion. This has given a faster and more severe pH decrease *post mortem* resulting in structural changes of the meat followed by paler meat with higher reflectance values. However, often no effect of either production system (Gandemer *et al.*, 1990; van der Wal, 1991; van der Wal *et al.*, 1993; Dufey, 1995) or spontaneous activity (Petersen *et al.*, 1997b) on either pigmentation or meat-structure related paleness is found.

To summarise, the colour of pork is influenced by pigment content, chemical form of the pigment and by meat structure. Organic production and spontaneous activity may affect meat colour in different ways, either leading to a darker more pigmented meat or a paler, structurally affected meat. In many cases, however, none of the effects are pronounced; indeed, they might even counterbalance each other.

Shear force

Tenderness, here estimated as shear force, is an important trait. Some consumers we met were concerned about how tenderness is affected by alternative production systems. Also, it is difficult to directly state how tenderness is affected by both extensive and intensive production forms. In Study I, no effect of rearing environment on shear force was seen, whereas in Study III meat of organically produced pigs whithout the RN^- allele (rn^+/rn^+) required more shear force for penetration (Table 2). Several explanations could be plausible for the higher shear force values in organically produced meat than in conventionally produced meat. Firstly, the lower IMF of the organic meat could result in higher shear force values. However, IMF did not correlate with shear force, and meat of organic non-carriers of the allele did not have lower levels of IMF compared to meat of organic RN^- carriers or conventionally produced pigs. Secondly, a slower daily growth rate could have caused slower protein turnover in the muscle of the more extensively raised animals. Indeed, it has been debated whether such a slow protein turnover, more than IMF, may explain tougher meat (Wood, *et al.*, 1992). However, in Study III organically raised animals had a slightly higher daily weight gain than conventionally raised animals. Thirdly, if slower *post mortem* proteolytic tenderisation due to slower protein turnover rate does not cause the increased shear force, perhaps the properties of intramuscular collagen do. Collagen can adapt to functional demands such as physical activity, and a tendency towards an increased amount of heat-stable collagen has been reported in LD of pigs exposed to physical activity (Petersen *et al.*, 1997a). Any one or a combination of these factors may play a role in the increased shear force in organic rn^+/rn^+ meat.

Higher shear force in extensively produced meat was reported by van der Wal et al. (1993) and Enfält et al. (1997) whereas Gentry et al (2002c) found lower shear force values in meat of pigs finished outdoors compared with indoors. Spontaneous activity did not affect tenderness in BF and LD in two Danish studies (Petersen *et al.*, 1997a; Petersen *et al.*, 1997b).

Notably, no effect of production system on shear force was observed within RN^- carriers in Study III. Earlier studies in conventional production systems have shown that carriers of the allele produce more tender meat with lower maximum shear force values than meat of non-carriers (Lundström *et al.*, 1996; van Laack *et al.*, 2001). As for pH_u and drip loss, meat with extremely high glycogen levels, in some respects, seems more robust than normal meat; its quality is less affected by different types of pre-slaughter handling, usually influencing meat quality.

After all, the higher shear force in the meat of non-carriers produced organically in Study III, seemed to have limited practical significance at consumption as neither a descriptive sensory test of the attribute tenderness, nor a consumer preference test, both preformed on meat from the same animal material as Study III, could discriminate between meat from the two production systems (Jonsäll *et al.*, 2002).

Table 2. *The effect of various treatments in Study I-III on technological quality attributes of BF and LD (least-squares means ± standard errors)*

	Study I, BF			**Study II, LD**			**Study III, LD**		
	Indoor	*Free range*	*p-value*	*Conventional diet*	*Organic diet*	*p-value*	*Conventional production system*	*Organic production system*	*p-value*
pH_u	5.46 ± 0.01	5.46 ± 0.01	0.772	5.50 ± 0.02	5.48 ± 0.02	0.486	5.48 ± 0.01	5.43 ± 0.01	*i*
Drip loss, %	**5.8 ± 0.4**	**7.0 ± 0.4**	**0.047**	8.5 ± 0.6	8.2 ± 0.5	0.662	8.3 ± 0.4	9.6 ± 0.4	*i*
Cooking loss, %	28.0 ± 1.0	27.3 ± 0.9	0.624	34.8 ± 0.7[b)]	34.6± 0.7[b)]	0.914	**23.0 ± 0.4**	**19.7 ± 0.4**	**0.001**
Shear force	3.7 ± 0.15[c)]	3.5 ± 0.13[c)]	0.463	-	-	-	32.1 ± 1.3[d)]	36.0 ± 1.3[d)]	*i*
Water content %	75.0 ± 0.2	75.0 ± 0.2	0.762	75.1± 0.2	74.8 ± 0.2	0.279	**75.3 ± 0.1**	**74.8 ± 0.1**	**0.004**
Crude protein, %	**21.6 ± 0.2**	**21.0 ± 0.2**	**0.010**	22.1 ± 0.3	22.4 ± 0.3	0.356	**21.3 ± 0.1**	**22.2 ± 0.1**	**0.001**
IMF, %	2.4 ± 0.1	2.1 ± 0.1	0.122	2.2 ± 0.2	2.6 ± 0.2	0.246	**2.0 ± 0.1**	**1.6 ± 0.1**	**0.030**
Res. glycogen[a)]	-	-	-	45.6 ± 2.5	47.0 ± 2.4	0.691	35.0 ± 1.5	36.3 ± 1.5	0.569
Ash, %	1.2 ± 0.03	1.1 ± 0.03	0.216	1.1 ± 0.02	1.1 ± 0.02	0.808	**1.0 ± 0.01**	**1.1 ± 0.01**	**0.028**

a) Residual glycogen = glycogen, glucose and G-6-P (μmol/g)
b) Measured in fried patties of minced meat
c) WB, kg/cm^2, Warner-Bratzler apparatus
d) WB, max shear force, N, Stable Micro Systems Texture Analyser
i) Significant interaction between the fixed factors, production system and *RN* genotype.

Chemical composition, nutritional and safety food aspects

The effects of alternative production systems on the chemical composition of pig meat are not consistent when comparing Studies I, II and III. For the outdoor reared animals in Study I, the crude protein content of BF was lower ($p=0.010$) and the water/protein ratio higher ($p= 0.013$) compared to the pigs kept indoor whereas the opposite was seen in Study III, comparing LD of organic pigs with that of conventional pigs. Further, for IMF, no differences between production forms were found in Study I whereas meat of organically produced pigs had lower levels of IMF than that of conventionally produced pigs in Study III. No difference in ash contents were found between the different treatments in Study I, whereas organic pigs in Study III had higher ash contents than the conventionally produced animals. In Study II, where animals were fed either conventional or organic feed no effects were observed on any of the chemical components measured (Table 2).

Generally, spontaneous activity/physical training affects meat quality traits related to the muscle metabolic state at slaughter and *post mortem* pH decline and is therefore less likely to alter the chemical composition of meat (Hawrysh *et al.*, 1974; Lewis *et al.*, 1989; Enfalt *et al.*, 1993; Petersen *et al.*, 1998b; Gentry *et al.*, 2002b). Thus, if the chemical composition of the meat is altered due to production system, this is often attributable to differences in management level, *i.e.* feed intake and energy expenditure for maintenance. Relatively few studies have focused on how alterative production systems affect chemical properties of meat.

A higher crude protein and lower water/protein ratio in meat from pigs raised extensively has been reported previously (Dworschák *et al.*, 1995; Enfält *et al.*, 1997b). Lower IMF in outdoor reared animals is rather commonly reported (Enfält *et al.*, 1997b; Sather *et al.*, 1997; Claudi-Magnussen, 1999) whereas no difference was reported by others (van der Wal *et al.*, 1993; Dworschák *et al.*, 1995). Gentry *et al*. (2002b) found that moderate exercise did not affect IMF in LD whereas Enfält *et al*. (1993) reported lower IMF values in BF in exercised pigs compared to controls.

The higher ash content found in the meat of the organically raised animals compared to the conventional ($p= 0.028$) in Study III is perhaps not an important result but the underlying causes evoke some curiosity. Dworschák *et al.* (1995) found higher levels of zinc in loin, neck-end and liver, and higher levels of copper and iron in neck-end and liver of naturally kept pigs compared to a control group. They concluded that the metal binding capacity of proteins could be higher in the muscles of these animals. Other studies reported no effect on ash content in the meat of outdoor fattened pigs (Dufey, 1995; Enfält *et al.*, 1997b; Bridi *et al.*, 1998). Other explanations for the increased ash content could be higher iron content due to a higher pigment content and capillarisation of LD in the exercised animals. Lindén *et al.* (2001) showed, by measuring the cadmium content of the kidneys of the same organically raised animals as those examined in Study III, that outdoor raised pigs can accumulate minerals in their body (kidneys) through rooting. This may also play a role in the higher ash content of the meat.

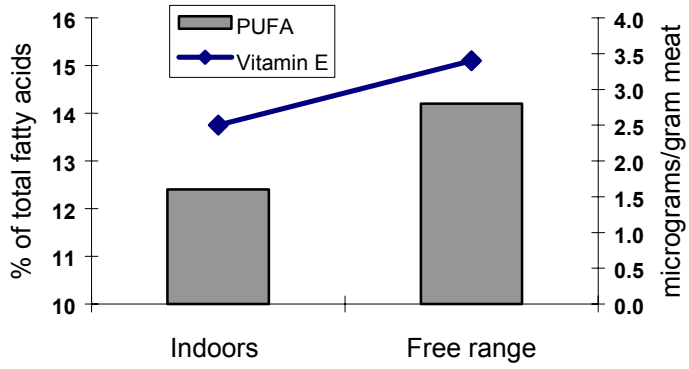

Figure 5. The effect of production system on the levels of polyunsaturated fatty acids ($p = 0.026$) and vitamin E ($p = 0.030$) in pig meat.

Fatty acid composition, antioxidants and shelf life

Study I shows that alternative production systems have a potential to increase the nutritional value of pig meat by improving the fat quality through pigs consuming green feed. In this study, pigs with access to green feed in the form of growing barley, peas and oats produced meat with higher levels of PUFA compared with those in a control group that were not fed green feed ($p=0.026$, Figure 5). Because both the n-3 and n-6 fatty acids increased somewhat in the meat of the outdoor reared animals, no effect on the n-6:n-3n ratio was noted. It is well known that the fatty acid composition in muscles of monogastric animals is relatively easily manipulated through dietary means as reviewed by *e.g.* Jakobsen (1995). Thus, if pigs have access to pasture, which is rich in linoleic acid (18:3 n-3) this may be reflected in the fatty acid composition of the meat improving the n-6:n-3 ratio (Jakobsen, 1995; Muriel *et al.*, 2002). However, these are complex relationships, as for example, neutral and polar lipid fractions may be affected differently and genotype and sex interactions occur (Högberg *et al.*, 2001a; Högberg *et al.*, 2001b). Climatic conditions and temperature changes also affect the fatty acid composition, at least of back fat; colder temperatures increase the unsaturation of the back fat (Lefaucheur *et al.*, 1991; Lebret *et al.*, 2002). Stewart *et al.* (2001) showed that pork with high content of PUFAs lowers LDL cholesterol in women and they concluded that modifications of the fatty acid composition of traditional animal foods, such as pork, may be a useful approach to lowering the amount of saturated fat consumed.

In Study I, the changes in fatty acid composition due to the consumption of green feed decreased the oxidative stability of the meat only for lean, outdoor reared females that were carriers of the RN^{-} allele. This may partly be explained by the higher ($p = 0.030$) levels of the natural antioxidant α-tocopherol (vitamin E) that was detected in meat from the free-range reared animals (Figure 5).

Table 4. *p-values for the effect of RN genotype (rn^+/rn^+ or RN^-/rn^+) on technological meat quality characteristics in Study I, II and III*

	Study I, BF	Study II, LD	Study III, LD
pH_u	0.745	**0.001**	*i*
Drip loss, %	0.059	**0.022**	*i*
Cooking loss, %	**0.009**	**0.004**	**0.001**
Shear force	0.053	-	*i*
Water content, %	0.140	**0.003**	**0.001**
Crude protein, %	**0.023**	**0.006**	**0.001**
Intramuscular fat, %	0.099	0.943	0.560
Residual glycogen[a)]	-	**0.001**	**0.001**
Ash, %	0.242	**0.001**	**0.001**

a) Residual glycogen = glycogen, glucose and G-6-P (µmol/g)
i) Significant interaction between the fixed factors, production system and *RN* genotype.

Table 5. *p-values for the effect of sex (castrated males or females) on technological meat quality characteristics in Study I, II and III*

	Study I, BF	Study II, LD	Study III, LD
pH_u	0.282	0.292	0.726
Drip loss, %	0.367	0.754	0.889
Cooking loss, %	0.530	0.342	0.724
Shear force	0.143	-	0.499
Water content, %	0.242	0.074	0.262
Crude protein, %	0.080	0.130	0.644
Intramuscular fat, %	0.126	0.200	0.666
Residual glycogen[a)]	-	0.444	0.571
Ash, %	0.141	0.702	0.143

a) Residual glycogen = glycogen, glucose and G-6-P (µmol/g).

The influence of the RN^- allele and sex on technological meat quality

Besides the effects of production system, the influence of RN genotype and sex on technological meat quality was also studied in Study I-III. The results, presented in table 4 and 5 show that *RN* genotype substantially influenced most technological traits, especially when measured in the glycolytic LD, whereas the sex of pigs did not influence these traits.

The natural variation of precursors of HCAs in a defined animal material (Study II)

The concentrations of the precursors glucose, creatine and free amino acids vary between animal species (Vikse & Joner, 1993; Pais *et al.*, 1999) and these variations may affect the mutagenic compounds formed upon heating. Little is known, however, about the natural variation of precursor in a muscle within

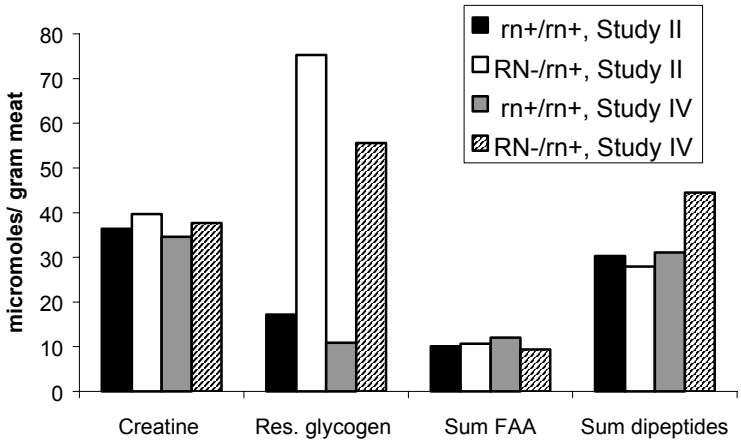

Figure 6. The concentration of important HCA precursors in meat of carriers and non-carriers of the RN^- allele in study II and IV

animal species. In Study II the effects of RN genotype, feed (organic or conventional) and sex on the level of precursors were studied.

The natural variations of the precursors for the formation of HCAs were large. In Study II, the concentration of residual glycogen varied 13-fold, between 7 and 91 µmol/g meat whereas creatine content varied 1.5-fold, from 29.1 to 44.2 µmol/g meat. Summarising the concentrations of all free amino acids, they ranged between 8.0 and 16.7 µmol/g meat. The dipeptides, anserine and carnosine, also varied about twofold in the ranges of 0.5 – 1.1 and 20.3 – 35.2 µmol/g meat, respectively.

An important explanation for these large variations is the occurrence of the RN^- allele. In Study II, meat originating from RN^- carriers had significantly higher concentrations of residual glycogen and creatine whereas the level of the dipeptides anserine and carnosine was lower in this meat compared to that of non-carriers (Figure 6). Diet and sex had no, or very limited, influence on these precursors. For the concentrations of various free amino acids, a few were affected by *RN* genotype or diet but no effect of sex was observed. Neither *RN* genotype, nor feed nor sex affected the total sum of free amino acids. Except for the sum of free amino acids and dipeptides, these relations were true also when comparing only one animal of each *RN* genotype in Study IV (Figure 6).

The mechanisms behind the biochemical differences of RN^-/rn^+ compared to normal meat are not yet fully understood. For the well documented increased glycogen levels, either a dominant negative mutation inhibiting AMP (adenosine monophosphate) activation and glycogen degradation, or a gain-of-function mutation leading to an increased glucose transport, and/or glycogen synthesis has been put forward as possible explanations (Milan et al., 2000). For the increased creatine levels found in Study II and IV, differences in the *post mortem* glycolytic patterns between carriers-and non-carriers of the RN^- allele (Josell *et al.,* 2003b)

may be part of the explanation. Generally the levels of creatine in lean meat are relatively constant (Dvorák, 1981). Nonetheless, a possible effect of feeding high or low energy feedstuffs on the free amino acid content of pig muscle was observed by Koga *et al.* (1985). Further, Cornet & Bousset (1999) reported that the muscle content of several amino acids is closely related to the metabolic types of fibres of the muscles. Higher concentrations of carnosine have been analysed in glycolytic muscles compared to more oxidative muscles (Cornet & Bousset, 1999). The possible increase in the relative areas of oxidative IIA and IIBr fibres in muscle of RN^- carriers (Lebret *et al.*, 1999a) might be an explanation to the lower carnosine/dipeptide levels in RN^-/rn^+ compared to rn^+/rn^+ meat.

To summarise, due to the occurrence of the RN^- allele, the variation in the level of HCA precursors within this pig material is almost as large as that found between species (Vikse & Joner, 1993; Pais *et al.*, 1999).

The formation and exposure of HCAs in relation to pig meat composition and consumer cooking practices and preferences (Study II and IV)

The observed variations in precursor levels directly affect formation of HCAs (Table 6). After frying meat of the two *RN* genotypes under very similar time/temperature conditions it was clear that considerably lower levels of HCAs were formed in meat of RN^- carriers. In Study II, including 13 animals of each *RN* genotype, half the amount of HCAs were formed in the RN^- meat and in Study IV, comparing only one pig of each genotype, barely 12% of the HCAs formed in the rn^+/rn^+ meat were found in the RN^- carrier. These studies are the first systematic investigations on the natural variation of HCA precursors and their effect on the formation of HCAs within the same species and muscle. By including meat of both *RN* genotypes an extremely wide range of precursors, especially residual glycogen, was obtained. This created an ideal design for investigations on the importance of these variations on the formation of HCAs. These types of investigation have formerly mostly been performed in model systems or by addition of *e.g.* glucose to the meat. For HCA formation, model system studies suggests an optimal relationship of mono- or disaccharides at about half the molar amount of creatine and free amino acids. When the concentrations of sugar increase above this optimal level, the formation of mutagens decrease (Skog & Jägerstad, 1990; Skog *et al.*, 1992). The same relationship, with inhibiting effects of high sugar concentrations, seems to be valid also in meat and may expalin the considerably lower amounts of HCAs found in meat of RN^- carriers, in which the residual glycogen content is higher than in the rn^+/rn^+ meat. The effects of differences in creatine, free amino acid and dipeptide concentrations, however, are probably overshadowed by the large variations in residual glycogen levels in Study II and IV.

In Study II, the mutagenic/carcinogenic HCAs MeIQx, 4,8-Di MeIQx and PhIP, along with the comutagens harman and norharman, were formed. In Study IV, the same HCAs were detected except for 4,8-Di MeIQx which was not found, and IQx, which was found in the RN^-/rn^+ meat fried at medium and high temperatures. The

reason for this difference may be because two different methods of analysis were used in Study II and IV or because cooking procedures differed.

Besides the precursors mentioned above, other so-called modulators for the formation of HCAs in meat are known. The presence and relative amounts of various enhancers, inhibitors, lipids and pro/antioxidants as well as the pH-value and water content of the meat may play a role in the formation of HCAs in meat fried under identical conditions (Jägerstad *et al.*, 1998; Felton *et al.*, 2000).

Both the lipid content of the meat as well as the amount and type of fat used for cooking may affect the formation of HCAs. In our data set (Study II), the intramuscular fat content explains little of the variance and has not been considered important for HCA formation. However, the role of fat has not been clarified and studies on how fat content and frying fat influence the formation of food mutagens report inconsistent results (Johansson *et al.*, 1995).

Iron is known to act as a pro-oxidant in lipid oxidation during the formation of free radicals that in turn may potentiate the Maillard reaction (Johansson & Jägerstad, 1993). The addition of iron (Fe 2+ and Fe 3+) to a liquid creatinine, glycine and glucose model system increased the formation of IQx, MeIQx and DiMeIQx about twofold whereas haemoglobin both increased and decreased the yield of HCAs depending on heating time (Johansson, & Jägerstad, 1996). To my knowledge, no studies on the difference in iron content between carriers and non-carriers of the RN^- allele have been published. When comparing the minor elements in LD of one carrier and one non-carrier of the RN^- allele no large differences were observed (Table 7). Concerning iron, rn^+/rn^+ meat contained 5.45 mg/kg and RN^-/rn^+ 5.13 mg/kg. Because these values are only from two animals, it is not possible to draw any conclusions from them. Considreing the often reported higher ash contents as well as the fibre type differences between the genotypes described by Lebret *et al.* (1999a), somewhat more iron in RN^- carriers compared to non-carriers might be expected if measured in a larger material.

Water is important for the transport of water-soluble precursors within meat and the transport of precursors can be restricted by the addition of water binding compounds *e.g.* salt, soy protein and starch, thereby reducing the formation of HCAs in cooked meat (Jägerstad *et al.*, 1998; Persson *et al.*, 2003). Due to lower pH_u and protein levels in combination with a higher glycogen concentration, the meat of RN^- carriers loses more weight during cooking than normal meat. Additionally, myoproteins in meat of *RN* carriers are suggested to be more heat sensitive, and to denaturate more easily upon heating (Deng *et al.*, 2002; Bertram *et al.*, 2004a). Preliminary results from our laboratory show that the RN^-/rn^+ meat juice released during cooking contains considerable amounts of glycogen, glucose and G-6-P (Enfält & Hullberg, 2004). A large cooking loss has been found to be related to the formation of large amounts of HCAs (Skog & Jägerstad, 1990; Skog *et al.*, 1992; Skog *et al.*, 1995).

Table 6. *The effect of RN genotype feed and sex on the formation of HCAs*

	RN genotype			Feed			Sex		
	rn+/rn+	RN⁻/rn+	p-value	Conventional	Organic	p-value	Castrated male	Female	p-value
MeIQx	1.9 ± 0.2 (n = 14)	1.5 ± 0.3 (n = 12)	0.273	1.6 ± 0.3 (n = 12)	1.8 ± 0.2 (n = 14)	0.530	1.5 ± 0.3 (n = 13)	1.9 ± 0.2 (n = 13)	0.247
4,8-DiMeIQx	0.4 ± 0.1 (n = 14)	0.2 ± 0.1 (n = 12)	0.063	0.3 ± 0.1 (n = 12)	0.3 ± 0.1 (n = 14)	0.695	0.3 ± 0.1 (n = 13)	0.3 ± 0.1 (n = 13)	0.479
PhIP	**1.9 ± 0.3** (n = 14)	**0.2 ± 0.3** (n = 11)	**0.001**	1.0 ± 0.3 (n = 11)	1.1 ± 0.3 (n = 14)	0.873	0.8 ± 0.3 (n = 12)	1.4 ± 0.3 (n = 13)	0.157
Sum mutagenic HCAs	**4.2 ± 0.5** (n = 14)	**2.0 ± 0.5** (n = 11)	**0.006**	3.0 ± 0.5 (n = 11)	3.2 ± 0.5 (n = 14)	0.775	2.6 ± 0.5 (n = 12)	3.6 ± 0.5 (n = 13)	0.167
Harman	0.7 ± 0.5 (n = 12)	1.6 ± 0.5 (n = 11)	0.217	0.4 ± 0.6 (n = 10)	1.9 ± 0.5 (n = 13)	0.053	0.9 ± 0.7 (n = 9)	1.3 ± 0.5 (n = 14)	0.659
Norharman	1.9 ± 0.6 (n = 11)	3.4 ± 0.6 (n=11)	0.094	1.8 ± 0.7 (n = 9)	3.6 ± 0.6 (n = 13)	0.059	2.9 ± 0.7 (n = 8)	2.5 ± 0.5 (n = 14)	0.650

Bold font denotes a significant ($p<0.05$) effect of the fixed factors *RN* genotype, feeding regime or sex.

Table 7. *Mineral and trace element content (mg/kg) of meat from one non-carrier and one carrier of the RN^- allele*

	rn^+/rn^+	RN^-/rn^+
K	4256	4208
P	2448	2366
Na	483	478
Mg	280	256.3
Ca	34.7	36.9
Zn	13.4	17.4
Fe	5.45	5.13
Cu	0.44	0.39
Pb	0.008	0.008
Mo	0.007	0.01
Cr	0.006	0.006
Cd	0.004	0.004
Ni	0.008	0.009
Total	**7521.1**	**7368.2**

Recently, Persson *et al.* (2003) concluded that a high cooking loss bringing about considerable transport of precursors of the meat to the surface is more favourable for HCA formation than retention of water and increased surface temperature. For RN^-/rn^+ meat, the efficient transport of glucose to the meat surface resulted in inhibition of formation of HCAs.

In minced compared to whole meat, the formation of HCAs might be affected by a higher leakage of water-soluble precursors to the pan due to disruption of the cellular structure during mincing (Johansson, 1995). To compare minced and intact LD regarding cooking loss and amount of HCAs formed, minced patties of the rn^+/rn^+ meat in Study IV were fried at medium temperature under the same conditions as the chops. The minced meat patties and intact chops respectively contained 1.26 and 0.43 ng MeIQx/g cooked meat, 1.31 and 2.87 ng PhIP/g cooked meat and no IQx in either minced nor intact LD. The mean cooking loss was higher for the minced meat, 26.5% than in the intact meat, 22.7%. It is difficult to draw conclutions from this small experiment but it seems that somewhat more meat juice is lost during cooking of minced compared to whole meat and that the formation of HCA might be affected by mincing.

The concentration of reducing sugars is clearly related to the surface browning of pig meat, and the higher residual glycogen content in the RN^-/rn^+ meat probably explains the darker crust colours found in Study II and IV, through a more pronounced Maillard reaction (Pearson *et al.*, 1962; Bowers *et al.*, 1968).

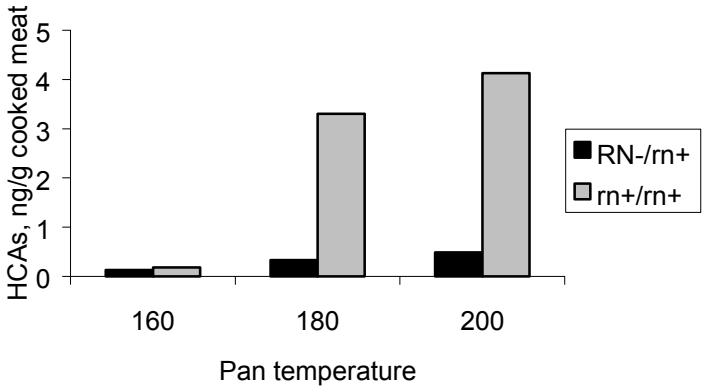

Figure 7. *The varying effect of pan temperature (°C) on the formation of mutagenic HCAs in meat of different RN genotypes.*

In Study IV, three temperature regimens (initial pan temperature 160, 180 or 200º C) were chosen to mimic various domestic frying practices. By frying pork chops from one carrier and one non-carrier of the RN^- allele at these different temperatures, we found the mutagenic HCAs, MeIQx, PhIP and IQx, in low concentrations in the crust. IQx was detected only in the RN^-/rn^+ crust whereas the comutagens, harman and norharman, were detected in the crust of the rn^+/rn^+ chops but not in the RN^-/rn^+ meat. Normal (rn^+/rn^+) meat fried at an initial pan temperature of 200ºC gave more than 20 times the amount of mutagenic HCAs compared to that fried at 160ºC, 4.13 compared to 0.18 ng/g cooked meat. PhIP was the main HCA formed. Also noticeable was the dramatic increase in the total content of mutagenic HCAs in the fried rn^+/rn^+ chops when raising the initial pan temperature from 160 to 180º C (Figure 7). In a study by Augustsson *et al.* (1997) a 20-fold increase of PhIP levels was typically seen when increasing the frying temperature for several meat dishes from 175ºC to 225º C, and a general temperature dependency of the formation of HCAs has been repeatedly reported (*e.g.* Skog *et al.*, 1995; Sinha *et al.*, 1998).

When respondents looked at colour photographs, they preferred fried chops with medium crust colour, rejecting the very pale crust of the non-carrier of the RN^- allele fried at low temperatures as well as the dark brown of the carrier fried at medium/high temperature. Most respondents chose fried chops from the non-carrier, which, based on information given in a questionnaire, would result in an markedly higher average contribution to the monthly HCA intake of 359 ± 402 ng (mean ± SD) compared to 35 ± 60 ng/month for consumers who preferred the RN^-/rn^+ chops. Overall, the total monthly intake of mutagenic HCAs derived from consuming pork chops was moderate, on average 256 ng, ranging from 0 to 1982 ng/month. A mean intake of 256 ng/month would mean a limited daily contribution of about 9 ng from fried pork chops, well in agreement with an earlier Swedish study which estimated a daily contribution of 10.1 ng/day from fried pork chops and gravy (Voskuil *et al.*, 1999). This intake rate should be related to the assessed total daily intake of 200 and 120 ng HCAs for Swedish men and women,

respectively (Augustsson *et al.*, 1997). Besides the Swedish study, data on the specific contribution of pork to the intake of HCAs are sparse. According to estimates by Layton *et al.* (1995) the intake of HCAs originating from pork would be 51 ng/day for a man or woman weighing 70 kg. The corresponding figures for beef and chicken would be 542 and 173 ng/day, respectively. The estimated daily intake in this study was however high, 1820 ng/day compared to 160 ng/day in a Swedish study (Augustsson *et al.*, 1997).

As Study IV involves respondents from a small regional area and a relatively homogenous population group, it is not suited to extrapolate its findings to general exposure of HCAs in the Swedish society. However, it does contribute to better knowledge about the level and formation of HCA in domestically prepared pork chops as well as understanding of the complexity of using different exposure indicators, such as the degree of surface browning, in the assessment of HCA intake through diet. The study shows that the raw material is important and that small adjustments in the domestic cooking practices may help reduce the formation and intake of HCAs.

To estimate total HCA exposure, epidemiologists try to identify markers for the factors cooking time and temperature. Doneness of meat and crust browning have been put forward as a reasonable indirect measurement of mutagenic activity (Steineck *et al.*, 1993; Sinha *et al.*, 1999). However, our data show that care should be taken when using colour photographs to determine cooking preferences for assessment of HCA intake because background factors, such as the occurrence of the RN^{-} allele in pigs, may influence the results. The *RN* genotype of pigs affects both colour formation and HCA content and thereby makes crust colour inappropriate for estimation of HCA content in cooked pig meat carrying the RN^{-} allele.

Concluding remarks

The results of this thesis show that combinatory effects of *RN* genotype, production system and to some extent, sex affect pig meat quality and are important for the level of precursors and the formation of HCAs.

Satisfactory technological meat quality is a prerequisite for producer and consumer acceptance and future development of the organic meat market. In general, alternative production systems for pig meat that were studied resulted in satisfactory carcass and meat quality. If pigs are adequately fed, alterations in the production system comparable to those in organic production should not substantially influence meat quality. However, in meat with normal levels of glycogen, *i.e.* in non-carriers of the RN^- allele, the alternative systems with increased spontaneous activity caused inferior technological quality of the pork in terms of reduced water-holding capacity and increased shear force. However, these negative effects on technological meat quality may be of little importance for eating quality. Assessing the same animals, only minor differences between the two types of meat could be detected by a trained sensory panel, and a consumer preference test showed that consumers cannot discriminate between meat produced according to the organic standards of KRAV and conventional meat (Jonsäll et al., 2002).

The high glycolytic potential of RN^- carriers to some extent prevented the effects of extrinsic factors that usually affect meat quality. The results emphasis that production and pre-slaughter factors may become more important in the future if it is decided to exclude the RN^- allele from the Swedish slaughter pig population.

The average daily consumption of dishes with pig meat is 22 g/day for women and 29 g/day for men, making it the most consumed of all meats in Sweden (SLV, 2002). Nutritional improvements of pig meat may be one of several future ways to address the public health problems. Modifications of the fatty acid composition of traditional animal foods, such as pork, may be a useful approach to lower the amount of saturated fat consumed. It was shown that access to pasture may lead to higher PUFA and α-tocopherol levels in pork. The higher levels of PUFA resulted in decreased oxidative stability of frozen meat only in lean outdoor reared females that were carriers of the RN^- allele.

A well-defined animal material gave an unique opportunity to study the effects of *RN* genotype, production system and sex on the formation of HCAs. The natural variation of the concentrations of HCAs precursors in pork was 13-fold for residual glycogen, 1.5-fold for creatine, and two-fold for both total free amino acids and dipeptides. These variations were best explained by the RN genotype of the animals, whereas neither feeding regime nor sex significantly contributed to the variation. High concentrations of residual glycogen were analysed in meat from pigs that were carriers of the dominant RN^- allele. The increased residual glycogen levels resulted in browner crust colour after frying compared to meat of non-carriers. Further, high glycogen significantly reduced the yield of total HCAs, in particular PhIP, and resulted in about 50 % lower amounts of total HCAs in cooked meat of RN^- carriers than in cooked meat from normal pigs.

The results further show that care should be taken when using colour photographs to determine cooking preferences for assessment of HCA intake because background factors, such as the occurrence of the RN^- allele in pigs, may influence the results. The *RN* genotype of pigs affects both colour formation and HCA content, and thereby makes crust colour inappropriate for estimation of HCA content in cooked pig meat carrying the RN^- allele. Data also indicate that although fried pork chops is a popular dish on Swedish dinner tables, it only marginally contributes to the daily intake of HCAs. By choosing meat from pigs carrying the RN^- allele and by frying the meat at lower temperatures, the contribution of pork chops to the total intake of HCAs could be even further reduced.

References

Adamson, R. H., Thorgeirsson, U. P. & Snyderwine, E. G. 1990. Carcinogenicity of 2-amino-3-methylimidazo-(4,5-f)quinoline in nonhuman primates: induction of tumours in three macaques. *Japanese Journal of Cancer Research* 81, 10-14.

Alarik, M. 2003. Slaktkropparnas kvalitet i ekologisk uppfödning 2002. *Ekokött, Svenskt ekologiskt kött*, 1-26.

Allen, C. E. & Foegeding, E. A. 1981. Some lipid characteristics and interactions in muscle foods - a review. *Food Technology* 35, 253-257.

Andresen, N. 2000. The foraging pig. Resource utilisation, interaction, performance and behaviour of pigs in cropping systems. *Doctoral thesis, Agraria 227*, Swedish University of Agricultural Sciences.

Andersson, K. 1980. Studies on crossbreeding and carcass evaluation in pigs. *Doctoral thesis*, Swedish University of Agricultural Sciences.

Andersson, K., Andersson, I. & Essén-Gustavsson, B. 1990. Grisarna ute eller inne? *Department of animal nutrition and management, SLU*, *Fakta Husdjur 12*.

Andersson, K. H., Olsson, V., Hullberg, A. & Lundström, K. 2003. Effects of Sex, Feed and Pre-slaughter Routines on Technological Meat Quality in Carriers and Non-carriers of the RN- allele. *Acta Agriculturae Scandinavica, A* 53, 147-154.

Augustsson, K., Skog, K., Jägerstad, M. & Steineck, G. 1997. Assessment of the human exposure to heterocyclic amines. *Carcinogenesis* 18, 1931-1935.

Augustsson, K. & Steineck, G. 2000. Cancer risk based on epidemiological studies. In M. Nagao and T. Sugimura, *Food borne carcinogens, heterocyclic amines.* Wiley. Chichester. 332-348 pp.

Avery, N. C., Sims, T. J., Warkup, C. & Bailey, A. J. 1996. Collagen Cross-linking in Porcine M. longissimus lumborum: Absence of a Relationship With Variation in Texture at Pork Weight. *Meat Science* 42, 355-369.

Barton-Gade, P. & Blaabjerg, L. O. 1989. Preliminary observations on the behaviour and meat quality of free range pigs. In *35:th International Congress of Meat Science and Technology,* 1002-1005.

Barton-Gade, P., Demeyer, D., Honikel, K. O., Joseph, R. L., Poulanne, E., Severine, M., Smulders, F. & Tornberg, E. 1994. Final version (I) of reference methods for water holding capacity in meat and meat products: Procedures recommended by an OECD working group. In *40:th Congress of Meat Science and Technology,* The Hague, The Netherlands.

Barton-Gade, P. 2002. Welfare of animal production in intensive and organic systems with special reference to Danish organic pig production. *Meat Science* 62, 353-358.

Bejerholm, C. & Barton-Gade, P. 1986. Effect of intramuscular fat level on eating quality of pig meat. In *32nd European meeting of meat research workers,* Ghent, Belgium. 389-391.

Bendall, J. R. 1951. The shortening of rabbit muscles during rigor mortis: its relation to the breakdown of adenosine triphosphate and creatinphosphate and to muscular contraction. *Journal of Physiology* 114, 71-88.

Bendall, J. R. & Wismer-Pedersen, J. 1962. Some properties of the fibrillar proteins of normal and watery pork muscle. *Journal of Food Science* 27, 144-159.

Bendall, J. R. 1973. Post mortem changes in muscles. In G. H. Bourne, *Structure and function of muscle.* Academic Press. New York. 243-309 pp.

Bendall, J. R. & Swatland, H. J. 1988. A review of the relationships of pH with physical aspects of pork quality. *Meat Science* 24, 85-126.

Benfalk, C. 2003. System för utegående slaktsvin i ekologiskt lantbruk - djurmiljö, hushållning av växtnäring och arbetsmiljö. In *Ekologiskt Lantbruk,* Uppsala, Sweden.

Bertram, H. C., Karlsson, A. H., Rasmussen, M., Pedersen, O. D., Donstrup, S. & Andersen, H. J. 2001. Origin of multiexponential T-2 relaxation in muscle myowater. *Journal of Agricultural and Food Chemistry* 49, 3092-3100.

Bertram, H. C., Engelsen, S. B., Busk, H., Karlsson, A. H. & Andersen, H. J. 2004a. Water properties during cooking of pork studied by low-field NMR relaxation: effects of curing and the RN¯-gene. *Meat Science* 66, 437-446.

Bertram, H. C., Schäfer, A., Rosenvold, K. & Andersen, H. J. 2004b. Physical changes of significance for early post mortem water distribution in porcine M. longissimus. *Meat Science* 66, 915-924.

Bjeldanes, L. F., Morris, M. M., Timourian, H. & Hatch, F. T. 1983. Effects of meat composition and cooking conditions on mutagen formation in fried ground beef. *Journal of Agricultural and Food Chemistry* 31, 18-21.

Boccard, R., Buchter, L., Casteels, E., Cosentino, E., Dransfield, E., Hood, D. E., Joseph, R. L., MacDougall, D. B., Rhodes, D. N. & Schon, I. 1981. Procedures for measuring meat quality characteristics in beef production experiments. Report of a working group in the commission of the European communities' (CEC) beef production research programme. *Livestock production Science* 8, 385-397.

Bowers, J. A., Harrison, D. L. & Kropf, D. H. 1968. Browning and associated properties of porcine muscle. *Journal of Food Science* 33, 147-151.

Bridi, A. M., Mueller, L. & Ribeiro, J. A. R. 1998. Indoor vs. outdoor-rearing of pigs, performance, carcass and meat quality. In *44th International Congress of Meat Science and Technology,* Barcelona, Spain. 1056-1057.

Briskey, E. J. 1964. Etiological status and asociated studies of pale, soft, exudative porcine musculature. In C. O. Chichester, E. M. Mrak and G. F. Stewart, *Advances in Food Research.* Academic Press. London. 90-168 pp.

Bång, J., Nukaya, H. & Skog, K. 2002. Blue Chitin columns for the extraction of heterocyclic amines from cooked meat. *Journal of Chromatography A* 977, 97-105.

Cambero, M. I., Seuss, I. & Honikel, K. O. 1992. Flavor compounds of beef broth as affected ny cooking temperature. *Journal of Food Science* 57, 1285-1290.

Ciobanu, D., Bastiaansen, J., Malek, M., Helm, J., Woollard, J., Plastow, G. & Rothschild, M. 2001. Evidence for new alleles in the protein kinase adenosine monophosphate - activated subunit gene associated with low glycogen content in pig skeletal muscle and improved meat quality. *Genetics* 159, 1151-1162.

Clarke, S. D. 2000. Polyunsaturated fatty acid regulation of gene transcription:a mechanism to improve energy balance and insulin resistance. *British Journal of Nutrition* 83, 59-66.

Claudi-Magnussen, C. 1999. Ökologisk svineproduktion, sammenligning af ekologiske og konventionelle detailprodukters köd-och spisekvalitet. *Danish Meat Research Institute*, *17.258/01*, 1-28

Cornet, M. & Bousset, J. 1999. Free amino acids and dipeptides in porcine muscles: differences between 'red' and 'white' muscles. *Meat Science* 51, 215-219.

D´Souza, D. N., Warner, R. D., Dunshea, F. R. & Leury, B. J. 1998. Effect of on-farm and pre-slaughter handling of pigs on meat quality. *Australian Journal of Agricultural Research* 49,

Danielsen, V., Hansen, L. L., Möller, F., Bejerholm, C. & Nielsen, S. 2000. Production results and sensory meat quality of pigs fed different amounts of concentrate ad lib. Clover grass or clover grass silage. In *Ecological animal husbandry in the nordic countries. NJF seminar no. 303,* Horsens, Denmark. 79-86.

Dalrymple, R. H. & Hamm, R. 1973. A method for extraction of glycogen and metabolites from a single muscle sample. *Journal of Food Technology* 8, 439-444.

de Meester, C. 1995. Genotoxic potential of β-carbolines: a review. *Mutation Research* 33, 139-153.

del Campo, F., Gallego, B., Berregi, I. & Casado, A. 1998. Creatinine, creatine and protein in cooked meat products. *Food Chemistry* 63, 187-190.

Deng, Y., Rosenvold, K., Karlsson, A. H., Horn, P., Hedegaard, J., Steffensen, C. L. & Andersen, H. J. 2002. Relationship between thermal denaturation of porcine muscle proteins and water-holding capacity. *Journal of Food Science* 67, 1642-1647.

DH. 1994. Nutritional aspects of cardiovascular disease. *Department of Health (DH), Her Majesty´s Stationery Office*,

Draper, H. H., Squires, E. J., Mahmoodi, H., Wu, J., Agarval, S. & Hadley, M. 1993. A comparative evaluation of thiobarbituric acid methods for the determination of malondialdehyde in biological materials. *Free Radical Biology & Medicine* 15, 353-363.

Dufey, P.-A. 1995. Fleisch- und fettqualität bei schweinemast mit weidegang. *Agrarforschung* 2, 453-456.

Dutta, P. C., Helmersson, S., Kebedu, E., Alemaw, G. & Appelqvist, L.-Å. 1994. Variation of lipid composition of Niger Seed (Guizotia abyssinica cass.) samples collected from different regions in Ethiopia. *JAOCS* 71, 839-843.

Dvorák, Z. 1981. Creatine as an indicator of net muscle porteins. *Journal of the Science of Food Agriculture* 32, 1033-1036.

Dworschák, E., Barna, É., Gergely, A., Czuczy, P., Hóvári, J., Kontraszti, M., Gaal, Ö., Radnóti, L., Bíró, G. & Kaltenecker, J. 1995. Comparison of some components of pigs kept in natural (free-range) and large-scale condition. *Meat Science* 39, 79-86.

Enfält, A. C., Lundström, K., Hansson, I., Karlsson, A., Essengustavsson, B. & Hakansson, J. 1993. Moderate Indoor Exercise - Effect on Production and Carcass Traits, Muscle Enzyme-Activities and Meat Quality in Pigs. *Animal Production* 57, 127-135.

Enfält, A.-C., Lundström, K., Hansson, I., Johansen, S. & P-E., N. 1997a. Comparison of non-carriers and heterozygous carriers of the RN$^-$ allele for carcass composition, muscle distribution and technological meat quality in Hampshire-sired pigs. *Livestock production Science* 47, 221-229.

Enfält, A.-C., Lundström, K., Hansson, I., Lundeheim, N. & Nyström, P.-E. 1997b. Effects of outdoor rearing and sire breed (Duroc or Yorkshire) on carcass composition and sensory and technological meat quality. *Meat Science* 45, 1-15.

Enfält, A.-C., Hullberg, A. 2004. Glycogen, glucose and glucose-6-phosphate content in fresh and cooked meat and meat juice from carriers and non-carriers of the *RN*$^-$ allele. *Manuscript.*

Enser, M., Hallett, K., Hewitt, B., Fursey, G. A. J. & Wood, J. D. 1996. Fatty acid content and composition of english beef, lamb and pork at retail. *Meat Science* 42, 443-456.

Enser, M. 2000. Producing meat for healthy eating. In *46:th International Congress on Meat Science and Technology,* Buenos Aires, Argentina. 124-129.

Enser, M., Richardson, R. I., Wood, J. D., Gill, B. P. & Sheard, P. R. 2000. Feeding linseed to increase the n-3 PUFA of pork: fatty acid composition of muscle, adipose tissue, liver and sausages. *Meat Science* 55, 201-212.

Essén-Gustavsson, B., Lundström, K., Larsson, G., Lindholm, A., Nordin, A.-C., Hansson, I. & Tornberg, E. 1988. The effect during growth of moderate exercise on muscle metabolic charachteristics in vivo and relation to meat quality and sensory properties. In *34th International congress of meat science and technology,* Brisbane, Australia. 27-30.

Essén-Gustavsson, B. & Jensen-Waern, M. 1993. Muscle charachteristics and metabolic response at slaughter in domestic pigs reared indoors or outdoors. In *International Congress of Meat Science and Technology,* Calgary, Canada. F2P09.

Essén-Gustavsson, B., Karlsson, A., Lundström, K. & Enfält, A.-C. 1994. Intramuscular fat and muscle fibre lipid contents in hatothane-gene-free pigs fed high or low protein diets and its relation to meat quality. *Meat Science* 38, 269-277.

Essén-Gustavsson, B. & Blomstrand, E. 2002. Effect of exercise on concentrations of free amino acids in pools of type I and type II fibres in human muscle with reduced glycogen stores. *Acta Physiologica Scandinavica* 174, 275-281.

Estevez, M., Morcuende, D. & Lopez, R. C. 2003. Physico-chemical characteristics of M-Longissimus dorsi from three lines of free-range reared Iberian pigs slaughtered at 90 kg live-weight and commercial pigs: a comparative study. *Meat Science* 64, 499-506.

Estrade, M., Vignon, X., Rock, E. & Monin, G. 1993. Glycogen hyperaccumulation in white muscle fibres of RN- carrier pigs. A biochemical and ultrastructural study. *Comp. Biochem. Physiol.* 104B, 321-326.

Fay, L. B., Ali, S. & Gross, G. A. 1997. Determination of heterocyclic aromatic amines in food products: automation of the sample preparation method prior to HPLC and HPLC-MS quantification. *Mutation Research* 376, 29-35.

Felton, J. S., Knize, M. G., Shen, N. H., Lewis, P. R., Andersen, B. D., Happe, J. & Hatch, F. T. 1986. The isolation of a new mutagen from fried ground beef: 2-amino-1-methyl-6-phenyl-imidazo(4,5-b)pyridine (PhIP). *Carcinogenesis* 7, 1081-1086.
Felton, J. S. & Knize, M. G. 1991. Occurrence, Identification, and Bacterial Mutagenicity of Heterocyclic Amines in Cooked Food. *Mutation Research* 259, 205-217.
Felton, J. S., Malfatti, M. A., Knize, M. G., Salmon, C. P., Hopmans, E. C. & Wu, R. W. 1997. Health risks of heterocyclic amines. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis* 376, 37-41.
Felton, J. S., Jägerstad, M., Knize, M. G., Skog, K. & Wakabayashi, K. 2000. Contents in food, beverages and tobacco. In M. Nagao and T. Sugimura, *Food borne carcinogens, heterocyclic amines.* John Wiley & Sons Ltd. pp.
Ferguson, L. R. 2002. Natural and human-made mutagens and carcinogens in the human diet. *Toxicology* 181-182, 79-82.
Fernandez, X., Mågård, M. & Tornberg, E. 1992. The Variation in Pig Muscle Glycolytic Potential During Lairage - an In vivo Study. *Meat Science* 32, 81-91.
Fernandez, X., Monin, G., Talmant, A., Mourot, J. & Lebret, B. 1999. Influence of intramuscular fat content on the quality of pig meat - Composition of the lipid fraction and sensory characteristics of m. longissimus lumborum. *Meat Science* 53, 59-65.
Fujii, J., Otzu, K., Zorazo, F., DeLeon, S., Khanna, V. K., Weiler, J., O´Brien, P. J. & MacLennan, D. H. 1991. Identification of a mutation in porcine ryanodine receptor associated with malignant hyperthermia. *Science* 253, 448-451.
Gandemer, G., Pichou, D., Bouguennec, B., Caritez, J. C., Berge, P., Briand, E. & Legault, C. 1990. Influence du système d´élevage et du génotype sur la composition chimique et les qualités organoleptiques du muscle long dorsal chez le porc. *Journées de la Reserche Porcine en France* 22, 101-110.
García, C., Ventanas, J., Antequera, T., Ruiz, J., Cava, R. & Alvarez, P. 1996. Measuring sensorial quality of Iberian ham by Rasch model. *Journal of Food Quality* 19, 379-412.
Gariepy, C., Amiot, J. & Nadai, S. 1989. Ante-mortem detection of PSE and DFD by infrared thermography of pigs before stunning. *Meat Science* 25, 37-41.
Gentry, J. G., McGlone, J. J., Blanton, J. R. & Miller, M. F. 2002a. Alternative housing systems for pigs: Influences on growth, composition, and pork quality. *Journal of Animal Science* 80, 1781-1790.
Gentry, J. G., McGlone, J. J., Blanton, J. R. & Miller, M. F. 2002b. Impact of spontaneous exercise on performance, meat quality, and muscle fiber characteristics of growing/finishing pigs. *Journal of Animal Science* 80, 2833-2839.
Gentry, J. G., McGlone, J. J., Miller, M. F. & Blanton, J. R. 2002c. Diverse birth and rearing environment effects on pig growth and meat quality. *Journal of Animal Science* 80, 1707-1715.
Govindarajan, S. 1973. Fresh meat colour. *CRC Critical reviews in Food Technology* 1, 117-140.
Gross, G. A., Grüter, A. & Heyland, S. 1992. Optimization of the sensitivity of high-performance liquid chromatography in the detection of heterocyclic aromatic amine mutagens. *Food and Chemical Toxicology* 30, 491-498.
Gross, G. A., Turesky, R. J., Fay, L. B., Stillwell, W. G., Skipper, P. L. & Tannenbaum, S. R. 1993. Heterocyclic Aromatic Amine Formation in Grilled Bacon, Beef and Fish and in Grill Scrapings. *Carcinogenesis* 14, 2313-2318.
Hansen, L. L., Claudi-Magnussen, C. & Andersen, H. J. 2001. Köd- og spisekvalitet fra ekologisk producerede grise. *Ministeriet for födevarer, lantbrug og fiskeri, Danmarks Jordbrugsforskning*, *145*, 39-46
Hara, A. & Radin, N. S. 1978. Lipid extraction of tissues with a low-toxicity solvent. *Analytical Biochemistry* 90, 420-426.
Hawrysh, Z., Murray, D. & Bowland, J. 1974. Effects of exercise on palatability and cooking characteristics of pork. *Can. J. Anim. Sci.* 54, 191-195.
Hedrick, H. B. 1994. *Principles of meat science*. Third edition. Kendall/Hunt Publishing Company. Dubuque. pp.

Henckel, P., Karlsson, A., Oksbjerg, N. & Soholm Petersen, J. 2000. Control of post mortem pH decrease in pig muscles: experimental design and testing of animal models. *Meat Science* 55, 131-138.

Henckel, P., Karlsson, A., Jensen, M. T., Oksbjerg, N. & Petersen, J. S. 2002. Metabolic conditions in Porcine longissimus muscle immediately pre-slaughter and its influence on peri- and post mortem energy metabolism. *Meat Science* 62, 145-155.

Herderich, M. & Gutsche, B. 1997. Tryptophan-derived bioactive compounds in food. *Food Rev. Int.* 13, 103-135.

Hofmann, K. 1994. What is meat quality? Definition, measurement and evaluation of meat quality. *Meat Focus International* February, 73-81.

Honikel, K. O. 1998. Reference methods for the assessment of physical characteristics of meat. *Meat Science* 49, 447-457.

Högberg, A., Pickova, J., Babol, J., Andersson, K. & Dutta, P. C. 2001a. Muscle lipid, vitamins E and A, and lipid oxidation as affected by diet and RN genotype in female and castrated male Hampshire crossbred pigs. *Meat Science* In press,

Högberg, A., Pickova, J., Dutta, P. C., Babol, J. & Bylund, A. C. 2001b. Effect of rearing sytem on muscle lipids of gilts and castrated male pigs. *Meat Science* 58, 223-229.

IARC. 1993. Monographs on the Evaluation of Carcinogenic Risk to Humans. *International Agency for Research on Cancer*, Vol. 56

IDF. 1991. Anhydrous milk fat, determination of peroxide value. *Standard method 74A:1991*,

Jakobsen, K. 1995. Fatty acids, possibilities of enriching meat with n-3 fatty acids. *Meat Focus International* July 1995, 286-289.

Johansson, M., Fredholm, L., Bjerne, I. & Jagerstad, M. 1995. Influence of frying fat on the formation of heterocyclic amines in fried beefburgers and pan residues. *Food and Chemical Toxicology* 33, 993-&.

Johansson, M. & Jägerstad, M. 1993. Influence of oxidized deep-frying fat and iron on the formation of food mutagens in a model system. *Food and Chemical Toxicology* 31, 971-979.

Johansson, M. A. E. & Jägerstad, M. 1996. Influence of pro- and antioxidants on the formation of mutagenic-carcinogenic heterocyclic amines in a model system. *Food Chemistry* 56, 69-75.

Jones, S. D. M. 1993. Free range pigs. *Meat Focus International* 2, 396-398.

Jonsäll, A., Johansson, L. & Lundström, K. 2000. Effects of red clover silage and RN genotype on sensory quality of prolonged frozen stored pork (*M. Longissimus dorsi*). *Food Quality and Preference* 11, 371-376.

Jonsäll, A., Johansson, L. & Lundström, K. 2001. Sensory quality and cooking loss of ham muscle (M. biceps femoris) from pigs reared indoors and outdoors. *Meat Science* 57, 245-250.

Jonsäll, A., Johansson, L., Lundström, K., Andersson, K. H., Nilsen, A. N. & Risvik, E. 2002. Effects of genotype and rearing system on sensory characteristics and preference for pork (M. Longissimus dorsi). *Food Quality and Preference* 13, 73-80.

Josell, Å., Martinsson, L., Borggaard, C., Andersen, J. R. & Tornberg, E. 2000. Determination of RN- phenotype in pigs at slaughter-line using visual and near-infrared spectroscopy. *Meat Science* 55, 273-278.

Josell, Å., Enfält, A. C., von Seth, G., Lindahl, G., Hedebro-Velander, I., Andersson, L. & Lundström, K. 2003a. The influence of RN genotype, including the new V199I allele, on the eating quality of pork loin. *Meat Science* 65, 1341-1351.

Josell, Å., Martinsson, L. & Tornberg, E. 2003b. Possible mechanism for the effect of the RN- allele on pork tenderness. *Meat Science* 64, 341-350.

Jägerstad, M., Laser Reuterswärd, A., Öste, R., Dahlqvist, A., Olsson, K., Grivas, S. & Nyhammar, T. 1983. Creatine and Maillard reaction products as precursors of mutagenic compounds formed in fried beef. In G. R. a. F. Waller, M.S., *The Maillard reaction in foods and nutrition.* American chemical society. Washington DC. 507-519 pp.

Jägerstad, M., Skog, K., Arvidsson, P. & Solyakov, A. 1998. Chemistry, formation and occurence of genotoxic heterocyclic amines identified in model systems and cooked foods. *Z Lebensm Unters Forsch A* 207, 419-427.

Karlsson, A., Enfält, A.-C., Essen-Gustavsson, B., Lundström, K., Rydmher, L. & Stern, S. 1993. Muscle histochemical and biochemical properties in relation to meat quality during selection for increased lean tissue growth rate in pigs. *Journal of Animal Science* 71, 930-938.

Kauffman, R. G., Eikelenboom, G., van der Wal, P. G., Merkus, G. & Zaar, M. 1986. The use of filter paper to estimate drip loss of porcine musculature. *Meat Science* 18, 191-200.

Kauffman, R., Sybesma, W., Smulders, F. J. M., Eikelenboom, G., Engel, B., van Laack, R. L. J. M., Hoving-Bolink, A. H., Sterrenburg, P., Nordheim, E. V., Walstra, P. & van der Wal, P. G. 1993. The effectiveness of examining early post-mortem musculature to predict ultimate pork quality. *Meat Science* 34, 283-300.

Keating, G. A. & Bogen, K. T. 2001. Methods for estimating heterocyclic amine concentrations in cooked meats in the US diet. *Food and Chemical Toxicology* 39, 29-43.

Klont, R. E., Brocks, L. & Eikelenboom, G. 1998. Muscle fibre type and meat quality. *Meat Science* 49, S219-S229.

Klont, R. E., Hulsegge, B., Hoving-Bolink, A. H., Gerritzen, M. A., Kurt, E., Winkelman-Goedhart, H. A., de Jong, I. C. & Kranen, R. W. 2001. Relationships between behavioral and meat quality characteristics of pigs raised under barren and enriched housing conditions. *Journal of Animal Science* 79, 2835-2843.

Knize, M. G., Andresen, B. D., Healy, N., Shen, N. H., Lewis, P. R., Bjeldanes, L. F., Hatch, F. T. & Felton, J. S. 1985. Effects of temperature, patty thickness and fat content on the production of mutagens in fried ground beef. *Food and Chemical Toxicology* 23, 1035-1040.

Knize, M. G., Dolbeare, F. A., Carroll, K. L., Moore, I., D. H. & Felton, J. S. 1994. Effect of cooking time and temperature on the heterocyclic amine content of fried beef patties. *Food and Chemical Toxicology* 32, 595-603.

Koga, K., Fukunaga, T., Ohiki, Y. & Kawaida, H. 1985. Free amino acids and carnosine contents in the lean meats (Longissimus dorsi and Biceps femoris)from the strain and the strain-cross pigs. *Bulletin of the faculty of agriculture* 35, 65-73.

KRAV. 2004. KRAV standards - January 2004.

Kristensen, L., Therkildsen, M., Riis, B., Sorensen, M. T., Oksbjerg, N., Purslow, P. P. & Ertbjerg, P. 2002. Dietary-induced changes of muscle growth rate in pigs: Effects on in vivo and postmortem muscle proteolysis and meat quality. *Journal of Animal Science* 80, 2862-2871.

Lambooij, E., Hulsegge, B., Klont, R. E., Winkelman-Goedhart, H. A., Reimert, H. G. M. & Kranen, R. W. 2004. Effects of housing conditions of slaughter pigs on some post mortem muscle metabolites and pork quality characteristics. *Meat Science* 66, 855-862.

Laser Reuterswärd, A., Skog, K. & Jägerstad, M. 1987. Mutagenicity of pan-fried bovine tissues in relation to their content of creatine, creatinine, monosaccharides and free amino acids. *Food and Chemical Toxicology* 25, 755-762.

Lawrie, R. 1992. Conversion of muscle to meat. In D. E. Johnston, M. K. Knight and D. A. Ledward, *The chemistry of muscle based foods.* Royal Society of chemistry. Cambridge. 43-61 pp.

Layton, D. W., Bogen, K. T., Knize, M. G., Hatch, F. T., Johnson, V. M. & Felton, J. S. 1995. Cancer Risk of Heterocyclic Amines in Cooked Foods - an Analysis and Implications for Research. *Carcinogenesis* 16, 39-52.

Le Roy, P., Naveau, J., Elsen, J. M. & Sellier, P. 1990. Evidence for a new major gene influencing meat quality in pigs. *Genetical Research, Cambridge* 55, 33-40.

Le Roy, P., Elsen, J. M., Caritez, J.-C., Talmant, A., Juin, H., Sellier, P. & Monin, G. 2000. Comparison between the three porcine genotypes for growth, carcass composition and meat quality traits. *Genet. Sel. Evol.* 32, 165-186.

Lebret, B., Le Roy, P., Monin, G., Lefaucheur, L., Caritez, J. C., Talmant, A., Elsen, J. M. & Sellier, P. 1999a. Influence of the three RN genotypes on chemical composition, enzyme activities, and myofiber characteristics of porcine sceletal muscle. *Journal of Animal Science* 77, 1482-1489.

Lebret, B., Lefaucheur, L. & Mourot, J. 1999b. La qualité de la viande de porc. Influence des facteurs d´élevage non génétiques sur les caractéristiques du tissu musculaire. *INRA Prod. Anim* 12, 11-28.

Lebret, B., Massabie, P., Granier, R., Juin, H., Mourot, J. & Chevillon, P. 2002. Influence of outdoor rearing and indoor temperature on growth performance, carcass, adipose tissue and muscle traits in pigs, and on the technological and eating quality of dry-cured hams. *Meat Science* 62, 447-455.

Ledward, D. A. 1992. Colour of raw and cooked meat. In J. D.E., M. K. Knight and D. A. Ledward, *The chemistry of muscle based foods.* Royal Society of Chemistry. Cambridge. 128-144 pp.

Lefaucheur, L., Ledividich, J., Mourot, J., Monin, G., Ecolan, P. & Krauss, D. 1991. Influence of Environmental-Temperature on Growth, Muscle and Adipose-Tissue Metabolism, and Meat Quality in Swine. *Journal of Animal Science* 69, 2844-2854.

Leseigneur-Meynier, A. & Gandemer, G. 1991. Lipid composition of pork muscle in relation to the metabolic type of the fibres. *Meat Science* 29, 229-241.

Lewis, P. K., Rakes, L. Y., Brown, C. J. & Noland, P. R. 1989. Effect of exercise and pre-slaughter stress on pork muscle characteristics. *Meat Science* 26, 121-129.

Lien, R., Hunt, M. C., Anderson, S., Kropf, D. H., Loughin, T. M., Dikeman, M. E. & Velazco, J. 2002. Effects of endpoint temperature on the internal color of pork patties of different myoglobin form, initial cooking state, and quality. *Journal of Food Science* 67, 1011-1015.

Lindahl, G., Lundström, K. & Tornberg, E. 2001. Contribution of pigment content, myoglobin forms and internal reflectance to the colour of pork loin and ham from pure breed pigs. *Meat Science* 59, 141-151.

Lindahl, G., Enfält, A.-C., von Seth, G., Josell, A., Hedebro-Velander, I., Andersen, H. J., Braunschweig, M., Andersson, L. & Lundström, K. 2004. A second mutant allele (V199I) at the PRKAG3 (RN) locus- Effect on technological meat quality of pork loin. *Meat Science* 66, 609-619.

Lindén, A., Andersson, K. & Oskarsson, A. 2001. Cadmium in organic and conventional pig production. *Archives of Environmental Contamination and Toxicology* 40, 425-431.

Lundström, K., Essén-Gustavsson, B., Rundgren, M., Edfors-Lilja, I. & Malmfors, G. 1989. Effect of halothane genotype on muscle metabolism at slaughter and its relstionship with meat quality:a within-litter comparison. *Meat Science* 25, 251-263.

Lundström, K., Andersson, A. & Hansson, I. 1996. Effect of RN gene on technological and sensory meat quality in crossbred pigs with Hampshire as terminal sire. *Meat Science* 42, 145-153.

Lundström, K. & Enfält, A.-C. 1997. Rapid prediction of phenotype in pigs by means of meat juice. *Meat Science* 45, 127-131.

Lundström, K., Enfält, A.-C., Hansson, I., Essén-Gustavsson, B., Johansson, M. & Tornberg, E. 1998a. Technological meat quality in carriers and non-carriers of the RN-allele in Hampshire crosses with a low or high lean meat content. In *The 44:th International Congress of Meat Science and Technology,* Barcelona, Spain. 910-911.

Lundström, K., Enfält, A.-C., Tornberg, E. & Agerhem, H. 1998b. Sensory and technological meat quality in carriers and non-carriers of the RN- allele in Hampshire crosses and in purebred Yorkshire pigs. *Meat Science* 48, 115-124.

MacDougall, D. B. 1982. Changes in the colour and opacity of meat. *Food Chemistry* 9, 75-88.

Mayoral, A. I., Dorado, M., Guillén, M. T., Robina, A., Vivo, J. M., Vázquez, C. & Ruiz, J. 1999. Development of meat and carcass quality characteristics in Iberian pigs reared outdoors. *Meat Science* 52, 315-324.

Milan, D., Jeon, J.-T., Looft, C., Amarger, V., Robic, A., Thelander, M., Rogel-Gaillard, C., Paul, S., Iannuccelli, N., Rask, L., Ronne, H., Lundström, K., Reinsch, N., Gellin, J., Kalm, E., Le Roi, P., Chardon, P. & Andersson, L. 2000. A mutation in PRKAG3 associated with excess glycogen content in pig skeletal muscle. *Science* 288, 1248-1251.

Miller, K. D., Ellis, M., Bidner, B., McKeith, F. K. & Wilson, E. R. 2000. Porcine longissimus glycolytic potential level on growth performance, and carcass and meat quality characteristics. *Journal of Muscle Foods* 11, 169-181.

Millet, S., Hesta, M., Seynaeve, M., Ongenae, E., De Smet, S., Debraekeleer, J. & Janssens, G. P. J. in press. Performance, meat and carcass traits of fattening pigs with organic versus conventional housing and nutrition. *Livestock production Science* In Press, Corrected Proof.

Monin, G. & Sellier, P. 1985. Pork of low technological quality with a normal rate of muscle pH fall in the immediate post-mortem period: The case of the Hampshire breed. *Meat Science* 132, 49-63.

Mottram, D. S. 1998. Flavour formation in meat and meat products: a review. *Food Chemistry* 62, 415-424.

Muriel, E., Ruiz, J., Ventanas, J. & Antequera, T. 2002. Free-range rearing increases (n-3) polyunsaturated fatty acids of neutral and polar lipids in swine muscles. *Food Chemistry* 78, 219-225.

Murkovic, M., Weber, H. J., Geiszler, S., Frohlich, K. & Pfannhauser, W. 1999. Formation of the food associated carcinogen 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) in model systems. *Food Chemistry* 65, 233-237.

Nagao, M. E. 2000. *Food borne carcinogens, heteocyclic amines*. John Wiley & Sons Ltd. 373 pp.

Naveau, J. 1986. Contribution a l´étude du déterminisme génétique de la qualité de viande porcine, herabilité du rendement technologique Napole. *Journées de la Reserche Porcine en France* 18, 265-276.

NFA. http://www.slv.se/. 2003. (accessed 031228).

Ngapo, T. M., Dransfield, E., Martin, J.-F., Magnusson, M., Bredahl, L. & Nute, G. R. 2004. Consumer perceptions: pork and pig production. Insights from France, England, Sweden and Denmark. *Meat Science* 66, 125-134.

Nilzén, V., Aurasaari, S., Lundström, K., Jägerstad, M. & Skog, K. 1999. A screening of selected pig meat components and some precursors of heterocyclic amines as related to feeding regime, RN genotype and sex. In *45th International Congress on Meat Science and Technology,* Yokohama, Japan. 722-723.

NMKL. 1991. Moisture and ash. Gravimetric determination in meat and meat products. *Nordic committee on Food Analysis*, *No 23, 3rd ed. UDC 637.5*,

Nyhammar, T. 1986. Studies on the Maillard reaction and its role in the formation of food mutagens. *Doctoral thesis*, Swedish University of Agricultural Sciences.

Offer, G. & Knight, M. K. 1988a. The structural basis of water-holding in meat. Part 2: Drip losses. In R. Lawrie, *Developments in meat science 4*. Elsevier Applied Science Publication. London. 172-234 pp.

Offer, G. & Knight, P. 1988b. The structural basis of water-holding in meat. Part 1: general principles and water uptake in meat processing. In R. Lawrie, *Developments in meat Science 4*. Elsevier Applied Science Publication. London. 63-157 pp.

Offer, G., Knight, P., Jeacocke, R., Almond, R., Cousins, T., Elsey, J., Parsons, N., Sharp, A., Starr, R. & Purslow, P. P. 1989. The structural basis of the water-holding, appearance and toughness of meat and meat products. *Food Microstructure* 8, 151-170.

O'Halloran, G. R., Troy, D. J., Buckley, D. J. & Reville, W. J. 1997. The role of endogenous proteases in the tenderisation of fast glycolysing muscle. *Meat Science* 47, 187-210.

Ohgaki, H., Takayama, S. & Sugimura, T. 1991. Carcinogenicities of Heterocyclic Amines in Cooked Food. *Mutation Research* 259, 399-410.

Pais, P., Salmon, S. P., Knize, M. G. & Felton, J. S. 1999. Formation of mutagenic/carcinogenic heterocyclic amines in dry-heated model systems, meats, and meat drippings. *Journal of Agricultural and Food Chemistry* 47, 1098-1108.

Pais, P., Tanga, M. J., Salmon, C. P. & Knize, M. G. 2000. Formation of the mutagen IFP in model systems and detection in restaurant meats. *Journal of Agricultural and Food Chemistry* 48, 1721-1726.

Pearson, A. M., Harrington, G., West, R. G. & Spooner, M. E. 1962. The browning produced on heating of fresh pork. I. The relation of intensity of browning to chemical constituents and pH. *Journal of Food Science* 27, 177-182.

Pearson, A. M., Tarladgis, B. G., Spooner, M. E. & Quinn, J. R. 1966. The browning produced on heating fresh pork. II: Nature of the reaction. *Journal of Food Science* 31, 184-190.

Pearson, A. M., Chen, C., Gray, J. I. & Aust, S. D. 1992. Mechanism(s) involved in meat mutagen formation and inhibition. *Free Radical Biology & Medicine* 13, 161-167.

Penny, I. F. 1969. Protein denaturation and water-holding capacity in pork muscle. *Journal of Food Technology* 4, 269-273.

Persson, E., Sjöholm, I. & Skog, K. 2003. Effect of high water-holding capacity on the formation of heterocyclic amines in fried beefburgers. *Journal of Agricultural and Food Chemistry* 51, 4472-4477.

Petersen, J. S., Berge, P., Henckel, P. & Sorensen, M. T. 1997a. Collagen characteristics and meat texture of pigs exposed to different levels of physical activity. *Journal of Muscle Foods* 8, 47-61.

Petersen, J. S., Henckel, P., Maribo, H., Oksbjerg, N. & Sorensen, M. T. 1997b. Muscle metabolic traits, post mortem pH-decline and meat quality in pigs subjected to regular physical training and spontaneous activity. *Meat Science* 46, 259-275.

Petersen, J. S., Henckel, P., Oksbjerg, N. & Sorensen, M. T. 1998a. Adaptations in muscle fibre characteristics induced by physical activity in pigs. *Animal Science* 66, 733-740.

Petersen, J. S., Oksbjerg, N., Jorgensen, B. & Sorensen, M. T. 1998b. Growth performance, carcass composition and leg weakness in pigs exposed to different levels of physical activity. *Animal Science* 66, 725-732.

Pickova, J., Dutta, P. C., Larsson, P.-O. & Kiessling, A. 1997. Early embryonic cleavage pattern, hatching success, and egg-lipid fatty acid composition: comparison between two cod (Gadus morhua) stocks. *Canadian Journal of Fisheries and Aquatic Sciences* 54, 2410-2416.

Quali, A. 1992. Proteolytic and physicocohemical mechanisms involved in meat texture development. *Biochimie* 74, 251-265.

Riccardi, G. & Rivellese, A. A. 2000. Dietary treatment of the metabolic syndrome - the optimal diet. *British Journal of Nutrition* 83, S143-S148.

Risvik, E. 1994. Sensory properties and preferences. *Meat Science* 36, 67-77.

Rosenvold, K., Laerke, H. N., Jensen, S. K., Karlsson, A. H., Lundström, K. & Andersen, H. J. 2001a. Strategic finishing feeding as a tool in the control of pork quality. *Meat Science* 59, 397-406.

Rosenvold, K., Petersen, J. S., Laerke, H. N., Jensen, S. K., Therkildsen, M., Karlsson, A. H., Moller, H. S. & Andersen, H. J. 2001b. Muscle glycogen stores and meat quality as affected by strategic finishing feeding of slaughter pigs. *Journal of Animal Science* 79, 382-391.

Rosenvold, K. & Andersen, H. J. 2003. Factors of significance, for pork quality - a review. *Meat Science* 64, 219-237.

Sather, A. P., Jones, S. D. M., Schaefer, A. L., Colyn, J. & Robertson, W. M. 1997. Feedlot performance, carcass composition and meat quality of free range reared pigs. *Canadian Journal of Animal Science* 77, 225-232.

Scholderer, J., Asger Nielsen, N., Bredahl, L., Claudi-Magnussen, C. & Lindahl, G. 2004. Organic in the head? Separating the effects of label information and actual meat type on consumer perceptions on pork quality. In *2004 Food Congress: Life Style Challenges to Food Science & Technology,* Copenhagen, Denmark.

Schäfer, A., Rosenvold, K., Purslow, P. P., Andersen, H. J. & Henckel, P. 2002. Physiological and structural events postmortem of importance for drip loss in pork. *Meat Science* 61, 355-366.

Sellier, P. & Monin, G. 1994. Genetics of pig meat quality: A review. *Journal of Muscle Foods* 5,

Sellier, P. 1998. Genetics of meat and carcass traits. In M. F. Rothschild and A. Ruvinsky, *The genetics of the pig.* CAB International. Cambridge. 463-510 pp.

Sheard, P. R., Enser, M., Wood, J. D., Nute, G. R., Gill, B. P. & Richardson, R. I. 2000. Shelf life and quality of pork and pork products with raised n-3 PUFA. *Meat Science* 55, 213-221.

SIGMA. 1995. Glucose (HK). Procedure No. 16-UV. *SIGMA diagnostics*.

Sinha, R., Knize, M. G., Salmon, C. P., Brown, E. D., Rhodes, D., Felton, J. S., Levander, O. A. & Rothman, N. 1998. Heterocyclic amine content of pork products cooked by different methods and to varying degrees of doneness. *Food and Chemical Toxicology* 36, 289-297.

Sinha, R., Chow, W. H., Kulldorff, M., Denobile, J., Butler, J., Garcia-Closas, M., Weil, R., Hoover, R. N. & Rothman, N. 1999. Well-done, Grilled Red Meat Increases the Risk of Colorectal Adenomas. *Cancer Res* 59, 4320-4324.

Skog, K. & Jägerstad, M. 1990. Effects of monosaccarides and disaccarides on the formation of food mutagens in model systems. *Mutation Research* 230, 263-272.

Skog, K. & Jägerstad, M. 1991. Effects of glucose on the formation of PhIP in a model system. *Carcinogenesis* 12, 2297-2300.

Skog, K., Jägerstad, M. & Laser Reuterswärd, A. 1992. Inhibitory effect of carbohydrates on the formation of mutagens in fried beef patties. *Food and Chemical Toxicology* 30, 681-688.

Skog, K., Steineck, G., Augustsson, K. & Jägerstad, M. 1995. Effect of cooking temperature on the formation of heterocyclic amines in fried meat products and pan residues. *Carcinogenesis* 16, 861-867.

Skog, K., Augustsson, K., Steineck, G., Stenberg, M. & Jägerstad, M. 1997. Polar and non-polar heterocyclic amines in cooked fish and meat products and their corresponding pan residues. *Food and Chemical Toxicology* 35, 555-565.

Skog, K., Johansson, M. & Jägerstad, M. 1998. Carcinogenic heterocyclic amines in model systems and cooked foods: A review on formation, occurrence and intake. *Food and Chemical Toxicology* 36, 879-896.

Skog, K. 2002. Problems associated with the determination of heterocyclic amines in cooked foods and human exposure. *Food and Chemical Toxicology* 40, 1197-1203.

SLV. 2002. Riksmaten 1997-98. Kostvanor och näringsintag i Sverige. *Swedish National Food Administration*,

Solyakov, A. & Skog, K. 2002. Screening for heterocyclic amines in chicken cooked in various ways. *Food and Chemical Toxicology* 40, 1205-1211.

Staessen, L., DeBacquer, D., DeHenauw, S., DeBacker, G. & VanPeteghem, C. 1997. Relation between fat intake and mortality: an ecological analysis in Belgium. *European Journal of Cancer Prevention* 6, 374-381.

Stecchini, M. L., Mascarello, F. & Falaschini, A. 1990. Influence of breeding systems on pH and histochemical properties of muscle fibres in porcine M. semimembranosus. *Meat Science* 28, 279-287.

Steineck, G., Gerhardsson de Verdier, M. & Övervik, E. 1993. The epidemiological evidence concerning intake of mutagenic activity from the fried surface and the risk of cancer cannot justify preventive measures. *European Journal of Cancer Prevention* 2, 293-300.

Stern, S., Heyer, A., Andersson, A. K., Rydmher, L. & Lundström, K. 2003. Production Results and Technological meat quality for pigs in indoor and outdoor rearing systems. *Acta Agriculturae Scandinavica, Sect. A* 53, 166-117.

Stewart, J. W., Kaplan, M. L. & Beitz, D. C. 2001. Pork with a high content of polyunsaturated fatty acids lowers LDL cholesterol in women. *American Journal of Clinical Nutrition* 74, 179-187.

Stoier, S., Aaslyng, M. D., Olsen, E. V. & Henckel, P. 2001. The effect of stress during lairage and stunning on muscle metabolism and drip loss in Danish pork. *Meat Science* 59, 127-131.

Sugimura, T., Nagao, M. & Wakabayashi, K. 1982. Metabolic aspects of the comutagenic action of norharman. In R. Snyder, D. V. Parke, J. J. Kocsis, D. J. Jollow, G. C. Gibson and C. M. Witmer, *Biological reactive intermediates - II. Chemical mechanisms and biological effects.* Plenium Press. pp. 1011-1025.

Sugimura, T. 2000. Nutrition and dietary carcinogens. *Carcinogenesis* 21, 387-395.

Sulser, H. 1978. Chemie det extraktstoffe. In H. Sulser, *Die extractstoffe des fleisches.* Wissenschiftliche Verlagsgesellschaft MBH. Stuttgart. 1-37 pp.

Wahlefeld, A. W., Holz, G. & Bergmeyer, H. U. 1974. Creatine. In H. U. Bergmeyer, *Methods of enzymatic analysis.* Verlag Chemie & Academic press. Weinheim, New York and London. 1786-1790 pp.

Wakabayashi, K., Nagao, M., Esumi, H. & Sugimura, T. 1992. Food-Derived Mutagens and Carcinogens. *Cancer Research* 52, S2092-S2098.

van der Wal, P. G. 1991. Free range pigs: carcass characteristics and meat quality. In *35th International Congress of Meat Science and Technology,* Copenhagen, Denmark. 202-205.

van der Wal, P. G., Mateman, G., de Vries, A. W., Vonder, G. M. A., Smulders, F. J. M., Geesink, G. H. & Engel, B. 1993. "Scharrel" (free range) pigs: carcass composition, meat quality and taste-panel studies. *Meat Science* 34, 27-37.

van Laack, R. L. J. M., Kauffman, R. G., Sybesma, W., Smulders, F. J. M., Eikelenboom, G. & Pinheiro, J. C. 1994. Is colour brightness (L-value) a reliable indicator of water-holding capacity in porcine muscle? *Meat Science* 38, 193-201.

van Laack, R., Stevens, S. G. & Stalder, K. J. 2001. The influence of ultimate pH and intramuscular fat content on pork tenderness and tenderization. *Journal of Animal Science* 79, 392-397.

van Oeckel, M. J., Warnants, N. & Boucque, C. V. 1999. Pork tenderness estimation by taste panel, Warner-Bratzler shear force and on-line methods. *Meat Science* 53, 259-267.

Warnants, N., Van Oeckel, M. J. & Boucque, C. V. 1996. Incorporation of dietary polyunsaturated fatty acids in pork tissues and its implications for the quality of the end products. *Meat Science* 44, 125-144.

Warner, R. D., Kauffman, R. G. & Russel, R. L. 1993. Quality attributes of major porcine muscles: A comparison with the Longissimus Lumborum. *Meat Science* 33, 359-372.

Warren, K. E., Hunt, M., Kropf, D. H., Hague, M. A., Waldner, C. L., Stroda, S. L. & Kastner, C. L. 1996. Chemical properies of ground beef patties exhibiting normal and premature brown internal cooked color. *Journal of Muscle Foods* 7, 303-314.

Warriss, P. D., Kestin, S. C. & Robinson, J. M. 1983. A note on the influence of rearing environment on meat quality in pigs. *Meat Science* 9, 271-279.

Warriss, P. D., Bevis, E. A. & Ekins, P. J. 1989. The Relationships between Glycogen Stores and Muscle Ultimate Ph in Commercially Slaughtered Pigs. *British Veterinary Journal* 145, 378-383.

Vestergaard, M., Oksbjerg, N. & Henckel, P. 2000. Influence of feeding intensity, grazing and finishing feeding on muscle fibre characteristics and meat colour of semitendinosus, longissimus dorsi and supraspinatus muscles of young bulls. *Meat Science* 54, 177-185.

Vikse, R. & Joner, P. E. 1993. Mutagenicity, Creatine and Nutrient Contents of Pan Fried Meat from Various Animal Species. *Acta Veterinaria Scandinavica* 34, 363-370.

Wismer-Pedersen, J. 1988. *Köd som levnedsmiddel*. DSR Forlag. Kopenhagen. 250 pp.

Wood, J. D., Warriss, P. D. & Enser, M. B. 1992. Effects of production factors on meat quality in pigs. In D. E. Johnston and M. K. Knight, *The chemistry of muscle-based foods.* Royal Society of Chemistry. London. 3-14 pp.

Wood, J. D., Brown, S. N., Nute, G. R., Whittington, F. M., Perry, A. M., Johnson, S. P. & Enser, M. 1996. Effects of breed, feed level and conditioning time on the tenderness of pork. *Meat Science* 44, 105-112.

Wood, J. D., Richardson, R. I., Nute, G. R., Fisher, A. V., Campo, M. M., Kasapidou, E., Sheard, P. R. & Enser, M. 2004. Effects of fatty acids on meat quality: a review. *Meat Science* 66, 21-32.

Voskuil, D. W., Augustsson, K., Dickman, P. W., van't Veer, P. & Steineck, G. 1999. Assessing the Human Intake of Heterocyclic Amines: Limited Loss of Information Using Reduced Sets of Questions. *Cancer Epidemiol Biomarkers Prev* 8, 809-814.

Yaylayan, V. A. & Lachambre, S. 1990. Pyrylium betaines as reactive intermediates in maillard reaction. *Journal of Food Science* 55, 1124-1126.

Zimmerli, R., Rhyn, P., Zoller, O. & Schlatter, J. 2001. Occurrence of heterocyclic aromatic amines in the Swiss diet: analytical method, exposure estimation and risk assessment. *Food Additives & Contaminants* 18, 533-551.

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