# Development of empirical models for pork quality

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**Declaration**

I hereby declare that this thesis is of my own work and all assistance has been duly acknowledged. The results presented herein have not previously been submitted for any other degree or qualification.

Laszlo Trefan

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**Abstract**

Pork quality is an important issue for the whole meat chain, from producers, abattoirs, retailers through to costumers and is affected by a web of multi-factorial actions that occur throughout the pork production chain. A vast amount of information is available on how these diverse factors influence different pork quality traits. However, results derived from individual studies often vary and are in some cases even contradictory due to different experimental designs or different pork quality assessment techniques or protocols. Also, individual influencing factors are often studied in isolation, ignoring interacting effects. A suitable method is therefore required to account for a range of interacting factors, to combine the results from different experiments and to derive generic response-laws. The aim of this thesis was to use meta-analyses to produce quantitative, predictive models that describe how diverse factors affect pork quality over a range of experimental conditions.

***Chapter 1*** provides the necessary definitions of the pork quality traits, together with the descriptions of known influencing factors and the meta-analysis methodology. For carrying out the meta-analyses construction of a database was required, ***Chapter 2*** describes this database in detail.

It was beyond the scope of this thesis to examine the role of all known factors that affect pork quality. Therefore a subset of relevant factors needed to be selected. Selection criteria included (i) the relevance of a factor to current pork quality science and EU regulations, (ii) the availability of information about this factor as well as the (iii) feasibility of carrying out a meta-analysis according to the criteria defined in Chapter 1.

Vitamin E is used as an industrial-wide antioxidant supplement and is known to improve meat quality. However, although various experiments have been carried out to study its effect on various pork quality traits, no exact suggestion concerning the required amount of supplementation and duration have been established. In ***Chapter 3 & 4***, results of three meta-analyses, examining the effects of dietary vitamin E supplementation on pork quality, are provided and discussed. These meta-analyses are built upon published results of 13, 10 and 5 experiments, respectively. In the first meta-analysis the relationship between dietary vitamin E supplementation and accumulation as α-tocopherol in pork was studied. The relationship was found to be non-linear, thus different types of nonlinear models were tested. The best fitting nonlinear model was the Gompertz model with one random experimental factor. The analysis provided an estimate for the asymptotic value of the maximum α-tocopherol concentration accumulated in *M. longissimus* that could be achieved through dietary vitamin E supplementation (6.4 µg /g tissue). As oxidation of lipids is a major cause of deterioration in the quality of muscle foods, the second meta-analysis investigated the effect of vitamin E on lipid oxidation. Lipid oxidation levels were described as milligram of malonaldehyde equivalent per kg tissue, measured by distillation based on the Thiobarbituric Acid Reacting Substances (TBARS) method and expressed as TBARS values. The statistical analysis (using mixed models) resulted in two models for lipid oxidation. The first model predicts how TBARS change with storage time, using *M. longissimus* α-tocopherol concentration and vitamin E supplementation time as covariates. The second model describes how TBARS values are affected by vitamin E dose and storage time. Considering time trends both models suggested that TBARS increase during the initial 3-4 days post slaughter, after which an asymptotic

value is approached. In the third meta-analysis the effects of supplementary vitamin E on pork colour as an important attribute of appearance was studied. The statistical analysis (using mixed models) found significant effects of tissue α-tocopherol concentration in *M. longissimus dorsi*, as well as of storage time and storage light on pork redness (a*) and its change over time. The relationship between pork redness and α-tocopherol concentration was found to be linear, whereas the relationship between redness and storage time was more accurately described by a polynomial of third degree. Storage light was found to have a strong influence on redness.

Gender/sex is an inseparable part of an animal, and current technological attempts to manipulate gender, such as immuno castration and sperm sexing, have stimulated many studies to examine the effect of gender on pork quality. In ***Chapter 5*** the results of a meta-analysis of effects of gender, in combination with carcass weight and breed, on various pork quality traits are described and discussed. Altogether, published results of 43 references were used in this meta-analysis. Swine genders were defined as intact/entire male (EM), surgically castrated male (SM) immuno castrated male (IM) and entire female (EF). The studied traits were pH of pork at 24hr (pH24hr), pH at 45 minutes (pH45min), objective colour attributes lightness (L*), redness (a*), yellowness (b*) (in CIE colour system), colour and marbling scores, drip loss, intra muscular fat content (IMF) and P2 backfat thickness as well as sensory scores of juiciness and tenderness. Data from two different muscles, *M. longissimus* and *M. semimebranosus* were used. After standardisation of scaled traits (colour, marbling scores, juiciness, tenderness) and accounting for cold carcass weight (CW) the statistical analysis was carried out using mixed models. The analysis found a general effect of gender on each of the traits and pair-wise

comparisons identified significant differences between the genders for L* (lightness), marbling scores, IMF, P2 in *M. longissimus* and pH24hr in *M. semimebranosus*. Carcass weight dependence was found to be non-linear (quadratic) for a* (redness), and marbling scores and linear for b* (yellowness) and colour scores in *M. longissimus* and pH24hr in *M. semimebranosus*. The analysis identified significant breed effects for all traits and thus confirmed the known impact of diverse breeds on specific traits.

All of the above described meta-analyses were based on frequentist statistical methods. In **Chapter 6** the effect of gender on a subset of pork quality traits (i.e. objective colour attributes, IMF, drip loss) was additionally studied using a Bayesian approach. The findings of this approach strongly agreed with the findings of the frequentist approach.

**Chapter 7** briefly discusses the main conclusions obtained from the meta-analyses carried out and provides recommendations for future work related to pork quality.

## Publications

### Peer reviewed publications

Trefan L, Bünger L, Blom-Hansen J, Rooke JA, Salmi B, Larzul C, Terlouw C and Doeschl-Wilson A 2011. Meta-analysis of the effects of dietary vitamin E supplementation on α-tocopherol concentration and lipid oxidation in pork. Meat Science 87, 305-314. (based on **Chapter 3**)

Trefan L, Bünger L, Blom-Hansen J, Rooke JA, Salmi B, Larzul C, Terlouw C and Doeschl-Wilson A 2010. Meta-analysis of effects of dietary vitamin E and post slaughter storage conditions on changes of redness (a*) of pork. Archives Animal Breeding 53, 564-577. (based on **Chapter 4**)

Trefan L, Bünger L, Rooke JA, Blom-Hansen J, Terlouw C and Doeschl-Wilson A 2011. Meta-analysis of the effects of gender in combination with carcass weight and breed on pork. Journal of Animal Science in preparation. (based on **Chapter 5**)

### Conference proceedings

Trefan L, Bünger L and Doeschl-Wilson A 2009. Using meta-analysis to determine the influence of dietary factors on pork quality . Proceedings of the British Society of Animal Science, Southport March 2009, 107.

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# Chapter 1: General introduction

## 1.1 Introduction

Pork and pork products are an important part of the diet in the European Union. The EU produces more than one fifth of the world production of pork, which makes the pork industry an important socio-economic factor in the EU (Vidal, 2002). However, after the beginning of the new millennium EU expectations for pork production were for a slight decline, while other major pork producers in the world market (USA, Canada, Brazil and China) would keep increasing production (Eurostat, 2006). In reaction to this situation and to fulfil the consumers increasing demands for high standards regarding quality, safety, diversity, and healthiness of foods and their concerns about the production conditions (welfare aspects), the EU launched a large scale, collaborative project, named Q-Porkchains in 2007 (Q-Porkchains, 2007). The overall objective of this project is to 'improve the quality of pork and pork products for the consumer: by the development of innovative, integrated and sustainable food production chains providing high quality pork products; and; to inform and teach all participants about the latest achievements and techniques. The ultimate aim is to develop high quality pork products in sustainable production systems with low environmental impact' (Q-Porkchains, 2007).

This thesis is part of Module VI of this complex project. The objective of this module is to develop prediction models for pork quality, safety and welfare as a consequence of the production systems. In particular, the objective of the Module VI work package, to which this thesis contributes, is to integrate existing and upcoming knowledge to produce quantitative models for technological and sensory pork quality, based on animal, production and slaughter information. Therefore the aim of

this PhD-thesis is to develop quantitative models that describe how different factors in the pork production system influence various important pork quality traits.

This chapter will provide a definition of pork quality traits, a short qualitative review of known influencing factors and present the methodology of meta-analysis as a quantitative tool for integrating findings from various experiments into a comprehensive prediction model. The specific research objectives of this thesis are described in more detail in the final section of this chapter.

## 1.2 Pork quality

### *1.2.1 Concept of pork quality*

Pork quality is a complex, composite trait comprising a great multitude of traits. It covers inherent properties decisive for the suitability of the meat for further processing and storage (including retail display) and for human consumption (Rosenvold and Andersen, 2003, Kocwin-Podsiadla *et al.*, 2006). The types of traits, important for processing and storage are called *technological* pork quality traits, whereas those important for consumption are called *sensory/palatability* pork quality traits. Pork quality traits which can be assessed instrumentally are called objective and those by human sensory involvement, are called subjective pork quality traits. However it is important to note that participants in the pork production chain may have different perceptions concerning quality (e.g. carcass quality traits, wholesomeness, traceability) and may assign different importance to different traits (e.g. Andersen *et al.*, 2005).

### *1.2.2 Technological pork quality traits*

The main attributes of technological pork quality traits are acidity/alkalinity, water holding capacity, fat content and composition, colour, oxidative stability and tenderness (Rosenvold and Andersen, 2003, Kocwin-Podsiadla *et al.*, 2006).

***Measurement of the acidity or alkalinity (pH):*** One of the most important post-mortem changes is the lowering of pH in muscle due to the accumulation of lactic acid as a consequence of anaerobic metabolic processes after slaughter (Hedrick *et al.*, 1994). pH influences the enzymatic activity in the meat, the electric charges of the muscle proteins and when the pH decline is rapid and substantial it can lead to denaturation of the muscle proteins associated with loss of water binding capacity (Miller, 2002).

The pH of meat is usually measured by instrumental methods. However, pH can be measured at different time points post-slaughter (e.g. 0, 30 minutes, 1 h, 48 h); the two pH measurements commonly used are initial pH (pH45min), measured at 45 minutes post-mortem, and ultimate pH (pH24hr), measured at 24 hours post-mortem. Usually pH decreases from a value of approximately 7.2 in living muscle to an ultimate pH24hr between 5.1 and 5.5 (Kocwin-Podsiadla *et al.*, 2006).

***Water Holding Capacity:*** Water Holding Capacity (WHC) is the ability of pork to retain moisture during storage or processing. The water content in the fresh muscle is ~65-80 %. Water is held in three different ways, forming bound-, immobilised-, and

free- layers of water in the muscle (Hedrick *et al.*, 1994). During post-mortem changes this water content is released at different extent.

WHC decreases with decreasing pH until reaching the isoelectric point of the myofibrillar proteins in the pH range of 5.1 to 5.5. At higher pH electrostatic charges attract water leading to less expressed juice and lower cooking loss (Kocwin-Podsiadla *et al.*, 2006).

Despite harmonisation efforts, there are still several methods following different protocols for WHC related characteristics in use (Honikel, 1998). One usually distinguishes between two classes of methods to measure pork WHC, based on the process to which the meat has been previously subjected: (i) d*rip loss* from raw meat, and (ii) water loss from cooked meat, called *cooking loss.* More detailed description of the existing methods can be found in the Appendix.

The comparisons of the different WHC measuring methods showed that 'bag-method' gives systematically higher ~1.2% drip loss values than both 'EZ-tray method' at 24h post slaughter (Christensen, 2003) and 'tray-method' at 48h post slaughter (Lundström and Malmfors, 1985) alongside *M. longissimus.* Furthermore Christensen, (2003) and Otto *et al.* (2004) found that drip loss values measured by 'EZ-tray method' at 24 h and 48 h post slaughter, respectively depend on the transverse (dorsal, superficial, ventral) sampling positions of *M. longissimus.* Therefore these measures require special consideration in the statistical analysis.

***Pork fat content and composition:*** Historically, animal products were considered to be wholesome, versatile foods for humans and important for human health. From the 1960s however, attitudes towards fatty food began to

change and animal fats were linked to onset of cardiovascular and other diseases (Moloney, 2002), consumers currently have a greater demand for lean meat (EFSA, 2004). Fat is an essential component of meat for the consumers' perception of healthiness and sensory expectations, which influence a consumer to purchase a particular meat product. At the same time fat in meat also supplies fatty acids that cannot be synthesised by humans (Moloney, 2002).

*Intra-, intermuscular and subcutaneous fat content:* Intramuscular fat (IMF) is deposited within the muscle in loose networks of connective tissue in close proximity to blood vessels; the visible form of this type of fat is called marbling. Intermuscular fat (seam fat), in contrast, is deposited between individual muscles and contains variable quantities of adipose tissue (Hedrick *et al.*, 1994). The most visible type of pork fat is the external fat that can be separated by dissection (trimming off) from the lean part of the meat. This fat type is called subcutaneous fat and can be gauged as the fat depth at the position of last rib, at approximately 4.5 cm, 6.5 cm, 8.0 cm from the midline of the *M. longissimus* and called P1, P2, P3 backfat thickness, respectively (Whittemore, 2003). For gauging subcutaneous fat of the different parts of the animals other measures are also in use (e.g. Gispert *et al.*, 2010).

*Fat composition:* Based on chemical analysis carried out for fats as lipids, fat content of pork can be characterised by P/M/S ratios, which refers to the relative proportions of polyunsaturated fatty acids (PUFA) to monounsaturated fatty acids to saturated fatty acids, the chain length of the saturated fatty acids and omega 6 vs.

omega 3 PUFA content (Purchas, 2000). These measures of pork fat composition bear importance on how pork consumption affect different human blood parameters (i.e. level of cholesterol, blood clot mechanism) (Purchas, 2000). However, all herein mentioned fatty acids were studied (e.g. Wood, 1973, Wood *et al.*, 1978, Cameron *et al.*, 2000) and reviewed (Wood *et al.*, 2004, Wood *et al.*, 2008) in their detailed structures in pork, there were not sufficient published data to carry out any analysis of these traits in this thesis.

***Colour:*** Colour is a very important meat quality attribute as it influences the consumer in the choice of meat (Faustman and Cassens, 1990). Colour of pork can be characterised as *meat colour* and *fat colour* (Purchas, 2000).

*Meat colour:* Colour may be assessed subjectively using a standard colour chart or objectively, using a reflectometry based instrument. (Kerry *et al.*, 2002). One of the most common subjective standard colour systems is the Japanese Colour Standard (JPC) (Nakai *et al.*, 1975). Objectively the internal or surface reflectance is measured as the light scattering properties of meat proteins, which are related to the physical structure of the muscle fibres. The method measures the lightness or luminescence, L* (with value black=0 and white=100); the redness, a* (with value negative=green and positive=red)); and the yellowness, b* (with value negative=blue and positive=yellow). The colour may also be expressed in hue angle, H (colour itself with 0°=red, 90°=yellow, 180°=green and 270°=blue), and chroma (intensity or purity or saturation) values. Increased chroma values indicate increased colour intensity (CIE, 1976, HunterLab, 2008).

*Fat colour/Marbling scores:* For evaluation of marbling scores, amount of marbling in pork is subjectively determined by trained persons, based on photographs of standards of the longissimus muscle cross-section of the 10th rib (Hedrick *et al.*, 1994). One of the most common standards for assessing marbling scores is that of the (American) National Pork Producers Council (NPPC, 1999). By chemical analysis the content of IMF (marbling) in pork can be assessed objectively (Aaslyng, 2002).

***Oxidative stability:*** Lipid oxidation, along with microbial spoilage, is the primary cause of loss of quality of pork and thus determines the shelf life of pork products. Oxidation leads to the production of off-flavours and odours (Morrissey *et al.*, 1994). Indicators of the lipid oxidation can be the concentration of the products of the oxidation process themselves, one of these products is malonaldehyde (malondialdehyde) which is formed mainly when reactive oxygen species degrade polyunsaturated fatty acids. Malonaldehyde and other thiobarbituric reactive substances condense with two equivalents of thiobarbituric acid to give a fluorescent red derivative that can be assayed (Tarladgis *et al.*, 1960). Several methods (Tarladgis *et al.*, 1960, Witte *et al.*, 1970, Tarladgis *et al.*, 1964, Raharjo *et al.*, 1993) have been developed for assaying malonaldehyde content in different types of meat (Vyncke, 1975, Salih *et al.*, 1987) and meat products (Ke *et al.*, 1977).

The rate of discolouration of meat is also believed to depend on both oxidative processes and enzymatic metmyoglobin reducing systems (Faustman and Cassens, 1990).

***Mechanical tenderness:*** Although tenderness is part of the sensory characteristics of pork quality, it is also a trait with technological characteristics, and can be measured by objective, instrumental methods. Objective tenderness measurements are intended to mimic the forces produced during biting and mastication, and can be used for raw and cooked meat: in the latter case good correlation with subjective tenderness is expected (Hedrick *et al.*, 1994). The most widespread instrumental methods of measuring tenderness; are (i) 'Tensile test method', based on complete rupture of the specimen; and, (ii) 'Warner-Bratzler shear force', based on the deformation of the sample. In both cases the value of tenderness relates to the measured peak force required to achieve a certain degree of deformation (Honikel, 1998).

### *1.2.3 Sensory/palatability pork quality traits*

Sensory characteristics of pork are made up of appearance, texture, juiciness, tenderness, and flavour (Andersen *et al.*, 2005).

***Appearance:*** Consumers expect raw meat to have an attractive colour, which is some shade of red. Dark colour is often associated with lack of freshness, even though it usually indicates just coming from an older animal, or one that was slaughtered under stress. Such an impression reduces expectations for, or prejudices perception of, flavour when meat is consumed. With respect to the fat, the most desired colour is a creamy white.

The colour of the cooked products has impact on consumer enjoyment. The interior colour of many cuts also influences palatability reactions, depending on preference for rare (pink), medium (light pink to gray), or well done (uniform gray) products (Hedrick *et al.*, 1994).

***Texture:*** Textural properties of cooked meat affect its appearance and impart sensory impressions related to adhesion, meal alikeness, or fragmentation. Overcooked meat may be stringy in appearance and is associated by previous experience with dryness and lack of flavour (Claus, 1995).

***Juiciness:*** The principal sources of juiciness in meat, as detected by consumers, are intramuscular lipids and water. In combination with water, melted lipids constitute a broth that, when retained in meat, is released upon chewing. This broth also may stimulate flow of saliva and thus improve apparent juiciness (Andersen *et al.*, 2005).

***Sensory tenderness:*** The sensation of tenderness is mainly the force required to bite through a sample and beside that has several aspects, these are: softness to tongue and cheek; resistance to tooth pressure; ease of fragmentation; meal alikeness; adhesion of fibres; residue after chewing.

Major components of meat that contribute to tenderness, or lack of it, may be divided into three groups: connective tissue, muscle fibres, and adipose tissue (Miller, 2002).

***Flavour/Aroma:*** Flavour and aroma sensations result from a combination of factors that are difficult to separate. Physiologically, perception of flavour involves detection of four basic sensations (salty, sweet, sour, and bitter) by nerve endings on the surface of the tongue. Aroma is detected when numerous volatile materials stimulate nerve endings in the lining of the nasal passages. The total sensation is a combination of gustatory (taste) and olfactory (smell) stimuli (Hedrick *et al.*, 1994).

In case of pork beside the "normal" flavour, and overall palatability, categories like off-flavour, odour, warm-over-flavour or flavour of boar-taint are also in use (e.g. Jeremiah and Sather, 1999, Font i Furnols *et al.*, 2008).

***Sensory panel:*** The sensory characteristics described above are evaluated in a frame of sensory panel. Over time different sensory panel systems have been implemented, these are: difference-, paired comparison-, triangular-, alternative forced choice/3-AFC-,duo-trio-, 'A'-'not A'-, ranking-, two from five-tests, the conditions for the assessment of the sensory panel should satisfy certain requirements (Nute, 2002). Members of the panel would be trained persons (e.g. Wood *et al.*, 1986, Font i Furnols *et al.*, 2009), pork consumers (e.g. Kempster *et al.*, 1986, Font i Furnols *et al.*, 2008) or even households (e.g. Jeremiah and Sather, 1999). Comparison of four different types of rating scales: nine-point category scales, line marking, magnitude estimation and a hybrid category and line scale found that all methods are able to find significant differences between products, however category scales had a small advantage over the other methods (Lawless and

Malone, 1986), that is why for analysis, category scaled palatability traits are going to be used in this thesis.

### *1.2.4 Pork quality classes*

Based on the previously defined quality traits, four different types of pork can be characterised: *RFN*: red, firm, normal; *RSE*: redish, soft, exudative; *PSE*: pale, soft, exudative; *DFD*: dark, firm, dry. Table 1.1 shows representative values for the four different pork quality categories (Lee *et al.*, 2000).

**Table 1.1** Representative values for pork quality classes.

| Pork quality class | pH24hr | Drip loss (%) | Lightness (L*) |
|---|---|---|---|
| PSE | 5.36 (0.1) | 9.6 (1.9) | 52.8 (0.1) |
| RSE | 5.48 (0.2) | 8.1 (1.1) | 47.5 (1.2) |
| RFN | 5.63 (0.1) | 4.0 (1.3) | 45.3 (2.0) |
| DFD | 6.18 (0.2) | 1.3 (0.3) | 39.2 (2.2) |

*PSE*: pale, soft, exudative; *RSE*: redish, soft, exudative; *RFN*: red, firm, normal; *DFD*: dark, firm, dry; Drip loss is measured by 'bag-method', L* measured using CIE colour system (CIE, 1976) on pork loins at 24 h post-mortem.

## 1.3 Factors affecting pork quality

There is a substantial knowledge on factors influencing pork quality traits (Rosenvold and Andersen, 2003, Andersen *et al.*, 2005, Pisula and Florowski, 2006, Bonneau and Lebret, 2010). Although the number of these factors is tremendous,

they can be grouped into some major categories. These include ‘Animal’ related factors like gender, breed, genotype which determine the growth potential of an animal. This growth potential can be modified by factors belonging to the ‘Farming system’ or management factors, i.e. the conditions under which an animal grew up. These factors include nutrition, housing conditions and production systems. The ‘Pre-slaughter’ conditions affecting pork quality comprise factors such as pre-slaughter fasting, transport, lairage conditions (e.g. resting time, stocking density, temperature). Other factors belong to the group of ‘Slaughter’ related procedures, which include procedures how the animals are moved to the point of kill and the stunning methods. All of these factors can modify the pork quality considerably through exposing the animals to different stress levels. Finally ‘Post-slaughter handling’ of carcasses, including electrical stimulation of the carcass, or chilling protocols are also of importance for the final pork quality. Figure 1.1 shows these major groups of factors and how they relate to the meat quality traits. Brief descriptions of elements of these major factors are given in the next sections, with emphasis on the factors considered in this thesis, which were animal and nutrition related.

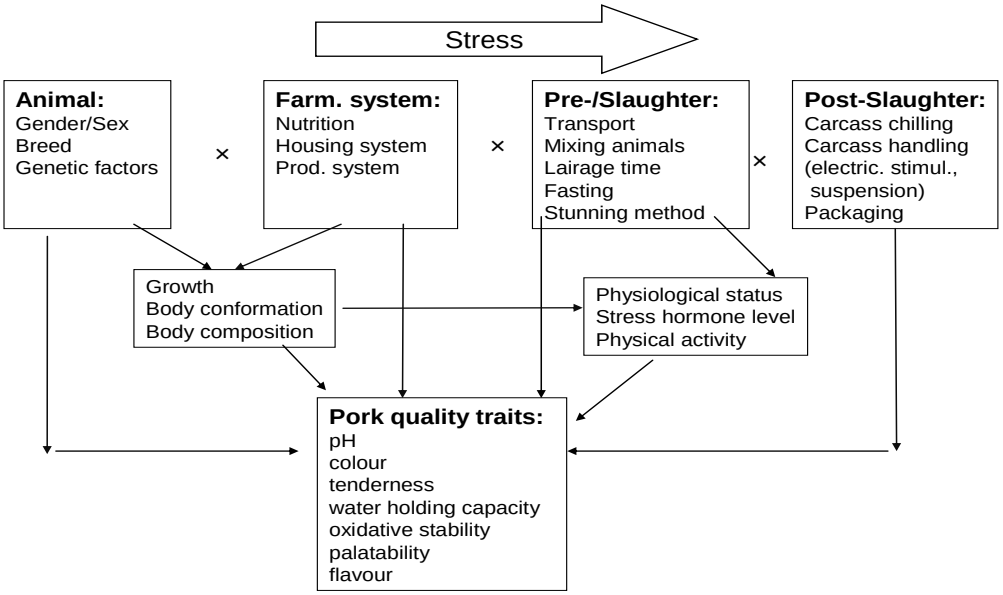


**Figure 1.1** Summary of major factors affecting pork quality.

### *1.3.1 Animal*

***Gender/sex:*** Gender/sex is an intrinsic part of an animal. It is known that boars have better feed conversion ratio than gilts or castrates (Whittemore, 1980) but develop often the so-called boar taint when left intact and slaughtered after maturity age. Surgical castration of male pigs used for meat production has been widely practised for centuries to avoid aggressive behaviour and partly because of the higher propensity of castrates to deposit fat, a commodity that had been in demand until quite recently (1960's) (EFSA, 2004). In addition to surgical castration there are new, alternative ways emerging that modify regulation of releasing of sex hormones in male pigs, one of them is based on active immunisation against gonadotropin releasing (GnRH) regulatory hormone, called immunocastration. Immunocastration

offers the possibility to exploit the growth potential of boars and simultaneously avoid boar taint (Bonneau and Lebret, 2010). Earlier studies assessing gender effects on pork quality mainly compared traits related to boars, gilts and castrates (e.g. Malmfors and Nilsson, 1978, Ellis *et al.*, 1983). Research nowadays concentrates on the effects of immunocastration (e.g. Pauly *et al.*, 2009, Zamaratskaia *et al.*, 2008). Comparison of effects of all available gender types including immunocastrates on pork quality is sparse (Gispert *et al.*, 2010).

***Breed:*** The genetic basis for the majority of contemporary commercial pig breeds around the world has been derived from relatively few breeds. Most of these have emerged in Europe and North America in the early 1900s. These breeds are: Large White, Yorkshire, Landrace, Duroc, Pietran in Europe and Hampshire, British Hampshire in North America (Whittemore and Kyriazakis, 2006). However, while for commercial pork production as business-based farm enterprise, pigs from Europe and North America are predominant, there are remaining indigenous types of breeds throughout China, South-Asia and parts of Africa and Europe. Breed comparisons regarding pork quality, including indigenous and local breeds and crosses were subject of several studies (e.g. Chang *et al.*, 2003, Edwards *et al.*, 2006, Poto *et al.*, 2007, Juárez *et al.*, 2009). Pork of some breeds like Chinese (e.g. Meshian) or Berkshire breeds have extremely high fat content, meanwhile Duroc is known to have higher content of IMF compared to the white breeds like Landrace and Large White (Aaslyng, 2002).

***Genetic effects on pork quality:*** Genetic variation among breeds or animals within a breed can be caused by a large number of genes with small effect (Fisher's infinitesimal model, Falconer, 1983), also known as polygenic inheritance, and/or a few major genes with larger effect. The first mode of inheritance seems responsible for the moderate heritability (0.15-0.30) of most pork quality traits (Sellier and Monin, 1994). Although the heritability of IMF and fat tissues is high (0.50 and 0.69, respectively) the genetic correlation between them is very low (0.11, Wood, 1990), suggesting selection for high IMF in lean carcasses. There are two well known examples for major genes: one major gene is known as halothane gene -this is a mutation of regulatory region of chromosome 6, which is responsible for the regulation of the calcium ion receptor in the muscles- causing PSE in stress exposed homozygous animals (Larzul *et al.*, 1997); the second major gene is known as Rendement Napole (RN-) gene –this is a mutation in the regulatory region of the genome, which is responsible for the regulation of energy level sensors in the muscles- associating with high glycogen storage and lower pH24hr subsequently, higher L* and inferior WHC in homo- and heterozygous animals (Le Roy *et al.*, 2000). By methods of scanning the porcine genome for loci affecting quantitative traits, called QTLs, mainly growth and carcass related traits have been identified (Bidanel *et al.*, 2001, Milan *et al.*, 2002, Mohrmann *et al.*, 2006), but some studies have also identified QTL for meat quality (Grindflek *et al.*, 2001, Ovilo *et al.*, 2002, Nii *et al.*, 2005, Lee *et al.*, 2006).

### *1.3.2 Farming system*

***Nutrition:*** Pigs' lean growth requires a certain concentration of energy: typical dietary values for mega joules of digestible energy per kilogram feed (MJ DE/kg feed) range between 12.0-15.0 MJ DE/kg feed, digestible energy (DE) per crude protein (CP) ratio ranges between 9-14 and biological value of protein (BV), which is the lysine in diet (g/kg) per CP in the diet (g/kg), ranges between 0.6-0.8 (Whittemore, 1980). Pigs are monogastric animals and many dietary components are consequently directly transferred from to feed to the muscle and fat tissues, which subsequently affect pork quality. This is true for the fatty acid composition in the diet, vitamin and mineral compositions and components giving rise to off flavours such as fishmeal (Rosenvold and Andersen, 2003). For many years there has been a strong interest in modifying animal fat composition through feeding to meet the dietary recommendations for humans, i.e. optimal ratio between saturated, monounsaturated and polyunsaturated fatty acids (Jakobsen, 1999). In monogastric animals like pigs, dietary fatty acids are absorbed unchanged from the intestine and incorporated into tissue lipids. The polyunsaturated fatty acids linoleic and α-linolenic cannot be synthesised in situ, thus tissue concentrations respond rapidly to dietary changes. In contrast, saturated and monounsaturated fatty acids are de novo synthesised, hence their concentrations are less readily influenced by diet (Wood and Enser, 1997). A consequence of the efforts for leaner carcasses resulted high content of PUFA in pork and pork products (Cameron *et al.*, 1999, Warnants *et al.*, 1998), which make them to be prone to oxidation and thus reduce shelf life (Sheard *et al.*, 2000). A strong inverse

correlation was found between the amount of fat and the concentration of PUFA (Wood and Enser, 1997).

Several metabolic modifiers have been found to have effects on pork quality traits (Pettigrew and Esnaola, 2001, Dunshea *et al.*, 2005, Dikeman, 2007). These are either vitamins -vitamin $D_3$, vitamin A and vitamin E or 'designer' lipid -conjugated linoleic acid (CLA) or minerals -chromium, manganese or hormones -porcine growth hormone or metabolic enhancers -β-agonist, although the administrations of the latter two ones are prohibited in the EU. Either CLA or vitamin E supplementation was suggested to overcome the previously described challenges set by increased PUFA content of pork (Rosenvold and Andersen, 2003) and for CLA supplementation Dunshea *et al.* (2005) established quantitative relationship between the supplementation and pork quality.

***Housing conditions:*** The housing or rearing of animals prior to slaughter can affect meat quality. These effects are mainly due to the lack of stress or the level of stress inflicted on the animal due to the rearing environment (Miller, 2002). Several indoor-, outdoor or combination of these housing types do exist in present pork production systems (Whittemore and Kyriazakis, 2006, Bonneau and Lebret, 2010). In indoor housing conditions stress factors might be: (i) temperature - animals are exposed to too high/low temperature, or inappropriate air conditioning (Whittemore, 1980) (ii) animals are kept under over-crowded conditions, there may be limited access to feed and water and animals exhibit undesirable social behaviours such as fighting, chewing and inability to rest properly. In these situations animal growth

will be affected and the subsequent meat may be lower in overall fatness and meat from these animals may have a higher incidence of quality problems related to stress during slaughter (Miller, 2002).

In outdoor rearing conditions pigs would have access of feed-stuff (forages) that may result in off-flavour in their pork (Miller, 2002).

***Production system:*** Modern intensive production systems are able to provide favourable conditions for animal growth and pork quality, and the exchange of genetic material and collaboration between countries have resulted in more homogenous systems than the traditional ones (Rosenvold and Andersen, 2003). During the past decade consumers have become more concerned about ethical aspects, animal welfare, organic farming and especially sensory characteristics of meat. This led to a change from confined to free range or other environmentally rich form of production systems in EU and North America (Andersen *et al.*, 2005), with only a few traditional production systems surviving, e.g. Iberian pig production, or the certified Italian Parma ham production. However it was found that variation of carcasses and meat quality from these 'new' production systems are not due to the rearing system alone, but genetic factors (i.e. breed), applied feeding strategies and pre-slaughter handling are just as important (Andersen *et al.*, 2005, Bonneau and Lebret, 2010).

### *1.3.3 Pre-slaughter handling*

For reducing the risk of microbial contamination during slaughter, 12-15 h fasting pre-slaughter is common practice in several countries (Pisula and Florowski, 2006). Furthermore, it is known that pigs should not be fed immediately prior to transport, as it will cause higher mortality. It was found that more than 24 h are required to find significant effect of fasting on pork quality (Eikelenboom *et al.*, 1991). Other pre-slaughter factors affecting the pork quality are mixing of unfamiliar animals, loading, transport and abattoir lairage (Faucitano, 1998). Mixing of unfamiliar animals should be avoided as it leads to additional fighting between them, because pigs in groups develop social hierarchies and mixing them requires rank of order fights to re-establish these hierarchical structures. Loading of animals at the farm and off loading them at the abattoir is the most stressful parts of animal transport. During transport the quality of vehicle, ventilation, stocking densities and travel distance are of importance for stress levels induced in pigs (Rosenvold and Andersen, 2003). Lairage time was also found to affect stress level in pigs (Faucitano, 1998).

Pre-slaughter stress can be divided into long-term stress (farm handling, mixing, loading, transport) and short-term stress (lairage conditions, driving to stunner). Long-term stress mainly leads to DFD, short-term stress to RSE or PSE pork (Rosenvold and Andersen, 2003).

### *1.3.4 Slaughter procedure*

It is a legal requirement that during slaughter all animals are rendered insensible and remain in this state until there is a complete loss of brain responsiveness due to

exsanguination (CEE, 1993). The industry considers the effect of different stunning systems on (i) meat quality, (ii) presence of haemorrhages and (iii) bone fractures. For pigs the most widely used stunning methods are carbon dioxide or electrical stunning (Rosenvold and Andersen, 2003), however applications of alternative methods (e.g. captive bolt) are still subject of scientific debate (Gregory, 1994). The biggest impact of the different slaughtering procedures is believed to be on WHC through pH decline (Rosenvold and Andersen, 2003).

### *1.3.5 Post-slaughter handling*

Chilling rate influences pork quality, as the latter one depends on the pH/temperature history of the muscle. However, several studies investigated the effects of accelerated or delayed chilling on pork quality (Maribo *et al.*, 1998), the results are still ambiguous (Rosenvold and Andersen, 2003). Another problem that might arise during accelerated chilling is cold shortening, which is a severe shortening (~60%) of the length of unrestrained muscles and can occur when temperature decreases too rapidly while the energy level in the muscle is still high (Hedrick *et al.*, 1994). Cold shortening was found to affect WHC and tenderness of pork (Rosenvold and Andersen, 2003).

However electrical simulation and suspension of pig carcasses is not as common as for beef and lamb carcasses, as it was shown that electrical simulation together with rapid chilling does not reduce WHC (Taylor and Tantikov, 1992, Taylor and Martoccia, 1995). The hanging method has also effect on pork quality. Suspension of

carcasses from the aitch bone (pelvis) shortly after slaughter, but before onset of rigor mortis was found to result in greater sarcomere length, higher tenderness and improved WHC in the stretched muscles (e.g. Taylor *et al.*, 1995).

## 1.4 Description of meta-analysis

A vast amount of studies examining the effects of various factors on diverse pork quality traits have been published over the last decades. However, most of the present knowledge is based on studies investigating the influence of a single or sometimes a few factors on pork quality, and some of the studies are of low statistical power. It has been shown that for satisfying the needs of future pork quality demands a better quantitative understanding is required of how production and slaughter factors influence pork quality and perhaps most importantly, how they interact (Rosenvold and Andersen, 2003). Substantial knowledge gain could be expected by combining the results of individual studies. In statistics, a meta-analysis combines the results of several studies that address a set of related research hypotheses. Meta-analysis analyses the results from a group of studies and is an attempt to overcome the problem of reduced statistical power in studies with small sample sizes, thus improving prediction accuracy.

Meta-analysis is a collective terminology that comprises gathering, storing and analysing data derived from different experiments, and using adequate statistical methods to integrate the findings in a robust, quantitative and comparable way

(Wang and Bushman, 1999). Unlike traditional research methods meta-analysis may use the summary statistics from individual studies as the data points. A key assumption of this analysis is that each study provides a differing estimate of the underlying relationship within the population. This way by accumulating results across studies, researchers gain a more accurate representation of the population compared to individual study estimators (Glass, 1977). Sauvant *et al.* (2008), Normand (1999) and Cook *et al.* (1995) outlined the main steps of meta-analysis.

These are:

- Setting up the aims of the analysis: what effect(s) on what trait(s) are of interest.

- Building up a database where the relevant information and data are stored. The database should satisfy certain conceptual and structural requirements (Vernet and Ortigues-Marty, 2006).

- Visual analysis of the data for detecting trends and possible inconsistencies or sources of bias, for identifying missing pieces of information and for evaluating the data distribution.

- Development of the 'meta-design', which refers to the transformation of data or new, better descriptive choice of variables if required.

- Setting up a statistical model and trying to apply it on the traits of interest with all available, accountable parameters.

– After reaching a significant statistical model, validation of the model, using independent data or contingency methods.

Since meta-analysis is partly an empirical method (Sauvant *et al.*, 2008) each of its steps has a "feedback" step, pointing to the previous one, for reaching the best final results.

Figure 1.2 shows the above described main steps of meta-analysis.

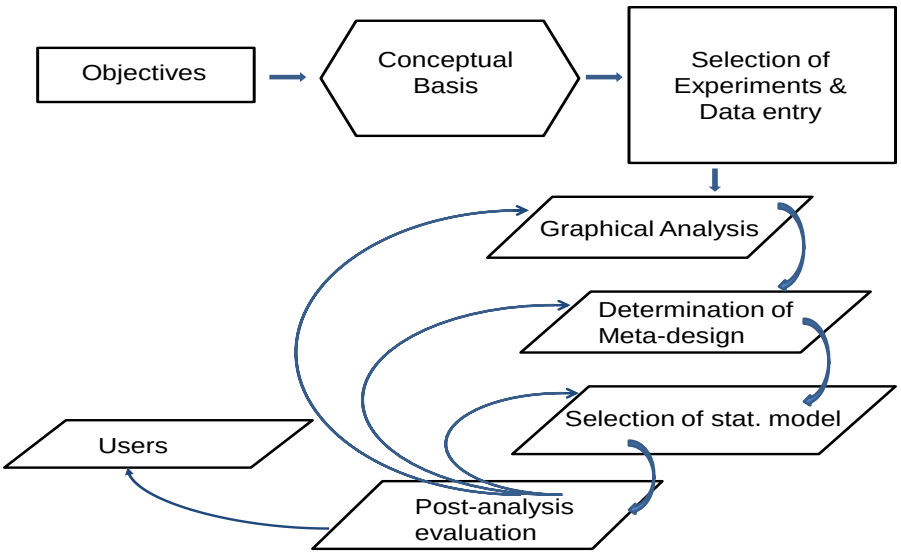


**Figure 1.2** Main steps of meta-analysis (Source: Sauvant *et al.*, 2008)

Although meta-analysis is originally of educational- and medical-research origin nowadays it is used more and more in meat- and animal science as a valuable tool for reviews (Dunshea *et al.*, 2005), or to model and to demonstrate effects of certain treatments (Schmidely *et al.*, 2008, Glasser *et al.*, 2008, Bermingham *et al.*, 2008).

In the practice of meta-analysis both classical i.e. frequentist and Bayes Theorem based methods (i.e. empirical Bayesian or full Bayesian methods), can be applied in the statistical models for parameter estimations (Normand, 1999).

### 1.5 Thesis aims

The aim of the introduction was to outline a concept of pork quality by defining pork quality traits and (qualitatively) reviewing the main factors of influence. A tremendous amount of data had been gathered and published on these factors over the last decades. This was considered to be the right time to subject existing knowledge and data to several meta-analyses, which in combination should give new insight into this complex topic. It is noteworthy that this PhD project is complemented within Q-Porkchains by a PhD project at the French National Institute of Agricultural Research (INRA) with high liaison to this thesis. Both PhD projects aimed at the study and quantification of the effects of factors on pork by meta-analytic approaches. The Scottish Agricultural College (SAC) component of this project was to focus on the influence of factors from the categories 'Animal' (excluding genetics) and 'Farming system' in Figure 1.1, whereas INRA focused on the influence of animal genes and pre-slaughter/slaughter conditions.

Selection criteria for the considered factors in both theses were:

i. Factor(s) should be relevant to current pork quality science and of interest or potential interest to the industry.

ii. A wide range of relevant factors across the whole pork production chain should be covered.

iii. No previous meta-analysis has been carried out on the (combination of) selected factor(s) and affected trait(s).

iv. Studied factor(s) should be in accordance with relevant EU regulations (i.e. regarding applied methods, supplementations). For example effects of porcine growth hormone or ractophamine as metabolic modifier were excluded as these substances are not allowed in the EU to enhance pork production.

v. The available quantitative information on the selected factor(s) must be sufficient to carry out a statistical analysis with robust statistical power.

### 1.6 Thesis outline

A pre-requisite for all statistical analyses was the gathering, handling and storing of all relevant information available about all the factors and the related experiments. Hence the first aim of this thesis was to design, establish and fill a database with relevant information on factors affecting pork quality presented in the corresponding publications. This database is described in **Chapter 2**.

The further objectives of this thesis are outlined in the following chapters:

**Chapter 3 and Chapter 4: Effect of dietary vitamin E supplementation on pork quality.** As a consequence of the efforts to obtain lean carcasses in the

past decades, the concentration of polyunsaturated fatty acids has increased and enhanced oxidative instability of pork (Rosenvold and Andersen, 2003). To overcome this challenge, supplementation with vitamin E, as industry wide used antioxidant, above maintenance level was recommended (Kerry *et al.*, 2002) and Rosenvold and Andersen (2003) suggested the existence of an optimum threshold to improve oxidative stability and colour of pork. Therefore the aim of the first meta-analysis was to study the relationship between dietary vitamin E supplementation and its accumulation in pork (Chapter 3). The second meta-analysis investigated the effect of vitamin E on lipid oxidation (Chapter 3) and the third meta-analysis aimed to study the effect of vitamin E supplementation on colour (Chapter 4).

**Chapter 5: Effect of gender, breed and slaughter weight on pork quality.** Although gender/sex is an intrinsic part of an animal, and known to affect growth, body composition and therefore also pork quality, quantitative comparisons of pork quality between different gender types in individual studies are relatively sparse (e.g. Gispert *et al.*, 2010). Often gender effects are studied together with some breed effects (e.g. Channon *et al.*, 2004). However, when effect of (live/carcass) weight is studied at individual research level only particular genders are taken account (e.g. Knudson *et al.*, 1985). Although surgical castration of male pigs has been practiced for centuries, it has lately raised animal welfare issues in the EU (EFSA, 2004). Immunocastration has been a recently approved practice in the EU as an alternative way to surgical castration (Gispert *et al.*, 2010), and has been subject of several recent studies (e.g. D'Souza and Mullan, 2003, Pauly *et al.*, 2008, Pauly *et al.*, 2009).

Therefore the aim of the fourth meta-analysis of this thesis was to provide quantitative models of the effect of gender, including immunocastrates in combination with carcass weight and breed on pork quality and to establish general relationships for several pork quality traits.

**Chapter 6: A Bayesian meta-analysis of the effects of gender, breed and carcass weight on pork quality.** The above described meta-analyses are carried out using classical, frequentist statistical models. In this chapter Bayes Theorem based statistical model was applied on a subset of pork quality traits, related to effect of gender in combination with carcass weight and breed. Bayesian methods have proved useful if limited information is available, as uncertainties about the data can be incorporated directly in the statistical models. The aim of the analysis of this chapter was to asses whether new insights regarding the relationships between gender, breed, slaughter weight and pork quality traits, or more accurate estimates, can be gained by application of this alternative approach.

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# Chapter 2: Meta-analysis database

**Abstract**

For integrating diverse factors influencing pork quality traits and the definitions of numerous traits together with relevant information about the origin and derivation, a database is required to handle these multiple pieces of information. In animal science, a meta-analysis related database has been constructed, and implemented on a special platform (Microsoft) to served specific purposes (Vernet and Ortigues-Marty, 2006). A new conception of meta-analysis database is being described here, implemented on an open source multiplatform, which can be access freely through the internet and serves as a generic platform for statistical models.

The reliability of this approach was proved by several meta-analyses using the database.

**Keywords:** meta-analysis, database

## 2.1 Introduction

Collecting information from a large variety of literature sources and re-assembling the information in new ways is fundamental to meta-analysis. Therefore a database is required to store the data for subsequent meta-analysis models.

Relational databases store information in tables and establish relations between the tables through indices and keys (primary-, secondary-, foreign key) (Date, 1993). In agricultural research relational databases have been used for different purposes (Lescourret *et al.*, 1992a, Lescourret *et al.*, 1992b) and a relational database has also been described by Vernet and Ortigues-Marty (2006) which is used for the purpose of meta-analysis. This latter database served special purposes by managing data for meta-analyses related only to ruminant metabolisms. Intense coding was required to fulfill missing dietary information in that database and specialised knowledge and permission for data entry.

In this work a new conception of a database is given, which was suitable for meta-analysis. The database itself was designed to store information related to every aspects of pork production e.g. animal welfare, consumer, quality, and to be available for every module member, located all over in Europe with equal permission rights. In this chapter only elements of this approach which were relevant to meta-analyses on pork quality are discussed.

## 2.2 Materials and methods

Important pieces of information related to the references, pork quality traits and factors were identified by the three partner organisations collaborating on this EU project: SAC (Scottish Agricultural College), INRA (French National Institute for Agricultural Research) and DMRI (Danish Meat Research Institute). Information was also gathered about the users' requirements (SAC, INRA) and the DMRI's own expertise knowledge about meat quality databases. This information established the ground for the specifications.

### *2.2.1 Specifications for the construction of the meta-analysis database*

The database was designed according to the following specifications:

1. The user should be able to enter all the different types of information available in the publications that could be relevant for subsequent analyses.
2. The database has to be able to store significant quantities of information, in a reliable way, and to easily integrate and retrieve information.
3. It must be possible to quantitatively describe all accountable factors influencing pork quality.
4. The database has to enable both the storage of statistical summary data and raw data for meta-analytic purposes.

5. Statistical values have to be traceable. It was a requirement to design the database to be able to store interactions and statistical values related to a model.
6. Data input or retrieval from the database has to be time efficient and straightforward.

### *2.2.2 Specifications for database accessibility*

The project, within framework this work had been carried out, required close cooperation between participants located all over Europe. Therefore the database had to be made globally available through internet. Additionally, since different institutions were involved in the project, with different information technological backgrounds, platform independence and use of open source software was required. This resulted in a database that could be accessed by diverse internet browsers for data input and output.

### *2.2.3 Publications used in meta-analysis database*

The publications included into the database generally referred to a single experiment. However, some publications were composed of separate experiments (e.g. Buckley *et al.*, 1989, Pauly *et al.*, 2009), and in some cases different aspects of results using the same groups of animals were found in separate publications (e.g. Chang *et al.*, 2003, Wood *et al.*, 2004). Therefore the base for identification of the results for data management was always the experiment.

The inputs for all meta-analyses, which have been carried out in this thesis, were results of the statistical models used in the original publications. Therefore, only publications that provided results of models in statistical forms of either as least square means (LS) means and standard errors of LS means (SEM) or means and standard deviations (SD), were considered for data entry.

### *2.2.4 Data modelling*

The collected information related to the references, quality traits and influencing factors offered the basis for the definition of entities. A standard entity-relationship and normalization procedure was then applied to the identified entities, which led the definition of the database tables, their keys and their relations.

## 2.3 Results

Since all meta-analyses of this thesis used published data of references, it was crucial what pieces of information made up an entity, eventually a database table related to a reference.

### *2.3.1 References in the database*

The following information was identified and defined in the database table related to a reference. First of all each reference has a unique identifier (‘idReference’). The table contains the authors’ name (‘Authors’), title (‘Title’), name (‘SourceName’),

year (‘SourceYear’) and volume (‘SourceVolume’) of the reference. Further entries refer to the starting page (‘PageFrom’) and end page (‘PageTo’) of the reference used, and it is possible to store its ISBN number (‘ISBN’) in the table. It is also possible to upload the abstract (‘Abstract’), location (‘Link’) and, if the publication was open source, the file containing the reference (‘Filename’) into named fields. For an alternative to the abstract, a field (up to 255 characters long) was defined for comments or short summary of the reference (‘Description’). It is also possible to define keywords (‘Keywords’) as combination of several predefined keywords (idKeyword0 … idKeyword10) related to the reference. The database administrated which user (‘InsertedBy’) and what date (‘InsertDate’) the information was inserted into the table. Each reference table related to a project (‘idProject’). The description of the table named ‘Ref’ can be found in Table A.1 of the Appendix.

### *2.3.2 Pork quality traits and influencing factors in the database*

From a database point of view both pork quality traits and factors were handled as common entities, defining them as ‘Methods group’. Each ‘Methods group’ was made up by related ‘Method’s and eventually each ‘Method’ contained the individual quality traits or factors having a common terminology ‘Attributes’. Short descriptions, and a few examples of what pieces of information made up the implementation of these entities in the database, are given in the following two paragraphs. The descriptions of the related tables of the ‘Methods group’, ‘Method’ and ‘Attributes’ as ‘MethodGroup’, ‘Method’ and ‘Attrib’, respectively can be found in Table A.2, A.3, A.4 in the Appendix.

*2.3.2.1 Pork quality traits in the database*

Pork quality traits were entered into the 'Methods group', named 'Meat Quality Measurements', which contained the following 'Methods': 'pH', 'Colour', 'Drip loss', Glycogen', 'Lactate', Water Holding Capacity', 'Sensorial quality', 'Cooking loss', 'Fat', 'Intra Muscular Fat', 'Marbling scores', 'Shear force', 'Dry-ashed', 'Soluble protein', 'Lean percentage'.

For example, 'Method' 'pH' contained the following pork quality traits as 'Attributes': pH-as general term, pH0min –pH measured at 0 minute slaughter, pH30min –pH measured at 30 minutes after slaughter, pH45min –pH measured at 45 minutes after slaughter, pH6hr –pH measured at 6 hours after slaughter, pH12hr –pH measured at 12 hours after slaughter, pH24hr –pH measured at 24 hours after slaughter, pH40hr -–pH measured at 40 hours after slaughter.

'Method' 'Colour' contained the following pork quality traits: Colour –stands for subjective colour scores, Colour_reflect, Colour_red, Colour_yellow –stand for lightness (L*), redness (a*) and yellowness (b*) of the pork, measured by reflectance based objective method given in CIE (CIE, 1976) or Hunter colour system (HunterLab, 2008).

'Method' 'Drip loss' contained the following traits as 'Attributes': Driploss_24 -drip loss measured by unidentified method at 24 hour after slaughter, Driploss_48 -drip loss measured by unidentified method at 48 hour after slaughter, Driploss_bag, Driploss_cent, Driploss_EZ_tray, Driploss_filt, Driploss_tray stand for drip loss

measured by bag, centrifugation, EZ-tray, filter paper, tray method respectively. (Description of the relevant methods can be found in the Appendix).

*2.3.2.2 Factors in the database*

The following group of relevant factors as 'Methods group' described the accountable factors of the influence of: 'Breeding', 'Feeding', 'Stables and production systems', 'Transport', 'Slaughter process', 'Classification and weight', 'Chilling', 'Packing'.

For example, 'Methods Group' 'Breeding' contained only one 'Method', named 'Breed, gender', which contained the following individual factors as 'Attributes': Breed –breed of the animals e.g. Yorkshire, Landrace etc., FathersBreed, MothersBreed -breed of sire, dam, respectively, Crossbred –coded as F0 pure breed, F1 first cross, F2 second cross, Genotype –referring to major genes as halothane, Rendement Napole (RN-) genes.

'Methods group' 'Feeding' contained two 'Method's, named 'Feeding' and 'Fasting'. 'Feeding' contained all pigs' food related factors as 'Attributes': GE, DE, ME, NE –gross, digestible, metabolisable, net energy in unit of MJ/kg feed, CP_content –crude protein content in the diet in unit of %, CF_content –crude fat content in diet in unit of %, Lysine –lysine percentage in diet in unit of %, etc.; 'Fasting' contained all to pigs' fasting practices related factors as 'Attributes': Fasting Duration –duration of fasting before slaughter, Water access -–predefined as Yes/No.

‘Methods group’ ‘Stables and production systems’ contained only one ‘Method’, named ‘Housing’, which contained the following factors as ‘Attributes’: Group size-group size of animals in the experiment, Pen floor type –predefined as indoor straw/slats, access to courtyard, Temperature control –predefined as Yes/No, Temperature –internal temperature of pigsty in unit of ºC etc.

The flexibility of the database allowed us to define any type of traits or factors.

### *2.3.3 Data management*

***Data input:*** The first step of each data input was to store the details of the reference which studied the effect(s) of factor(s) and contained the required results. It meant inserting a new record into table ‘Ref’. After defining the reference, a related model was defined, which meant insert a new record into table ‘Model’. When a reference contained results of more than one experiment, new records were defined for each experiment in table ‘Model’. After defining a record of the model, studied traits of the model were defined in the table ‘AttribModel’. After the definitions of studied traits, descriptions of influencing factors in the database were handled by inserting factors into ‘ModelFactor’ tables. After defining the factors, their levels should be defined by inserting values into table ‘ModelFactorLevels’. A software function generated the possible combinations of factors with their interactions, and inserted the combinations as records in the table ‘ModelCombi’. The user selected factor combinations within a model and a software function generates the possible combinations of factor levels for each selected combination of factors inserting the

combinations as records into the table ‘ModelRow’. The user can now insert published values of a statistical model i.e. least square (LS) means or means (‘Estimate’), standard error of LS means (‘Stderr’) or standard deviation of means (‘Stddev’), number of data points used (‘N’), degree of freedom (‘DF’) and p-value (‘P Value’) into table ‘ModelMean’. Figure 2.1 shows in flow chart diagram steps of the described data input with the related database tables involved. Descriptions of these tables can be found in Tables A.5 up to A.11 in the Appendix.

Each step was administrated by the database, so that each data input related to a model, which was eventually related to a user.

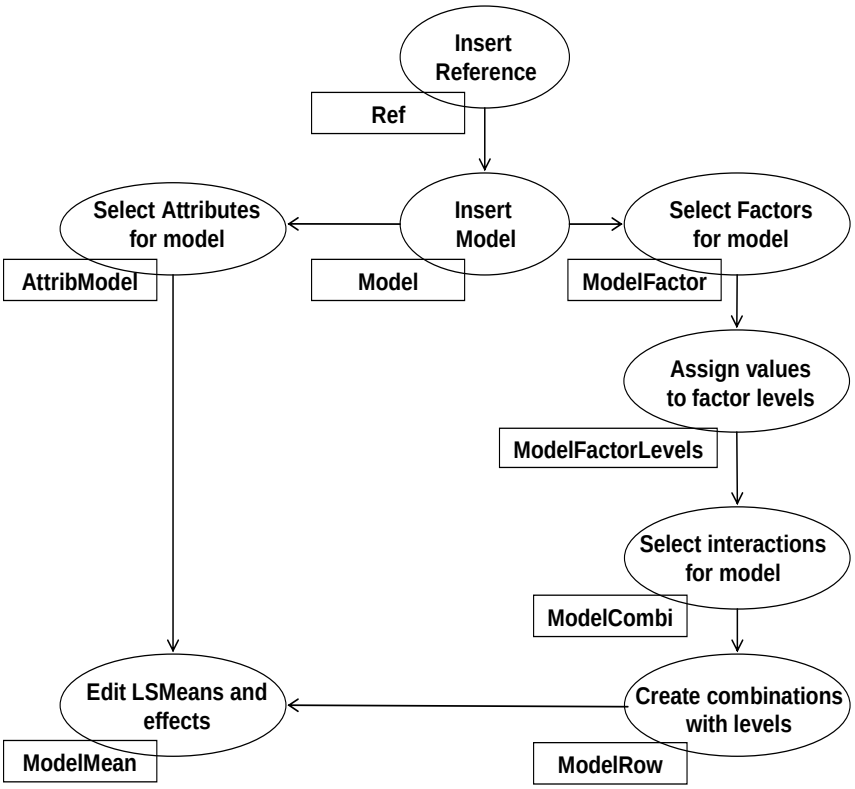


**Figure 2.1** Main steps of data input into meta-analysis database with the related tables of the database.

***Data entry:*** For satisfying the specifications of data management, making it as flexible as possible, data entry was developed in two ways: either through manually typing the data into table 'ModelMean' through a web browser or by importing the data in one of four alternative platform independent formats -tabulate, comma, semicolon or space separated ASCII file formats.

***Data output:*** Data retrieval from the database is done by queries, which produce outputs in tabulate separated ASCII file format.

### *2.3.4 Development of the rational and physical database*

Entities and relationships were translated into computer tables. The database is a relational database, which implies that the data are stored in many tables, related by index fields. For implementing the tables, a widely used language, Structured Query Language (SQL), had been chosen. After definition of tables in SQL, a conceptual database was created and users carried out functional testing of the features of the database i.e. data input, output, definition of test records with relevant traits and factors. Parallel with the user tests, tests of different versions of SQL server, operation system and web server had been carried out with special consideration placed on the speed, safety and the reliability of the database. During these tests the final version of the operating system, web and database server had reached its final shape.

Finally the open source MySQL server version 5.0.27 is used as database server. The server is running on a Linux server (Zend engine version 2.2.0) as operating system,

user access is handled by Apache 2.0 software, and the interface is programmed in PHP version 5.2.0. Wherever it was possible open source software were used.

## 2.4 Discussion

This work described a new conception of a database for meta-analytic purposes. The aim of the creation of such a database was to gather and manage information from publications related to factors affecting pork quality, and to enable the establishment of response laws relating to the different factors using meta-analysis.

The choice was made to create a relational database instead of storing the data on spreadsheets for four reasons. Firstly the expected data volumes were very high -30-40000 of records and spreadsheet software cannot handle this amount of data. Secondly, the project, the framework the database was created within, was an international project, which required close cooperation of participants considering sharing information through internet. Thirdly, the database offered the possibility of crossing influencing factors and pork quality traits, whereas spreadsheets did not have this option. Finally, databases are in everyday use in information technology and open software versions are available for any purposes related to creating and managing the databases.

There were distinguishable differences between the database described in this chapter and the previously described meta-analysis database (Vernet and Ortigues-Marty, 2006). First of all, that database used method MERISE (Lescourret *et al.*, 1992b) to define entities into tables. Firstly for the database described here, identification of pork quality traits, influencing factors and their relations were

established, based on literature (Rosenvold and Andersen, 2003, Dunshea *et al.*, 2005, Dikeman, 2007) and the DMRI's own knowledge, then for the attributes of both factors and pork quality traits normalization procedures were applied, resulting in optimal relational database tables for data storage. Secondly, in the database described by Vernet and Ortigues-Marty (2006), each meta-analysis required a new (cross) table to be defined and intense coding carried out for each table. For the purposes of our meta-analyses only conversion of units to standard units, with no intensive coding, was required, furthermore cross tables were defined using the previously pre-defined tables. Thirdly, for the implementation of the tables the previously described database used only one platform (Microsoft) and one particular software (Access), which limits its usability. The database, described in this chapter was developed to be reached by platform independent browsers, and for its implementation, open sourced software was widely used. The advantage of using the latter implementation approach was that the database could store considerably more data than the Access implemented one, because Access limits its size to 2Gygabyte.

## 2.5 Conclusion

A description of a meta-analysis database was given, which was created on multiplatform approach and served diverse purposes of the users. The reliability of this approach has been proven by the several meta-analyses which used this database for gathering information of publications (Salmi *et al.*, 2010, Trefan *et al.*, 2010, Trefan *et al.*, 2011, Chapter 5).

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# Chapter 3: Meta-analysis of the effects of dietary vitamin E supplementation on α-tocopherol concentration and lipid oxidation in pork

**Abstract**

Meta-analyses were carried out to quantify the effect of dietary vitamin E on α-tocopherol accumulation and on lipid oxidation in porcine *M. longissimus*. Published results of 13 (vitamin E accumulation) and 10 (lipid oxidation) experiments respectively were used for the analyses. After a number of standardisation procedures, a nonlinear relationship was found between the supplementary vitamin E and the accumulation of α-tocopherol in pork which approached a maximum value of 6.4 μg/g tissue. Pork lipid oxidation levels were described in terms of Thiobarbituric Acid Reacting Substances (TBARS) values. The statistical analysis revealed significant effect of vitamin E dose, muscle α-tocopherol concentration and supplementation time on TBARS, resulting in two prediction models for lipid oxidation. The first model predicts how TBARS change with storage time, using *M. longissimus* α-tocopherol content and vitamin E supplementation time as covariates with negative slopes values -0.0477(0.0073) and -0.00178 (0.0008), respectively. The second model describes how TBARS affected by vitamin E dose and storage time. Considering effect of dose, modelled least square (LS) means of TBARS decrease from 0.52(0.03) to 0.23(0.05) as dose increases from 0 IU to 300 IU. Considering time trends, both models estimated LS means of TBARS increase during the initial 3-4 days post slaughter after which an asymptote is reached and found significant differences between TBARS estimates related to short storage (≤4 days) and long (≥7 days) storage time.

Meta-analysis has proven to be a valuable tool for combining results from previous studies to quantify the effects of dietary vitamin E. Further studies, carried out with standardised experimental protocols would be beneficial for model validation and to increase the predictive power of the derived models.

**Keywords:** Vitamin E, α-tocopherol, lipid oxidation, pork quality, TBARS, meta-analysis

## 3.1 Introduction

Oxidation of lipids is a major cause of deterioration in the quality of muscle foods. Once oxidation is initiated, it continues as free radicals catalyse additional free-radical generating reactions (Jadhav *et al.*, 1996, Abuja and Albertini, 2001). These oxidative processes lead to the deterioration of flavour, odour and colour of meat (Liu *et al.*, 1995, Faustman and Cassens, 1990). Many studies have therefore aimed to delay the initiation of oxidation and subsequent loss of quality. In contrast to most synthetic antioxidants, dietary vitamin E can be directly fed to the animals and absorbed by the tissues (Buckley and Conolly, 1980). The role of vitamin E in particular, as a major lipid-soluble antioxidant, has been investigated for several decades and shown to stabilize animal products as far back as 1946 (Tsai *et al.*, 1978). The stabilizing effect of vitamin E on flavour, colour, texture and nutritive value has since been confirmed for chicken meat, beef and pork (Buckley and Conolly, 1980, Gray *et al.*, 1996).

While there is a substantial amount of literature that investigates the effects of dietary vitamin E on pork quality traits and on subcellular membranes (mitochondria, microsomes) (Buckley *et al.*, 1989, Monahan *et al.*, 1994, Asghar *et al.*, 1991) under specific experimental conditions, there have been few attempts (Apple, 2007) to unify diverse experimental findings in a statistically robust way. Review articles (Dikeman, 2007, Pettigrew and Esnaola, 2001, Buckley *et al.*, 1995, Dunshea *et al.*, 2005) have compiled relevant literature data providing thereby a good qualitative overview, but do not offer quantitative relationships.

Meta-analysis has been established as a useful statistical tool to overcome the difficulty of extrapolating general response equations from single experiments and to gain statistically reliable, robust response laws for animal science (Sauvant *et al.*, 2008, St-Pierre, 2001, Phillips, 2005). The benefits of the meta-analytic approach are that response rates can be quantified over a range of experimental conditions, thus limiting the bias that differences in experimental conditions may cause for the derivation of prediction equations (Vernet *et al.*, 2005). Whereas meta-analysis is commonly used in medical and genetic studies, its use in animal science is relatively recent with the main focus on metabolic processes in ruminants (Bermingham *et al.*, 2008, Eugène *et al.*, 2004, Dragomir *et al.*, 2008, Schmidely *et al.*, 2008) or pigs (Schulin-Zeuten *et al.*, 2007). In contrast, meta-analysis for meat quality traits has been relatively sparse in the past (Dunshea *et al.*, 2005).

The aims of this chapter were to use meta-analysis to establish quantitative relationships between different dietary vitamin E levels and their accumulation in pork in the form of α-tocopherol and to quantify the effect of dietary vitamin E supplementation on lipid oxidation in pork, which is a fundamental process for determining shelf life (Gray *et al.*, 1996).

## 3.2 Materials and methods

### *3.2.1 Data sourcing*

A list of potential studies for use in the meta-analysis was established by search of journals, conference proceedings, book articles and abstracts from various

electronic databases. Indices used in the search were combinations of the terms ‘vitamin E’, ‘alpha-tocopherol’, ‘α-tocopherol’, ‘pork’, ‘dietary’, ‘pig’, ‘swine’, ‘hog’ and any plurals thereof. In the search published information written in English from all over the world from 1970 to 2009 was included. Relevant additional information on experimental procedures and results was sometimes obtained by personal communication with the authors of the original studies and specialists in the field.

The literature search resulted in the identification initially of 60 references, which described the effect of dietary vitamin E supplementation on pork and pigs. Some of these examined aspects of vitamin E utilization (e.g. blood α-tocopherol concentration) or pork quality related issues at a cell or lower level (e.g. mitochondrial lipid changes), which were not of interest in our analysis. As a conceptual base, 40 references remained, which studied how different amounts of dietary vitamin E affected various pork quality traits (e.g. pH45min, pH24hr, colour, taste panel scores, saturated/unsaturated fatty acid ratio changes) in various muscles (*M. longissimus dorsi*, *M. psoas major*, ham, shoulder etc.) under different storage conditions (storage light, storage temperature, sample packaging). Among these references there were studies in which diets contained fat higher than 5% on a dry matter (DM) base in various fat supplements. This amount of fat supplementation might change the saturated and unsaturated fatty acid ratio in the pork, making it more susceptible to oxidation (Buckley et al., 1995). In some experiments additional antioxidants (e.g. vitamin C) were supplied or minerals (Se, Cu) added, which could interact with vitamin E utilization in meat. Since this study focused on the pure antioxidant effect of

vitamin E, we excluded all references that, to our knowledge, used treatments that could interfere or modify the effect of the vitamin.

These selection criteria together with the standardisation criteria outlined in the next section generated 13 references for the relationship between dietary vitamin E and α-tocopherol accumulation in pork and 10 references for pork lipid oxidation. Table 3.1 and Table 3.2 respectively show these references, including the main experimental details. There were 6 references among the chosen studies which studied both relationships.

**Table 3.1** References used for meta-analysis of the effect of dietary vitamin E supplementation on pork α-tocopherol concentration.

| No. of pigs/ supplementation level | Vitamin E levels (IU)* | Pig breed | Gender | Length of supplementation (days) prior to slaughter | Reference |
|---|---|---|---|---|---|
| 8/8/8 | 0/100/200 | Not published | barrow & gilt | 98 | Asghar *et al.*, 1991 |
| 15/15 | 0/100 | Hybrid | barrow & gilt | 84 | Cannon *et al.*, 1996 |
| 5/6 | 0/500 | BLxLW | not published | 46 | Cheah *et al.*, 1995 |
| 8/8/8 | 50/100/300 | Hybrid | barrow & gilt | 60 | Corino *et al.*, 1999 |
| 16/16/16/16/16 | 0/100/150/300/600 | Hybrid | barrow & gilt | 42 | Hasty *et al.*, 2002 |
| 20/20/20 | 100/200/700 | DL | boar & gilt | 105 | Jensen *et al.*, 1997 |
| 4/4/4/4 | 0/50/200/250 | 0.5Dx0.25LWx0.25L | gilt | 105 | Lanari *et al.*, 1995 |
| 12/12 | 50/200 | LxLW | boar & gilt | 14 | Monahan *et al.*, 1990a |
| 16/16 | 0/200 | LxLW | not published | 122.5 | Monahan *et al.*, 1990b |
| 29/28/29 | 0/100/300 | LW | barrow & gilt | 61 | Niculita *et al.*, 2007 |
| 9/9 | 0/200 | Pix(PixHa) | gilt | 107 | Nuernberg *et al.*, 2002 |
| 38/35 | 50/200 | Hybrid | barrow & gilt | 62 | Pfalzgraf *et al.*, 1995 |
| 14/14 | 50/400 | Dx(DLxDY) | gilt | 22 | Rosenvold *et al.*, 2002 |

BLxLW: British Landrace x Large White; DL: Danish Landrace; 0.5Dx0.25LWx0.25L: composite breed of 50% Duroc x 25% Large White x 25% Landrace; LxLW: Landrace x Large White; LW: Large White; Pix(PixHa): published in this form, no further information is available; Dx(DLxDY): Duroc x (Danish Landrace x Danish Yorkshire)
*Supplementary vitamin E rounded and expressed as IU mg/kg food

**Table 3.2** References used for meta-analysis of pork lipid oxidation.

| No. of pigs/ supplementation level | Vitamin E levels (IU)* | Pig breed | Gender | Length of supplementation (days) prior to slaughter | Reference |
|---|---|---|---|---|---|
| 6/6** | 0/200 | Not published | barrow & gilt | 28 | Buckley *et al.*, 1989 |
| 6/6** | 0/200 | Not published | barrow & gilt | 70 | Buckley *et al.*, 1989 |
| 15/15 | 0/100 | Hybrid | barrow & gilt | 84 | Cannon *et al.*, 1996 |
| 8/8/8 | 50/100/300 | Hybrid | barrow & gilt | 60 | Corino *et al.*, 1999 |
| 36/35 | 0/200 | Yx(FLxDuL) | barrow & gilt | 84 | Hoving-Bolink *et al.*, 1998 |
| 4/4/8 | 0/50/200 | 0.5Dx0.25LWx0.25L | gilt | 105 | Lanari *et al.*, 1995 |
| 12/12 | 50/200 | LxLW | boar & gilt | 14 | Monahan *et al.*, 1990a |
| 16/16 | 0/200 | LxLW | not published | 122.5 | Monahan *et al.*, 1990b |
| 29/28/29 | 0/100/300 | LW | barrow & gilt | 61 | Niculita *et al.*, 2007 |
| 12/12/12 | 0/50/100 | PIC crossbreed | barrow & gilt | 84.4 | Waylan *et al.*, 2002 |

Yx(FLxDuL): Yorkshire x (Finnish Landrace x Dutch Landrace); 0.5Dx0.25LWx0.25L: composite breed of 50% Duroc x 25% Large White x 25% Landrace; LxLW: Landrace x Large White; LW: Large White; PIC crossbreed: L326/327 boars x C22 sow of PIC, Franklin, KY

*Supplementary vitamin E rounded and expressed as IU mg/kg food

**In (Buckley *et al.*, 1989) different animals were supplemented with vitamin E for different supplementation time. They were considered here as two different experiments

### *3.2.2 Meta-analysis database*

The first pre-requisite to generate robust relationships was to establish a comprehensive database, which satisfied certain conceptual and structural requirements and described in details in Chapter 2, for meta-analytic purposes. The database includes a detailed description of each reference (e.g. type, name and date of publication, title, author names), animals used in the experiments (e.g. gender, breed, beginning or slaughter weight), diet (e.g. gross energy, crude protein, fat content), rearing conditions (e.g. indoor/outdoor, group size), slaughter processes (e.g. stunning method), pork quality traits studied in the experiment, sample size and treatment (e.g. storage temperature) as well as results from statistical analyses performed on the datasets from individual experiments.

### *3.2.3 Meta-design*

A prerequisite for meta-analysis is that the factors considered in the statistical analysis are uniform across the experiments (Vernet *et al.*, 2005). This was achieved by applying the standardisation procedures described in the three sections that follow.

#### *3.2.3.1 Dose definition for expressing vitamin E supplementation*

Vitamin E is a generic term for eight chemical compounds, namely α-, ß-, γ-, δ-tocopherol, and α-, ß-, γ-, δ-tocotrienol (Lauridsen *et al.*, 2002) and the biological

activity of each can be expressed on α-tocopherol base (Debier and Larondelle, 2005, Jensen *et al.*, 1988). α-tocopherol was used as the unified measure for vitamin E concentration in the present study. Therefore in one reference (Rosenvold *et al.*, 2002) where supplementary vitamin E was analysed and published as fed α- and γ-tocopherol, bioactivity of the latter was converted to its α-tocopherol equivalent. The base of this conversion was that 1 mg all-rac-α-tocopheryl (DL-α-tocopheryl) acetate, which is an equimolar mixture of eight synthetic α-tocopherol stereoisomers (Lauridsen *et al.*, 2002) in acetate form, has bioactivity conversion factor 1.0 and defines 1 International Unit (IU) of α-tocopherol. Furthermore, all-rac-α-tocopheryl (DL-α-tocopheryl) acetate is the industrial form of vitamin E supplementation and this was the form of supplementation used in all references. For the three different terminologies referring to α-tocopherol that had been used in these references to define vitamin E administration, i.e. vitamin E (e.g. Buckley *et al.*, 1989), α-tocopherol (e.g. Hoving-Bolink *et al.*, 1998) or analysed fed α-tocopherol (e.g. Lanari *et al.*, 1995), the amount of supplementation could thus be expressed in IU of α-tocopherol.

In some studies (e.g. Monahan *et al.*, 1990b) different amounts of vitamin E were fed for different time periods throughout the period of dietary supplementation. In these cases we defined the administrated amount of vitamin E (in IU) by calculating weighted averages with the individual time periods as weighting factors.

The different experiments varied in their definition of control vitamin E levels and in the accuracy with which supplementary vitamin E concentrations were reported. To minimise the potential bias introduced by this variation, we rounded administrated vitamin E in units of 50 IU/kg feed e.g. 25 IU/kg feed was defined as vitamin E dose

0 IU/kg feed, 35 IU/kg feed was defined as vitamin E dose 50 IU/kg feed etc. Henceforth the rounded measure is denoted as *vitamin E dose*.

*3.2.3.2 Standardisation procedures related to pork α-tocopherol concentration*

In describing the origin of the samples used to measure accumulated α-tocopherol concentration, authors used either general terms like 'ham', 'shoulder' or provided the exact muscle such as *M. longissimus lumborum, M. longissimus thoracis*, *M. longissimus dorsi*. Since 95% of the samples analysed for muscle α-tocopherol concentration were obtained from one part of the *M. longissimus* (i.e. *M. longissimus lumborum, M. longissimus thoracis* or *M. longissimus dorsi*) only data originating from this group of muscles were included in our analysis and a common term *M. longissimus* was used.

All α-tocopherol measurements in the original studies were taken between carcass chilling times of 24 and 48 hours after slaughter, or from frozen samples, and there was little variation in other storage conditions between experiments. There was no evidence in the literature that suggested these differences in chilling time and storage conditions would have a significant influence on the measured α-tocopherol levels.

Further, all the experiments considered used the same method to measure muscle α-tocopherol concentration, in which α-tocopherol was extracted from the meat sample, saponified, separated by high performance liquid chromatography and quantified with reference to an α-tocopherol standard (e.g. Buttris and Diplock, 1984).

*3.2.3.3 Analytical and standardisation procedures related to pork lipid oxidation*

In the experiments contributing to this meta-analysis lipid oxidation was measured as Thiobarbituric Acid Reacting Substances (TBARS) (Tarladgis *et al.*, 1960, Tarladgis *et al.*, 1964, Vyncke, 1975, Witte *et al.*, 1970, Raharjo *et al.*, 1993), which expressed lipid oxidation as milligram malonaldehyde (MA) equivalent / kg tissue or muscle (TBARS values). Two different TBARS methods were present in the different experiments, i.e. the distillation or the cold extraction methods. Although it is known that TBARS values obtained by cold extraction methods are approximately 0.6 of values of those obtained by distillation (Dr R. I. Richardson personal communication), a precise conversion formula does not exist. Therefore only publications where TBARS values were measured by distillation (Tarladgis *et al.*, 1960, Witte *et al.*, 1970) were included in meta-analysis. This method was used by 90% of the references. As in the case of pork α-tocopherol concentration, only TBARS values from *M. longissimus dorsi* were considered, which was provided in 90% of our data sources.

Since storage conditions may affect the rate of lipid oxidation process (Dunshea *et al.*, 2005) we selected only data where samples were: a.) kept in the dark or under natural light or where based on the storage description we could assume that samples were not exposed to illumination; b.) stored at 4 °C or based on the description we could assume that this temperature was not lower than 0 °C or higher than 5 °C; c.) wrapped in oxygen permeable material (PVC) or based on the description of storage conditions we could assume that samples were not wrapped at all.

In addition, it was necessary to standardise the time scales provided in different experiments to a uniform scale. We considered the starting point of the storage time as the time of slaughter, and expressed the storage time in units of days post slaughter, counting the day of slaughter as day zero.

### *3.2.4 Statistical analysis*

The inputs for meta-analysis were results of the statistical models used in the original publications in the form of least square (LS) means or means (if LS means were not provided) and corresponding standard errors of LS means (SEM) or standard deviations (SD) where SEM were not provided.

#### *3.2.4.1 Statistical analysis of M. longissimus α-tocopherol concentration*

Visual inspection of the published α-tocopherol concentrations in *M. longissimus* indicated a nonlinear relationship between vitamin E dose and α-tocopherol concentration in *M. longissimus* (Figure 3.1).

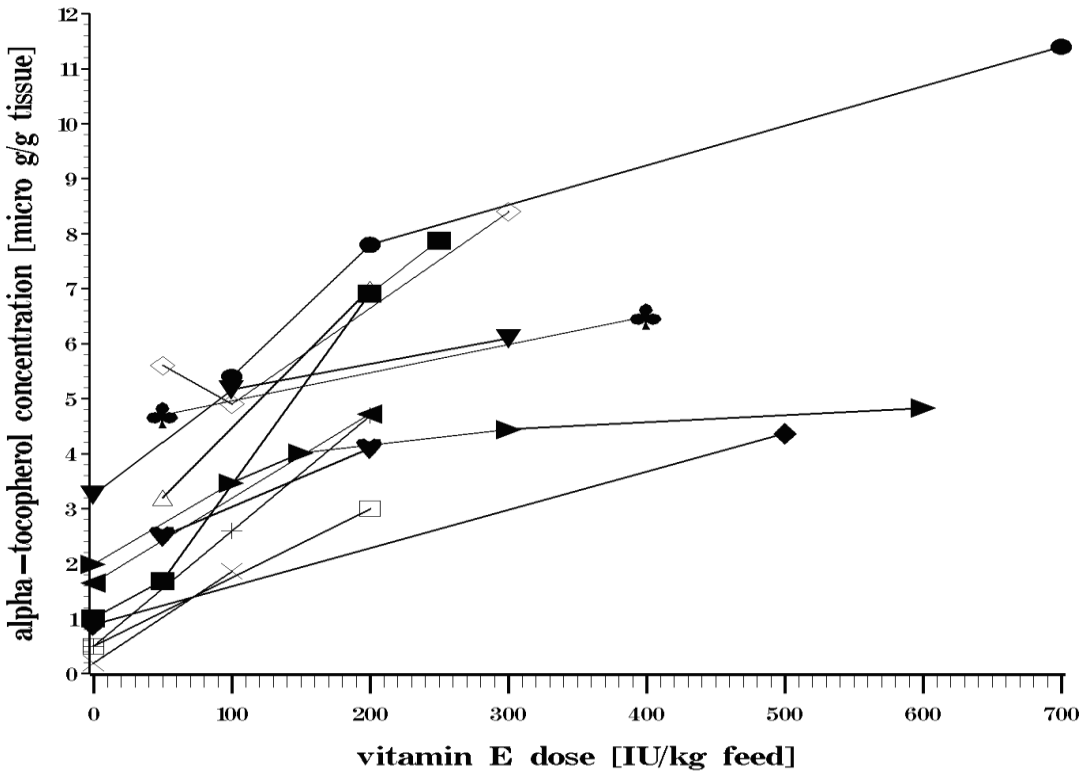



**Figure 3.1** Changes in concentrations of α-tocopherol in *M. longissimus* in response to dietary vitamin E doses. Data from 13 different studies: Asghar *et al.*, 1991 (+); Cannon *et al.*, 1996 (X); Cheah *et al.*, 1995 (♦); Corino *et al.*, 1999 (◊); Hasty *et al.*, 2002 (►); Jensen *et al.*, 1997 (●); Lanari *et al.*, 1995 (■); Monahan *et al.*, 1990a (Δ); Monahan *et al.*, 1990b (◄); Niculita *et al.*, 2007 (▼); Nuernberg *et al.*, 2002 (□); Pfalzgraf *et al.*, 1995 (♥); Rosenvold *et al.*, 2002 (♣).

Three nonlinear functions, i.e. Gompertz, logistic and exponential, commonly used to describe biological (often growth) processes that approach an asymptotic behaviour (Lambe *et al.*, 2006) were fitted to the data using the PROC NLMIXED procedure of SAS (SAS, 2000). The mathematical models and the interpretations of their parameters are shown in Table 3.3. Experiment-specific random effects were added to the initial fixed effects models as linear perturbations of one of the model parameters at a time or a combination of model parameters. Distribution of random effects was assumed to be normal. All three mathematical functions require three

parameters and were expressed in a form that provides a biological interpretation for each model parameter (Table 3.3, Lambe *et al.*, 2006).

For the Gompertz- and logistic models the estimated parameters and their respective interpretation were A: estimated final α-tocopherol concentration (µg/g tissue); B: maximum α-tocopherol concentration increase (µg/g tissue) / vitamin E dose (IU/kg feed); C: vitamin E dose at maximum α-tocopherol concentration increase (IU/kg feed).

For the exponential model the estimated parameters can be described as A: estimated final α-tocopherol concentration (µg/g tissue); $B_E$: maximum α-tocopherol concentration increase (µg/g tissue) at vitamin E dose 0 (IU/kg feed); $C_E$: initial α-tocopherol concentration (µg/g tissue).

**Table 3.3** Models used to describe the relationship between *M. longissmus* α-tocopherol concentration and vitamin E dose.

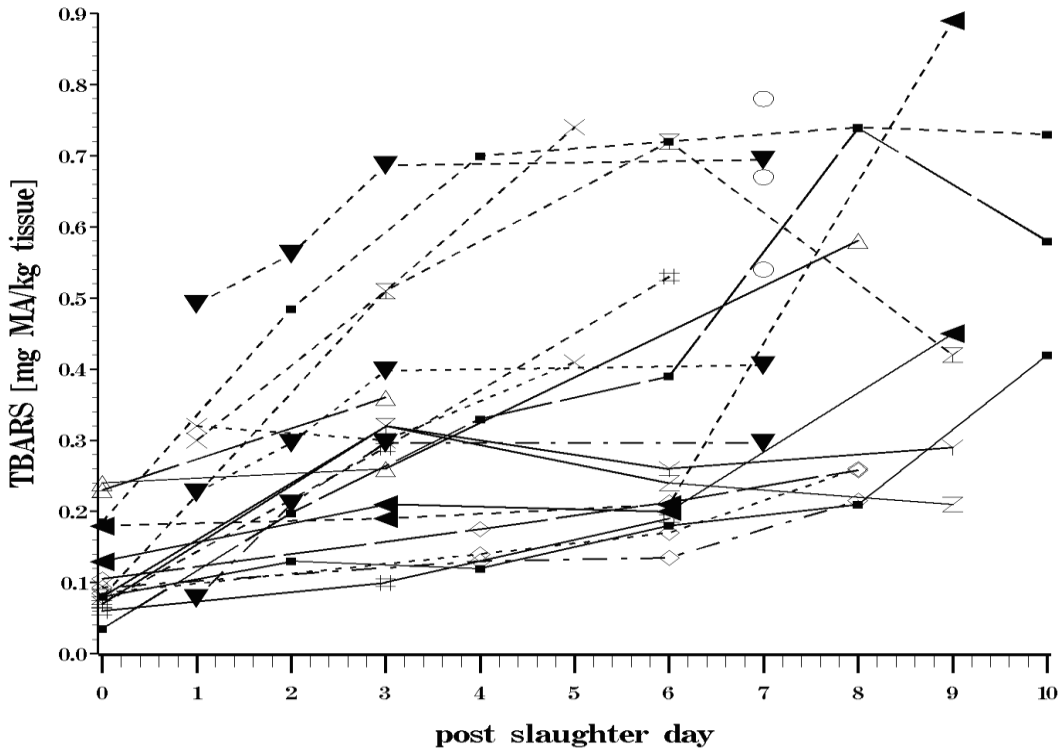
| Function name | Estimated parameter | Nonlinear function |
|---|---|---|
| Gompertz | A, B, C | $A \exp\{-\exp[Be^1(C-Dose)/A]\}$ |
| Logistic | A, B, C | $A\{1+\exp[4B(C-Dose)/A]\}^{-1}$ |
| Exponential | A, $B_E$, $C_E$ | $A-(A-C_E)\exp((-B_E*Dose)/(A-C_E))$ |

$e^1$ = Euler's number (=2.7182818)
A: estimated final α-tocopherol concentration (µg/g tissue); B: maximum α-tocopherol concentration increase/vitamin E dose (µg/g tissue/IU); C: vitamin E dose at maximum α-tocopherol concentration increase (IU); $B_E$: maximum α-tocopherol concentration increase (µg/g tissue) at vitamin E dose 0 (IU); $C_E$: initial α-tocopherol concentration (µg/g tissue) (Lambe *et al.*, 2006)

To compare the accuracy of fit of the models Akaike's information criteria (AIC) (SAS, 2000), Pearson's correlation (R) between the predicted and observed values (i.e. LS means of original experiments), RSD (residual standard deviation) and CD (coefficient of determination) were calculated. RSD was calculated as $[\Sigma\Sigma(\varepsilon_{ij})^2/(n-p)]^{1/2}$ where $\varepsilon_{ij}$ is the residual value of i-th experiment at j-th dose level, n is the number of observations and p is the number of parameters in the model. CD was calculated as $1 - \Sigma\Sigma(\varepsilon_{ij})^2/ (\Sigma\Sigma(Y_{ij})^2)$ where $\varepsilon_{ij}$ is the same as in RSD and $Y_{ij}$ is the predicted value i-th experiment at j-th dose level (Bünger and Herrendörfer, 1994). In addition visual analysis of predicted values and normality test of residuals were carried out.

*3.2.4.2 Statistical analysis of effect of vitamin E on lipid oxidation in M. longissimus dorsi*

After visual inspection of the data one outlier with an extremely high TBARS value: of 1.6 mg MA/ kg tissue (Monahan *et al.*, 1990a) was removed. Figure 3.2 shows how the remaining TBARS values of *M. longissimus dorsi* changed over time in the 10 selected experiments for different doses of vitamin E. TBARS values were below 1 mg MA/ kg tissue (Figure 3.2), because samples of the experiments considered were not exposed to illumination and were stored at standard temperature (≤ 5 ºC) therefore during the studied period of time the oxidation process has not been accelerated.




**MA: malonaldehyde**

**Figure 3.2** Changes in lipid oxidation (TBARS) in *M. longissimus dorsi* with storage time for various supplementary vitamin E doses from 10 different studies: Buckley *et al.*, 1989 (Y)*; Buckley *et al.*, 1989 (Z)*; Cannon *et al.*, 1996 (X); Corino *et al.*, 1999 (◊); Hoving-Bolink *et al.*, 1998 (#); Lanari *et al.*, 1995 (■); Monahan *et al.*, 1990a (Δ); Monahan *et al.*, 1990b (◄); Niculita *et al.*, 2007 (▼); Waylan *et al.*, 2002 (○);Vitamin E dose levels (IU):0:……;50:____ ____;100:__ __ __;200:_____; 300:____ _.
*Different animals were supplemented with vitamin E over time periods of different duration. These were considered here as two experiments.

For meta-analysis of changes in TBARS LS means with time the PROC MIXED procedure of SAS (SAS, 2000) was used where autocorrelation between repeated measurements could be taken into account (Verbeke and Molenberghs, 1997, Whitehead, 2002). The maximum fitted model contained a general intercept, α-tocopherol concentration of *M. longissimus* and vitamin E supplementation time as covariates, as well as breed, gender, vitamin E dose and storage time as fixed effects,

and interaction terms between fixed effects and between covariates and fixed effects. The random experimental effect was included as a random intercept. For those studies that did not provide measures of muscle α-tocopherol concentration we used the predicted LS mean value derived from the best fitting, nonlinear model described earlier. Autocorrelations between repeated measurements were examined using the REPEAT statement of PROC MIXED, where vitamin E dose was nested within experiment identifier and various residual covariance matrix structures (SAS, 2000) were explored (e.g. AR[1] – first-order autoregressive; SP(EXP) – exponential spatial covariance; UNR – unstructured correlated; VC – standard variance components). A stepwise approach was adopted, starting from a model that included all the fixed effects and covariates described earlier together with their interactions, followed by stepwise removal of statistically non-significant factors.

Comparisons of the different models were based on the quantities AIC, R, RSD and CD defined earlier for effects of vitamin E dose on pork α-tocopherol concentration. In addition, for each model normality tests of residuals and visual examinations of the residuals and predicted values (Sauvant *et al.*, 2008) were carried out.

No weighting of individual experiments could be applied here, because optimal weighting of the observation would require identical expression of the variability of the studied parameters (Schmidely *et al.*, 2008). Unfortunately, this was not the case in our studies: variability was expressed either as standard error of LS means (SEM), or standard deviation (SD) for the mean of each treatment group, or SD of the difference between means; in some publications variations were not even reported.

## 3.3 Results

### *3.3.1 Effect of dietary vitamin E on M. longissimus α-tocopherol concentration*

There was little difference (Table 3.4) in the goodness-of-fit measures between the three nonlinear models for *M. longissimus* α-tocopherol concentration when differences between experiments were not included as random effects. For the Gompertz and exponential functions, the goodness-of-fit increased when experiments were included as random effects in one of the model parameters, whereas for the logistic model, inclusion of a random experimental effect did not improve the goodness-of-fit (Table 3.4). Using more than one random effect in the parameters did not lead to convergence. The model producing the best goodness-of-fit based on all indicators (AIC, R, RSD and CD) was the Gompertz function, although the measures were similar to those corresponding to the exponential function, i.e. AIC values for Gompertz and exponential function, respectively, were 125 compared to 129.5, R was 0.96 compared to 0.95, RSD was 0.54 compared to 0.67 and CD was 0.98 compared to 0.97 (Table 3.4).

**Table 3.4** Comparison of goodness-of-fit indicators for each nonlinear model for *M. longissmus* α-tocopherol concentration and vitamin E dose without and with one random effect, including Akaike's information criteria values (AIC), Pearson's correlation between predicted and observed values (R), residual standard deviation (RSD) and coefficient of determination (CD).

| Nonlinear model name | AIC | R | RSD | CD |
|---|---|---|---|---|
| Gompertz | 141.7 | 0.76 | 2.92 | 0.889 |
| Logistic | 142.4 | 0.76 | 2.97 | 0.888 |
| Exponential | 141 | 0.77 | 2.86 | 0.893 |
| Gompertz -random effect in parameter A | 125 | 0.96 | 0.54 | 0.983 |
| Logistic -random effect in parameter C | 144.3 | 0.76 | 2.97 | 0.889 |
| Exponential -random effect in parameter A | 129.5 | 0.95 | 0.67 | 0.980 |

There was also little difference (Table 3.5) between the three nonlinear functions for the estimate of the α-tocopherol asymptote A with the random exponential model giving the smallest estimates (6.25 µg/g tissue) and both the fixed and random effect logistic models the largest (6.78 µg/g tissue). Inclusion of a random experimental effect in the parameter A resulted in slightly lower estimates of LS means (i.e. 6.44 vs. 6.91 for the Gompertz model and 6.25 vs. 7.28 for the exponential function, Table 3.5). In addition, the estimates of parameter B (maximum α-tocopherol concentration increase/ vitamin E dose) differed little between the Gompertz and logistic models. The logistic model however provided larger values for the parameter C (vitamin E dose at maximum increase α-tocopherol concentration) than the Gompertz model (i.e. C = 79.75 µg/g tissue for the random logistic model compared to C = 40.07 µg/g tissue for the random Gompertz model). The estimates for the parameters $B_E$ (maximum α-tocopherol concentration increase at dose 0) and $C_E$ (initial α-tocopherol concentration) of the exponential model cannot be compared

with the other two models due to their different biological meanings. However, the estimates for both fixed and random effects models were biologically realistic.

**Table 3.5** Estimated parameters of Gompertz-, logistic- and exponential models.

| | Gompertz | | Logistic | | Exponential | |
|---|---|---|---|---|---|---|
| Estimated parameters | Estimation (SE) | P-value | Estimation (SE) | P-value | Estimation (SE) | P-value |
| A | 6.91(0.87) | <0.0001 | 6.78(0.79) | <0.0001 | 7.28(1.13) | <0.0001 |
| B | 0.025(0.008) | 0.003 | 0.023(0.007) | 0.002 | | |
| C | 40.07(21.12) | 0.0661 | 79.59(29.13) | 0.0098 | | |
| $B_E$ | | | | | 0.036(0.014) | 0.0129 |
| $C_E$ | | | | | 1.41(0.55) | 0.0148 |
| $\varepsilon_{ij}$ | 2.67(0.64) | 0.0002 | 2.72(0.65) | 0.0002 | 2.67(0.63) | 0.0002 |
| $^{x}A$ | 6.44(0.86) | <0.0001 | 6.78(0.81) | <0.0001 | 6.25(0.87) | <0.0001 |
| B | 0.024(0.005) | 0.0002 | 0.023(0.007) | 0.0006 | | |
| $^{y}C$ | 28.27(12.78) | 0.0471 | 79.75(30.14) | 0.0213 | | |
| $B_E$ | | | | | 0.052(0.009) | 0.0002 |
| $C_E$ | | | | | 1.24(0.32) | 0.002 |
| $\varepsilon_{ij}$ | 0.73(0.23) | 0.0068 | 2.70(0.88) | 0.0097 | 0.91(0.29) | 0.0092 |
| $u_{ij}$ | 6.15(2.96) | 0.0597 | 10.41(1461.6) | 0.9946 | 6.60(3.48) | 0.082 |

A: estimated final α-tocopherol concentration (µg/g tissue); B: maximum α-tocopherol concentration increase/vitamin E dose (µg/g tissue/IU); C: vitamin E dose at maximum α-tocopherol concentration increase (IU); $B_E$: maximum α-tocopherol concentration increase (µg/g tissue) at dose 0 (IU); $C_E$: initial α-tocopherol concentration (µg/g tissue)

SE: standard error of estimation; $\varepsilon_{ij}$: the residual value of i-th experiment at j-th dose level; $u_{ij}$: variance of random effect of i-th experiment at j-th dose level

$^{x}$ random effect in parameter A; $^{y}$ random effect in parameter C

Finally, taking all the criteria mentioned into account, the Gompertz model with one random effect (in its parameter A) was chosen as the best fitting model. Figure 3.3 shows LS means of pork α-tocopherol concentrations predicted by this model.

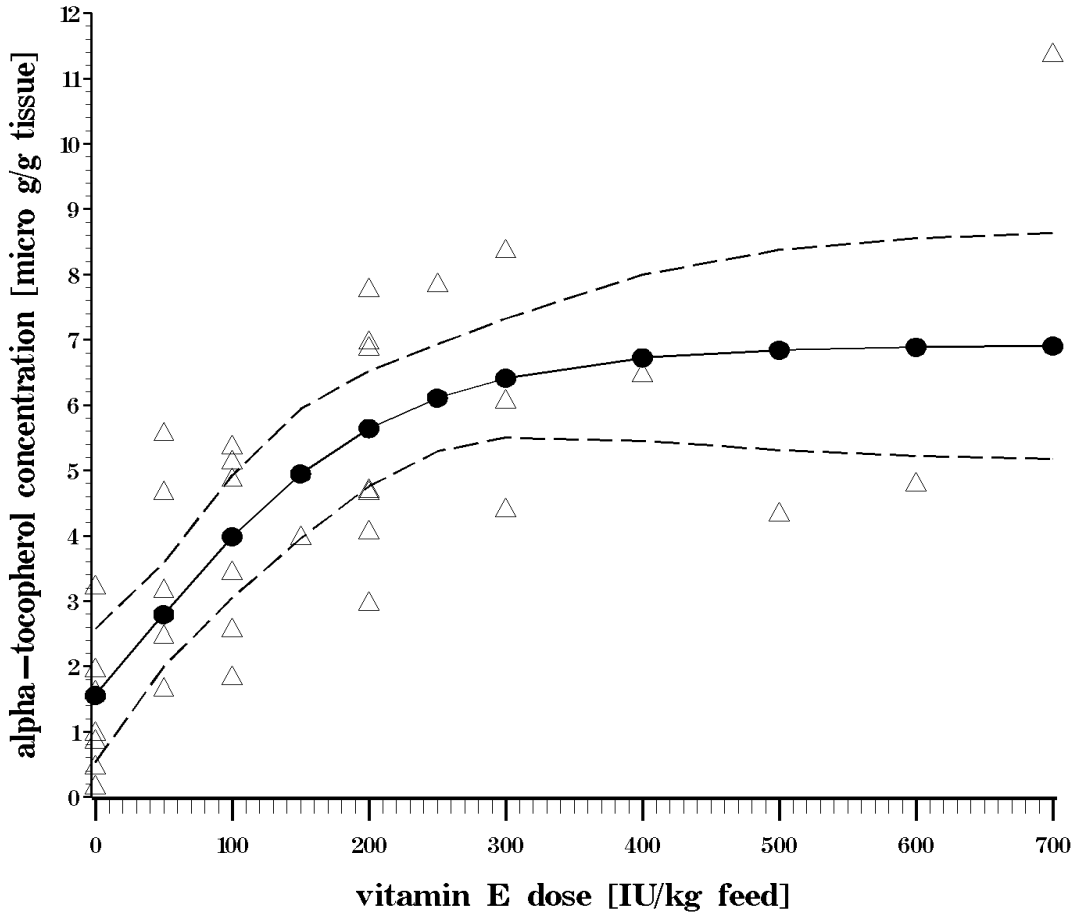



**Figure 3.3** Predicted changes in α-tocopherol concentrations in *M. longissimus* (filled circles, solid line) with 95% confidence bands (dashed lines) for increasing dietary vitamin E doses based on the best fitting, Gompertz model. (Triangles symbolise the original data.)

The lengths of vitamin E supplementation ranged from 14 to 122.5 days prior to slaughter (Table 3.1). A possible dependence of parameters on these times was studied using the best fitting Gompertz-model. Lengths of supplementation were split up into two (<70 and >70 days prior to slaughter) in the first or three (<50, >50 and <100, >100 days prior to slaughter) supplementation intervals in the second analysis. Comparisons of the parameters relating to the different supplementation intervals within one analysis were made using the NLMIXED procedure of SAS. Both analyses resulted in similar estimates for A (asymptotic α-tocopherol concentration), 5.78-7.64 and B (maximum α-tocopherol concentration increase/vitamin E dose), 0.016-0.031 with (the best fitting) original Gompertz-model. Comparisons of these parameters showed no significant differences between the different lengths of supplementation time categories. For parameter C (vitamin E dose at maximum α-tocopherol concentration increase) both analyses yielded higher estimates for longer supplementation times (>70 and >100 days) than the original model, 82.44 and 73.61, respectively vs. 28.27, and only these estimates were significantly different from zero at 95% confidence level. Comparisons showed significant difference between these parameters belonged to higher (>70 or >100 days) and lower (<70 or <50 days) lengths of supplementation, respectively (Table 3.6).

**Table 3.6** Parameters and their comparisons estimated by best fitting Gompertz model for different categories of vitamin E supplementation time.

| | Length of vitamin E supplementation (prior to slaughter) in days | | Length of vitamin E supplementation (prior to slaughter) in days | | |
|---|---|---|---|---|---|
| | <70 | >70 | <50 | >50 & <100 | >100 |
| Estimated parameters | Estimation (SE) | Estimation (SE) | Estimation (SE) | Estimation (SE) | Estimation (SE) |
| A -random effect in parameter | 6.44(1.11)[a] | 6.96(1.23)[a] | 5.78(1.43)[a] | 6.62(1.89)[a] | 7.64(1.52)[a] |
| B | 0.021(0.007)[a] | 0.031(0.005)[a] | 0.027(0.013)[a] | 0.016(0.005)[a] | 0.031(0.005)[a] |
| C | ***-8.49(15.18)[a] | 82.44(14.81)[b] | ***-0.71(18.59)[a] | ***19.43(36.11)[a,b] | 73.61(17.69)[b] |

SE: standard error of estimation

*** Estimated parameter is significantly not different from zero ($p<0.05$)

*a, b: common characters in the superscripts refer to no significant differences ($p<0.05$); comparisons between estimates of the same parameter belong to different vitamin E supplementation times (days prior to slaughter) within a model*

### *3.3.2 Effect of dietary vitamin E on time trend of TBARS measures of M. longissimus dorsi*

Stepwise removal of non-significant factors from the statistical model for TBARS led to model (1) and (2), with the lowest AIC (-59.1, -75.2), and highest correlation between the predicted and observed values (R), 0.82, 0.84 and coefficients of determination (CD) 0.93, 0.90, respectively. In these models either the combination of muscle α-tocopherol concentration at slaughter and vitamin E supplementation time or vitamin E dose alone had a significant effect on change in TBARS over time. Model (1) described the change of TBARS over time including muscle α-tocopherol concentration (at slaughter) and length of supplementation as covariates:

$$TBARS_{ijk} = ß_1 TOC\_cont_{ij} + ß_2 Tsupp_{ij} + StoreTime_k + a_i + e_{ijk} \qquad (1)$$

where $TBARS_{ijk}$: TBARS values of i-th experiment of j-th dose level at the k-th storage time point; $TOC\_cont_{ij}$: α-tocopherol concentration of *M. longissimus* of the i-th experiment of j-th dose level and $ß_1$ its slope; $Tsupp_{ij}$: supplementation time of the i-th experiment of j-th dose level and $ß_2$ its slope; $StoreTime_k$: effect of time at k-th storage time point; $a_i$: the random effect ("random intercept") of the i-th experiment and $e_{ijk}$: denotes the residuals. There were no significant interactions between the different factors.

The slopes $ß_1$ and $ß_2$ for muscle α-tocopherol concentration and supplementation time, respectively, were both significantly different from zero ($p<0.0001$ and

$p<0.042$, respectively) and negative ($ß_1$ = -0.0477(0.0073) and $ß_2$ = -0.00178 (0.0008)). Tests and comparisons of the different covariance structures of $e_{ijk}$ residuals showed no indication of autocorrelation within the data and the best fitting covariance structure was found to be the standard variance components (VC) structure, which assigns equal variance for each experiment.

Table 3.7 shows results of least square (LS) means of TBARS values estimated from models (1) and (2).

Predicted LS means of TBARS (Table 3.7) increased from 0.15 (0.04) to 0.46 (0.10) over the first 5 days post slaughter, after which they remained approximately stable until day 8 before they again increased. Except for storage day 9, with a very high estimate for TBARS of 0.73, there was no significant difference between TBARS measures corresponding to two or often more consecutive days (Table 3.7). However, as indicated by the superscripts in Table 3.7, TBARS LS means corresponding to few storage days are significantly lower than TBARS LS means corresponding to storage of a period of a week or longer.

Model (2) describes the impact of dietary vitamin E dose and time on TBARS values:

$$TBARS_{lmn} = Dose_m + StoreTime_n + a_l + e_{lmn} \qquad (2)$$

where $TBARS_{lmn}$: TBARS values of l-th experiment of m-th dose level at the n-th time point; $Dose_m$: effect of m-th level of dose; $StoreTime_n$: effect of time at n-th storage time point; $a_l$ : the random effect (“random intercept”) of the l-th experiment

and $e_{lmn}$ denotes the residuals. No interaction terms were statistically significant. As for model (1), there was no indication of autocorrelation between values of TBARS on successive days and the standard variance components (VC) structure was used.

Considering the dose (model (2), Table 3.7), predicted LS means of TBARS gradually decrease as vitamin E doses increase. For a dose of 0 IU, predicted LS mean of TBARS is 0.52 (0.03) whereas it is less than half of that (i.e. 0.23 (0.05)) for dose 300 IU. There were significant differences for lipid oxidation between dose 0 IU and all higher doses and between dose 50 and doses 200, 300 IU (Table 3.7). However, no significant differences between LS means for TBARS were found between any of the doses equal or above 100 IU (Table 3.7).

The estimated changes with time for LS means of TBARS corresponding to model (2) were similar to that described by (1) with TBARS generally increasing over the first 5 storage days post slaughter and fluctuating between 0.33 and 0.52 thereafter. In contrast to model (1), model (2) did not produce higher predicted TBARS LS means for 9 and 10 storage days (Table 3.7). Multiple comparisons of LS means of TBARS between the different storage days generally showed no significant differences between successive storage days, but significant differences between the estimates corresponding to short (i.e. four days or less) and long (above a week) storage (Table 3.7).

**Table 3.7** Least squares (LS) means of TBARS and their comparisons, based on models (1) and (2).

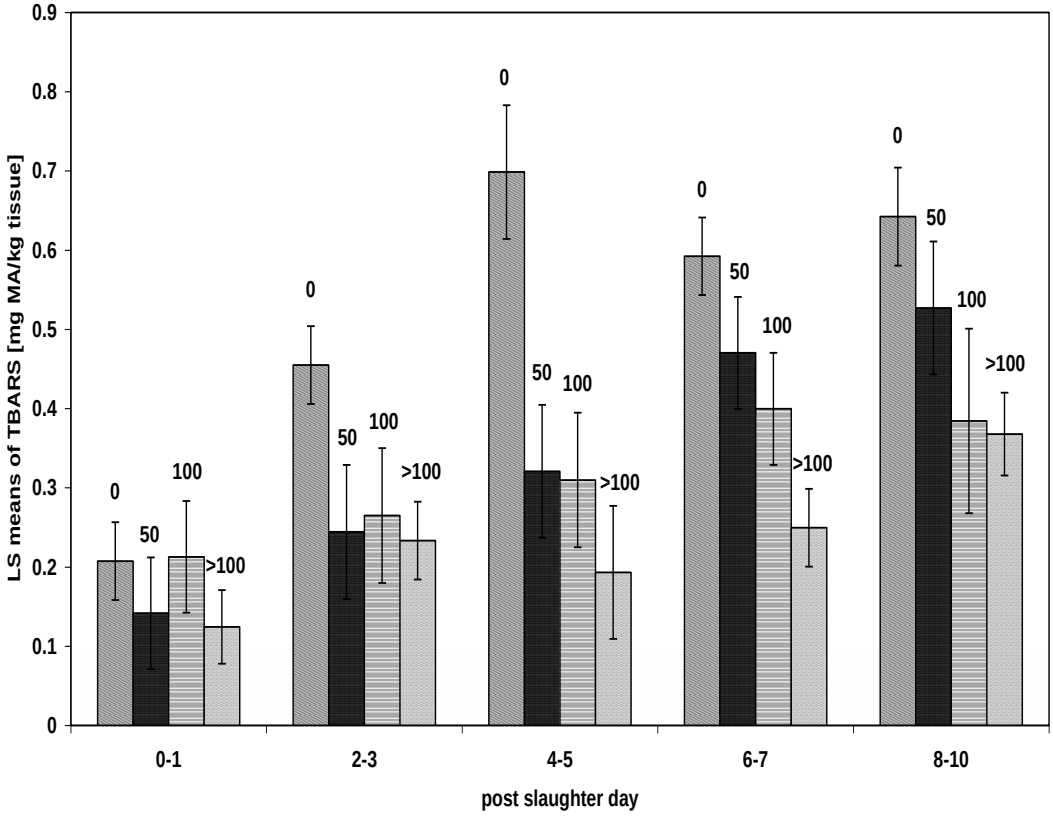
| | | Model (1) | Model (2) |
|---|---|---|---|
| Effect | | LS mean (SEM) | LS mean (SEM) |
| Vitamin E dose (IU) | 0 | | 0.52 (0.03)[a] |
| | 50 | | 0.39 (0.04)[b] |
| | 100 | | 0.33 (0.04)[b,c] |
| | 200 | | 0.29 (0.03)[c] |
| | 300 | | 0.23 (0.05)[c] |
| Storage time | 0 | 0.15 (0.04)[a] | 0.09 (0.04)[a] |
| (days post slaughter) | 1 | 0.22 (0.06)[a,b] | 0.24 (0.06)[b] |
| | 2 | 0.28 (0.06)[b,c] | 0.27 (0.05)[b] |
| | 3 | 0.29 (0.04)[b,c] | 0.31 (0.04)[b,c] |
| | 4 | 0.32 (0.06)[b,c,d] | 0.27 (0.05)[b] |
| | 5 | 0.46 (0.10)[c,d,e] | 0.50 (0.10)[c,e] |
| | 6 | 0.34 (0.04)[b,d] | 0.33 (0.04)[b,d] |
| | 7 | 0.46 (0.06)[d,e,] | 0.51 (0.05)[e] |
| | 8 | 0.48 (0.05)[e] | 0.44 (0.05)[d,e] |
| | 9 | 0.73 (0.10)[f] | 0.40 (0.06)[b,e] |
| | 10 | 0.56 (0.08)[e,f] | 0.52 (0.08)[e] |

SEM: standard error of LS means

*a, b, c, d, e, f: common characters in the superscripts refer to no significant differences ($p<0.05$); comparisons between different vitamin E doses/ post slaughter days within a model*

Both models (1) and (2) indicated that both dietary vitamin E dose and supplementation time affect TBARS values at different storage times, but do not explain how TBARS progress over time (i.e. corresponding interactions with storage time were not significant). However, in model (2) the interaction between dose and storage time approached statistical significance ($p=0.0667$). To test the hypothesis that this lack of interaction could be due to the relatively low number of data points compared to the number of levels of each factor, the following simplifications were carried out: a.) combination of post slaughter storage times into intervals comprising 2-3 days with medians of corresponding TBARS values of each experiment for each storage time interval; b.) combination of several doses to one category (e.g. 0 and 50 IU, or all doses >100 IU). Following this simplification interactions between dose and storage time became marginally significant ($p=0.055$), allowing several interesting observations in this framework.

As illustrated in Figure 3.4, a consistent effect of vitamin E dose on TBARS values (higher vitamin E doses led to lower TBARS values) only occurred after 4 days post slaughter storage. There were significant differences between estimated LS means of TBARS for dose 0 and doses ≥100 IU/kg feed from the second interval (2-3 days post slaughter) onward. There was no significant difference between estimated TBARS LS means for doses 50 and 100 IU/kg feed, for any storage time interval, between doses 0 and 50 IU/kg feed with the exception of interval 2-3 and 4-5 days post slaughter.




Error bars show standard errors of interaction terms
MA: malonaldehyde

**Figure 3.4** Estimated least square means for TBARS of dose category*storage time interaction in *M. longissimus* for different supplementary dose categories at different storage time intervals after slaughter according to model (2) with interaction term included.

## 3.4 Discussion

In this study meta-analyses were performed to determine relationships between dose and duration of vitamin E supplementation and evolution of oxidative processes in post-slaughter muscle of pigs. We analysed both the effects on vitamin E

accumulation in muscle tissue, and on TBARS values, a much used indicator of lipid oxidation.

The first meta-analysis determined quantitative relationships between dietary vitamin E and its accumulation in muscle and estimates for biologically relevant parameters were given. The relationship found was nonlinear and an asymptotic value for vitamin E accumulation in muscle, estimates for maximum possible α-tocopherol accumulation per unit dose and for the vitamin E dose range associated with maximum accumulation could be obtained.

The second meta-analysis quantitatively established relationships between changes over time in lipid oxidation and factors influencing it. These have been represented by two different models describing the influence of different factors on lipid oxidation. The first model described how pork lipid oxidation was affected by α-tocopherol accumulated in pork, the period over which vitamin E was fed and by pork storage time. Beside supplementation- and storage time, this model established a direct relationship between the pork α-tocopherol concentration as antioxidant and the oxidation process. The second model described how dietary vitamin E and storage time affected lipid oxidation. Besides storage time this model described the effect of the daily dose of vitamin E fed on the oxidation process. Regarding changes with time, both models predicted a general increase of TBARS over the first 5 storage days post slaughter and some fluctuation thereafter. Interactions between accumulated α-tocopherol, supplementation time and dietary vitamin E with storage time were found to be not significant within the original data set. A simplification of levels of data was required to get marginally significant interactions between simplified vitamin E doses and storage time intervals.

### *3.4.1 Pork α-tocopherol concentration*

Several studies (e.g. Cheah *et al.*, 1995, Cannon *et al.*, 1996, Corino *et al.*, 1999) reported an increase in muscle α-tocopherol concentration with increasing daily vitamin E dose, but an exact relationship between these measures had not been established. Hasty *et al.* (2002) reported a linear relationship between pork α-tocopherol and fed vitamin E. In contrast Jensen *et al.* (1997) and Hoppe *et al.* (1993) reported a linear relationship between α-tocopherol concentration and logarithm of daily vitamin E dose. The meta-analysis presented in this study showed a nonlinear relationship between supplementary vitamin E and muscle α-tocopherol accumulation in *M. longissimus* with an asymptotic limit ca. 6.4 μg α-tocopherol/g tissue, implying a maximum capacity for α-tocopherol accumulation. The nonlinear functions used to describe the relationship between vitamin E dose and *M. longissimus* α-tocopherol concentration are some of those most commonly used for describing animal growth (Lambe *et al.*, 2006) and as indicated by the model fit criteria, describe correctly this relationship. The present study used summary statistics and aimed to establish response laws and consequently, simple models were preferred. Therefore other possible candidate functions, such as Richards, Bridges, Brody, and Michaelis-Menten models have not been used as they contain either more parameters or lacked direct biological interpretations for the model parameters (Lambe *et al.*, 2006, Lorenzo Bermejo *et al.*, 2003).

The tested models gave similar, biologically realistic estimates for the α-tocopherol asymptote A, which implied that *M. longissimus* has a maximum capacity for α-

tocopherol accumulation, between 6.3 and 7.3 α-tocopherol µg/g tissue for supplementary vitamin E doses between 0 and 700 IU/kg feed.

Both, the Gompertz and logistic models provided similar estimates for (B) the maximum α-tocopherol accumulation per unit dose (µg/g tissue/IU) suggesting a highest possible α-tocopherol accumulation in *M. longissimus* under this vitamin E supplementation form (dietary and α-tocopheryl acetate) was between 0.023 and 0.025 (±0.008) µg/g tissue/IU.

The logistic model produced larger estimates than the Gompertz model for the vitamin E dose associated with the maximum accumulation of α-tocopherol in muscle (i.e. estimated value for parameter C in the logistic and Gompertz models were 79.8 and 28.3 IU/kg feed, respectively). However these differences are largely due to the fact that the inflection point of logistic model is fixed at A/2, whereas the Gompertz model fixes this value at A/e (e=Euler number, 2.718281818) (Lambe *et al.*, 2006). Therefore this difference is an artefact of the mathematical model and not of biological origin.

For the exponential model, the estimate for the α-tocopherol concentration corresponding to dose 0 (IU/kg feed) was significantly greater than zero, i.e. $C_E$ = 1.24 for the model with random experimental effect on the asymptote A. This may be due to the fact that diets with vitamin E dose classified as 0 IU/kg feed referred to diets containing 8-25 mg/kg of vitamin E, that is, those satisfied the minimum requirements of (NRC, 1998), 8-15 mg vitamin E/kg feed, and those containing levels up to 25 mg/kg feed before rounding.

Comparisons of the parameters considered for different lengths of vitamin E supplementation, estimated by the best fitting Gompertz-model showed no

significant differences for the maximum α-tocopherol concentration (A) and maximum α-tocopherol accumulation per unit dose (B). Significant differences were only found for the Gompertz parameter C, i.e. vitamin E dose associated with the maximum accumulation of α-tocopherol, with significantly different estimates corresponding to long (>70 or >100 days) and short (<70 or <50 days) lengths of supplementation, respectively. However the estimations for corresponding to the short supplementation duration did not differ significantly from zero at the 95% confidence level. These results thus suggest that when vitamin E supplementation occurs over a shorter time period, the strongest difference in α-tocopherol accumulation occurs between no supplementation and small doses of vitamin E. In contrast, if supplementation occurs over a longer time period, the actual supplementation dose appears to matter more. This albeit weak effect of supplementation time on the actual α-tocopherol accumulation (i.e. Gompertz parameters A and B) could be explained by earlier studies which showed that blood α-tocopherol concentration of pigs stabilised after 2 weeks vitamin E supplementation and there was a positive linear relationship between blood- and tissue α-tocopherol concentration at slaughter (Hoppe *et al.*, 1993). Furthermore among the references considered, the experiment (Monahan *et al.*, 1990a) of the shortest supplementation time (14 days pre slaughter) found ~2.5 fold increase in both plasma and tissue α-tocopherol concentration in 14 days between supplementation of 200 IU/kg feed and basal diet (50 IU/kg feed). The same magnitudes of increase were found for these measures in the experiment (Monahan *et al.*, 1990b) of the longest supplementation time (122.5 days pre slaughter) under similar supplemenatation (200 IU/kg feed vs. 0) conditions (Table 3.1). The duration

of vitamin E supplementation was equal to or longer than 14 days pre slaughter in all references used for this meta-analysis (Table 3.1). Thus tissue α-tocopherol concentrations might have reached equilibrium with vitamin E supply, which might explain why the contribution of supplementation time was significant only to one parameter of the best fitting model.

A meta-analysis was also carried out using those references that provided fed α-tocopherol levels. The same nonlinear relationships were found and the estimated parameters of the models were in good agreement with the model parameters discussed earlier, confirming reliability of our results.

The present meta-analysis was based on the results obtained on a specific muscle, *M. longissimus*. To the best of our knowledge only Tsai *et al.* (1978), Jensen *et al.* (1997) and Cheah *et al.* (1995) have published meat α-tocopherol concentration of other muscles e.g. *M. triceps*, *M. psoas major* and *M. masseter* respectively. Although there are too few data to conduct a meta-analysis, the results obtained on the *M. triceps*, *M. psoas major* show also nonlinear trends for α-tocopherol accumulation for several doses and α-tocopherol concentration published for *M. triceps* and for *M. masseter* are within the range of predicted values by our nonlinear model.

### *3.4.2 Pork lipid oxidation*

The present study started with the use of a general model to take into account effects of all available factors and interaction terms on lipid oxidation. A stepwise simplification approach was subsequently adopted because models with many

parameters (e.g. due to non-significant interaction terms) did not converge or some of the less relevant factors (e.g. gender) were not significant. During the simplification procedure vitamin E dose was found to be influential as fixed factor to such an extent that when included in the model, apart from storage time, no other factor was significant. The fact that vitamin E dose and accumulated α-tocopherol were not simultaneously significant in the statistical models could be explained by the collinear relationship between vitamin E dose and accumulated α-tocopherol, which has been established in this study.

The meta-analysis models of pork lipid oxidation considered a time period between 0 and 10 days storage post slaughter. Several authors (Pfalzgraf *et al.*, 1995, Boler *et al.*, 2009) reported a sharp increase in pork TBARS after 7 days storage post slaughter in a high oxygen ($80\%O_2/20\%CO_2$), modified atmosphere package. Similar results were reported for beef TBARS after 9 days storage post slaughter (Campo *et al.*, 2006) under similar packaging conditions. These results indicate that after a certain storage time, lipid oxidation process accelerates. This is coherent with the knowledge that once oxidation is initiated, it continues as free radicals catalyse additional free-radical generating reactions (Jadhav *et al.*, 1996, Abuja and Albertini, 2001). All the TBARS data analysed in the present paper were derived from samples stored at atmospheric oxygen levels and not with artificially elevated oxygen levels. Although most data used in the present study did not reach this acceleration point, a few experiments showed a larger increase at days 8 and 9 post slaughter (Figure 3.2). Buckley *et al.* (1995) reported that rate and extent of lipid oxidation in pork depend on the α-tocopherol concentration in tissues, but did not quantify this relationship. In contrast Corino *et al.* (1999) found a linear (negative) relationship between TBARS

α-tocopherol concentration in *M. longissimus dorsi* (TBARS=0.21875-0.00757*α-tocopherol) and a correlation between muscle α-tocopherol concentration and TBARS of -0.44 (p=0.0328) on day 6 post slaughter and no correlation on day 8 post slaughter. Model (1) described a general linear relationship between TBARS and muscle α-tocopherol concentration and predicted a higher negative slope value -0.0477 than that suggested by Corino *et al.* (1999).

Dunshea *et al.* (2005) reported that vitamin E supplementation at doses of 200 IU/kg provided over 84 to 130 days pre-slaughter and higher doses of 700-800 IU/kg for shorter periods of 14 days prior to slaughter significantly reduced muscle lipid oxidation in pork and pork products, but no time relationship was quantified. Model (1) described a general linear relationship between TBARS and supplementation time for doses ranging from 0 to 300 IU/kg feed and predicted a negative slope -0.00178 for this relationship. Since the highest dose in our analysis was 300 IU, model (1) does not describe time dependence for high doses of 700-800 IU/kg feed.

Our analysis did not provide exact estimates for the minimum required dose of vitamin E for which a significant difference in TBARS can be observed. Model (2) would suggest a dose between 50 and 100 IU/kg feed. This is in agreement with the results of the models quantifying the relationship between dietary vitamin E dose and α-tocopherol, which estimated the dose corresponding to highest accumulation of α-tocopherol to be between 39 and 80 IU/kg feed.

The predicted time trends for LS means of TBARS corresponding to both models were found to be similar i.e. modelled LS means of TBARS generally increased over the first five storage days post slaughter and fluctuated between 0.33 and 0.52 thereafter. The fluctuation was likely rather due to variable numbers of data points

belonging to the different storage time points than to the progress of the oxidation process.

In the mixed models (1) and (2) no time autocorrelation was found. When autocorrelation terms were included into these models, we assumed that TBARS values of an experiment at the same dose level (at different time points) are derived from the same piece of muscle. Thorough examination of experimental descriptions of the studies showed that in many experiments different sides (right/left) of carcasses, but the same type of muscle from different animals might have been used as samples for TBARS measurement at a certain dose level for different time points, so these TBARS values could not have produced strong longitudinal data, which would explain the lack of autocorrelation.

Furthermore random intercepts were found not to be significant, implying that experimental conditions of the individual studies as random effects statistically did not give significant contribution to the modelled values.

Again, present results refer to one particular muscle *M. longissimus dorsi*. To the best of our knowledge just a few individual references (Tsai *et al.*, 1978, Houben *et al.*, 1998, Phillips *et al.*, 2001, Guo *et al.*, 2006) published TBARS of *M. triceps*, Boston-butt, shoulder, ham, respectively and only two (Buckley and Conolly, 1980, Jensen *et al.*, 1997) for *M. psoas major* –the latter one in logarithmically transformed form. Although this low number of references is insufficient for meta-analysis of vitamin E effect on lipid oxidation in muscles other than *M. longissimus dorsi*, results of these studies showed similar effects and tendencies to those discussed in the present study.

Information about breeds in most of the experiments were available (Table 3.1 and Table 3.2), but breed effects could not be assessed in this study, because different cross breeds were used with insufficient replicates.

In meta-analysis of pork α-tocopherol concentration it seemed also relevant to investigate the effects of certain genotypes, but only two studies investigated this aspect, Rosenvold *et al.* (2002) used halothane gene free (NN) and Cheah *et al.* (1995) halothane heterozygote animals (Nn), the latter for the particular experiment which we used for our analysis. However segregation of this allele in other experiments could not be excluded, and accounting for it was not possible due to lack of the relevant information.

## 3.5 Conclusion

This study found meta-analysis a powerful tool for establishing quantitative relationships between dietary vitamin E and its accumulation in pork and its effects on pork lipid oxidation. For α-tocopherol accumulation an asymptotic value of ca. 6.4 µg α-tocopherol/g tissue was established as the maximum capacity for α-tocopherol accumulation. This level could be reached in *M. longissimus* of hybrid crossbreeds under diet regimes which contain less than 5% fat (on DM base) and 0-700 IU supplementary vitamin E /kg feed. The maximum increase for α-tocopherol accumulation, which is ca. 0.024 µg α-tocopherol /g tissue/IU for this particular muscle, could be reached by supplementation between 39 and 80 IU supplementary vitamin E /kg feed. The duration of supplementation only affects the vitamin E dose associated with maximum α-tocopherol increase, with the greatest increase occurring

at low doses when supplementation times were short and greatest increase occurring at higher doses for longer supplementation times.

In the second meta-analysis the effect of vitamin E supplementation on lipid oxidation was studied. Based on this meta-analysis accumulated α-tocopherol, supplementation time, vitamin E dose and storage time were all found to be important factors affecting lipid oxidation and thus determine various pork quality characteristics. The results also indicated that at least 100 IU vitamin E /kg feed is required to gain a significant decrease of lipid oxidation and every 1 μg α-tocopherol/g tissue decreases lipid oxidation by ca. 0.05 TBARS values in *M. longissimus dorsi*. The magnitude of the effect of length of vitamin E supplementation is lower than the magnitude of the accumulated α-tocopherol. Under the described storage conditions this meta-analysis found gradually increased lipid oxidation for a few (3-4) days storage post slaughter and then its stabilisation for the remaining storage time up to the 10th day post slaughter. The present analysis suggests that administrated vitamin E dose and storage time interact i.e. the actual vitamin E dose affects how lipid oxidation changes over time, but more data is required to confirm this interaction. Further studies are required, using standardised methods to confirm findings of these meta-analyses and to extend results to other muscles.

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# Chapter 4: Meta-analysis of effects of dietary vitamin E and post-slaughter storage conditions on changes of redness (a*) of pork

**Abstract**

A meta-analysis was carried out to quantify the effects of dietary vitamin E and storage conditions on colour changes of pork from *M. longissimus dorsi*. After standardisation procedures, redness of pork (CIE colour specification a*), one of the most important objective colour attributes, was used as an indicator for colour changes in this analysis. The analysis was based on results from 5 experiments, which met selection criteria. Analysis of changes of other objective colour attributes, lightness (L*) and yellowness (b*) was not possible due to lack of published data.

The statistical analysis (using mixed models) found significant effects of tissue α-tocopherol concentration in *M. longissimus dorsi*, simplified supplemented vitamin E levels as well as storage time and storage light on redness of pork and its changes over time. The relationship between redness and α-tocopherol concentration was found to be linear, and between redness and storage time was non-linear (third degree polynomial) in one model. This model suggested that an increase of 1 μg of α-tocopherol in the muscle led to an expected increase a* value of 0.11. Another model identified significant interactions about 0.28 between α-tocopherol concentration and storage time in late storage periods. A third model found a significant difference of -0.48 between predicted a* values at lower (≤50 IU/kg feed) and higher supplemented vitamin E levels (≥100 IU/kg feed). The models predicted an initial increase for 3 days, a stable period for 5 days and then a decrease for a* values over storage time. The a* values were significantly lower by about 1.4 when samples were exposed to light in the models, the effect of light found to be constant over time.

Further studies, carried out with standardised methods, are needed to increase the predictive power of the derived models and to validate the models for other muscles.

**Keywords:** Vitamin E, α-tocopherol, colour, redness, pork quality, meta-analysis

## 4.1 Introduction

Visual appearance is an important characteristic of meat quality, as it strongly influences the consumer's purchase decision. From the various factors which contribute to the visual appearance of meat, colour is the main factor that affects the acceptability of meat products (Faustman and Cassens, 1990). The rate of discolouration of meat is related to several factors including ultimate pH, storage temperature, lipid oxidation and illumination (Faustman and Cassens, 1990). To maintain acceptable fresh meat colour over prolonged periods of time, it is necessary to delay pigment oxidation and/or enhance reduction of oxidized myoglobin (Gray *et al.*, 1996). The supplementation of feed with vitamin E, a well known antioxidant was found to significantly reduce the oxidation process (Buckley *et al.*, 1995). For beef, vitamin E supplementation resulted in an extension of retail display life from1.6 to 5 days without compromising microbiological quality and similar trends have been reported for pork products (Gray *et al.*, 1996). There is strong evidence from various studies, review papers and meta-analysis that dietary vitamin E supplementation decreases lipid oxidation in pork (Buckley *et al.*, 1995, Dikeman, 2007, Dunshea *et al.*, 2005, Lahucky *et al.*, 2000, Lahucky *et al.*, 2005, Pettigrew and Esnaola, 2001, Rosenvold and Andersen, 2003, Chapter 3). However, results from individual studies on the effect of vitamin E on pork colour changes are ambiguous (Rosenvold and Andersen, 2003). While some papers have reported improved colour stability with vitamin E supplementation (e.g. Asghar *et al.*, 1991, Monahan *et al.*, 1992), several others found no significant effect (e.g. Cannon *et al.*, 1996, Jensen *et al.*, 1997, Phillips *et al.*, 2001, Krska *et al.*, 2001). To resolve this

ambiguity and to determine quantitatively whether vitamin E influences colour changes in pork using a cross-experimental approach, meta-analysis may be used.

In animal science, meta-analysis has recently been described as a useful statistical tool to objectively integrate the results of individual experiments and to establish general response laws that are valid over a range of conditions (Sauvant *et al.*, 2008, St-Pierre, 2001, Phillips, 2005).

The aim of this chapter was to use meta-analysis to quantify the effects of different dietary vitamin E levels and post-slaughter storage conditions (e.g. storage time, storage light) on the changes of pork colour during storage. This work focuses on the analysis of redness (a*) as an attribute of objective colour measurement of pork using the CIE L*,a*,b* colour system (CIE, 1976).

## 4.2 Materials and methods

### *4.2.1 Data sourcing and construction of meta-analysis datasets*

The literature search used published information written in English from 1970 to 2009, from various scientific sources including journals, conference proceedings, book articles and abstracts from various electronic databases. Indices used in the search were combinations of the terms ‘vitamin E’, ‘alpha-tocopherol’, ‘α-tocopherol’, ‘pork’, ‘dietary’, ‘pig’, ‘swine’, ‘hog’ and plurals of these. In some cases, authors were contacted directly to complete missing information.

The literature research identified 60 initial references, related to the effect of dietary vitamin E supplementation on pork and pigs. Some studies reported aspects of dietary vitamin E utilization (e.g. blood α-tocopherol concentration), which were not relevant to the analysis and were therefore excluded. Since this study focused purely on the antioxidant effect of vitamin E, references using additional treatments which could possibly interfere with or modify the antioxidant effect of the vitamin E were also excluded. This was the case for experiments using diets with a fat to dry matter (DM) ratio of more than 5%, as this may change saturated and unsaturated fatty acid ratio in pork making it more susceptible to oxidation (Buckley *et al.*, 1995). Experiments using diets containing in addition to vitamin E other antioxidants (e.g. vitamin C) or minerals (e.g. Se) above the normal maintenance level, which may interact with the vitamin E (Oldfield, 2003), were also excluded. These selection criteria together with the standardisation criteria outlined below led to a drastic reduction of acceptable studies and the following references remained for inclusion in the meta-analysis (Dirinck *et al.*, 1996, Hoving-Bolink *et al.*, 1998, Lanari *et al.*, 1995, Phillips *et al.*, 2001, van Heugten *et al.*, 2002). Table 4.1 summarizes experimental details of these studies.

**Table 4.1** References used for meta-analysis of changes of redness (a*) of pork.

| No .of pigs/treat-ment group | Vitamin E levels (IU)* | Pig breed | Gender | Length of supplem. (days) prior to slaughter | Reference |
|---|---|---|---|---|---|
| 24 | 50/200 | Danis | gilt | 105 | Dirinck *et al.*, 1996 |
| 36/35 | 0/200 | Yx(FLxDuL) | barrow & gilt | 84 | Hoving-Bolink *et al.*, 1998 |
| 4/4 | 0/200 | 0.5Dx0.25LW x0.25L | gilt | 105 | Lanari *et al.*, 1995 |
| 10 | 50/200 | YxY | boar & gilt | 42 | Phillips *et al.*, 2001 |
| 48 | 0/100/150 300/600 | Hybrid | barrow & gilt | 42 | van Heugten *et al.*, 2002 |

Danis: published in this form, no further information is available; Yx(FLxDuL): Yorkshire x (Finnish Landrace x Dutch Landrace); 0.5Dx0.25LWx0.25L: composite breed of 50% Duroc x 25% Large White x 25% Landrace; YxY: Yorkshire crossbred
*Supplementary vitamin E levels rounded and expressed in IU/kg feed

Experimental data were entered into a database that had been constructed according to certain conceptual and structural requirements and described in details in Chapter 2. The database includes a detailed description of all relevant information on references (e.g. type, name and date of publication, title, author names), animals used in the experiments (e.g. gender, breed, beginning or slaughter weight), diet (e.g. gross energy, crude protein, fat concentration), rearing conditions (e.g. indoor/outdoor, group size), slaughter processes (e.g. stunning method ), pork quality traits studied, sample size and treatment (e.g. storage temperature) as well as results from statistical analyses performed on the datasets from individual experiments.

### *4.2.2 Meta-design*

For powerful statistical analysis standardisation of the factors was required.

#### *4.2.2.1 Dose definition and storage time standardisation*

All experiments used in the analysis supplied vitamin E in form of all-rac-α-tocopheryl (DL-α-tocopheryl) acetate, which is an equimolar mixture of eight synthetic α-tocopherol stereoisomers in acetate form (Lauridsen *et al.*, 2002). 1mg of this chemical compound has a bioactivity conversion factor of 1.0 and corresponds to 1 International Unit (IU) of α-tocopherol. For the three different terminologies referring to α-tocopherol that had been used in the studies to define vitamin E administration, i.e. vitamin E (e.g. Hoving-Bolink *et al.*, 1998), α-tocopherol (e.g. Dirinck *et al.*, 1996) or analysed fed α-tocopherol (e.g. Lanari *et al.*, 1995) the amount of supplementation could thus be expressed in IU of α-tocopherol.

In one study (Phillips *et al.*, 2001) different amounts of vitamin E had been administrated for different time periods throughout the supplementation time. We defined the administrated vitamin E (in IU) in this case by calculating weighted averages with the individual time periods as weighting factors.

Furthermore the different experiments varied in their definition of control or treatment vitamin E levels and in the accuracy of the provided vitamin E measures. To minimise the potential bias introduced by this variation, we rounded administrated vitamin E in units of 50 IU/kg feed. For example amounts of supplementation below 25 IU/kg feed were defined as vitamin E dose 0 IU/kg feed,

and supplementations between 25 and 75 IU/kg feed were defined as vitamin E dose 50 IU/kg feed. Henceforth the rounded measure is denoted as *vitamin E dose*.

#### *4.2.2.2 Standardisation procedures related to colour of pork*

Objective colour parameters of meat based on reflectance method can be given in different systems (e.g. Hunter (HunterLab, 2008) or CIE (CIE, 1976)). All references used in the present study provided pork colour in term of the CIE colour system, which describes colour by three parameters: lightness (L*), redness (a*), and yellowness (b*) (CIE, 1976). However only the redness (a*) parameter of this colour system was assessed in this study, because of lack of sufficient data for the other two colour attributes. The value as a* value of this colour parameter is going to be equally referred as redness of pork in this chapter.

The muscles used in the studies referred to *M. longissimus dorsi*, *M. longissimus lumborum* or *M. psoas major*. Pork redness data were available from *M. longissimus dorsi/M. longissimus lumborum* in all five references, whereas less data were available for the other muscle. Since *M. longissimus lumborum* is part of *M. longissimus dorsi*, the present study uses the term *M. longissimus dorsi* for both *M. longissimus dorsi* and *M. longissimus lumborum*.

As storage conditions affect the rate of colour changes (Faustman and Cassens, 1990) redness data were considered from studies in which samples were: (i) stored at 4-7 °C; (ii) wrapped in oxygen permeable material (PVC, plastic foil); and (iii) stored under atmospheric conditions i.e. non oxygen/carbon-dioxide elevated or vacuumed storage environment. If samples were exposed to illumination, redness data received

the attribute ‘light’ and if samples were kept either in dark or where the description of storage conditions demonstrated that samples were not exposed to light, they received the attribute ‘no-light’ in the meta-analysis database.

In addition, it was necessary to standardise the time scales provided in different experiments to a uniform scale. The time of slaughter was considered as the starting point of storage (day 0) and expressed the storage time in units of days post slaughter.

### *4.2.3 Statistical analysis*

The aim of this meta-analysis was to derive statistical models which describe how redness of pork changes over time, and how the trend is affected by factors e.g. dietary vitamin E dose, storage conditions.

The inputs for the meta-analysis were the results of the statistical models used in the original publications in the form of least square (LS) means or means (if LS means were not provided) and the corresponding standard errors of LS means (SEM) or standard deviations (SD) where SEM were not provided.

Meta-analysis was performed using the PROC MIXED procedure of SAS (SAS, 2000) where the effects of different covariates and fixed effects as well as individual study effects and autocorrelation between repeated measurements could be taken into account (Verbeke and Molenberghs, 1997, Whitehead, 2002). The fitted models contained α-tocopherol concentration of *M. longissimus dorsi* as covariate, vitamin E dose, vitamin E supplementation time and storage light as fixed effects, as well as interaction terms between covariates and fixed effects. Effect of storage time was

examined separately in the models either as covariate or as fixed effect. Including storage time in the model as a continuous covariate provides a description of the time trend of redness, whereas inclusion as a fixed effect lends itself better to the statistical comparison of redness associated with different stages during storage and of interactions between storage time and other factors influencing it. The random experimental effect was included as a "random intercept". For one study (Dirinck *et al.*, 1996) that did not report muscle α-tocopherol concentrations, LS means predicted from the best fitting (Gompertz) nonlinear model (Chapter 3, Trefan *et al.*, 2011) were used. Autocorrelations between repeated measurements were examined using the REPEAT statement of PROC MIXED, where vitamin E dose was nested within experiment identifier and various residual covariance matrix structures (SAS, 2000) were explored.

To compare the models, the following model fitting criteria were used: correlation between the predicted and observed values through Pearson correlation R; Akaike's information criteria (AIC) (SAS, 2000); RSD (residual standard deviation), $RSD=[\Sigma\Sigma(\varepsilon_{ij})^2/(n-p)]^{1/2}$ where $\varepsilon_{ij}$ is the residual value of i-th experiment at j-th dose level, n is the number of observations and p is the number of parameters in the model; CD (coefficient of determination) $CD=1-\Sigma\Sigma(\varepsilon_{ij})^2/\Sigma\Sigma(Y_{ij})^2$ where $\varepsilon_{ij}$ is the same as in RSD and $Y_{ij}$ is the predicted value i-th experiment at j-th dose level (Bünger and Herrendörfer, 1994). In addition, visual inspection of predicted values, residuals and normality tests of residuals were carried out (Sauvant *et al.*, 2008).

Since research designs and accuracy varied across studies, weighting factors based on the published experimental errors were included in the models. The weighting factor was the inverse of the square of these errors divided by their mean value (St-

Pierre, 2001). In one study (Dirinck *et al.*, 1996) neither SEM nor SD were published, and error terms were estimated based on the coefficient of variations of the other experiments. Weighting of models was performed using the WEIGHT statement of PROC MIXED procedure of SAS (SAS, 2000)

### 4.3 Results

Figure 4.1 shows the (LS) means of redness (a*) measured in *M. longissimus dorsi* at different storage time points from the 5 selected experiments for the various doses of supplementary vitamin E. a* values showed intial increase up to 8th days post slaughter which was not expected based on general knowledge about post-mortem changes in colour of pork, exposed to oxygen.

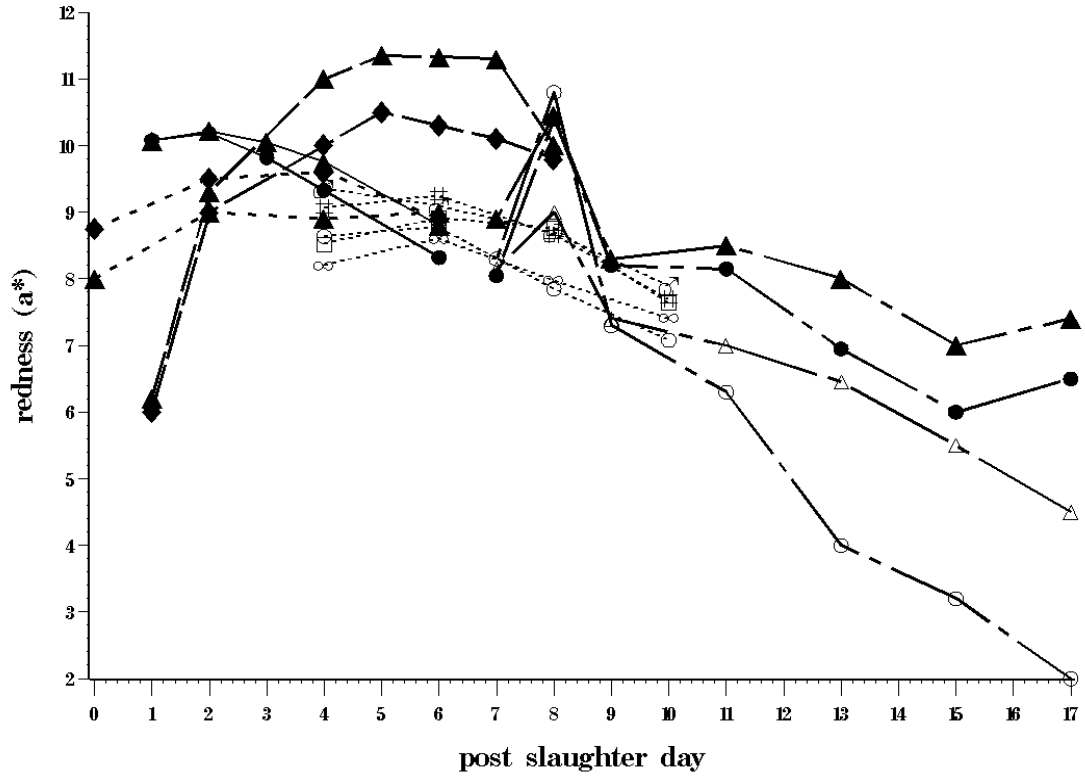


**Figure 4.1** Changes in redness (a*) in *M. longissimus dorsi* with storage time for various supplementary vitamin E doses from 5 different studies: Dirinck *et al.*, 1996 (___ ___); Hoving-Bolink *et al.*, 1998 (_____); Lanari *et al.*, 1995 (____ _ ); Phillips *et al.*, 2001 ( _ _ _ ); van Heugten *et al.*, 2002 (……). Vitamin E dose levels: 0 IU (○/●), 50 IU (♦),100 IU (□), 150 IU (♂), 200 IU (Δ/▲), 300 IU (#); 600 IU (∞). Open and closed symbols refer to storage conditions in which samples were exposed to illumination ('light'), or kept in darkness ('no light'), respectively.

After stepwise removal of statistically non-significant factors influencing redness and comparison of different assumptions for storage time dependence (i.e. linear, quadratic, cubic), the statistical model (1) provided the best fit out of all models where storage time was a continuous variable (AIC: 227.8, R: 0.85, RSD: 0.96, CD: 0.99). According to this model accumulated α-tocopherol concentration in pork (at slaughter), storage time and storage light have significant effects on redness of pork:

$a^*_{ijkl} = ß_0 TOC\_cont_{ij} + ß_1\ Time_{ijk} + ß_2\ Time^2_{ijk} + ß_3\ Time^3_{ijk} + StoreLight_l + b_i + e_{ijkl}$ (1)

where $a^*_{ijkl}$ is the a* value of i-th experiment of j-th dose level at the k-th storage time point under l-th storage light condition; $TOC\_cont_{ij}$ is the α-tocopherol concentration in *M. longissimus dorsi* of the i-th experiment of j-th dose level and $ß_0$ its slope; $Time_{ijk,}$, $Time^2_{ijk}$ , $Time^3_{ijk}$ are the storage time, square and cube of k-th storage time point of the i-th experiment of j-th dose level, respectively, $ß_{1,}$ $ß_2$ and $ß_3$ their corresponding coefficients; $StoreLight_l$ is the effect of storage light (l=0 refers to 'no light' and l=1 refers to 'light'); $b_i$ is the random effect ("random intercept") of the i-th experiment and $e_{ijkl}$ denotes the residuals. There were no significant interactions between the different factors.

The slope $ß_0$ for muscle α-tocopherol concentration and the coefficients $ß_{1,}$ $ß_2$ and $ß_3$ for linear, quadratic and cubic storage time terms as continuous variables were significantly different from zero ($p<0.0309$, $p<0.0001$, $p<0.0001$ and $p<0.0011$, respectively). Muscle α-tocopherol concentration was found to have a positive effect on redness with a slope value $ß_0$ =0.11 (SE: 0.05). The linear, quadratic and cubic storage time coefficients $ß_1$, $ß_2$ and $ß_3$, respectively were $ß_1$ = 1.04 (0.21), $ß_2$ = -0.13 (0.03) and $ß_3$ = 0.004 (0.001). A significant difference of 1.35 (SEM: 0.23) ($p<0.0001$) was found for the predicted redness with lower a* values when light was present during storage.

Figure 4.2 shows the least square (LS) mean values for redness (a*) estimated from model (1) for different vitamin E doses, storage conditions and storage times.

Confidence intervals of the individual predicted a* values ranged from 0.34 to 0.92. Distribution of the residuals was uniform over storage time.

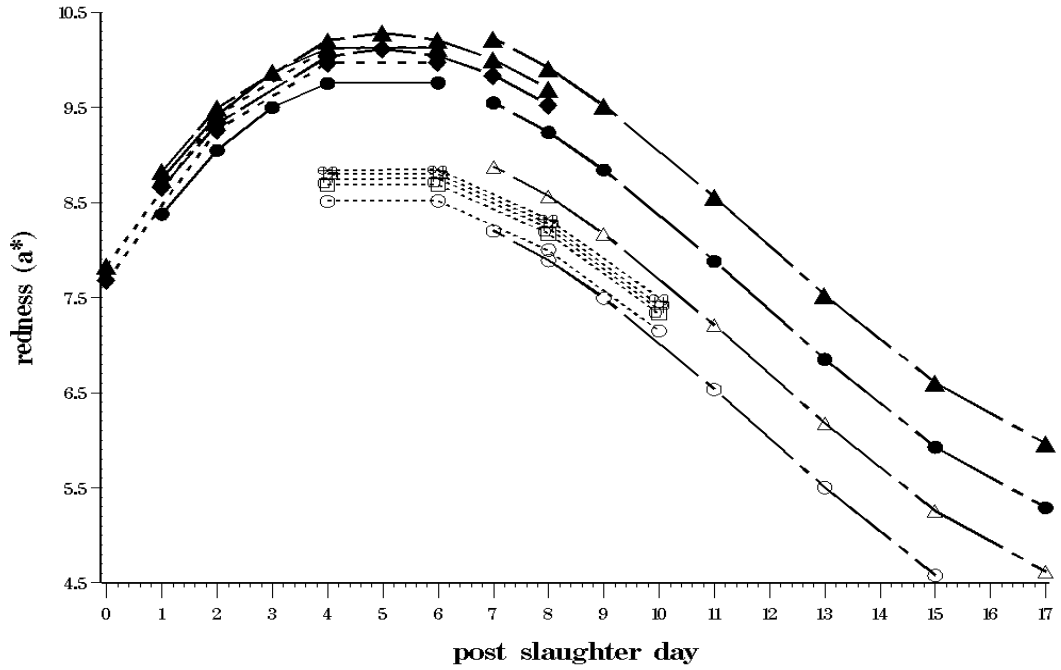


**Figure 4.2** Model (1) predicted redness (a*) in *M. longissimus dorsi* for different supplementary vitamin E doses and storage light conditions. Vitamin E dose levels: 0 IU (○/●), 50 IU (♦),100 IU (□), 150 IU (♂), 200 IU (Δ/▲), 300 IU (#); 600 IU (∞). The predicted values are presented according to whether pork samples were stored in dark (●,♦,▲) or exposed to illumination (○,□,♂,Δ,#,∞).

In model (1) storage time was considered as a continuous variable. This provided a quantitative description for the change in redness of pork with time, but did not allow statistical comparisons of predicted redness between different storage time points. Therefore an alternative model was applied to the data where storage time was considered as a fixed effect. The resulting best fit model (AIC: 238.7, R: 0.91, RSD: 0.95, CD: 0.99) was:

$$a^*_{mnpq} = (TOC\_cont*StoreTime)_{mnp} + StoreTime_p + StoreLight_q + b_m + e_{mnpq} \quad (2)$$

where $a^*_{mnpq}$ is the a* value of m-th experiment of n-th dose level at the p-th storage time point under q-th type storage light condition; $TOC\_cont*StoreTime_{mnp}$ is the interaction between α-tocopherol concentration of *M. longissimus dorsi* of the m-th experiment of n-th dose level and p-th storage time point; $StoreTime_p$ is the effect of time at p-th storage time point; $StoreLight_q$ is the effect of storage light (q=0 refers to 'no light' and q=1 refers to 'light'); $b_m$ is the random effect, as random intercept of the m-th experiment and $e_{mnpq}$ denotes the residuals. Detailed analysis of the $TOC\_cont*StoreTime_{mnp}$ interaction at individual storage times showed that the interaction was significant at 1, 6 and the last 3 days post slaughter storage times and their LS means were -0.70 (0.25), 0.39 (0.19), 0.30 (0.12), 0.28 (0.12), 0.28 (0.12), respectively, implying that α-tocopherol concentrations at slaughter affect the change in redness at rather later stages of the oxidation process. This coincides with the period in which redness changes most as LS means of a* values estimated from model (2) increased from 7.58 (0.54) to 9.30 (0.63) over the first 3 days post slaughter, after which values remained approximately stable until day 8 after which they gradually decreased to 5.05 (0.37). Multiple comparisons of LS means of redness between the different storage days generally showed no significant differences between successive storage days, but significant differences were found between estimates corresponding to short (i.e. four days or less) and long (above a week) storage. As in the case of model (1) a significant difference of 1.43 (0.19) ($p<0.0001$) was found for predicted redness with lower a* values when illumination was present during storage. Confidence intervals of the individual predicted redness

varied between 0.47 and 1.47. Distribution of the residuals was uniform over storage time.

In the derivation of the statistical models vitamin E dose as a multilevel fixed effect as well as its accumulation in the form of α-tocopherol were introduced into the models. However, α-tocopherol concentration was found to be the better predictor for redness, and the effect of vitamin E was not significant at the 95% confidence limit and hence not included in these models. In order to answer the question whether supplementation of vitamin E has a direct effect on the change of redness of pork, a further model was tested in which vitamin E dose was attributed to one of two dose categories corresponding to doses either equal to or less than 50 IU/kg feed (control) or greater than or equal to 100 IU/kg feed (supplementation), respectively. The statistical model applied to the data contained the two simplified dose categories, storage time and storage light as fixed effects. Although the fit of the model did not improve (AIC: 232.7, R: 0.88, RSD: 0.97, CD: 0.99), the model was:

$$a^*_{rstv} = simpDose_{rs} + StoreTime_t + StoreLight_v + b_r + e_{rstv} \quad (3)$$

where $a^*_{rstv}$ is the a* value of r-th experiment of s-th simplified dose level at the t-th storage time point under v-th type storage light condition; $simpDose_{rs}$ effect of s-th evel of simplified vitamin E dose of r-th experiment; $StoreTime_t$ is the effect of time at t-th storage time point; $StoreLight_v$ is the effect of storage light (v=0 refers to 'no light' and v=1 refers to 'light'); $b_r$ is the random effect, as random intercept of the r-th experiment and $e_{rstv}$ denotes the residuals. All fixed effects were found to be significant, and interactions between fixed effects were found to be non-significant at

the 95% confidence limit. Model (3) gave similar estimation for changes of LS means of redness over time as model (2) e.g. estimated a* values increased from 7.64 (0.60) to 9.20 (0.60) on the first 3 days post slaughter, after which values remained approximately stable until day 8 after which they gradually decreased to 5.10 (0.42). Furthermore multiple comparisons of estimated redness between the different storage days showed the same patterns as in the case of model (2) and a significant difference of 1.47 (0.22) ($p<0.0001$) with the same magnitude of model (1) & (2) was found between light and dark storage conditions. In particular a significant difference of -0.48 (0.19) ($p<0.0162$) was found between predicted redness at lower (≤50 IU/kg feed) and higher vitamin E doses (≥100 IU/kg feed). Confidence intervals of the individual predicted a*values varied between 0.55 and 1.20. Distribution of the residuals was uniform over storage time.

Random intercepts were found to be not significant in either model, implying that experimental conditions of the individual studies did not contribute to the modelled values.

Weighting did not improve the fitting of the models, nor did it change general tendencies in results and only marginally modified predicted values. Hence, weighting factors were excluded from the final models.

The present meta-analysis was based on the period between days 0 and 17 post slaughter. However, data between days 11 and 17 originated from a single reference (Lanari *et al.*, 1995). To test for potential bias in the statistical results caused by this single reference for later storage times, the meta-analysis was also carried out using only data from the period between days 0 and 11 post slaughter. Removal of the data

referring to the later storage times was however found to have only negligible influence on the statistical model results.

### 4.4 Discussion

In this study a meta-analysis was performed to determine the effects of dietary vitamin E as an antioxidant on the changes of redness of pork during storage.

The meta-analysis combined results from five independent studies to establish quantitative relationships between the changes in pork redness with time and influencing factors of significant importance. To the best of the authors' knowledge this study is the first to quantitatively describe the time dependence of the discolouration process of pork when related to dietary vitamin E and storage in different light conditions.

Model (1) revealed a significant influence of accumulated α-tocopherol concentration in *M. longissimus dorsi*, as well as storage time and storage light on redness and its change over time. The relationship between redness and α-tocopherol concentration was linear, whereas the change of redness with storage time was best described by a third order polynomial. The moderate but positive slope value associated with α-tocopherol concentration in model (1) suggested that an increase of 1 μg of α-tocopherol in the muscle as a result of dietary vitamin E led to an expected increase of 0.11 in redness across all storage times.

A second model was derived using storage time as a fixed effect. Model (2) found an interaction between α-tocopherol concentration of *M. longissimus dorsi* and storage time, indicating that muscle α-tocopherol concentration (and hence vitamin E

supplementation) affects the evolution of redness over time. This influence was stronger after 6 days of storage. Model (2) further shows that redness decreases only slowly over time, and that differences become significant only after about a week of storage.

A third model was developed to establish quantitative relationship between supplemented vitamin E dose, storage conditions and changes in redness. In model (3) definition of dose categories was required to quantify the effect of dose. This simplification may have been due to the relatively low number of data points (80) from 5 references compared to the available number of vitamin E dose levels (7), however this model predicted the changes of redness over time as model (2).

Considering redness changes over time the initial increase in redness during storage is coherent with findings of earlier studies unrelated to the effects of vitamin E (Lindahl *et al.*, 2006, Li *et al.*, 2009). While these two studies found that after the initial increase, redness remained stable, other studies found a decrease in redness over several days of storage (Juncher *et al.*, 2001, Tikk *et al.*, 2008), which is in accordance with the results of this meta-analysis.

To correctly predict redness, all models needed to take into account whether samples were exposed to illumination. This result is a good agreement with the existing literature (Faustman and Cassens, 1990, Honikel, 1998) which shows that redness of pork and pork products decrease during exposure to light (Calkins *et al.*, 1986), (Andersen *et al.*, 1988). Storage light was found to have a significant influence on redness of pork with a difference of about 1.4 between light and dark storage conditions and this effect was found constant over all storage time by this meta-analysis.

Several other factors that may influence the relationship between vitamin E and colour changes could not be assessed in the present analysis. Unlike in Chapter 3, the length of time supplementary vitamin E was fed was identified as a non-significant factor in the models. Earlier studies established that blood α-tocopherol concentration of pigs stabilise after more than 2 weeks of vitamin E supplementation and a linear relationship was found between blood- and tissue α-tocopherol concentration at slaughter (Hoppe *et al.*, 1993). The duration of vitamin E supplementation was considerably longer than 14 days pre slaughter in all references used for the present meta-analysis (Table 4.1). Thus tissue α-tocopherol concentrations may have reached equilibrium with vitamin E supply, which might explain why supplementation times did not significantly contribute to the models. Furthermore, although studies included in the meta-analysis gave information on the breeds used, mainly modern crossbreds (Table 4.1), any breed effect could not be assessed. The reason is that pigment (myoglobin) content of muscle fibres is primarily responsible for meat colour (Hedrick *et al.*, 1994, Faustman and Cassens, 1990) and no marked difference in muscle fibre numbers and size of the longissimus muscle were apparent between modern meat-type pig breeds and crosses (Pas, 2004). Similarly, the present meta-analysis concerns only redness of a specific muscle, *M. longissimus dorsi*. Among the references used in this analysis only two (Hoving-Bolink *et al.*, 1998, Phillips *et al.*, 2001) published changes of a* values of *M. psoas major* over time. This dataset was too small to carry out a meta-analysis for this muscle. Finally among the references, which satisfied our selection criteria, only 2 references (Dirinck *et al.*, 1996, van Heugten *et al.*, 2002) contained information on time dependent changes in lightness (L*) and only one (van Heugten *et al.*, 2002) on

time dependent changes in yellowness (b*) following different amounts of vitamin E administration.

In summary the present study used meta-analysis based on a small number of carefully selected experiments to establish quantitative relationships for the effect of dietary vitamin E supplementation on change of redness of pork under different storage conditions.

## 4.5 Conclusion

In conclusion, the three statistical models together suggest that vitamin E supplementation affects redness of pork, but only when supplementation of dose exceeds 100 IU/kg and after 6 days post slaughter.

The number of experiments included in this meta-analysis was small, as the aim was to produce statistically robust estimates rather than less reliable estimates based on a larger number of studies but inconsistent experimental conditions.

Further studies would be necessary to confirm the results, to establish and validate the found relationships for other muscles and colour attributes.

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# Chapter 5: Meta-analysis of effects of gender in combination with carcass weight and breed on pork quality

**Abstract**

Meta-analyses have been carried out to quantify the effects of gender in combination with carcass weight and breed on pork quality. Altogether published results of 43 references were used. The analysed traits were: pH24hr, pH45min, objective colour attributes lightness (L*), redness (a*), yellowness (b*) (in CIE colour system), colour- and marbling scores, drip loss, intra muscular fat content (IMF), P2 backfat thickness as well as sensory scores of juiciness and tenderness. Data of two muscle types *M. longissimus* and *M. semimembranosus* were used for the analysis.

Swine genders were defined as intact/entire male (EM), surgically castrated male (SM) immuno castrated male (IM) and entire female (EF). After standardisation of scaled traits (colour-, marbling scores, juiciness, tenderness) and accounting for cold carcass weight (CW) the statistical analysis was carried out using mixed model where breed was included as random effect. The analysis found a general effect of gender on each traits and pair-wise comparisons identified significant differences between the genders for L* (lightness), marbling scores, IMF, P2 in *M. longissimus* and pH24hr in *M. semimembranosus*. Carcass weight dependence were found to be non linear (quadratic) for a* (redness), P2 (backfat thickness) and marbling scores and linear for b* (yellowness) and colour scores in *M. longissimus* and pH24hr in *M. semimembranosus*. The analysis identified significant breed effects for all traits and confirmed the known impact of diverse breeds on specific traits.

**Keywords:** meta-analysis, pork quality, gender, sex

## 5.1 Introduction

Gender or sex is an inseparable part of an animal and sexual dimorphisms are known for many traits. The gender in mammals is based on X and Y chromosomes, with XX animals being female and XY animals being male. However, secondary sexual traits can be manipulated postnatally by surgical removal of sexual organs or other methods. Manipulations of gender of different species of domestic animals have been practiced for thousands of years (Amann and Schanbacher, 1983) and it can be applied to both males (e.g. geld horse) and females (e.g. spay bitches).

Surgical castration of male pigs used for meat production has been widely practiced for centuries, mainly for easier control their behaviour (entire males tend to be more aggressive) and avoiding boar taint, but also because of the higher propensity of castrates to deposit fat, a commodity that had been in high demand until quite recently (EFSA, 2004). Emergence of new, alternative techniques to surgical castration i.e. administration of exogenous hormones (Busch *et al.*, 1979), passive- and active immunization against pituitary (LH) or hypothalamic hormones (GnRH) (Xue *et al.*, 1994, Schneider *et al.*, 1998), which regulate release of sex hormones (Amann and Schanbacher, 1983) in male pigs may make possible a wider definition of pigs' genders. Technological attempts which allow manipulation of gender by sexing of sperm (Johnson *et al.*, 1987, Johnson and Clark, 1998) or manipulation of sexes of preborn animals by semen selection (Rath *et al.*, 2003) or in IVF (Rath *et al.*, 1997) potentially offer the possibility of homogenous sexes in herds in the future make require a review the effect of pig genders.

Gender should be considered in every scientific paper because it is an important attribute of the animal. Publications studying effect of sex on pigs concentrate on growth of pigs (e.g. Quiniou *et al.*, 1999, Schinkel *et al.*, 2002, Hamilton *et al.*, 2003) or that study only one particular aspect of pork quality (e.g. Zamaratskaia and Squires, 2009). Individual papers are sparse where effects of all gender types are studied and compared (Gispert *et al.*, 2010) and very often they quantify the effects for a particular breed or carcass weight. Review papers about the effects of gender discuss only one particular sex (e.g. Lundström *et al.*, 2009) or two (e.g. Xue *et al.*, 1996) but not all genders together, also they offer good qualitative overview, but do not establish quantitative relationships.

In animal science, meta-analysis has been described as a useful statistical tool to objectively integrate the results of individual experiments and to establish general response laws (St-Pierre, 2001, Sauvant *et al.*, 2008). The meta-analysis is widely used in animal science for genome wide genetic association studies and in the past the subjects of successive analyses were metabolic processes of ruminants (e.g. Bermingham *et al.*, 2008, Dragomir *et al.*, 2008), but recently several papers have been published on either pigs (Schulin-Zeuten *et al.*, 2007) or pork quality (Trefan *et al.*, 2010, Trefan *et al.*, 2011, Salmi *et al.*, 2010) using different methods for meta-analysis.

The aim of this chapter was to use meta-analysis to quantify the effects of different pig gender types on traits describing pork quality across a range of carcass weights and breeds. Boar taint was not studied because it is a subject of an ongoing meta-analysis running in Agroscope in Switzerland by Dr Giuseppe Bee (Dr Olena Doran personal communication).

## 5.2 Materials and methods

### *5.2.1 Data collection*

The literature search used published information written in English from 1950's to 2010, from various scientific sources including reports, journals, conference proceedings, book articles and abstracts from various electronic databases. Indices used in the search were combinations of the terms 'sex', 'gender', 'carcass weight', 'pork', ,'pork quality' and 'pig', 'swine', 'hog' and plurals of these. In some cases, authors were contacted directly to complete missing information.

The initial literature search identified 300 references, related to the effects of sex/gender and some factor(s) (e.g. carcass weight category, breed, lysine level in diet, major gene). In the majority of these references where effect of sex was studied with another factor(s), effects of these factor(s) were not available at gender/sex level either it was effect of diet (e.g. Serrano *et al.*, 2009b) or carcass weight (e.g. Piao *et al.*, 2004) or genotype (e.g. Serrano *et al.*, 2008) or major gene (e.g. Hamilton *et al.*, 2000) or penning environment (e.g. Mandell *et al.*, 2006), so the gender effect was not quantified. Since the aim of this study was to quantify how gender affects various pork quality traits with influencing factors, all of these references were excluded from the analysis. These selection criteria together with the standardisation criteria outlined below led to a drastic reduction (~60) of potential studies for meta-analysis.

### *5.2.2 Meta-analysis database*

Details of the experiments chosen for the analysis were inserted into a database, which had already been used for previous meta-analyses (Salmi *et al.*, 2010, Trefan *et al.*, 2010, Trefan *et al.*, 2011) and described in details in Chapter 2. The records stored in the database contained detailed description of each reference (e.g. type, name and date of publication, title, author names), animals used in the experiments (e.g. gender, breed, beginning-, live-, slaughter-, carcass weight), diet (e.g. gross energy, crude protein, fat content, lysine level), rearing conditions (e.g. indoor/outdoor, pen area, group size), slaughter processes (e.g. stunning method), pork quality traits studied in the experiment, sample size, origin (e.g. muscle) and treatment (e.g. storage time and temperature) as well as results from statistical analyses performed on the datasets from individual experiments.

### *5.2.3 Definition of pig genders*

Due to the complexity of scenarios related to gender in different publications, redefinition of historical classification of pig sex as boar, gilt and barrow was required in this work. Considering female animals, publications are available where the effects of gilts' castration (Serrano *et al.*, 2009a), the times of given birth to piglets (Heyer *et al.*, 2004) or the length of lactation (Ellis *et al.*, 1996) on pork quality were studied. However, due to the low number of references investigating these particular effects only data of intact females were included in the present meta-analysis and therefore these gilts were defined as *entire females* (EF). Surgical

castration of male animals by removal of the two testicles is the most common way of castration (EFSA, 2004). Considering this type of castration literature data exist which studied how late age (~80 kg live weight) affects pork quality. In this study only data of surgically castrated animals were taken into consideration where the castration of animals had happened at an early age (i.e.1-8 weeks of age) and therefore the term *surgically castrated males* (SM) will be used for these animals. An alternative way of surgical castration is active immunization against the gonadotropin releasing hormone (GnRH), known as immunocastration. Immunization against GnRH reduces the concentrations of testicular steroids, including androstenone, the size of reproductive organs, sperm numbers and aggressive behaviour in male animals (Zamaratskaia *et al.*, 2008). In this study only data were analysed where this active immunization process had taken place twice, at early stage (~8-16 weeks of age) and at a late stage (~18-20 weeks of age) in the animals. These animals will be called *immuno castrated males* (IM) and intact boars will be called *entire males* (EM) in this work.

### *5.2.4 Analysed pork quality traits*

***pH45min & pH24hr:*** The meta-analysis included studies, in which tissue pH values were measured by portable standard industrial equipment at 45 minutes and 24 hour after slaughter in various muscles of the carcasses. Storage temperatures of the carcasses in the considered studies were between 1 and 4 ºC.

***Objective colour:*** All references used in the present study provided pork colour in term of the CIE colour system (CIE, 1976), which describes colour by three parameters: lightness (L*), redness (a*), and yellowness (b*).

***IMF:*** Intramuscular fat content (IMF) was measured in connective tissue, subcutaneous- and intermuscular fat free samples by standard chemical method (e.g D'Souza and Mullan, 2002), (near) infrared spectroscopy (e.g. van Oeckel *et al.*, 1996) or CT image analysis (e.g. Faucitano *et al.*, 2005) in the included references. Intramuscular fat content was expressed as the weight percentage (%) of wet muscle tissue.

***P2 backfat thickness:*** backfat thickness was the fat depth measured at the position of last rib, approximately 6.5 cm from the midline (P2) of the *M. longissimus* (Whittemore, 2003) in all of the considered publications.

***Drip loss:*** Drip loss of pork was measured by bag method (Honikel, 1998). (See. in Appendix)

***Colour scores:*** In the references subjective colour scores were assessed using either standard colour systems such as Japanese Colour Standard (JPC) (Nakai *et al.*, 1975), National Pork Producers Council (NPPC)'s standards (NPPC, 1983, 1991) with scales 1-6, 1-5, respectively or own systems (Candek-Potokar *et al.*, 1998) with scale 1-7.

***Marbling scores:*** For evaluation of marbling scores, amounts of marbling in pork were assessed either using different editions of NPPC standards (NPPC, 1983, 1991, 1999, 2000) with scales 1-10, 1-5, 1-7, 1-10, respectively or alternative systems (Candek-Potokar *et al.*, 1998) with scale 1-7.

***Juiciness & tenderness:*** In all references, samples of sensory evaluation for juiciness and tenderness were either grilled (e.g. D'Souza and Mullan, 2002) or kept in a water bath (e.g. Kim *et al.*, 2009). The internal temperature of the samples was 70-80 ºC and the sensory assesements have been carried out by consumers or trained panellists. Juiciness and tenderness scores were assessed in different scales in the publications: 1-100 (e.g. D'Souza and Mullan, 2002), 1-8 (e.g. Coker *et al.*, 2009), 1-6 (e.g. Kim *et al.*, 2009), 1-7 (e.g. Malmfors and Nilsson, 1978) and 0-7 (e.g. Nadeje *et al.*, 2000), the latter one only for tenderness.

### *5.2.5 Meta-design*

For robust statistical analysis standardisation procedures were applied to the data (Vernet *et al.*, 2005).

#### *5.2.5.1 Standardisation procedures related to pork quality traits*

Weights of the animals were published in different forms, either live weight as pre slaughter weight (LW) or slaughter weight as weight after bleeding off the animal (SW) or hot carcass weight (hot CW) or cold carcass weight (CW). In order to derive

a uniform measure to quantify the effect of weight on the pork quality traits, the different available weights were expressed as cold carcass weight. The respective conversion factors were either publications (Friesen *et al.,* 1994 and Tikk *et al.,* 2008), where all these measures were available or literature data (Whittemore, 2003). The conversion ratios of CW/LW, CW/SW and CW/hot CW were 0.77, 0.8 and 0.98, respectively.

To standardise subjective colour- and marbling score scales the actual published values were divided by the corresponding maximum values, which were 5, 6, 7 for colour scores and 5, 7, 10 for marbling scores, and multiplied by 10. This way both types of scores were expressed as tenth of percentage of the maximum of the scale. The same method was applied for the standardisation of juiciness and tenderness scores but the maximum values of the scales were divided by 100, since maximum scores were 6, 7 and 100, expressing these scores as percentage of the maximum of the scale.

*5.2.5.2 Standardisation procedures related to data selection*

Only experiments were considered for the final analysis where digestible energy were 12.7-15.4 MJ/kg feed, fat content of the pigs' food was less than 5% at dry matter (DM) base. In the majority of experiments that satisfied pre-selection criteria animals were fed *ad libitum*. Exceptions were (Zamaratskaia *et al.,* 2008) *semi ad libitum* –accordance with animals' appetite, or (Wood *et al.,* 2004) 90% of *ad libitum*, or (Corino *et al.,* 2002) where the weight of food was 9% of LiveWeight$^{0.75}$, and (Ellis *et al.,* 1983) *feed to appetite* where diet regime

was with an upper daily limit of 2.3 kg. The latter two ones fit with standards of commercial practices and were not considered as restrictions for the animals (Whittemore, 1980). Lysine levels of analysed experiments were approximately 0.94% and in references (e.g. Friesen *et al.*, 1994), where effect of different lysine levels were studied, data related to this lysine level were considered into the analysis.

In vast majority of the published experiments, animals were kept in indoor rearing systems except of one study (Heyer *et al.*, 2004) where animals were kept in seasonal outdoor environment. Where data were published related to outdoor/indoor rearing environments (Högberg *et al.*, 2004) or organic/conventional diet (Kim *et al.*, 2009), only data of the indoor systems and conventional dietary regimes were taken into account. Where data were available related to a major gene (Heinze and Mitchell, 1988) data of halothane gene non-homozygote animals were used and in one experiment (Bee *et al.*, 2007) where insulin-like growth factor I (IGF1) transgenic and non transgenic animals were used, data of the latter ones were taken into consideration.

Where it was published, dietary levels of supplemented vitamins (e.g. vitamin C, A, E) and minerals (e.g. Se, Cr, Cu) which might have effect on pork quality (Pettigrew and Esnaola, 2001, Rosenvold and Andersen, 2003, Dunshea *et al.*, 2005, Dikeman, 2007) or growth were compared and checked to established levels (NRC, 1998, Whittemore, 2003) for normal maintenance.

### *5.2.6 Statistical analysis*

The aim of this meta-analysis was to derive a statistical model for each pork quality trait on how different genders affect the trait, with influencing factors.

The inputs for meta-analysis were results of the statistical models used in the original publications in the form of least square (LS) means or means (if LS means were not provided) and corresponding standard errors of LS means (SEM) or standard deviations (SD) where SEM were not provided.

For the meta-analysis the PROC MIXED procedure of SAS (SAS, 2000) was used which was suggested by St-Pierre, (2001) and Sauvant *et al.* (2008).

All traits were submitted to careful graphical examination at all stages of the analysis and a model, including all possible influencing factors and their interactions, was applied to the data of each trait. The visual inspection showed curvilinear tendencies on dependence of carcass weight for several traits, so the general model contained a general intercept, quadratic and linear terms of cold carcass weight as covariates, as well as gender, muscle types (e.g. *M. longissimus, M. psoas major*) or muscles within a muscle type (*M. longissimus dorsi, M. longissimus thoracis, M. longissmus lumborum*) where applicable, as fixed effects, and all possible interaction terms. Comparisons between the different gender types were carried out using the CONTRAST statement of PROC MIXED procedure (SAS, 2000). In particular, two types of comparisons were carried out for each trait: a.) pair-wise comparisons between EM, SM, IM and EF and b.) comparison between a ‘male’ group, containing EM, SM and IM and between a ‘female’ group, containing only EF.

Studies used wide range of breeds from different pure breeds (e.g. pure Duroc, Tamworth) through special far east breeds (e.g. Korean Black Big) to a large variety of combinations of crosses (e.g. Large White x (Landrace x Large White)). This led to a high number of breed levels (~10-15) compared to the data points (~30-56). From previous meta-analyses (Trefan *et al.*, 2010 and Trefan *et al.*, 2011) it was known that in the case of usage of (ANOVA based) PROC MIXED, a simplification of breed levels to a few ones would have been required, if we had wanted to use them as fixed effect. Since this simplification was not possible, in conclusion breed was used as a random effect, assuming a study specific statistics for each breed (Normand, 1999). The effect of breed was studied in different covariance matrix structures (e.g. UN – unstructured; TOEP – Toeplitz; VC – standard variance components) (SAS, 2000) by the RANDOM statement of PROC MIXED, where the SUBJECT of this statement was the experiment identifier. In these structures no correlation was assumed between the breeds, because they belonged to independent experiments. A random experimental effect was also assumed and included as a random intercept into the models.

A stepwise approach was adopted, starting from a model for each pork quality trait that included all the above described fixed effects and covariates together with their interactions, followed by stepwise removal of statistically non significant factors. To compare the accuracy of fit of the models Akaike's information criteria (AIC) (SAS, 2000), Pearson's correlation (R) between the predicted and observed values (i.e. LS means of original experiments), RSD (residual standard deviation) and CD (coefficient of determination) were calculated. RSD was calculated as $[\Sigma\Sigma(\varepsilon_{ij})^2/(n-p)]^{1/2}$ where $\varepsilon_{ijk}$ is the residual value of i-th experiment at j-th gender level of the k-th

breed, n is the number of observations and p is the number of parameters in the model. CD was calculated as $1-\Sigma\Sigma(\varepsilon_{ijk})^2/\ \Sigma\Sigma(Y_{ijk})^2$ where $\varepsilon_{ij}$ is the same as in RSD and $Y_{ijk}$ is the predicted value i-th experiment at j-th gender level of the k-th breed (Bünger and Herrendörfer, 1994). In addition, for each model normality tests of residuals and visual examinations of the residuals and predicted values (Sauvant *et al.*, 2008) were carried out.

Since research designs and accuracy varied across studies, weighting factors based on the published experimental errors were included in the models. The weighting factor was the inverse of the square of these errors divided by their mean value (St-Pierre, 2001). In studies where neither SEM nor SD was published error terms were estimated based on the coefficient of variations of the other experiments. Weighting of models was performed using the WEIGHT statement of PROC MIXED procedure of SAS (SAS, 2000).

### *5.2.7 Validation of models*

For validation of (best fitting) models of each trait, data of non-analysed traits of previous meta-analyses (Trefan *et al.*, 2010, Trefan *et al.*, 2011) and control data of experiments which studied the same effects as this analysis but the animals were treated e.g. hormones (White *et al.*, 1993) were used.

## 5.3 Results

### *5.3.1 Description of the selected datasets for meta-analysis*

The selection criteria together the standardization criteria outlined before led to 43 acceptable studies. The following references that remained for inclusion in the meta-analysis together with relevant experimental details are shown in Table 5.1.

Table 5.2 summarises which references were used for different analyses.

**Table 5.1** References used for meta-analysis of the effects of gender in combination of carcass weight and breed on pork quality traits. EM: entire male; SM: surgically castrated male; EF: entire female; IM: immuno castrated male.

| No. of pigs/ gender | Gender | Pig breed | Muscle | Reference |
|---|---|---|---|---|
| 36/36 | EM/EF | 0.75Lx0.25LW | *M. longissimus (dorsi)* | Beattie *et al.*, 1999 |
| 14/12 | EM/EF | DK43 hybrid | *M. longissimus* | Bee *et al.*, 2007 |
| 19 | SM | Dx(LxLW) | *M. longissimus thoracis* | Candek-Potokar *et al.*, 1998 |
| 24/35/32 | SM/EM/EF | Belgian Landrace | *M. longissimus dorsi* | Casteels *et al.*, 1974 |
| 12/12/11 | SM/EM/EF | Pietrain | | |
| 24* | EM | D-PIC<br>LW-PIC<br>Tamworth<br>Berkshire | *M. longissimus dorsi*<br>*M. psoas major* | Chang *et al.*, 2003 |
| 14/14 | SM/EF | Large White<br>0.5Dx0.5LW<br>Duroc | *M. longissimus thoracis* | Channon *et al.*, 2004 |
| 5 | EM | YxHampxD | *M. longissimus dorsi* | Coker *et al.*, 2009 |
| 10 | SM | Large White | *M. longissimus lumborum* | Corino *et al.*, 2002 |
| 10/10 | SM/EF | Dx(LxLW) | *M. longissimus thoracis* | Correa *et al.*, 2006 |
| 12/12/12 | SM/EF/IM | LWxLx0.5D | *M. longissimus thoracis* | D'Souza and Mullan, 2002 |
| 12/12/12 | SM/EF/IM | LWxLx0.25D | *M. longissimus thoracis* | |
| 20/20/20 | SM/EM/IM | LWxLxD | *M. longissimus thoracis* | D'Souza and Mullan, 2003 |
| 50/65/65 | SM/EM/EF | LWx(LWxL) | *M. longissimus dorsi*<br>*M. semimembranosus*<br>*M. semispinalis capitis* | Ellis *et al.*, 1983 |

| | | | | |
|---|---|---|---|---|
| 9 | EF | LWx(LWxL) | *M. longissimus dorsi* | Ellis *et al.*, 1996 |
| 28/22 | SM/EM | Dx(YxL) | *M. longissimus dorsi* | Faucitano *et al.*, 2004 |
| 20 | EF | Large White<br>LWxM<br>SG 3000 pure | *M. longissimus* | Faucitano *et al.*, 2005 |
| 8 | SM | DxL<br>Tia MesxL | *M. longissimus lumborum* | Fernandez *et al.*, 1999 |
| 9 | EF | L326 | *M. longissimus* | Friesen *et al.*, 1994 |
| 23/35/24/36 | SM/EM/EF/IM | (LxD)xPi | *M. longissimus thoracis*<br>*M. semimembranosus* | Gispert *et al.*, 2010 |
| 17 | EF | South African Landrace | *M. longissimus thoracis* | Heinze and Mitchell, 1988 |
| 14 | EF | LWxD | *M. longissimus dorsi* | Heyer *et al.*, 2004 |
| 7/7 | SM/EF | (Hamp)x(Swed LWxD) | *M. longissimus dorsi* | Högberg *et al.*, 2004 |
| 10/10 | SM/EF | Large White pure<br>Landrace pure<br>Duroc pure<br>Cz (Bel LxD)x(Bel LxH) | *M. longissimus thoracis* | Jeleniková *et al.*, 2008 |
| 10 | SM | Lampiño pure<br>Entrepelado pure<br>Retinto pure<br>Torbiscal pure<br>IBxSp D | *M. psoas major* | Juárez *et al.*, 2009 |
| 30 | SM | Korean Native Black | *M. longissimus dorsi* | Kim *et al.*, 2009 |
| 13/13 | SM/EM | Hybrid | *M. longissimus*<br>*M. brachialis* | Knudson *et al.*, 1985 |

| | | | | |
|---|---|---|---|---|
| | | | *M. semitendinosus* | |
| 40/40 | SM/EF | Dx(LxLW) | *M. longissimus*<br>*M. semimembranosus* | Latorre *et al.*, 2009 |
| 35/23 | EM/EF | Swedish Landrace pure | *M. quadriceps femoris* | Lundström *et al.*, 1987 |
| 30/30 | EM/EF | LxLW | *M. longissimus dorsi* | Magowan and McCann, 2006 |
| 187/95/175 | SM/EM/EF | Landrace | *M. longissimus dorsi* | Malmfors and Nilsson, 1978 |
| 40 | SM | Dx(LWxL) | *M. longissimus thoracis* | Martoccia *et al.*, 1995 |
| 17/32 | EM/EF | Landrace pure | *M. longissimus dorsi*<br>*M. adductor femoris* | Moss and Robb, 1978 |
| 20/20 | SM/EM | (LWxL)xH | *M. longissimus dorsi* | Nadeje *et al.*, 2000 |
| 10 | EM | Landrace pure<br>Korean Native Black | *M. longissimus dorsi* | Park *et al.*, 2007 |
| 12/12 | SM/EM | Swiss Large White | *M. longissimus* | Pauly *et al.*, 2008 |
| 13/13/13 | SM/EM/IM | Swiss Large White | *M. longissimus* | Pauly *et al.*, 2009 |
| 13/13 | EM/EM | Swiss Large White | | |
| 30/30 | SM/EF | Dan DxRetinto | *M. longissimus dorsi*<br>*M. semimembranosus* | Serrano *et al.*, 2009a |
| 10/10 | SM/EF | Dx(Dan LxDan Y) | *M. longissimus thoracis*<br>*M. semimembranosus* | Tikk *et al.*, 2008 |
| 66/49 | EM/EF | M-Y | *M. longissimus lumborum*<br>*M. semimembranosus* | van der Wal *et al.*, 1997 |
| 85/60 | EM/EF | V-Y | *M. longissimus lumborum*<br>*M. semimembranosus* | |
| 56/42 | EM/EF | Dutch Yorkshire | *M. longissimus lumborum*<br>*M. semimembranosus* | van der Wal *et al.*, 1999 |
| 11/11/9 | SM/EM/EF | Belgian Landrace | *M. longissimus thoracis* | van Oeckel *et al.*, 1996 |

| | | | | |
|---|---|---|---|---|
| 23/22/20 | SM/EM/EF | Belgian hybrid | | |
| 5/5 | SM/EF | Yorkshire | *M. longissimus dorsi* | White *et al.*, 1995 |
| 5/5 | SM/EF | Meishan | *M. longissimus dorsi* | |
| 24* | EM | D-PIC | *M. longissimus dorsi* | Wood *et al.*, 2004 |
| | | LW-PIC | *M. psoas major* | |
| | | Tamworth | | |
| | | Berkshire | | |
| 24/24/24 | SM/EM/IM | Swe Yx Swe L | *M. longissimus dorsi* | Zamaratskaia *et al.*, 2008 |
| | | | *M. biceps femoris* | |

* The same experiment but different traits were published

0.75Lx0.25LW: composite breed of 75% Landrace x 25% Large White; DK43 hybrid: hybrid of DeKalb Swine Breeders Inc., DeKalb, Ill.; Dx(LxLW): Duroc x (Landrace x Large White) crossbreed; D-PIC: Duroc based commercial breed of Pig Improvement Company; LW-PIC: Large White based commercial breed of Pig Improvement Company; 0.5Dx0.5LW: composite breed of 50% Duroc x 50% Large White; YxHampxD: Yorkshire x Hampshire x Duroc crossbreed; LWxLx0.5D: composite breed of Large White x Landrace and 50% Duroc; LWxLx0.25D: composite breed of Large White x Landrace and <25% Duroc; LWxLxD: Large White x (Landrace x Duroc) crossbreed; LWx(LWxL): Large White x (Large White x Landrace) crossbreed; Dx(YxL): Duroc x (Yorkshire x Landrace) crossbreed; LWxM: Large White x Meishan crossbreed; SG 3000 pure: Syntetic Genex 3000 -pure breed of Hypor Inc., Canada; DxL: Duroc x Landrace crossbreed; Tia MesxL: Tia Meslan (syntetic line contains Chinese blood) x Landrace crossbreed; L326: lean breed of Pig Improvement Company, Franklin, KY; (LxD)xPi: (Landrace x Duroc) x Pietrain; LWxD: Large White x Duroc; (Hamp)x(Swed LWxD): Hampshire x (Swedish Large White x Duroc) crossbreed; Cz (Bel LxD)x(Bel LxH): Czech meat pig -hybrid of Belgian Landrace x Duroc and Belgian Landrace x Duroc crosses; IBxSp D: Iberian Strain x Spanish Duroc crossbreed; LxLW: Landrace x Large White crossbreed; Dx(LWxL): Duroc x (Large White x Landrace) crossbreed; (LWxL)xH: hybrid combination of (Large White x Landrace) x H, published this form; Dan DxRetinto: Danish Duroc x Retinto crossbreed; Dx(Dan LxDan Y): Duroc x (Danish Landrace x Danish Yorkshire) crossbreed; M-Y: Lean Yorkshire line; V-Y: Fast growing Yorkshire line; Swe Yx Swe L: Swedish Yorkshire x Swedish Landrace crossbreed

**Table 5.2** References used for pork quality traits in the meta-analysis.

| Reference | pH45min | pH24hr | L* | a* | b* | IMF | P2 | Drip loss | Colour score | Marbling score | Juiciness | Tenderness |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Beattie *et al.*, 1999 | | X | X | X | X | | | X | | | | |
| Bee *et al.*, 2007 | | | | | | | X | | | | | |
| Candek-Potokar *et al.*, 1998 | | X | X | X | X | X | | X | X | | | |
| Casteels *et al.*, 1974 | | X | | | | | | | | | | |
| Chang *et al.*, 2003 | X | X | X | X | X | | | X | | | | |
| Channon *et al.*, 2004 | | | | | | X | X | | | | | |
| Coker *et al.*, 2009 | | | | | | | X | | X | X | X | X |
| Corino *et al.*, 2002 | X | X | X | X | X | | | | | | | |
| Correa *et al.*, 2006 | X | X | X | X | X | X | | | X | X | | |
| D'Souza and Mullan, 2002 | | X | X | | | X | X | | | | X | X |
| D'Souza and Mullan, 2003 | | X | X | X | X | X | X | | X | | X | X |
| Ellis *et al.*, 1983 | X | X | | | | | X | | | | | |
| Ellis *et al.*, 1996 | | X | | | | | X | | | | | |
| Faucitano *et al.*, 2004 | | | | | | X | | | | X | | |
| Faucitano *et al.*, 2005 | | | | | | X | | | | X | | |
| Fernandez *et al.*, 1999 | X | X | X | X | X | X | | | | | | |
| Friesen *et al.*, 1994 | | | | | | | | | X | X | | |
| Gispert *et al.*, 2010 | X | X | X | X | X | X | X | | X | X | | |
| Heinze and Mitchell, 1988 | X | X | | | | | | | | | | |
| Heyer *et al.*, 2004 | | X | X | X | X | | | | | | | |
| Högberg *et al.*, 2004 | | | | | | X | X | | | | | |
| Jeleniková *et al.*, 2008 | X | X | | | | X | | | | | X | X |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Juárez *et al.*, 2009 | | X | | | | X | | | | | | | |
| Kim *et al.*, 2009 | | | X | X | X | | | | | | X | | X |
| Knudson *et al.*, 1985 | | | | | | X | | | | | | | |
| Latorre *et al.*, 2009 | X | X | | | | X | | | | | | | |
| Lundström *et al.*, 1987 | | X | | | | | | | | | | | |
| Magowan and McCann, 2006 | | | | | | | X | | | | | | |
| Malmfors and Nilsson, 1978 | | | | | | X | | | | | X | | X |
| Martoccia *et al.*, 1995 | X | X | X | X | X | | | X | | | | | |
| Moss and Robb, 1978 | X | X | | | | | | | | | | | |
| Nadeje *et al.*, 2000 | | X | | | | | | | | | | | X |
| Park *et al.*, 2007 | | X | X | | | X | | | | | | | |
| Pauly *et al.*, 2008 | | X | X | X | X | | | X | | | | | |
| Pauly *et al.*, 2009 | | X | X | X | X | | | X | | | | | |
| Serrano *et al.*, 2009a | | X | | | | | | | | | | | |
| Tikk *et al.*, 2008 | X | X | X | | | | | | X | | | | |
| van der Wal *et al.*, 1997 | X | X | X | | | | X | | | X | | | |
| van der Wal *et al.*, 1999 | X | X | X | X | X | | X | | | | | | |
| van Oeckel *et al.*, 1996 | | | X | | | X | | | | | | | |
| White *et al.*, 1995 | | | | | | | X | | X | X | | | |
| Wood *et al.*, 2004 | | | | | | | X | | | | | | |
| Zamaratskaia *et al.*, 2008 | | X | | | | | | | | | | | |

### *5.3.2 Final statistical models*

Stepwise removal of non-significant factors from the general statistical model for gender and associated factors led to a model for each trait, which contained linear and quadratic terms of cold carcass weights as covariates, gender as fixed effects and breed as random effect. The final covariance structure for breed was the standard variance components (VC), which assigned different variances to the different breeds. Model (1) describes this model:

$$PQ_{ijkl} = ß_1 CW_{ij} + ß_2 CW^2_{ij} + Gender_j + Breed_k + Muscle_l + e_{ijkl} \qquad (1)$$

where $PQ_{ijkl}$: pork quality trait values of i-th experiment of j-th type of gender of the k-th breed in l-th type of muscle; $CW_{ij}$: cold carcass weight of the i-th experiment of j-th type of gender and $ß_1$ its slope; $CW^2_{ij}$: square of cold carcass weight of the i-th experiment of j-th type of gender and $ß_2$ its slope; $Gender_j$: fixed effect of the j-th type of gender; $Breed_k$: the random effect of the k-th type of breed; $Muscle_l$: fixed effect of the l-th type of muscle and $e_{ijkl}$: denotes the residuals. There were no significant interactions between the different factors and neither general nor 'random' intercept was significant. No significant differences had been found between *M. longissimus, M. longissimus dorsi, M. longissimus thoracis* and *M. longissimus lumborum,* terminologies referred to the origin of analysed data of any pork quality traits, so the most general term *M. longissimus* will be used describing the results.

Comparison between ‘male’ and ‘female’ group showed marginally significant differences for only two traits, colour lightness (L*) (p=0.0721) and P2 backfat thickness (p=0.0854), so furthermore only results of multiple comparisons are going to be shown.

### *5.3.3 Effect of gender in combination with CW and breed on pork quality traits*

***pH45min & pH24hr:*** No carcass weight dependence was found for pH45min and pH24hr in *M. longissimus*. No significant differences were found between the estimates of pH45min and pH24hr related to the different genders in this muscle.
For pH24hr in *M. semimembranosus* linear carcass weight dependence was found with slopes of 0.007 (0.002). No significant differences were found between the estimates of pH45min related to the different genders, significant differences were found of pH24 estimates of EM and each of the other genders in *M. semimembranosus* (Table 5.3).

***Colour:*** No carcass weight dependence was found for L* objective lightness of pork in *M. longissimus*. Linear, quadratic carcass weight dependence were found for objective pork redness (a*) and yellowness (b*) of this muscle with slopes of $ß_1$= -0.18 (0.06), $ß_2$= 0.0011 (0.0003) and $ß_1$= 0.019 (0.007), respectively. The same types of carcass weight dependence were found for subjective measurements of pork colour as colour- and marbling scores in *M. longissimus*. The slope, $ß_1$ for colour score was 0.025 (0.007) and for marbling scores $ß_1$, $ß_2$ were -0.11 (0.03), 0.0008 (0.0002) respectively (Table 5.3). Significant differences were found between

estimates of lightness of IM and EM, IM and EF and estimates of marbling scores of SM and EM and SM and EF. No significant differences were found between estimates of different gender types of redness, yellowness and colour scores (Table 5.3). The difference between lightness estimates of IM and SM approached the statistical significance (p=0.0694). Breed was found to have effect on each herein mentioned trait: Large White and crossing with Large White was found to increase estimated L*, a*, b* with values of around 4.14 (1.04), 1.95 (0.65), 3.67 (0.82), respectively and Meishan was found to change estimated standardised colour and marbling scores by 1.14 (0.39) and 0.82 (0.31), respectively.

***Drip loss:*** Carcass weight dependence and differences between the estimates of drip loss belonging to different genders in *M. longissimus* were found not significant. Breed was found to have effect on estimates of drip loss: one unidentifiable breed 'Hybrid' and Large White cross found to increase estimated drip loss by 2.12 (0.52) % and 2.63 (0.57) %, respectively.

***IMF:*** No carcass weight dependence was found for IMF in *M. longissimus*. Significant differences were found between estimated intramuscular fat contents of EM and SM, EM and EF, EM and IM and SM and EF (Table 5.3). The difference between IMF estimates of IM and EF approached the statistical significance (p=0.0736). Breed was found to have effect on estimated intramuscular fat content in *M. longissimus*: two pure breeds, Duroc and Landrace were found to increase estimated IMF by 2.49 (0.25) % and 0.87 (0.25) %, respectively and Large White to decrease it by 1.09 (0.25) %.

***P2:*** For P2 backfat thickness quadratic carcass weight dependence were found with a slope of $ß_2$= 0.0009 (0.0002). Significant differences were found between estimated P2 backfat thickness of EM and SM, EM and IM and SM and EF (alongside *M. longissimus)*. Breed was found to have effect on estimated P2 backfat thickness: breed Meishan was found to increase estimated P2 by 11.74 (1.82) (mm) and Large White or crossing Large White to decrease it by 4.33 (1.83) (mm).

***Juiciness & tenderness:*** No carcass weight dependence was found for (standardised) juiciness and tenderness scores of *M. longissimus* and no significant differences were found between the estimates of these scores belonging to the different gender types (Table 5.3). Breed was found to have effect on estimated (standardised) juiciness and tenderness scores of *M. longissimus*: pure Large White was found to decrease both scores by 19.71 (4.58) and 10.79 (5.19), respectively and pure Landrace to decrease the juiciness scores by 13.08 (4.58).

**Table 5.3** Estimations and standard errors of slopes (SE) of linear ($ß_1$), quadratic ($ß_2$) cold carcass weight dependence. Least square (LS) means and standard error of LS means (SEM) of the pork quality traits and their comparisons related to the different genders, based on the best fitting model -EM: entire male; SM: surgically castrated male; IM: immuno castrated male; EF: entire female.

| Trait | Muscle | $ß_1$ (SE) | $ß_2$ (SE) | LS mean (SEM) | |
|---|---|---|---|---|---|
| pH45min | *M. l.* | | | EM | 6.40 (0.04) |
| | | | | SM | 6.34 (0.04) |
| | | | | IM | 6.37 (0.08) |
| | | | | EF | 6.38 (0.04) |
| pH45min | *M. s.m.* | | | EM | 6.41 (0.06) |
| | | | | SM | 6.42 (0.06) |
| | | | | IM | 6.40 (0.06) |
| | | | | EF | 6.41 (0.06) |

| | | | | | |
|---|---|---|---|---|---|
| pH24h | *M. l.* | | | EM | 5.57 (0.02) |
| | | | | SM | 5.56 (0.02) |
| | | | | IM | 5.56 (0.03) |
| | | | | EF | 5.55 (0.02) |
| pH24hr | *M. s.m.* | 0.007 (0.002) | | EM | 5.76 (0.06)[a] |
| | | | | SM | 5.69 (0.06)[b] |
| | | | | IM | 5.67 (0.07)[b] |
| | | | | EF | 5.72 (0.06)[b] |
| Lightness (L*) | *M. l.* | | | EM | 53.69 (0.79)[b] |
| | | | | SM | 53.82 (0.79)[a,b] |
| | | | | IM | 54.68 (0.86)[a] |
| | | | | EF | 53.46 (0.79)[b] |
| Redness (a*) | *M. l.* | -0.18 (0.06) | 0.0011 (0.0003) | EM | 7.05 (0.26) |
| | | | | SM | 7.14 (0.26) |
| | | | | IM | 6.64 (0.36) |
| | | | | EF | 6.89 (0.28) |
| Yellowness (b*) | *M. l.* | 0.019 (0.007) | | EM | 5.72 (0.77) |
| | | | | SM | 5.59 (0.77) |
| | | | | IM | 5.83 (0.78) |
| | | | | EF | 5.56 (0.78) |
| Colour scores | *M. l.* | 0.025 (0.007) | | EM | 5.16 (0.34) |
| | | | | SM | 4.92 (0.31) |
| | | | | IM | 4.71 (0.62) |
| | | | | EF | 5.26 (0.28) |
| Marbling scores | *M. l.* | -0.11 (0.03) | 0.0008 (0.0002) | EM | 2.62 (0.29)[b] |
| | | | | SM | 3.36 (0.23)[a] |
| | | | | IM | 2.41 (0.56)[a,b] |
| | | | | EF | 2.57 (0.19)[b] |
| Drip loss (%) | *M. l.* | | | EM | 5.29 (0.46) |
| | | | | SM | 5.57 (0.48) |
| | | | | IM | 5.69 (0.59) |
| | | | | EF | 5.63 (0.62) |
| IMF (%) | *M. l.* | | | EM | 2.12 (0.19)[c] |
| | | | | SM | 2.55 (0.19)[a] |
| | | | | IM | 2.71 (0.25)[a,b] |
| | | | | EF | 2.35 (0.18)[b] |
| P2 (mm) | *M. l.* | | 0.0009 (0.0002) | EM | 15.48 (1.29)[b] |
| | | | | SM | 19.76 (1.44)[a] |
| | | | | IM | 18.99 (1.69)[a] |
| | | | | EF | 16.60 (1.29)[b] |
| Juiciness | *M. l.* | | | EM | 54.17(4.32) |
| | | | | SM | 53.09(3.50) |
| | | | | IM | 57.44(4.26) |
| | | | | EF | 51.51(3.76) |
| Tenderness | *M. l.* | | | EM | 57.77(4.46) |
| | | | | SM | 58.38(3.49) |
| | | | | IM | 62.67(4.93) |
| | | | | EF | 55.27(4.06) |

*M. l.: M. longissimus; M. s.m.: M. semimembranosus*
*a, b, c: common characters in the superscripts refer to no significant differences ($p<0.05$); comparisons between different genders within a trait*

### *5.3.4 Weighting and validation of models*

Weighting did not improve the fitting of the models, nor did it change general tendencies in results and only marginally modified predicted values. Hence, weighting factors were excluded from the final models.

Within the models carcass weight range, data points used for validation were between the 95% confidence limits of predicted values of the established models for each trait.

## 5.4 Discussion

In this study meta-analysis was performed to determine the effects of gender in combination of carcass weight and breed on several pork quality traits. The meta-analysis combined results from 43 independent studies to establish quantitative relationships between the effect of gender and influencing factors of significant importance on pork quality. Effects of gender on individual pork quality traits have been studied in numerous experiments, but to the best of the authors' knowledge this study is the first to quantitatively describe the relationship between several pork quality traits and gender categories across wide range of carcass weights and breeds. The meta-analysis used results of publications where the effects of gender together with the influencing factor(s) were available in quantified format. Data where these effects were not quantified at gender levels were excluded from the analysis, because the applied statistical method, frequentist approach required level(s) of each factor

for its calculations either the factor was taken account as fixed or random effect in the models. Using ad-hoc calculations as estimations of the effects of a factor as input would have undermined the precision of the calculations in the models and potentially led identification of fake factors in the analysis.

When gender categories were grouped into two groups as 'male' and 'female' in the analyses, no significant differences were found for any traits between these groups. It can be concluded that both analysed (surgical and immuno) types of castration are such a drastic intervention of the male animals' processes that from a pork quality point of view they should be treated as two different distinguishable genders therefore results of four different gender categories will be discussed.

General effect of gender was found for each studied pork quality traits, the pair-wise comparisons revealed significant differences between the estimated LS means related to the different gender types for the following traits: pH24 in *M. semimembranosus* and L*, marbling scores, IMF and P2 in *M. longissimus*. Carcass weight dependence was found either linear or quadratic for the following traits: a*, b*, colour-, marbling scores, P2 in *M. longissimus* and pH24hr in *M. semimembranosus*. (There was a model for pH45min in *M. semimembranosus* which had similar fit statistics of the one whose results presented in this study earlier (Table 5.3) and found linear carcass weigh dependence with $ß_1$= -0.009 (0.002) for pH45min in this muscle.) Breed was also found to be a significant factor on pork quality traits however in magnitude its effect (estimated value for a breed/estimated values of a trait) ranged from very low (e.g. ~10% - breed Large White for L* estimates) up to very high (e.g. ~100% - pure Duroc breed for IMF estimates).

Only one analysed reference (van der Wal *et al.*, 1997) identified factors as sources of variances for different pork quality traits, using linear regression for pH, (Hunter) colour values and threshold model for subjective pork colour and filter paper scores. Only definitions of analysis by linear regression are being discussed here. A number of dependent variables (Y variables) with various independent variables (explanatory or X variables) were fitted to the data in that reference. The largest set of X variables consisted of a factor for day of slaughter, supplemented with linear and squared variables for the resting period, combinations of scores about the intensity of the muscular contractions during stunning, shackling and exsanguination, sex and (genotype) lines. These X variables added significant information to the (regression) model. Smaller sets of X variables were also formed by omitting one or more factors or variables. Comparing the two models related to a trait, percentage variation explained (PVE) was defined as PVE = [(RSS2-RSS1)/RSS2]*100% = [(D2-D1)F/{D1+(D2-D1)F}]*100% where RSS1 is residual sum of squares of the 'largest model', RSS2 is residual sum of squares of the 'smallest model'; D1,D2 are the corresponding degrees of freedom of the residual sums of squares; F is the traditional F value for the various explanatory variables which are present in the largest models, but not in the smallest model. That particular reference found PVE 4.8%, 1.6% and 2.3% explained by sex (boar and gilt) for pH24hr in *M. semimembranosus*, water holding capacity measured by filter paper method (expressed in mg) and colour lightness (L*) in *M. longissimus (lumborum),* respectively. PVE of water holding capacity measured by filter paper method expressed in mg and scores, colour scores and L* in *M. longissimus (lumborum)* in extent of 4.4%, 3.2%, 2.6% and 2.6%, respectively was explained by two genotypes,

lean (M-Y) and fast growing (V-Y) Yorkshire lines. These findings are not contradictory the findings of this meta-analysis, because gender effect was found on each mentioned pork quality traits and modifying effect of breed has been also confirmed by this meta-analysis. However it is important to note that our results try to establish general laws, but the results of the paper discussed herein are valid for only two gender types and two genotypes under a particular experiment conditions.

Individual references (Beattie *et al.*, 1999, Candek-Potokar *et al.*, 1998, Coker *et al.*, 2009, Correa *et al.*, 2006, Latorre *et al.*, 2009, White *et al.*, 1995) which studied how weight affects pork quality just reported pork quality traits values at different weight levels but did not study their dependence on weight. This analysis found (cold) carcass weight dependence quadratic for a*, P2 and marbling scores and linear for b*, colour scores in *M. longissimus* and pH24hr in *M. semimembranosus*.

The results of the meta-analyses of this work offer the possibility to give a position for pork of IM animals. Based on the studied traits, pork of IM animals is distinguishably different from pork of natural genders (EM, EF) i.e. significant differences were found between estimates of IM and EM, EF for most of the pork quality traits (Table 5.3). The results also show that pork of IM is the lightest and lighter than pork of SM animals (in the latter case, the differences of the relevant estimates of L* approached the level of significance). Furthermore however no significant differences were found between IM and SM, but the results suggest higher intramuscular fat content (IMF), juiciness, tenderness and lower subcutaneous fat content for IM animals than for SM animals (Table 5.3). More experimental results are required to confirm these suggestions.

Because of the low number of IMs in studied traits, models were rerun without IM animals, the tendencies and differences between estimations for the three other genders did not change.

Apart from natural sexes EM, EF, data of surgically- and immunised against GnRH as immuno castrated animals were analysed. Data of other alternative methods to surgical castration (Giri *et al.*, 2002) were sparse and in the case of the immunocastration it has been shown that active immunization against hypothalamic hormone (GnRH) is more effective then active immunization against pituitary hormone (LH) (Falvo *et al.*, 1986) therefore the available published data of latter one was not enough for a meta-analysis. All IM animals used, were vaccinated twice during their growths with 2 ml Improvac© by Pfizer Ltd, which contained 200 μg GnRH-protein conjugate/ml in an aqueous adjuvant system.

In the statistical analysis breed was considered as random effect. The advantage of this that estimations for range of breeds could have been got, but could have not been studied whether gender effects differed between the breeds (i.e. could not have tested for interaction). Fitting experiment also as random effect was found not significant for any traits, implying that the individual experimental conditions did not give significant contribution to the estimated values.

For traits for which data were published related to more then one muscle (Table 5.1), effect of muscle type together with gender was studied using the different muscle types as fixed effect in the models. Muscle effects have been found for each of the examined traits but sufficient data for meta-analysis were only for two muscles i.e. for *M. longissimus, M. semimembranosus*.

For the traits IMF and marbling scores, where there were indications in the literature (Faucitano *et al.*, 2004) that the values of the trait may differ within a particular muscle (i.e. *M. longissimus),* the possible within muscle effect of the different parts of the muscle were studied, using the sections of the muscle i.e. *M. longissimus dorsi, M. longissimus thoracis, M. longissimus lumborum* as fixed effect in the models. Current results did not confirm indications of the literature neither for IMF nor for marbling scores, therefore the general term, *M. longissimus* with the corresponding LS means of the trait were assigned to.

Storage conditions affect pork quality (Faustman and Cassens, 1990, Rosenvold and Andersen, 2003), but deeper study of the storage conditions of the individual experiments showed that taking account of all available time measures reported in the references i.e. chilling time, carcass storage time, additionally to the published (sample) storage time, made all storage times close to 48 h post slaughter in each relevant experiment. Therefore storage time dependence of pork quality traits could not have been examined in this analysis for traits where literature suggested e.g. drip loss (Otto *et al.*, 2004) or there were indications from previous meta-analysis (Trefan *et al.*, 2010) (a*).

Water holding capacity of pork was described and analysed as drip loss measured by bag method in this study. Drip loss data measured by filter paper method (Kauffman *et al.*, 1986, Appendix) were available only in the case of 4 references (van der Wal *et al.*, 1997, van der Wal *et al.*, 1999, Juárez *et al.*, 2009, Kim *et al.*, 2009), measured by tray method or EZ-tray method (Christensen, 2003, Appendix) only in the case of 2 references (Channon *et al.*, 2004, Heyer *et al.*, 2004) and 1 reference (Correa *et al.*, 2006), respectively. The low number of data points did not offer the possibility to

carry out analyses for these data and in the case of 2 further references (Heinze and Mitchell, 1988, Park *et al.*, 2007) even the (drip loss) measuring methods were not described.

Cooking losses were published only in the case of 4 papers (Heinze and Mitchell, 1988, Beattie *et al.*, 1999, Nadeje *et al.*, 2000, Kim *et al.*, 2009) measured by standard method (Honikel, 1998, Appendix), in several other references samples were vacuum packed and frozen at -20 ºC (e.g. Pauly *et al.*, 2009) and even frozen period or storage times were not published, therefore there were not sufficient number of data points to carry out meta-analysis for cooking loss. Similar reasons to the cooking loss, data of Warner-Bratzler shear force were also not assessed in this study.

Only two sensory traits were analysed in this meta-analysis. Other sensory traits such as odour, abnormal flavour, off-flavour were not taken into the analysis because either the published sensory scores were impossible to standardise and compare or conditions of sample preparations differ in such extent (e.g. Jeremiah and Sather, 1999, Font i Furnols *et al.*, 2008, Font i Furnols *et al.*, 2009) that it might have affected the assessed scores (Wood *et al.*, 1995) and made them incomparable.

Information about major genes was available just in the case of a few references. Högberg *et al.* (2004) used Rendement Napole ($RN^-$) gene allele carrier animals, Heinze and Mitchell (1988) reported the results at halothane gene heterozygote and homozygote levels, Martoccia *et al.* (1995), Candek-Potokar *et al.* (1998) used halothane gene free, Fernandez *et al.* (1999) $RN^-$ gene free pigs and Bee *et al.* (2007) used insulin-like growth factor I (IGF1) transgenic and non transgenic animals. However existence and segregations of all of these genes and alleles in other

experiments could not be excluded, but accounting for them were not possible due to lack of the relevant information.

## 5.5 Conclusion

This study found meta-analysis a powerful tool for establishing quantitative relationships between gender, influencing factors and pork quality traits.

When pigs are fed with a standard diet of digestible energy 12.7-15.4 MJ/kg feed, which contain ~0.94 % lysine, less than 5% fat (on DM base), supplementary vitamins and minerals at levels of maintenance, gender has been found to be significant factor affecting pork quality traits of pH45min, pH24hr, objective colour attributes (L*, a*, b*), colour- and marbling scores, IMF, P2, drip loss, juiciness and tenderness in *M. longissimus*/*M. semimembranosus*. Carcass weight dependence has been found for a*, b*, colour-, marbling scores, P2 in *M. longissimus* and for pH24hr in *M. semimembranosus* and breed also has been found to have significant influence on all traits with different magnitude.

Validation of the models has confirmed the reliability of the established models.

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# Chapter 6: Meta-analysis of effects of gender in combination with carcass weight and breed on selected pork quality traits using a Bayesian approach

**Abstract**

Meta-analyses using a Bayesian approach have been carried out to validate the results of the previous chapter with an alternative statistical approach, and to asses whether a method that can take account of prior uncertainties in the data yields more accurate estimates for the effect of influencing factors on the traits of interest.

For this purpose a subset of data that was used for the previous frequentist meta-analysis (Chapter 5) has been selected to quantify the effects of gender, in combination with carcass weight and breed on pork quality. The studied traits were objective colour attributes lightness (L*), redness (a*), yellowness (b*), intramuscular fat content (IMF) and drip loss in *M. longissimus*. The statistical model was the meta-regression with a linear hierarchical model (multilevel model).

The estimates for gender effects and carcass weight dependence of the studied pork quality traits obtained from the Bayesian approach were in good agreements with the findings of the previous, frequentist approach.

**Keywords:** meta-analysis, Bayesian approach

## 6.1 Introduction

In the field of meta-analysis, Bayesian methods are well established (Normand, 1999, Phillips, 2005, Whitehead, 2002). In particular, several studies have demonstrated the usefulness of Bayesian meta-analyses assessing the influence of diverse factors (e.g. Phillips *et al.*, 2001, Salmi *et al.*, 2010). The essence of the Bayesian approach is that it combines known likelihoods of parameters with their assumed prior distributions and calculates posterior distributions for the estimations of these parameters in the applied models. The advantage of the Bayesian approach is the ability to account for uncertainty in all relevant sources of variability in the applied model. In a Bayesian analysis, the posterior density is fully evaluated and exact posterior standard deviations and credibility intervals can be obtained from the posterior distributions for each model parameter (Whitehead, 2002). In contrast, in the frequentist approach, model parameters are often computed using formulae that rely on the assumption that the variance components are known (Verbeke and Molenberghs, 1997).

Here a Bayesian approach was used to validate the results from the frequentist approach applied to study the effects of gender in combination with breed and carcass weight on pork quality (Chapter 5), and assess whether additional information on the relevant effects can be discovered. For this purpose a subset of previously analysed data was used.

Particular traits were chosen according to uncertainties about their dependence on gender or on gender and carcass weight found in the previous meta-analysis. Therefore IMF and drip loss were chosen, because both traits showed a significant

general gender effect, but significant differences between the different gender classes were only found for IMF (Table 5.3). Objective pork colour attributes were also chosen, because of a general gender influence on each attribute, although significant differences between the different gender classes were only found for L*. Also, the Bayesian approach was used to validate the different carcass weight dependencies for the colour attributes (Table 5.3).

## 6.2 Materials and methods

Data sourcing, inclusion criteria and detailed description of the used dataset can be found in (Chapter 5). In brief:

### *6.2.1 Description of the meta-analysis dataset and analysed traits*

All references used in the present study provided pork colour in terms of the CIE colour system (CIE, 1976), IMF was measured mainly by standard chemical method (e.g. Channon *et al.*, 2004) and drip loss by 'bag method' (Honikel, 1998, Appendix). Only data originating from *M. longissimus* were used.

In the references considered, dietary levels of pigs food constituents were at normal maintenance levels, fat content of <5% on DM base. Genders of the animals were defined as intact/entire male (EM) and female (EF), surgically castrated (SM) and immuno castrated male (IM) and weights of the animals were expressed as cold carcass weights (CW).

Further details of the analysed traits related references can be found in Table 5.1 and Table 5.2 in the previous chapter.

### *6.2.2 Statistical analysis*

The model presented below has been used for each pork quality trait considered and is called meta-regression with a linear hierarchical model, the latter one is also known as multilevel model. A quadratic carcass weight dependence was assumed based on the results of the analyses in Chapter 5.

$$pq_{ijk} \sim N(\mu_{ijk}, \sigma^2)$$

$$\mu_{ijk} = \alpha_k + ß_j \text{ x } gender_{ij} + \gamma_1 * cw_{ij} + \gamma_2 * cw^2_{ij}$$

$$\alpha_k \sim N(\mu_{k,} \sigma^2_k)$$

$$ß_j \sim N(\mu_j, \sigma^2_j)$$

$$\gamma_l \sim N(\mu_l, \sigma^2_l) \quad l=1,2$$

$$diff_{jm} = ß_j - ß_m \quad j \neq m$$

where $pq_{ijk}$ is the observed value of the pork quality trait of i-th experiment of j-th gender class of the k-th breed. The model assumes that these values represent $\mu_{ijk}$ true values of the trait of i-th experiment of j-th type of gender of the k-th breed, which is normally distributed with $\sigma^2$ (common) variance. The measures $cw_{ij}$, $cw^2_{ij}$ refer to cold carcass weight and its square value of the i-th experiment of j-th type of

gender, $\alpha_k$ is the intercept of a k-th breed ($\alpha_k$ is associated with the random effect), ßj is the coefficient of the j-th gender type (ßj is associated with fixed effect) and $\gamma_1$, $\gamma_2$ is the gradients of $cw_{ij}$, $cw^2_{ij}$, respectively, $diff_{jm}$ denotes the estimator of the difference between gender types of j-th and m-th.

$N(y, x^2)$ denotes a normal distribution with mean y and variance $x^2$, from which the effects were sampled.

Parameter estimations of the statistical model were performed by Markov chain Monte Carlo simulations, using Bayesian statistics with the Windows version of the BUGS software package, called WinBUGS (Lunn *et al.*, 2000). The software assumes a Bayesian or full probability model, in which all quantities are treated as random variables. The model consists of a defined joint distribution over all unobserved and observed quantities (parameters and the data, respectively). Marginalizing over the posterior distribution in order to obtain inferences on the main quantities of interest is carried out using a Monte Carlo approach to numerical integration (Gibbs sampling). The credible interval (CI) corresponds to a 95% probability region for the estimated parameters (Prankel *et al.*, 2004).

A stepwise approach was adopted, starting from a model for each pork quality trait that included all the above described effects, followed by stepwise removal of factors whose credible intervals included zero. To compare the accuracy of fit and complexity of the models, effective number of parameters (pD) and Deviance Information Criterion (DIC) (Spiegelhalter *et al.*, 2002) were used.

### *6.2.3 Model fitting*

Prior distributions were required for all nodes (parameters or unknown quantities) in the statistical models. For nodes that have parents, i.e. nodes that directly influence them, these are generated by forward sampling. For nodes that have no parents, it was necessary to define prior distributions.

The prior distributions chosen for the models were:

$1/\sigma^2 \sim \Gamma(0.1, 0.1)$

$ß_j, \gamma_1, \gamma_2 \sim N(0, 10^6)$

$\mu_k \sim N(0, 10^{-3})$

$1/\sigma^2_k \sim \Gamma(1.1001, 1.1001)$

Where $\Gamma(a, b)$ denotes a gamma distribution with shape parameter a and rate parameter b.

Starting values used were for $ß_j, \gamma_1, \gamma_2 = 0$ and for $1/\sigma^2=1$, and for the remaining parameters they were generated by the software.

Each model was fitted with a single chain and the Markov chain Monte Carlo algorithm was run for an initial 1000–3000 iterations (burn-in). Convergence was assessed visually and was obtained for each studied trait. Every sample was recorded. At each iteration of the Gibbs sampler, one realisation of each parameter was calculated. History, trace and (posterior) distribution of each parameter was studied visually. The median value and the quantiles (which are the running averages) of the resulting Markov chain were then taken as the estimates and the 95% CI for the parameter.

## 6.3 Results

### *6.3.1 "Significance" in Bayesian approach*

Significance testing in the classical Neyman–Pearson sense (Neyman and Pearson, 1933) is not well defined in a Bayesian paradigm. However, for readability, ''significance'' here is used to mean that the posterior 95% credible interval of the estimation of a coefficient of a parameter in the model ($\alpha_k$, $\beta_j$, $\gamma_{1,2}$) or a difference ($diff_{jm}$) does not include zero (Prankel *et al.*, 2004).

### *6.3.2 Effect of gender classes in combination with CW and breed on pork quality traits*

*Colour:* Effect of each gender type was found significant for each objective pork colour attribute in *M. longissimus* but estimates of differences between these gender types were not significant. No significant carcass weight dependence was found for L* objective lightness. Significant linear and quadratic carcass weight dependence was found for redness (a*) and yellowness (b*), and the associated 'slopes' were $\gamma_1$= -0.22 (0.08), $\gamma_2$= 0.001 (0.004) and $\gamma_1$= 0.015 (0.011), respectively (Table 6.1).

*IMF:* The effect of each gender class was found significant for IMF in *M. longissimus* but no significant carcass weight dependence was found (Table 6.1). Significant differences were found between gender types of EM and SM, EM and EF, EM and IM and SM and EF. Two pure breeds, Duroc, Landrace, and Korean Native Black were found to increase IMF estimates by 2.69 (0.31) % and 1.02 (0.29)

%, 2.36 (0.37) %, respectively, and breed Large White to decrease it by 0.93 (0.34) %.

*Drip loss:* Effect of each individual gender types were found significant for drip loss in *M. longissimus* and neither differences between the individual gender types nor carcass weight dependence were found (Table 6.1). Breeds, 'Hybrid' -unidentifiable and Large White cross were found to increase estimated drip loss by 2.19 (0.77) % and 2.67 (0.87), respectively.

**Table 6.1** Estimations of effects of individual gender types and carcass weight dependence in Bayesian and frequentist approach. The estimations are means and standard deviations (SD) of the posterior distributions of the model parameters in the Bayesian- and least square means and standard errors (SE) of the model parameters in the frequentist approach.

| **Bayesian approach** | | | | | | |
|---|---|---|---|---|---|---|
| Trait | Effect of gender (SD) | | | | Coefficients of | |
| | EM | SM | IM | EF | CW | $CW^2$ |
| L* (lightness) | 55.36 (0.63) | 55.11 (0.81) | 55.54 (0.94) | 54.46 (0.73) | | |
| a* (redness) | 15.42 (3.15) | 15.68 (3.21) | 15.10 (3.19) | 15.12 (3.19) | -0.22 (0.08) | 0.0013 (0.0004) |
| b* (yellowness) | 3.94 (0.84) | 3.84 (0.88) | 3.97 (0.90) | 3.57 (0.86) | 0.015 (0.011) | |
| IMF (intramuscular fat) (%) | 1.98 (0.23) | 2.41 (0.22) | 2.58 (0.29) | 2.22 (0.21) | | |
| Drip loss (%) | 5.11 (0.56) | 5.47 (0.64) | 5.53 (0.82) | 5.60 (0.81) | | |
| **Frequentist approach (mixed model)** | | | | | | |
| | Effect of gender (SE) | | | | Coefficients of | |
| | EM | SM | IM | EF | CW | $CW^2$ |
| L* (lightness) | 53.69 (0.79) | 53.82 (0.79) | 54.68 (0.86) | 53.46 (0.79) | | |
| a* (redness) | 14.01 (2.34) | 14.10 (2.36) | 13.60 (2.37) | 13.85 (2.36) | -0.18 (0.06) | 0.0011 (0.0003) |
| b* (yellowness) | 3.99 (0.95) | 3.88 (0.96) | 4.11 (0.97) | 3.84 (0.95) | 0.019 (0.007) | |
| IMF (intramuscular fat) (%) | 2.12 (0.19) | 2.55 (0.19) | 2.71 (0.25) | 2.35 (0.18) | | |
| Drip loss (%) | 5.29 (0.46) | 5.57 (0.48) | 5.69 (0.59) | 5.63 (0.62) | | |

EM: entire male; SM: surgically castrated male; IM: immuno castrated male; EF: entire female; CW: (cold) carcass weight; drip loss measured by 'bag method'

## 6.4 Discussion

This work described an alternative meta-analytic approach to that used in the previous chapters. Objective colour attributes (L*, a*, b*), IMF, drip loss pork quality traits were studied using a Bayesian approach. The applied statistical model was a meta-regression combined with a linear hierarchical/multilevel model in which all influencing factors were considered as random. Appropriate parameterisation of the factors in the Bayesian models made it possible to associate these factors with equivalent factors used in the classical, frequentist models. In this context breed could be considered as 'random effect', gender as 'fixed effect' and carcass weight as 'covariate', and the Bayesian estimates of these are comparable with estimates obtained by frequentist models. Comparisons of the estimates of the effect of gender, breed and coefficients of CW and $CW^2$, calculated by the different methods, showed very good agreement for each trait (Table 6.1). For traits where no carcass weight dependence was found (i.e. L*, IMF, drip loss) gender related differences calculated by the Bayesian approach could be compared with differences calculated by frequentist approach. The differences related to the different gender types were very similar for IMF and drip loss. Only for the colour trait L*, the gender class differences by the frequentist approach were not confirmed by the Bayesian approach. The corresponding least square (LS) mean estimates of L* are close to the lower bounds of credibility interval (95%) of the posterior distributions obtained in the Bayesian approach. However, the means (medians, modes) of L* in these distributions were slightly higher (~55.0) for all gender classes (Table 6.1). This

discrepancy could be an artefact of the different assumptions used in the alternative statistical approaches. The frequentist approach assumes normally distributed input data (SAS, 2000), whereas in the Bayesian approach no such assumption is made. Inspection of the L* input data showed slight skewness of the L* values with 60% of the input data reaching values above 55.0. The deviation from the normality assumption may introduce a downward bias in the frequentist LS mean estimates. Alternatively, the Bayesian estimates may be influenced by the choice of prior distributions. However, inspection of the results corresponding different flat prior distributions (e.g. dflat() – continous uniform ditribution) showed that the prior distributions had little influence on the posterior distributions. Direct comparison of differences between gender classes calculated by the two different methods was not possible when CW dependence is found, because the difference estimates obtained by the two approaches are not the same measures, as they include covariates in different ways.

The choice for the inverse variances ($1/\sigma^2_k$) of the prior distributions related to effect of breeds were gamma distributions with relatively high values (~1.1001) for both shape and scale parameters, so that the actual variances, which are distributed according to an inverse gamma distribution, can assume a wide range of values. This is in agreement with the choice of parameterisation of breed associated with 'random effect', because in the case of random effect, the effect is supposed to be derived from a super-population with a usually assumed high population variance (Normand, 1999).

Despite leading numerically to very similar results, the Bayesian method used in this study is fundamentally different from the previously used frequentist method

(Blasco, 2005). For the dataset used in this study the amount of information from the experiments considered was large relative to the factors considered, so the choice of prior distribution was not crucial. On the other hand, when there are only a small number of experimental data (or experiments in this case), the estimates of parameters related to a particular factor can be very imprecise (Whitehead, 2002). Hence we would expect greater discrepancies between the results of Bayesian and frequentist methods when availability of data of a pork quality trait is limited, as would be the case for drip loss data measured by other methods to the 'bag method'. (See discussion of Chapter 5). However, it should be acknowledged that the Bayesian methods still provide posterior distributions for parameters estimates, and their wide dispersion reflects the large degree of uncertainty in these estimates. In contrast, the frequentist approach may not yield parameter estimates at all. It is therefore not surprising that the Bayesian approach has been favoured for data sets with limited data in several biological studies (e.g. Reckhow, 1990).

## 6.5 Conclusion

The reliability of the method used in this study confirmed the usability of the Bayesian approach for further meta-analyses. Such meta-analysis would be fruitful in cases when the availability of a pork quality trait is limited or impossible for calculations of frequentist based models.

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# Chapter 7: General discussion

## 7.1 Objectives overview

A slight decline in pork production and an increase in the consumers' desire for products of high quality and safety have brought new challenges to the European pork production industry. In response to these challenges a large scale project was launched by the EU, with improvement of pork quality as one of the objectives. Although pork quality is determined by numerous factors, this thesis has concentrated on quantifying effects of nutritional and animal intrinsic factors, as other factors were considered by other project partners. Several sub-models with statistical power have been established describing how these factors have impact on a variety of important pork quality traits. These models, as such empirical models, have been obtained by a set of statistical analyses of data through sufficiently balanced meta-designs (Sauvant and Martin, 2006).

## 7.2 Key results and findings

The advantage of the meta-analytic approach compared to the 'classical' literature review is that it is quantitative and uses sound statistical treatments (Sauvant and Martin, 2006). Another strength of using meta-analysis was that the established models are valid over the cumulative scope of the individual experiments (Sauvant and Martin, 2006) and therefore provide relationships over a wider range of experimental conditions than the individual experiments themselves. This had also the advantage that new relations between factors were revealed that were not identified or were not the focus in the individual experiments. For example,

interactions were found between supplemented vitamin E or α-tocopherol content of pork and storage time as results of the employed meta-analyses.

The main results and findings of this thesis are summarised and discussed below in the order of the chapters.

**Chapter 1** provided a concept of pork quality traits and a short qualitative review of known influencing factors.

**Key results of Chapter 2:** The first result of this work was the establishment of a database (Chapter 2) that is able to store and manage data from various data sources and to make them available in platform independent form for meta-analytic purposes. This was a pre-requisite for the work carried out in this thesis but it was also required for the work and involvement of other project partners.

**Key findings for the effect of dietary vitamin E on pork (Chapter 3 and Chapter 4):** In the first set of meta-analyses effect of vitamin E on pork and its accumulation were studied and quantified. A nonlinear relationship between the supplemented amount of vitamin E and the accumulation of α-tocopherol in *M. longissimus* was established. For α-tocopherol accumulation an asymptotic value of ca. 6.4 µg α-tocopherol/g tissue was obtained as the maximum capacity for α-tocopherol accumulation. According to the models the maximum α-tocopherol accumulation increase, which is ca. 0.024 µg α-tocopherol /g tissue/ IU, can be reached by supplementation between 39 and 80 IU supplementary vitamin E /kg feed. The

duration of supplementation only affects the vitamin E dose associated with maximum α-tocopherol increase, with the greatest increase occurring at low doses when supplementation times are short and greatest increase occurring at higher doses for longer supplementation times (**Chapter 3**).

In the second and third meta-analysis the effect of vitamin E supplementation on lipid oxidation and redness in *M. longissimus dorsi* were studied (**Chapter 3 and Chapter 4**). Based on these analyses accumulated α-tocopherol, vitamin E dose and storage time were all found to be important factors affecting these traits. The results also indicated that at least 100 IU vitamin E /kg feed are required to gain a significant decrease of lipid oxidation and increase of pork redness. Also, the models estimate that every 1 μg α-tocopherol/g tissue decreases lipid oxidation by approximately 0.05 TBARS values and increase a* values by approximately 0.11. An interaction between supplemented vitamin E or accumulated α-tocopherol and storage time also was found by both meta-analyses which became significant at later stages of storage (after approximately 7 days post slaughter) i.e. when antioxidant effects of vitamin E become effective. Storage time dependence was found to be linear and non-linear (up to third order polynomial) for lipid oxidation and changes of redness, respectively. Effect of illumination was found to be constant, decreasing a* values by approximately 1.4 over the storage periods considered.

**Key findings of the effect of gender/breed and slaughter weight on pork quality traits (Chapter 5 and Chapter 6):** Gender is an inseparable part of an animal but can be manipulated in different ways. In the second sets of meta-analyses effect of four different swine genders, entire male (EM), surgically castrated male (SM),

immuno castrated male (IM), entire female (EF) in combination with carcass weight and breed was studied and quantified. Gender was found to be an overall significant factor affecting the pork quality traits of pH45min, pH24hr, objective colour attributes (L*, a*, b*), colour- and marbling scores, IMF, P2, drip loss, juiciness and tenderness in *M. longissimus* and pH45min, pH24hr in *M. semimbranosus*. Carcass weight dependence was found to be non-linear (quadratic) for a*, marbling scores and P2 and linear for b*, colour scores in *M. longissimus* and for pH24hr in *M. semimbranosus*. Breed had also significant effect of varying magnitude on all traits. However, when comparing effects between individual genders significant gender differences were only identified between mainly fat related traits i.e. IMF, P2, marbling scores and L* of the different gender types (**Chapter 5**). A subset of pork quality traits (i.e. objective colour attributes, IMF, drip loss) was additionally studied using a Bayesian approach, which is fundamentally different to the statistical methods used in Chapters 3-5. The findings of this approach strongly agreed with the findings of the previous, frequentist approach (**Chapter 6**).

These meta-analyses showed that the various manipulations of male sex do not directly affec the majority of the pork quality traits, but probably rather indirectly i.e. changes in the male reproductive organs cause changes in the hormonal regulations of the animals, which lead then to changes in body composition and eventually to differences in fat related pork quality traits.

For all above described results standard diet regimes were used, where pigs were fed with diets, which contained less than 5% fat and supplementation levels of vitamins

and minerals were at maintenance levels, except for the vitamin E for the relevant analyses.

### 7.3 Implications

The results of the meta-analyses described in this thesis bear implications for industrial practices. First of all, supplementing modern commercial breeds between 0-700 IU/kg feed vitamin E the results suggest a limit for vitamin E accumulation, therefore exceeding the upper amount of supplementation has no beneficial effect in the accumulation of this antioxidant. The results also suggest that at least 100 IU/kg feed vitamin E supplement is required at least for 14 days pre-slaughter to gain extension of pork shelf life. The results also show that the differences between the present practices in the modification of gender primarily affect fat related pork quality traits, while no significant effect of these differences was found for the other studied traits. These results bear importance for the pork industry when special market requirements should be satisfied, i.e. very low or very high desired fat content.

### 7.4 Limits of the of meta-analytic approach

In this work the meta-analytic approach was used, which is considered as a statistical modelling approach within the class of biological models (Haefner, 1996). All data used in these analyses were published results of experiments implying that the established models have predictive power only within the range for which data were

available (Sauvant and Martin, 2006). For the purpose of statistical robustness a conservative approach of the meta-analysis was applied in this thesis. There was a vast amount of information available about the effects of different experimental factors on various pork quality traits and the individual experiments often used different scientific protocols. For achieving the statistical robustness the focus of the analysis on a given specific topic and standardisations of individual experiments in the framework of the meta-design were required. These standardisation procedures covered standardisation of measurements of inputs of experiments, e.g. dose definition for vitamin E supplementation, definition of a standard weight, as well as standardisation of outputs of the individual experiments e.g. only TBARS measures were taken account which used comparable measuring methods. This way, harmonised gauges of both inputs and outputs of the individual experiments have been gained on a given scientific topic, which provided the grounds for further quantification. The approach itself relies on the standardisation of the experiments. Due to the exclusion of several experiments as resulting from the stringent standardisation criteria, the results are mainly valid for particular muscles (*M. longissimus (dorsi)*, *M. semimembranosus*) and quantify the effects of only a few factors and their interactions in the whole production chain.

In reality, a particular pork quality trait will be influenced by many factors and their dynamic interactions (Kerry *et al.*, 2002, Miller, 2002, Rosenvold and Andersen, 2003). Statistical models treat independent variables (i.e. influencing factors) as static effects, and are not well suited to establish cause and effect. Dynamic mechanistic models, similar to those established to model pig growth (Whittemore and Fawcett, 1976, Ferguson *et al.*, 1997, Green and Whittemore, 2003) may be

better suited to capture the dynamic nature of the comprehensive biological system underlying pork quality. However, it is noteworthy that for such models meta-analyses could provide valuable estimates for the underlying parameters or provide the means for validation of the models (Sauvant and Martin, 2006).

## 7.5 Further research

The results of the meta-analyses described and discussed in this thesis offer recommendations for further research in the form of experiments and meta-analyses.

### *7.5.1 Recommendations for further experiments*

- The first set of meta-analyses of this thesis established a minimum requirement of vitamin E supplementation, which was 100 IU/kg feed at least for 14 days pre-slaughter to extend pork shelf life. Modern type meat breeds were used in the experiments considered in these analyses. The second set of meta-analyses found effects of gender, animal weight and breed on mainly fat related pork quality traits. Future experiments would aim to confirm this minimum vitamin E requirement as fat-soluble vitamin supplement by measuring relevant pork quality traits (TBARS, L*, a*, b* -objective colour attributes) under the modifying effects of gender, weight and breed.

- In all models, which studied the effects of muscle α-tocopherol concentrations and storage time on pork quality traits (i.e. lipid oxidation, redness), α-tocopherol concentrations at slaughter were considered. There is indication in the literature that this accumulated α-tocopherol was very stable over the studied storage time (Pfalzgraf *et al.*, 1995, Hasty *et al.*, 2002, van Heugten *et al.*, 2002, Niculita *et al.*, 2007). In the future parallel with any pork quality traits, α-tocopherol concentrations should be measured along with storage time, which would reveal quantitatively more about how its antioxidant effect changing over time.
- Immunocastration is an alternative to surgical castration which means active immunisation against the gonadotropin realising hormone (GnRH) in male animals. Further experiments may aim to study the effect of passive immunisation against this hormone, because at the moment results related to this latter type of alternative castration are sparse (e.g. Schneider *et al.*, 1998, Xue *et al.*, 1994).

### *7.5.2 Recommendations for further meta-analyses*

- All diet regimes considered in this thesis contained <5% fat at DM base. New meta-analyses would aim how the studied properties of pork change under feeding regimes containing higher than this fat level(s).

- The established relationships of this study are mainly based on data of particular muscle(s), i.e. *M. longissimus (dorsi), M. semimembranosus*. In the future, if sufficient amount of data were available, new meta-analyses should be carried out for other important muscle types.
- Preliminary literature search would suggest that there could be sufficient data for meta-analysis about factors belonging to the 'Post-slaughter' (Figure 1.1) category i.e. carcass handling, carcass chilling, hanging methods.
- A common problem for meta-analysis in the frequentist method is that when the data are highly unbalanced is not possible to carry out the analysis. The advantage of the meta-analysis in the Bayesian approach is that it relaxes the restrictions imposed by the frequentist approach, i.e. any distribution can be assumed for parameter estimations in the applied models. This approach should be applied for future meta-analyses related to pork quality, especially when the available information is limited.
- Most of the pork quality traits, studied in this thesis were technical pork quality traits. Future meta-analyses would aim to study the sensory pork quality traits. The differences in sample preparation conditions/procedures and in sensory panel assesements may require application of Bayesian approach in these analyses.

In these experiments and analyses the established relationships of this thesis could be further validated or extended.

## 7.6 Conclusion

The results of this thesis suggest meta-analysis as a powerful tool for establishing quantitative relationships between factors that influence a variety of important pork quality traits. As it was illustrated in Figure 1.1, the factors of influence can classified into different categories, namely 'Animal', 'Production system', 'Pre-slaughter handling', 'Slaughter procedures' and 'Post-slaughter handling'. The relationships studied in this thesis belong to the major categories of 'Animal' (gender) and 'Farming system' which include 'Nutrition' (vitamin E supplementation). Complementary meta-analyses within the Q-Porkchains project quantified effects of a major gene, the halothane gene, which belongs to category 'Animal' on six pork quality traits and difference of carcass lean percentage (Salmi *et al.*, 2010) and also aimed to study pre-slaughter effects, such as fasting, lairage and transport time on pork quality. The meta-analyses for pork quality carried out within this Q-Porkchains project are therefore by no means comprehensive. The influence of additional factors or alternative pork quality traits to those considered here could be studied using a meta-analytic approach. A publication of meta-analysis on effects of chromium supplementation on carcass characteristics and meat-quality of growing-finishing swine is currently being under peer reviewing process (Sales and Jancik, 2011) and as it was recommended in the pervious section there might be sufficient data for meta-analysis about effects of post-slaughter factors on pork quality. Also, genome wide association studies identify more and more genes with significant effect on pork quality traits (e.g. Yu *et al.*, 1995). As genetic studies require a large sample size it is not surprising that meta-analysis becomes

increasingly established in the field of genetics (Alfonso, 2005, Khatkar *et al.*, 2004) and would be expected to be increasingly applied to pork quality (Silva *et al.*, 2011). Given the evidence of this and other studies, it would be expected that meta-analysis will continue to be used as a quantitative tool to study pork quality in the future, which will produce validated relationships between additional factors and pork quality traits. At the same time these analyses combined with the meta-analyses presented in this thesis may lead to more global response laws and data for a multi-factorial model of pork quality.

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# Appendix

**Drip loss measuring methods:**

a.) *Filter-paper* press *method* is a subjective method, where a filter paper is placed vertically on a 10 minute old slice of the muscle. The wetness of the filter paper is then scored on a scale from 0 to 5, where 0 denotes a dry filter paper and 5 means saturated (Kaufmann *et al.*, 1986).

b.) *Centrifugation method* is an instrumental, objective method where ~3-10g sample is minced and weighted, then centrifugation is applied for 30 minutes and the sample is reweighed. The whole process is being carried out at 4-6 °C (Fabry *et al.*, 1991). The drip loss is expressed as a percentage of the initial sample weight.

c.) *Bag method* is an objective method, where 24 h post-mortem, 2.5 cm thick pork slices, which are trimmed for connective tissue and external fat, are weighed and put into a net made of cotton (no dabbing with tissue), and both items are put into a plastic bag, avoiding the touch of the plastic bag. Then the bag is carefully closed and stored in a chilling room at 4-6 °C for 24 h/48 h. After 24h/48 h the piece of meat is gently dabbed with soft tissue and its weight is measured by the same calibrated scale. The drip loss is expressed as a percentage of the initial sample weight (Honikel, 1998, Christensen, 2003, Otto *et al.*, 2004).

d.) *Tray method* is an instrumental, objective method where 24 h post-mortem ~100g samples are weighted and placed with cut surface facing down in a tray supplied with a net bottom and squared inset. Then the tray is placed in a larger container, covered with an inverted container and kept at 4 °C. The samples are reweighed after 24h/48h storage. The drip loss is expressed as a percentage of the initial sample weight (Lundström and Malmfors, 1985).

e.) *EZ-tray method* is an instrumental, objective method where 24 h post-mortem sample is taken by a special knife, resulting about sample with weight about 10g and 2.5 cm diameter. Then the sample is weighted and put into a cylinder shaped special plastic container, and stored there under the closed lid of the container for 24h/48h in a chilling room, at 4-6 °C. Since there is no dubbing of the sample, the operator's influence may be reduced during the measurement (Christensen, 2003). After 24h/48h sample weight is measured, and the drip loss is expressed as a percentage of the initial sample weight (Christensen, 2003).

**Measurement of cooking loss:**

Samples should be freshly cut and weighed (initial weight). Individual standardized slices of 50 mm thick (maximum) and of a standard weight in thin-walled plastic bags are placed in a continuously boiling water-bath, with the bag opening extending above the water surface. Samples should be cooked to a defined internal temperature; 75°C is recommended. If other temperatures are used, these must be defined in the methodology. When the end-point temperature has been attained, samples should be removed from the water-bath, cooled in an ice slurry and held in chill conditions (1 to 5°C) until equilibrated. The meat is then taken from the bag, blotted dry and weighed. The cooking loss is expressed as a percentage of the initial sample weight (Honikel, 1998).

References cited here can be found in the reference list of Chapter 1.

**Table A.1** ***Ref*** -reference related database table.

| Field | Type | Null | Default | Comment |
|---|---|---|---|---|
| idReference | int(10) | No | | Unique Identifier<br>Auto incremented |
| idProject | int(10) | No | | *Key* to project table |
| Authors | varchar(255) | No | | |
| Title | varchar(255) | No | | |
| SourceName | varchar(255) | No | | |
| SourceYear | int(11) | No | | |
| SourceVolume | varchar(10) | No | | |
| PageFrom | varchar(10) | No | | |
| PageTo | varchar(10) | No | | |
| ISBN | varchar(20) | No | | |
| Abstract | text | No | | |
| InsertedBy | int(10) | No | | |
| InsertDate | datetime | No | 0000-00-00<br>00:00:00 | |
| Filename | varchar(255) | Yes | *NULL* | |
| Description | varchar(255) | Yes | *NULL* | |
| Link | varchar(90) | Yes | *NULL* | |
| idKeyword0 | int(10) | No | | |
| idKeyword1 | int(10) | No | | |
| idKeyword2 | int(10) | No | | |
| idKeyword3 | int(10) | No | | |
| idKeyword4 | int(10) | No | | |
| idKeyword10 | int(10) | No | | |
| idKeyword11 | int(10) | No | | |
| idKeyword12 | int(10) | No | | |
| idKeyword13 | int(10) | No | | |
| idKeyword14 | int(10) | No | | |
| Keywords | varchar(255) | No | | |

**Table A.2** ***MethodGroup*** –methods group related database table.

| Field | Type | Null | Default | Comment |
|---|---|---|---|---|
| idMethodGroup | int(10) | No | | Unique identifier<br>Auto incremented |
| idDepartment | int(10) | Yes | 0 | *Key* to department table |
| MethodGroup | int(10) | Yes | *NULL* | Sort order used for presentation |
| Initials | varchar(6) | Yes | *NULL* | |
| Name | varchar(45) | Yes | *NULL* | |
| idUser | int(10) | No | | *Key* to users table, to keep track of the user who inserted the record |

**Table A.3** ***Method*** –method related database table.

| Field | Type | Null | Default | Comment |
|---|---|---|---|---|
| idMethod | int(10) | No | | Unique identifier<br>Auto incremented |
| idMethodGroup | int(10) | No | 0 | *Key* to MethodGroup table |
| Method | int(10) | Yes | *NULL* | Sort order used for presentation |
| Name | varchar(45) | Yes | *NULL* | |
| idUser | int(10) | No | | *Key* to users table, to keep track of the user who inserted the record |

**Table A.4** ***Attrib*** –attributes related database table.

| Field | Type | Null | Default | Comment |
|---|---|---|---|---|
| idAttrib | int(10) | No | | Unique identifier<br>Auto incremented |
| idMethod | int(10) | Yes | *NULL* | *Key* to Method table |
| idProgram | int(10) | Yes | *NULL* | |
| Name | varchar(45) | Yes | *NULL* | Local name for the attribute |
| EN | varchar(45) | Yes | *NULL* | English name for the attribute |
| Description | varchar(45) | Yes | *NULL* | |
| ResultType | smallint(5) | Yes | *NULL* | The result type can be found in the result type table. |
| Attrib | int(10) | Yes | *NULL* | Sort order used for presentation |
| SampleLevel | int(10) | Yes | *NULL* | The level number for samples where you will enter data |
| idUser | int(10) | No | | |

**Table A.5** ***Model*** –model related database table.

| Field | Type | Null | Default | Comment |
|---|---|---|---|---|
| idProject | int(10) | No | 0 | *Key* to Projects table |
| Model | smallint(5) | No | 1 | Model # 1-n |
| ModelNumber | smallint(5) | No | 1 | The order of the model in presentations |
| Name | varchar(255) | No | | |
| Description | text | No | | |
| SavedData | char(1) | No | 0 | 0=No statistical data saved, model can be changed<br>1=Statistical data saved, model cannot be changed |
| idReference | int(10) | No | 0 | |
| idDepartment | int(10) | No | 1 | |
| idUser | int(10) | No | 0 | |

**Table A.6** ***AttribModel*** –trait related database table.

| Field | Type | Null | Default | Comment |
|---|---|---|---|---|
| idProject | int(10) | No | 0 | |
| Model | smallint(5) | No | 1 | |
| idAttrib | int(10) | No | 0 | |
| Attrib | int(10) | Yes | *NULL* | |
| Status | tinyint(3) | Yes | 0 | |
| LevelNumber | int(10) | No | 1 | |

**Table A.7** ***ModelFactor*** –experimental factor related database table.

| Field | Type | Null | Default | Comment |
|---|---|---|---|---|
| idProject | int(10) | No | 0 | |
| idFactor | tinyint(1) | No | 1 | |
| Model | smallint(5) | No | 1 | |
| LevelNumber | smallint(6) | No | 1 | |
| Name | varchar(45) | Yes | *NULL* | |
| idAttrib | int(10) | Yes | *NULL* | |
| ClassOrCont | char(1) | No | 0 | |
| Levels | tinyint(3) | No | 2 | |

**Table A.8** ***ModelFactorLevels*** –experimental factor levels related database table.

| Field | Type | Null | Default | Comment |
|---|---|---|---|---|
| idProject | int(10) | No | 1 | |
| Model | smallint(5) | No | 1 | |
| idFactor | tinyint(1) | No | 1 | |
| Item | smallint(5) | No | | |
| LevelNumber | smallint(5) | No | 1 | |
| Name | varchar(255) | No | | |

**Table A.9** ***ModelCombi*** –combination of experimental factor levels related database table.

| Field | Type | Null | Default | Comment |
|---|---|---|---|---|
| idProject | int(10) | No | 1 | |
| Model | smallint(5) | No | 1 | |
| idModelCombi | smallint(5) | No | 1 | |
| Mother | int(10) | No | 1 | |
| Levelnumber | int(10) | Yes | 0 | |
| Name | varchar(255) | No | | |
| InModel | char(1) | No | 0 | 0=the combination does not take part in the model<br>1=the combination does take part in the model |
| Item | smallint(5) | No | | |
| idFactorArray | varchar(255) | No | | The idFactor is separated by comma, a fast way of referencing the factors in the combination. |

**Table A.10** ***ModelMean*** –database table for model related statistics.

| Field | Type | Null | Default | Comment |
|---|---|---|---|---|
| idProject | int(10) | No | 0 | *Key* to projects table |
| Model | Smallint(5) | | | *Key* to Model table |
| idModelRow | int(10) | No | 0 | *Key* to ModelRow table |
| idAttrib | int(10) | No | | *Key* to Attrib table |
| idModelCombi | int(10) | No | 0 | *Key* to ModelCombi table |
| Estimate | double | Yes | *NULL* | |
| Stddev | double | Yes | *NULL* | |
| Stderr | double | Yes | *NULL* | |
| DF | int(10) | No | 0 | |
| P Value | double | Yes | *NULL* | |
| N | int(10) | No | | |
| TimeStamp | datetime | Yes | *NULL* | |
| idSignature | int(10) | No | 1 | *Key* to the Signatures table |

**Table A.11** ***ModelRow*** –user defined combination of experimental factor levels related database table.

| Field | Type | Null | Default | Comment |
|---|---|---|---|---|
| idProject | int(10) | No | 1 | |
| Model | smallint(5) | No | 1 | |
| idModelRow | int(10) | No | | |
| idFactor1 | tinyint(1) | No | 0 | |
| idFactor2 | tinyint(1) | No | 0 | |
| idFactor3 | tinyint(1) | No | 0 | |
| idFactor4 | tinyint(1) | No | 0 | |
| idFactor5 | tinyint(1) | No | 0 | |
| idFactor6 | tinyint(1) | No | 0 | |
| idFactor7 | tinyint(1) | No | 0 | |
| idFactor8 | tinyint(1) | No | 0 | |
| idFactor9 | tinyint(1) | No | 0 | |
| idFactor10 | tinyint(1) | No | 0 | |
| idFactor11 | tinyint(1) | No | 0 | |
| idFactor12 | tinyint(1) | No | 0 | |
| idFactor13 | tinyint(1) | No | 0 | |
| idFactor14 | tinyint(1) | No | 0 | |
| idFactor15 | tinyint(1) | No | 0 | |
| idFactor16 | tinyint(1) | No | 0 | |
| Item1 | smallint(5) | No | 0 | |
| Item2 | smallint(5) | No | 0 | |
| Item3 | smallint(5) | No | 0 | |
| Item4 | smallint(5) | No | 0 | |
| Item5 | smallint(5) | No | 0 | |
| Item6 | smallint(5) | No | 0 | |
| Item7 | smallint(5) | No | 0 | |
| Item8 | smallint(5) | No | 0 | |
| Item9 | smallint(5) | No | 0 | |
| Item10 | smallint(5) | No | 0 | |
| Item11 | smallint(5) | No | | |
| Item12 | smallint(5) | No | | |
| Item13 | smallint(5) | No | | |
| Item14 | smallint(5) | No | | |
| Item15 | smallint(5) | No | | |
| Item16 | smallint(5) | No | | |
| LevelValue1 | varchar(255) | No | | |
| LevelValue2 | varchar(255) | No | | |
| LevelValue3 | varchar(255) | No | | |
| LevelValue4 | varchar(255) | No | | |
| LevelValue5 | varchar(255) | No | | |
| LevelValue6 | varchar(255) | No | | |
| LevelValue7 | varchar(255) | No | | |
| LevelValue8 | varchar(255) | No | | |

| | | | | |
|---|---|---|---|---|
| LevelValue9 | varchar(255) | No | | |
| LevelValue10 | varchar(255) | No | | |
| LevelValue11 | varchar(255) | No | | |
| LevelValue12 | varchar(255) | No | | |
| LevelValue13 | varchar(255) | No | | |
| LevelValue14 | varchar(255) | No | | |
| LevelValue15 | varchar(255) | No | | |
| LevelValue16 | varchar(255) | No | | |
| idModelCombi | int(10) | No | 0 | |