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MODELLING TOOLS APPLIED TO THE CONTROL OF BEEF MEAT QUALITY AND SAFETY

Thesis for the degree of Doctor of Animal Science, Specialty in Technology of Animal Products submitted to Instituto de Ciências Biomédicas Abel Salazar da Universidade do Porto.

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To God, for all blessings

To my dear Parents and Grandparents

To my beloved Sister and my little prince Gabriel

To Nuno

“If you can't excel with talent, triumph with effort.”

Dave Weinbaum

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Declaration

By submitting this thesis, I declare that the entirety of the work contained is my own, original work, and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

This thesis represents a compilation of manuscripts where each chapter is an individual entity and some repetition between the chapters has, therefore, been unavoidable.

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List of Symbols and Abbreviations

% - Percentage

A – Age

AML – *Longissimus dorsi* muscle

ANOVA – Analysis of variance

ATP – Adenosine triphosphate

BIC – Bayesian Information Criterion

Class – Animal class

CookLoss – Cooking loss

CS – Cold-shortened

DFD – Dark, firm, dry

EFSA – European Food Safety Authority

EGS – Subcutaneous fat thickness

ES – Electrical Stimulation

EU – European Union

g – Gram

h – Hour

HCW – Hot carcass weight

Kg – Kilogram

kNN – k-nearest neighbours

k_{pH} – pH decay rate

k_{Temp} – Temperature decay rate

LD – *Longissimus dorsi*

LDA – Linear discriminant analysis

MSA – Meat Standards Australia

NSC – Nearest shrunken centroids

ºC – Degrees Celsius

OQ – Optimal quality

p.m. – Post-mortem

PDO – Protected Designation of Origin

PGI – Protected Geographical Indication

pH – Hydrogen potential

pH_0 – Initial pH

$pH_{1.5}$ – pH at 1.5 h

pH_{24} – pH at 24 h

$pH_{3.0}$ – pH at 3.0 h

$pH_{4.5}$ – pH at 4.5 h

$pH_{6.0}$ – pH at 6.0 h

pH_{∞}– Final pH

R^2 – Coefficient of determination

RLDA – Robust linear discriminant analysis

S – Sex

SEUROP – European Beef Carcass Classification System

SF – Shear-force

sHSP – Small heat shock proteins

SNPs – Nucleotide polymorphisms

SVM – Support vector machines

T_0 – Initial temperature

T_{∞}– Final temperature

$Temp_{1.5}$ – Temperature at 1.5 h

$Temp_{3.0}$ – Temperature at 3.0 h

$Temp_{4.5}$ – Temperature at 4.5 h

$Temp_{6.0}$ – Temperature at 6.0 h

$Temp_{pH6}$ – Temperature at which pH reached 6.0

$t_{Lairage}$ – Lairage time

$t_{pH6.0}$ – Time when pH reached 6.0

$t_{Transport}$ – Transport time

UK – United Kingdom

VTEC – Verotoxigenic *Escherichia coli*

WB – Warner-Bratzler

Abstract

The overall aim of this research work was to investigate and develop modelling tools to control and predict beef meat quality and safety, especially the pH and temperature patterns of the carcass to enhance decision-making and optimise the carcasses classification and grading systems. Meat and meat products are the main vehicles of foodborne diseases in humans caused by pathogens such as *Salmonella* spp., *Campylobacter* spp., *Listeria monocytogenes*, *Yersinia enterocolitica*, verotoxigenic *Escherichia coli* (VTEC) and *Staphylococcus aureus*. In order to prioritise research on those microbial hazards, a meta-analysis was conducted to summarise available microbial data on meats produced in Portugal. The overall occurrence rate of *Campylobacter* in Portuguese broiler meat (40%; 95% CI: 22.0–61.4%) was about ten times higher than that of *Salmonella* (4.0%; 95% CI: 1.4–10.8%); although these levels were comparable to current EU ranges. In Portuguese meat products, the incidence of *L. monocytogenes* (8.8%; 95% CI: 6.5–11.8%) and *Salmonella* spp. (9.7%; 95% CI: 7.0–13.4%) in the categories 'intended to be eaten cooked' and 'to be eaten raw', were considerably higher than EU levels for products in comparable categories. *S. aureus* was the pathogen with the highest prevalence (22.6%; 95% CI: 15.4–31.8%) in meat products. This meta-analysis also highlighted important gaps of knowledge, and may assist food safety authorities in the prioritisation of microbiological hazards, and the implementation of improved food safety assurance systems. Recognising that beef cattle carrying *Salmonella* spp. represents a risk for contamination of meat and meat products, an exposure assessment model elucidating the changes in *Salmonella* prevalence in beef along the processing stages was developed. Published incidences on *Salmonella* in Brazilian beef were assembled and conversion factors modelled based on beta distributions representing the effect of every production stage on the *Salmonella* incidence on beef carcasses. The results underscored the significant increase in *Salmonella* prevalence that can occur during evisceration/splitting and boning and also reinforced that, when hygienic slaughter procedures are properly implemented, the load of *Salmonella* can be reduced at dehiding, rinsing and chilling.

During beef carcass chilling, the eating quality of meat can be severely affected by either hot- or cold-shortening. Meat of optimal tenderness can be produced when *rigor mortis* (pH=6.0) is attained when carcass temperature falls between 12–35ºC. Our work aimed to predict meat quality from modelled pH/temperature decay descriptors and informative animal/carcass characteristics. Temperature and pH from a total of 126 beef carcasses were logged during 24 h *post-mortem*, and subsequently modelled by exponential decay equations

that estimated temperature (k_T) and pH (k_{pH}) decay rates. The exponential decay equation turned out to be an adequate model to describe the carcass' decay in both pH and temperature. Linear discriminant analysis and its robust variant yielded the best carcass quality classification performance, having the highest mean accuracy (0.941–0.951) and kappa (0.878–0.897) statistics. The model allows the classification of beef carcasses according to meat tenderness into cold-shortened and optimal quality by combining hot carcass weight and the carcasses' pH and temperature of at least the first three hours post-slaughter.

Subsequently, the combined effects of animal/carcass characteristics and pH/temperature decay descriptors on the two main eating quality attributes of meat – namely, tenderness (measured as shear-force) and juiciness (measured as cooking loss) – during chill ageing were assessed. The pH and temperature in *longissimus thoracis* muscle of 45 beef carcasses were recorded during 24 h *post-mortem*, and decay descriptors were obtained by fitting exponential models. The fitted mixed-effect models revealed that both meat tenderisation and cooking loss increased with ageing ($p<0.01$) although their rates slowed down in time ($p<0.05$). Nonetheless, the slower the pH decay rate, as happens in a cold-shortened carcass, the lower the potential for tenderisation ($p=0.038$) and water retention ($p=0.050$) during ageing. The good fitting quality of the shear-force ($R^2=0.847$) and cooking loss ($R^2=0.882$) models and their similarity among the different endpoints *post-mortem* indicated that both eating quality attributes can be approached by recording the pH decline of a beef carcass during the first three hours after slaughter.

The data of pH/temperature decay descriptors can be used to design a real time monitoring system to predict meat tenderness immediately after slaughtering. This system could have applicability for the objective classification and grading of beef carcasses, as well as for the definition of the individual carcasses' ageing period. This real time monitoring system would also be useful for the optimisation of the rate of pH/temperature decline early *post-mortem*, by controlling the abattoir's chilling protocol.

Resumo

O objetivo geral desta tese foi desenvolver modelos de previsão da qualidade e da segurança da carne de bovino, com especial enfoque nos padrões de declínio do pH e da temperatura das carcaças, que facilitem a tomada de decisão e a otimização dos sistemas de classificação das carcaças. A carne e os produtos cárneos são os principais veículos de doenças transmitidas pelos alimentos, e causadas por agentes patogénicos como a *Salmonella* spp., *Campylobacter* spp., a *Listeria monocytogenes*, a *Yersinia enterocolitica*, a *Escherichia coli* verotoxigénica (VTEC) e o *Staphylococcus aureus*. A fim de priorizar a investigação sobre os riscos microbianos, foi realizado um estudo de meta-análise onde se resumiram dados de presença dos referidos microrganismos em carnes e produtos cárneos produzidos em Portugal. Verificou-se que, na carne de frango, a taxa de ocorrência global de *Campylobacter* (40%; 95% IC: 22,0–61,4%) foi cerca de dez vezes, superior à de *Salmonella* (4,0%; 95% IC: 1,4–10,8%); sendo estes valores comparáveis aos valores médios apresentados pela UE. Nos produtos cárneos portugueses, a incidência de *L. monocytogenes* (8,8%; 95% IC: 6,5–11,8%) e *Salmonella* spp. (9,7%; 95% IC: 7,0–13,4%) nas categorias «consumidos cozinhados» e «consumidos crus», apresentaram valores consideravelmente superiores aos níveis da UE para os produtos de categorias semelhantes. Nos produtos cárneos portugueses, o *S. aureus* foi o agente patogénico com maior prevalência (22,6%; 95% CI: 15,4–31,8%). Este estudo de meta-análise destacou ainda, a existência de lacunas de conhecimento, pelo que pode auxiliar as autoridades de segurança alimentar na priorização dos perigos microbiológicos, bem como na melhoria de sistemas de garantia de segurança dos alimentos. Os bovinos contaminados com *Salmonella* spp., representam um risco para a contaminação da carne e dos produtos cárneos, pelo que foi desenvolvido um modelo de avaliação de exposição para explicar as alterações da prevalência de *Salmonella* spp. na carne de bovino ao longo das suas fases de transformação. Assim, foram reunidos resultados de vários estudos publicados sobre a incidência de *Salmonella* em bovinos, no Brasil, com o objectivo de estimar fatores de conversão modelados, baseados em distribuições beta, para representar o efeito da sua incidência em cada estádio de processamento das carcaças de bovinos. Os resultados não só sublinharam o aumento da prevalência de *Salmonella* spp. durante as etapas de evisceração/corte e de desossa, mas também reforçaram que, a correcta utilização dos procedimentos higiénicos de abate contribuem para reduzir a carga de *Salmonella* spp. na esfola, na lavagem e a refrigeração.

Durante a refrigeração das carcaças, a qualidade da carne pode ser severamente afetada pelo fenómeno designado por encurtamento pelo frio. Com base nos resultados de estudos prévios, sabemos que a tenrura máxima da carne é alcançada quando o *rigor mortis* (pH=6,0) é atingido, e se a temperatura da carcaça se encontrar entre 12–35ºC. O nosso trabalho teve como objetivo desenvolver modelos de previsão da qualidade da carne a partir de descritores de declínio de pH/temperatura e das características do animal/carcaça. Para tal, foram registados os valores de temperatura e de pH durante 24 h *post-mortem*, num total de 126 carcaças de bovinos, que, subsequentemente foram modelados por equações exponenciais de declínio para estimar as taxas de declínio da temperatura (k_T) e do pH (k_{pH}). A equação de declínio exponencial mostrou-se adequada para descrever a queda do pH e da temperatura das carcaças. A análise linear discriminante e sua variante robusta apresentaram o melhor desempenho na classificação da qualidade das carcaças, com precisão média (0,941–0,951) e coeficiente de concordância de escalas nominais (kappa 0,878–0,897). O modelo permite classificar carcaças bovinas, de acordo com a tenrura da carne, em "Óptima Qualidade" e em "Encurtadas pelo Frio", usando como variáveis independentes o peso da carcaça quente e os descritores do pH e da temperatura, recolhidos nas primeiras três horas após o abate.

Subsequentemente foram avaliados os efeitos combinados das características do animal/carcaça e dos descritores de declínio pH/temperatura nos dois principais atributos de qualidade da carne – a ternura (avaliada pela força de cisalhamento) e a suculência (avaliada pelas perdas de cozedura) – ao longo da maturação da carne. O pH e a temperatura do músculo *longissimus thoracis* foram registados, durante as 24 h *post-mortem*, em 45 carcaças de bovinos e os seus descritores de declínio foram modelados através do modelo de declínio exponencial. Os modelos mistos revelaram que a tenrura e as perdas por cozedura aumentaram ($p<0,01$) ao longo da maturação, no entanto as taxas diminuíram ($p<0,05$) ao longo do tempo. Assim, carcaças com taxas de declínio do pH mais baixo, como acontece nas carcaças encurtadas pelo frio, apresentam menor ($p<0,05$) potencial de maturação e de capacidade de retenção de água. A boa qualidade de ajuste dos modelos de previsão da força de cisalhamento ($R^2=0,847$) e das perdas por cozedura ($R^2=0,882$) e sua similaridade entre os diferentes tempos de maturação, mostra que ambos os atributos de qualidade da carne podem ser previstos usando como variáveis independentes os descritores de declínio do pH medidos durante as primeiras três horas após o abate.

Os descritores de declínio do pH e da temperatura, desenvolvidos nesta tese, poderão ser usados para projetar um sistema de monitorização, em tempo real, para prever a ternura da

carne nas primeiras horas após o abate. Este sistema poderá permitir a classificação das carcaças de bovinos de forma objectiva, bem como definir o período óptimo de refrigeração das carcaças. Um sistema de monitorização em tempo real também será útil para a otimização da taxa de declínio do pH e da temperatura, no início do *post-mortem*, uma vez que pode fornecer informação essencial ao ajustamento do protocolo de refrigeração dos matadouros.

1. General Introduction

1.1 BACKGROUND

A top priority factor in the success of meat industry relies on the ability to deliver specialities that satisfy the consumer's taste requirements (Cortez, 2006), and on the ability to attain a high standard of beef quality that ensures consumer's satisfaction and repurchase (Coleman *et al.*, 2016). However, the meat sector has been subject to constant demands, having to satisfy the interests of different stakeholders, such as health authorities, large supermarket chains and consumers (Troy, 1999; Simmons *et al.*, 2008). Consumers' awareness regarding organoleptic meat qualities, animal welfare issues in agricultural production systems as well as food safety issues has progressively increased in many countries, which has in turn led to intensification in the demand of high quality products, including safe and tenderer meat with lower fat content (Williams, 2008).

On the other hand, meat and meat products are the main vehicles of foodborne diseases in humans caused by pathogens such as *Salmonella* spp., *Campylobacter* spp., and Shiga Toxin-producing *Escherichia coli* (SVS, 2011; EFSA, 2013; Xavier *et al.*, 2014). This is an alarming fact since there is evidence that zoonotic *E. coli* and *Salmonella* spp. species are increasingly resistant to antimicrobials (Swartz, 2002; Magiorakos *et al.*, 2011). Therefore, it is necessary to know more about food safety and to pool efforts to prevent and control the pathogens in the food supply.

Meat quality is influenced by the interaction between the biological traits and the pre-slaughter and slaughter environmental conditions. This interaction has an impact on the biochemical processes that occur *post-mortem* as muscle is converted to meat (Lomiwes *et al.*, 2014). Appearance, tenderness and flavour are the main attributes by which consumers judge meat quality (Lomiwes *et al.*, 2014), with tenderness being considered the main determinant of consumer's overall satisfaction (Hopkins and Geesink, 2009). To achieve consistent meat tenderness has been a challenge for the beef industry, as tenderness is a complex trait influenced by many factors, such as marbling, muscle type, pH and temperature, ageing, breed, sex and environmental factors (Platter *et al.*, 2003; Listrat *et al.*, 2016; Khasrad *et al.*, 2017).

Ageing is a biochemical process where the beef's natural enzymes degrade the myofibrillar proteins causing the weakening of the muscle connective tissue, which leads to more tender beef (Dashdorj *et al.*, 2016). Typically, bovine carcasses are aged at 0–2ºC during a period of time that can vary between 7 to 21 days, however the ordinary ageing period applied is 14 days (Koohmaraie *et al.*, 2002; Hwang and Thompson, 2001). Nevertheless, despite the

common tenderisation through ageing, variability in tenderness of beef is still being observed due to other factors which are more difficult to control such as pre- and post-slaughter conditions, stress and age of the animals, etc. (Thompson, 2002; Dashdorj *et al.*, 2016).

After slaughter, the anaerobic glycolysis generates lactate that accumulates, lowering the intracellular pH, so that by 24 h *post-mortem*, the pH declines from 7.2 to about 5.4–5.7 (Wood, 1995). Several studies that investigated beef tenderness found that pH monitoring after slaughter was a quick and reliable method for judging meat quality (Mounier *et al.*, 2006; Hamoen *et al.*, 2013; Hopkins *et al.*, 2014; Warner *et al.*, 2014). Such recording was shown to be able to allow meat classification, thereby helping to determine if the meat is suitable for further processing or not. In general, 24 h after slaughter (ultimate pH - pH_{24}), pH values between 5.4 and 6.0 are considered to be within the normal range. However, it is well known that both ultimate pH and rate of pH fall have major consequences on beef meat quality (Maltin *et al.*, 2003; Kim *et al.*, 2014). Moreover, it has been demonstrated that the interactions of pH and temperature decline early *post-mortem* impact on the beef meat eating properties (Hamoen *et al.*, 2013; Kim *et al.*, 2014). In particular, carcasses with a slow pH decline (or high pH at low temperature) are susceptible to cold-shortening leading to meat toughness, while the application of a high temperature treatment during early *post-mortem* period improves the meat tenderness (Wipple *et al.*, 1990). Yet, a very rapid pH decline combined with a slow chilling regime also leads to an increase in meat toughness (Thomson *et al.*, 2008) due to the exhaustion of alfa-calpain, reducing the meat ageing potential during chilling (Takahashi *et al.*, 1987). Thus, during *rigor mortis* development, muscle pH and temperature interact continuously since both have an influence on the muscle physical shortening (Tornberg, 1996; Thomson *et al.*, 2008) and the proteolytic enzyme activity (Dransfield, 1992; Kim *et al.*, 2014). Hwang and Thompson (2001) showed that an intermediate pH decline (pH 5.9 to 6.2 at 1.5 hours *post-mortem*), or rigor temperature (29 to 30ºC at pH 6.0) produced the most tender meat in the striploin muscle after 14 days of ageing. Nonetheless, despite the abundant research undertaken to optimise meat quality and minimise its variability, the causes of the large variation in meat quality are still not fully understood (Lomiwes *et al.*, 2014). Moreover, the critical pH, temperature and time combinations affecting biochemical/physical modifications of pre-rigor muscle and subsequent meat quality changes have also not yet been clearly investigated (Kim *et al.*, 2014). Further research is still required to find early slaughter indicators that may be used as efficient predictors of meat quality.

In this work, we hypothesise that the measurement of both, pH and temperature after slaughter and the determination of its relationship would lead to more efficient predictors for

meat tenderness, and consequently meat quality than the sole measurement of ultimate pH. To investigate the relationship between pH and temperature decline early *post-mortem* and meat ageing – and subsequent meat tenderness – a research project was developed in partnership with the Municipal Slaughterhouse of Braganza, located in the industrial Zone of Cantarias, and with the Agropecuaria Mirandesa Cooperative, located in the industrial Zone of Vimioso, Portugal. Both entities played a crucial role in the performance of this work. The pH and temperature values of the carcasses were monitored at the Municipal Slaughterhouse of Braganza, where the animals were slaughtered, and subsequently refrigerated. The Agropecuaria Mirandesa Cooperative provided the Mirandesa meat samples, which were matured during different periods of time and later, their tenderness was evaluated by shear force tests. This research work was carried out with cattle from the Mirandesa breed and crossbreed as these are the predominant breeds in the region of Braganza.

Mirandesa cattle are an old local meat breed, with docile temperament and the animals are of medium size (1024 kg for males and 630 kg for females). They have a good maternal instinct and an excellent reproductive capacity, and they are hardy and resistant to the extreme climatic conditions in northern Portugal. They are predominantly kept by extensive management with medium herd sizes. The "traditional" management, with less than 10 cows, represent around 78% of all farms. The calves are sold at weaning at approximately 7 months for slaughter. Mirandesa meat has excellent organoleptic qualities. This set of qualities is due to the unique genetic characteristics of the breed, as well as to a feeding system based on grass and forage (Sousa and García, 2009).

1.2 AIM

The overall aim of this research work was to investigate and to develop modelling tools applied to the control of beef meat quality and safety, especially the pH/temperature trends and patterns of the carcass to enhance decision-making and optimise the carcasses classification and grading systems.

The specific objectives were:

i) To summarise available information on the incidence of pathogens in meats produced in Portugal;

ii) To build an exposure assessment model elucidating the changes in *Salmonella* prevalence in beef along the processing stages;

iii) To develop a discriminant analysis model to classify beef carcasses quality into "optimal quality" and "cold-shortened" according to pH and temperature decline ideal window rule; and

iv) To develop linear models to predict eating quality attributes of beef related to tenderness and juiciness, from animal characteristics, ageing time and early *post-mortem* pH and temperature decay descriptors.

1.3 OUTLINE

This thesis addresses two of the major themes in the meat sector: 1) Prevalence of Foodborne Pathogens, and 2) Carcass and Meat Quality. Hence, these themes are discussed throughout the four chapters which are the main core of this research work, being preceded by a General Introduction and finalised by General Discussion, Overall Conclusions and Future Perspectives.

Since in Portugal there is scattered information on foodborne zoonoses, and raw meat provides an ideal growth medium for a wide range of pathogens, in **Chapter 2.1** we carried out a meta-analysis on the occurrence of *Salmonella* spp. and *Campylobacter* in Portuguese fresh meats.

Foodborne pathogens are a major public health issue in many countries. **Chapter 2.2** addresses this issue of food safety in bovine carcasses. In partnership with Brazilian colleagues, an exposure assessment model on the prevalence of *Salmonella* spp. along the processing stages in the slaughterhouse was developed. In this published research, my contribution was (i) the creation of a database of *Salmonella* prevalence in Brazilian beef carcasses at different processing stages, and (ii) the realisation of the meta-analytical model for a transfer factor relating *Salmonella* incidence after dehiding to *Salmonella* incidence after carcass bleeding (shown as Figure 1) and the meta-analysis of the prevalence of this pathogen on beef hides after bleeding (shown as Figure 2 in the published article).

There are many biochemical and structural events that take place in the first 24 hour period after the animal is slaughtered and the muscle is converted to meat. This period affects meat tenderness and other meat quality characteristics, and is dependent on the time, temperature and pH interaction (Dransfield *et al.*, 1992; Tornberg, 1996; Aberle *et al.*, 2001; Hamoen *et*

al., 2013). The interactive effects between pH and temperature during chilling of beef carcasses and carcass grading according to meat quality are described in **Chapter 3.1**.

Chapter 3.2 addresses one of the main challenges faced by the meat industry: the meat quality. In this chapter, we describe the combined effects of the animal/carcass characteristics (i.e., breed, gender, age class, fat class, hot carcass weight, carcass conformation, among others) on the two main eating quality attributes of meat (tenderness and juiciness). The models we developed allow the prediction of the minimum ageing period of beef carcasses with known early *post-mortem* pH and temperature decay to reach optimal meat eating quality.

In **Chapters 4** and **5** of General Discussion and Overall Conclusions, respectively, we address the relevance of this thesis for the implementation of support decision systems for beef meat production systems, which will allow to obtain significant improvements in the safety and quality of beef carcasses. Finally, in **Chapter 6,** we point out some Future Perspectives that may be developed based on the results of this research work.

1.4 LIST OF PUBLICATIONS

This thesis was based on the following publications:

Papers Published in Peer-reviewed Journals

Xavier, C., Gonzales-Barron, U., Paula, V., Estevinho, L., Cadavez, V. 2014. Meta-analysis of the incidence of foodborne pathogens in Portuguese meats and their products. *Food Research International,* 55, 311-323.

Gonzales-Barron, U., Piza, L., **Xavier, C.**, Costa, E., Cadavez, V. 2014. An exposure assessment model of the prevalence of *Salmonella* spp. along the processing stages of Brazilian beef. *Food Science and Technology International*, 22 (1), 10-20.

Papers in Preparation for Submission in Peer-reviewed Journals

Xavier, C., Gonzales-Barron, U., Müller, A., Cadavez, V. 2017. Early *post-mortem* classification of beef carcasses according to meat quality using animal/carcass characteristics and pH/temperature decline descriptors. (In preparation for submission)

Xavier, C., Gonzales-Barron, U., Müller, A., Cadavez, V. 2017. Modelling meat eating quality traits during ageing as affected by beef carcass characteristics and early *post-mortem* pH decay descriptors. (In preparation for submission)

Papers Published in Conference Proceedings

Xavier, C., Gonzales-Barron, U., Muller, A., Cadavez, V. 2016. Classifying beef carcasses according to meat quality using animal/carcass characteristics and pH/temperature decline descriptors early post-mortem. In: *Proceedings of the International Conference on Simulation and Modelling in the Food and Bio-Industry – FOODSIM´2016.* Impe, J.F.M.V., Geril, P. (Editors), EUROSIS-ETI Publication, Ghent, Belgium, 147-152. ISBN: 9789077381922

Xavier, C., Gonzalés-Barron, U., Muller, A., Cadavez, V. 2015. Estudio de meta-análisis del efecto de estimulación eléctrica en la fuerza de corte de la carne de vacuno. In: *Proceedings of the XVI Jornadas sobre Producción Animal.* Asociación Interprofesional para el Desarrollo Agrario, (Editor), Zaragoza, Spain, 2, 609-611. ISBN: 9788460679714

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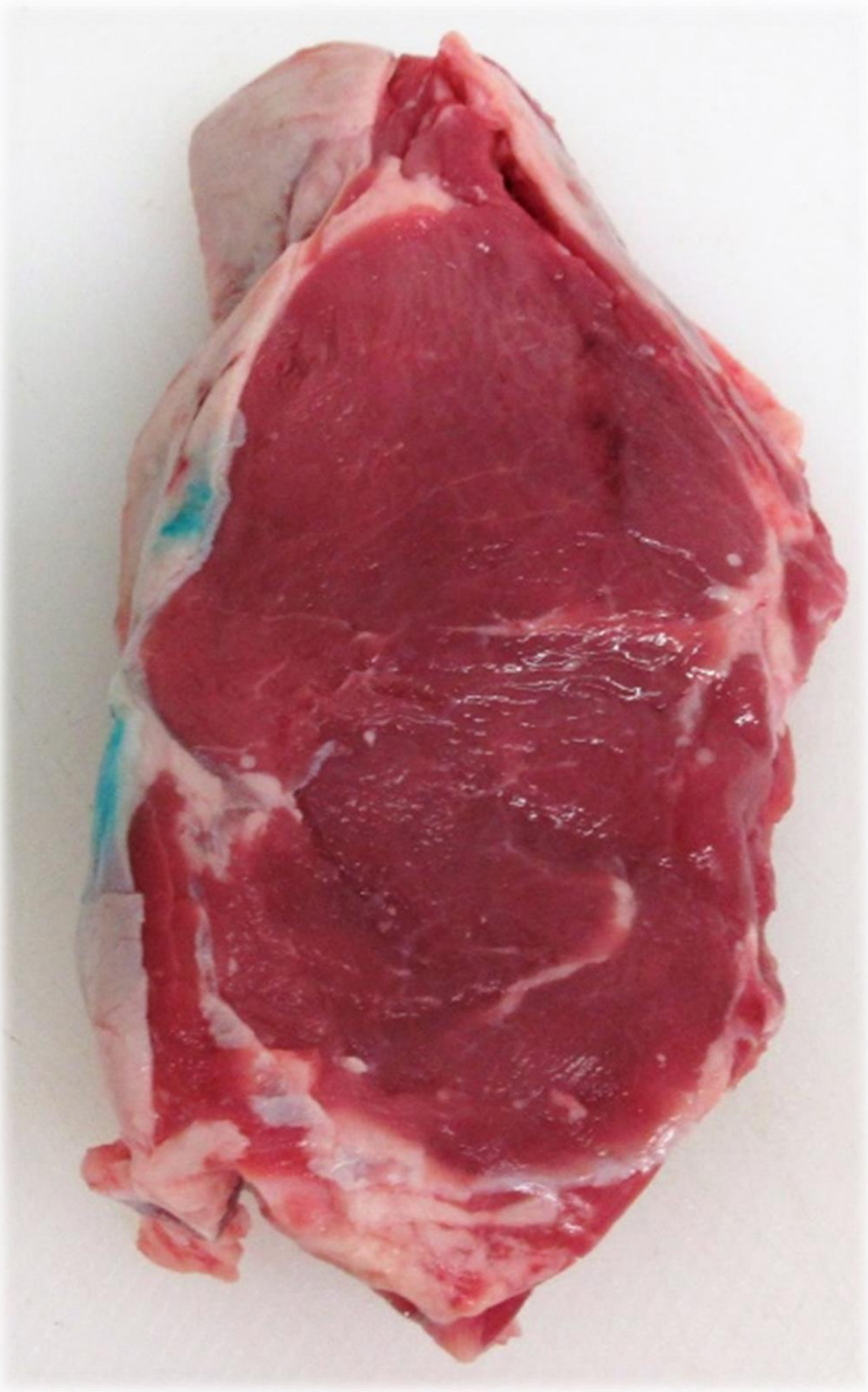
2. Prevalence of Foodborne Pathogens



(Source: Cristina Xavier)

2.1. Meta-analysis applied to meat microbiological safety: Meta-analysis of the incidence of foodborne pathogens in Portuguese meats and their products

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Meta-analysis of the incidence of foodborne pathogens in Portuguese meats and their products

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ABSTRACT

Meat and meat products are the main vehicles of foodborne diseases in humans caused by pathogens such as *Salmonella* spp., *Campylobacter* spp., *Listeria monocytogenes*, *Yersinia enterocolitica*, verotoxigenic *Escherichia coli* (VTEC) and *Staphylococcus aureus*. In order to prioritise research on those microbial hazards, a meta-analysis study was conducted to summarise available information on the presence of such pathogens in meats produced in Portugal. By using a logit-transformed proportion as effect size parameterisation, a number of multilevel random-effect meta-analysis models were fitted to estimate mean occurrence rates of pathogens, and to compare them among meat categories (i.e., bovine meat, broiler meat, pork, minced beef and minced pork), and among meat product categories (i.e., intended to be eaten cooked, to be eaten raw and cured meats). The mean occurrence rate of *Campylobacter* in Portuguese broiler meat (40%; 95% CI: 22.0–61.4%) was about ten times higher than that of *Salmonella* (4.0%; 95% CI: 1.4–10.8%); although these levels were comparable to current EU ranges. Nevertheless, in the other meat categories, the meta-analysed incidences of *Salmonella* were slightly to moderately higher than EU averages. A semi-quantitative risk ranking of pathogens in Portuguese-produced pork pointed *Salmonella* spp. as critical (with a mean occurrence of 12.6%; 95% CI: 8.0–19.3%), and *Y. enterocolitica* as high (6.8%; 95% CI: 2.2–19.3%). In the case of the Portuguese meat products, the non-compliance to EU microbiological criteria for *L. monocytogenes* (8.8%; 95% CI: 6.5–11.8%) and *Salmonella* spp. (9.7%; 95% CI: 7.0–13.4%) at sample units level, in the categories 'intended to be eaten cooked' and 'to be eaten raw', were considerably higher than EU levels for ready-to-eat products in comparable categories. *S. aureus* was the pathogen of greatest concern given its high occurrence (22.6%; 95% CI: 15.4–31.8%) in meat products. These results emphasised the necessity of Portuguese food safety agencies to take monitoring, and training actions for the maintenance of good hygiene practices during the production of the great variety of traditional meat products. This meta-analysis study also highlighted important gaps of knowledge, and may assist food safety authorities in the prioritisation of microbiological hazards, and the implementation of essential food safety assurance systems at primary production.

1. Introduction

Raw meat provides an ideal growth medium for a wide range of pathogens, and, if there is any malpractice in the handling, post-processing, storage or cooking of the product, illness can be a real possibility. Contamination of meat with foodborne pathogens is a major public health issue. In fact, in 2011, campylobacteriosis was the most commonly reported gastrointestinal bacterial pathogen in humans in the EU, followed by salmonellosis, with 220,209 and 95,548 confirmed cases, respectively While campylobacteriosis has increased significantly over the past four reported years (2008–2011), salmonellosis continues its decreasing trend since 2007 although it is the most reported cause of outbreaks (EFSA, 2013). In particular, the human cases caused by the two most common serovars, *Salmonella* Enteritidis (44%) and *Salmonella* Typhimurium (25%), diminished significantly since 2008. In foodstuffs, the highest proportion of *Campylobacter* positive samples was once again reported for fresh poultry (31.3% of positive samples), while *Salmonella* serovars were most often detected in fresh broiler (6.7%) and pig meat (0.7%). Furthermore, non-compliance with the EU *Salmonella* criteria has been most often observed in foods of meat origin, being higher for minced poultry (6.8% based on sample units) and minced meat from other species (1.1%) intended to be eaten cooked and minced meat and meat preparations intended to be eaten raw (1.6%) (EFSA, 2013).

In the case of the verotoxigenic *Escherichia coli* (VTEC) infections (9485 cases), a 2.6-fold increase was observed in comparison to 2010, while the confirmed EU cases of human listeriosis (1476) was slightly lower than previous years, yet with a high fatality rate of 12% (EFSA, 2013). Contaminated bovine meat (1.4% of contaminated sample units in 2011) continues to be considered the major source of VTEC infections in humans (EFSA, 2012, 2013), while non-compliance with the EU *Listeria monocytogenes* criteria is mostly observed in ready-to-eat (RTE) fishery products (6.7%) and of meat origin (2.4%). In 2011, although following a decreasing five-year trend, yersiniosis was the fourth most frequently reported zoonosis (7017 confirmed cases) in

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the EU. Pigs are considered to be a major reservoir while pork and poultry products are considered to be the most important source of pathogenic *Yersinia enterocolitica* infection in humans (Simonová, Vazlerová & Steinhauserová, 2007). In fact, in pig meat samples taken from four European countries, the overall incidence of *Y. enterocolitica* was 2.4% (EFSA, 2013).

In Portugal, there is considerably less information on zoonoses, and incidence of pathogens in meats. Because of the current compulsory national surveillance, and control programmes of *Salmonella* in foods, more information is available for this pathogen. According to the last EFSA report (EFSA, 2013), the notification rates of salmonellosis in 2011 (1.6 confirmed cases per 100,000) appeared lower than the EU average (20.7 per 100,000), and, peculiarly, well below other Western European countries such as Spain (32.8 per 100,000), France (13.4 per 100,000) and Denmark (21.0 per 100,000). However, as Portugal has one of the highest hospitalisation rates (84% as pointed out in the same report), this may indicate that, apart from a likely large number of citizens not seeking medical advice, there is also under-reporting by the surveillance systems which capture primarily the most severe cases. With regard to the foodstuff contaminated with *Salmonella* spp. in Portugal, pig meat has been identified as the most important likely source of infection, with a mean incidence of 5.0%, in comparison with the EU average of 0.6% (EFSA, 2013). Nevertheless, information with regard to other foodborne diseases is scarce (i.e., no Portuguese surveillance systems in place for campylobacteriosis, listeriosis, yersiniosis and VTEC infections), which leads to an inaccurate evaluation of the relative importance of each foodborne disease. Due to the limited zoonosis information, it is difficult to establish an evolution trend of the incidence of foodborne diseases as well as the occurrence of the main microbial contaminants in Portuguese foods in the last years (Veiga et al., 2012). Nonetheless, given (i) the strong association of foodborne diseases in humans with the consumption of contaminated meat and meat products, and (ii) the high consumption of meats (93 kg per Portuguese habitant in 2012 above the average 80 kg per EU citizen) and meat products (672 tonnes production in 2009 in Portugal), it is imperative to gather as much information as possible on the levels of foodborne pathogens in Portuguese meats and meat products in order to understand the current epidemiological situation, prioritise microbial hazards for risk analysis, and identify knowledge gaps to provide direction for further research.

Meta-analysis is a body of summarising statistical techniques whose objective is to synthesise, integrate and contrast the results from a large amount of primary studies investigating the same research question (Gonzales-Barron, Cadavez, Sheridan, & Butler, 2013). The primary objective of meta-analysis is to produce a more precise estimate of the effect size of a particular treatment, with increased statistical power, than is possible using only a single study (Sutton, Abrams, & Jones, 2001). Yet, with meta-analysis, it is also possible to explain differences in the study outcomes by coding study characteristics, such as: research design features, data collection procedures, type of samples or even year (Hox & De Leeuw, 2003). In the past few years, meta-analysis has increasingly been applied in food safety (Den Besten & Zwietering, 2012; Gonzales Barron, Bergin, & Butler, 2008; Gonzales-Barron & Butler, 2011; Gonzales-Barron et al., 2013; Grieg et al., 2012; McQuestin, Shadbolt, & Ross, 2009; Sánchez, Dohoo, Christensen, & Rajic, 2007). In food safety research, meta-analysis may be conducted to address a broad range of research questions such as disease incidence, prevalence of microorganisms in foods, effect of interventions pre- and post-harvest, risk ranking of pathogens and consumer practices, among others. Thus, the objectives of this research are: (i) to compile all publicly accessible information on the occurrence of *Salmonella* spp., *Campylobacter*, *L. monocytogenes*, VTEC, *Y. enterocolitica* and *Staphylococcus aureus* in Portuguese meats, and meat products grouped by categories; (ii) to quantitatively summarise, and compare the occurrence of pathogens according to available information by conducting separate meta-analysis models for meat and meat products; (iii) to appraise likely publication bias, a common artefact in meta-analysis studies (Viechtbauer, 2010); (iv) to conduct a semi-quantitative risk ranking of pathogens in pork using the characterisation of severity of hazards proposed in EFSA (2011a); and (iv) to identify knowledge gaps on the occurrence of pathogens in certain meat categories.

2. Methodology

The *problem statement* in this study was the estimation of the overall incidence or occurrence of foodborne pathogens in Portuguese meats. The *population* was specified as meat and meat products produced in Portugal while the *measured outcome* is the detection of pathogens in meats sampled either at processing plants or at retail. Following the systematic review protocol presented by Sargeant, Amezcua, Rajic, and Waddell (2005), electronic searches were carried out to identify official reports published by national and international organisations (such as World Health Organisation, WHO; European Food Safety Authority, EFSA; International Commission for Microbiological Specification in Foods, ICMSF) reporting occurrence values of *Salmonella* spp., *Campylobacter*, *L. monocytogenes*, VTEC, *Y. enterocolitica* and *S. aureus* in Portuguese meats (categorised as: fresh bovine, fresh broiler, fresh pork, minced beef and minced pork) and meat products (categorised as: intended to be eaten raw, intended to be eaten cooked, and cured meats). Literature search to identify suitable scientific articles was conducted using the ISI Web of Knowledge and Web of Science databases for papers indexed since 1990 as well as Google searches using both English and Portuguese terms for combinations of foodborne disease or zoonosis (e.g., salmonellosis) or the pathogen (e.g., *Salmonella*), and the meat under study (e.g., pig meat, pork, pork product, pork preparation, sausage). For inclusion in the meta-analyses, the papers had to meet two requirements: to be an original article, and to make use of an approved microbiological method for pathogen detection.

Following the formulation of the problem statement and data collection, a parameterisation or measure unit of the effect size needs to be determined. The parameter measuring the effect size is a common metric that permits direct comparison and summation of primary studies (Noble, 2006). The effect size (θ) refers to the degree to which the hypothetical phenomenon (i.e., pathogens in meats) is present in the population. Because the measured outcome is binary (i.e., a meat sample tests either positive or negative for the pathogen) and is given only for single groups, the only possible parameter to measure effect size is the *raw proportion p* (or incidence) and its *transformations*. In order to restrict the range of the effect size or pathogen's incidence to [0–1] and to stabilise the variance, the logit transformation of the raw proportion was used as the effect size measure θ (Viechtbauer, 2010). If the sample size n of the primary study is at least higher than 20, it is usually reasonable to assume that the sampling distribution of the outcomes is normal (Bryk & Raudenbush, 1992).

2.1. Description of data sets

After assessing all the information presented in every study, a total of 21 primary studies – encompassing international reports and scientific articles – were considered appropriate for inclusion in the meta-analysis models. The meta-analysis models for fresh meats were based on 16 primary studies (Antunes, Reu, Sousa, Pestana, & Peixe, 2002; Baptista, 2010; Borges, 2009; EFSA, 2005, 2006, 2007, 2009, 2010a, 2010b, 2011b, 2012, 2013; Esteves, Aymerich, et al., 2006; Esteves, Saraiva, Fontes, & Martins, 2006; Mena et al., 2004; Mena, Rodrigues, Silva, Gibbs, & Teixeira, 2008), while the ones for meat products were based on 7 primary studies (Almeida, Mena, & Carneiro, 1998; Esteves, Aymerich, et al., 2006; Esteves, Patarata, Saraiva, & Martins, 2008; Esteves, Saraiva, et al., 2006; Ferreira, Fraqueza, & Barreto, 2007; Mena et al., 2004; Mendes, 2013; Vaz-Velho, Almeida, Mena, Carneiro, & Freitas, 1998). From each of the primary studies (j), the number of samples (s) experiencing the event of interest (i.e., testing positive for a

Table 1
Number of incidence and observations (s/n) of food-borne pathogens in Portuguese fresh meats by category extracted from published survey studies.

Pathogen	Fresh bovine	Fresh broiler	Fresh pork	Minced beef	Minced pork
Salmonella	3 (11/180), (0/55), (0/1142)	7 (1/81), (4/216), (0/25), (2/50), (0/40), (44/421), (7/64)	12 (3/60), (25/105), (6/58), (2/61), (5/30), (62/1122), (39/256), (14/99). (16/99), (25/105), (13/101), (10/64)	2 (0/95), (3/135)	5 (13/130), (2/73), (5/186), (2/142), (3/120)
Campylobacter	0	8 (17/81), (21/108), (0/33), (296/421), (21/78), (39/78), (46/62), (24/38)	0	0	0
Y. enterocolitica	0	0	3 (0/61), (0/58), (1/61)	0	4 (13/25), (10/25), (2/75), (0/74)
VTEC	0	0	3 (0/74), (0/34), (4/25)	0	1 (5/50)
L. monocytogenes	1 (3/17)	2 (26/63), (9/15)	0	0	0
S. aureus	0	1 (28/64)	1 (32/64)	0	0

pathogen) and the total number of samples (n) were extracted. Information such as sample weight, production stage, sampling site of the carcass (if applicable) and year of the survey was also annotated from every primary study. For the Portuguese fresh meats, a total of fifty-one observations of incidence (s/n) of foodborne pathogens were extracted from the 16 primary studies, and are compiled according to meat categories in Table 1.

In the case of the Portuguese meat products, sixty-nine incidence observations on pathogens were found for 26 types of products. These were mainly traditional meat products elaborated with pig meat and fat, and were categorised as traditional products 'to be eaten raw' (encompassing different kinds of dry-fermented sausages) and 'to be eaten cooked' (including fresh sausages and blood sausages). A third category of 'cured meat products' was considered so as to comprise heat-treated meat products such as cooked sliced ham and *mortadella*. These three meat product categories are mutually exclusive, and the observations of incidence of pathogens per category are summarised in Table 2. *Y. enterocolitica*, VTEC and *Campylobacter* have not been listed in Table 2 due to the absence of incidence data of such pathogens in meat products.

2.2. Random-effects meta-analysis

In its simplest form, a meta-analysis can be carried out as a fixed-effects variant to make a *conditional inference* only about the J primary studies included (Hedges & Vevea, 1998). However, most meta-analyses are based on sets of studies that are not exactly identical in their methods and the characteristics of their samples, which may introduce variability (i.e., heterogeneity) among the true effects estimated by the primary studies. One way to model the heterogeneity is to treat it as purely random (Viechtbauer, 2010). In contrast to the fixed effects model, random models provide an *unconditional inference* about a larger set of studies from which the J studies included in the meta-analysis are assumed to be a random sample (Hedges & Vevea, 1998). It envisions a hypothetical population of studies that comprises studies that have been conducted, that could have been conducted or that may be conducted in the future. The random-effects model addresses the question: How large is the average true effect in this larger population of studies? In a random effects model, each primary study investigates its own true effect size Θ_j,

$$\theta_j = \Theta_j + \varepsilon_j = \overline{\Theta} + v_j + \varepsilon_j \tag{1}$$

where θ_j is the observed effect size in the primary study j, $\overline{\Theta}_j$ the mean true effect size, and ε_j the error due to sampling variance. The ε_j are assumed to be normally-distributed with mean zero and true variance ξ^2. The term v_j represents the deviation of the true study effect size Θ_j from the mean true effect size. The values of v_j are normally-distributed random effects with mean zero and variance τ^2. Thus, it follows that for a random-effects meta-analysis model, $\theta_j \sim Normal(\Theta,\tau^2 + \xi^2)$. In this approach, two sources of variation are distinguished: sampling variation (ξ^2), and variation between true effect sizes (τ^2). By including this additional component (τ^2), the standard error in the effect size estimates represents random variability at both the subject level and the study level. Notice that the nomenclature θ_j and Θ_j is a general notation that refers to any effect size measure for the observed and the true effect size, respectively. In our particular case, it refers to the logit transformation of the incidence or proportion p_j, which is calculated using the number of successes s_j (positive samples) and total sample size n_j taken from each of the primary studies.

$$\theta_j = logit\ p_j = \log\left(\frac{p_j}{1-p_j}\right) = \log\left(\frac{s_j}{n_j - s_j}\right). \tag{2}$$

The incidence or proportion p_j can be back-transformed as,

$$p_j = \frac{\exp\left(\theta_j\right)}{1 + \exp\left(\theta_j\right)}. \tag{3}$$

Table 2
Number of incidence and observations (s/n) of food-borne pathogens in Portuguese meat products by category extracted from published survey studies.

Pathogen	Intended to be eaten raw	Intended to be eaten cooked	Cured meats
L. monocytogenes	14 (1/12), (4/16), (1/15), (1/8), (1/8), (1/7), (1/8), (0/6), (0/10), (2/38), (1/30), (1/44), (1/27), (0/48)	11 (3/26), (3/24), (1/11), (0/10), (1/11), (0/10), (1/10), (1/10), (0/32), (10/96), (1/9)	3 (0/66), (0/42), (1/4)
Salmonella	10 (1/23), (1/7), (1/15), (0/6), (0/10), (1/38), (1/30), (0/6), (0/6), (0/3)	13 (0/19), (3/9), (1/1), (1/15), (1/2), (3/10), (0/11), (0/10), (0/10), (1/10), (2/32), (12/96), (0/10)	2 (1/107), (0/51)
S. aureus	6 (1/10), (8/38), (4/30), (2/6), (0/6), (1/3)	7 (1/10), (3/11), (0/10), (2/10), (0/10), (6/32), (48/96)	0

Apart from the values of θ_j from each primary study, the standard error of the effect size must be calculated. The variance of the sampling distribution of the transformed variable is known from statistical theory as

$$\sigma^2\left(\theta_j\right) = \frac{1}{n_j p_j\left(1-p_j\right)}. \quad (4)$$

In order to estimate the mean true effect size from Eq. (1), the observed effect sizes θ_j should be averaged. However, since primary studies usually differ from each other in the reliability of estimating the true effect size (for instance, due to differences in study sizes), a weighted average is preferred with weights w^*_j equal to the precision in estimating the population effect size.

$$w^*_j = \frac{1}{\sigma^2\left(\theta_j\right) + \tau^2}. \quad (5)$$

The variance τ^2 is estimated from the Q-statistic (DerSimonian & Laird, 1986),

$$\hat{\tau}^2 = \frac{Q-(J-1)}{\sum_j w_j - \left(\sum_j w_j^2 / \sum_j w_j{}^j\right)} \quad (6)$$

where w_j are the weights that would be assigned in a simple fixed-effects model, and Q is a popular statistic used to test the presence of heterogeneity in effect size across primary studies (Cochran, 1954).

$$w_j = \frac{1}{\sigma^2\left(\theta_j\right)} \quad (7)$$

$$Q = \sum_j \frac{\left(\theta_j - \hat{\theta}\right)^2}{\sigma^2\left(\theta_j\right)}. \quad (8)$$

When effect sizes across studies are homogeneous, Q follows a chi-square distribution with $(j-1)$ df. If the hypothesis is rejected, there is evidence that there are additional sources of variability (τ^2) other than within-study sampling error (ξ^2). It is then common practice either to examine moderating variables; to divide the studies in homogeneous groups to perform separate meta-analysis; or to use a random-effects or a multilevel model.

The mean true effect size Θ and its standard error $\sigma(\Theta)$ are now estimated from Eqs. (9) and (10) using instead the corrected weights w^*_j.

$$\hat{\theta} = \frac{\sum_j w^*_j \theta_j}{\sum_j w^*_j} \quad (9)$$

$$\hat{\sigma}\left(\hat{\theta}\right) = \frac{1}{\left(\sum_j w^*_j\right)^{0.5}}. \quad (10)$$

To evaluate whether the effect size is larger than zero, often a Wald test assuming normality for the dependent variable is used comparing the estimated weighted average divided by its standard error with a standard normal distribution ($U = \hat{\theta}/\hat{\sigma}\left(\hat{\theta}\right)$). The U statistic is compared with a chi-square distribution with one df.

2.3. Multilevel meta-analysis

A meta-analysis can be considered a special case of *multilevel analysis* using hierarchical linear models, with subjects between studies at the first level and studies at the second level. In a multilevel meta-analysis, as in any other multilevel analyses, one usually starts from the random-effects model (Van den Noortgate & Onghena, 2003). If the between-study variance is shown to be noteworthy, study characteristics or moderators can be added to the model to account for at least part of the heterogeneity in the true effects. This leads to the mixed-effects model given by:

$$\theta_j = \Theta_j + \varepsilon_j = \beta_0 + \sum_{s=1}^{S} \beta_s X_{sj} + v_j + \varepsilon_j \quad (11)$$

with S (X_1 to X_S) study characteristics. This model treats the moderator effects β_s as fixed, and v_j as random effects that distribute normally with a mean zero and a variance of τ^2. Yet, τ^2 now denotes the amount of residual heterogeneity among the true effects, or the variability among the true effects that is not accounted for by the S moderators included in the model. The goal of the analysis is then to examine to what extent the moderators influence the size of the average true effect size Θ. The resulting model is thus more general than the ones commonly used in classical meta-analysis. If no predictors are included, the model of Eq. (11) simplifies to the random-effects model (Eq. (1)), or if the variance in true effects is zero, to the fixed-effects model. The use of a regression equation ($\beta_0 + \sum_{s=1}^{S} \beta_s X_{sj}$) for the study characteristics is appealing for several reasons. First, different predictors can easily be investigated together; meaning that even possible inter-correlations can be taken into account, which is not the case when separate meta-analyses are performed to investigate the moderating effects of study characteristics. Secondly, regression is a general approach that can be used for continuous as well as for categorical moderator variables (Van den Noortgate & Onghena, 2003).

2.4. Fitting of models

To estimate the parameters, maximum likelihood estimation (MLE) procedures are most frequently used. In MLE, residuals on both levels (v_j and ε_j of Eq. (1)) are assumed to be independently distributed. To test the fixed parameters of the model (β_s), the Wald test is used like in the traditional approaches, comparing the parameter estimate by the standard error with a standard normal distribution. Apart from the Q statistic, other measures can be computed to facilitate the interpretation of the estimated amount of between-study heterogeneity (τ^2). The I^2 statistics or intra-class correlation estimates the proportion of between-study variance from the total variance. This is analogous to using the proportion of explained variance in standard regression models to indicate the importance of specific predictor variables. Hunter and Schmidt (1990) pointed out that, when the number of studies is small, a lack of significance for τ^2 does not imply that the outcomes are homogeneous. So, they proposed a 25% rule of thumb; this is, if the intra-class variance I^2 is higher than 25% of the total variance, the variance between studies can be deemed as large enough to attempt to model it using available study characteristics. The I^2 statistic is just a monotonic transformation of τ^2 (see Higgins & Thompson, 2002).

For models including moderators, an omnibus or moderators test (QM test) of all model coefficients is conducted that excludes the intercept. By default, the test statistics of the individual coefficients in the model are based on the normal distribution, while the moderators test is based on a chi-square distribution with S degrees of freedom (S being the number of moderators tested). Finally, after attempting to explain the heterogeneity among studies using the study characteristics,

the QE test can be performed to test the non-explained (residual) variance using the statistic (Raudenbush & Bryk, 1985)

$$QE = \sum_j \frac{\left(\theta_j - \hat{\beta}_0 - \sum_{s=1}^{S} \hat{\beta}_s w_{sj}\right)^2}{\sigma^2\left(\theta_j\right)} \quad (12)$$

which follows a chi-square distribution with *J–S–1°* of freedom.

As there was considerable dispersion in the number of incidence observations among the meat category – pathogen combinations (Tables 1 and 2), separate meta-analysis studies were conducted on suitable data groups of the combinations presenting more observations. For the meat categories, these were: (i) a meta-analysis on the incidence of *Salmonella* spp. across meat categories; (ii) a meta-analysis on the incidence of *Salmonella* spp., *Campylobacter* and *L. monocytogenes* in fresh broiler; and (iii) a meta-analysis on the incidence of *Salmonella* spp., *Y. enterocolitica*, VTEC and *S. aureus* in pork (fresh and minced) for a semi-quantitative risk ranking. For each of the three meta-analyses of pathogens in meats, a random-effects meta-analysis was fitted. The objective of this meta-analysis was to estimate the mean effect size (mean incidence) as well as to assess between-study heterogeneity. Subsequently, multilevel meta-analysis models (Gonzales-Barron et al., 2013) were fitted to each of the three studies using categorical variables, defined as 'meat category' for study (i), and 'pathogen' for studies (ii) and (iii). The general statistical notation of the multilevel model (Eq. (11)) became either $\theta_j = \beta_0 + (\beta_1 X_{1j} + \beta_2 X_{2j} \ldots + \beta_p X_{pj}) + \nu_j + \varepsilon_j$ or $\theta_j = \beta_0 + (\beta_1 X_{1j} + \beta_2 X_{2j} \ldots + \beta_m X_{mj}) + \nu_j + \varepsilon_j$, where the vector $(\beta_1, \beta_2, \ldots, \beta_p)$ or $(\beta_1, \beta_2, \ldots, \beta_m)$ refers to the shift in effect size coefficients of each pathogen or each meat category for the multilevel model either with pathogens or with meat categories as subgroups, respectively. The coded variable X_p or X_m takes the value of 1 for the pathogen or meat category subgroup. The objective of fitting meta-analyses with a moderator was to assess any statistical difference between subgroups (H_0: $\beta_1 = \beta_2 \ldots = \beta_{p/m} = 0$). Notice that, in this work, the subscript *j* used in the above equations is general and indistinctly refers to the incidence entry unit for each of the meta-analysis (random-effects and multilevel).

In the case of the meat products, three separate meta-analysis studies were performed on the incidence of each of the three pathogens, *L. monocytogenes*, *Salmonella* spp., and *S. aureus*. For each of the three studies, a random-effects meta-analysis was first adjusted; and subsequently, a multilevel meta-analysis model using 'meat product' as a categorical moderating variable of three levels ($\theta_j = \beta_0 + (\beta_1 X_{1j} + \beta_2 X_{2j} + \beta_3 X_{3j}) + \nu_j + \varepsilon_j$). Meta-analysis models were adjusted in R version 2.14.2 (R Development Core Team) using the 'metafor' package (Viechtbauer, 2010), which provides functions for fitting the various models described above as well as meta-analytical graphs (forest plots, funnel plots, etc.).

2.5. Semi-quantitative risk ranking of pathogens in Portuguese raw pork

With the available information of occurrence rates of some pathogens in Portuguese raw pork, a preliminary semi-quantitative risk ranking of *Salmonella* spp., *Y. enterocolitica*, VTEC and *S. aureus* was conducted to identify the top pathogens in terms of public health importance as related to raw pork and potential foodborne infections. The FAO risk assessment grid (FAO, 1998) that qualitatively ranks risk in terms of two variables: severity and likelihood of occurrence, was employed in this study. The categorisation used for the *severity of disease* was the one developed by EFSA (2011a), where the severity of human infection was assessed using lethality among confirmed cases as an indicator, without taking into account whether pork was identified as a source of infection of a case. Using this definition, the severity of consequences is "high" when the human cases are higher than 10/100,000 and case-fatalities are lower than 0.1%. The severity of consequences is "medium" when the human cases are between in 1 and 10/100,000, and case-fatalities are lower than 0.1%. Lastly, the severity of consequences in pork is low in two cases: when the human cases are lower than 1/100,000 and case-fatalities higher than 0.1%, or when the human cases are lower than 1/100,000 and case-fatalities lower than 0.1% (EFSA, 2011a). According to this severity categorisation, *Salmonella* spp. belongs to high severity, *Y. enterocolitica* to medium severity, while VTEC belongs to low severity. EFSA (2011a) gives *S. aureus* a qualitative category of *unknown* because of the lack of data on frequency and case fatality of confirmed cases.

The second variable is the *likelihood of occurrence* of the pathogen, which ideally should be measured at the point of consumption. However, in practice this type of data is not available, and instead prevalence data at a slaughterhouse or at retail level was used. Occurrence – or even better, concentration – data for the pathogen either at retail level or at another point further up the chain would give some measure of the likelihood of occurrence of the pathogen for the purposes of the risk ranking grid. Thus, in our research, the likelihood of occurrence of a pathogen was replaced by a quantitative estimation of its incidence, obtained by summarising all the occurrences from the primary studies. Box plots of the incidence rates were approached by fitting a beta distribution ($s_T + 1$, $n_T - s_T + 1$) to the incidence data of each of the pathogens (Vose, 2008), where s_T is the total of pooled number of successes (total number of positive samples from all primary studies) and n_T the total sample size.

3. Results and discussion

Although there is a perception that a high incidence of foodborne diseases may have various causes such as inadequate manipulation, preparation and distribution of foods along the food chain, it is also true that quantitative risk assessment leading to efficient prevention demands data availability. Overall, in Portugal, data on microbial contaminants in meats and epidemiology is relatively scarce. For instance, at the level of national compulsory surveillance of infectious diseases, salmonellosis and brucellosis are the only food-related illnesses caused by bacteria subject to notification. In relation to zoonotic agent outbreaks, in 2011, Portugal reported 8 confirmed outbreaks due to toxins of *S. aureus* and *Clostridium* spp. which affected a total of 101 people (EFSA, 2013). Nevertheless, it is not possible to obtain any information about the types of food that mostly contributed to the notified individual cases and outbreaks. The most recent register of the associated foods dates back from 2000, and is found in a report of the European monitoring programme for foodborne disease control (WHO, 2003). According to this study, the foodstuffs mostly associated to the outbreaks in Portugal, in the period 1999–2000, belonged to meat and meat products (21%), followed by baked products (14%) and ready-to-eat meals (22%). Despite the 12-year difference, EFSA (2013) reinforced that meat and meat products – although at EU level – were still the main vehicle of foodborne diseases, responsible for 32% of the strong evidence outbreaks in 2011. In this way, whether eating habits in Portugal have remained basically unchanged in the past decade or the Portuguese situation is well approached by the EU trend, it is imperative to gather as much information as possible on the foodborne pathogens present in Portuguese meats in order to understand the current epidemiological situation, and to allocate resources to prioritise microbial hazards for risk analysis.

The systematic review conducted in this study indicated that currently the information on the level of occurrence of certain pathogens in meats produced in Portugal is sparse. The foodborne pathogens whose presence in meats have not been extensively surveyed are *Campylobacter* (with only 8 published studies identified), *Y. enterocolitica* (7 primary studies), VTEC and *L. monocytogenes* (3 primary studies) and *S. aureus* (2 primary studies). For these pathogens, there is no knowledge (Table 1) on their occurrence or levels in fresh bovine meat, except for one study found for *L. monocytogenes*, and generally no knowledge of their levels in any minced type of meat (i.e., minced beef, minced poultry,

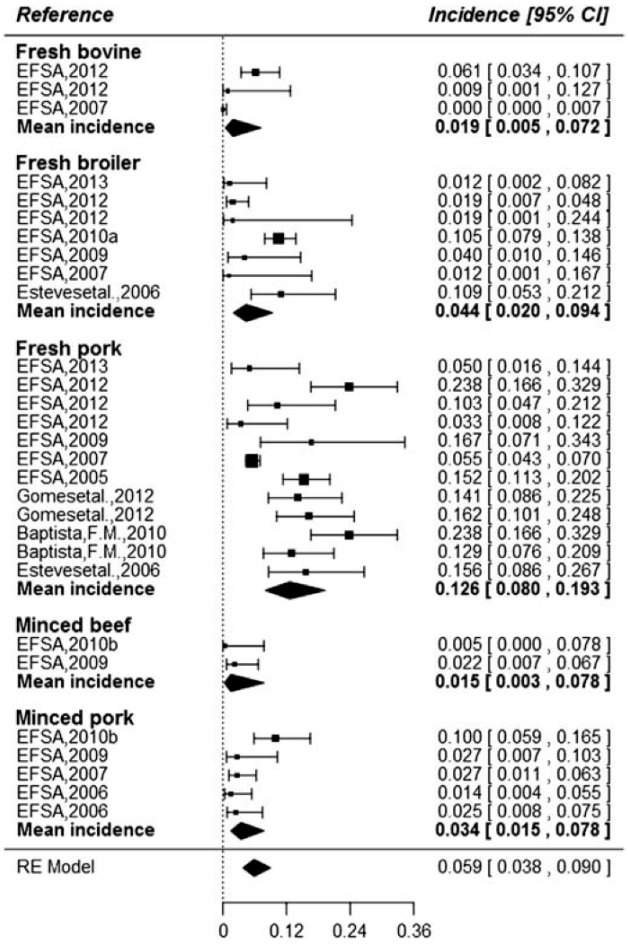

Fig. 1. Forest plot of the multilevel random-effects models of incidence of *Salmonella* spp. in Portuguese meats.

minced pork). Likewise, no single study reporting incidence or numbers of *Y. enterocolitica* in broiler meat or *Campylobacter* in pork have been found. Except for minced broiler meat, *Salmonella* spp. comes out as the only pathogen that has been widely examined in most of the meat categories with 29 primary studies retrieved. This data availability has been prompted by the current national programmes in place to control *Salmonella* in bovine, pig and broiler meat sampled at processing plants and retail. Moreover, the EU mandatory *Salmonella* monitoring programmes at primary production, as well as the enforced food safety criteria for *Salmonella* (Reg. No. 1441/2007) setting limits for specific food categories, have in turn prompted more scientific research of this pathogen with an integrated food-chain approach.

3.1. Incidence of pathogens in Portuguese meats

The first random-effects meta-analysis indicated that the incidence of *Salmonella* spp. in Portuguese meats is 6% (CI: 4–9%). As displayed in the forest plot (Fig. 1), considerable variability in reported incidence were observed among studies, which was proven by the significant test of heterogeneity ($Q = 171$; $p < 0.001$ in Table 3). The between-study variance decreased from $\tau^2 = 1.21$ to 0.58 when meats were grouped in categories by the multilevel model, although there was still some unexplained residual heterogeneity ($QE = 130$; $p < 0.001$ in Table 3). The omnibus moderator test (QM) showed that the mean incidence of at least one meat category differed significantly from the others (Table 3). Despite fresh pork corresponds to the category presenting the higher between-study variability (Fig. 1), it is undoubtedly the one with the highest incidence of *Salmonella* spp., diverging significantly from fresh bovine and fresh broiler meat. In contrast with the EU average occurrence for *Salmonella* in fresh pork (0.7%; EFSA, 2013) and minced pork (0.6%; EFSA, 2012), the corresponding occurrence values for Portugal, 12.6% (95% CI: 8.0–19%) and 3.4% (95% CI 1.5–7.8%) are much higher. This finding may be due to the fact that in Portugal there is no bacteriological or serological control programme of pigs on farm that could enable the application of risk management strategies at primary production. Because *Salmonella* is primarily located in the gastrointestinal tract of sub-clinically infected pigs, they introduce the pathogen to the slaughterhouse through their internal organs, skin, faeces, and the cross-contamination of carcasses is basically a matter of redistributing *Salmonella* bacteria from positive pigs during the various slaughter processes. In a risk assessment model, Gonzales Barron et al. (2009) underscored the need to target *Salmonella* contamination at swine production, calculating that on average 77% of the variability in the total contaminated carcasses at the point of evisceration is explained by the contamination from the carrier animals entering the slaughter lines. Thus, although in Portugal there is a national monitoring programme for *Salmonella* in pig meat based on sampling at slaughterhouse and meat cutting plants, the incidence of *Salmonella* in pork is still high, as its control requires rather a systematic approach from farm to fork with specific risk management strategies in place also at farm level. This fact, along with the high consumption of pork in Portugal (43 kg/habitant/year), calls for the contemplation of the implementation of a national *Salmonella* monitoring programme of pig herds. Referring to serovars, the most common ones present in Portuguese fresh pork include Typhimurium (37, 70%), Derby (11, 14%) and Rissen (7, 14%) (as recovered by Baptista, 2010; Gomes-Neves et al., 2012), which belong to the most frequent serovars in pigs and pig meat isolated in the EU (EFSA, 2013).

In relation to the fresh bovine meat and minced beef produced in Portugal, the meta-analysed occurrences of *Salmonella* are 1.9% (95% CI: 0.5–7.2%) and 1.5% (95% CI: 0.3–7.8%), respectively (Fig. 1), which

Table 3
Results of the meta-analysis models for the logit-transformed incidence of *Salmonella* in Portuguese meats. Effect size and CI values correspond to back-transformed values. Between-study variability (τ^2), intra-class variability (I^2), and tests for heterogeneity (Q), residual heterogeneity (QE) and moderators (QM) are presented. Superscript letters denote statistical differences among meat categories.

Meta-analysis type	Effect size Incidence (CI)	τ^2	I^2 (%)	QM (F)	Q/QE
Overall random-effects	0.059 (0.038–0.090)***	1.21	91.6*	–	171*** (df = 28)
Multilevel					
Fresh bovine	0.019 (0.005–0.072)^{a}	0.58**	–	49.7*** (df1 = 5, df2 = 24)	130*** (df = 24)
Fresh poultry	0.044 (0.020–0.094)^{ab}				
Fresh pork	0.126 (0.080–0.193)^{c}				
Minced beef	0.015 (0.003–0.078)^{a}				
Minced pork	0.034 (0.015–0.078)^{ab}				

Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '·' 0.1 ' ' 1 'ns' Non-significant.

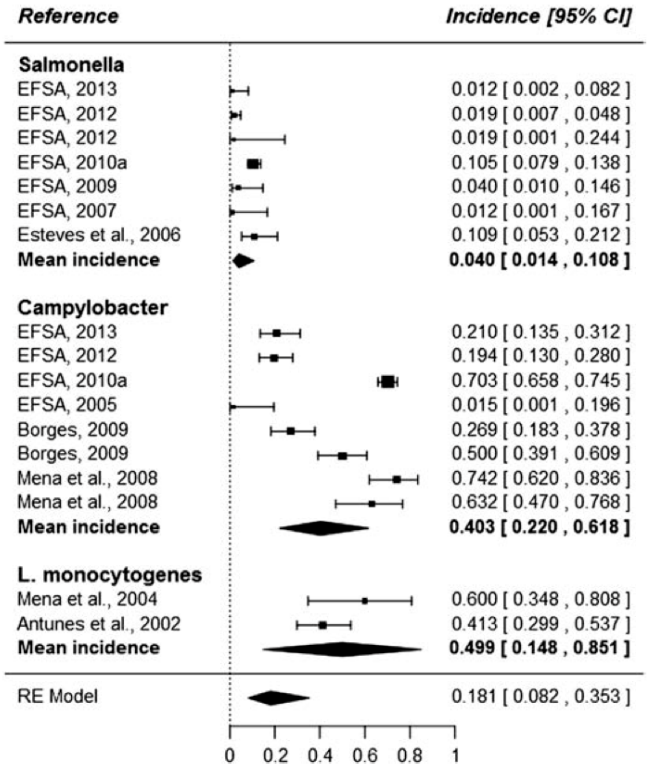

Fig. 2. Forest plot of the multilevel random-effects models of incidence of *Salmonella* spp., *Campylobacter* and *L. monocytogenes* in fresh broiler produced in Portugal.

are values moderately higher than the current EU average (0.2% and 0.4%, respectively; EFSA, 2013). On the contrary, the mean *Salmonella* occurrence in fresh broiler produced in Portugal (4.4%; 95 CI: 2.0–9.4%) appeared to be within the EU level (5.9%). A knowledge gap was found with regard to the *Salmonella* serovars most frequently recovered from Portuguese bovine and broiler meats.

Our study also summarised the occurrence of some pathogens in fresh broiler meat produced in Portugal. The discrepancy among the incidence values of the different pathogens can be visualised in the multilevel meta-analysis forest plot (Fig. 2). As expected, this led to a high between-study heterogeneity ($\tau^2 = 3.22$; $Q = 453$ with $p < 0.001$ in Table 4) which still remained significant after categorising the effect sizes by pathogen ($\tau^2 = 1.16$; $QE = 178$ with $p < 0.001$). Although a higher between-study variability was observed for *Campylobacter* in comparison with both *Salmonella* and *L. monocytogenes* incidence values (Fig. 2), still the omnibus moderator test (QM) showed that the mean incidence of at least one pathogen differed significantly from the others (Table 4). The fact that *Salmonella* spp. in broiler meat had at least ten times lower incidence than *Campylobacter* and *L. monocytogenes* (Table 4) is believed to be due to the positive impact of the national *Salmonella* control programmes at the primary production; this is, in flocks of laying hens, breeding flocks and broiler flocks, with risk reduction targets set in 2007. It is also deemed that the EU compulsory food safety criteria for *Salmonella* (Reg. No. 1441/2007) setting limits for products of meat origin may have contributed to the overall reduction of this pathogen in poultry. The same cannot be said for *Campylobacter* in fresh broiler as its high incidence in Portugal (40.3%; CI: 22.0–61.8%) follows the high EU average trend (31.3%; EFSA, 2013). Since 2005, campylobacteriosis continues to be the most commonly reported foodborne disease in the EU, with broiler meat considered to be the major source of the disease. EFSA (2013) concluded that handling, preparation and consumption of broiler meat may account for 20–30% of human campylobacteriosis in the EU, whilst 50–80% may be attributed to the chicken reservoir as a whole. Moreover, 46% of the *Campylobacter* outbreaks in the EU in 2011, in which the implicated food vehicle was provided, were associated to broiler meat (EFSA, 2013). Nevertheless, it is unfeasible to explore such a link in Portugal since campylobacteriosis cases are neither notified nor its food vehicle investigated. Some research has shown that *Campylobacter jejuni* (15,70%) and *Campylobacter coli* (40,70%) are the two species of highest incidence in Portuguese fresh broiler (as extracted from Borges, 2009; Mena et al., 2008). While there continues to be a lack of epidemiological and risk assessment studies of *Campylobacter* in Portuguese broiler meat, control measures can only be directed to the consumers. Through food labelling and education campaigns, Portuguese consumers should be made aware that adequate cooking will assure safety of meats but serious undercooking or cross contamination from a raw to a cooked product in the kitchen are thought to be major routes of infection.

The association of *L. monocytogenes* as a pathogen of high prevalence in poultry meat, would be considered by many as surprising. However, due to the fact that the high level of mean occurrence of 50% (CI: 14.8–85.1%; Fig. 2) has been obtained from only two available primary studies (Antunes et al., 2002; Mena et al., 2004), at present the main recommendation can be for enabling actions to address the data gap. Djeniyi, Wegener, Jensen, and Bisgaard (1996) indicated that because *L. monocytogenes* is not frequently isolated from chickens, it is likely that the live animals may only contribute little to the total contamination of the abattoir, and that the pathogen may be introduced from dirty transport crates. Even though, poultry has not been associated to a great extent with human listeriosis, variable incidence rates of this pathogen have been found in the past in Denmark (23%; Djeniyi et al., 1996), USA (30%; Cox, Bailey, & Berrang, 1997), Ireland (6–30%; Whyte, McGill, Monahan, & Collins, 2004), Northern Ireland (17%; Soultos, Koidis, & Madden, 2003) and Brazil (28%; Loura, Almeida, & Almeida, 2005). Thus, these and the Portuguese results highlight that the presence of *L. monocytogenes* in Portuguese broiler meat would not be uncommon; and hence, maintaining good hygiene practices in production, processing and the consumer's kitchen cannot be overemphasized.

Within the pork category, occurrence data were available for *Salmonella* spp., *Y. enterocolitica*, *S. aureus* and VTEC (Table 1). The multilevel meta-analysis conducted in this data group also showed that there was significant between-study heterogeneity ($Q = 250$; $p < 0.001$ in Table 5).

Table 4
Results of the meta-analysis models for the logit-transformed incidence of pathogens (*Salmonella* spp., *Campylobacter* and *L. monocytogenes*) in Portuguese fresh broiler. Effect size and CI values correspond to back-transformed values. Between-study variability (τ^2), intra-class variability (I^2), and tests for heterogeneity (Q), residual heterogeneity (QE) and moderators (QM) are presented. Superscript letters denote statistical differences among incidence of pathogens.

Meta-analysis type	Effect size Incidence (CI)	τ^2	I^2 (%)	QM (F)	Q/QE
Overall random-effects	0.181 (0.082–0.353)***	3.22**	97.6*	–	453*** (df = 16)
Multilevel					
Salmonella	0.040 (0.014–0.108)[a]	1.16	–	13.8*** (df1 = 3, df2 = 14)	178*** (df = 14)
Campylobacter	0.403 (0.220–0.614)[bc]				
L. monocytogenes	0.499 (0.148–0.851)[c]				

Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '·' 0.1 ' ' 1 'ns' Non-significant.

Table 5
Results of the meta-analysis models for the logit-transformed incidence of pathogens (*Salmonella* spp., *Y. enterocolitica* and VTEC) in Portuguese fresh and minced pork. Effect size and CI values correspond to back-transformed values. Between-study variability (τ^2), intra-class variability (I^2), and tests for heterogeneity (Q), residual heterogeneity (QE) and moderators (QM) are presented. Superscript letters denote statistical differences among incidence of pathogens.

Meta-analysis type	Effect size Incidence (CI)	τ^2	I^2 (%)	QM (F)	Q/QE
Overall random-effects	0.085 (0.053–0.134)***	1.54**	92.6	–	250*** (df = 28)
Multilevel					
Salmonella	0.086 (0.048–0.152)^{a}	1.45	–	1.5* (df1 = 3, df2 = 25)	173*** (df = 25)
Y. enterocolitica	0.068 (0.022–0.193)^{a}				
VTEC	0.056 (0.012–0.221)^{a}				
S. aureus	0.500 (0.073–0.927)^{b}				

Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '·' 0.1 ^{ns} Non-significant.

Furthermore, while the mean incidence rates of both *Y. enterocolitica* and VTEC in pork were numerically lower than the *Salmonella* mean incidence, yet statistical differences among these occurrence rates could not be revealed (Table 5). Pig meat and pork products are considered to be the most important source of pathogenic *Y. enterocolitica* infections in humans (EFSA, 2012, 2013; Simonová, Vázlerová, & Steinhauserová, 2007). The meta-analysed mean value of this pathogen in Portuguese pork (6.8%; 95% CI: 2.2–19.3%) was moderately higher than the overall incidence rate of 2.4% (30 out of 1146 samples) reported for four EU countries in 2011 (EFSA, 2013). It is the ability of this organism to grow at 4 °C which makes refrigerated pork preparations with a relatively long shelf-life a probable source of infection (EFSA, 2005). Although the occurrence of VTEC in Portuguese pork was meta-analysed from only four primary studies, like *Y. enterocolitica*, VTEC's mean occurrence (5.6%; 95% CI: 1.2–22.2% in Table 5) turned out to be higher than the EU average reported over the years 2007–2010 (EFSA, 2012). Many of the investigations reported to EFSA in those years did not yield any positive findings, except for six countries which found VTEC incidence in fresh pig meat at very low levels (0.1–2%). VTEC O157:H7 was detected in three of these national surveys, and the highest proportion of positive samples was reported by Spain (1.2%; EFSA, 2012). On the other hand, although bovine meat is believed to be a major source of foodborne VTEC infections for humans, no microbiological survey of VTEC in bovine meat has been identified in Portugal. This is an issue that merits attention as VTEC infections in humans have been following an increasing trend since 2006 in a number of European countries such as the Netherlands, Austria, Denmark, Finland, France and Luxembourg (EFSA, 2012).

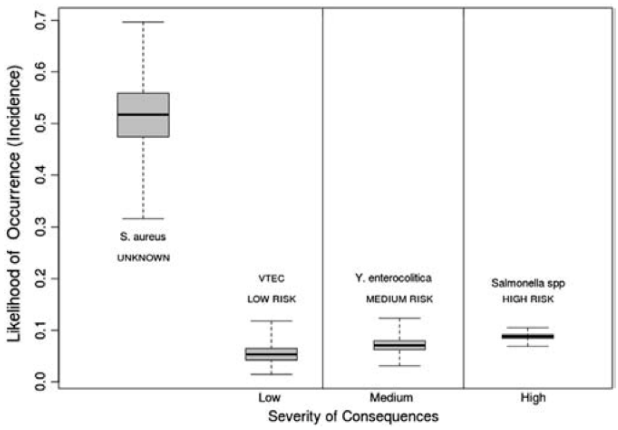

Fig. 3. Semi-quantitative risk ranking of pathogens associated with Portuguese fresh pork. The categorisation of severity of consequences was taken from EFSA (2011a).

3.2. Risk ranking of pathogens in Portuguese raw pork

A representation of the semi-quantitative risk ranking is shown in Fig. 3, where the confidence intervals of the box plots do not depict the uncertainty around the mean incidence (which is described by the confidence intervals of the meta-analysed mean incidences of Table 5), but instead the total uncertainty around the true incidence in a Bayesian framework. For this reason, the confidence intervals of the box plots (Fig. 3) are larger than those obtained by meta-analysis in Table 5. The pathogens were then categorised regarding their incidence or frequency of occurrence as per the criterion suggested in EFSA (2011a). This criterion establishes that the frequency of detection can be high, medium or low if the prevalence is higher than 5%, between 0.1 and 5% and lower than 0.1%, respectively. In our case, all four pathogens were assigned to the "high" frequency of detection category as at least 50% quartile of their box plot were above the cut-off criterion of 5%.

Bringing together the two variables, severity of disease and likelihood of occurrence, using the risk assessment grid from FAO (1998), it was found that the significance of *Salmonella* spp. in the fresh pork produced in Portugal is *critical* while the significance of *Y. enterocolitica* and VTEC is *major* and *minor*, respectively. In the case of *S. aureus*, there was a lack of data on intoxication severity, and also scarce knowledge on the likelihood of occurrence in Portugal (as only one primary study was retrieved). While such study (Esteves, Saraiva, et al., 2006) reported a high mean incidence of 50% in pork cuts, there is no knowledge on isolated strains capable of toxin formation. It is known that pork-derived products may remain a potential source of meticillin-resistant *S. aureus* (MRSA), with CC398 being the MRSA lineage most commonly associated to intensively-reared food-producing animals. However, so far there is no evidence for increased risk of human colonisation or infection following contact or consumption of food contaminated by CC398 (Smith et al., 2011). Furthermore, as the main risk factors of *S. aureus* include cross contamination and growth at processing, retail and domestic levels, EFSA (2011a) ranked the significance of *S. aureus* in chilled pig carcasses as low or minor risk. In our preliminary assessment, the microbiological hazards ranking in descending order were then: *Salmonella* spp., *Y. enterocolitica*, VTEC, while *S. aureus* was assigned to an unknown risk category (Fig. 3). A totally different outcome was found when using the ICMSF (2002) criteria for the severity characterisation of pathogens. In such a case, all three pathogens VTEC, *Salmonella* and *Y. enterocolitica* represented major risks. Thus, it is imperative that national resources and efforts be allocated to the implementation of a system for control and prevention to reduce, in the first place, the current high levels of *Salmonella* and *Y. enterocolitica* in the Portuguese pig herds and at processing level.

3.3. Incidence of pathogens in Portuguese meat products

L. monocytogenes has not only been detected in a variety of raw meats, but also in food contaminated post-processing such as cheeses and RTE meats, both of which have been implicated internationally in

Table 6
Results of the three meta-analysis models for the logit-transformed incidence of pathogens (*L. monocytogenes*, *Salmonella* spp., *S. aureus*) in Portuguese meat products. Effect size and CI values correspond to back-transformed values. Between-study variability (τ^2), intra-class variability (I^2), and tests for heterogeneity (Q), residual heterogeneity (QE) and moderators (QM) are presented. Superscript letters denote statistical differences among meat product categories.

Meta-analysis type	Effect size Incidence (CI)	τ^2	I^2 (%)	QM (F)	Q/QE
L. monocytogenes					
Fixed-effects	0.088 (0.065–0.118)***	0	0	–	22.2^{ns} (df = 27)
Multilevel					
To be cooked	0.098 (0.066–0.144)^{a}	–	–	0.87^{ns} (df1 = 2, df2 = 25)	20.8^{ns} (df = 25)
To be eaten raw	0.083 (0.051–0.131)^{a}				
Cured	0.042 (0.010–0.162)^{a}				
***Salmonella* spp.**					
Fixed-effects	0.097 (0.070–0.134)***	0	0	–	32.5^{ns} (df = 24)
Multilevel					
To be cooked	0.134 (0.092–0.190)^{c}	–	–	8.13*** (df1 = 2, df2 = 22)	18.7^{ns} (df = 22)
To be eaten raw	0.057 (0.028–0.113)^{b}				
Cured	0.009 (0.002–0.045)^{a}				
S. aureus					
Random-effects	0.226 (0.154–0.318)***	0.23	39.5**	–	33.5*** (df = 12)
Multilevel					
To be cooked	0.258 (0.157–0.393)^{b}	0.24	–	13.5*** (df1 = 2, df2 = 11)	24.0* (df = 11)
To be eaten raw	0.184 (0.097–0.319)^{a}				

Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '·' 0.1 '^{ns} Non-significant.

outbreaks. In contrast to the meta-analysis in Portuguese meats, which presented a high variability among studies (see spread of measured outcomes in Fig. 1), the meta-analysis of *L. monocytogenes* in meat products did not have a significant between-study variability ($Q = 22$, $p > 0.05$; Table 6), as can be also visualised in the respective forest plot (Fig. 4). The reduced spread in the measured incidences (Fig. 4) across the great variety of meat products, may be due to the fact that *L. monocytogenes* enters the chain mainly through the raw meat; and subsequently its survival is affected by common ingredients and processing steps (i.e., salt, spices, thermal processing of some ingredients/meats, fermentation, smoking and drying), in a way that such combined effects may be comparable among meat products. Thus, the random-effects model of Eq. (1), with a between-study variability of $\tau^2 = 0$, reduced to a fixed-effects model. The twenty-six meat products were grouped in three categories: to be cooked, to be eaten raw and cured meats, in order to conduct the multilevel fixed-effects meta-analysis. The first two sub-groups contained mainly traditional meat products, many of them bearing quality labels, and the last sub-group was comprised of products elaborated with curing salts and produced to a larger scale. Although discrepancies among the mean incidence values of *L. monocytogenes* in the three categories were not large enough to cause statistical significance (i.e., the number of primary studies for the cured meats were few in comparison to the other two categories, which brought about a greater uncertainty around its mean estimate), still the mean incidence for the cured meats was numerically lower than the others (Table 6). This lower rate (4.2%; 95% CI: 1.0–16.2%) may be due to the fact that these products contain nitrites and undergo some heat treatment; yet it can be said that this value is within the order of magnitude of the overall EU incidence of 2.4% for RTE products of meat origin except fermented sausages reported in 2010 (EFSA, 2012). It is worthwhile to mention that this EU mean incidence is taken within the context of compliance to the microbiological criterion of *L. monocytogenes* in RTE foods, expressed as a percentage of single samples and not of batch units; and in both, Portuguese studies and EFSA results, the stipulated sample weight of 25 g was used. The mean incidence of *L. monocytogenes* in Portuguese meat products intended to be eaten cooked (9.8%; 95% CI: 6.6–14.4%) was high and not different ($p > 0.05$) from the mean occurrence in fermented sausages (8.3%; 95% CI: 5.1–13.1%; Table 6). Although the doses (concentration) of *L. monocytogenes* in these products may as well be low, this is an outcome that merits further investigation since such fermented meats are of high consumption and commonly eaten in Portugal without any further cooking that would reduce the risk of contracting the pathogenic agent by the consumers. Although it may not be directly comparable, at EU level the non-compliance of *L. monocytogenes* in fermented sausages sampled at processing plants was of 1.0% in 2011

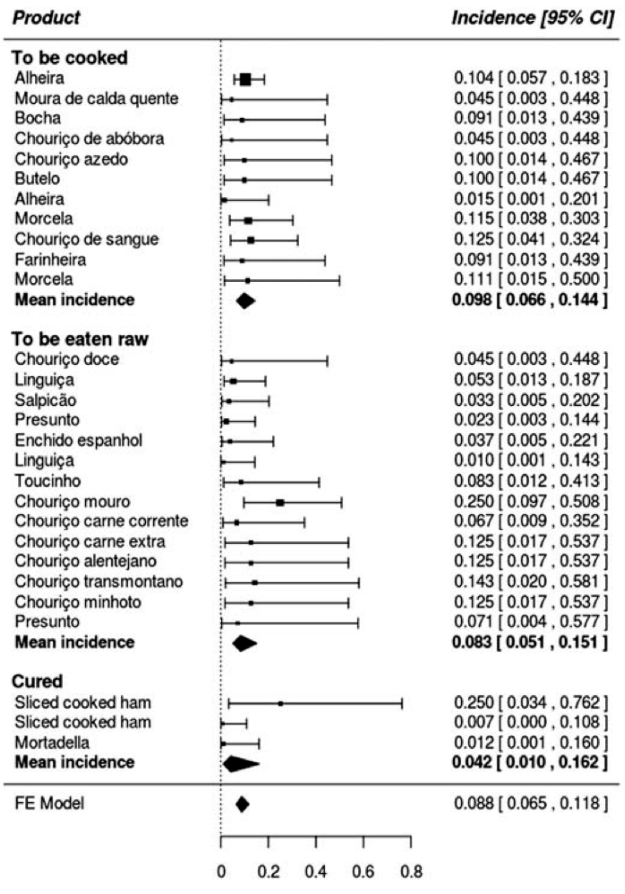

Fig. 4. Forest plot of the multilevel random-effects model of incidence of *L. monocytogenes* in Portuguese meat products.

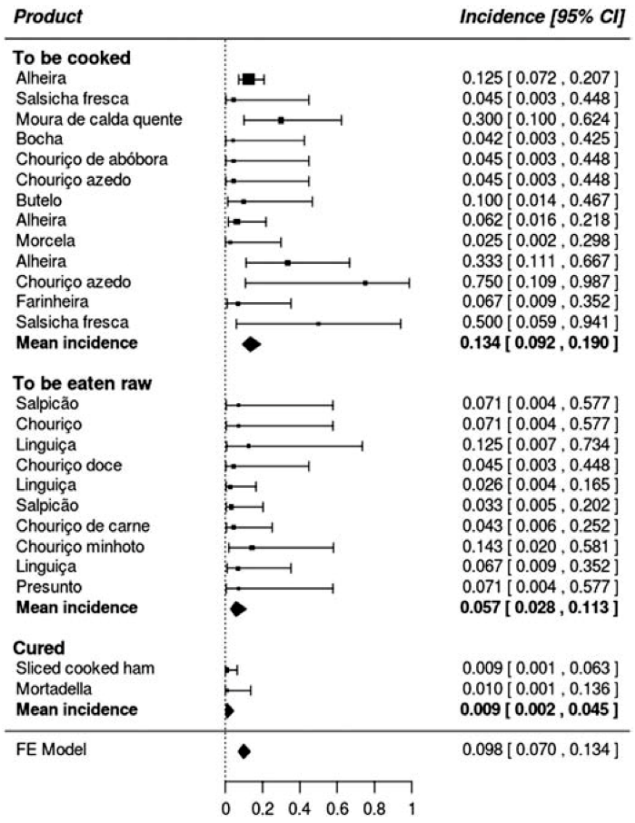

Fig. 5. Forest plot of the multilevel random-effects model of incidence of *Salmonella* spp. in Portuguese meat products.

(EFSA, 2013), which is a level fairly below the meta-analysed estimate for Portugal. These results underscore the necessity, firstly, to understand the risk factors in the processing of traditional meat products contributing to the pathogen's high prevalence; and secondly, to assess suitable risk-based control measures along production.

The meta-analysis of *Salmonella* spp. in meat products, like the meta-analysis of *L. monocytogenes* referred above, also led to a fixed-effects solution, as there was no significant variability among products

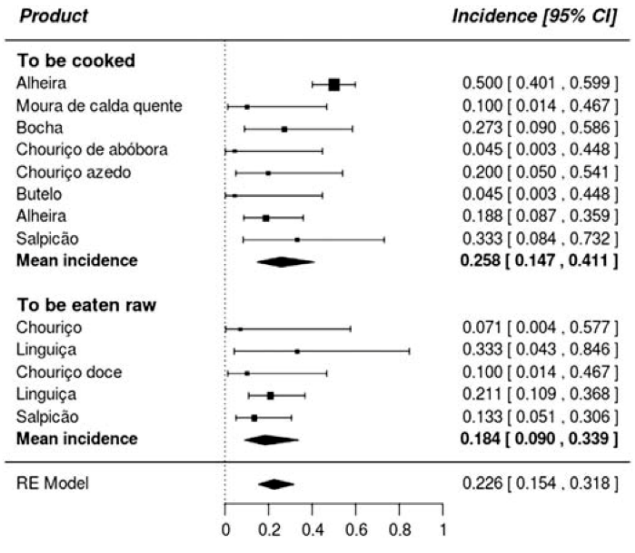

Fig. 6. Forest plot of the multilevel random-effects model of incidence of *S. aureus* in Portuguese meat products.

($Q = 32$, $p > 0.05$; and hence $\tau^2 = 0$ in Table 6). Again, the homogeneity in the measured incidences of *Salmonella* spp., despite the great variety of meat products considered, (Fig. 5) was noteworthy. The fact, that the combined effects of ingredients and processing on the viability of *Salmonella* along production may be comparable among the different meat products, could explain the absence of between-study heterogeneity in *Salmonella* occurrence. The overall mean incidence of *Salmonella* in Portuguese meat products was high (9.7%; 95% CI: 7.0–13.4%) and did not differ ($p > 0.05$) from the mean incidence of *L. monocytogenes* (8.8%; 95% CI: 6.5–11.8% in Table 6). In the case of cured meats, the incidence rate of *Salmonella* spp. (0.9%; 95% CI: 0.2–4.5%) was considerably lower than the incidence of *L. monocytogenes* (4.2%; 95% CI: 1.0–16.2%), which may be due to the lower resistance of *Salmonella* spp. to curing agents, heat treatment and cold ripening (Hwang et al., 2009). Among the three meat product categories compared by the multilevel meta-analysis (Table 6), there were significant differences in mean *Salmonella* occurrence, with the traditional meat products intended to be eaten cooked having the highest incidence (13.4%; 95% CI: 9.2–19.0%). This level of non-compliance to the *Salmonella* EU microbiological criterion (in single samples unit) was higher than the EU levels reported in 2011 for the following related categories (EFSA, 2013): poultry meat preparations intended to be eaten cooked (6.8%), meat preparations from other species than poultry intended to be eaten cooked (1.1%), and meat products from poultry intended to be eaten cooked (1.1%). Given such a high incidence of *Salmonella* in the Portuguese meat products to be cooked, and the severity of this pathogen, actions should be enabled to lower *Salmonella* prevalence in the pork industry as well as to instruct consumers of the importance of fully cooking the raw traditional meat products and avoiding cross contamination. On the other hand, the meat products intended to be eaten raw presented a lower *Salmonella* incidence of 5.7% (95% CI: 2.8–11.3% in Table 6), which, nonetheless, was still numerically higher than the EU average for the meat preparations intended to be eaten raw in 2011 (from 0 to 1.4%; EFSA, 2013). It is worthy to mention that since 2008 the highest level of non-compliance with the EU *Salmonella* microbiological criteria generally occurred in food of meat origin with minced meat and meat preparations from poultry intended to be eaten cooked having the highest level of non-compliance.

A different scenario was noticed for the meta-analysis conducted on the presence of *S. aureus* in Portuguese meat products (Fig. 6). There, a significant heterogeneity among products was observed ($Q = 33$, $p < 0.05$) with an I^2 suggesting that ~40% of the total variability in measured occurrences can be attributed to the variability product to product. Even after meat products were categorised in 'to be eaten raw' and 'to be eaten cooked', there was some remaining between-product variability (residual $QE = 24$, $p < 0.05$) in *S. aureus* incidence. Unlike *L. monocytogenes* and *Salmonella* spp., whose incidence rates were rather homogeneous among the meat products, the heterogeneity in the occurrence of *S. aureus* can only reveal the variability in hygiene among the different meat products during manufacturing. On average, 22.6% (95% CI: 15.4–31.8%; Table 6) of the Portuguese meat product samples are expected to be contaminated with this pathogen. The high occurrence of *S. aureus* in these meat products is an indicator of hygiene deficiency during processing. For instance, for Alheira (a raw meat product, first in Fig. 6), a product whose processing implies extensive manipulation, the incidence of *S. aureus* was the highest (50%). In the EU, staphylococcal enterotoxins were the causative agent of 6% (435 outbreaks) of all outbreaks reported in 2011; and along with other toxins produced by *Clostridium* and *Bacillus*, they ranked second as responsible agents of all the foodborne outbreaks in the EU, only after salmonellosis. In Portugal, and for the same year, 6 confirmed outbreaks due to staphylococcal toxins occurred, involving a total of 90 human cases. It is also known that the largest proportion of strong-evidence outbreaks caused by staphylococcal toxins is attributed to mixed food including meats (40%) (EFSA, 2013). Giletto and Fyffe (1998) indicated that the food vehicles most frequently involved in intoxication by *S. aureus*, are cooked

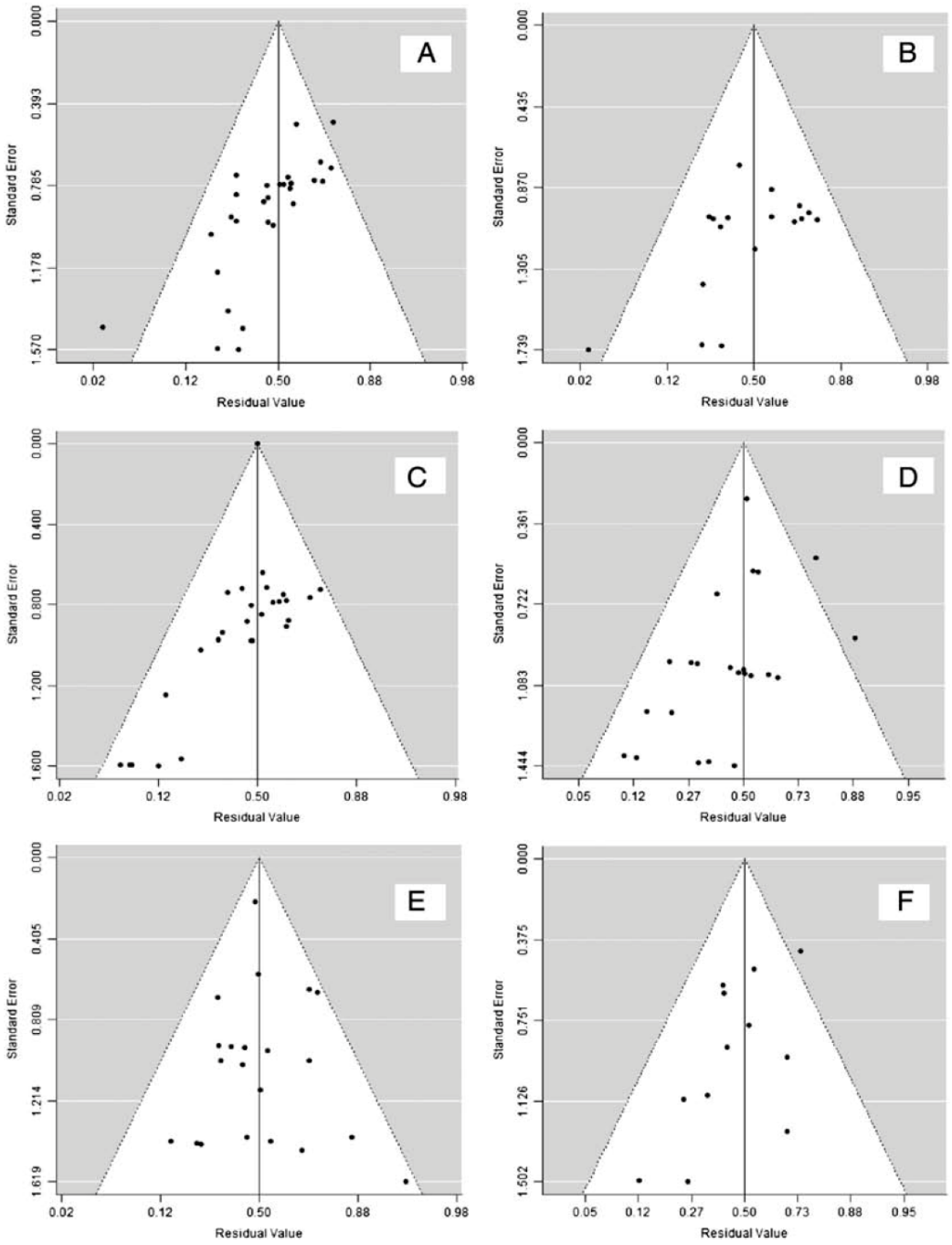

Fig. 7. Funnel plots of the incidence of *Salmonella* spp. in Portuguese fresh meat (A); the incidence of *Salmonella* spp., *Campylobacter* and *L. monocytogenes* in fresh broiler (B); the incidence of *Salmonella*, VTEC, *Y. enterocolitica* and *S. aureus* in pork (C); and the incidence of *L. monocytogenes* (D), *Salmonella* spp. (E) and *S. aureus* (F) in Portuguese meat products.

refrigerated meats and meat products, such as turkey meat, roasted meat, ham and fermented meat products as well as bakery products containing cream. Thus, the outcome of this meta-analysis, which points towards a very high incidence of *S. aureus* in both meat products to be cooked (25.8%; 95% CI: 15.7–39.3%) and meat products to be eaten raw (18.4%; 95% CI: 9.7–31.9%) may support the current epidemiological situation of foodborne outbreaks in Portugal; at least to some extent as also other foods can be vehicles of staphylococcal enterotoxins. Furthermore, considering that the background microflora might limit the growth of *S. aureus* and therefore, the toxin production, further research should be carried out from a dose-response perspective.

As part of this study, publication bias was also investigated for each of the six multilevel meta-analyses conducted. Firstly, this was done by the construction of a funnel plot, which relates the mean incidence value from each primary study (or its residual after removing the covariate effects in the case of a multilevel meta-analysis) with its respective standard error as a measure of the level of confidence in the results of such primary study (for further details on the funnel plot, refer to Whitehead, 2002). There was a general tendency (Fig. 7) in having very few or a total absence of publications reporting high incidence values (higher residuals in the x-axis of the funnel plot) from small sample sizes (higher standard errors). This can be verified by the blank right

bottom area in most funnel plots (Fig. 7). Nevertheless, some caution should be taken in identifying this phenomenon directly as a proof of publication bias, since it is quite common that a small sample size will fail to detect any pathogen if this is present in lower concentrations. Said otherwise, it is likely that a sample size consisting of five sample units will not have the statistical power to detect *Salmonella* spp. if the true prevalence of this pathogen in the food under question is very low. For this reason, in microbiological surveys of absence/presence of pathogens in foods, in order to accurately estimate the pathogen's prevalence, a large sample size is commonly required when its concentration in food is known to be low. This is the same phenomenon which produces the high number of zero counts normally observed in microbial data (Gonzales-Barron & Butler, 2011). Thus, some caution should be taken when interpreting the funnel plots of incidence data as the blank right bottom area is not necessarily a proof of publication bias. Another approach to test publication bias is to investigate the effect of the study size directly by including the total sample size of a study as explanatory variable in a multilevel meta-analysis. Results of these multilevel meta-analyses (not shown) suggested that there was no significant effect of sample size on the observed incidences. Hence, as the presence of unpublished small-size studies reporting high prevalence is very unlikely, it is highly probable that the effect size outcomes (mean incidences) presented in these meta-analysis are not affected by publication bias.

4. Conclusion

The systematic review conducted in this research allowed to recognise the sparseness of knowledge on the incidence of pathogens in meats and meat products produced in Portugal. This meta-analysis study provided the first pooled incidence estimates for pathogens in specific meat categories, which are more robust and reliable than single study estimates. For the meta-analyses conducted on Portuguese meats categorised by origin, a greater number of incidence observations from primary studies were sourced for *Salmonella* in the different meats and *Campylobacter* in broiler meat than in all the other pathogen-meat combinations. The meta-analysed mean occurrence rate of *Campylobacter* in broiler meat (40%) was found to be nearly ten times higher than that of *Salmonella* (4.4%), although both levels were well within EU ranges. The lower incidence of *Salmonella* in broiler meat may be explained by the positive impact of the national control programmes in flocks of laying hens, breeding flocks and broiler flocks in place from 2008. In other fresh meat categories (i.e., bovine meat, pork, minced beef and minced pork), the *Salmonella* mean incidence values for Portugal were in all cases slightly to moderately higher than EU averages, being lowest in fresh bovine (1.9%) and highest for pig meat (12.6%). Furthermore, the semi-quantitative risk ranking in Portuguese pork assigned to *Salmonella* spp. and *Y. enterocolitica* (6.8% occurrence) the risk categories of critical and major, respectively. Considering that, in Portugal the meat of pig origin has the highest consumption per capita, it is essential that a comprehensive pork carcass safety assurance be implemented. The current monitoring programme, consisting in testing for *Salmonella* in pig carcasses at the slaughterhouses, per se will not lead to any risk mitigation unless reduction targets are set to be achieved for *Salmonella* and *Y. enterocolitica* in/on chilled pig carcasses. It is equally necessary that, earlier in the food chain, risk management strategies such as differentiation of both pig batches and abattoirs as well as on-farm strategies such as categorisation of pig herds and herd health programmes be implemented in Portugal. It was surprising to find few primary studies reporting high occurrences of *L. monocytogenes* in Portuguese broiler (50%) and *S. aureus* in pork (50%), which are issues of concern that should be addressed by further research. This meta-analysis also highlighted the areas where there are gaps of knowledge such as the *Salmonella* serovars in bovine and broiler meats, the occurrence of VTEC in fresh bovine meat, *Y. enterocolitica* in fresh bovine and broiler meats, *Campylobacter* in pork, and in general the lack of information on the presence of toxin-producing pathogens in raw meats.

In the case of the Portuguese meat products, incidence data was only available for *L. monocytogenes*, *Salmonella* spp. and *S. aureus*. Data gaps were recognised for toxin-producing microorganisms such as *Clostridium* spp. and *Bacillus cereus*. The overall incidence of *S. aureus* (22.6%) in the Portuguese meat products was significantly higher than those of *L. monocytogenes* (8.8%) and *Salmonella* (9.7%), which indicates a breakdown in processing hygiene. Bringing together the great variety of meat products produced in Portugal, it was found that the meat products 'intended to be eaten cooked' had mean incidences generally higher than the meat products 'to be eaten raw' for the three pathogens assessed. Nevertheless, since the mean occurrence rates or non-compliance rates to the EU microbiological criteria for both *L. monocytogenes* (9.8% in products to be cooked and 8.3% in products to be eaten raw) and *Salmonella* (13.4% in products to be cooked and 5.7% in products to be eaten raw) were considerably higher than EU levels for RTE products in comparable categories, it is essential that Portuguese food safety agencies take actions for the maintenance of good hygiene practices in the production and processing of traditional meat products. Putting all information together, this meta-analysis work revealed a clearer picture of the state of knowledge on the incidence of the most important foodborne pathogens in Portuguese-produced meats and meat products. It may also assist national food safety authorities and policy makers in the prioritisation of microbiological hazards in the specific meat type categories, and can equally aid researchers to provide direction for future investigation.

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2.2. Meta-analysis applied to microbiological risk assessment: An exposure assessment model of the prevalence of *Salmonella* spp. along the processing stages of Brazilian beef

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An exposure assessment model of the prevalence of *Salmonella* spp. along the processing stages of Brazilian beef

Ursula Gonzales-Barron[1], Luciana Piza[2], Cristina Xavier[1], Ernane Costa[2] and Vasco Cadavez[1]

Abstract

Beef cattle carrying *Salmonella* spp. represents a risk for contamination of meat and meat products. This study aimed to build an exposure assessment model elucidating the changes in *Salmonella* prevalence in Brazilian beef along the processing stages. To this effect, the results of a number of published studies reporting *Salmonella* incidences were assembled in order to model conversion factors based on beta distributions representing the effect of every production stage on the *Salmonella* incidence on beef carcasses. A random-effects meta-analysis modelled the hide-to-carcass transfer of *Salmonella* contamination. The Monte Carlo simulation estimated the *Salmonella* prevalence in beef cuts from processing plants to be ~6.1% (95% CI: 1.4–17.7%), which was in reasonable agreement with a pool ($n = 105$) of surveys' data of *Salmonella* in Brazilian beef cuts (mean 4.9%; 95% CI: 1.8–11.5%) carried out in commercial establishments. The results not only underscored the significant increase in *Salmonella* prevalence that can occur during evisceration/splitting and boning but also reinforced that, when hygienic slaughter procedures are properly implemented, the load of *Salmonella* can be reduced at dehiding, rinsing and chilling. As the model was based on a systematic review and meta-analysis, it synthesised all available knowledge on the incidence of *Salmonella* in Brazilian beef.

Keywords

Simulation, meta-analysis, systematic review, slaughterhouse, beef cuts

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INTRODUCTION

Foodborne salmonellosis is a major public health issue in all countries and requires concerted efforts to prevent and control the pathogen in the food supply. In Brazil, a remarkable increase in salmonellosis has been reported. According to the Sanitary Surveillance of the Brazilian Department of Health, from a total of 6791 reported foodborne outbreaks occurring during 1999 to 2010 in Brazil, 46% of them were caused by *Salmonella* spp., red meat being the vehicle in 12% of the outbreaks occurred during the same period (SVS, 2011). On the other hand, since 2005, Brazil has become one of the top producers of beef meat in the world with one of the highest annual export rates of over 1.5 millions of tons, which in 2010, represented a share of US$4.1 billion to the national economy (ABIEC, 2011).

Due to its importance in the international market, the quality and safety of Brazilian beef have to meet

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stringent international standards. On the other hand, the sparse information on the occurrence of pathogens in the Brazilian beef meat may have an impact on the international trade, leaving the country in some disadvantage in relation to other large producers such as United States and Australia. In the past few years, meta-analysis has been increasingly applied to synthesize food safety information, such as prevalence of microorganisms in foods, effect of processing steps and interventions, risk ranking of pathogens and disease incidence (Den Besten and Zwietering, 2012; Grieg et al., 2012; Gonzales-Barron et al., 2013; Xavier et al., 2014), and has been used as a tool for exposure assessment models of pathogens in foods (Gonzales-Barron and Butler, 2011; Gonzales Barron et al., 2009). Thus, this study aimed to bring together all available information, reported in the literature, on the effects of the different beef processing stages on the occurrence of *Salmonella*, in order to build an exposure assessment model that could be validated for Brazil. The simulation model was constructed using transfer, reduction and contamination factors modelling the effect of dehiding, evisceration and splitting, rinsing, chilling and boning on the *Salmonella* occurrence on beef. To model the distribution of the simulation's input variable, the prevalence of *Salmonella* on Brazilian beef hides post-bleeding, a random-effects meta-analysis was conducted to combine the occurrence rates from different Brazilian abattoirs' surveys. In the particular case of the dehiding process, given the many published articles reporting *Salmonella* occurrence values before and after the operation, a second random-effects meta-analysis was applied to get an overall relative risk with improved precision. Appraisal of the model's ability to produce accurate predictions was performed by comparing the prevalence output estimated by the model against a pool of *Salmonella* occurrences recovered from Brazilian beef cuts reported in Almeida et al. (2010), Colvara et al. (2007) and Xavier and Joele (2004).

METHODOLOGY

Hill et al. (2003) devised a slaughterhouse exposure assessment model to estimate, stage by stage, the prevalence of *Salmonella* on pig carcasses produced in the UK. Such a model employed contamination and reduction factors based on beta distributions modelling uncertainty around prevalence. Those factors were modelled bringing together available results from published literature. In this work, we build upon this type of prevalence modelling by incorporating meta-analytical techniques. However, because there was a data gap on the effect of bleeding on the occurrence of *Salmonella* on beef hides, the present model assumed that there was no effect of stunning and bleeding on the prevalence of *Salmonella* on beef hides. The processing stages contemplated by the model were dehiding, evisceration and splitting, rinsing, chilling and boning. Literature identification was conducted using electronic search through Google including combinations of the terms '*Salmonella*', 'beef', 'carcass' and a beef processing stage (i.e. 'dehiding') in both English and Portuguese. Suitable scientific articles indexed since 1990 were also identified from bibliographic databases such as PubMed, Science Direct and Scopus using the same keywords. For modelling the contamination and reduction factors, the papers included in this work had to meet two requirements: to present occurrence values before and after a processing stage; and to make use of an approved microbiological method for *Salmonella* detection clearly stating the extent of the carcass swabbed area. Apart from the latter requirement, for modelling the input variable and for validation, an additional prerequisite was that the study had to be conducted in a Brazilian abattoir.

Transfer factor of *Salmonella* for the dehiding operation

Six published studies (Brichta-Harhay et al., 2008; Gandra, 2011; Lanna et al., 2011; Lopes, 2011; Minuzzi et al., 2012; Silva, 2011) were found to report the occurrence values on beef hides after bleeding and on carcasses after dehiding for the same animals. The outcome data from the published studies were available on n_T beef hides in the post-dehiding group (treated group) and n_C beef carcasses in the pre-dehiding group (control group). The number of successes (*Salmonella*-positive carcasses or hides) in the post-dehiding and pre-dehiding group is represented by s_T and s_C, respectively, and they are compiled in Table 1. In order to combine all these binary results, a meta-analysis was conducted on the effect size parameterization of the natural logarithm of relative risk (log *RR*). *RR* is defined as the probability of encountering *Salmonella*-positive beef carcasses after dehiding relative to the probability of encountering *Salmonella*-positive hides before dehiding. The meta-analysis procedure to obtain an overall (weighted average) log *RR* was the one explained in detail in Gonzales-Barron et al. (2013). The same nomenclature was used. Given the significant variability (i.e. heterogeneity) in the outcomes among individual studies, a random-effect solution was opted, which led to an overall log *RR* (i.e. effect size of dehiding) of −0.984 and a standard error of 0.262. Hence, the distribution of the hide-to-carcass transfer factor (T_D) was modelled as

$$T_D = \exp(\text{Normal}(-0.984, 0.262))$$

Table 1. Occurrence of *Salmonella*-positive bovine carcasses before and after the dehiding operation per sampled batch as detected by six primary studies

	Pre-dehiding group (control)		Post-dehiding group (treated)	
Sources	s_C	n_C	s_T	n_T
Brichta-Harhay et al. (2008)	106	121	9	18
	83	90	21	31
	99	106	1	5
	108	127	46	84
Gandra (2011)	2	38	0	38
	1	22	0	22
Lanna et al. (2011)	11	135	1	135
Lopes (2011)	31	200	7	200
Minuzzi et al. (2012)	11	200	0	200
	6	200	0	200
Silva (2011)	4	120	0	120

The uncertainty about the *Salmonella* prevalence on beef carcasses after dehiding (P_D) was calculated as

$$P_D = P_B \times T_D$$

where P_B is the prevalence of *Salmonella* on beef hides after bleeding before dehiding.

Contamination factor of *Salmonella* for the evisceration and splitting operations

Occurrence values of *Salmonella* spp. on beef carcasses were recovered from three published studies (Lanna et al., 2011; Minuzzi et al., 2012; Narvaez-Bravo et al., 2013). The following occurrence data were extracted (s/n): 1/135, 0/200 and 13/237 before evisceration; and 4/135, 3/200 and 17/237 after splitting from the respective sources. In all cases, a carcass area of 100 cm^2 was swabbed. Knowing that the uncertainty about a true prevalence value can be modelled by a beta distribution and that such information can be easily updated through Bayes' theorem (i.e. if the prior opinion about the prevalence is $Beta(a, b)$, and the observed number of successes is s out of a sample size of n, the posterior distribution turns out to be a $Beta(a+s, b+n-s)$), the procedure used to model the prevalence about the *Salmonella* occurrence on pre-eviscerated carcasses (Vose, 2008) is described as follows: (i) assuming a non-informed prior of $Beta(1,1)$, the posterior distribution of *Salmonella* prevalence after the conduction of the study of Lanna et al. (2011) will be a $Beta(1+1, 1+135-1) = Beta(2, 135)$; (ii) now the distribution $Beta(2, 135)$ can be assumed to be a prior and updated using the new occurrence data (Minuzzi et al., 2012), which will produce a posterior $Beta(2+0, 135+200-0) = Beta(2, 335)$; and (iii) once again, this $Beta(2, 335)$ can be presumed to be a prior distribution that can be updated using the results from Narvaez-Bravo et al. (2013), which yields a posterior $Beta(2+13, 335+237-13) = Beta(15, 559)$. The $Beta(15, 559)$ represents the uncertainty about the *Salmonella* prevalence on beef carcasses before evisceration. Exactly the same procedure was used to model the uncertainty about the *Salmonella* prevalence after splitting, leading to a $Beta(25, 549)$. Because both beta distributions originated from paired results (i.e. the same primary studies reported incidence values before and after evisceration), the contamination factor of *Salmonella* for evisceration and splitting (C_S) was built as a ratio of the two prevalence uncertainty distributions

$$C_S = \frac{Beta(25, 549)}{Beta(15, 559)}$$

The prevalence of *Salmonella* on Brazilian beef carcasses after evisceration and splitting (P_S) was then estimated as

$$P_S = P_D \times C_S$$

Conversion factor of *Salmonella* for the rinsing operation

As in the previous sub-section, an after-to-before rinsing conversion factor of the prevalence of *Salmonella* on beef carcasses was modelled using available results from three published articles. In the Brazilian studies, rinsing had no appreciable effect on the occurrence of *Salmonella* on beef carcasses: Swabbing a total of 200 beef carcasses, Minuzzi et al. (2012) found that before rinsing three samples were positive while after rinsing four samples were positive. Similarly, Lanna et al. (2011) swabbed a total of 135 carcasses and recovered four positive before rinsing and five positive after rinsing. In the Venezuelan study considered (Narvaez-Bravo et al., 2013), rinsing the beef carcasses had some beneficial effect for the reduction of *Salmonella*. Sampling 237 carcasses, Narvaez-Bravo et al. (2013) found that rinsing decreased the number of positive swabs from 17 to 11. To consider the possibility that the rinsing operation could increase, decrease or have no appreciable effect on the occurrence of *Salmonella* on beef carcasses, the conversion factor for rinsing (C_R) was modelled integrating the incidence results from the

three studies in the same way as done for the evisceration and splitting conversion factor. This led to

$$C_R = \frac{Beta(21, 553)}{Beta(25, 549)}$$

The proportion of *Salmonella*-positive Brazilian beef carcasses after rinsing (P_R) was estimated as

$$P_R = P_S \times C_R$$

Reduction factor of *Salmonella* for the chilling operation

In the case of chilling, only one American study (Ruby et al., 2007) was found to report the occurrence of *Salmonella* on beef carcasses before and after this operation. Making use of a large sample size ($n = 5355$), this study demonstrated that chilling has a decreasing effect on the recovery of *Salmonella* from beef carcasses. Assuming that such reduction effect obtained in the American abattoirs under evaluation is on average comparable to the one achieved in a common Brazilian beef abattoir, a reduction factor of *Salmonella* due to chilling (R_{ch}) was modelled using the results from such study; that is, the positive samples at the entrance to the chiller were 123 out of 5355 and the positive samples after 24 h chilling were 53 out of 5300 beef carcasses. Using the uninformed *Beta*(1, 1) as priors for the distributions of prevalence before and after chilling, the R_{ch} was

$$R_{Ch} = \frac{Beta(53 + 1, 5355 - 53 + 1)}{Beta(123 + 1, 5355 - 123 + 1)}$$

The proportion of *Salmonella*-positive Brazilian beef carcasses after chilling (P_{Ch}) was estimated as

$$P_{Ch} = P_R \times R_{Ch}$$

Contamination factor of *Salmonella* for the boning operation

At the point that contaminated carcasses enter the processing plant, the number of contaminated surfaces in the line increases sharply. If contaminated carcasses enter the deboning plant, cross-contamination of conveyor belts, cutting boards and other contact surfaces can occur. The results from the only Brazilian study (Sigarini, 2004) available investigating the effect of deboning on the microbiological quality of beef meat were used to model the contamination in the boning halls. According to Sigarini (2004), by analysing pieces of rump, they observed that the occurrence of *Salmonella* on beef meat due to boning increased slightly from 0.125 (10 out of 71) to 0.20 (16 out of 80). Using the uninformed *Beta*(1, 1) as a prior for both the prevalence before and after boning, the contamination factor (C_C) due to boning was modelled as

$$C_C = \frac{Beta(16 + 1, 80 - 16 + 1)}{Beta(10 + 1, 71 - 10 + 1)}$$

The proportion of *Salmonella*-positive beef cuts (P_C) was then estimated as

$$P_C = P_{Ch} \times C_C$$

Model validation using Brazilian data

The input of the stage-by-stage simulation model was the occurrence of *Salmonella* on Brazilian beef hides after the bleeding operation (P_B). Data of interest were found from six individual studies and are presented in Table 2. The total number of beef hides swabbed after bleeding is represented by n_B while the number of *Salmonella*-positive samples is given by s_B. These binary data were combined on the basis that all these studies were conducted in Brazil and that the microbiological methods for determining *Salmonella* were comparable. Nonetheless, the microbiological protocol from these published studies differed in the hide swabbed area, which was 400 cm^2 for some and 100 cm^2 for other studies. Since the greater the swabbed area, the higher the likelihood of detecting

Table 2. Data sources utilised for the approximation of the incidence of *Salmonella* on Brazilian beef hides after the bleeding operation

Sources	Number of *Salmonella* (+) samples (s_B)	Total number of samples (n_B)	Hide swabbed area (cm^2)
Souza et al. (2010)	5	52	400
Lopes (2011)	31	200	400
Lanna et al. (2011)	11	135	400
Gandra (2011)	2	38	100
	1	22	100
Silva (2011)	4	120	100
Minuzzi et al. (2012)	11	200	100
	6	200	100
Pooled data	71	967	

contamination, and hence, the higher the occurrence rate; the difference in swabbed area among primary studies had to be accounted for in the integration of the results (Table 2). This integration was done by conducting a separate meta-analysis on the effect size parameterization of the logit transformation of the proportion p_j of *Salmonella*-positive hides after bleeding, which was calculated using the number of successes s_{Bj} and the sample size n_{Bj} taken from each of the primary studies j (Table 2). In the regression, the quantitative variable 'swabbed area' (A) was included as a moderating variable of the meta-analysis model, in order to assess statistically whether area had any effect on the *measured* occurrence rates of *Salmonella* on beef hides. The model fitted was of the form

$$logitp_j = log\left(\frac{p_j}{1-p_j}\right) = \beta_0 + \beta_1 A_j + v_j + \varepsilon_j$$

where β_0 is an intercept, β_1 is the fixed effect of the swabbed area, v_j the random effects due to the variability among the logit of the true prevalence estimated by each of the primary studies and ε_j the error due to sampling variance. For further details on the procedure to fit a random-effects model with a moderating variable, see Xavier et al. (2014). Since the meta-analysis model confirmed that the swabbed area had a significant effect ($p < 0.001$) on the measured prevalence, the expected *Salmonella* prevalence on Brazilian beef hides was estimated on the basis of a *fail-safe* 400 cm^2 (mean prevalence 0.125 with a 95% CI: 0.095–0.164) (Figure 2). With this prevalence estimate, it is possible to calculate the *most likely* total number of beef hides s'_B that would have tested positive from a 400-cm^2 swab, knowing that a total of 967 animals were tested (Table 2). The s'_B estimate would then be $0.125 \times 967 = 121$ total *Salmonella*-positive beef hides.

The *most likely* total number of contaminated beef hides s'_B attempts to quantify the sum of the number of *Salmonella*-positive hides from the individual studies that would have been likely to be obtained if 400 cm^2 of hide samples were swabbed in all individual studies. The most likely s_B' and the total n_B from the six studies were then combined using the Bayes' theorem for updating the beta distribution for prevalence, as explained above. Thus, assuming a prior of *Beta*(1, 1), the uncertainty about the occurrence of *Salmonella* on Brazilian beef hides after bleeding (P_B) was

$$P_B = Beta(121 + 1, 967 - 121 + 1)$$

For validation, the output of the model (P_C), fed by Brazilian data, had to be compared to some actual estimate of the occurrence of *Salmonella* in Brazilian beef cuts. For this, Brazilian survey studies reporting *Salmonella* occurrences were sought. Three published studies were found (Almeida et al, 2010; Colvara et al., 2007; and Xavier and Joele, 2004) and their results were integrated. Uncertainty was modelled by a beta distribution, as performed for the model's input P_B. The meta-analysis models were adjusted in R version 2.14.2 (R development Core Team) using the 'metafor' package. The simulation model of the exposure assessment was developed in Microsoft Excel using the @Risk add-in (Industrial Edition version 4.5.2, Palisade, NY), and run for 10,000 iterations using Latin Hypercube sampling without any separation of uncertainty and variability. A sensitivity analysis was carried out in order to identify the key parameters that influence the model's output. The sensitivity of the *Salmonella* prevalence in beef joints to input values was measured by regression whereby the higher the Pearson correlation coefficient between the input and the output, the more significant the input is in determining the output's value.

RESULTS AND DISCUSSION

Many studies on the microbiological hygiene (*Escherichia coli* O157:H7 and *Salmonella* spp.) of beef cattle at slaughter have shown that animal's faeces and hides are the primary sources of contamination (Arthur et al., 2007; Bell, 1997). The microbial levels of the hides are strongly correlated with carcass contamination, as a result of cross-contamination during processing (Barkocy-Gallagher et al., 2003). Although previous work has shown that there is a substantial transfer of pathogens from hide to carcass during the dehiding operation (McEvoy et al., 2003; Puyalto et al., 1997), no previous attempt was done to quantify such a transfer. In this work, a separate meta-analysis model was used to statistically represent the transfer of contamination from exsanguinated hides to pre-eviscerated beef carcasses. The meta-analysis not only confirmed that, within slaughter batches, there is an association between *Salmonella*-positive hides and *Salmonella*-positive carcasses (i.e. a significant transfer of *Salmonella* from hides to carcass, as indicated by the significant overall log *RR*) but also showed that the *Salmonella* occurrence on carcasses is lower ($p < 0.01$) than the occurrence on hides. Note that, in the forest plot of Figure 1, all the log *RR* estimates from the primary studies are negative. For the simulation model, the random-effects solution was utilised because of the significant heterogeneity ($p < 0.01$) in the measured occurrences from the primary studies.

Because in the Brazilian literature, there is a data gap on the prevalence of *Salmonella*-positive hides before bleeding, the input of the present model was

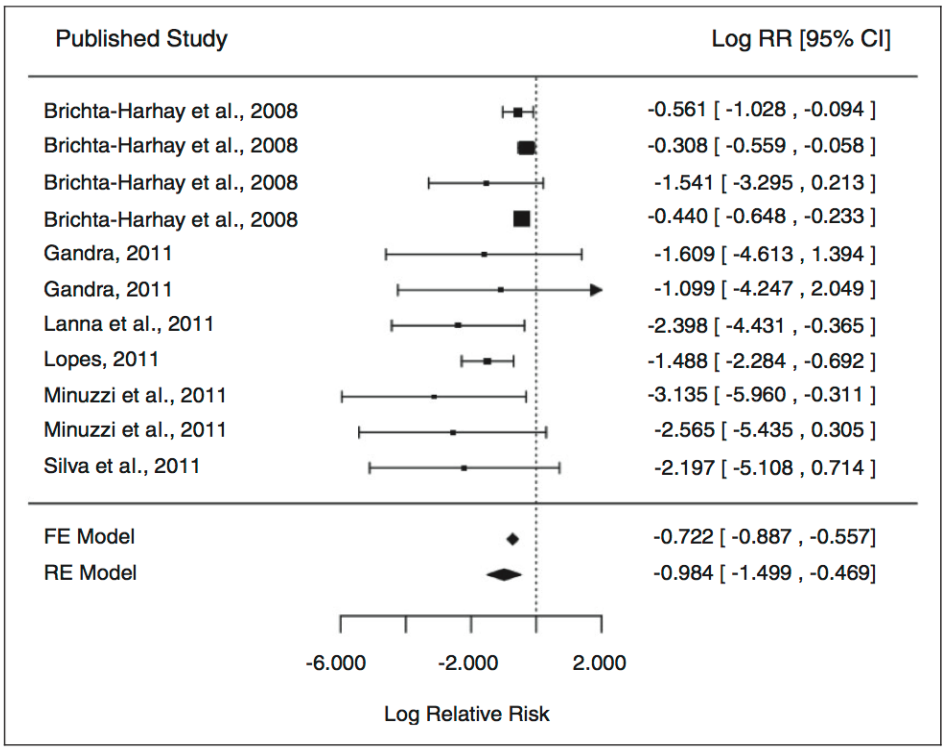

Figure 1. Forest plot of the risk of *Salmonella* incidence on beef carcasses after dehiding relative to the *Salmonella* incidence on beef hides after bleeding, among surveyed abattoirs. Size of the square markers reflects the weight assigned to primary studies. Individual estimates and overall relative risk by a fixed-effects (FE) and a random-effects (RE) model are shown with 95% confidence intervals.

the *Salmonella* prevalence on exsanguinated hides. The separate meta-analysis conducted on this input's variable corroborated that the extent of swabbed area has a significant influence ($p < 0.001$) on the recovery of *Salmonella*-positive beef hides. The forest plot of Figure 2 suggests that based on a small swabbed area of 100 cm^2, the expected *Salmonella*-prevalence on Brazilian beef hides would be very low at 4.3% (95% CI: 2.9–6.3%). However, the estimate of *Salmonella*-prevalence on Brazilian beef hides post-exsanguination used as input of the present simulation model corresponded to the greater area of 400 cm^2 (12.5%; 95% CI: 9.5–16.4%). This estimate appears to be lower than estimates in the range of 18–94% reported for other countries by Bacon et al. (2002), Brichta-Harhay et al. (2008), Fegan et al. (2005) and Reid et al. (2002). It is also lower than the occurrence of 36.7% of *Salmonella* on hides from three large Venezuelan abattoirs, recently surveyed by Narvaez-Bravo et al. (2013). Among other reasons, such as differences in sampling sites, method of *Salmonella* detection or seasonality, the relatively low value of *Salmonella* prevalence in Brazilian hides could be partly explained by the extent of the area swabbed in the Brazilian studies which was lower (100–400 cm^2 in Table 2) than in the studies mentioned above (hide areas ranging from 750 to 1000 cm^2). It is worthy to mention that if the difference in swabbed areas had not been accounted for by means of the meta-analysis procedure, and instead, the model's input had been modelled by simply adding together the outcomes of the primary studies (viz. using the total $s_B = 71$ and total $n_B = 967$ from Table 2) in a beta $(71+1,\ 967-71+1)$ distribution, the *Salmonella* prevalence on exsanguinated beef hides produced in Brazil would have been considerably lower at 7.4% (95% CI: 5.9–9.2%). The use of this value as the simulation model's input would have produced biased estimates of *Salmonella*-prevalence all along the processing stages.

The model estimated that, after dehiding, the occurrence of *Salmonella* on Brazilian beef carcasses is significantly lower at an average of ~4.8% (95% CI: 2.7–8.0% in Table 3). This model prediction was in good agreement with the results from two Brazilian surveys (Lanna et al., 2011; Lopes, 2011) where *Salmonella* was recovered with frequencies of 0.7% and 3.5% from 135 and 200 carcasses at this point of the chain, respectively. The model estimate was also comparable to the occurrence of *Salmonella* (5.5%) in 237 pre-eviscerated Venezuelan beef carcasses, surveyed by

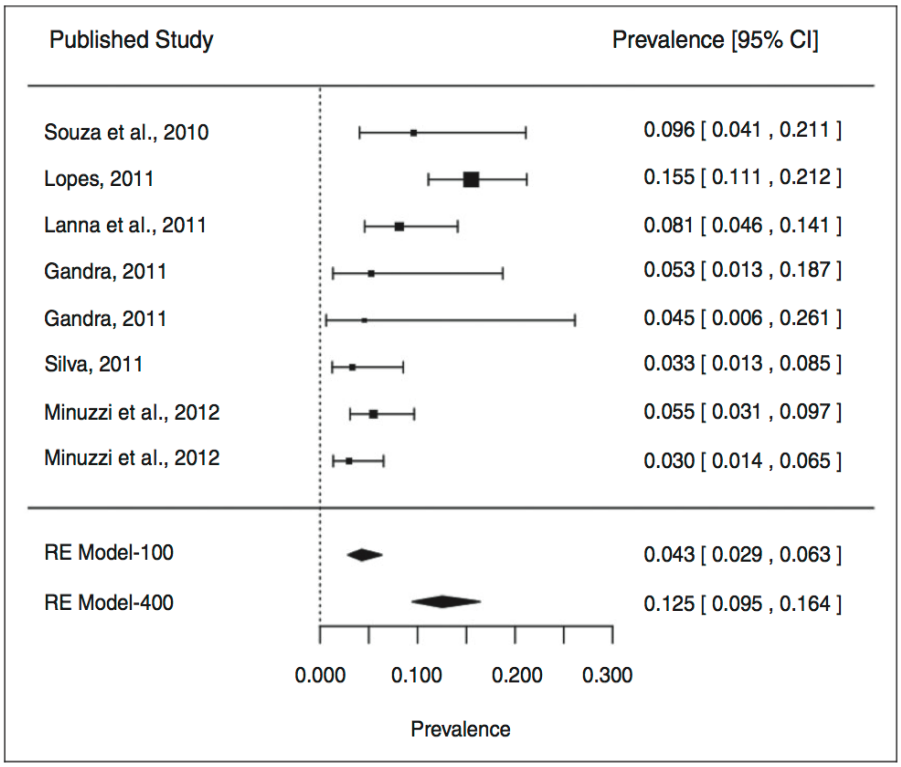

Figure 2. Forest plot of the prevalence of *Salmonella* spp. in Brazilian beef hides after bleeding. Mean prevalence values based on swabbed areas of 100 and 400 cm^2 estimated by a random-effects (RE) meta-analysis models are shown.

Table 3. Means, standard deviations and confidence intervals of the model's outputs for the incidence of *Salmonella* spp. on Brazilian beef carcasses at the different processing stages

Prevalence on hide/carcass	Mean (SD)	95% CI
After bleeding (input)	0.125 (0.011)	0.095–0.164
After dehiding	0.048 (0.014)	0.027–0.080
After evisceration/splitting	0.086 (0.039)	0.035–0.179
After rinsing	0.075 (0.042)	0.024–0.182
After chilling	0.036 (0.021)	0.011–0.091
After boning	0.061 (0.045)	0.014–0.177

SD: standard deviation; CI: confidence levels.

Narvaez-Bravo et al. (2013). Following carcass evisceration and splitting, the model predicted a significantly higher *Salmonella* occurrence of 8.6% (95% CI: 3.5–17.9% in Table 3), which was expected as there is a positive correlation between the presence of *Salmonella* in the intestinal faeces from asymptomatic animal carriers and subsequent contamination of carcasses. Through risk factor analysis, Narvaez-Bravo et al. (2013) found that *Salmonella* carrier animals had eight times higher likelihood of testing positive on hides, three times higher likelihood to test positive on pre-eviscerated carcasses, and two times greater likelihood to test positive on carcasses post-evisceration. The simulation model estimated that the carcass washing step, before carcass entry into the cooler, has little effect on the *Salmonella* occurrence within slaughter groups. Although, on average, there is apparently a numerical reduction from 8.6% to 7.5%, a comparison between the confidence intervals for *Salmonella* on eviscerated carcasses (95% CI: 3.5–17.9%) and washed carcasses (95% CI: 2.4–18.2% in Table 3) evidences that, taking groups of carcasses, the washing operation could either increase or decrease the contamination. Buncic and Sofos (2012) explained that carcass washing per se could even further spread the microbial contamination to uncontaminated areas of the carcass if there was not previous removal of the contaminated area by knife trimming. On the other hand, Koohmaraie et al. (2005) explained that further reductions in contamination can be attained by a series of carcass washing steps such as pre-evisceration wash of hot water or organic acid, rinsing with heated water or steam after splitting and a heated organic acid rinse before carcasses enter the final sales cooler. The model's estimate after rinsing was again in reasonable agreement with the *Salmonella* occurrence rates reported for Brazil by Lanna et al. (2011), Lopes (2011), Minuzzi et al. (2012) and Souza et al. (2010), who recovered, respectively,

3.7% (5/135), 3.0% (6/200), 2.0% (4/200) and 1.9% (1/52) of *Salmonella*-positive carcass swabs after final rinsing. Nonetheless, the prevalence of *Salmonella* in pre-chill beef carcasses produced in Brazil, as estimated by the model (7.5%), is higher than the mean occurrence from Spanish (3.8%) and Italian (3.2%) slaughterhouses (EFSA, 2012), which were the countries reporting the highest contamination of *Salmonella* in beef in the European Union. The average occurrence of *Salmonella* on pre-chill beef carcasses from European slaughterhouses is quite low at 0.2% (EFSA, 2012).

The model suggested that the process of cooling and chilling has a significant effect on the recovery of *Salmonella* cells from beef carcasses, reducing the occurrence approximately by half on average (3.6%; 95% CI: 1.1–9.1%; Table 3). At this stage, the model's output could not be assessed with the results of Brazilian studies given the absence of *Salmonella* surveys on post-chill beef carcasses. However, our model's output is comparable to the results of a survey from a Mexican slaughterhouse where *Salmonella* was recovered in 6% of the beef carcasses sampled after 24 h of dry chilling (Narvaez-Bravo et al., 2010). Slightly lower occurrences were surveyed from two American studies involving very large surveys at beef abattoirs: Rose et al. (2002) and Ruby et al. (2007) found that 3% (125/4042) and 1% (53/5355) of post-chill beef carcasses tested positive for *Salmonella* spp. The simulation model estimated that the prevalence of *Salmonella* in Brazilian beef cuts is on average 6.1%. Figure 3 shows the histogram of the model's output representing the uncertainty around that average value (6.1%). Superimposed is the best-fit Gamma distribution, Gamma (2.0990, 0.0271). For validation, this final output was compared to a pool of surveys' data extracted from Almeida et al. (2010), Colvara et al. (2007) and Xavier and Joele (2004) who tested a total of 103 deboned beef cuts from Brazilian commercial beef processing plants using an excision microbiological protocol. Although our model's output implicitly expresses the prevalence based on positive swabs while the validation results express the occurrence in terms of positive excised meat, both outcomes are still comparable. The pool of the validation surveys' data (mean 4.9%; 95% CI: 1.8–11.5%) was well within the 95% confidence interval (1.4–17.7% in Table 3) of the model's final output (Figure 3). The distribution shape found by simulation, considerably wide and skewed, may reflect the substantial variation in the prevalence on *Salmonella* among production batches and among slaughterhouses. If the model's input variable had not

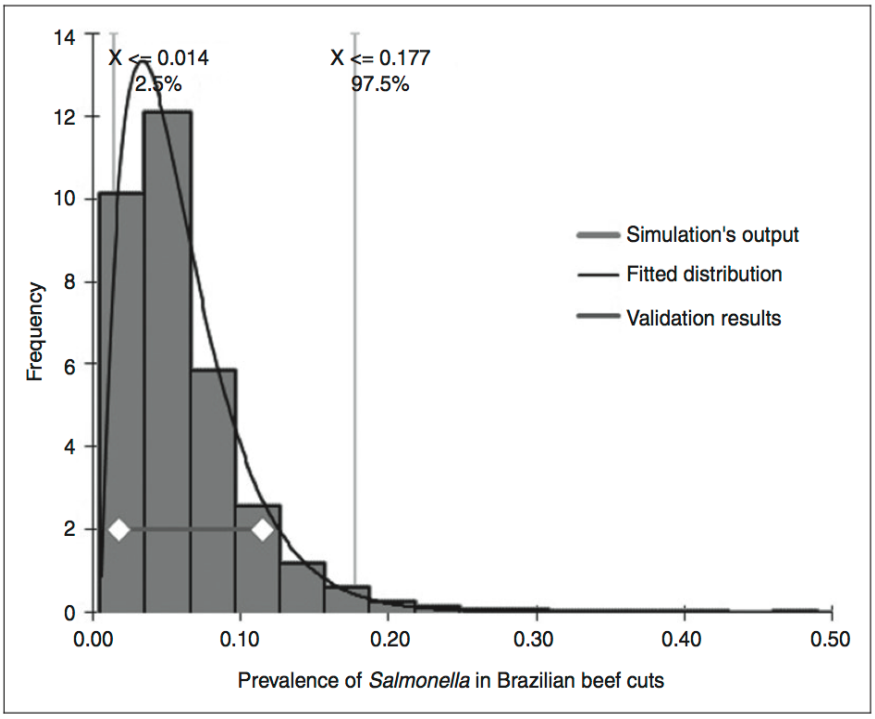

Figure 3. Output distribution of the prevalence of *Salmonella* spp. in beef cuts produced in Brazil showing 95% confidence interval as estimated by the stage-by-stage simulation model. The 95% confidence interval of the validation results is superimposed.

been corrected for the difference in swabbed areas, the output of the simulation model would have been 3.6% (95% CI: 0.8–10.6%) of *Salmonella*-positive Brazilian beef cuts, suggesting that the model would have slightly underestimated the validation surveys' data.

The sensitivity analysis performed on the model's output warned that the increase in *Salmonella* prevalence that can be attained during boning and evisceration may have a stronger influence on the final prevalence on beef cuts than the reduction in contamination that can occur during dehiding, rinsing and chilling (Figure 4). Depending upon how rinsing is performed, it may not have a strong impact ($r = -0.132$) on the reduction of *Salmonella* prevalence on beef carcasses. The high correlation between the model's output with the contamination factor due to boning ($r = 0.423$) and the contamination factor due to evisceration and splitting ($r = 0.392$) reinforces the notion that good manufacture and good hygiene practices should at all times be observed during such critical stages. Reassuringly, the initial prevalence of *Salmonella* on beef hides (model's input) seemingly does not determine the contamination in the beef cuts at the end of processing (low $r = 0.112$). This implies that, if contaminated bovine hides entered the abattoir, and yet good practices were observed throughout slaughter, the occurrence of *Salmonella* in the final product can still be lowered during dehiding, rinsing and chilling. Koohmaraie et al. (2005) sustained that, generally, the prevalence of pathogens on hides is much higher than the carcass contamination rates; and that, although such carcass contamination rate is the highest immediately after hide removal, it consistently declines during processing.

Because of data gaps, this exposure assessment model did not assess quantitatively the effects of intervention strategies to bring down the prevalence of *Salmonella* in Brazilian-produced beef. Nonetheless, it highlighted that such prevalence is relatively high (in comparison to estimates for America and the EU) and action measures should be taken. Intervention strategies such as hide washing after exsanguination, pre-evisceration carcass washing, pre-evisceration organic

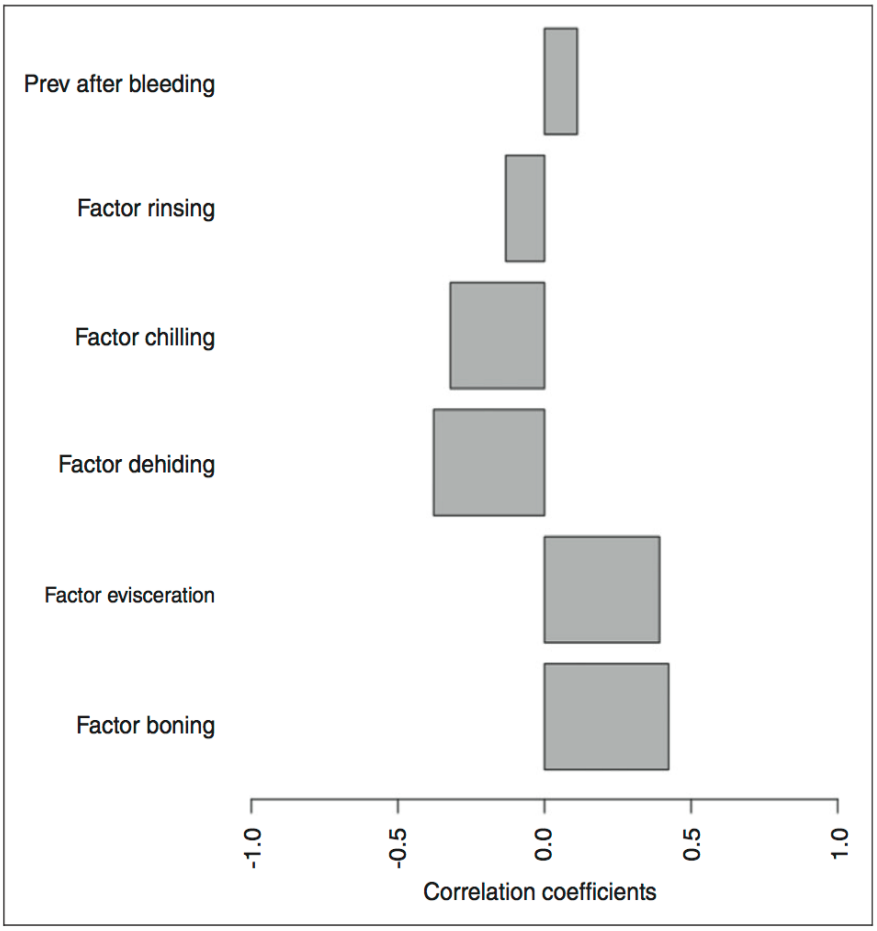

Figure 4. Sensitivity of the prevalence of *Salmonella* in beef cuts to the total uncertainty of individual variables.

acid solution rinsing, hot water carcass washing, post-evisceration final carcass washing or post-evisceration organic acid solution rinsing should be considered (Koohmaraie et al., 2005). Other data gaps encountered were the occurrence of *Salmonella* on beef hides before stunning and bleeding, and the limited information to model the effect of jointing on the occurrence of *Salmonella* on beef as well as the cross-contamination in processing plants. Furthermore, because of data gaps, the model was not developed for any specific *Salmonella* serovar, although the most common in Brazilian beef has been found to be Infantis, Enteritidis, Newport, Saintpaul and Anatum (Lopes, 2011; Silva, 2011). The exposure assessment model was developed considering only *Salmonella* occurrence values due to the limited information available on concentrations or most probable number values in Brazilian beef. Nevertheless, the model met the objective of synthesising all available research to date on *Salmonella* during processing of beef in Brazil. Finally, given the good agreement between the model predictions of *Salmonella* prevalence and the outcomes from Brazilian surveys along the different processing stages, it can be said that this model, integrating input distributions justified by published studies, approximates fairly well the contamination of *Salmonella* in Brazilian beef abattoirs.

CONCLUSIONS

Increasing our understanding of the variation in *Salmonella* contamination present on the beef hides and carcasses during processing is an important prerequisite for risk analysis and process control assessment. Our exposure assessment model, which integrated all up-to-date knowledge on *Salmonella* in Brazilian beef along processing, predicts a *Salmonella* prevalence of 6.1% (95% CI: 1.4–17.7%) in beef produced at Brazilian processing plants, which was in close agreement with the occurrence estimates from surveys at commercial establishments (4.9%; 95% CI: 1.8–11.5%). The model also underscored that the stages of evisceration/splitting and boning are highly critical, as they may largely amplify the contamination of *Salmonella* spp. Although the hides of animals carrying *Salmonella* constitute the major source of contamination, the spread of this pathogen during the process can be still minimised by the correct implementation of food safety programs. To this respect, the model demonstrated that the load of *Salmonella* spp. that initially enters the plant with the live animals does not determine the extent of contamination in the final product. When hygienic slaughter procedures and sanitary programs are working properly, the initial *Salmonella* load can still be decreased at the stages of dehiding, rinsing and chilling.

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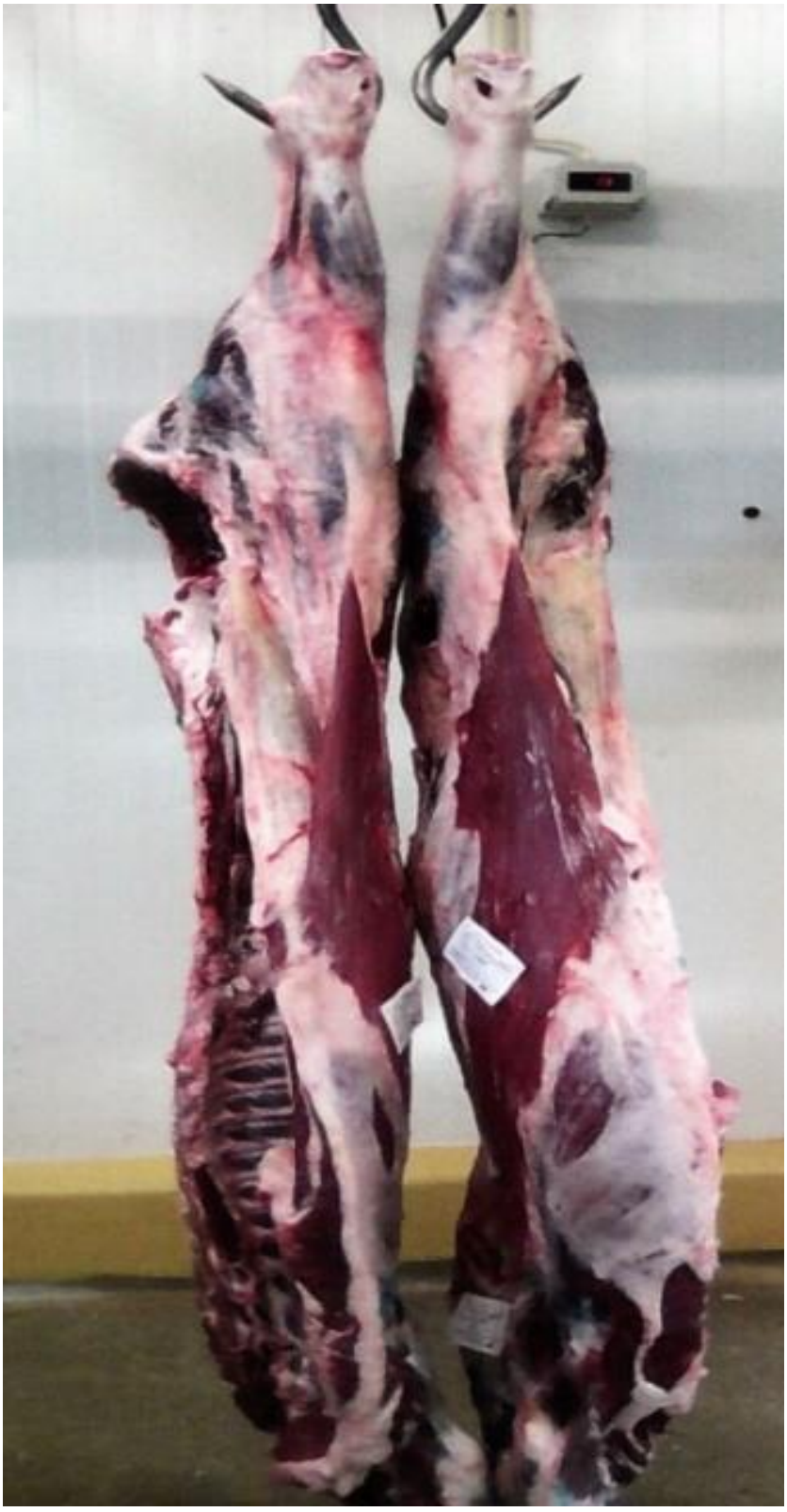
3. Carcass and Meat Quality



(Source: Cristina Xavier)

3.1. Early *post-mortem* classification of beef carcasses according to meat quality using animal/carcass characteristics and pH/temperature decline descriptors

C. Xavier, U. Gonzales-Barron, A. Müller and V. Cadavez

In preparation for submission (2017)

ABSTRACT

During beef carcass chilling, the eating quality of meat can be severely affected by either hot- or cold-shortening. With basis on previous knowledge that meat of optimal tenderness can be produced when *rigor mortis* (pH=6.0) is attained when carcass temperature falls between 12-35ºC, the objective of this study was to predict meat quality from modelled pH/temperature decay descriptors and informative animal/carcass characteristics. Temperature and pH from a total of 126 beef carcasses were logged during 24 h *post-mortem*, and subsequently modelled by exponential decay equations that estimated temperature (k_T) and pH (k_{pH}) decay rates. In addition, a number of pH/temperature decay descriptors were estimated from the fitted models. From linear models adjusted to each of these descriptors, it was found that, generally, hot carcass weight, age, breed, gender, age class (calf, vealer, yearling), fat cover, conformation and transport and lairage time had significant influence on pH/temperature decay. Thus, bringing together the orthogonal variables k_T and k_{pH}, and the aforementioned animal/carcass characteristics as linear predictors of discriminant functions, a classification analysis was performed.

INTRODUCTION

The inconsistency in the eating-quality characteristics of meat is one of the problems faced by the meat industry worldwide. Moreover, the importance of providing end-users with a food product of consistent quality has never been greater (Simmons *et al.,* 2006). Thus, a top priority factor in the success of meat industry relies on the ability to deliver specialities that satisfy the consumer's taste requirements (Cortez *et al.,* 2006). For the consumers, the most important beef sensory attribute, in their individual assessments of overall eating satisfaction, is tenderness (Ferguson, 2004). Consumers are even willing to pay more for beef of higher or guaranteed tenderness. However, meat tenderness is a complex trait that is influenced by a variety of factors, many of which can be managed systematically to reduce the incidence of tenderness problems in the final product (Platter *et al.,* 2003). Sources of tenderness variation in beef may be attributed to carcass factors such as marbling, muscle type, effect of pH and temperature, breed and genetic differences, and environmental factors such as age, sex, time on feed and *ante-mortem* stress.

The muscle shortening that occurs during *rigor mortis* causing meat to toughen has led to the realisation that *post-mortem* treatments may outweigh live-animal factors, such as breed, age and carcass weight in determining meat quality. Diverse *post-mortem* treatments have been applied to improve tenderness; namely, ageing, electrical stimulation, *Tenderstretch*

carcass suspension from the obturator foramen of the pelvis, severance of the skeleton, high pressure processing, mechanical tenderisation, blade tenderisation, marinade or injection with organic acids, salt, phosphates, calcium chloride and/or ammonium hydroxide (Smith *et al.,* 2008). Nonetheless, the two main determinants of *post-mortem* processing outcomes are the rates of pH and temperature decline (Simmons *et al.*, 2006). Temperature and pH control is extremely important since it affects meat tenderness, and varies with cooling rate and the stress level of the animal before harvesting (Bianchini *et al.,* 2007).

Muscle pH and temperature decline continuously interact during rigor development to affect both muscle contracture and proteolytic enzyme activity (Tornberg, 1996). During the first 24h *post-mortem*, the rate of temperature decline affects the biochemical and structural changes during the conversion of muscle to meat. High temperature accelerates the pH decline in muscle and during this period, the rate of decrease in pH and the ultimate pH of meat are highly variable (Kahraman *et al.,* 2012). The combination of a very rapid pH decline with a slow chilling regime causes heat shortening, which is an increase in toughness due to sooner exhaustion of μ-calpain at high carcass temperatures, leaving less potential for ageing. On the other end, the phenomenon of cold-shortening emerges if the pH decline is too slow, remaining high while the temperature falls (Roça, 2000). Cold-shortened carcasses produce tougher meat than hot-shortened ones do.

Hwang et al. (2003) showed that, in order to minimise cold-shortening – to enhance tenderisation, the muscle temperature should not be lower than 11°C before muscle pH reaches 6.1–6.3. When the process of glycolysis develops slowly, the initial pH (right after slaughter), which is about 7.0, goes down to 6.4–6.8 after 5 hours, and subsequently to 5.5 – 5.9 after 24 hours (Roça, 2000). If, due to a deficiency of glycogen, the final pH (after 24 hours) remains high, above 6.2, the muscle turns into DFD (dark, firm, dry) meat so the limit value for emergence of DFD meat is pH 6.0 (Bianchini *et al.,* 2007). Because of the closeness of the pH threshold of the activation of calpain to the pH at rigor, it has been proposed to take the rigor temperature, Temp(pH=6.0)=$Temp_{pH6.0}$, as an indicator for meat tenderness (Thompson, 2002). This has led to the concept of pH/temperature “window” to be used as a specification to describe the relationship between carcass pH and temperature from slaughter to when the ultimate pH is reached (Thompson, 2002; Ibarburu *et al.,* 2007). The window requires the carcass pH to be greater than 6.0 while the carcass temperature is above 35°C and below 6.0 before the temperature falls below 12°C. If the rate of pH temperature decline does not fall through this ideal window, the carcass tenderness is compromised, either by hot- or cold-shortening (Figure 3.1.1). Temperature and pH decay

rates are not only good predictors of meat quality, but also of colour and drip loss of meat (Ibarburu *et al.,* 2007).

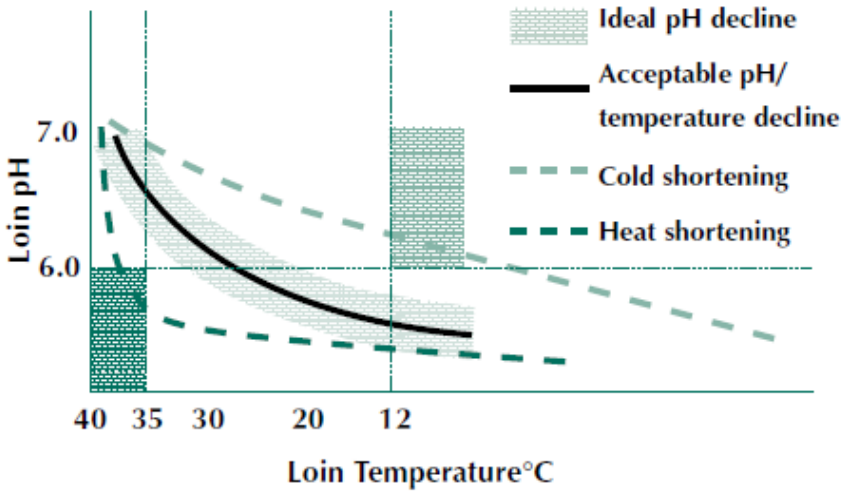


Figure 3.1.1. "Ideal window" describing the relationship between carcass pH and temperature (Extracted from Meat Standards Australia, 2011)

Thus, the objective of this study is three-fold. The first objective is to model the decrease in temperature and pH during chilling of beef carcasses early *post-mortem* so that pH and temperature decline rates can be accurately estimated. The second objective is to evaluate the extent of influence of live-animal/carcass characteristics (i.e., sex, weight, age, breed, class, fat cover, conformation, and transport and lairage time) on the pH and temperature decline rates; or, say otherwise, if the muscle shortening occurring during *rigor mortis* outweighs the effects of live-animal factors in determining meat quality. Once the significant live-animal/carcass characteristics affecting pH and temperature decline are identified, linear discriminant analysis that classifies beef carcasses quality (i.e., tenderness) into optimal quality and cold-shortened is to be developed taking into account the "ideal window" rule (Figure 3.1.1).

METHODOLOGY

Beef animals

In this study, a total of 126 beef animals (74 cross-breed and 52 Mirandesa breed), 85 males and 41 females, slaughtered in a local abattoir were sampled. The animals had an average age of 10.1 ± 2.32 months when slaughtered at one abattoir located in the Northeast of

Portugal. The animals were transported by truck to the abattoir; and, at arrival, they were kept in individual stalls until slaughtering. They were not fed but did receive water *ad libitum*. After electric stunning, animals were slaughtered and dressed sequentially. Carcasses had an average hot carcass weight of 209.7 ± 65.60 kg. For each of the animals/carcasses, the following live-animal/carcass characteristics were annotated: sex, age, breed, transport time, lairage time, hot carcass weight, age class (calf, vealer or yearling), the SEUROP class from the European beef carcass classification scheme for conformation and degree of fat cover of the carcass (1, 2, 3 or 4).

Temperature and pH recording

Approximately two hours after slaughter, pH and temperature were recorded at intervals of 10 min during 24 h of carcass chilling, in *longissimus thoracis* muscle at the level of the 4th rib. The pH and temperature measurements were made using a weather-resistant wireless-transmitter CRISON pH probe (Crison Instruments SA, Barcelona, Spain) and wireless-transmitter OMEGA temperature probe Pt 100 (Omega Engineering Limited, Manchester, United Kingdom) connected to an OMEGA UWTC-REC1 wireless channel receiver/host (Omega Engineering Limited, Manchester, United Kingdom).

Statistical modelling

i) Fitting exponential decay models

The experimental curves of pH and temperature decline *post-mortem* (p.m.) were modelled as a function of time using the parameterisation of the exponential decay function proposed earlier by Hwang and Thompson (2001). For modelling pH measured as a function of time pH(t), the three-parameter decay function was defined as,

$$\mathrm{pH(t)} = \mathrm{pH0} + (\mathrm{pH0} - \mathrm{pH\infty})\exp(-\mathrm{kpHt}) \qquad (1)$$

where pH_∞ is the final pH; pH_0 is the initial pH; k_{pH} is the exponential constant of pH decay; and t is the time in hours after slaughtering. Time zero was set to the time of slaughter. The three-parameter model for temperature as a function of time (T(t)) was defined by the same equation,

$$\mathrm{T(t)} = \mathrm{T0} + (\mathrm{T0} - \mathrm{T\infty})\exp(-\mathrm{kTempt}) \qquad (2)$$

where T_∞ is the final temperature (ºC); T_0 is the initial temperature (ºC); and k_{Temp} (h^{-1}) is the exponential constant of temperature decay. Equations (1) and (2) were fitted to each of the experimental decay curves originated from the 126 beef muscles. Models' adequacy was assessed by examining normality of residuals and heteroscedasticity. Using the model parameters (pH_0, pH_∞, k_{pH}, T_0, T_∞ and k_{Temp}), the following pH/temperature decay descriptors were computed for each of the curves: the pH at 1.5 h ($pH_{1.5}$), at 3.0 h ($pH_{3.0}$), at 4.5 h ($pH_{4.5}$), at 6.0 h ($pH_{6.0}$) and at 24.0 h (pH_{24}); the temperature at 1.5 h ($Temp_{1.5}$), at 3.0 h ($Temp_{3.0}$), at 4.5 h ($Temp_{4.5}$), and at 6.0 h ($Temp_{6.0}$); the time when pH reached 6.0 ($t_{pH6.0}$), and the temperature at which pH reached 6.0 ($Temp_{pH6}$). Thus, these fitted descriptors were available for every carcass.

ii) Effect of live-animal/carcass characteristics on pH/temperature decay curve descriptors

In order to evaluate whether live-animal/carcass characteristics affect the pace at which pH and temperature decline in a beef carcass early *p.m*., analyses of variance (ANOVA) were conducted separately on each of the temperature/pH decay curve descriptors (namely, $pH_{1.5,}$ $pH_{3.0}$, $pH_{4.5}$, $pH_{6.0}$, pH_{24}, $Temp_{1.5}$, $Temp_{3.0}$, $Temp_{4.5}$, $t_{pH6.0}$, $Temp_{pH6}$, k_{pH} and k_{Temp}) as response variables with live-animal/carcass characteristics as explanatory variables. The animal/carcass characteristics considered as regressors were: sex, age, breed, hot carcass weight (HCW), transport time ($t_{Transport}$), lairage time ($t_{Lairage}$) and animal class (Class). Whenever a categorical factor (sex, breed or class) was significant in the ANOVA ($Pr(F)<0.10$), least-squares means were computed and contrasted among the factor levels. Because information on SEUROP classification and fat cover was not available for all 126 carcasses, but for 95 carcasses, a second run of ANOVAs was carried out on the data subset considering only SEUROP and Fat as factors for all of the temperature/pH decay curve descriptors. As with the first ANOVA, least-squares means for SEUROP and Fat levels were computed and contrasted when these factors turned out to be significant ($\alpha=0.10$).

iii) Carcass classification by quality compliance

Taking into account the ideal window rule, an additional class variable named 'Compliance' was created in the dataset to assign carcasses to one of two classes (i.e., cold-shortened "CS" and optimal quality "OQ") which were quality categories known *a-priori* from the experimental data. A carcass was classified as "OQ" if $Temp_{pH6.0}$ was between 12º and 35ºC, and as "CS" if $Temp_{pH6.0}$ was lower than 12. In the 126 carcass dataset, since no carcass underwent hot-shortening (i.e., $Temp_{pH6.0}$ was never higher than 35ºC), a third class "hot-shortened" could not be included. Thus, the problem reduced to predicting meat quality

(either optimal or cold-shortened) from selected animal/carcass characteristics and pH/temperature decay descriptors.

As a first step, a principal component analysis was performed to the full data set in order to ascertain the variables (i.e., animal/carcass characteristics and pH/temperature decay descriptors) having the greatest capacity to discriminate between quality compliance classes. Once these variables were identified, a series of modelling functions for classification were tested and compared; namely: linear discriminant analysis (LDA), robust linear discriminant analysis (RLDA), k-nearest neighbours (kNN), support vector machines (SVM) and nearest shrunken centroids (NSC). The comparison was based on classification efficacy, and was accomplished by two different procedures:

a) Each of the classification models was trained using the entire data set. Distributions of model performance measures (accuracy and kappa statistics) were obtained by k-fold cross-validation resampling (k=6) with 100 iterations. All the classification functions were set to select the tuning parameters with the largest value of the mean kappa statistic computed from the held-out samples.
b) Each of the classification models was trained using ~70% of the data (89 samples) and was later used to generate predictions for new samples (remaining ~30% of the data or 37 samples). Thus, a ~70/30% stratified random split of the data was first created. Models were then trained as described above, and tested using the separate data set to characterise their classification efficacy. Models' performance was characterised by obtaining confusion matrix, accuracy and kappa statistics. For further details on classification training and algorithms, refer to Kuhn (2008).

Total accuracy is the sum of true positives and true negatives divided by the total number of items. The kappa statistic measures agreement relative to what would be expected by chance; hence a value of 1 indicates perfect agreement.

While exponential models and linear models were fitted using the *nlme* and *MASS* packages, respectively; principal component analysis was conducted using the *FactoMineR* package, and the classification training and testing using the *caret* package; all of them implemented in the software R (R Core Development Team, 2011). In addition, graphs were produced by the *factoextra* package.

RESULTS AND DISCUSSION

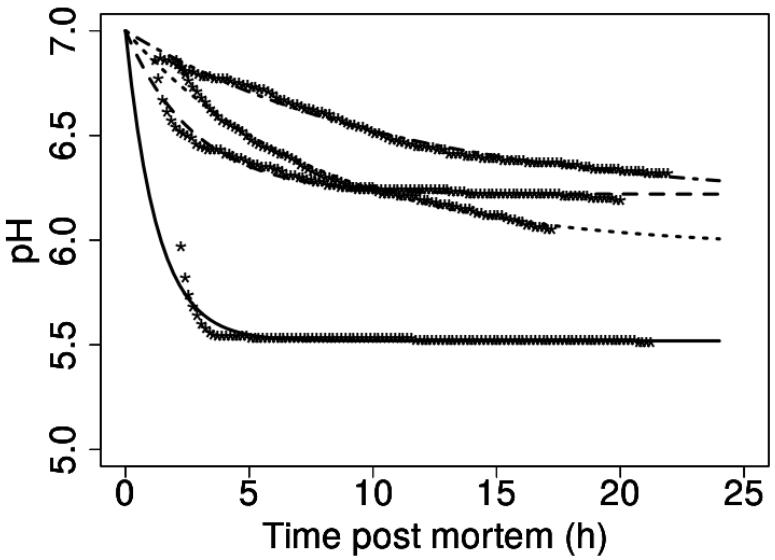
Data description and modelled pH and temperature decay

As beef carcasses in the abattoir were chosen randomly, the sample sizes across the animal/carcass characteristics' levels were different. Moreover, for some factors such as SEUROP and fat cover, data were not available for all classes: no beef animals with conformation "S" or "E" and with fat cover "1" or "4" were slaughtered in the abattoir (Table 3.1.1). Similarly, in terms of meat quality compliance, no carcass underwent hot-shortening, so the compliance classes that could be derived were "cold-shortened" and "optimal quality". From the data set, ~39% of the monitored carcasses did not undergo an optimal pH/temperature decline, but had either a slow pH fall or a rapid temperature drop (Table 3.1.1). The data description statistics for age and HCW by factor indicates that Mirandesa-breed animals were younger (9 months) and not as heavy (152 kg) as cross-breed ones (10.5 months and 238 kg; Table 3.1.1). As expected, heavier (232 kg) and older animals (11 months) were associated to greater fat cover (class "3"). While heavier carcasses (221 kg) belonged to male animals, the data also showed that higher SEUROP conformation classes were assigned to older and heavier animals. Thus, the live-animal/carcass characteristics measured and extracted from the abattoir's records were highly interrelated. Such interrelationships were taken into account when developing the linear and multivariate models of Subsections ii) and iii).

All of the pH and temperature experimental curves obtained from the 126 carcasses could be closely depicted by the chosen exponential decay models. There were no convergence problems and residuals from each model could be approximated to normal distributions. Even the experimental pH decay curves from beef muscles that did not reach the *rigor mortis* pH (6.0) within the 24-hour monitored period, which amounted to 36 out of 126 curves, were well adjusted by the model. For illustration, three of these curves are shown in Figure 3.1.2 (top). Notice also that both pH_0 and T_0 were fixed, respectively, at 7.0 (Figure 3.1.2, top) and 39.0ºC (Figure 3.1.2, bottom) at time zero, which represented the time of slaughter in the models. pH_0 (Equation (1)) and T_0 (Equation (2)) were not estimated as models' parameters because many experimental decay curves produced unrealistic estimates of pH_0 (>>7.0) and T_0 (>>40ºC) which in turn led to poorer goodness-of-fit measures. Generally, fitting the exponential functions with known pH_0 and T_0 provided a useful means of describing pH and temperature changes up to 24 h *post-mortem* (Figure 3.1.2).

Table 3.1.1. Mean slaughter age and hot carcass weight (standard deviation in brackets) by live-animal/carcass characteristics

Characteristic	Level	Sample size	Age (months)	Hot carcass weight (kg)
Gender	Female	41	9.75 (2.12)	165.0 (46.58)
	Male	85	9.95 (2.01)	221.0 (62.77)
Breed	Cross-breed	74	10.47 (2.10)	238.3 (56.02)
	Mirandesa	52	9.06 (1.63)	152.1 (30.86)
Age class	Calf	30	7.78 (0.42)	159.7 (42.29)
	Vealer	74	10.27 (0.93)	215.2 (57.54)
	Yearling	22	14.21 (1.42)	265.6 (67.32)
Fat class	2	43	10.28 (1.76)	210.0 (64.09)
	3	41	11.04 (2.03)	231.8 (57.75)
SEUROP	U	8	11.38 (1.51)	309.6 (32.87)
	R	31	11.09 (2.18)	255.8 (41.02)
	O	33	10.57 (1.95)	204.4 (53.17)
	P	23	9.65 (1.33)	161.2 (27.27)
Compliance	CS	49	9.83 (1.70)	190.2 (52.95)
	OQ	77	9.94 (2.32)	210.7 (68.58)



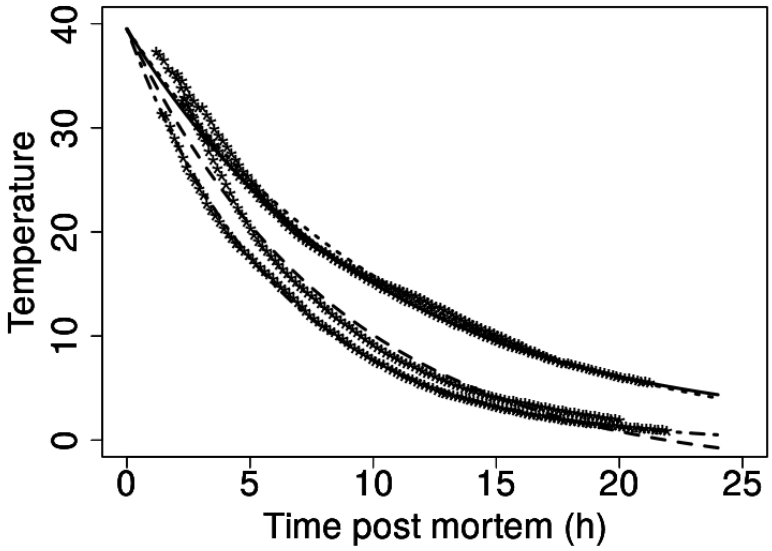


Figure 3.1.2. pH (top) and temperature (bottom) decline experimental curves from four sampled carcasses showing fitted exponential models in dashed lines

Descriptive statistics of the pH and temperature decay descriptors (models' fitted parameters and estimated values) are compiled in Table 3.1.2 Sampled muscles' pH decreased steadily from ~7.0 to median values of 6.53, 6.25, 6.07 and 5.95, at the corresponding median temperatures of 33.1, 28.0, 23.7 and 20.1ºC after 1.5, 3.0, 4.5 and 6 hours *p.m*, respectively (Table 3.1.2). In the sampled carcasses, on average, monitored muscles attained the pH of 6.0 after 6 hours (see $pH_{6.0}$). Nonetheless, wide ranges in muscle pH and temperature values were obtained at each sampling time, as shown in Figure 3.1.2 and Table 3.1.2. For both the pH and temperature estimates at different sampling times, the variation (i.e., range) between carcasses increased as time elapsed (Table 3.1.2). As a consequence, the estimated time to reach *rigor mortis* was highly variable, ranging from 1.52 to 20.2 hours; although 36 carcasses did not attain pH of 6.0 by the end of the 24 h recorded period). Similarly, the rigor temperature ($Temp_{pH6.0}$) had a wide range between 1.96 and 35ºC. Contrasting this finding with the ideal window rule (viz. muscle should reach rigor while in the temperature range 12-35ºC), it can be deduced that, whereas both phenomena hot- and cold-shortening are likely to occur in the beef meat produced in the commercial abattoir surveyed, cold-shortening takes place at a much higher frequency. Only a proportion of ~61% of the sampled carcasses exhibited an optimal pH/temperature decay for becoming meat of good eating quality. From our data, the ultimate pH (pH_{24}) median was 5.74, a value very close to the maximum final pH ($5.20 < pH_{24} < 5.70$) recommended by Meat Standards Australia for meat of sufficient juiciness. Moreover, a high proportion (~39%) of the sampled muscles reached

an ultimate pH above 6.0 (see maximum value of pH_{24}=6.51 in Table 3.1.2), implying that these carcasses would be cold-shortened and tough, as for pH_{24}>6.0, the drip loss is constant and minimal (Hamoen *et al.,* 2013). Ultimate pH of meat is a major quality determinant since it has been demonstrated to explain 79% of the variation in meat colour, 57% of the variation in drip loss, and 77% of the variation in purge loss (Bidner *et al.,* 2004).

Table 3.1.2. Mean, median and range of pH and temperature decline descriptors

Estimated values and model parameters	Mean	Median	Min	Max
$pH_{1.5}$	6.52	6.53	6.01	6.93
$pH_{3.0}$	6.24	6.25	5.62	6.87
$pH_{4.5}$	6.08	6.07	5.45	6.81
$pH_{6.0}$	6.00	5.95	5.38	6.76
pH_{24}	5.78	5.74	5.30	6.51
$Temp_{1.5}$ (ºC)	32.9	33.1	27.2	36.4
$Temp_{3.0}$ (ºC)	27.8	28.0	19.2	33.9
$Temp_{4.5}$ (ºC)	23.7	23.7	13.8	31.5
$Temp_{6.0}$ (ºC)	20.1	20.1	10.1	29.3
k_{pH} (h^{-1})	0.335	0.344	0.079	0.697
k_{Temp}(ºC/h)	0.113	0.101	0.022	0.256
$Time_{pH6.0}$ (h)	4.92	3.85	1.52	20.2
$Temp_{pH6.0}$ (ºC)	24.4	25.7	1.96	35.0

The considerable carcass-to-carcass variation in pH and temperature decay under commercial conditions can be also appreciated from the wide range of the fitted slopes k_{pH} (0.079 – 0.697 1/h) and k_{Temp} (0.022 – 0.256 ºC/h; Table 3.1.2). All the studied live-animal/carcass characteristics were found to affect the rate of temperature decline (k_{Temp}) while for the pH decay rate (k_{pH}), gender, breed and fat class had no discernible effect (Table 3.1.3). In the sampled beef muscles, no correlation was found between pH (k_{pH}) and temperature (k_T) decay rates (r=0.10; Table 3.1.4), which corroborated earlier results in electrically-stimulated beef carcasses (Hwang and Thompson, 2001). The fact that these decay rates are randomly distributed and independent (Figure 3.1.3) is advantageous from a statistical modelling viewpoint since they can be used as orthogonal (i.e., independent) variables in the development of a multivariate algorithm for carcass quality classification.

Table 3.1.3. Mean and standard deviation (in brakets) of fitted pH and temperature decay rates (i.e., k_{pH}, k_T) by live-animal/carcass characteristics

Characteristic	Level	k_{pH}	k_T
Gender	Female	0.335 (0.147)	0.124 (0.050)
	Male	0.334 (0.135)	0.108 (0.054)
Breed	Cross-breed	0.339 (0.142)	0.085 (0.035)
	Mirandesa	0.328 (0.134)	0.152 (0.050)
Age Class	Calf	0.367 (0.127)	0.141 (0.050)
	Vealer	0.308 (0.144)	0.105 (0.049)
	Yearling	0.371 (0.114)	0.072 (0.041)
Fat Class	2	0.322 (0.158)	0.117 (0.051)
	3	0.323 (0.129)	0.084 (0.037)
SEUROP	U	0.453 (0.175)	0.058 (0.021)
	R	0.306 (0.132)	0.074 (0.029)
	O	0.317 (0.128)	0.106 (0.047)
	P	0.298 (0.151)	0.145 (0.034)
Compliance	CS	0.241 (0.120)	0.121 (0.048)
	OQ	0.394 (0.114)	0.108 (0.056)

From the correlation matrix analysis (Table 3.1.4), it was deduced that $pH_{3.0}$ was a descriptor suitable for inclusion in the classification analysis because it contains information of both rigor time and rigor temperature (correlation of 0.86 with $Time_{pH6.0}$, and -0.83 with $Temp_{pH6.0}$); the ultimate pH of the carcass (correlation of 0.83 with pH_{24}); and the pH decay rate (correlation of -0.84 with k_{pH}). Thus, $pH_{3.0}$ descriptor represents a measurement that, although taken earlier during chilling, can predict with good accuracy the remaining pH decline trend and the ultimate pH. In a similar fashion, the muscle temperature after 3 hours *p.m.* ($Temp_{3.0}$) is a suitable descriptor for inclusion in the classification analysis, because, despite taken very early during carcass monitoring, it contains most of the information of the temperature decline rate (correlation of -0.98 with k_{Temp}).

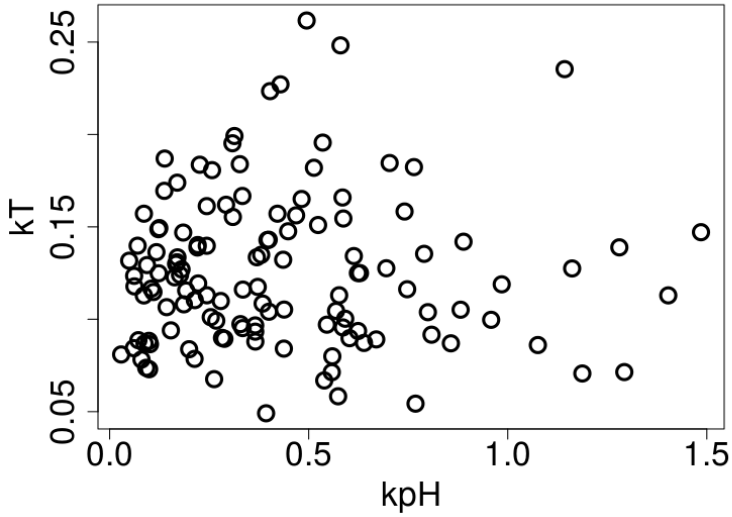


Figure 3.1.3. Lack of association between pH (k_{pH}) and temperature (k_T) exponential decay rates

Table 3.1.4. Correlation matrix of selected pH/temperature descriptors and model parameters

	$pH_{3.0}$	$pH_{4.5}$	pH_{24}	$Temp_{3.0}$	$Temp_{4.5}$	k_{pH}	k_{Temp}	$Time_{pH6}$	$Temp_{pH6}$
$pH_{3.0}$	1.00								
$pH_{4.5}$	0.99	1.00							
pH_{24}	**0.83**	0.88	1.00						
$Temp_{3.0}$	0.13	0.14	0.18	1.00					
$Temp_{4.5}$	0.12	0.13	0.17	0.99	1.00				
k_{pH}	**-0.84**	-0.78	-0.42	-0.09	-0.08	1.00			
k_{Temp}	-0.15	-0.16	-0.20	**-0.98**	-0.98	0.10	1.00		
$Time_{pH6.0}$	**0.86**	0.88	0.78	0.15	0.15	-0.68	-0.17	1.00	
$Temp_{pH6.0}$	**-0.83**	-0.84	-0.72	0.32	0.33	0.66	-0.29	**-0.85**	1.00

Effect of live-animal/carcass characteristics on pH/temperature decay curve descriptors

In the analyses of variance for the pH estimates at different time points, $pH_{1.5}$, $pH_{3.0}$, $pH_{4.5}$, $pH_{6.0}$ and pH_{24}, the variables related to animal size – hot carcass weight (HCW), age class and SEUROP – were consistently significant (Table 3.1.5). The inverse relationship between carcass weight and pH at the different times (as indicated by the negative regression coefficients -0.001 in Table 3.1.5) suggests that a heavier beef carcass can be associated to

higher muscle glycogen reserves, which in turn prompts a faster pH drop. The same explanation applies to the SEUROP variables, whereby beef carcasses of increasingly better conformation (from "P" to "U") attained progressively lower pH values. For instance, after 1.5 hours *p.m.* ($pH_{1.5}$), carcasses classified as "U" attained on average a pH of 6.359, whilst "P" carcasses had still a pH of 6.587 (Table 3.1.5). The same trend in least-squares mean estimates for SEUROP classes was observed in the subsequent time points $pH_{3.0}$, $pH_{4.5}$, $pH_{6.0}$ and pH_{24}. Age class displayed also some effect on the estimated pH values: younger animals (calves) consistently reached lower pH values than vealers and yearlings at all time points. For instance, the mean final pH (pH_{24}) of calves was 5.651 which was significantly lower than those of vealers (5.846) and yearlings (5.865; Table 3.1.5). This result was unexpected as younger animals tend to have higher pH values, and more likely to lead to DFD meat due to the fact that they have increased energy requirements, and they are more difficult to adapt to the effects caused by stress (Araújo, 2005). Nonetheless, previous results from Sánchez et al. (1997) and McGeehin et al. (2001) were also faced with higher pH values in older animal carcasses. There is no strong evidence that adult animals have lower pH values, although it is known that the muscle glycogen content and pH drop rate increase with the age of the animal. The buffer capacity of the muscles and their water retention capacity also influence the value of ultimate pH (Araújo, 2005).

As with the pH estimates at the different time points, both SEUROP conformation and age class influenced the pH decay rate (k_{pH}). The conformation class "U" produced higher rates of pH decline (p=0.008), while the vealers as a group presented a slower pH decay (p=0.025; Table 3.1.5). The numerically higher value of k_{pH} (0.371) for the yearlings (12-24 month-aged) can be explained by the fact that this category includes also females, which due to their likely greater size and glycogen reserves are associated to a faster decay in pH. On the contrary, carcasses of poorer conformation corresponding to class "P" exhibited the (at least numerically) slowest decay in pH (k_{pH}=0.298).

Unlike the pH-related descriptors, in addition the variables breed, gender and fat cover affected the temperature-related descriptors. Age (as a continuous variable) and transport time, nonetheless, could not be proven to be relevant in any of the pH or temperature decay descriptors. In the case of temperature decay rate (k_{Temp}), once again animal characteristics related to animal size such as hot carcass weight (p<.0001), breed (p=0.043), gender (p=0.108), fat cover (p<.0001) and SEUROP (p<.0001) greatly determined the rate of temperature decay in the monitored muscle samples (Table 3.1.5). Hot carcass weight (slope estimate -0.001) and fat cover (slope estimate -0.029) were inversely correlated with temperature decay rate because, in smaller animals, heat is more rapidly liberated. Fatter

carcasses (0.080) had a significantly lower temperature decay rate than leaner carcasses (0.109). Such retarding effect of fat on heat transfer became more evident in the linear models for carcass temperature at different time points. Carcasses of fat cover "2" (28.15, 24.02 and 20.25ºC) reached lower temperatures than those of greater fat cover "3" (29.89, 26.16, and 22.88ºC) after 3.0, 4.5 and 6.0 h, respectively, following slaughter. Said otherwise, a fatter carcass requires longer time to reach the lower temperature that a leaner carcass would reach at the same time point. Carcasses of Mirandesa-breed origin cooled down significantly faster (k_{Temp}=0.122) than those of cross-breed (0.102) as they were slaughtered at a younger age (i.e., smaller carcasses). Similarly, as female animals were overall larger than male ones, the mean temperature decay rate in females was significantly lower (0.105). The temperature decay was also slower in carcasses having better conformation (from "P" to "U") and in those from older animals (from "calf" to "yearling").

As with k_{Temp}, carcass weight, breed, gender, class, fat and SEUROP conformation consistently moderated the carcass temperature after 1.5, 3.0, 4.5 and 6.0 hours *p.m*. Since female animals are associated to greater carcass size and fat deposits than males, following the reasoning above, it is not unexpected that recorded muscles samples from females presented higher mean temperatures than the ones from males (notice that least-square mean estimates for $Temp_{1.5}$, $Temp_{3.0}$ $Temp_{4.5}$ and $Temp_{6.0}$ for females are systematically higher than for males; Table 3.1.3). Carcasses of greater conformation and higher age class (i.e., larger carcasses) presented higher mean temperatures at the different time points, $Temp_{1.5}$, $Temp_{3.0}$ $Temp_{4.5}$ and $Temp_{6.0}$. For instance, 3 h after slaughter, calves, vealer and yearlings cooled down up to a mean of 26.45, 27.59 and 29.01ºC, respectively; while the conformation classes "P", "O", "R" and "U" attained consistently-increasing mean temperatures of 26.27, 28.10, 30.14 and 31.57ºC, respectively, in the sampled muscle (Table 3.1.5). Unexpectedly, lairage time was positively associated to the carcass temperature at the different time points (p=0.019–0.090). This could have been rather an effect of the abattoir logistics of slaughtering smaller animals first.

In the linear models for the descriptors related to *rigor mortis*, the only variable that statistically influenced the time to reach pH 6.0 was the fat cover, although at a significance level of 20%. The smaller carcasses of animals aged between 8-12 months, took the longest to attain *rigor mortis* ($Time_{pH6.0}$=5.39 h), because smaller carcasses may be associated to lower glycogen levels, which retards pH drop. Contrarily, carcasses from animals aged 12-24 months took on average a significantly shorter time to reach *rigor mortis* ($Time_{pH6.0}$=4.75 h). The thickness of the subcutaneous fat has an important role, since it acts as a thermal insulator, protecting the muscles of adverse effects of direct exposure to cooling

temperatures. It also allows the muscles to have a slower cooling and optimises the proteolytic enzyme activity, reducing the likelihood of cold-shortening and improving meat tenderness (Zhou *et al.,* 2010). Ferguson et al. (2001) stated that carcasses with fat thickness between 3 and 4 have adequate capacity to achieve the *rigor mortis* and are less likely to suffer the phenomenon of cold-shortening. Fat cover associated with the degree of finish has a very positive impact on the beef quality characteristics (tenderness, colour, flavour and juiciness), and consequently, on the quality profile of the final product (Kinsella *et al.,* 2006).

The carcass temperature at *rigor mortis* ($Temp_{pH6.0}$) was influenced by the hot carcass weight, the age class and the SEUROP conformation. The positive coefficient with carcass weight (0.039; Table 3.1.5) implies that in heavier carcasses, the pH drops faster while temperature is still high, in comparison to lighter carcasses where pH drops at a slower rate while they get cooler. The same reasoning can be applied to age class: the larger carcasses from yearlings reached *rigor mortis* at a mean temperature of 29.05ºC, significantly higher than rigor temperature of the smaller carcasses from calves (23.54ºC). Similarly, greater conformation from "P" to "U" resulted in progressively higher mean rigor temperatures from 20.73 to 27.93ºC, respectively (Table 3.1.5).

Table 3.1.5. Estimates of live-animal/carcass characteristics as significant explanatory variables in the linear models fitted to pH and temperature decay descriptors

pH/Temperature decay descriptors	Live-animal/carcass characteristics(1)	Mean	St. deviation	P>|t|
pH at 1.5 h ($pH_{1.5}$)	HCW	-0.001	0.0004	0.024
	Class			
	Vealer	0.143	0.0496	0.005
	Yearling	0.098	0.0817	0.230
	SEUROP			
	O	-0.025	0.0641	0.693
	R	-0.046	0.0650	0.479
	U	-0.227	0.0969	0.021
	[2]Class			
	Calf	6.425[a]	0.0394	-
	Vealer	6.568[b]	0.0276	-
	Yearling	6.523[ab]	0.0658	-

	[3]SEUROP			
	P	6.587[a]	0.0492	-
	O	6.561[a]	0.0411	-
	R	6.541[a]	0.0424	-
	U	6.359[b]	0.0835	-
pH at 3.0 h ($pH_{3.0}$)	HCW	-0.001	0.0005	0.036
	Class			
	Vealer	0.206	0.0690	0.004
	Yearling	0.149	0.1137	0.193
	SEUROP			
	O	-0.035	0.0904	0.696
	R	-0.072	0.0916	0.436
	U	-0.296	0.1366	0.033
	Class			
	Calf	6.110[a]	0.0548	-
	Vealer	6.317[b]	0.0385	-
	Yearling	6.259[ab]	0.0916	-
	SEUROP			
	P	6.343[a]	0.0694	-
	O	6.308[a]	0.0579	-
	R	6.272[a]	0.0598	-
	U	6.047[b]	0.1180	-
pH at 4.5 h ($pH_{4.5}$)	HCW	-0.001	0.0006	0.028
	Class			
	Vealer	0.231	0.0762	0.003
	Yearling	0.176	0.1250	0.162
	SEUROP			
	O	-0.038	0.1005	0.707
	R	-0.085	0.1018	0.407
	U	-0.306	0.1518	0.047
	Class			
	Calf	5.933[a]	0.0604	-
	Vealer	6.165[b]	0.0423	-
	Yearling	6.110[ab]	0.1008	-
	SEUROP			
	P	6.194[a]	0.0771	-
	O	6.156[a]	0.0644	-
	R	6.109[ab]	0.0664	-
	U	5.889[b]	0.1307	-
	HCW	-0.001	0.0006	0.022
	Class			
	Vealer	0.238	0.0780	0.003

pH at 6.0 h ($pH_{6.0}$)	Yearling	0.192	0.1280	0.135
	SEUROP			
	O	-0.036	0.1030	0.725
	R	-0.090	0.1048	0.388
	U	-0.295	0.1563	0.062
	Class			
	Calf	5.830[a]	0.0617	-
	Vealer	6.069[b]	0.0433	-
	Yearling	6.023[ab]	0.1032	-
	SEUROP			
	P	6.099a	0.0794	-
	O	6.063a	0.0663	-
	R	6.008a	0.0684	-
	U	5.804b	0.1346	-
pH at 24 h (pH_{24})	HCW	-0.001	0.0005	0.008
	Class			
	Vealer	0.195	0.0686	0.005
	Yearling	0.213	0.1127	0.061
	SEUROP			
	O	-0.006	0.0905	0.940
	R	-0.071	0.0917	0.440
	U	-0.213	0.1368	0.100
	Class			
	Calf	5.651[a]	0.0544	-
	Vealer	5.846[b]	0.0381	-
	Yearling	5.865[b]	0.0908	-
	SEUROP			
	P	5.864[a]	0.0695	-
	O	5.857[a]	0.0580	-
	R	5.793[ab]	0.0598	-
	U	5.651[b]	0.1178	-
pH decay rate (k_{pH})	Class			
	Vealer	-0.058	0.0266	0.025
	Yearling	0.004	0.0421	0.297
	SEUROP			
	O	0.018	0.0378	0.624
	R	0.008	0.0383	0.832
	U	0.155	0.0571	0.008
	Class			
	Calf	0.367[a]	0.0212	-
	Vealer	0.309[b]	0.0161	-

	Yearling	0.371[ab]	0.0363	-
	SEUROP			
	P	0.298[a]	0.0290	
	O	0.316[a]	0.0242	-
	R	0.306[a]	0.0250	-
	U	0.453[b]	0.0492	-
Temperature decay rate (k_{Temp})	HCW	-0.001	0.0001	<.0001
	Breed – Mirandesa	0.020	0.0097	0.043
	Gender – Male	0.014	0.0085	0.108
	Class			
	Vealer	-0.012	0.0078	0.877
	Yearling	-0.028	0.0133	0.831
	Fat – 3	-0.029	0.0070	<.0001
	SEUROP			
	O	-0.037	0.0094	<.0001
	R	-0.066	0.0095	<.0001
	U	-0.088	0.0141	<.0001
	Breed			
	Cross	0.102[a]	0.0055	-
	Mirandesa	0.122[b]	0.0072	-
	Gender			
	Female	0.105[a]	0.0068	-
	Male	0.119[b]	0.0050	-
	Class			
	Calf	0.134[a]	0.0064	-
	Vealer	0.118[b]	0.0052	-
	Yearling	0.095[c]	0.0111	-
	Fat			
	2	0.109[a]	0.0053	-
	3	0.080[b]	0.0057	-
	SEUROP			
	P	0.143[a]	0.0072	-
	O	0.105[b]	0.0061	-
	R	0.076[cd]	0.0063	-
	U	0.055[d]	0.0121	-
Time to pH 6.0 (h) ($Time_{pH6.0}$)	Fat – 3	1.379	1.0880	0.200
	Fat			
	2	4.518[a]	0.8060	-
	3	5.896[b]	0.7315	-
	HCW	0.039	0.0146	0.009
	Class			
	Vealer	-2.160	1.8907	0.410

	Yearling	0.332	3.2032	0.918
	SEUROP			
Temperat. at pH 6.0 (ºC) ($Temp_{pH6.0}$)	O	3.620	2.6400	0.176
	R	6.370	2.5600	0.016
	U	7.200	3.5000	0.044
	Class			
	Calf	23.54[a]	1.2800	-
	Vealer	24.02[a]	1.0560	-
	Yearling	29.05[b]	2.2900	-
	SEUROP			
	P	20.73[a]	1.975	-
	O	24.36[a]	1.755	-
	R	27.11[b]	1.631	-
	U	27.93[b]	2.891	-
	HCW	0.022	0.0035	<.0001
	Breed – Mirandesa	-0.429	0.3613	0.240
	Gender – Male	-0.543	0.3170	0.090
	Class			
	Vealer	0.073	0.2910	0.800
	Yearling	0.026	0.4938	0.960
	Lairage	0.584	0.3410	0.090
Temperat. at 1.5 h (ºC) ($Temp_{1.5}$)	SEUROP			
	O	1.236	0.3770	0.002
	R	2.571	0.3790	<.0001
	U	3.191	0.5670	<.0001
	Breed			
	Cross	33.13[a]	0.1954	-
	Mirandesa	32.71[b]	0.2655	-
	Gender			
	Female	33.16[a]	0.2579	-
	Male	32.69[b]	0.1670	-
	Class			
	Calf	32.04[a]	0.2955	-
	Vealer	32.74[b]	0.1731	-
	Yearling	33.57[c]	0.3207	-
	SEUROP			
	P	31.82[a]	0.2875	-
	O	33.05[b]	0.2426	-
	R	34.39[c]	0.2482	-
	U	35.01[c]	0.4897	-
	HCW	0.036	0.0056	<.0001

Temperat. at 3.0 h (ºC) ($Temp_{3.0}$)	Breed – Mirandesa	-0.568	0.5816	0.330
	Gender – Male	-0.862	0.5103	0.094
	Class			
	Vealer	0.116	0.4685	0.804
	Yearling	0.075	0.7946	0.925
	Lairage	1.062	0.5200	0.044
	Fat – 3	1.746	0.4420	<.0001
	SEUROP			
	O	1.832	0.5840	0.002
	R	3.865	0.5870	<.0001
	U	5.303	0.8630	<.0001
	Breed			
	Cross	28.22[a]	0.3329	-
	Mirandesa	27.65[b]	0.4336	-
	Gender			
	Female	28.37[a]	0.4110	-
	Male	27.51[b]	0.3018	-
	Class			
	Calf	26.45[a]	0.3958	-
	Vealer	27.59[b]	0.3220	-
	Yearling	29.01[c]	0.6880	-
	Fat			
	2	28.15[a]	0.3260	-
	3	29.89[b]	0.3481	-
	SEUROP			
	P	26.27[a]	0.4400	-
	O	28.10[b]	0.3811	-
	R	30.14[c]	0.3850	-
	U	31.57[c]	0.7470	-
Temperat. at 4.5 h (ºC) ($Temp_{4.5}$)	HCW	0.045	0.0068	<.0001
	Breed – Mirandesa	-0.521	0.7063	0.460
	Gender – Male	-1.025	0.6200	0.100
	Class			
	Vealer	0.137	0.5692	0.810
	Yearling	0.131	0.9653	0.890
	Lairage	1.426	0.6440	0.029
	Fat – 3	2.143	0.5480	<.0001
	SEUROP			
	O	2.189	0.7240	0.003

	R	4.731	0.7270	<.0001
	U	6.556	1.0700	<.0001
	Breed			
	Cross	24.05[a]	0.4043	-
	Mirandesa	23.52[a]	0.5286	-
	Gender			
	Female	24.30[a]	0.4993	-
	Male	23.27[b]	0.3666	-
	Class			
	Calf	21.92[a]	0.4837	-
	Vealer	23.33[b]	0.3935	-
	Yearling	25.15[c]	0.8404	-
	Fat			
	2	24.02[a]	0.4041	-
	3	26.16[b]	0.4315	-
	SEUROP			
	P	21.72[a]	0.5454	-
	O	23.91[b]	0.4724	-
	R	26.46[c]	0.4772	-
	U	28.28[c]	0.9259	-
Temperat. at 6.0 h (ºC) ($Temp_{6.0}$)	HCW	0.05	0.0053	<.0001
	Gender – Male	-1.215	0.6060	0.047
	Class			
	Vealer	0.127	0.6161	0.836
	Yearling	0.105	1.0325	0.919
	Lairage	1.703	0.7150	0.019
	Fat – 3	2.337	0.6080	<.0001
	SEUROP			
	O	2.304	0.8020	0.005
	R	5.126	0.8060	<.0001
	U	7.180	1.1860	<.0001
	Gender			
	Female	20.96[a]	0.4887	-
	Male	19.74[b]	0.3931	-
	Class			
	Calf	17.72[a]	0.5978	-
	Vealer	20.46[b]	0.4773	-
	Yearling	23.28[c]	1.0192	-
	Fat			
	2	20.25[a]	0.4482	-
	3	22.88[b]	0.4786	-
	SEUROP			

	P	18.06[a]	0.6049	-
	O	20.37[b]	0.5239	-
	R	23.19[c]	0.5293	-
	U	25.24[c]	1.0269	-

[1]Model estimates, standard deviations and P-values are shown only for animal/carcass characteristics that were significant in the ANOVA (Pr(F)<0.10)

[2]Least-squares means and standard deviations of factor levels were computed only when categorical variables (i.e, breed, gender, class) were significant in the ANOVA. Significant differences in means are denoted by different superscript letters within a factor.

[3]Shaded boxes present least-squares means and standard deviations computed from a separate ANOVA where the only variables tested were Fat and SEUROP.

Carcass classification by quality compliance

Two orthogonal principal components were found to explain 87.7% of the total variability in selected animal/carcass characteristics and pH/temperature decay descriptors (Figure 3.1.4). The first component, which accounted for 48.6% of the data variability, was negatively correlated with k_{Temp}, while positively and highly correlated with the temperature decay descriptors $Temp_{1.5}$, $Temp_{3.0}$, $Temp_{4.5}$, $Temp_{6.0}$ and, to a lesser extent, with the animal's age and hot carcass weight. As the series of temperatures at different time points were highly correlated one to another, it was clear that using them all would provide redundant information to the classification algorithms. Thus, only $Temp_{3.0}$ was selected for inclusion. The second principal component accounted for 39.1% of the total variability, and was highly correlated with the pH decay descriptors $pH_{1.5}$, $pH_{3.0}$, $pH_{4.5}$ and $pH_{6.0}$. As occurred in the first component, all of those pH descriptors contained very similar information as suggested by their high pairwise correlation coefficients (Figure 3.1.4). Thus, from all these pH at different time points, it was decided to choose the pH after three hours of slaughter ($pH_{3.0}$) for inclusion in the classification analyses, as this was also the only variable highly correlated with both rigor time and rigor temperature (see Subsection **Data description and modelled pH and temperature decay**).

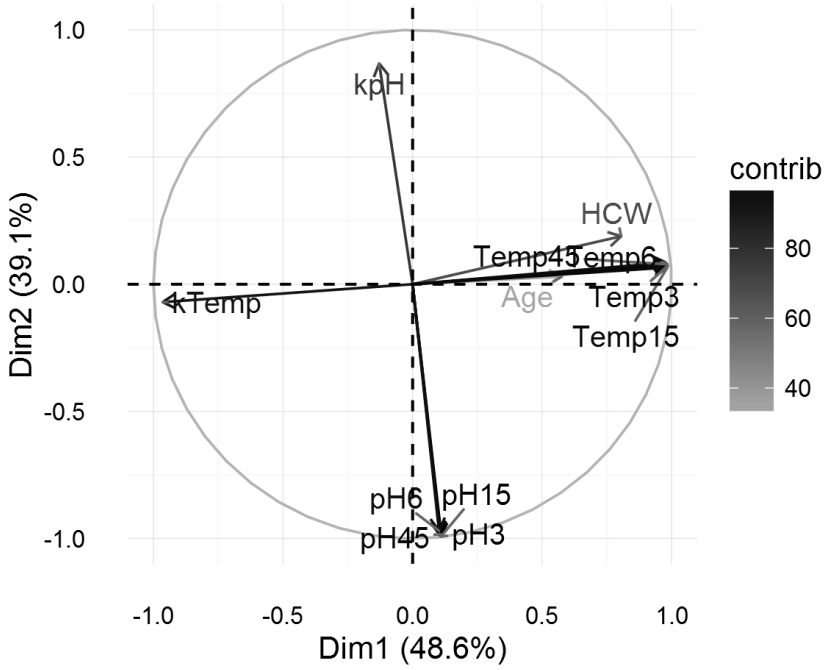


Figure 3.1.4. Loadings map for a two principal component solution showing contribution (R^2) of variables

The variables containing most information that were considered for the prediction of meat quality compliance – according to the ideal window rule, were: hot carcass weight, k_{pH}, k_{Temp}, $pH_{3.0}$ and $Temp_{3.0}$. With the reduced number of variables, the principal component analysis was re-run, and a score plot of the individual samples was obtained in order to visually assess the separability between cold-shortened and optimal quality carcasses (Figure 3.1.5). The fact that carcasses were distinguished mainly along the y-axis (positive y-axis for optimal quality carcasses and negative y-axis for cold-shortened carcasses) underlines that the pH decay has a much greater contribution than the temperature decay in determining the carcass quality. Said otherwise, carcasses with slower pH decay (lower k_{pH}) – and hence with higher $pH_{3.0}$ – tend to be classified as cold-shortened. It was calculated that if the pH of a beef carcass three hours post slaughter is lower than 6.25, it is very likely to become meat of optimal quality (Figure 3.1.6).

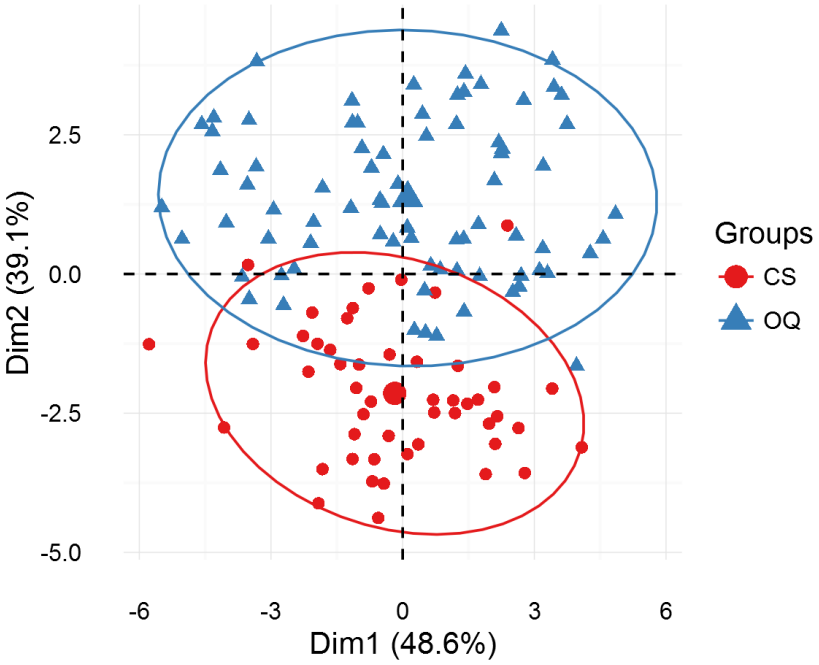


Figure 3.1.5. Scores plot for the two principal component solution showing separability between cold-shortened (CS) and optimal quality (OQ) carcasses

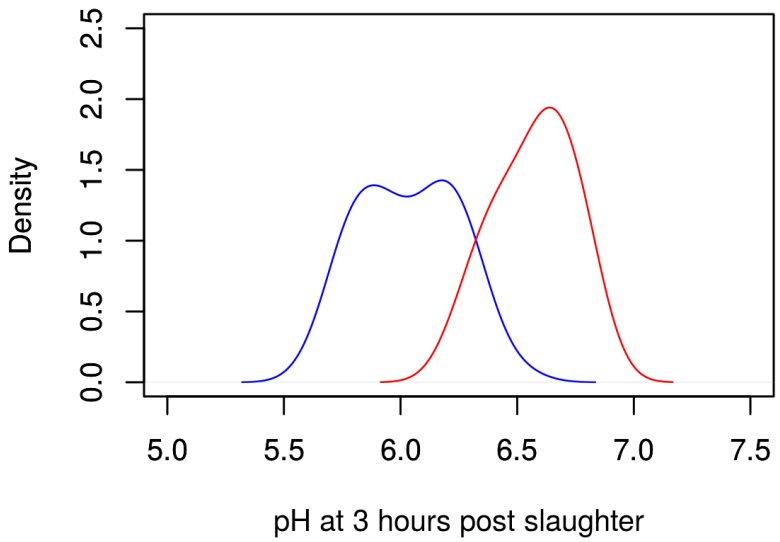


Figure 3.1.6. Density plots of pH values at 3.0 hours post slaughter from cold-shortened (red line) and optimal quality (blue line) carcasses. Distributions overlap at a $pH_{3.0}$ ~6.25

By (cross-validation) resampling 100 times the entire data set, it was possible to obtain 100 values of accuracy and kappa statistics for each of the classification algorithms tested. To assess the efficacy of the classification algorithms, box plots of the accuracy and kappa statistics are shown in Figure 3.1.7. The NSC by far presented the worst classification performance, with not only the lowest mean values of accuracy (0.919) and kappa (0.828) but also the widest range (0.667 – 1.000 and 0.238 – 1.000; respectively. A wide confidence interval stems from the fact that, in many iterations, the proportion of misclassification was very high. On the other hand, the SVM and kNN algorithms displayed a similar classification efficacy with comparable mean accuracy (0.914 – 0.919) and mean kappa (0.818 – 0.828; Figure 3.1.7). A better classification efficacy was provided by the LDA and the RLDA algorithms in terms of the highest accuracy and kappa statistics, and their narrowest deviations. LDA presented the highest total accuracy (0.951), and when the effect of chance was removed, still had the best performance (kappa=0.897); although these statistics were not significantly better than those produced by the RLDA method (accuracy=0.941; kappa=0.878).

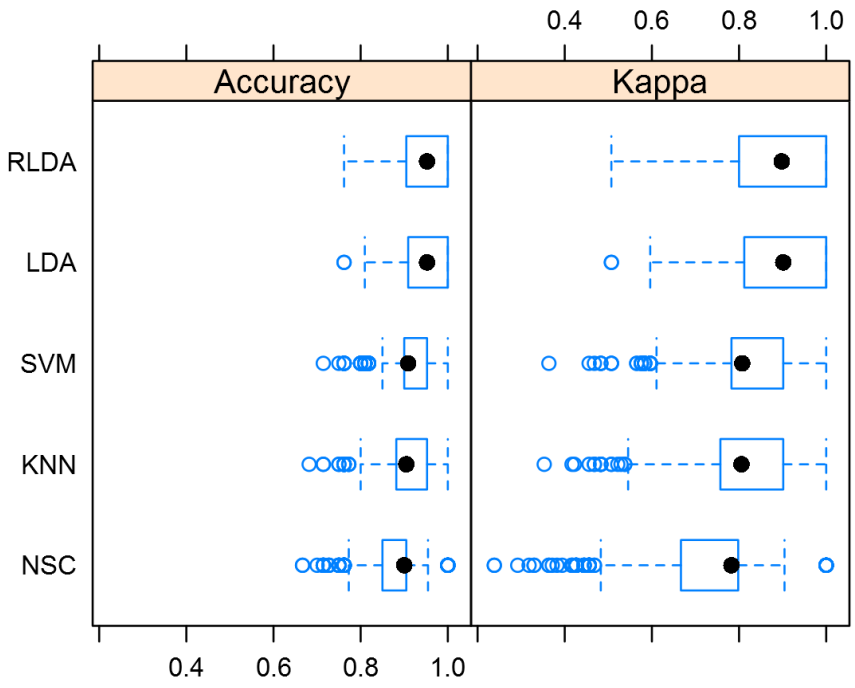


Figure 3.1.7. Box plots of the accuracy and kappa statistics for each of the classification algorithms tested – robust linear discriminant analysis (RLDA), linear discriminant analysis (LDA), k-nearest neighbours (kNN), support vector machines (SVM) and nearest shrunken centroids (NSC) – obtained from resampling the entire data set

When the models' performance was compared by randomly splitting the data into train (70%) and test data set (30%), the classification efficacy of the algorithms ranked similarly (Table 3.1.7). NSC was the algorithm with the worst accuracy (0.864) and kappa (0.700) statistics. Both kNN and SVM misclassified two cold-shortened carcasses as optimal quality carcasses, and two optimal quality carcasses as cold-shortened carcasses. The same confusion matrix produced by both classification algorithms led to the same mean accuracy (0.892) and kappa (0.770) statistics. Once again, the LDA and RLDA techniques presented comparable classification efficacy, although, for this particular test data set, RLDA performed slightly better than LDA, as indicated by their total accuracy (0.946 and 0.919, respectively) and kappa (0.885 and 0.830, respectively) statistics (Table 3.1.7). The RLDA classification algorithm mistakenly assigned one optimal quality carcass to the cold-shortening category while another cold-shortened carcass was mistakenly assigned to the optimal quality category.

Table 3.1.6. Confusion matrices, accuracy and kappa indices of the predictions of carcass quality compliance by robust linear discriminant analysis (RLDA), linear discriminant analysis (LDA), k-nearest neighbours (kNN), support vector machines (SVM) and nearest shrunken centroids (NSC), using a separate test data set

Algorithm	Prediction	Reference		Accuracy (95% CI)	Kappa
		CS	OQ		
RLDA	CS	13	1	0.946	0.885
	OQ	1	22	(0.818 – 0.993)	
LDA	CS	13	2	0.919	0.830
	OQ	1	21	(0.781 – 0.983)	
kNN	CS	12	2	0.892	0.770
	OQ	2	21	(0.746 – 0.970)	
SVM	CS	12	2	0.892	0.770
	OQ	2	21	(0.746 – 0.970)	
NSC	CS	10	1	0.864	0.700
	OQ	4	22	(0.712 – 0.955)	

CONCLUSIONS

Under the commercial conditions of the surveyed abattoir, there was considerable variation in rigor time (1.5–20.2 h) and rigor temperature (2.0–35.0ºC), which allowed both testing the adequacy of the pH/temperature decay models, and appraising the significant animal/carcass characteristics that affect pH/temperature decay. As per the rigor temperature range observed in the sampled carcasses, the quality of meat tenderness could be either optimal (~61%) or cold-shortened (~39%). Whereas it was highly unlikely for the

abattoir to produce hot-shortened meat, the ultimate pH of some carcasses (pH_{24} range=5.30–6.51) did not fall within the recommended values for meat of sufficient juiciness ($5.20 < pH_{24} < 5.70$).

The exponential decay equation turned out to be an adequate model to describe the carcass' decay in both pH and temperature. Linear models adjusted to key descriptors extracted from the fitted pH/temperature decay curves revealed that more animal/carcass characteristics modulated the carcass temperature decay than the pH decay. While the temperature-related descriptors were moderated by hot carcass weight, age class, SEUROP conformation, breed, gender and fat cover; the only three factors affecting the pH-related descriptors were hot carcass weight, age class and SEUROP conformation. Rigor time was found to be influenced only by fat cover.

A two-dimensional principal component analysis showed that five variables – hot carcass weight, pH decay rate, temperature decay rate, and pH and temperature at three hours post-slaughter – can be used to distinguish the beef meat quality into cold-shortened and optimal quality. Linear discriminant analysis and its robust variant yielded the best meat quality classification performance, having the highest mean accuracy (0.941 – 0.951) and kappa (0.878 – 0.897) statistics. This work underscores that it is feasible to classify meat tenderness into cold-shortened and optimal quality from beef carcasses' pH and temperature monitoring information of at least the first three hours post slaughter and hot carcass weight, a simple feature annotated in a commercial abattoir.

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3.2. Modelling meat eating quality traits during ageing as affected by beef carcass characteristics and early *post-mortem* pH decay descriptors

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ABSTRACT

Previous work has demonstrated that beef carcasses can be promptly and accurately classified into optimal quality and cold-shortened in accordance to the concept of pH/temperature “ideal window” by using carcass characteristics and early *post-mortem* pH/temperature decay descriptors. The objective of this study was to assess the combined effects of the aforementioned variables on the two main eating quality attributes of meat – namely, tenderness (measured as shear-force) and juiciness (measured as cooking loss) – during chill ageing. The pH and temperature in *longissimus thoracis* muscle of 45 beef carcasses were recorded during 24 h *post-mortem*, and decay descriptors were then obtained by fitting exponential models. Measures of Warner-Bratzler shear-force and cooking loss were obtained from cooked meat after 3, 8 and 13 days of cold ageing. The fitted mixed-effect models revealed that both meat tenderisation and cooking loss increased with ageing ($p<0.01$) although their rates slowed down in time ($p<0.05$). Beef carcasses with a higher pH (obtained at different endpoints: 1.5, 3.0, 4.5 or 6.0 h *post-mortem*) produced aged meat with increased tenderness ($p=0.013$) and increased water retention during cooking ($p=0.016$) than those of lower pH. Nonetheless, the slower the pH decay rate, as happens in a cold-shortened carcass, the lower the potential for tenderisation ($p=0.038$) and water retention ($p=0.050$) during ageing. Whereas sex affected shear-force, with females producing meat of higher tenderness, aged meat of increased water retention was produced by heavier beef carcasses ($p<0.001$). The good fitting quality of the shear-force ($R^2=0.847$) and cooking loss ($R^2=0.882$) models and their similarity among the different endpoints *post-mortem* indicated that both eating quality attributes can be approached by recording the pH decline of a beef carcass during the first 3.0 hours after slaughter.

INTRODUCTION

An ongoing challenge for the red meat industry is to produce high value table cuts of consistent good quality, since currently the variability in tenderness has been the greatest problem faced by meat producers (Lomiwes *et al.*, 2014). Consumer acceptance of meat depends on quality characteristics such as tenderness, colour, appearance, flavour and palatability attributes, which are influenced by a series of factors, ranging from physical and chemical to histological properties and meat-processing procedures (Kahraman *et al.*, 2014).The quality of meat can be seen as the result of interactions between the biological traits of the live animal and the biochemical processes that occur *post-mortem* as muscle is converted to meat and during storage (Lomiwes *et al.*, 2014).

Factors *ante-mortem* such as age, sex, nutrition, exercise, stress before slaughter; and *post-mortem* factors such as electrical stimulation, *rigor mortis*, chilling and maturation affect significantly meat quality (Roça, 2006; Koohmaraie *et al.*, 2002). The rates of pH and temperature decline during rigor development are probably two of the most important *post-mortem* factors affecting meat quality in terms of colour, water-holding capacity and tenderness (Savell *et al.*, 2005; Thompson *et al.*, 2006; Huff-Lonergan and Lonergan, 2007).

However, from all meat quality traits, tenderness and juiciness are the most important. Juiciness is an important sensory attribute inversely correlated to cooking loss. A high cooking loss results in low juiciness, giving then an expectation of a less optimal eating quality (Toscas *et al.*, 1999). Essentially, sarcomere length, connective tissue content and proteolysis of myofibrillar proteins account for most of the explainable variation observed in tenderness of aged meat. Sarcomere shortening during rigor development is the cause of *longissimus* toughening up to 24 h *post-mortem*, while proteolysis of key myofibrillar proteins, beginning soon after slaughter, is the cause of meat tenderisation. Connective tissue rather determines the background toughness, accounting for little of the variation in tenderness of the *longissimus* after 14 days *post-mortem* storage. However, the relative contribution of each of the three components of tenderness is muscle dependent (Koohmaraie *et al.*, 2002). A fourth biochemical factor affecting meat tenderness has been proposed to be the activity of small heat shock proteins (sHSP), which are synthesised to prevent unnecessary apoptosis (i.e., programmed cell death induced by adverse environmental conditions). Lomiwes et al. (2013) explained that the presence of bioactive sHSP in intermediate pH meat (pH=5.8-6.2), combined with the suboptimal proteolytic activities of calpains and cathepsins at that pH range, contribute to maintaining the integrity of myofibrillar proteins, leading to the higher meat toughness observed in intermediate pH meat. However, the role that sHSPs may contribute to meat toughness is restricted to their interactions with muscle cells either pre-rigor or even pre-slaughter, where sHSPs are known to be up-regulated in stressed muscle (Paulsen *et al.*, 2007).

As explained in the previous chapter, the concept of pH/temperature “ideal window” is a specification to describe the relationship between carcass pH and temperature from slaughter to when the ultimate pH is reached (Ibarburu *et al.*, 2007). The window requires the carcass pH to be greater than 6.0 (rigor) while the carcass temperature is above 35°C and below 6.0 before the temperature falls below 12°C. If the rate of pH/temperature decline does not fall through this ideal window, the carcass tenderness is said to be compromised, either by hot- or cold-shortening. Nonetheless, beef carcasses that fall within the “ideal window” (i.e. reach rigor when temperature is between 12 – 35ºC) are still subject to variable levels of

tenderness. For instance, Hwang and Thompson (2001b) found that the most tender beef meat after 14 days of ageing was achieved when the rigor temperature was 29 – 30ºC. As muscle pH and temperature impact on both physical sarcomere shortening and proteolytic enzyme activity (viz. the two factors explaining most of the variation in beef meat tenderness during ageing), the first objective of this chapter was to assess the combined effects of early *post-mortem* pH and temperature decline and animal/carcass characteristics on the two main eating quality attributes of meat – namely, tenderness (quantified as shear-force) and juiciness (quantified as cooking loss) – during chill ageing. The ultimate aim is to build practical models that can be used to predict the minimum ageing period of a beef carcass with known early *post-mortem* pH and temperature decay to reach optimal meat eating quality.

MATERIALS AND METHODS

Beef animals

In this study, a total of 45 Mirandesa breed animals, 30 males and 15 females, slaughtered in a local abattoir were sampled. The animals had an age of 8.58 ± 0.995 months when killed at one abattoir located in the Northeast of Portugal. The animals were transported by truck to the abattoir; and, at arrival, they were kept in individual stalls until slaughtering. They were not fed but did receive water *ad libitum*. After electric stunning, animals were slaughtered and dressed sequentially. Resulting carcasses had an average hot carcass weight of 145.8 ± 27.06 kg. For each of the animals, the following live-animal/carcass characteristics were annotated: sex, age, hot carcass weight and animal class (either calf or vealer).

Early *post-mortem* temperature and pH recording

Approximately two hours after slaughter, the carcasses' pH and temperature were recorded at intervals of 10 min during 24 h of carcass chilling, in *longissimus thoracis* muscle at the level of the 4th rib. The pH and temperature measurements were made using a weather resistant wireless transmitter CRISON pH probe (Crison Instruments SA, Barcelona, Spain) and wireless transmitter OMEGA temperature probe Pt 100 (Omega Engineering Limited, Manchester, UK) connected to an OMEGA UWTC-REC1 wireless channel receiver/host (Omega Engineering Limited, Manchester, UK).

Sampling of meat and eating quality measurements

After slaughter, the beef carcasses remained in the chilling room for 24 hours before cutting. Meat samples were obtained by cutting *longissimus thoracis et lumborum* muscle from the 12th thoracic vertebrae to the 3rd lumbar vertebrae; and three blocks of approximately 250 g (5 cm width each) were cut for laboratory measurements. These blocks were vacuum packed (1, 2 or 3) and randomly assigned to one of three ageing periods (3, 8 and 13 days) and held at 1ºC for the relevant ageing period until analysis.

On each of the ageing time points, three measurements were obtained: thickness of subcutaneous fat, cooking loss and shear-force. On the day of analysis, the sample was removed from the vacuum pack, and the thickness of subcutaneous fat was measured with a digital calliper (Owim GmbH & Co. KG, Neckarsulm, Germany). Fat was then trimmed off to leave only the loin. The loin sample was placed in plastic bags, was weighed and then cooked in a 2.2-L water bath (Memmert GmbH & Co. KG, Schwabach, Germany) at 70°C until the sample reached an internal temperature of 70°C (varying from 1.5 to 2.0 hours), as measured by a thermometer (Amarell GmbH & Co. KG, Kreuzwertheim, Germany). After cooking, the samples were again weighed to determine the cooking loss (%), and placed on a tray to cool (~6 h) to room temperature (20ºC).

While meat tenderness can be determined by compression, tensile strength, cut and shear, the instrumental evaluation by shear-force has been the main tool used in studies involving meat texture (Pinto *et al.*, 2010). The combination of compression and shear methods simulating the action of chewing is generally assessed by the method of Warner-Bratzler (Pereira, 2012). Briefly, the shear-force samples were prepared by using a 1 cm cork-borer to give the maximum number of sub-samples. Depending on the size of the muscle sample, ten to fifteen replicates of 1 cm^2 cross-sectional area were obtained. The force required to shear a sample was measured perpendicular to fibre orientation using a TA.XT*Plus* texture analyser (Stable Micro Systems Ltd, Surrey, United Kingdom) fitted with a standard shear blade Warner-Bratzler (WB) that sheared down through the samples. For the WB test, the blade is firmly held by means of the holder which screws directly into the texture analyser. The slotted blade insert is located directly into the HDP/90 Heavy Duty Platform and acts as a guide for the blade whilst providing support for the meat sample. The cross-head speed of the analyser was 5 mm/s. Shear-force values for each sample were expressed as kgF. The instrumental texture analysis was performed taking into account the procedures described by Wheeler et al. (1997) and Hopkins et al. (2014).

Statistical analyses

i) Modelling pH and temperature decay

The experimental curves of pH and temperature decline *post-mortem* were modelled as a function of time using an exponential decay function. Once models were fitted, the following fitted pH/temperature decay descriptors were obtained for each of the pH and temperature decay curves: the exponential pH decay rate (k_{pH}), the exponential temperature decay rate (k_{Temp}), the pH at 1.5 h ($pH_{1.5}$), at 3.0 h ($pH_{3.0}$), at 4.5 h ($pH_{4.5}$) and at 6.0 h ($pH_{6.0}$) *post-mortem*, the temperature at 1.5 h ($Temp_{1.5}$), at 3.0 h ($Temp_{3.0}$), at 4.5 h ($Temp_{4.5}$), and at 6.0 h ($Temp_{6.0}$) *post-mortem*. In this way, the aforementioned descriptors were linked to every carcass. The procedures for model fitting and estimation of pH/temperature descriptors were detailed in the previous chapter.

ii) Modelling shear-force during ageing

The studied variables influencing shear force of aged beef meat were determined by linear mixed-effects models. Four linear mixed models were developed for different early *post-mortem* endpoints matching the information available from the time of slaughter until 1.5, 3.0, 4.5 and 6.0 hours post-slaughter. This was done in order to determine whether it is feasible to predict meat tenderness from early *post-mortem* information (animal characteristics and pH/temperature values monitored until a given endpoint); and, if so, to evaluate how promptly after slaughter meat tenderness can be predicted. In the linear mixed models, the response variable was shear-force (SF) and the predictors tested were: ageing time (Ageing), animal/carcass characteristics – namely, sex (S), Age (A), hot carcass weight (HCW) and animal class (Class) – and pH/temperature decay descriptors – namely, k_{pH}, k_{Temp}, and depending upon the endpoint of the model (1.5, 3.0, 4.5 or 6.0 hours *post-mortem*), $pH_{1.5}$, $Temp_{1.5}$; $pH_{3.0}$, $Temp_{3.0}$; $pH_{4.5}$, $Temp_{4.5}$ or $pH_{6.0}$, $Temp_{6.0}$. The independent variables and two-way interactions predicting shear-force were added to the basic model (only ageing time as fixed-effect) one by one and tested by assessing the parameter significance, the improvement in goodness-of-fit (by the Bayesian Information Criterion BIC) and the behaviour of the residuals.

The best-fit and most parsimonious linear mixed-effects model that was fitted to the four endpoints was,

$$SF_{ij} = \beta_{0j} + \beta_{1j} \times Ageing_{ij} + \beta_2 \times Ageing_i^2 + \beta_3 \times pH_{ep} + \beta_4 \times k_{pH} \times Ageing_i + \beta_5 \times Sex + \varepsilon_{ij}$$
$$\beta_{0j} = \overline{\beta_0} + u_j$$
$$\beta_{1j} = \overline{\beta_1} + v_j \quad (1)$$

Where SF_{ij} is the shear-force (KgF) measured in cooked *longissimus* muscle belonging to the beef carcass *j* after *i* days of cold storage (i = 3, 8, 13). Depending upon the model's endpoint (ep), pH_{ep} stands for $pH_{1.5}$, $pH_{3.0}$, $pH_{4.5}$ or $pH_{6.0}$. The random-effects terms u_j and v_j were added to the mean of the intercept β_0 and time slope β_1 to account for random shifts due to carcass *j*. The two random effects were assumed to follow normal distributions with means zero and covariance matrix [s^2_u, s^2_{uv}; s^2_{uv}, s^2_v]. The residual error ε_{ij} followed a normal distribution with mean zero and variance s^2.

iii) Modelling cooking loss during ageing

The procedure for developing a linear mixed-effects model for cooking loss was the same as for shear-force, detailed in the previous sub-section. The same variables were assessed in their capability to estimate cooking loss (CookLoss), considering as well the four different early *post-mortem* endpoints from the time of slaughter until 1.5, 3.0, 4.5 and 6.0 hours post-slaughter. The best-fit and most parsimonious linear mixed-effects model that was fitted to each of the four endpoints was,

$$CookLoss_{ij} = \beta_{0j} + \beta_{1j} \times Ageing_{ij} + \beta_2 \times Ageing_i^2 + \beta_3 \times pH_{ep} + \beta_4 \times k_{pH} \times Ageing_i + \beta_5 \times HCW + \varepsilon_{ij}$$
$$\beta_{0j} = \overline{\beta_0} + u_j$$
$$\beta_{1j} = \overline{\beta_1} + v_j \quad (2)$$

where $CookLoss_{ij}$ is the cooking loss (%) measured in cooked *longissimus* muscle belonging to the beef carcass *j* after *i* days of cold storage (i = 3, 8, 13). The terms pH_{ep}, u_j, v_j and ε_{ij} hold the same meanings as those of Equation (1) while HCW stands for hot carcass weight (Kg). Exponential models and linear mixed-effects models were fitted using the *nlme* and *MASS* packages while exploratory graphs were produced using the *gplots* package, all implemented in the software R (R Core Development Team).

RESULTS AND DISCUSSION

Shear-force during meat ageing

The shear-force trend during beef ageing found in this study concurred with previous results (Hwang and Thompson, 2001a,b; Koohmaraie *et al.*, 2002), whereby the rate of meat tenderisation is higher in the beginning and slows down as ageing time elapses (Figure 3.2.1). The shear-force values measured on the 3rd day after slaughter had a mean of 4.72 KgF (range [3.04 – 7.50 KgF]), which decreased to 4.02 KgF (range [2.23 – 6.19 KgF]) on the 8th day, and finally to 3.74 KgF on the 13th day (range [1.94 – 6.12 KgF]). These values were considerably lower than those measured by Lomiwes et al. (2013) on comparable *post-mortem* days (5.6 – 16.3 KgF), probably because in our study meat from younger animals was evaluated. As stated by Hopkins et al. (2007), heavier carcasses are prone to have higher rates of pH decline, which leads to higher incidence of heat-toughened meat. In our study, the heat-shortening phenomenon was not observed in any of the carcasses.

Tenderisation was found to be influenced by both animal's sex and age class. On average, at each ageing time point, meat from females (Figure 3.2.1, top left) and vealers (Figure 3.2.1, top right) were significantly more tender than meat from males and calves, respectively. In both cases, the lower shear-force values obtained may be at least partly explained by the higher intramuscular fat present in females and older animals (vealers as opposed to calves).

In addition, carcasses were categorised according to final pH (pH_{24}) into high (pH_{24}>=6.2, n=16), intermediate (5.8=<pH_{24}<6.2, n=12) and low (pH_{24}<5.8, n=15), in an attempt to assess the effect of ultimate pH on tenderisation. As shown in Figure 3.2.1 (bottom left), tenderness is highly influenced by the carcass' final pH: carcasses of low final pH produced the least tender meat throughout the ageing period; although, considering that the upper limit of acceptable tenderness in beef is 11 KgF (Bickerstaffe *et al.*, 2001), the low final pH meat was still acceptable seemingly after a day following slaughter. Low pH meat was initially tough but with a sharp decline in shear-force attained tenderness levels at 13 days post-slaughter comparable to those of high pH meat. By contrast, high and intermediate final pH produced more tender meat although with a slower decline in shear-force during ageing (i.e., less ageing potential) (Figure 3.2.1, bottom left).

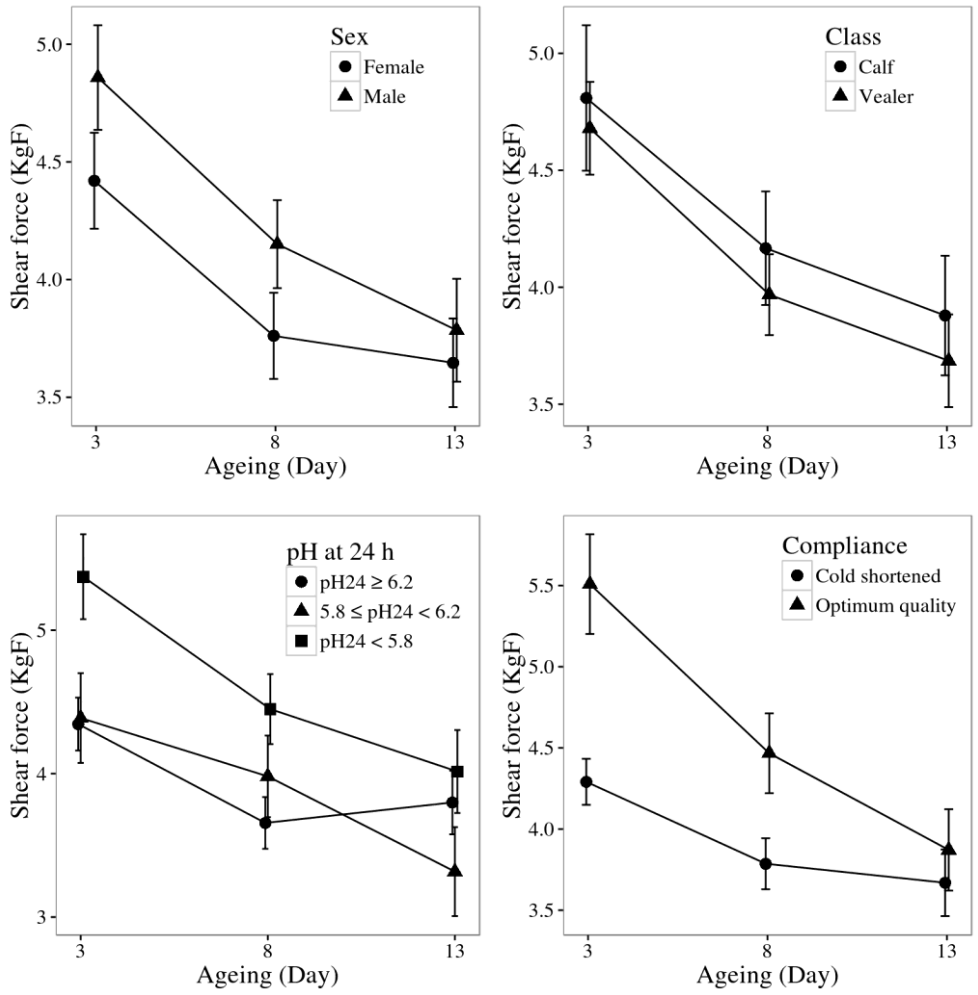

Figure 3.2.1. Evolution of shear-force during beef meat ageing, as affected by animal`s sex (top left), animal`s age class (top right), final pH class (bottom left) and carcass quality compliance (bottom right)

In muscle, the pH remains high when glycogen reserves are rapidly depleted. The depletion of muscle glycogen may be caused by a variety of severe pre-slaughter stresses including transport exhaustion, fear, climatic stress, aggressive behaviour with young bulls, hunger, prolonged with holding of feed prior to slaughter, mixing of unfamiliar animals and extreme adrenaline excitement (Gomide *et al.*, 2009). As a result, the *rigor mortis* eventually settles in the first hours after slaughter, often before the carcasses enter into the cooling chamber because their energy reserves are not sufficient to sustain the anaerobic metabolism and produce lactic acid. The lower production of lactic acid causes a minor decrease in pH *post-mortem* (i.e., high final pH meat), which is the necessary condition for the appearance of DFD meat (Feiner, 2006). Although DFD meats are considered defective, when cooked and subjected to shear-force tests, they have lower values (i.e., are more tender) than normal pH

meat, as attested also in this study. Earlier, Dransfield et al. (1992) proposed a decay model for μ-calpain by which the reduction in μ-calpain activity accelerates when muscle pH approaches ~6.2, and afterwards the rate of decline accelerates exponentially with increases in temperature. Thus, the tenderisation rate is faster in high pH meat because the myofibrillar proteolysis by μ-calpain is restricted to early *post-mortem* when the inherent pH of muscle is near physiological levels. Having concomitant results, Dransfield (1996) and Silva et al. (1999) explained that it is normal that meat of high final pH be more tender than normal pH meat due to the higher proteolytic activity responsible for the breakdown of myofibrils. More recently, Lomiwes et al. (2013) verified that, as a result of the early activation of the enzyme and autolysis, in high pH meat the μ-calpain activities greatly decreased 2 days *post-mortem*, and remains with a significantly lower activity in the following days, in comparison to low and intermediate pH meat.

Tenderisation trends obtained by the cold shortened (linked to DFD) and optimum quality carcasses (Figure 3.2.1, bottom right) were, as expected, analogous to those produced by low pH and intermediate/high pH meat (Figure 3.2.1, bottom left), respectively. These two tenderisation trends are corresponding because beef carcasses that attain rigor pH at a temperature between 12º to 35ºC (i.e., optimal quality) produce meat of lower final pH than those that attain rigor at a lower temperature (i.e., cold shortened). This takes place as a consequence of slower pH drop (i.e., lower pH decay rate k_{pH}) of the cold-shortened carcasses. As explained above, when muscle is maintained at high/intermediate pH for longer, it is the earlier activation of μ-calpain that produces meat of higher tenderness. The degradation of titin and filamin was found to be most rapid in high pH meat, with intact titin and filamin no longer detectable in high pH meat at 2 and 7 days *post-mortem*, respectively, while titin and filamin degradation products were detected in low pH meat at 1 and 2 days *post-mortem*, respectively (Lomiwes *et al.*, 2013). Rapid tenderisation in cold-shortened meat (higher final pH meat) is due to the fragmentation of intra-myofibrillar linkages in the I-band where titin has been found to degrade (Boyer-Berri and Greaser, 1998). In contrast, in meat of optimum quality (i.e., lower final pH meat), there is a delayed fragmentation of nebulin and desmin. Huff-Lonergan et al. (2010) argued that the tenderisation in lower pH meat is due to the disruption of the inter-myofibrillar linkages and costameres with in muscle cells. In this work, with the longer period of ageing (13 days), meat of low final pH (or from optimal quality carcasses) was still less tender than that of intermediate/high final pH meat (or from cold-shortened carcasses) (Figure 3.2.1, bottom left and right). Nonetheless, with prolonged ageing (beyond two weeks), the ultimate tenderness of the low pH meat may become similar to that of higher pH meats.

The parameter estimates of the mixed-effects models for estimating shear-force at different endpoints post-slaughter (i.e., 1.5, 3.0, 4.5 and 6.0 h) are shown in Table 3.2.1. There were no statistical differences between the endpoint models, as implied by the similar values of residuals and goodness-of-fit measures (BIC and R^2) and the p-values of the parameter estimates (Table 3.2.1). This suggests the possibility of estimating meat tenderness, as early as 3 h after slaughter, from logging pH decline of carcasses during this time. In general, all of the different endpoint models yielded a good agreement between observed and predicted values, as well as residuals that distributed as a normal distribution. This is graphically shown for the model whose endpoint was 3 h post-slaughter (Figure 3.2.2). The significance of the linear (p=0.012–0.017) and quadratic (p=0.045) ageing stemmed from the curvilinear relationship between shear-force and ageing time, as shown in Figure 3.2.1. The only animal characteristic tested that was found to have a significant impact on meat tenderness was sex. Sex affected only the intercept of the relationship between shear-force and ageing. In the shear-force models, the intercept for male animals (mean weight 157 kg) was higher than the intercept for females (mean weight 127 kg) for all the endpoints (Table 3.2.1), meaning that meat hardness values were significantly lower for female carcasses. Nonetheless, the effect of animal's sex on meat tenderness has shown contradictory results. While some authors stated that sex was a significant factor influencing beef tenderness (Johnson *et al.*, 1995; Pipek *et al.*, 2003; Jeleníková *et al.*, 2008; Hanzelková *et al.*, 2011); others did not achieve the same results (Wulf *et al.*, 1996; Araújo, 2006). The fact that female animals were linked to tender meat in our work may be explicated by lower collagen ratio, larger amount of white fibers, larger amount of fat, and even greater protection against cold shortening in female carcasses.

Table 3.2.1. Parameters estimates and goodness-of-fit measures (Bayesian Information Criterion BIC and coefficient of determination R^2) of the best-fit models estimating shear-force (KgF) of aged beef *longissimus* using animal characteristics and pH decay descriptors obtained at different endpoints after slaughter (1.5, 3.0, 4.5 and 6.0 hours)

Endpoint	Parameters	Mean	SE	P-value	BIC / R^2
1.5 hours	Fixed-effects				
	Sex – F	15.85	4.126	<.001	380 / 0.882
	Sex – M	16.16	4.105	<.001	
	Ageing	-0.171	0.070	0.017	
	Ageing^2	0.008	0.004	0.045	
	$pH_{1.5}$	-1.617	0.618	0.013	
	Ageing×k_{pH}	-0.195	0.082	0.020	
	Random-effects				
	Intercept (s_u)	0.915			
	Ageing (s_v)	0.069			
	Residuals (s)	0.538			
3.0 hours	Fixed-effects				
	Sex – F	11.92	2.617	<.001	381 / 0.882
	Sex – M	12.24	2.593	<.001	
	Ageing	-0.174	0.070	0.015	
	Ageing^2	0.008	0.004	0.045	
	$pH_{3.0}$	-1.061	0.403	0.012	
	Ageing×k_{pH}	-0.184	0.081	0.025	
	Random-effects				
	Intercept (s_u)	0.907			
	Ageing (s_v)	0.069			
	Residuals (s)	0.538			
4.5 hours	Fixed-effects				
	Sex – F	10.66	2.157	<.001	381 / 0.882
	Sex – M	10.97	2.132	<.001	
	Ageing	-0.177	0.070	0.013	
	Ageing^2	0.008	0.004	0.045	
	$pH_{4.5}$	-0.882	0.337	0.012	
	Ageing×k_{pH}	-0.174	0.079	0.031	
	Random-effects				
	Intercept (s_u)	0.904			
	Ageing (s_v)	0.069			
	Residuals (s)	0.538			
6.0 hours	Fixed-effects				
	Sex – F	10.06	1.954	<.001	382 / 0.882
	Sex – M	10.37	1.927	<.001	
	Ageing	-0.179	0.070	0.012	
	Ageing^2	0.008	0.004	0.045	
	$pH_{6.0}$	-0.797	0.308	0.013	
	Ageing×k_{pH}	-0.165	0.078	0.038	
	Random-effects				
	Intercept (s_u)	0.905			
	Ageing (s_v)	0.069			
	Residuals (s)	0.538			

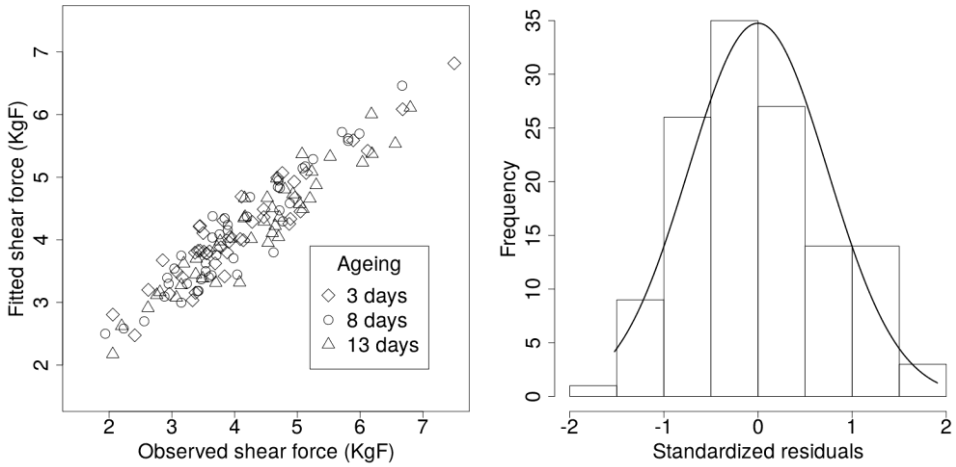

Figure 3.2.2. Scatter plot of shear-force observations and fitted values (left), and normality of residuals (right) for the mixed-effects model whose *post-mortem* endpoint is 3.0 h

In this work, the pH decay descriptors had a much stronger impact on the measured shear-force than the temperature decay descriptors did, since no temperature decay descriptor was found to significantly affect meat tenderness. This was probably an artifact of the chilling protocol being the same for all sampled carcasses. The early *post-mortem* pH decay of a carcass modulated both the intercept (as implied by the significant pH_{ep}, p=0.012–0.013) and the time slope (as implied by the significant interaction with k_{pH}, p=0.020–0.038) of the relationship between meat's shear-force and ageing (Table 3.2.1). As suggested by the negative sign of the pH_{ep} estimate, the higher the carcass pH at any endpoint (i.e., $pH_{1.5}$, $pH_{3.0}$, $pH_{4.5}$ or $pH_{6.0}$), the lower the shear-force (i.e., the higher the tenderness). This phenomenon has been explained above by the higher proteolytic activity that takes place in high pH meat. It is interesting that in Hwang and Thompson's (2001b), $pH_{1.5}$ was also a significant predictor of tenderness, and the optimal $pH_{1.5}$ to produce the most tender meat after 14 days of ageing was in the range [5.9 – 6.2]. This pH range coincided with the glycolysis rate that produced the longest sarcomeres.

The negative estimate of the interaction between the ageing time and the pH decay rate (Ageing×k_{pH}) signifies that beef carcasses undergoing a faster pH drop early *post-mortem* (i.e., higher k_{pH}) tend to have a higher tenderisation rate (i.e. lower negative Ageing slope). This can be better understood analysing Figure 3.2.1 (bottom right), because optimal quality carcasses (related to faster pH decay) have steeper drops in shear-force, between 3 and 8 days, and 8 and 13 days of ageing, than the cold shortened carcasses (related to slower pH decay). Said otherwise, the significant interaction Ageing×k_{pH} statistically corroborates that

carcasses of faster pH decay early *post-mortem* have more ageing potential than those undergoing a slower pH decay. Our results concurred with those of Melody et al. (2004), who found that muscles that experience a slightly accelerated pH decline also exhibit an accelerated rate of tenderisation. In the ageing potential, both calpain and cathepsin play a role. In Lomiwes et al. (2013), μ-calpain activity was numerically higher in carcasses with faster pH decay (i.e., low pH meat) until 14 days *post-mortem*. In this type of meats, μ-calpain autolysis was initially detected at 1 day *post-mortem*, and by 14 days, only the 76 kDa subunit was detected. In addition, cathepsin B activities are higher in low pH meat (i.e., faster pH decay) throughout the ageing period. At 2 days *post-mortem*, a marked rise of cathepsin B activities has been found in low pH meat, which signals its release from the lysosomes as the muscle pH declines to acidic values. Cathepsin B are optimally active at pH values ranging from 5.0 to 6.0 (Ouali, 1992), and the increased activity of this enzyme coincides with the further degradation of desmin and progressive decline in shear-force. Sarcoplasmic cathepsin B activities in low pH meat progressively increase with ageing such that the highest cathepsin B activities have been found at 28 days *post-mortem* (Lomiwes *et al.*, 2013). By contrast, the activity of cathepsin B in high pH meat (i.e., linked to slower pH decay rate) remains relatively unchanged throughout the ageing period (Huff-Lonergan *et al.*, 2010).

Cooking loss during meat ageing

The cooking loss is a combination of liquid and soluble matters lost from the meat during cooking. The water is lost due to heat induced protein denaturation during cooking of meat, which causes less water to be entrapped within the protein structures held by capillary forces. During ageing, cooking losses were found to increase from a mean of 23.02% (range [12.40 – 31.50%]) on the 3rd day *post-mortem* to a mean of 26.02% (range [15.30 – 33.50%]) on day 8, and they stabilised when measured on the 13th day *post-mortem* (mean 25.44%, range [13.20 – 33.00%]). The variability in cooking loss was high, yet comparable to the values (15 – 32%) measured by Aaslyng et al. (2003) when assessing the influence of intramuscular fat, carcass weight, pH, drip loss and cooking procedures on cooking loss of 3-day-aged beef steaks heated until a centre temperature of 70ºC. Exploratory analysis further revealed that, on average, lower cooking losses were registered in meat from males (Figure 3.2.3, top left) and in larger/older animals (Figure 3.2.3, top right). Meat from vealers, as opposed to calves, is likely to hold more water during cooking because of its higher intramuscular fat (Aaslyng *et al.*, 2003).

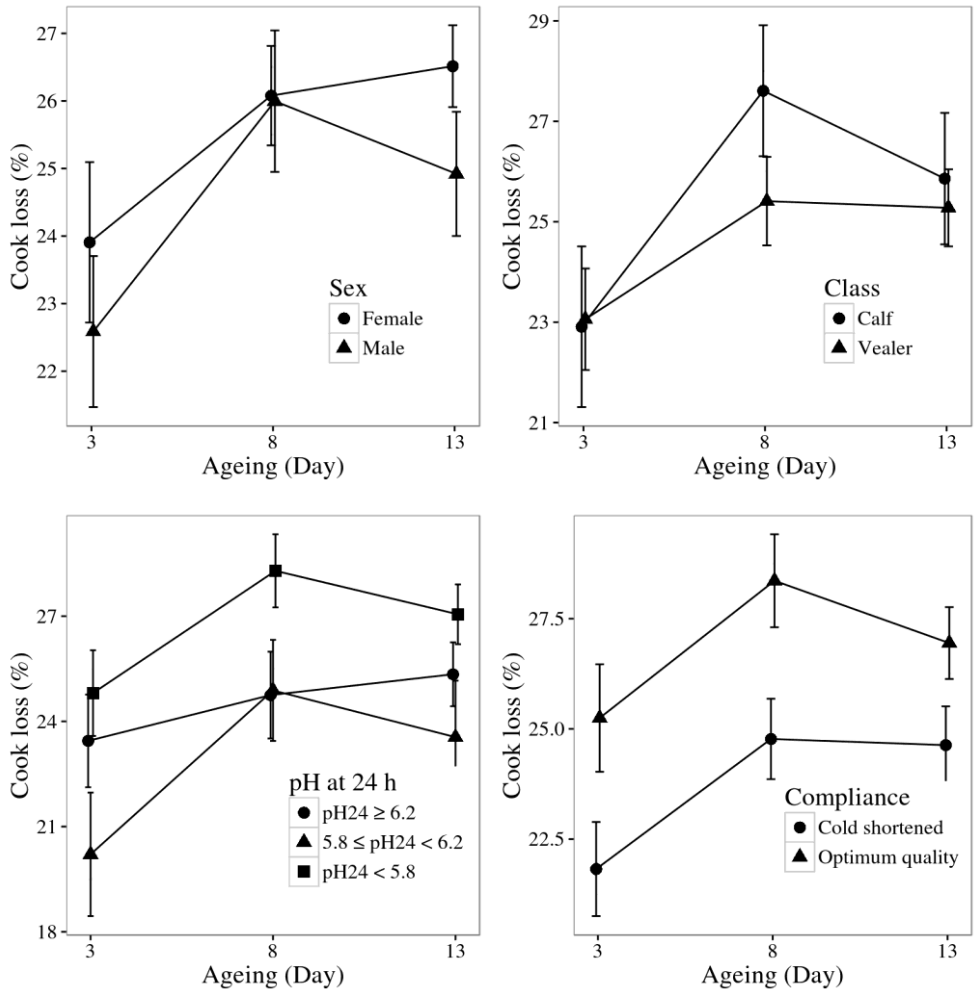

Figure 3.2.3. Evolution of cooking loss during beef meat ageing, as affected by animal`s sex (top left), animal`s age class (top right), final pH class (bottom left) and carcass quality compliance (bottom right)

Meat of low final pH released more water when cooked than that of intermediate and high final pH (Figure 3.2.3, bottom left). This outcome was not unexpected since in DFD meat, the muscle is called “firm” precisely due to the high water holding capacity meat, and “dry” because the water is tightly held within the muscle, which prevent cooking losses and maintain juiciness (Roça, 2000). In Aaslyng et al. (2013), pig meat from the high pH class had lower cooking losses, irrespective of the tested cooking procedures. Similarly, Eikelenboom et al. (1996) encountered a high correlation (r=0.68) between meat final pH and a sensory trait derived from cooking loss, juiciness. On the contrary, when muscle experiences a rapid decrease in pH at high temperatures, this would impact on protein functionality, with a reduction in the ability of muscle to retain water (Bee *et al.*, 2007). As a consequence, the meat from optimal quality carcasses (i.e., linked to lower final pH) exhibited a significantly higher water loss (25.2 – 28.0%) throughout ageing than the meat

from the defective cold-shortened carcasses (22.3 – 24.8% in Figure 6.3, bottom right). Very similar mean cooking losses were found by Schneider et al. (2006), although in chicken meat, whereby cooked DFD breast fillets lost 22% liquid, while normal fillets reached a mean cooking loss of 25% and PSE fillets a mean of 26%.

In our work, *post-mortem* ageing affected cooking loss as a linear ($p<0.001$) and a quadratic ($p<0.001$ in Table 3.2.2) predictor. Notice in Figure 3.2.1 that the relationship between these two variables is indeed not linear; instead the cooking loss rate slows down with increased meat ageing time. Apart from this similarity with the shear-force model (i.e., quadratic effect of ageing), the same pH descriptors selected for the shear-force model (i.e., pH_{ep} and k_{pH}) had a significant effect on the cooking loss of beef meat, shifting both the relationship's intercept (pH_{ep} with a $p=0.011–0.016$) and the time slope (k_{pH} in interaction with Aging, $p=0.025–0.050$ in Table 3.2.2). It is noteworthy that, despite these similarities in the model's explanatory variables, the correlations between shear force and cooking loss at the different ageing time points were not high (Figure 3.2.4). In general, the variables explained up to 84.7% (Table 3.2.2) of the variance in cooking loss. The only animal/carcass characteristic selected as a significant predictor of cooking loss was the hot carcass weight. By affecting only the model's intercept ($p<0.001$), higher hot carcass weights are expected to shift downwards the cooking loss trend (i.e., lower cooking loss). This strong inverse relationship between pig carcass weight and cooking loss was also measured by Aaslyng et al. (2003) ($r=0.53–0.68$). This is not an unexpected finding since a heavier animal will tend to have more intramuscular fat deposits, and the inverse correlation of this eating quality trait with intra-muscular fat ($r=0.33$) has been already shown (Eikelenboom *et al.*, 1996). At 60ºC and 70ºC beef steak centre temperature, cooking losses were found to be lower in the pork meat group with the highest intramuscular fat content (Aaslyng *et al.*, 2003).

Table 3.2.2. Parameters estimates and goodness-of fit measures (Bayesian Information Criterion BIC and coefficient of determination R^2) of the best-fit models estimating cooking loss (%) of aged beef *longissimus* using animal characteristics and pH decay descriptors obtained at different endpoints after slaughter (1.5, 3.0, 4.5 and 6.0 hours)

Endpoint	Parameters	Mean	SE	P-value	BIC / R^2
1.5 hours	Fixed-effects				
	Intercept	77.89	17.97	<.001	751 /
	Ageing	1.601	0.328	<.001	0.846
	Ageing^2	-0.072	0.019	<.001	
	$pH_{1.5}$	-7.185	2.704	0.011	
	HCW	-0.073	0.019	<.001	
	Ageing×k_{pH}	-0.715	0.314	0.025	
	Random-effects				
	Intercept (s_u)	4.354			
	Ageing (s_v)	0.217			
	Residuals (s)	2.576			
3.0 hours	Fixed-effects				
	Intercept	59.58	11.55	<.001	751 /
	Ageing	1.585	0.327	<.001	0.847
	Ageing^2	-0.072	0.019	<.001	
	$pH_{3.0}$	-4.518	1.750	0.013	
	HCW	-0.075	0.019	<.001	
	Ageing×k_{pH}	-0.661	0.307	0.034	
	Random-effects				
	Intercept (s_u)	4.356			
	Ageing (s_v)	0.217			
	Residuals (s)	2.576			
4.5 hours	Fixed-effects				
	Intercept	54.05	9.673	<.001	753 /
	Ageing	1.573	0.327	<.001	0.847
	Ageing^2	-0.072	0.019	<.001	
	$pH_{4.5}$	-3.690	1.457	0.015	
	HCW	-0.077	0.020	<.001	
	Ageing×k_{pH}	-0.619	0.300	0.043	
	Random-effects				
	Intercept (s_u)	4.361			
	Ageing (s_v)	0.218			
	Residuals (s)	2.576			
6.0 hours	Fixed-effects				
	Intercept	51.68	8.876	<.001	753 /
	Ageing	1.564	0.326	<.001	0.847
	Ageing^2	-0.072	0.019	<.001	
	$pH_{6.0}$	-3.325	1.326	0.016	
	HCW	-0.078	0.020	<.001	
	Ageing×k_{pH}	-0.587	0.296	0.050	
	Random-effects				
	Intercept (s_u)	4.367			
	Ageing (s_v)	0.218			
	Residuals (s)	2.576			

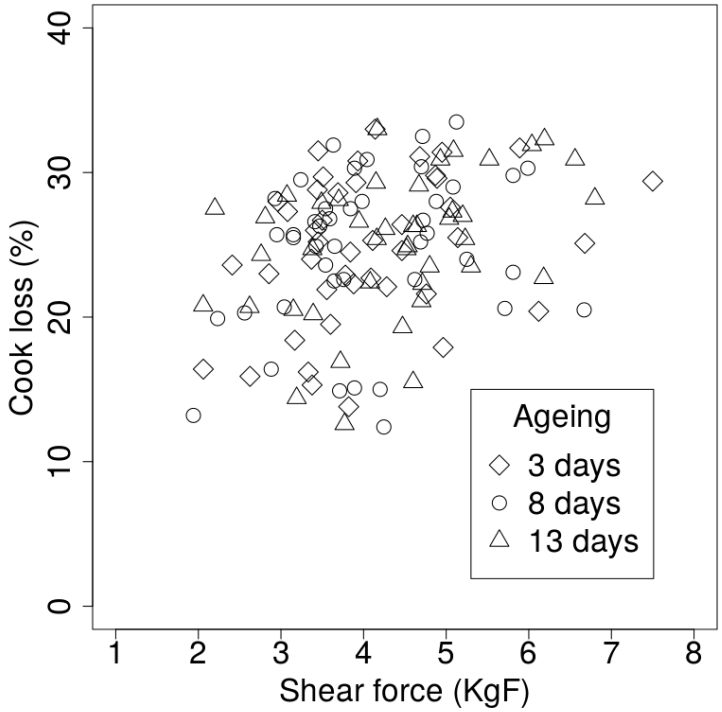

Figure 3.2.4. Scatter plot of shear-force and cooking loss in beef meat obtained at 3 days (r=0.386), 8 days (r=0.555) and 13 days (r=0.468) *post-mortem*

The carcass pH decay strongly influenced the cooking loss at two levels: at one level, the relationship between cooking loss and meat pH_{ep} was inverse (Table 3.2.2), meaning that carcasses with a higher pH at any endpoint *post-mortem* will retain more water during cooking. Next to this, the significant negative interaction Aging×pH_{ep} implies that a carcass with a faster pH drop early *post-mortem* (i.e., higher k_{pH}) will tend to have a lower increase in cooking loss during ageing in comparison to one that undergoes a slower pH decay. Said otherwise, meat from a carcass with a slower pH decay (viz. a cold-shortened carcass) tends to uphold more water; although, in time, it can release more water, in proportion, than a carcass with a faster pH decay. Other factors like concentration of glycogen could also influence cooking loss as an increased concentration of glycogen will increase the juiciness in beef with a normal pH (between 5.5 and 5.75) (Immonen *et al.*, 2000). However, this factor was not examined in the present study.

All of the different endpoint cooking loss models presented good fitting capacity and adhered to the assumption of normality of residuals, although graphs for assessment of model's adequacy are only shown for the endpoint of 3 h *post-mortem* (Figure 3.2.5). The similarity in the residuals and goodness-of-fit measures of the cooking loss models for the different

endpoints (Table 3.2.2) indicated that, like shear-force, cooking loss values can also be estimated by logging the pH decline of a beef carcass during the first 3.0 hours after slaughter. This is very important in the meat industry, as high cooking losses are viewed negatively by consumers because it affects meat consistency, leaving meat dry after cooking and impairing its palatability.

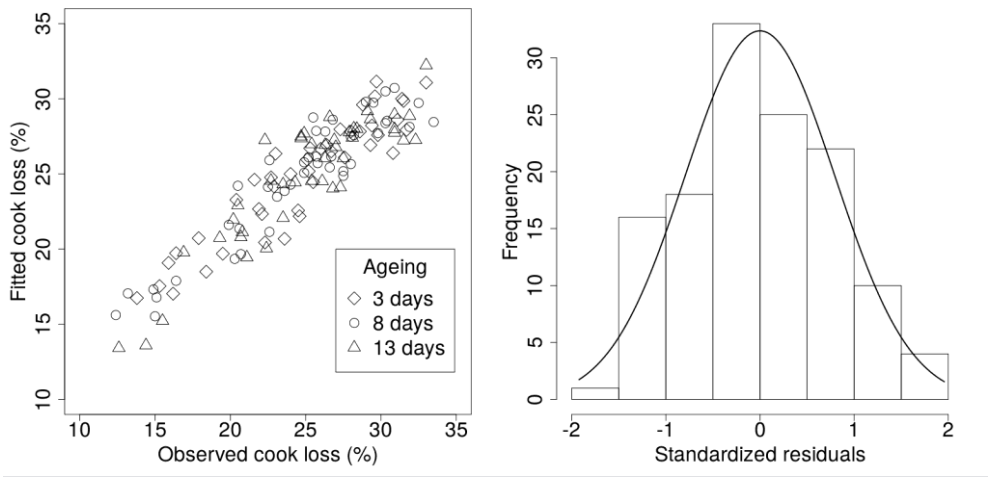

Figure 3.2.5. Scatter plot of cooking loss observations and fitted values (left), and normality of residuals (right) for the mixed-effects model whose *post-mortem* endpoint is 3 h

CONCLUSIONS

The properties of fresh meat determine its usefulness for merchant, his attraction to the consumer and their suitability for further processing. Thus, both the tenderness and the cooking losses are two of the most important eating quality attributes of meat. The rate of tenderisation is higher in the early *post-mortem* carcasses and slows down as ageing time elapses for the carcasses. Tenderisation was found to be influenced by both animal´s sex and age class, since meat from females and vealers were more tender than meat from males and calves. Sex was the only animal characteristic tested, for shear-force, which was found to have a significant impact on meat tenderness. Analysing the intercept of males and females, in the shear-force models for all of endpoints, was found that meat hardness values were lower for females, so female animals are linked to tender meat.

Carcasses of low final pH produced the least tender meat throughout the ageing period but with a sharp decline in shear-force attained tenderness levels at 13 days *post-mortem*

comparable to those of high pH meat, thus it appears that meat tenderness is highly influenced by the meat final pH. By contrast, carcasses with high and intermediate final pH produced more tender meat although with a slower decrease in shear-force during ageing. However, a high final pH produces DFD meat, which is considered as defective (meat), when cooked and subjected to shear-force tests, this meat is more tender than normal pH meat. We can conclude that with longer periods of ageing, meat from optimal quality carcasses (low final pH) was still less tender than meat from cold-shortened carcasses (intermediate/high final pH). Thus, muscles that experience a slightly accelerated pH decline also exhibit an accelerated rate of tenderisation.

In this work, comparing the influence in meat tenderness of the pH and temperature decay descriptors, the pH decay descriptors had a much stronger impact on the measured shear-force than the temperature decay descriptors. The early *post-mortem* pH decay of the carcass modulated both the intercept and the time slope of the relationship between meat´s shear-force and ageing. Thus, the higher is the value of the carcass final pH at any endpoint, the lower is the value of the shear-force and consequently, the greater the tenderness of the meat.

Relatively to the studies of cooking loss, a lower cooking loss were registered in meat from males and in larger/older animals; and meat from vealers, as opposed to calves, is likely to hold more water during cooking. Meat of low final pH released more water when cooked than that of intermediate and high final pH. Hot carcass weight was the only animal/carcass characteristic selected as a significant predictor of cooking loss. By affecting only the model´s intercept higher hot carcass weights are expected to shift downwards the cooking loss trend (lower cooking loss).

The relationship between *post-mortem* ageing and cooking loss is not linear, once the cooking loss rate slows down with increased meat ageing time. It appears that, like in the same pH descriptors selected for the shear-force model, they had a significant effect on the cooking loss of beef meat, shifting both the relationship´s intercept and the time slope. In general the correlations between shear-force and cooking loss at the different ageing time points were not higher.

The carcass pH decay strongly influenced the cooking loss once the carcasses with a higher pH at any endpoint *post-mortem* will retain more water during cooking. It also concludes that, meat from a carcass with slower pH decay (cold-shortened carcass) tends to uphold more water; although in time, it can release more water, in proportion, than a carcass with faster

pH decay. That is, carcass with a faster pH drop early *post-mortem* will tend to have a lower increase in cooking loss during ageing in comparison to one that undergoes slower pH decay.

This work underscores that with de good fitting quality of the shear-force and cooking loss models and their similarity among the different endpoints *post-mortem* indicated that both eating quality attributes of beef (tenderness, measured as shear-force and juiciness, measured as cooking loss) can be approached by recording the pH decline of a beef carcass during the first 3.0 hours after slaughter.

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4. General Discussion

In view of the proposed objectives for this thesis, **Chapters 2.1** and **2.2** summarised available information on the incidence of pathogens in meats and meat products produced in Portugal; and an exposure assessment model elucidating the changes in *Salmonella* prevalence in beef along the processing stages. In **Chapters 3.1** and **3.2**, a discriminant analysis model was developed to classify beef carcasses quality into “optimal quality” and “cold-shortened” according to the pH and temperature decline “ideal window rule”. Additionally, linear models to predict eating quality attributes of beef related to tenderness and juiciness were constructed from animal characteristics, ageing time and early *post-mortem* pH and temperature decay descriptors.

4.1 PREVALENCE OF FOODBORNE PATHOGENS IN MEAT

Globally, the threat of epidemic infections has increased due to the greater movement of people, animals and goods within and between countries, and also due to the globalization of the food supply, since consumers require more fresh foods all year round (Tauxe *et al.*, 2010; Tadesse and Tessema, 2014). Salmonellosis continues to be a major public health problem worldwide. Most cases of human salmonellosis are foodborne diseases, but some infections are acquired through direct or indirect contact with infected animals (Pui *et al.*, 2011; Hoelzer *et al.*, 2011). Beef cattle carrying *Salmonella* spp. represent a risk for contamination of meat and meat products. These products are also the main vehicles of foodborne diseases in humans caused by other pathogens such as *Campylobacter* spp., *Listeria monocytogenes*, *Yersinia enterocolitica*, verotoxigenic *Escherichia coli* (VTEC) and *Staphylococcus aureus* (EFSA, 2013; Xavier *et al.*, 2014). Basic hygiene practices and the implementation of correct management strategies can efficiently reduce the risks of carcass contamination associated with animal slaughtering (Tadesse and Tessema, 2014). In **Chapters 2.1** and **2.2,** we use meta-analytical methods to study and estimate the prevalence of *Salmonella*, and other pathogens, in meat and meat products in Portugal and Brazil, respectively.

As raw meat is one of the ideal growth medium for various pathogens, negligence in handling, post-processing, storage or cooking, makes illness a real possibility. Although salmonellosis reporting rates in Portugal are lower than the EU average, it is the country with the highest hospitalization rates and where pig meat has been identified as the most important likely source of *Salmonella* infection (EFSA, 2013). Nonetheless, in Portugal, there is little information on foodborne zoonoses as well as the incidence of the main microbial pathogens in meats and meat products (Veiga *et al.*, 2012). Due to the strong association between foodborne diseases in humans and the consumption of contaminated meat and

meat products, and the high consumption of meat in Portugal, it was considered useful to collect and analyse existing information on the occurrence of foodborne pathogens in Portuguese meats.

In **Chapter 2.1** we presented a meta-analysis that summarised and compared the occurrence of *Salmonella* spp. and *Campylobacter* in Portuguese fresh meats, in order to understand the current epidemiological situation of the country and prioritise its microbial risks. With this study, we could verify the sparseness of knowledge on the incidence of pathogens in meats and meat products produced in Portugal. Although both levels of *Campylobacter* and *Salmonella* were within EU ranges (~5.9%), the mean occurrence rate of *Campylobacter* in broiler meat (~40%) was found to be nearly ten times higher than that of *Salmonella* (~4.4%). This fact can be explained by the positive impact of the national *Salmonella* control programmes in place for poultry production since 2008. In other fresh meats, such as bovine and pig meat, the mean *Salmonella* prevalence was ~1.9% and ~12.6%, respectively. In our semi-quantitative risk ranking for pork, we ranked *Salmonella* spp. and *Y. enterocolitica* in the risk categories of critical and major, respectively. As pig meat is highly consumed in Portugal, it is essential that a pork carcass safety assurance be implemented. The incidence of *S. aureus* (~22.6%) in meat products was significantly higher than those of *L. monocytogenes* (~8.8%) and *Salmonella* (~9.7%), which signalled a poor hygiene process. This work demonstrated the necessity of Portuguese food safety agencies to take urgent monitoring and training actions for the maintenance of good hygiene and manufacturing practices during the production of the meat products. It may also assist national food safety authorities and policy makers in the prioritisation of microbiological hazards in the specific meat type categories.

Since Brazil has become one of the world's largest producers of beef, its meat must meet stringent international standards of quality and safety. However, the combination of a notable increase in salmonellosis in Brazil and the sparse information on the occurrence of pathogens in Brazilian beef, leave the country in disadvantage compared to other large producers such as the USA and Australia. Thus, it was desirable to build an exposure assessment model elucidating the changes in *Salmonella* prevalence in Brazilian beef. Through the percentage of *Salmonella* spp., in bovine carcasses at different stages of processing, **Chapter 2.2** demonstrated that the stages of evisceration/splitting (10.8%) and boning (7.80%) may amplify the contamination of *Salmonella* spp., but rinsing (9.50%) and chilling (4.60%) may decrease it. This work confirmed that, although faeces and hides of carrier animals are major sources of contamination, the spread of this pathogen during the process can be minimised by the correct implementation of food safety programs. When

hygienic slaughter procedures and sanitary programs are working properly, the initial *Salmonella* load can further be decreased at the critical stages of dehiding, rinsing and chilling. We concluded that the load of *Salmonella* spp. that initially enters the plant with the live animals does not necessarily determine the extent of contamination in the final product.

4.2 PREDICTION OF BEEF CARCASS QUALITY

After 24 hours of slaughter, the pH declines from 7.2 to normal values between 5.4 to 5.7 (Wood, 1995). The rate of pH decline and the ultimate pH (Maltin *et al.*, 2003) and the interactions between pH and temperature decline early *post-mortem* have all major impact on the beef meat quality (Hwang and Thompson, 2001). Thompson (2002) and Warner et al. (2014) showed that the pH and temperature relationship at the onset of *rigor mortis* has an impact on beef meat tenderness, reaching the "ideal" tenderness when temperature in the muscle is between 12 and 35ºC in the moment when pH reached 6.0 (*rigor mortis*). Thus, the rate of *post-mortem* glycolysis is dependent on the coolant temperature, as higher temperatures accelerate the lowering of the pH, reaching the final pH in shorter time. These two aspects show the importance of synchronising cooling with decreasing pH so that the meat is not still warm when the pH is low, or too cold when the pH is still high (Lombard, 2009). The temperature difference between the inside and outside of chilled muscles determines the cooling rate of the meat. This is important in a practical sense in carcasses from animals with less fat coverage. Kempster (1992) reported that rapid chilling of carcasses so that muscle temperature reaches approximately 10ºC when the pH is still above 6.0 and therefore in the pre-rigor state, can seriously toughen beef. Later, Rosenvold and Andersen (2003) described that rapidly chilled meat was tougher than conventionally chilled meat indicative of cold-shortening. Bovine carcasses are highly susceptible to the cold-shortening phenomenon due to their high amount of red fibres, which contain more mitochondria, and the less developed sarcoplasmic reticulum (Gil, 1996; Prates, 2000). Cold-shortening and its negative effects on water holding capacity can, under specific conditions, counterbalance the beneficial effects of rapid chilling on protein denaturation.

In **Chapter 3.1**, a predictive model was developed to carry out carcass classification and grading according to meat quality, taking into account the pH and temperature decay during chilling of beef carcasses. From the experiments, we verified that under abattoir chilling conditions, there is a considerable variation in the pattern of the pH and temperature decline among carcasses, leading to variation in meat tenderness. The results of this chapter demonstrated that as per the rigor temperature range observed in our sampled carcasses (1.96-35ºC), the carcasses quality according to the predicted meat tenderness could be

classified to either optimal (~61%) or cold-shortened (~39%). Thus, through the use of pH and temperature sensors that have monitored the carcasses, from entering the refrigeration chamber until reaching their final pH (pH_{24}), we demonstrated that the carcasses' pH and temperature evolution during cooling can be used to classify and grade the carcasses. We also attested that, in addition to the possibility of classifying the carcasses according to their meat quality (optimal or cold-shortened quality), it is also possible to predict this classification using information acquired only during the first 3 hours of carcass chilling. Whereas it was highly unlikely for the abattoir to produce hot-shortened meat, the ultimate pH of some carcasses (pH_{24} ranged from=5.30 to 6.51) did not fall within the recommended values for meat of optimum quality ($5.20<pH_{24}<5.70$). These carcasses (~39% of the samples) presented pH values above 6.0 and suffered the phenomenon of cold-shortening and originated dark, firm and dry (DFD) meat. DFD meat is known to be related to problems of prolonged pre-slaughter stress where all the muscle glycogen stores are depleted. This inhibits the production of lactic acid making it impossible to lower the meat pH *post-mortem*. So, DFD meat, which has high pH values, also has higher tenderness values (but only in the first days of ageing due to the high pH that influences the fast performance of the enzymes in the process of meat maturation), but this negatively affects the meat shelf life and as they cannot be stored for long, should be consumed in short time after slaughtering or should be used to produce meat processed products.

These results demonstrated that the formation of DFD meat during the chilling of carcasses is still a serious and worrying problem since it entails economic damage to both slaughterers and producers. However, we cannot positively conclude what are the real reasons that led to the appearance of this meat defect, because our studies did not focus on this subject. Nevertheless, we presume that its origin may derive from problems related to slaughterhouse protocols, as well as to the various intrinsic factors (breed, stress susceptibility, etc.) and extrinsic factors (environmental factors, handling, pre-slaughter management, shipping and transport, feeding, etc.). The need for a carcass monitoring system is therefore important to find answers to these problems and to provide information to both slaughterers and producers so that they can avoid the occurrence of DFD meat. Thus, we agree that this question still needs further investigation in this sense. However, through the results obtained, that both the duration and the speed of pH fall directly determine other physical factors of meat quality, such as water-holding capacity and cooking loss, tenderness, and occurrence of DFD meat, we can confirm that pH descriptors are the main objective measures that enables the differentiation of beef meat quality.

4.3 PREDICTION OF BEEF MEAT QUALITY

The variability in meat tenderness has been of great concern for producers, since consumers consistently rank tenderness high among the quality attributes that define an enjoyable eating experience. Since not all beef is equally tender, meat scientists have sought a myriad of ways to remedy this inconsistency. Over the years, the ageing of beef and other meats has been applied to improve tenderness. Nowadays, carcasses are allowed in a chiller for a period of time (usually between 14 and 21 days) while natural enzyme activity enhances tenderness. However, aging beef properly has economic consequences. Many processors complain that holding beef in refrigeration facilities with controlled humidity and airflow for long periods is too onerous. In addition, longer ageing periods may result in increased product shrinkage and weight loss (Lombard, 2009; Ramos, 2012).

Chapter 3.2 develops the issue of predicting the minimum ageing period of a beef carcass with known early *post-mortem* pH and temperature decay descriptors to reach meat with optimal eating quality. The animal characteristics that significantly affected tenderisation were animal's sex and age class; in that meat from female vealers were tenderer than meat from male calves. The higher intramuscular fat present in females and older animals (vealers) may be one the possible explanations for these values of shear-force. Sex was the only characteristic that exerted significant impact on meat tenderness, as female meat (3.90 KgF ± 0.781) had lower shear-force values than male meat (4.30 KgF ± 1.204). It was found out that the female animal's meat presents higher tenderness than the males': this difference is notorious on the third day of ageing, but on day 13, the shear-force values between sexes was no longer present (although the male meat continues to be statistically harder). On day 13, samples were softer than samples from day 3; hence ageing did result in softening of muscles. However, on day 3 of ageing, cold-shortened carcasses (4.3 KgF ± 0.750) exhibited lower shear-force values than optimal quality carcasses (5.5 KgF ± 1.189). Thus, optimal quality carcasses exhibit superior shear-force values of 1.2 KgF when compared to cold shortened carcasses. These results were not quite expected because they showed that cold-shortened carcasses, that originate DFD meat, present more tender meat than the optimal quality carcasses. This may take place because, cold-shortened carcasses have high pH, and muscles that experience a slightly accelerated pH decline also exhibit an accelerated rate of tenderisation. Note that, the accelerated tenderisation early post rigor is desirable in terms of meat tenderness. However, the conditions that prompt this phenomenon are expected to cause more water loss from the muscle; undesirably reducing during the ageing period, the shear-force values of the optimal quality carcasses decreased, and on day 13 there were no significant differences in tenderness between cold-shortened

carcasses (3.7 KgF ± 1.086) and optimal quality carcasses (3.9 KgF ± 0.971). However, with longer period of ageing, meat from optimal quality carcasses (low final pH) become less tender than meat from cold-shortened carcasses (intermediate/high final pH). Thus, we recommend the practice of ageing meat until day 13.

With regards to cooking loss, it was observed that from day 3 to day 8 of ageing, the cooking losses suffered an increase of 3 percentage units, which translates to an increase of 13%. From day 8 to day 13, there were no differences in cooking losses. This is due to the fact that meat from vealers hold more water during cooking because of its higher intramuscular fat. Hot carcass weight was the only characteristic selected as predictor of cooking loss. So, higher hot carcass weights are expected to reduce the cooking loss. Meat of optimal quality carcasses, with low final pH, released more water (27.1% ± 3.302) when cooked than meat of cold-shortened carcasses, with intermediate (23.6% ± 5.581) and high final pH (25.3% ± 3.647) on day 13 of ageing. This means that, carcasses with a slower pH drop early *post-mortem* (cold-shortened carcass) tends to uphold more water, although, during ageing, it can release more water in proportion than a carcass with a faster pH decay. Since the cooking losses adversely affect the meat's consistency, leaving it dry after cooking and damaging its palatability, the expected results would be the opposite. However, this is due to the fact that the DFD meat has a high water holding capacity and that water is tightly bound into the muscle, which prevents cooking losses and maintains its juiciness. However, precisely because of these characteristics, DFD meat is more susceptible to contamination and development of pathogenic microorganisms due to its high humidity content and its high pH and consequently.

Using the shear-force and cooking loss models, it is possible to predict, by recording the pH decline of a beef carcass during the first 3.0 hours after slaughter, the eating quality attributes of meat. The search for biological predictors of tenderness and other quality traits of meat is a necessity, in order to enhance valorisation of carcasses by directing them shortly after slaughter towards an optimal use on the basis of their potential qualities.

After concluding this investigation, we demonstrated that the application of this model is a useful tool for the meat sector, since, although it still has some limitations, it represents a way for further investigations that bring benefits and added value to beef meat sector. The application of these models in the slaughterhouse can already be put into practice, however, the shortage of supply of suitable equipment at low cost, can make its use still limited. Therefore, it is advisable to apply these pH/temperature measurements only in carcasses

that are at "high risk" of leading to poor quality meat (specifically, in carcasses more susceptible to the cold-shortening and the occurrence of DFD meat).

4.4 PRACTICAL APPLICABILITY OF THE MODELS

With this research, in the near future, we hypothesise the development of low-cost sensors for pH and temperature recording, which will make it possible to monitor all carcasses in the abattoirs. Through the development of these new sensors, it will be possible to create a database system to store and analyse data in real time, which allows us to understand and explain the reason for their phenotypic/genetic variability, and act as support decision system in a way to increase the quality of the meat. With the existence of this database system, it will be possible to evaluate and adjust the refrigeration protocols of slaughterhouses taking into account the characteristics of the carcasses.

This support decision system will also allow the grading the carcasses according to their characteristics, namely: carcass weight, sex, subcutaneous fat thickness, carcass conformation (SEUROP), etc., thus ensuring that their refrigeration meets the requirements of the "ideal window" of quality as described by Meat Standards Australia (Meat Standards Australia, 2011). The formation of these lots will reduce the inconsistency of meat quality. Nevertheless, the model developed in this research is already applicable and it is a useful tool in the classification of carcasses. So, this model makes possible to direct beef carcasses for suitable processing, giving rise to, e.g., steaks, minced meat, sausages, cured hams or hamburgers.

Knowing that meeting the preferences of consumers and their personal taste are key-criteria for the success of the meat industry, the creation of a monitoring system, based on this research model, could be a useful tool for the future design of a carcass and meat quality classification system. It is true that the meat sector has already adopted certifications in a large part of their products, namely Protected Designation of Origin (PDO) and Protected Geographical Indication (PGI), providing important guarantees of genuineness and traceability for consumers. However, these are certifications based on geographic and origin, have nothing to do, for example, with pH/temperature descriptors, as they do not provide any kind of information related to the quality of the products they certify. Since, producing beef that consistently provides a satisfactory eating experience meets consumer demand and adds value to cattle, the introduction of new certifications that give assurance of meat quality would be an excellent innovation for the sector and would serve as a complement for the existing certifications. This new certification would be based on the creation of a quality

assurance label that would identify the meat category, the maturation period and the ideal preparation methods for the specific cut.

This classification system would allow the meat to be paid according to its "quality level", thus high quality meat will be paid at fair price. The development and implementation of such a decision support system would give the opportunity to move from meat quality specifications to quality assurance of beef eating quality. Its implementation would be further facilitated since, as it was demonstrated throughout this work, knowledge of measurements of pH and temperature, shear-force and chilling rate would allow the monitoring of meat quality within a supply chain providing accurate information to abattoirs and producers. Essentially, such a decision support system will be focused in the assurance of meat quality and in monitoring the slaughterhouses chilling protocols.

Considering the results obtained in this research work, it is possible to develop a system to objectively predict the meat quality based on the carcass characteristics and pH/temperature decay descriptors. So, the models developed in this study have applicability to the objective classification and grading system of beef carcasses, as well as in the definition of the individual carcasses ageing period, and will be also useful for the optimisation of the rate of pH and temperature decline *post-mortem* in beef carcasses, by monitoring and adapting the abattoirs chilling protocol. Thus, our research results, despite it still has some limitations, may be beneficial to abattoirs, suppliers and consumers, as it provides a notorious contribution in the current demand for solutions to one of the major problems affecting the meat industry, beef meat tenderness.

4.5 REFERENCES

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5. Overall Conclusions

The meta-analyses conducted in **Chapters 2.1** and **2.2** confirmed the importance of the study of foodborne diseases for their prevention along the food processing chain of meat and meat products. Since foodborne diseases have a negative economic impact – due to the costs of surveillance investigation, treatment and prevention of illness – the implementation of safety measures in slaughterhouses and meat processing plants, as well as the education of the consumers, are essential to reduce the risk of transmission of *Salmonella* and other pathogens responsible for foodborne diseases.

Results from **Chapter 3.1** showed that a few carcass characteristics and pH decay descriptors are useful to objectively grade carcasses of different quality based on the prediction of meat tenderness. In addition, it was demonstrated that hot carcass weight, age class, carcass conformation (SEUROP) affected both temperature and pH decay descriptors (i.e, k_{pH}, k_{Temp}, etc.), whereas the temperature-decay descriptors were influenced by breed, gender and subcutaneous fat cover. Finally, we proved that it is feasible to classify carcasses according into cold-shortened and optimal quality carcasses, using the temperature and pH decay descriptors taken in the first three hours post-slaughter. This is of great importance since this early prediction allows an efficient carcass grading according to the expected meat quality. This information can also be used to define the optimal period of ageing and direct the carcasses to specific products according to the market's needs.

Subsequently, in **Chapter 3.2** a step forward was taken: models were developed, again using early slaughter measurements such as animal/carcass characteristics (sex, age, hot carcass weight, carcass class), and pH/temperature decay descriptors to estimate tenderness and drip loss of aged meat. Thus, with little information collected immediately after slaughter it is possible to predict the eating quality attributes of beef (as shear-force and cooking loss), and it is possible also to examine the evolution during the ageing period.

Both **Chapters 3.1** and **3.2** underscored that both temperature/pH decay descriptors, together with their interrelationships, are efficient predictors of meat quality than the sole ultimate pH measurement (pH at 24 hours post-slaughter). So, the prediction of meat tenderness can be made by recording the pH and temperature decline early *post-mortem* (during the first 3 hours after slaughter) of beef carcasses. Thus, the foremost hypothesis of this investigation was confirmed since there are strong interrelationships between pH and temperature decay descriptors early *post-mortem* with meat tenderness at different aging periods.

The pH/temperature decay logged data can be used to design a real-time monitoring system for beef meat quality. Along with other carcasses descriptors, this system should have applicability for the objective classification and grading of beef carcasses, as well as for the definition of the individual carcasses' ageing period. In cases where the abattoir's chilling conditions can be controlled, this system could also be useful for the versatile optimisation of the rate of pH and temperature decline early *post-mortem* in beef carcasses.

6. Future Perspectives

During the course of this research, a few questions and ideas for future work emerged. We already pointed out that the availability of information concerning foodborne hazards is essential to food safety authorities, producers and consumers. In this regard, our meta-analysis studies revealed that in Portugal such information is still limited. For instance, we found only a few primary studies reporting high occurrences of *Listeria monocytogenes* in broiler and *Staphylococus aureus* in pork. These two are pathogens of concern, whose occurrence and survival in these meats should be evaluated in further research work.

Under abattoir's chilling conditions, considerable variation among carcasses in the pattern of the pH and temperature decline early *post-mortem* can be expected, which will in turn result in great variation in meat tenderness. In spite of the abundant research undertaken to optimise meat quality and minimise its variability, the causes of the large variation in meat quality is still not fully understood. Moreover, the critical pH, temperature and time combinations affecting the biochemical/physical modifications of pre-rigor muscle and subsequent meat quality changes have not yet been clearly understood. Hence, this requires further investigation. A real time monitoring system of beef meat quality with the ability of storing and analysing the pH/temperature evolution will prove useful to enhance decision-making and optimise the carcasses classification and grading systems. Thus, a cheap and expeditious method to monitoring pH/temperature decline immediately after slaughtering bestows high-added value to the beef meat industry, since it will allow the study of genetic and environmental effects on the pH/temperature evolution in large scale giving information to decision support and to genetic improvement of these traits.

At the end of this work, we can state that the results obtained open up the way for the development of decision support, as well as an objective carcass classification system based on meat quality indicators. In this way, the results allow us to put forward two themes for future research and technological development, namely: (i) the effect of genotype (race, nucleotide polymorphisms - SNPs) on the evolution of the descriptors of pH/temperature decay and meat quality (viz. tenderness); and (ii) the development of an integrated system for collecting pH and temperature decline data to classify carcasses on the basis of objective meat quality parameters. The information collected may be used by producers to understand the reasons why the carcasses they produce do not meet the quality criteria. On the other hand, this information will allow the definition of strategies to correct eventual management failures, responsible for the occurrence of carcasses that do not meet the optimal quality criteria.

Annex

Annex I: Estudio de meta-análisis de las correlaciones entre las medidas de los tejidos obtenidas por ultrasonidos y sus homólogas de la canal de bovinos

C. Xavier, U. Gonzales-Barron, A. Müller and V. Cadavez
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ESTUDIO DE META-ANALISIS DE LAS CORRELACIONES ENTRE LAS MEDIDAS DE LOS TEJIDOS OBTENIDAS POR ULTRASONIDOS Y SUS HOMÓLOGAS DE LA CANAL DE BOVINOS

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INTRODUCCIÓN

Glass (1976) empleó por primera vez el término meta-análisis para referirse al análisis estadístico de resultados de diferentes ensayos clínicos para evaluarlos conjuntamente. El meta-análisis es una metodología estadística de un conjunto de publicaciones, con el objetivo de agregar y de comparar los resultados obtenidos por diversos estudios sobre el mismo tema (Viechtbauer, 2010). Así, los estudios de meta-análisis permiten combinar los resultados de varios estudios y reconocer patrones en los resultados de trabajos independientes (Gonzales-Barron *et al.*, 2012). Los estudios de meta-análisis pueden ser utilizados para estimar el efecto promedio y las diferencias de los efectos de varios estudios. Los trabajos que estudian las correlaciones de las medidas espesor de la grasa subcutánea (EGS) y del área del músculo *Longissimus dorsi* (AML), obtenidas por ultrasonido y las medidas homólogas efectuadas en la canal, presentan resultados muy variables y por veces contradictorios. De hecho, las correlaciones de cada uno de los trabajos son estimaciones y por tanto son portadores de imprecisiones. En este trabajo presentamos los resultados de un estudio de meta-análisis, de 9 estudios empíricos publicados en revistas internacionales, realizado con el objetivo de estimar el efecto promedio de las correlaciones de varios estudios.

MATERIAL Y MÉTODOS

Este trabajo de meta-análisis fue realizado con el paquete metafor (Viechtbauer, 2010) del software R (R Development Core Team, 2011). Para ello se utilizaron 9 artículos con información relativa a los coeficientes de correlación de Pearson entre las medidas de espesor de la grasa subcutánea (EGS) y del área del músculo *Longissimus dorsi* (AML) obtenidas por ultrasonido, a nivel de la 12-13ª costillas, en el animal vivo y las medidas homólogas efectuadas en la canal de bovinos. El tamaño del efecto promedio del coeficiente de correlación para las medidas EGS y AML fue determinado usando modelos de meta- análisis de correlaciones de efectos aleatorios y de efectos fijos basados en la transformación Z de Fisher. El sesgo de publicación fue evaluado por el gráfico de embudo, que es un diagrama de dispersión de las correlaciones estimadas *versus* el tamaño de muestra (*n*). Los estimados serán precisos cuando mayor fuera el tamaño de muestra. Laexistencia de heterogeneidad entre estudios fue evaluada por el índice I^2 que evalúa laproporción de la variación total que es atribuible a la heterogeneidad (Higgins and Thompson, 2002): $I^2 = T^2/(T^2 + \sigma^2)$ donde T^2 corresponde a la variabilidad entre estudios y σ^2 a la variabilidad interna en los estudios.

RESULTADOS Y DISCUSIÓN

Los resultados del meta-análisis para la medida EGS se presentan en la Figura 1. El tamaño del efecto medio presenta un valor elevado (0,78) y se puede considerar como significativa. Así, existe una relación elevada y positiva entre las medidas de EGS obtenidas por ultrasonido y las homólogas hechas en la canal. Se observó ausencia de homogeneidad enlos estudios y cerca de 78% (I^2 = 77,9%) de la variación total es atribuible a la heterogeneidad entre los estudios.

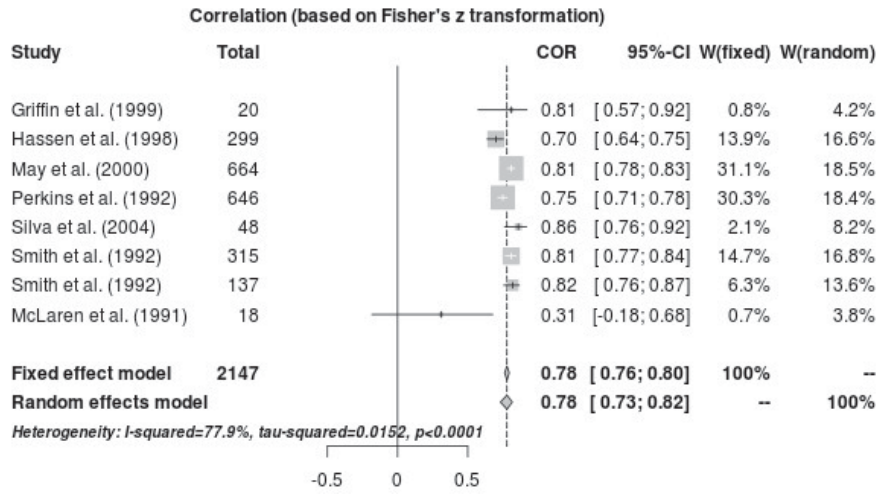

Figura 1. *Resultados del meta-análisis para la medida EGS*

Los resultados del meta-análisis para la medida AML se presentan en la Figura 2. El tamaño del efecto promedio presenta un valor de 0,54 que se puede considerar como significativo. Así, existe una relación elevada y positiva entre las medidas de AML obtenidas por ultrasonido y las homólogas efectuadas en la canal. Del mismo modo, se observó ausenciade homogeneidad entre estudios y cerca de 79% (I^2 = 78,3%) de la variación total esatribuible a la heterogeneidad entre los mismos.

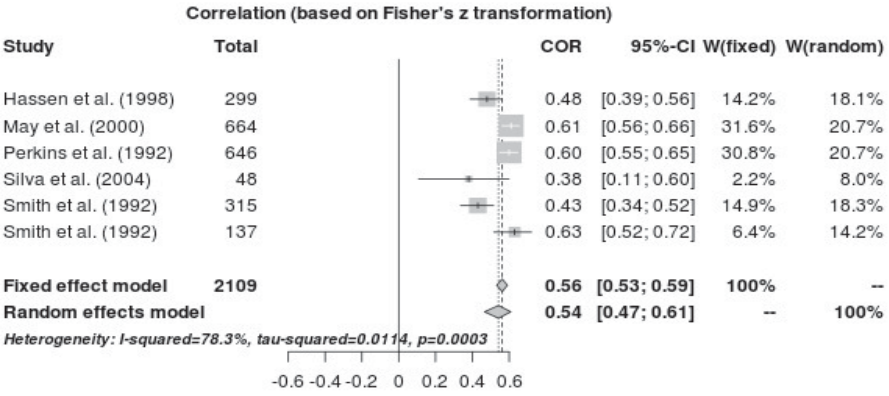

Figura 2. *Resultados del meta-análisis para la medida AML*

En ambas medidas (EGS y AML), se observó una elevada variabilidad entre estudios, y las correlaciones son influenciadas por efectos específicos de los estudios. Así, la variabilidad entre ellos se podrá corregir con la utilización de variables moderadoras que expliquen dicha heterogeneidad.

El presente estudio de meta-analisis confirmó las correlaciones positivas y significativas de las medidas de EGS y de AML, efectuadas por ultrasonido y las homólogas de la canal de bovinos. Los resultados contradictorios y variables resultan de condiciones específicas de los estudios como: raza y peso vivo de los animales y a los efectos de los operadores que realizan las mediciones de EGS y AML.

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META-ANALYSIS STUDY OF CORRELATIONS BETWEEN CARCASS TISSUES MEASUREMENTS OBTAINED BY ULTRASOUND AND TAKEN ON THE CARCASS OF BOVINES

ABSTRACT: The objective of this work was to use meta-analysis to estimate the effect size of the correlations between carcass tissues measurements obtained by ultrasounds and taken on the carcass of bovines. Eight idependent studies of correlations between carcass tissues measurements obtained by ultrasounds and taken on the carcass of bovines were used. In each study the correlations and the sample size were obtained and a correlations random-effects meta-analysis model was applied. The estimated correlations effect size was 0.78 (95% CI: 0.73-0.82), for the EGS measurement, and 0.54 (95% CI: 0.47-0.51), for the AML measurement, and the studies were hetereogeneous for both carcass tissues measuments. These results confirmedthe high and positive correlations between carcass tissues measurements obtained by ultrasounds and taken on the carcass of bovines.

Keywords: Bovines, Carcasses, Ultrasounds, Meta-analysis.

Annex II: Meta-analysis of *Salmonella* and *Campylobacter* in Portuguese fresh meats

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Méta-analyse de l'occurrence de *Salmonella* et de *Campylobacter* dans les viandes fraîches Portuguaises

Meta-analysis of *Salmonella* and *Campylobacter* in Portuguese fresh meats

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Résumé
La viande est un des principaux véhicules de maladies d'origine alimentaire chez l'homme causées par des pathogènes tels que *Salmonella* spp. et *Campylobacter* spp. Afin de prioriser la recherche sur les dangers microbiens, selon les catégories de viande, une méta-analyse à multiniveaux a été effectuée pour résumer l'information disponible sur l'incidence de ces agents pathogènes dans les viandes fraîches produites au Portugal (à savoir, la viande bovine, de poulet; du porc, la viande bovine et porcine hachée). Le taux moyen d'occurrence de *Campylobacter*, dans la viande de poulet frais Portugais (40%), était d'environ dix fois plus élevé que celui de *Salmonella* (4%), bien que les deux niveaux étaient comparables aux valeurs actuelles de l'UE. Cependant, pour les autres catégories de viande, la méta-analyse a révélé que l'incidence de *Salmonella* étaient légèrement à modérément plus élevé que la moyenne de l'UE, en présentant le viande du porc la plus haute occurrence de *Salmonella* (12,6%). Ces résultats appellent à la mise en œuvre des mesures de contrôle de la chaîne de production de viande de porc surtout au niveau de l'exploitation.
Mots-clés: incidence, prévalence, pathogènes, multiniveaux, effets aléatoires

Abstract
Meat is one of the main vehicles of food-borne diseases in humans caused by pathogens such as *Salmonella* spp. and *Campylobacter* spp. In order to prioritise research on those microbial hazards according to meat categories, a multilevel meta-analysis was conducted to summarise available information on the incidence of such pathogens in fresh meats produced in Portugal (i.e., bovine meat, broiler meat; pork, minced beef and minced pork). The mean occurrence rate of *Campylobacter* in Portuguese fresh broiler meat (40%) was about ten times higher than that of *Salmonella* (4%); although both levels were comparable to current EU ranges. Nevertheless, in the other meat categories the meta-analysed incidences of *Salmonella* were slightly-to-moderately higher than EU averages, presenting the Portuguese-produced pork the highest occurrence of *Salmonella* (12.6%). These results call for the implementation of on-farm control measures in the pork production chain.
Keywords: incidence, prevalence, pathogens, multilevel, random effects

1. Introduction

In Portugal, there is limited information on zoonoses as well as the occurrence of the main microbial contaminants in foods in the past years (Veiga et al., 2012). Nonetheless, given (i) the known strong association of food-borne diseases in humans with the consumption of contaminated meat and meat products, and (ii) the high consumption of meats (106 kg per habitant in 2012) in Portugal, it is imperative to gather as much information as possible on the occurrence of food-borne pathogen in Portuguese meats in order to understand the current epidemiological situation and prioritise microbial hazards for further study. Thus, the objective of this work was to quantitatively summarise and compare the occurrence of *Salmonella* spp., and *Campylobacter* in Portuguese fresh meats by conducting separate multilevel random-effects meta-analysis models.

2. Methodology

The *problem statement* in this study was the estimation of the overall incidence or occurrence of *Salmonella* spp. and *Campylobacter* spp. in Portuguese meats categorised as: fresh bovine, fresh broiler, fresh pork, minced beef and minced pork. The *population* was specified as meat produced in Portugal while the *measured outcome* is the detection of pathogens in meats sampled either at processing plants or at retail. Electronic searches were carried out to identify the relevant *primary studies* reporting the sought data. From each of the primary studies (j), the number of samples (s) experiencing the event of interest (i.e., testing positive for a pathogen) and the total number of samples (n) were extracted. While occurrence data of *Salmonella* could be found for all meat categories, occurrence values of *Campylobacter* could be only found for fresh broiler meat. For this reason, two data sets were created: (i) incidence values of *Salmonella* across meats, and (ii) incidence of *Salmonella* and *Campylobacter* in fresh broiler. Meta-analysis models were then fitted separately for each data set.

To measure the effect size θ (i.e., degree to which the hypothetical phenomenon – i.e., pathogens in meats – is present in the population, the logit transformation of the raw proportion $p=s/n$ was used,

$$\theta_j = logit\ p_j = \log\left(\frac{p_j}{1-p_j}\right) = \log\left(\frac{s_j}{n_j - s_j}\right) \qquad (1)$$

As most meta-analyses are based on sets of studies that are not exactly identical in their methods, which normally introduce variability (i.e., heterogeneity) among the true effects estimated by the primary studies, a random-effects meta-analysis model is more appropriate. In such a model, each primary study investigates its own true effect size Θ_j,

$$\theta_j = \Theta_j + \varepsilon_j = \overline{\Theta} + \nu_j + \varepsilon_j \qquad (2)$$

where θ_j is the observed effect size in the primary study j, $\overline{\Theta}$ the mean true effect size, and ε_j the error due to sampling variance. It is assumed that the ε_j have a normal distribution with mean zero and a true variance ξ^2. The term ν_j represents the deviation of the true study effect size Θ_j from the mean true effect size. The values of ν_j are normally-distributed random effects with mean zero and variance τ^2

(between-study variability). In this approach, two sources of variation are distinguished: sampling variation (ξ^2) and variation between true effect sizes (τ^2).

If the between-study variance is shown to be noteworthy, study characteristics or moderators can be added to the model to account for at least part of the heterogeneity in the true effects. This leads to the multilevel meta-analysis model. Thus, after fitting the random-effects meta-analysis model (Equation 2), the following multilevel meta-analysis models

$$\theta_j = \beta_0 + \left(\beta_1 X_{1j} + \beta_2 X_{2j} \ldots + \beta_p X_{pj}\right) + v_j + \varepsilon_j \qquad (3)$$

$$\theta_j = \beta_0 + \left(\beta_1 X_{1j} + \beta_2 X_{2j} \ldots + \beta_m X_{mj}\right) + v_j + \varepsilon_j \qquad (4)$$

were fitted using categorical moderating variables, defined as 'pathogen' p (for the data set of pathogens in broiler meat), or as 'meat category' m (for the data set of *Salmonella* across meat categories), respectively. The vector (β_1, β_2... β_p) or (β_1, β_2... β_m) refers to the shift in effect size coefficients of each pathogen or each meat category for the multilevel model either with pathogens or with meat categories as subgroups, respectively. The coded variable X_p or X_m takes the value of 1 for the pathogen or meat category subgroup. The objective of fitting meta-analyses with a moderator was to assess any statistical difference between subgroups (Ho: $\beta_1 = \beta_2 \ldots = \beta_{p/m} = 0$). This model treats the v_j as random effects that distribute normally with a mean zero and a variance of τ^2. Yet, τ^2 now denotes the amount of residual heterogeneity among the true effects, or the variability among the true effects that is not accounted for by the p or m moderators included in the model. Meta-analysis models were adjusted in R version 2.14.2 (R Development Core Team) using the 'metafor' package

3. Results and Discussion

The systematic review conducted in this study indicated that currently the information on the level of occurrence of certain pathogens in meats produced in Portugal is sparse. For instance, there is no knowledge on the incidence of *Campylobacter* in pork or beef. Except for minced broiler meat, *Salmonella* spp. comes out as the only pathogen that has been widely inspected in most of the meat categories with 29 primary studies retrieved. This data availability obeys to current national programmes in place to monitor *Salmonella* in bovine, pig and broiler meat sampled at processing plants and retail.

3.1 Incidence of *Salmonella* in Portuguese meats

The overall random-effects meta-analysis of the first data set indicated that the incidence of *Salmonella* spp. in Portuguese meats is 5.9% (CI: 3.8 – 9.0%). As displayed in the forest plot (Figure 1), considerable variability in reported incidence were observed among studies, which was proven by the significant test of heterogeneity ($p<0.001$). The between-study variance decreased from $\tau^2=1.21$ to 0.58 when meats were grouped in categories by the multilevel model, although there was still some unexplained residual heterogeneity. Fresh pork corresponds to the category presenting the highest incidence of *Salmonella* spp. diverging significantly from fresh bovine and fresh broiler meat. In contrast with the EU average occurrence for *Salmonella* in fresh pork (0.7%; EFSA, 2013) and minced pork (0.6%; EFSA, 2012), the corresponding occurrence values for Portugal (0.126; 95% CI: 0.08 –

0.19, and 0.034; 95% CI 0.015 – 0.078, respectively, in Figure 1) are much higher. This finding may be due to the fact that in Portugal there is no bacteriological or serological control programme of pigs on farm that could enable the application of risk management strategies at primary production. In a risk assessment model, Gonzales-Barron et al. (2009) underscored the need to target *Salmonella* contamination at swine production, calculating that on average 77% of the variability in the total contaminated carcasses at the point of evisceration is explained by the contamination from the carrier animals entering the slaughter lines. Thus, although in Portugal there is a national monitoring programme for *Salmonella* in pig meat based on sampling at slaughterhouse and meat cutting plants, the incidence of *Salmonella* in pork is still high as its control requires rather a systematic approach from farm to fork, with specific risk management strategies in place also at farm level. Referring to serovars, the most common ones present in Portuguese fresh pork include Typhimurium (37-70%), Derby (11-14%) and Rissen (7-14%) (Gomes et al., 2012; Baptista, 2010), which coincide to the most frequent serovars in pigs and pig meat isolated in the EU (EFSA, 2013).

In relation to the fresh bovine meat and minced beef produced in Portugal, the meta-analysed occurrences of *Salmonella* are 1.9% (95% CI: 0.5 – 7.2%) and 1.5% (95% CI: 0.3 – 7.8%), respectively (Figure 1), which are values moderately higher than the current EU average (0.2% and 0.4%, respectively; EFSA, 2013). On the contrary, the mean *Salmonella* occurrence in fresh broiler produced in Portugal (4.4%; 95 CI: 2.0 – 9.4%) appeared to be within the EU level (5.9%).

3.2 Incidence of *Salmonella* and *Campylobacter* in Portuguese broiler meat

The second meta-analysis study summarised the occurrence of *Salmonella* and *Campylobacter* in fresh broiler meat produced in Portugal. The discrepancy among the incidence values of the two pathogens can be visualised in the multilevel meta-analysis presented in Table 1. There was a high between-study heterogeneity (τ^2=3.22; Q=453 with p<0.001) which still remained significant after categorising the effect sizes by pathogen (τ^2=1.16; Q=178 with p<0.001). Although higher between-study variability was observed for *Campylobacter* in comparison with *Salmonella* (not shown), still the omnibus moderator test (QM) showed that the mean incidence of one pathogen differed significantly from the other (Table 1). The fact that *Salmonella* spp. in broiler meat had at least ten times lower incidence than *Campylobacter* (Table 1) is believed to be due to the positive impact of the national *Salmonella* control programmes at the primary production; this is, in flocks of laying hens, breeding flocks and broiler flocks, with risk reduction targets set in 2007. The same cannot be said for *Campylobacter* in fresh broiler as it high incidence in Portugal (40.3%; CI: 22.0 – 61.8%) follows the high EU average trend (31.3%; EFSA, 2013). Since 2005, campylobacteriosis continues to be the most commonly reported food-borne disease in the EU, with broiler meat considered to be the major source of the disease. It has been concluded that handling, preparation and consumption of broiler meat may account for 20-30% of human campylobacteriosis in the EU, whilst 50-80% may be attributed to the chicken reservoir as a whole. Moreover, 46% of the *Campylobacter* outbreaks in the EU in 2011, in which implicated food vehicle was provided, were associated to broiler meat (EFSA, 2013). Nevertheless, it is unfeasible to explore such a link in Portugal since campylobacteriosis cases are neither notified nor its food vehicle investigated. Some research has shown that *C. jejuni* (15-70%) and *C. coli* (40-70%) are the two species of highest incidence in Portuguese fresh broiler (Mena et al., 2008; Borges, 2009). Even though currently there is a lack of epidemiological and risk analysis studies of *Campylobacter* in Portuguese broiler meat, control measures should be directed to the consumers. Through food labelling and education campaigns, Portuguese consumers should be made aware that adequate cooking will assure safety of meats but serious undercooking or cross contamination from a raw to a cooked product in the kitchen are thought to be major routes of infection.

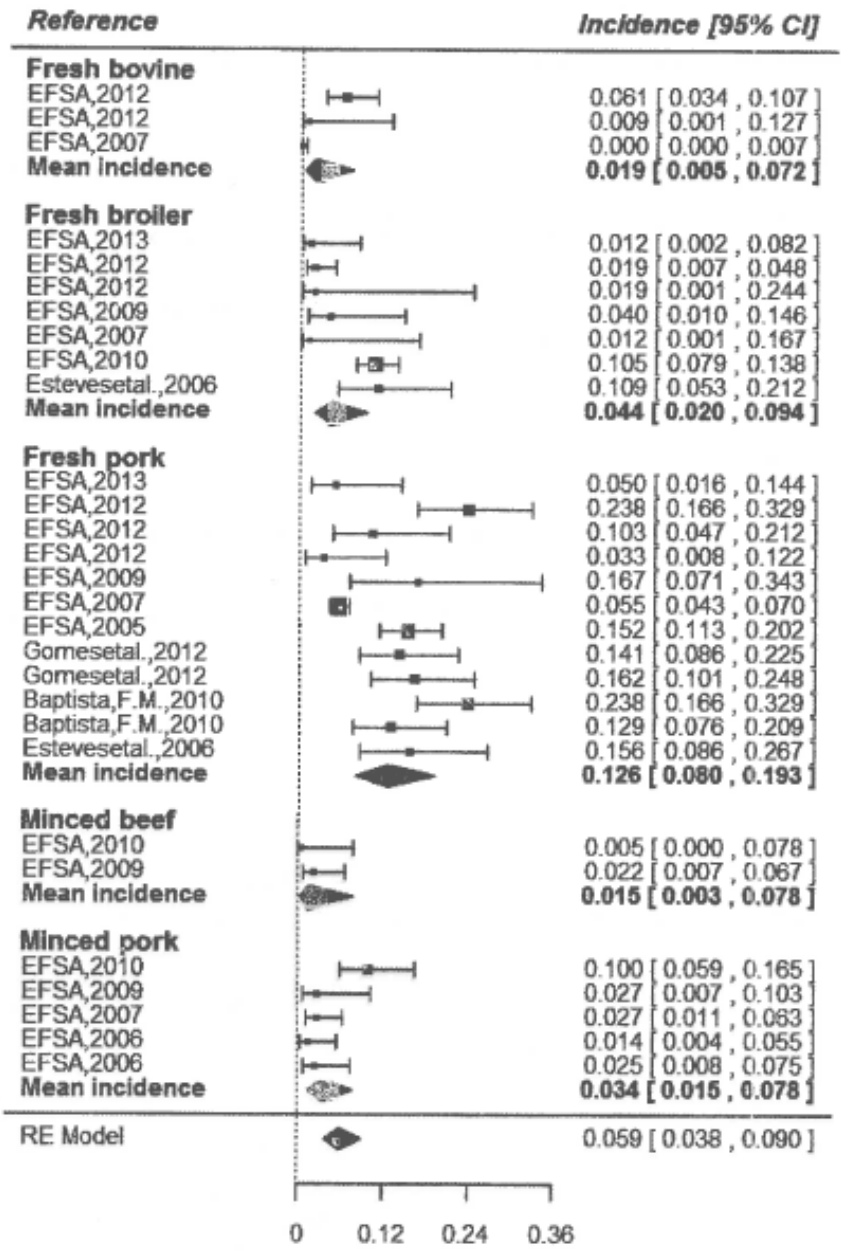

Figure 1: Forest plot of the multilevel random-effects model of incidence of *Salmonella* spp. in Portuguese meats

4. Conclusion

The results emphasised the necessity of Portuguese food safety agencies to take urgent monitoring and training actions for the maintenance of good hygiene and manufacturing practices during the production of the great variety of meat categories. The meta-analysis studies also highlighted important gaps of knowledge, and may assist food safety authorities in the prioritisation of microbiological hazards, and the implementation of essential food safety assurance systems at pork primary production.

Meta-analysis type	Effect size Incidence (CI)	τ^2	I^2 (%)	QM (F)	Q/QE
Overall random-effects	0.181 (0.082 – 0.353)***	3.22	97.6	-	453*** (df=15)
Multilevel					
Salmonella	0.040 (0.014 – 0.108)[a]	1.16	-	13.8*** (df_1=2, $_{df2}$=14)	178*** (df=14)
Campylobacter	0.403 (0.220 – 0.614)[b]				

[a,b] Superscript letters denote statistical differences between incidence of pathogens

Table 1: Results of the overall and multilevel meta-analysis models for the incidence of pathogens (*Salmonella* spp., and *Campylobacter*) in Portuguese fresh broiler

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Annex III: An exposure assessment model of the prevalence of *Salmonella* spp. along the production of Brazilian beef

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Un modèle d'évaluation de l'exposition pour la prévalence de *Salmonella* spp. le long de la production de boeuf brésilien

An exposure assessment model of the prevalence of *Salmonella* spp. along the production of Brazilian beef

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Résumé
L'objectif de cette étude était de construire un modèle d'évaluation de l'exposition pour la prévalence de *Salmonella* dans la viande bovine brésilienne. A cet effet, les facteurs de conversion fondés sur les distributions bêta ont été modélisées pour représenter l'effet de chaque étape de la production sur l'incidence de *Salmonella* et pour le dépouillement un facteur de transfert de la peau à la carcasse a été modélisé par méta-analyse. Un modèle de simulation de Monte Carlo a estimé la prévalence de *Salmonella* en les morceaux de viande désossé en moyenne de 7,8% (IC 95% : 1,9 à 21,9 %), qui est en accord avec des données de l'enquête (n = 4239) de Salmonella dans les morceaux de bœuf brésilien (moyenne de 4,2%, IC 95%: 3.6 à 4.8%) réalisée dans les abattoirs commerciaux. Les résultats ont souligné l'augmentation de la prévalence de *Salmonella* pendant l'éviscération/division et le désossage.
Mots-clés: simulation, méta-analyse, traitement, coupes de bœuf

Abstract
The objective of this study was to build an exposure assessment model elucidating the changes in *Salmonella* prevalence in Brazilian beef. To this effect, conversion factors based on beta distributions were modelled to represent the effect of every production stage on the *Salmonella* incidence and, for the dehiding process, a hide-to-carcass transfer factor was modelled by meta-analysis. The Monte Carlo simulation model estimated the *Salmonella* prevalence in beef cuts from boning halls to be on average 7.8% (95% CI: 1.9-21.9%), which was in agreement with a pool (n=4239) of survey's data of *Salmonella* in Brazilian beef cuts (mean 4.2%; 95% CI: 3.6 – 4.8%) carried out in commercial beef abattoirs. The results underscored the increase in *Salmonella* prevalence during evisceration/splitting and boning.
Keywords: simulation, meta-analysis, processing, beef cuts

1. Introduction

According to the Sanitary Surveillance of the Brazilian Department of Health, from a total of 6791 reported food-borne outbreaks occurring during 1999 to 2010 in Brazil, 46% of them were caused by *Salmonella* spp, being red meats the vehicle in 12% of the outbreaks occurred during the same period.

On the other hand, since 2005, Brazil has been one of the top producers of beef meat in the world with one of the highest annual export rates of over 2500 millions of tons (ABIEC, 2011). The limited amount of information on the occurrence of pathogens in the Brazilian beef meat has an impact on the international trade, leaving the country in some disadvantage in relation to other large producers such as United States and Australia. Thus, this study aimed to bring together all the incidence information, reported in the literature, of *Salmonella* in Brazilian beef along the different processing stages, in order to build an exposure assessment model.

2. Methodology

2.1 Transfer factor of *Salmonella* for the dehiding operation

Six studies (Brichta-Harhay et al., 2008; Gandra, 2011; Lanna et al., 2011; Lopes, 2011; Minuzzi et al., 2012; Silva, 2011) were found to report the incidence values on beef hides before bleeding and on carcasses after bleeding for the same animals. These binary results were combined by meta-analysis (Gonzales-Barron et al., 2013) on the effect size parameterization of relative risk, defined as the probability of encountering *Salmonella*-positive beef carcasses after dehiding relative to the probability of encountering *Salmonella*-positive hides before dehiding. The overall effect size of dehiding was estimated using a random-effects model, and then the distribution of the hide-to-carcass transfer factor (T_D) was modelled as $T_D = exp(Normal(-0.726, 0.169))$. The *Salmonella* prevalence on beef carcasses after dehiding (P_D), was calculated as $P_D = P_B \times T_D$, where P_B is the prevalence of *Salmonella* on beef hides after bleeding before dehiding.

2.2 Contamination factor of *Salmonella* for the evisceration and splitting operations

Incidence values of *Salmonella* spp. both before evisceration and after splitting were recovered from three studies (Narvaez-Bravo et al., 2013; Minuzzi et al., 2012; Lanna et al., 2011). The positive and the total number of samples were added together and a ratio of beta distributions modelling the contamination factor of *Salmonella* for evisceration and splitting (C_S) as, $C_S = Beta(24 + 1{,}572 - 24 + 1)/Beta(14 + 1{,}572 - 14 + 1)$. The prevalence of *Salmonella* after evisceration and splitting (P_S) was estimated as $P_S = P_D \times C_S$.

2.3 Conversion factor of *Salmonella* for the rinsing operation

A rinsing conversion factor of the prevalence of *Salmonella* was modelled using the results of three articles. While in the Brazilian studies (Minuzzi et al., 2012; Lanna et al., 2011), rinsing slightly increased the incidence of *Salmonella*, in the Venezuelan study (Narvaez-Bravo et al., 2013), rinsing had reduction effect. To consider that rinsing could increase, decrease or have no effect on the incidence of *Salmonella*, the ratio (C_R) was modelled by adding together the binary data of the three studies as $C_R = Beta(20 + 1{,}572 - 20 + 1)/Beta(24 + 1{,}572 - 24 + 1)$. The proportion of *Salmonella*-positive beef carcasses after rinsing (P_R) was estimated as $P_R = P_S \times C_R$.

2.4 Reduction factor of *Salmonella* for the chilling operation

For chilling, only one American study (Ruby et al., 2007) was found. Making use of a large sample size (n=5355), this study demonstrated that chilling has a decreasing effect on the recovery of *Salmonella* from beef carcasses. Assuming that such reduction effect obtained in the American abattoirs under evaluation is on average comparable to the one achieved in a common Brazilian beef

abattoir, a reduction factor of *Salmonella* due to chilling (R_{ch}) was modelled $R_{Ch} = Beta(53 + 1{,}5355 - 53 + 1)/Beta(123 + 1{,}5355 - 123 + 1)$; where 123 is the positive samples at the chiller entrance and 53 is the positive samples after 24 h chilling. The proportion of *Salmonella*-positive Brazilian beef carcasses after chilling (P_{Ch}) was estimated as $P_{Ch} = P_R \times C_{Ch}$.

2.5 Contamination factor of *Salmonella* for the boning operation

The results from a Brazilian study (Sigarini, 2004) investigating the effect of deboning on the microbiological quality of beef meat were used to model the contamination in the boning halls. Sigarini (2004), by analysing pieces of rump, observed that the incidence of *Salmonella* on beef meat due to boning increased slightly from 0.125 (10 out of 71) to 0.200 (16 out of 80). The contamination factor (C_D) due to boning was modelled as $C_D = Beta(16 + 1{,}80 - 16 + 1)/Beta(10 + 1{,}71 - 10 + 1)$. The proportion of *Salmonella*-positive Brazilian beef cuts (P_D) was estimated as $P_D = P_{Ch} \times C_D$.

2.6 Model validation using Brazilian data

The input of the stage-by-stage simulation model was the incidence of *Salmonella* on Brazilian beef hides after the bleeding operation (P_B). Data of interest were found from six individual studies (Table 1). The total number of beef hides swabbed after bleeding is represented by n_B while the number of *Salmonella*-positive samples is given by s_B. These binary data was combined on the basis that all these studies were conducted in Brazil and that the microbiological methods for determining *Salmonella* were comparable. Nonetheless, the microbiological protocol from these published studies differed in the hide swabbed area, which was 400 cm^2 for some and 100 cm^2 for other studies. Thus, it was deemed appropriate to allow for this difference by correcting the underestimated s_B values with a value of relative sensitivity as proposed by Logue and Nde (2007). The corrected s_B' and n_B of the six studies were added together, and the uncertainty about the incidence of *Salmonella* on Brazilian beef hides after bleeding (P_B) was modelled using a beta distribution as $P_B = Beta(119+1{,}967-119+1)$.

Source	Number of *Salmonella* (+) samples (s_B)	Total number of samples (n_B)	Hide swabbed area (cm^2)	Relative sensitivity of swabs (Se)	Corrected number of (+) samples (s_B')
Lopes (2011)	31	200	400	1.00	31
Souza et al. (2010)	5	52	400	1.00	5
Lanna et al. (2011)	11	135	400	1.00	11
Gandra (2011)	2	38	100	0.33	6
	1	22	100	0.33	3
Silva (2011)	4	120	100	0.33	12
Minuzzi et al. (2012)	11	200	100	0.33	33
	6	200	100	0.33	18
Pooled data		967			119

Table 1: Data sources utilised for the approximation of the incidence of *Salmonella* on Brazilian beef hides after the bleeding operation

For validation, the output of the model (P_D) was compared to the results of *Salmonella* incidence in beef cuts extracted from Brazilian published studies. The prevalence exposure assessment model was developed in Microsoft Excel using the @Risk add-in, and run for 10 000 iterations using Latin Hypercube sampling without any separation of uncertainty and variability.

3. Results and Discussion

The microbial levels of the hides are strongly correlated with carcass contamination, as a result of cross-contamination during processing (Barkocy-Gallagher et al., 2003). Thus, a transfer factor was meta-analytically modelled using the results from a few published studies to represent the transfer of contamination from hides to pre-eviscerated carcasses. The meta-analysis not only confirmed that a transfer of *Salmonella* from hides to carcass but also showed that the *Salmonella* incidence on carcasses is lower than the incidence on hides. The Brazilian literature presents a data gap on the prevalence of *Salmonella*-positive hides before bleeding, thus the input of the present model was the *Salmonella* prevalence on bled hides. The pool of the data (Table 1) suggested that the incidence of *Salmonella* on hides after the bleeding operation was 12.4% (95% CI: 10.4 – 14.6%). This estimate appears to be lower than other estimates (from 18 to 94%) reported by Fegan et al. (2005) and Brichta-Harhay et al. (2008). It is also lower than the incidence of 36.7% of *Salmonella* on hides from three large Venezuelan abattoirs surveyed by Narvaez-Bravo et al. (2013). Among other reasons, such as differences in sampling sites, method of *Salmonella* detection or seasonality, the relatively low value of *Salmonella* prevalence in Brazilian hides could be partly explained by the extent of the area swabbed in the Brazilian studies which was lower (100 – 400 cm^2 in Table 1) than in the studies mentioned above (hide areas ranging from 750 to 1000 cm^2). The model estimated that, after dehiding, the incidence of *Salmonella* on Brazilian beef carcasses is lower ($P < 0.05$) at an average of ~6.1% (95% CI: 4.1 – 8.6%; Table 2). This prediction was in reasonable agreement with the results from two Brazilian survey´s (Lopes, 2011; Lanna et al., 2011) where *Salmonella* was recovered with frequencies of 3.5% and 0.7% at this point of the chain.

Following carcass evisceration and splitting, the model predicted a higher ($P < 0.05$) *Salmonella* incidence of 10.8% (95% CI: 4.90 – 21.3%; Table 2). Through risk factor analysis, Narvaez-Bravo et al. (2013) found that *Salmonella* carrier animals had eight times higher likelihood of testing positive on hides, three times higher likelihood to test positive on pre-eviscerated carcasses, and two times greater likelihood to test positive on carcasses post-evisceration. The simulation model estimated that the carcass washing step, before carcass entry into the cooler, did not have any effect ($P < 0.05$) on the *Salmonella* incidence within slaughter groups. Although, on average, there is apparently a numerical reduction from 10.8% to 9.5%, a comparison between the confidence intervals for *Salmonella* on eviscerated carcasses (95% CI: 4.90 – 21.3%) and washed carcasses (95% CI: 3.3 – 21.5%, Table 2) evidences that, taking groups of carcasses, the washing operation could either increase or decrease the contamination. Buncic and Sofos (2012) explained that carcass washing per se could even further spread the microbial contamination to uncontaminated areas of the carcass if there was not previous removal of the contaminated area by knife trimming. The model´s estimate at this point of the beef production was again in reasonable agreement with the *Salmonella* occurrence rates (ranging from 1.9 to 3.7%) reported for Brazil by Minuzzi et al. (2012), Lopes (2011), Lanna et al. (2011) and Souza et al. (2010). Nonetheless, the estimated prevalence of *Salmonella* (9.5%) in pre-chill beef carcasses is higher than the mean incidence from Spanish (3.8%) and Italian (3.2%) slaughterhouses (EFSA, 2012).

Prevalence on hide/carcass	Mean (%)	95% CI (%)
After bleeding (input)	12.4	[10.4 – 14.6]
After dehiding	6.09	[4.10 – 8.60]
After evisceration/splitting	10.8	[4.90 – 21.3]
After rinsing	9.50	[3.30 – 21.5]
After chilling	4.60	[1.50 – 10.9]
After boning	7.80	[1.90 – 21.9]

Table 2: Means and confidence intervals of the model´s outputs for the incidence of *Salmonella* spp. on Brazilian beef carcasses at the different processing stages

The model suggested that the process of cooling and chilling has an effect ($P < 0.05$) on the recovery of *Salmonella* cells, reducing the incidence approximately by half on average (4.6%; 95% CI: 1.5 – 10.9%; Table 2). Our model´s output is comparable to the results of a survey from a Mexican slaughterhouse where *Salmonella* was recovered in 6% of the beef carcasses sampled after 24 hours of dry chilling. Slightly lower incidences were surveyed from two American studies involving very large surveys at beef abattoirs: 1% by Ruby et al. (2007) and 3% for Rose et al. (2002). The simulation model estimated that the prevalence of *Salmonella* in Brazilian beef cuts is on average 7.8% (95% CI: 1.9 – 21.9%). This final output was well supported by a pool (mean 4.9%; 95% CI: 1.8 – 11.5% of survey's data extracted from Almeida et al. (2010), Colvara (2007) and Goldschmidt (2004) who tested a total of 103 Brazilian deboned beef from commercial beef abattoirs. Figure 1 shows the distribution of the total uncertainty around this value. The distribution shape found by simulation, considerably wide and skewed, may reflect the substantial variation in the prevalence on *Salmonella* among production batches.

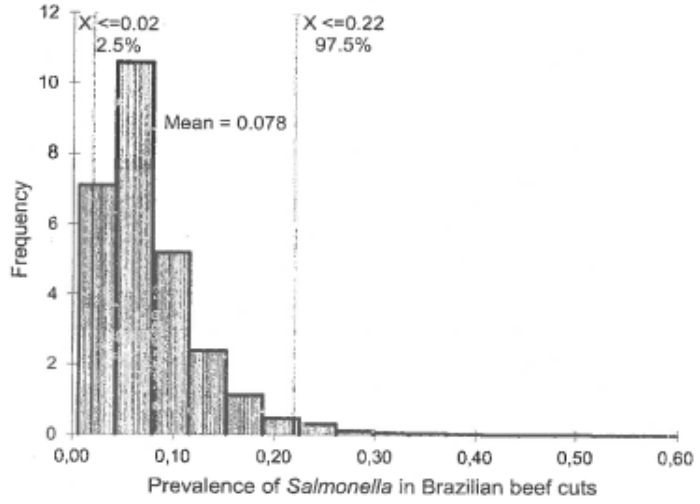

Figure 1: Prevalence of *Salmonella* spp. in beef cuts produced in Brazil showing mean and 95% confidence interval as estimated by the simulation model

4. Conclusions

Our meta-analytical prevalence model has shown that, while the stages of evisceration/splitting and boning may amplify the contamination, rinsing and chilling may decrease the contamination of *Salmonella* spp. Although faeces and hides of carriers animal are major sources of contamination, the spread of this pathogen during the process can be still minimised by the correct implementation of food safety programs. When hygienic slaughter procedures and sanitary programs are working properly, the ideal is that the load of pathogens that initially enter the plant with the live animals decreases at the critical stages of dehiding, trimming, rinsing and chilling.

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Annex IV: Modelling the temperature and pH decline early *post-mortem* of beef carcasses

C. Xavier, U. Gonzales-Barron, A. Müller and V. Cadavez
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MODELLING THE TEMPERATURE AND pH DECLINE EARLY *POST-MORTEM* OF BEEF CARCASSES

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KEYWORDS
pH, temperature, decline, meat, exponential decay

ABSTRACT

The objective of this work was to model the pH and temperature decline early *post-mortem* on beef carcasses and to study the effect of gender, genotype and weight class on the pH and temperature decline patterns. A total of 24 beef animals slaughtered in a local abattoir were sampled. pH and temperature were recorded using an OMEGA wireless receiver/host (UWTC-REC1). The decline of pH and temperature was modelled using one parameterisation of the exponential decay function, and its parameters were estimated using the software R. The fitted models were used to predict pH and temperature at 1.5 h, at 3.0 h and at 24 h; the time when pH reached 6.0, and the temperature at which pH reached 6.0. The rate parameters of the exponential decay function for pH (K_{pH}) and temperature (K_T) were found to be independent (r=0.35, P>0.05). The correlation between pH at 3 h and final pH (at 24 h) was very high (r=0.930, P<0.01). The K_T was influenced by the time elapsed from slaughter until the first recording, and by the carcass weight. In opposition, those variables did not affect the K_{pH}. The exponential decay function was able to model the early *post-mortem* decline of both pH and temperature, and the pH at 3 hours can be used as predictor of the final pH of beef meat.

INTRODUCTION

Temperature and pH control is extremely important since it affects meat tenderness, and varies with animal species, cooling rate and the stress level that the animal is subjected to before harvesting [Bianchini et al. 2007].
During the first 24 h *post-mortem*, the rate of temperature decline affects the biochemical and structural changes during the conversion of muscle to meat. High temperature accelerates the pH decline in muscle [Kahraman et al. 2012]. During this period, the rate of decrease in pH and the final pH of meat are highly variable. When the process of glycolysis develops slowly, the initial pH (right after slaughter), which is about 7.0, goes down to 6.4-6.8 after 5 hours, and subsequently to 5.5-5.9 after 24 hours [Roça 2000].
If, due to a deficiency of glycogen, the final pH (after 24 hours) remains high, above 6.2, the muscle turns into DFD meat (dark, firm, dry or dark-cutting). Thus, the pH 6.0 is considered as the limit value for the emergence of DFD meat [Bianchini et al. 2007]. In general, for optimal eating quality, beef should reach a pH of 6.0 while the carcass temperature is between 15ºC and 35ºC [Thompson 2002]. In addition, the storage temperature of carcasses is another factor that can cause significant changes in the rate of chemical reactions *post-mortem*. One of the most significant effects of storage temperature is the phenomenon of cold shortening, which consists in accelerating muscle metabolism with low temperatures (0-10ºC) [Roça 2000].
Studies have shown that temperature and pH (initial, final and decline rates) are good predictors of meat quality (flavour, juiciness, tenderness and texture). Also, pH is a good predictor of the colour and drip loss of meat [Ibarburu et al. 2007]. Therefore, given the importance of pH and temperature for the meat quality, it would be of great benefit for meat processors to know in advance how they are likely to behave. This would allow the introduction of rapid chilling systems, for example, to reduce evaporative weight losses without adversely affecting meat tenderness.
Thus, the objective of this study was to model the decrease in pH and temperature during chilling of beef carcasses in order to assess their predictability from an earlier stage. A second objective was to verify whether sex, breed and weight class have any influence on the pH and temperature decline rates.

MATERIALS AND METHODS

Animals

In this study, a total of 24 beef animals (17 crossbred, undefined breed, and 7 Mirandesa), 18 males and 6 females, slaughtered in a local abattoir were sampled. The carcasses had an average hot carcass weight (hcw) of 203.4 ± 52.61 kg. Carcasses were assigned to a "light" category if weight was below 200 kg and to a "heavy" category if the weight was above this value.
The animals were transported by truck to the slaughterhouse when they were 9.7 ± 1.91 months old. At arrival, they were led to individual stalls, where they were kept until slaughtering. They were not fed at the slaughter plant, but they did receive water *ad libitum*.

Temperature and pH recording

Two hours after slaughter, the pH and temperature decline were recorded, at intervals of 10 min during 24 h of carcass chilling, in *longissimus thoracis* muscle at the level of the 4th rib. The pH and temperature measurements were made using an OMEGA wireless receiver/host (UWTC-REC1) equipped with a weather resistant wireless pH/temperature transmitter and temperature probe Pt100.

Temperature and pH decay modelling

The pH and temperature decline *post-mortem* (p.m.) were modelled as a function of time using the parameterisation of the exponential decay function proposed by Hwang and Thompson (2001), as follows:

$$Y_{(t)} = A_{(u)} + (A_{(i)} - A_{(u)})e^{-Kt}$$

where $Y_{(t)}$ is the pH or temperature at time t; $A_{(u)}$ is the final pH or temperature; $A_{(i)}$ is the initial pH or temperature; K is the exponential constant of decay; and t is the time in hours after slaughtering.

The parameters ($A_{(u)}$, $A_{(i)}$ and K) were estimated using the non-linear least squares (nls) function implemented in the software R (R Core Team, 2014). The fitted models were used to predict the pH at 1.5 h ($pH_{1.5}$), at 3.0 h ($pH_{3.0}$) and at 24 h (pH_{24}), the time when pH reached 6 (t_{pH6}), the temperature at 1.5 h ($T_{1.5}$), at 3.0 h ($T_{3.0}$), and at 24 h (T_{24}) and the temperature at which pH reached 6 (T_{pH6}).

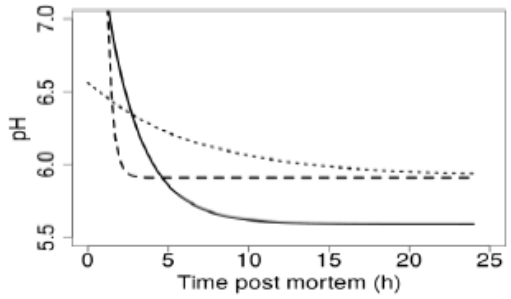
Data analysis

Data were analysed using correlation and analysis of variance models. One way analysis of variance was used to investigate the effects of gender (male and female), breed (Crossbred and Mirandesa breed), and carcass weight class (V – Veal and Z - Steer). Regression analysis was used to study the relationship between $pH_{3.0}$ and pH_{24}.

RESULTS AND DISCUSSION

Modelling the pH and temperature decline

Figure 1 presents the pH decline of three beef carcasses during cooling showing different patterns. The k_{pH} varied between 0.092 and 1.149, and the results clearly indicated that large differences in pH decline are likely to occur among carcasses. It can be observed that the pH decline rate was higher during the first few hours after slaughtering (approximately 5 hours). After 5.0 hours, the pH continues to decrease but at a slower rate, becoming relatively constant. A poor pH decline was observed in 68% of the carcasses, with a final pH higher than 5.8, which is a reason for concern considering the meat quality obtained from those carcasses [Pearce et al., 2010].



Figures 1: pH decline of three beef carcasses during cooling showing different patterns

In this study, the pH decline can be considered overall as suboptimal since a slow pH–temperature decline was observed. Thus, the slaughterhouse chilling regime needs to be improved so that carcasses attain the quality target of pH = 6 at 18 ºC.

Figure 2 presents the temperature decline of three beef carcasses during cooling showing different patterns. The k_T varied between 0.074 and 0.235, showing that also the temperature decline was highly variable among carcasses, and this variability was associated with high (> 35 ºC) and low (< 15 ºC) temperatures when pH reached the critical value of 6. The mean temperature at pH 6 was 22.0 ºC, however 26% of the carcasses presented a final pH higher than 6.

The prediction of the temperature decline curve can be used to adjust the cooling rate in order to force carcasses' temperature lay between 35 and 15 ºC when pH reach the critical value of 6.

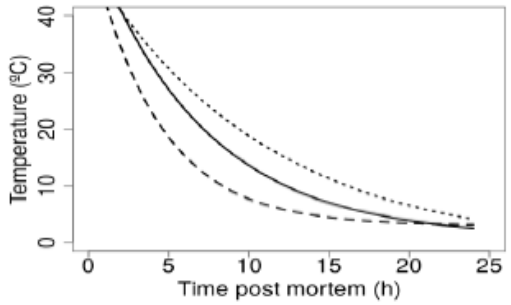


Figure 2: Temperature decline of three beef carcasses during cooling showing different patterns

Figure 3 shows the relationship between the K_{pH} and K_T parameters. These two parameters presented a low and non-significant correlation (0.35, $p<0.05$), which corroborated previous results of Hwang et al. (2001) who stated that they are independent parameters. The small data set used in this study and occurrence of some influential points may bias this relationship. Hence, results should be interpreted with some caution. More data should be collected in order to clarify this relationship.

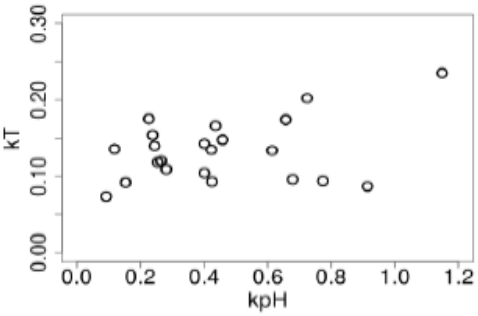


Figure 3: Relationship between the K_{pH} and K_T parameters

The simple linear correlations and the k-adjusted linear correlations among $pH_{1.5}$, $pH_{3.0}$ and pH_{24} are compiled in Table 1. The correlation between the $pH_{3.0}$ and pH_{24} was very high and highly significant (0.930, $P<0.01$) as suggested in Figure 4. These results show that $pH_{3.0}$ can be used to predict the final pH (pH_{24}) of beef meat. It is an interesting finding, as using data obtained early post-mortem carcasses could be classified according to their final pH.

Table 1: Correlations among $pH_{1.5}$, $pH_{3.0}$ and pH_{24}: a) simple correlations below the ones-diagonal; and b) k-adjusted correlations above the ones-diagonal

	$pH_{1.5}$	$pH_{3.0}$	pH_{24}
$pH_{1.5}$	1	0.59	-0.15
$pH_{3.0}$	0.48*	1	0.62
pH_{24}	-0.07ns	0.62**	1

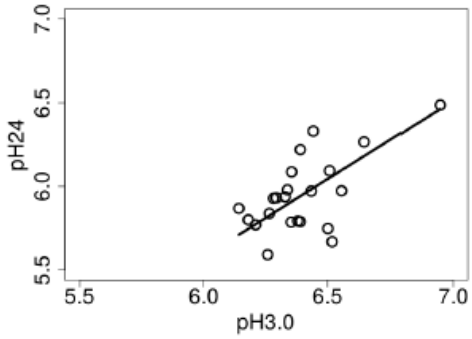


Figure 4: Relationship between $pH_{3.0}$ and pH_{24}

Effect of gender, genotype and class on temperature and pH decline

Table 2 shows the effect of gender on the K_{pH}, K_T, $pH_{1.5}$, $pH_{3.0}$, pH_{24}. No differences were found ($P > 0.05$) in these parameters between females and males. The last pH_{24} presented a wide range of values equally in males (5.59 to 6.09) and females (5.67 to 6.49). Similar patterns were found for the effects of genotype presented in Table 3, and class presented in Table 4.

Table 2: Effect of gender on K_{pH}, K_T, $pH_{1.5}$, $pH_{3.0}$, pH_{24}

	Female (n=16)	Male(n=6)
K_{pH}	0.355 ± 0.1771^a	0.487 ± 0.2995^a
K_T	0.132 ± 0.1318^a	0.134 ± 0.0472^a
$pH_{1.5}$	6.68 ± 0.2351^a	6.98 ± 0.5872^a
$pH_{3.0}$	6.30 ± 0.1186^a	6.43 ± 0.1837^a
pH_{24}	5.83 ± 0.1688^a	5.99 ± 0.2285^a

CONCLUSIONS

Beef carcasses showed high variation in the pH and temperature decline patterns early post-mortem, which results in high variability observed in the ultimate pH_{24}. The exponential decay model showed a good fit to the pH and temperature data, and parameters K_{pH} and K_T were found to be independent. This model can be used to predict the meat quality indicators $pH_{3.0}$, pH_{24}, T_{pH6}. The predicted pH at three hours after slaughtering ($pH_{3.0}$) seems to be a good predictor of the ultimate pH (pH_{24}) in beef meat. This study clearly shows that a quality control system should be implemented in the slaughter-house to ensure an optimal meat quality.

Table 3: Effect of genotype on K_{pH}, K_T, $pH_{1.5}$, $pH_{3.0}$, pH_{24}

	Crossbred (n=16)	Mirandesa(n=6)
K_{pH}	0.456 ± 0.3119^a	0.441 ± 0.1893^a
K_T	0.122 ± 0.0395^a	0.158 ± 0.0316^a
$pH_{1.5}$	6.96 ± 0.6204^a	6.76 ± 0.2156^a
$pH_{3.0}$	6.38 ± 0.1982^a	6.43 ± 0.1248^a
pH_{24}	5.91 ± 0.2238^a	6.03 ± 0.2122^a

Table 4: Effect of class on K_{pH}, K_T, $pH_{1.5}$, $pH_{3.0}$, pH_{24}

	Veal (n=16)	Steer (n=6)
K_{pH}	0.491 ± 0.2954^a	0.442 ± 0.2774^a
K_T	0.135 ± 0.0364^a	0.133 ± 0.0421^a
$pH_{1.5}$	6.80 ± 0.7561^a	6.84 ± 0.4755^a
$pH_{3.0}$	6.44 ± 0.1082^a	6.39 ± 0.1893^a
pH_{24}	5.87 ± 0.1217^a	5.97 ± 0.2389^a

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BIOGRAPHY

CRISTINA XAVIER is a Portuguese national, graduated in Animal Science Engineering at the School of Agriculture of the Polytechnic Institute of Braganza, Portugal, in 2011. Currently, she is on her 3rd year of PhD in Animal Science, with an investigation whose general objective is to develop a novel monitoring system of pH and temperature decline post-mortem for beef meat carcasses.

URSULA GONZALES-BARRON is a Peruvian-Irish national with a first degree in Food Engineering and a PhD in Biosystems Engineering from University College Dublin, Ireland. She conducts her research on the development of novel modelling methods for food safety and quality, covering predictive microbiology, risk assessment and meta-analysis. She has published circa. 50 peer-reviewed papers.

VASCO CADAVEZ is a Portuguese national with a first degree in Animal Husbandry Engineering, a Masters degree in Animal Production, and a PhD degree focused on meat quality. He is currently a Professor at the Animal Science Department of the Polytechnic Institute of Braganza. He is currently developing methods in meta-analysis, dynamic modelling and predictive microbiology; and has published over 30 articles.

Annex V: Estudio de meta-análisis del efecto de estimulación eléctrica en la fuerza de corte de la carne de vacuno

C. Xavier, U. Gonzales-Barron, A. Müller and V. Cadavez
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AIDA (2015), XVI Jornadas sobre Producción Animal, Tomo II, 609-611

ESTUDIO DE META-ANÁLISIS DEL EFECTO DE ESTIMULACIÓN ELÉCTRICA EN LA FUERZA DE CORTE DE LA CARNE DE VACUNO

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INTRODUCCIÓN

La terneza de la carne de vacuno es el principal atributo de calidad (Hopkins y Fogarty, 1998) y su variación resulta en pérdidas de valor al ser rechazada por los consumidores. Este atributo puede ser evaluado objetivamente por la fuerza de corte, simulando la acción de la masticación, y se determina generalmente por el método de Warner-Bratzler (Huff- Lonergan y Lonergan, 2005). Así, la fuerza de corte requerida para cortar una muestra de carne es inversamente proporcional a la terneza de la carne.

La estimulación eléctrica (EE) se basa en el envío de una corriente eléctrica a la canal, la cual acelera el rigor mortis. La utilización de la EE previene el endurecimiento por el frio, porque induce el uso de ATPs antes del inicio del *rigor mortis*, acelera la glicólisis anaeróbica e incrementa la caída del pH. Así, la carne sometida a la EE es más tierna como resultado de la menor contracción del musculo en el *rigor mortis*. Diversos autores (Aalhus *et al.*, 1994; Strydom y Frylinck, 2014; Strydom *et al.*, 2005; Hwang y Thompson, 2001; Kim *et al.*, 2007; Kuttinarayanan y Ramanathan, 2010; White *et al.*, 2006; Hopes-Jones *et al*., 2010) estudiaron el efecto de la EE en la terneza de la carne de vacuno; sin embargo, ningún trabajo hasta el momento resumió los resultados obtenidos con este método. El meta-análisis es una metodología estadística que permite comparar los resultados obtenidos por diversos estudios sobre un determinado tema (Viechtbauer, 2010), y permite combinar los resultados de varios trabajos independientes y reconocer patrones en los resultados de estos trabajos (Gonzales-Barron *et al.*, 2012). Este trabajo tuvo como objetivo efectuar un estudio de meta-análisis sobre el efecto de la EE en la terneza de la carne de vacuno.

MATERIAL Y MÉTODOS

Este estudio de meta-análisis fue realizado usando el paquete *metafor* (Viechtbauer, 2010) del software R (R Development Core Team, 2011). Para esto, se utilizaron 8 artículos con información relativa a la media y errores estándar de la fuerza de corte de la carne obtenida de canales no estimuladas (Control) y estimuladas eléctricamente (Tratamiento). De este modo, el estudio se basa en la comparación de la diferencia media de la terneza del músculo *Longissimus dorsi* y *Longissimus lumborum*, de la carne de vacuno sometidas o noa la EE. El tamaño del efecto promedio total fue determinado usando modelos meta-analíticos de efectos aleatorios y los resultados se visualizaron a través de un “forest plot”.

La existencia de heterogeneidad entre los estudios publicados fue evaluada por el índice I^2, definida como la proporción de la variación total que es atribuible a la variación entre estudios (Higgins y Thompson, 2002).

RESULTADOS Y DISCUSIÓN

Para este estudio meta-analítico, se definió un tamaño de efecto como la diferencia media entre la terneza promedio de la carne de canales estimuladas (tratamiento) y la terneza promedio de aquéllas no estimuladas (Control). Así, un tamaño de efecto negativo indica que las carnes de las canales estimuladas eléctricamente presentan valores de fuerza de corte más bajos, mientras que un valor positivo indica lo contrario. Aunque el modelo de efectos aleatorios indica la presencia de heterogeneidad (I^2=83.4%) entre estudios, se puede concluir que la EE contribuye a aumentar (P<0.001) la terneza de la carne de vacuno con un tamaño de efecto estimado de -1.34kgf. El “forest plot” (Figure 1) muestra también que los estudios presentan diferente precisión (intervalo de confianza diferentes) y que el estudio de Strydom *et al.* (2005) presentó una mayor contribución para el tamaño de efecto promedio, como se observa por la mayor dimensión del cuadrado. Por otro lado, el trabajo de White *et al.* (2006) presentó el mayor tamaño del efecto (-3.15 kgf), aunque exhibió también la mayor variación (CI: -7.37 a 1.07).

La EE contribuye a un aumento global de la terneza de la carne de vacuno, ya que estimula directamente el músculo después de la muerte del animal, provocando la aceleración del proceso de *rigor mortis*, y la caída inmediata del pH (Lombard, 2009). La EE contribuye para que el *rigor mortis* ocurra a una temperatura elevada y evita la ocurrencia del endurecimiento por frío en el músculo. Asimismo, también acelera el proceso de maduración de la carne (Simmons *et al*., 2008). En resumen, la EE mejora las características de calidad (color, terneza, etc.) de la carne de vacuno y puede ser utilizada para reducir las variaciones en los atributos de calidad de la carne, resultante de los efectos ambientales tales como edad, nutrición y estrés del animal (Lombard, 2009).

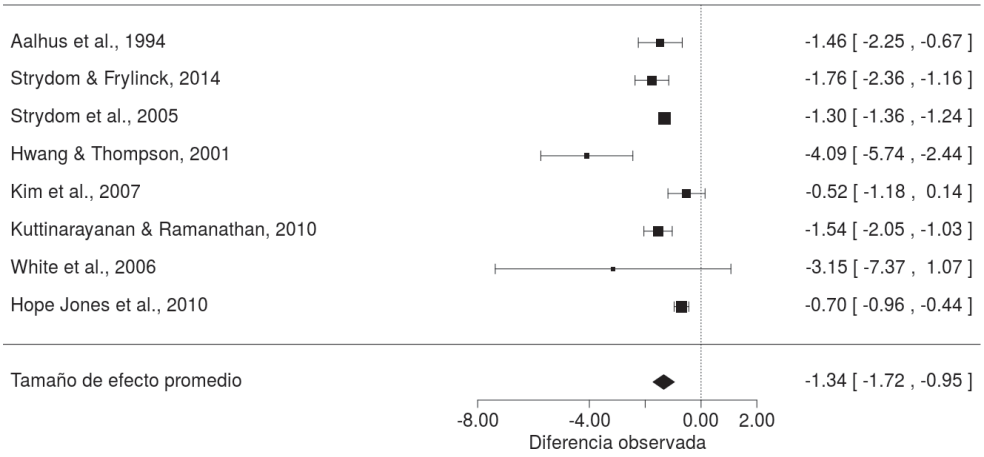

Figura1. *Gráfico de "forest plot" del efecto de la estimulación eléctrica sobre la fuerza de corte de carne de vacuno. En paréntesis se muestran los intervalos de confianza al 95%.*

El tamaño del efecto desplegó una gran variación entre estudios; variación esperada pues resulta de las diferentes condiciones experimentales de los mismos. Sin embargo, aplicando un modelo de tipo aleatorio, dicha variabilidad entre estudios fue extraída, y el tamaño del efecto promedio final confirmó el efecto positivo de la estimulación eléctrica en la terneza de la carne de vacuno.

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META-ANALYSIS STUDY OF THE ELECTRICAL STIMULATION EFFECT ON BEEF MEAT TENDERNESS

ABSTRACT: The objective of this work was to use meta-analysis to estimate the effect size of the electrical stimulation on beef tenderness through the study of the measurements of shear force. Eight independent studies were used based on comparison of shear force measurements on the *Longissimus dorsi* and *Longissimus lumborum* in cattle carcasses subject to electrical stimulation and unstimulated carcasses. For each study, the mean effect size and standard error was calculated in order to apply a random-effects meta-analysis model. The meta-analysis demonstrated that the electrical stimulation on beef carcasses decreases the values of shear-force of meat by an average of 1.34 kgf. Thus, this study confirmed the positive effect of the electrical stimulation on the beef meat tenderness. However, the effect size displayed high variation among studies which can be attributed to differences in their experimental conditions.

Keywords*:* beef meat, shear force, electrical stimulation, meta-analysis.

Annex VI: Classifying beef carcasses according to meat quality using animal/carcass characteristics and pH/temperature decline descriptors early *post-mortem*

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CLASSIFYING BEEF CARCASSES ACCORDING TO MEAT QUALITY USING ANIMAL/CARCASS CHARACTERISTICS AND pH/TEMPERATURE DECLINE DESCRIPTORS EARLY *POST-MORTEM*

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KEYWORDS
Fat, Shortening, Tenderness, Classification, Modelling .

ABSTRACT
During beef carcass chilling, the eating quality of meat can be severely affected by either hot- or cold-shortening. With basis on previous knowledge that meat of optimal tenderness can be produced when *rigor mortis* (pH=6.0) is attained when carcass temperature falls between 12-35ºC, the objective of this study was to predict meat quality from modelled pH/temperature decay descriptors and informative animal/carcass characteristics. Temperature and pH from a total of 103 beef carcasses were logged during 24 h post mortem, and subsequently modelled by exponential decay equations that estimated temperature (k_T) and pH (k_{pH}) decay rates. In addition, a number of pH/temperature decay descriptors were estimated from the fitted models. From linear models adjusted to each of these descriptors, it was found that, generally, hot carcass weight, age, gender and class (male, female, young animals) had significant influence on pH/temperature decay. Thus, bringing together the orthogonal variables k_T and k_{pH}, and the aforementioned animal/carcass characteristics as linear predictors of discriminant functions, a classification analysis was performed. While cold-shortened and hot-shortened carcasses were classified correctly for all samples, optimal quality carcasses were correctly classified in 87.5% of the samples.

INTRODUCTION

The inconsistency in the eating-quality characteristics of meat, predominantly tenderness, is one of the problems faced by the meat industry worldwide. Moreover, the importance of providing end-users with a food product of consistent quality has never been greater (Simmons et al. 2006). Thus, a top priority factor in the success of meat industry relies on the ability to deliver specialities that satisfy the consumer's taste requirements (Cortez et al. 2006). Since for the consumers the beef sensory attribute is most important in their individual assessments of overall eating satisfaction, tenderness is the primary consideration (Fergurson 2004).

However, meat tenderness is a complex trait that is influenced by a variety of factors, many of which can be managed systematically to reduce the incidence of tenderness problems in the final product (Platter et al. 2003). Muscle pH and temperature decline continuously interact during *rigor* development to affect both muscle contracture and proteolytic enzyme activity (Tornberg 1996). High temperature accelerates the pH decline in muscle and during this period, the rate of decrease in pH and the ultimate pH of meat are highly variable (Kahraman et al. 2012). The combination of a very rapid pH decline with a slow chilling regime causes heat shortening, which is an increase in toughness due to sooner exhaustion of μ-calpain at high carcass temperatures, leaving less potential for ageing. On the other end, the phenomenon of cold shortening emerges if the pH decline is too slow, remaining high while the temperature falls (Roça 2000). Cold-shortened carcasses produce tougher meat than hot-shortened ones do.

Hwang et al. (2003) showed that, in order to minimise cold-shortening – to enhance tenderisation, the muscle temperature should not be lower than 11°C before muscle pH reaches 6.1–6.3. When the process of glycolysis develops slowly, the initial pH (right after slaughter), which is about 7.0, goes down to 6.4-6.8 after 5 hours, and subsequently to 5.5-5.9 after 24 hours (Roça 2000). If, due to a deficiency of glycogen, the final pH (after 24 hours) remains high, above 6.2, the muscle turns into DFD meat (dark, firm, dry), so the limit value for emergence of DFD meat is pH 6.0 (Bianchini et al. 2007). The concept of ideal "window" was conceived to be used as a specification to describe the relationship between carcass pH and temperature from slaughter to when ultimate pH is reached (Thompson 2002; Ibarburu et al. 2007). The window requires the carcass pH to be greater than 6.0 while the carcase temperature is above 35°C and below 6.0 before the temperature falls below 12°C. If the rate of pH temperature decline does not fall through this ideal window, the carcass tenderness is compromised, either by hot- or cold-shortening.

Thus, the objective of this study is three-fold. The first objective is to model the decrease in temperature and pH during chilling of beef carcasses early *post-mortem* so that pH and temperature decline rates can be accurately estimated. The second objective is to evaluate whether live-animal/carcass characteristics (i.e., sex, weight, age, breed, class, fat, conformation, and transport and lairage time) have any influence on pH and temperature decline rates; or, instead, the muscle shortening occurring during *rigor mortis* outweighs the effects of live-animal factors in determining meat quality. Once the significant live-animal/carcass characteristics affecting pH and temperature decline are identified, linear discriminant analysis that classifies beef carcasses quality (i.e., tenderness) into (i) optimal quality, (ii) cold-shortened and (iii) hot-shortened,

is to be developed taking into account the ideal "window" rule.

MATERIALS AND METHODS

Beef animals

In this study, a total of 103 beef animals (68 cross-breed and 35 of Mirandesa breed), 68 males and 35 females, slaughtered in a local abattoir were sampled. The animals had an age of 10.1 ± 2.32 months when killed at one abattoir located in the Northeast of Portugal. The animals were transported by truck to the abattoir; and, at arrival, they were kept in individual stalls until slaughtering. They were not fed but did receive water *ad libitum*. After electric stunning, animals were slaughtered and dressed sequentially. Resulting carcasses had an average hot carcass weight of 209.7 ± 65.60 kg. For each of the animals/carcasses, the following live-animal/carcass characteristics were annotated: sex, age, breed, transport time, lairage time, hot carcass weight, animal class, the SEUROP class from the European beef carcass classification scheme for conformation and degree of fat cover of the carcass.

Temperature and pH recording

Approximately two hours after slaughter, pH and temperature were recorded at intervals of 10 min during 24 h of carcass chilling, in *longissimus thoracis* muscle at the level of the 4th rib. The pH and temperature measurements were made using a weather resistant wireless transmitter CRISON pH probe (Crison Instruments, SA, Spain, SP) and wireless transmitter OMEGA temperature probe Pt 100 (Omega Engineering Limited, United Kingdom, UK) connected to an OMEGA UWTC-REC1 wireless channel receiver/host (Omega Engineering Limited, United Kingdom, UK).

Statistical modelling

Fitting exponential decay models

The pH and temperature decline *post-mortem* (p.m.) were modelled as a function of time using the parametrisation of the exponential decay function proposed by Hwang and Thompson (2001). For modelling pH measured as a function of time pH(t), the three-parameter decay function was defined as,

$$pH(t) = pH_0 + (pH_0 - pH_\infty)\exp(-k_{pH}t) \quad (1)$$

where pH_∞ is the final pH; pH_0 is the initial pH; k_{pH} is the exponential constant of pH decay; and t is the time in hours after slaughtering. Time zero was set to match the time of slaughter. The three-parameter model for temperature (ºC) as a function of time (T(t)) was defined as,

$$T(t) = T_0 + (T_0 - T_\infty)\exp(-k_{Temp}t) \quad (2)$$

where T_∞ is the final temperature (ºC); T_0 is the initial temperature (ºC); and k_{Temp} (h^{-1}) is the exponential constant of temperature decay. Equations (1) and (2) were fitted to each of the experimental decay curves originated from the 103 beef muscles. Models' adequacy was assessed by examining normality of residuals and heterocedasticity. Using the model parameters (pH_0, pH_∞, k_{pH}, T_0, T_∞ and k_{Temp}), the following pH/temperature decay descriptors were computed for each of the curves: the pH at 1.5 h ($pH_{1.5}$), at 3.0 h ($pH_{3.0}$), at 4.5 h ($pH_{4.5}$) and at 24.0 h (pH_{24}); the temperature at 1.5 h ($Temp_{1.5}$), at 3.0 h ($Temp_{3.0}$), at 4.5 h ($Temp_{4.5}$), and at 24.0 h (T_{24}); the time when pH reached 6.0 ($t_{pH6.0}$), and the temperature at which pH reached 6.0 ($Temp_{pH6}$). Thus, these predicted descriptors were available for every carcass.

Effect of live-animal/carcass characteristics on pH/temperature decay descriptors

In order to evaluate whether live-animal/carcass characteristics affect the pace at which pH and temperature decline in a beef carcass early *p.m.*, analyses of variance (ANOVA) were conducted separately on each of the temperature/pH decay descriptors (i.e., $pH_{1.5}$, $pH_{3.0}$, $pH_{4.5}$, pH_{24}, $Temp_{1.5}$, $Temp_{3.0}$, $Temp_{4.5}$, $t_{pH6.0}$, $Temp_{pH6}$) as response variables with live-animal/carcass characteristics as explanatory variables. The characteristics related to the animal/characteristics considered as regressors were: sex, age, breed, hot carcass weight (HCW), transport time ($t_{Transport}$), lairage time ($t_{Lairage}$), SEUROP classification for conformation (Conf), degree of fat cover (Fat) and animal class (A=male aged 12-24 months, E=female aged between 12-24 months and Z=either sex aged 8-12 months).

Linear discriminant analysis

Taking into account the ideal window rule, an additional class variable named 'Quality' was created in the dataset to assign carcasses to one of three classes (i.e., cold-shortened, hot-shortened and optimal quality) which were quality categories known from the experimental data. A carcass was classified as "hot-shortened" or "cold-shortened" if $Temp_{pH6.0}$ was higher than 35 or lower than 12, respectively. For carcasses in between, the classification of "Optimal" was given. Linear discriminant analysis was then performed in order to investigate whether selected pH/temperature decay descriptors and animal/carcass characteristics could accurately classify carcasses by quality into the three classes. Misclassification rates were then computed. While exponential models and ANOVA were fitted using the n*lme* and *MASS* packages, multivariate analyses were conducted using the *cluster* and *psych* packages, all of them implemented in the software R (R Core Development Team).

RESULTS AND DISCUSSION

All the pH and temperature experimental curves obtained from the 103 carcasses could be closely depicted by the chosen exponential decay models. There were no convergence problems and residuals from each model could be approximated to normal distributions. The models' fitting quality can be appreciated in Fig. 1 for four muscle samples. Notice that both pH_0 and T_0 were fixed at 7.0 and 39.0ºC, since time zero represents the time of slaughter in the models. Descriptive statistics of the pH and temperature decay descriptors are compiled in Table 1. Sampled muscles' pH decreased steadily from ~7.0 to median values of 6.41, 6.10 and 5.90, at the corresponding median temperatures of 34.1, 29.2 and 25.0ºC after 1.5, 3.0 and 4.5 hours p.m, respectively (Table 1). Nonetheless, a considerable variation in pH and temperature decay between carcasses under commercial conditions was observed (Fig. 1).

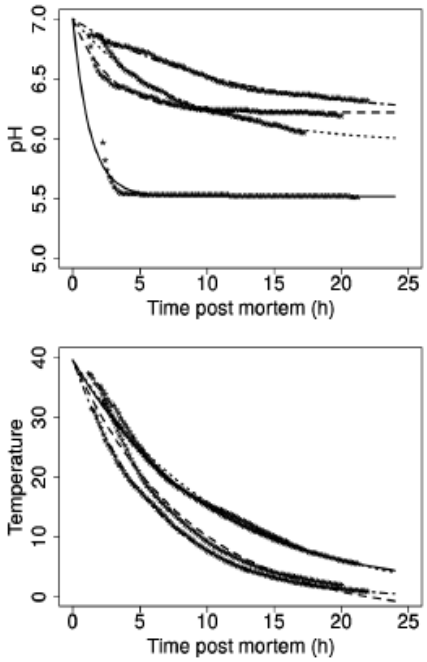

Figure 1: pH (top) and Temperature (bottom) Decline Curves from Four Sampled Carcasses Showing Fitted Exponential Model

Among the pH and temperature decay descriptors, the lowest variation between carcasses was registered at 1.5 h ($Temp_{1.5}$, $pH_{1.5}$). The time at which carcasses reached pH 6.0 was highly variable and ranged between 2.2 and 43.2 hours (i.e., some carcasses did not attain pH=6.0 by the end of the 24-h recorded period). Similarly, the temperature at which carcasses reached pH 6.0 had a wide range between 5.9 and 36.0ºC (Table 1). Taking into account the ideal window rule, it can be deduced that both phenomena hot- and cold-shortening occurred in the surveyed commercial abattoir, although the former at a much lower frequency (3.2%) than the latter (27.9%). Only a proportion of 68.8% of the sampled carcasses presented pH/temperature optimal decay for becoming meat of good eating quality.

Table 1: Mean, Median and Range of pH and Temperature Decline Descriptors (Predicted Values)

Predicted	Mean	Median	Min	Max
$pH_{1.5}$	6.48	6.41	6.00	6.90
$pH_{3.0}$	6.18	6.10	5.90	6.90
$pH_{4.5}$	6.02	5.90	5.60	6.80
$Temp_{1.5}$ (ºC)	33.6	34.1	27.1	38.8
$Temp_{3.0}$ (ºC)	28.8	29.2	18.9	34.3
$Temp_{4.5}$(ºC)	24.5	25.0	13.8	31.9
$Time_{pH6.0}$ (h)	5.25	2.96	2.17	42.3
$Temp_{pH6.0}$ (ºC)	26.6	29.2	5.89	36.0

In the sampled beef muscles, no correlation was found between pH (k_{pH}) and temperature (k_T) decay rates (r=-0.05; Table 2), which corroborated earlier results in electrically-stimulated beef carcasses (Hwang and Thompson 2001). The fact that these decay rates are randomly distributed (Fig. 1) is advantageous from a statistical modelling viewpoint since they can be used as orthogonal (i.e., independent) variables in the development of a multivariate algorithm for carcass quality classification. From the correlation analysis (Table 2), it was deduced that also $pH_{3.0}$ was a descriptor suitable for inclusion in classification analysis because it contains information of both the time and temperature at which *rigor mortis* takes place (correlation of 0.80 with $Time_{pH6.0}$, and -0.87 with $Temp_{pH6.0}$) and the ultimate pH of the carcass (correlation of 0.86 with pH_{24}). Thus, $pH_{3.0}$ descriptor represents a measurement that, although taken earlier during chilling, can predict with some accuracy the remaining pH decline trend.

Table 2: Correlation Matrix of pH/Temperature Decay Rates and Selected Decay Descriptors

	k_{pH}	k_{Temp}	$Time_{pH6}$	$Temp_{pH6}$	$pH_{3.0}$	pH_{24}
k_{pH}	1.00					
k_{Temp}	-0.05	1.00				
$Time_{pH6.0}$	-0.54	-0.09	1.00			
$Temp_{pH6.0}$	0.67	-0.26	-0.76	1.00		
$pH_{3.0}$	-0.73	0.13	**0.80**	**-0.87**	1.00	
pH_{24}	-0.39	0.13	0.76	-0.70	**0.86**	1.00

In the analyses of variance for the pH decay descriptors $pH_{1.5}$, $pH_{3.0}$, $pH_{4.5}$ and pH_{24}, the hot carcass weight (HCW) was consistently significant (Table 3). The inverse relationship between carcass weight and pH at the different times (as indicated by the negative sign of the regression coefficients in Table 3) suggests that a beef carcass that is larger and heavier can be associated to higher muscle glycogen reserves, which in turn prompts a faster pH drop. As opposed to hot carcass weight, breed had barely any impact on the pH/temperature descriptors, except for pH_{24}. From the least square estimates, beef carcasses from Mirandesa breed seemingly reached, on average, a lower ultimate pH (pH_{24}=5.611) than those from cross-breed animals (pH_{24}=5.778; Table 3).

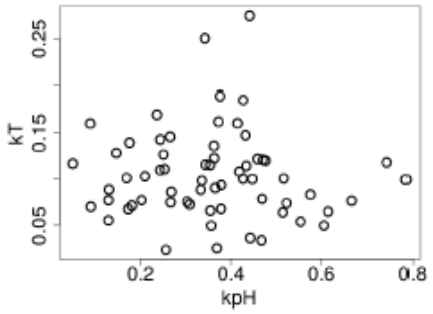

Figure 2: Distribution of the Fitted Exponential Decay Rates for pH (k_{pH}) and Temperature (k_T)

Carcass characteristics associated to animal size (Fat, Gender and Class) were found to have a strong impact on the pH decay rate. Least-square mean estimates revealed the trend that as thickness of fat cover increases, from class 1 (log

Table 3: Estimates of Live-Animal/Carcass Characteristics as Significant Explanatory Variables in the Linear Models Fitted to pH and Temperature Decay Descriptors

pH/Temperature decay descriptors	Live-animal/carcass characteristics[1]	Mean	St. deviation	P>|t|
pH at 1.5 h ($pH_{1.5}$)	HCW	-0.001	0.001	0.078
pH at 3.0 h ($pH_{3.0}$)	HCW	-0.001	0.001	0.078
pH at 4.5 h ($pH_{4.5}$)	HCW	-0.001	0.001	0.084
pH at 24 h (pH_{24})	HCW	-0.002	0.001	0.004
	Breed	-0.169	0.111	0.133
	Breed[2] Cross	5.778	0.049	-
	Mirandesa	5.611	0.092	-
pH decay rate ($\log k_{pH}$)	Fat 2	0.133	0.086	0.127
	Fat 3	0.082	0.090	0.365
	Fat 4	0.178	0.104	0.094
	Gender – Male	0.086	0.050	0.092
	Class E	0.170	0.098	0.091
	Class Z	0.046	0.067	0.490
	Fat[2] 1	0.255	0.087	-
	2	0.388	0.040	-
	3	0.337	0.036	-
	4	0.432	0.065	-
	Gender[2] female	0.310	0.047	-
	male	0.396	0.042	-
	Class[2] A	0.281	0.070	-
	E	0.450	0.068	-
	Z	0.328	0.029	-
Temperature decay rate ($\log k_{Temp}$)	HCW	-0.0015	0.0004	<.0001
	Age	-0.0247	0.0119	0.042
Time to pH 6.0 (h) ($\log Time_{pH6.0}$)	Class E	-0.175	0.166	0.300
	Class Z	0.055	0.115	0.630
	Class[2] A	0.511	0.106	-
	E	0.335	1.127	-
	Z	0.567	0.042	-
Temperature decay rate ($\log k_{Temp}$)	HCW	-0.0015	0.0004	<.0001
	Age	-0.0247	0.0119	0.042
Time to pH 6.0 (h) ($\log Time_{pH6.0}$)	Class E	-0.175	0.166	0.300
	Class Z	0.055	0.115	0.630
	Class[2] A	0.511	0.106	-
	E	0.335	1.127	-
	Z	0.567	0.042	-
Temperat. at pH 6.0 (°C) ($Temp_{pH6.0}$)	HCW	0.025	0.016	0.1308
	Lairage time	5.784	2.463	0.0230
Temperat. at 1.5 h (°C) ($\log Temp_{1.5}$)	HCW	0.0002	0.00004	<.0001
	Gender – Male	-0.0096	0.00048	0.052
	Gender[2] female	1.532	0.004	-
	male	1.522	0.003	-
Temperat. at 3.0 h (°C) ($\log Temp_{3.0}$)	HCW	0.0005	0.00007	<.0001
	Gender – Male	-0.0183	0.00949	0.059
	Gender[2] female	1.466	0.007	-
	male	1.448	0.006	-
Temperat. at 4.5 h (°C) ($\log Temp_{4.5}$)	HCW	0.001	0.001	<.0001
	Gender – Male	-0.007	0.016	0.653
	Fat 2	0.025	0.026	0.339
	Fat 3	0.041	0.028	0.148
	Fat 4	0.066	0.033	0.054
	Gender[2] female	1.388	0.011	-
	Male	1.381	0.009	-
	Fat[2] 1	1.350	0.026	-
	2	1.376	0.010	-
	3	1.392	0.009	-
	4	1.416	0.019	-

[1]Model estimates, standard deviations and P-values are shown only for animal/carcass characteristics that were significant in the analysis of variance (Pr(F)<0.10)

[2]Least-squares means and standard deviation of factor levels were computed only when categorical variables (i.e, breed, fat, gender, class) were significant

k_{pH}=0.255, then k_{pH}=1.79) to class 4 (log k_{pH}=0.432, then k_{pH}=2.70), the rate of pH decay increases (Table 3). Likewise, 12-24 month-aged female animals (Class E) also

produced a faster pH decay (log k_{pH}=0.450, then k_{pH}=2.80) than male (Class A, k_{pH}=1.91) and younger animals (Class Z, k_{pH}=2.13).

In the case of temperature decay, once again animal characteristics related to animal size such as hot carcass weight ($p<.0001$) and age ($p=0.042$) moderated the rate of muscle temperature decay (Table 3). Hot carcass weight (slope estimate -0.0015) and age (slope estimate -0.0247) were inversely correlated with temperature decay rate because, in smaller animals, heat is more rapidly liberated and, in addition, heat transfer does not get slowed down by greater fat levels. Such retarding effect of fat on heat transfer rates became more evident in the linear model for temperature at 4.5 h. Progressively fatter carcasses (from fat classes 1, 2, 3 and 4) reached increasingly higher average temperatures after 4.5 h p.m. (22.4, 23.8, 24.7 and 26.1ºC, respectively; taking the antilogarithm of the least square mean estimates presented in Table 3). Said otherwise, a fatter carcass requires longer time to reach the lower temperature of a leaner carcass at the same time. Both animal gender and hot carcass weight strongly influenced the temperature decay p.m., as denoted by the consistent statistical significance of these variables in the linear models for $Temp_{1.5}$, $Temp_{3.0}$ and $Temp_{4.5}$. As female animals are associated to greater carcass size and fat deposits than males, following the reasoning above, it is not unexpected that recorded muscles samples from females presented higher temperatures than the ones from males (notice that least-square mean estimates for $Temp_{1.5}$, $Temp_{3.0}$ and $Temp_{4.5}$ for females are systematically higher than for males; Table 3).

In the linear models for the descriptors related to rigor mortis, the only variable that statistically influenced the time to reach pH 6.0 was the carcass class. However, the carcass class may be thought of being an interaction between animal age and gender. The smaller carcasses of animals aged between 8-12 months, class E, took the longest to attain rigor mortis (log $Time_{pH6.0}$=0.567, so $Time_{pH6.0}$=3.7 h in Table 3), because, as explained earlier, smaller carcasses are associated to lower glycogen levels, which retards pH drop. Contrarily, carcasses from females aged 12-24 months took on average a significantly shorter time to reach rigor mortis (for Class E, log $Time_{pH6.0}$=0.567, so $Time_{pH6.0}$=2.2 h) than the ones from males (for Class A, log $Time_{pH6.0}$=0.511, so $Time_{pH6.0}$=3.2 h). Unexpectedly, lairage time was positively associated to the carcass temperature at rigor mortis ($p=0.023$). This could have been an effect of the abattoir logistics of slaughtering smaller animals first. The carcass temperature at *rigor mortis* was influenced by the hot carcass weight. The positive coefficient (0.025; Table 3) implies that in heavier carcasses, the pH drops faster while temperature is still high, in comparison to lighter carcasses where pH drops at a slower pace while they get cooler.

From the correlation analysis between pH/temperature decay descriptors (Table 2) and the assessment of animal/carcass characteristics capable of regulating the pH/temperature decay descriptors (Table 3), the variables containing most information that were considered for the prediction of meat tenderness – according to the ideal window rule, were: hot carcass weight, age, gender, class, k_{pH}, k_{Temp}, $pH_{1.5}$, $pH_{3.0}$, $Temp_{1.5}$ and $Temp_{3.0}$. Figure 2 shows the distribution of the paired values obtained from the two linear discriminant functions that were derived from the variables mentioned above, and evaluated for 93 carcasses. While cold-shortened (26/26) and hot-shortened carcasses (3/3) were classified correctly for all samples, optimal quality carcasses were correctly classified in 87.5% of the samples (48/56). Three optimal quality carcasses were mistakenly assigned to the cold-shortening category while other five optimal quality carcasses wrongly assigned to the hot-shortened category.

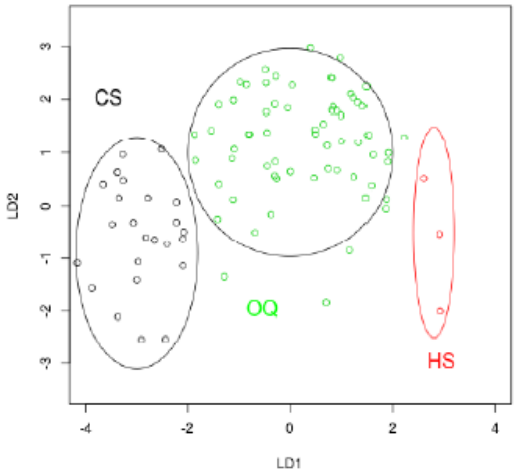

Figure 3: Discriminant Analysis of Meat Quality as Predicted by Two Linear Discriminant (LD) Functions of pH/Temperature Decay Descriptors and Animal/Carcass Characteristics; and Clustered by Classes: Cold-Shortened (CS), Hot-Shortened (HS) and Optimal Quality (OQ)

Under the commercial conditions of the surveyed abattoir, there was considerable variation in rigor time (2.2 – 42.3 h) and rigor temperature (5.9 – 36.0ºC), which allowed both modelling of pH and temperature decay, and the assessment of significant animal/carcass characteristics affecting pH/temperature decay. The exponential decay equation turned out to be an adequate model to describe the carcass decay in both pH and temperature. Linear models adjusted to key descriptors extracted from the fitted pH/temperature decay curves revealed that animal class, gender, age, hot carcass weight and degree of fat coverage are important traits that modulate the carcass pH and temperature decay. Although further work is still required, results from this investigation corroborated the feasibility to classify the quality of meat into cold-shortened, hot-shortened and optimal quality from early *post mortem* pH/temperature decline information and animal/carcass characteristics that are regularly annotated in a commercial abattoir.

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