



## **MID TERM SUMMARY REPORT**

**Covering Period 0 to 36 Months**

FOOD-CT-2006-36241

***ProSafeBeef***

Advancing Beef Safety and Quality through Research and Innovation

Integrated Project in FP6

**Start date of project:** March 2007

**Duration:** 5 years

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**Coordinating Body:** Ashtown Food Research Centre, Teagasc, Dublin, Ireland.

## **Pillar 1: Quantitative risk assessment of microbial and chemical hazards to maximise beef safety**

### **WP 1.1 Tools for online detection of biological contamination on beef carcasses**

**DNA-based diagnostic methods for indicator micro-organisms:** DNA technology was used to identify suitable bacterial indicators of faecal contamination. Groups of bacterial species consistently present in faecal material were identified and the presence of these bacteria validated in faeces from a large number of animals. Target genes in these bacteria are now being employed and tested in a PCR detection platform.

**Spectroscopic imaging:** Chlorophyll is ubiquitous in green plants and thus livestock diets. During digestion in the gut chlorophyll is partially degraded to coloured and fluorescent intermediates. *In vivo* studies initially in sheep have been used to identify the best chlorophyll breakdown products for use as markers of faecal contamination. Chlorophyll metabolites (combination of phaeophytin and phaeophorbide) from animals fed a range of diets have been found to be detected in faecal samples using spectral imaging techniques. Four feed additives based on chlorophyll were identified as potential additives which increased the fluorescence intensity of faeces when fed at 1 g/d. The most effective marker (PX) was assessed in a large scale beef production study with animals on two contrasting diets based on either concentrate and straw or grass silage. Measurements of faecal fluorescence have been recorded and investigation of faecal contamination carried out in a beef production study in the abattoir using the developed techniques (AbU, Nofima). A patent has been filed on the marker. The research work is now completed within *Prosafefbeef* but further funding was sought and obtained under a nationally funded project to continue refinement of the method. The technology is being investigated as a demonstration item in Pillar 6.

### **Workpack 1.2 Persistence and virulence of key microbial pathogens in the beef chain:**

**Characterisation:** Isolates of *Listeria*, *Campylobacter*, *Salmonella* and VTEC from the beef chain (hides, carcasses, minced beef, ready-to-eat beef) in different countries and geographic regions have been characterised (serotyping, antibiotic resistance testing, virulence gene profiles, molecular sub-typing) to gain knowledge of the diversity, resistance and human virulence potential of common beef chain pathogens. Some regional differences in pathogen characteristics have been noted in relation to antibiotic resistance and virulence. In relation to VTEC a key finding is that there is a huge diversity of serotypes but only a small portion have human virulence potential. An established project strain database is continuously updated. The database isolates are available to all consortium members.

**Biofilms** tools to evaluate bacterial adherence to abiotic surfaces and biofilm formation have been developed and optimised. Biofilm formation of a panel of *L. monocytogenes*, VTEC, *Salmonella* and slaughter house surface isolates has been studied using these methodologies. The effects of relevant environmental factors, also including effect of other bacteria present in slaughter house environments, on survival and biofilm formation of pathogens have been and will be further studied to obtain knowledge on how various factors affect survival, biofilm formation and persistence of pathogens in the beef chain. Increased survival and biofilm formation of VTEC were observed in the presence of the background micro-flora under dynamic conditions, illustrating effects of environmental bacteria on pathogen survival and persistence.

### ***Basis for persistence, stress adaptation and virulence of pathogens***

Virulence gene profiling of beef and clinical VTEC isolates has been performed using genome arrays. Virulence gene profiling of beef *Campylobacter* isolates by PCR has been performed. Studies have been initiated to study the effect of selected relevant stresses on VTEC and *Campylobacter* to gain more knowledge on associations between genotype and phenotype (virulence, stress response, fitness) of pathogens in the beef chain. Studies on molecular markers and proteins involved in adherence and biofilm formation of *L. monocytogenes* and *Salmonella* are in progress. Studies include mutant library generation of *L. monocytogenes* and proteomics of planktonic and sessile cells of *L. monocytogenes* and *Salmonella*. A close link between T1.2.2 and T1.2.3 activities is here evident. The effect of acid stress on molecular and physiological responses in *L. monocytogenes* using microarray experiments is ongoing.

## **WP 1.3 Quantitative risk assessment of key pathogens**

### ***Baseline microbiological study***

Baseline surveillance/tracking study have been conducted for a range of pathogens on bovine hide and carcasses of corresponding beef animals continued (1352 animals has been tested) and is completed now by Teagasc and NVRI but will continue by AUTH and Brazil in the next period. At retail level, 542 ground beef products have been tested for four pathogens and 200 ready-to-eat beef products have been tested for *Listeria monocytogenes*.

Data so far indicates that VTEC (serogroup O157) prevalence on hides ranged from 7- 15 % and on pre chill carcasses (1 - 4 %) while non O157 serogroups were lower ranged from 1-6 % on hides and 0-1% on carcasses. *Campylobacter* had a highly variable prevalence on both hide from (28 to 51 %) and on carcasses (0 to 15 %). *Listeria monocytogenes* was present on up to 13 to 26.5 % of examined hides and 2.9 - 14% of carcasses. *Salmonella* has been present at on 1-7% of hides and < 2% of carcasses. The number of all pathogens recovered were generally low <Log 2.00 CFU/100cm<sup>2</sup>. Overall the results indicating that the rate of cross transmission from hide to carcass was < 10%. At retail level, data on raw mince beef indicate that the prevalence of the selected pathogens were higher to those seen on carcass particularly for *L. monocytogenes* were prevalence and numbers increased significantly in samples at the retail stage on the chain (19 to 23 %)(at number sup to Log<sub>10</sub>2.00CFU/ g). All isolates recovered are been characterised in T1.2.1

### ***Beef chain conditions***

Surveys of domestic refrigerator and freezer temperature as well as on cooking temperatures and practices for home-made beef patties were performed and will be used in exposure assessment models

### ***Development and validation of predictive models for pathogen behaviour in beef products***

Models for growth and thermal death of beef pathogens based on literature data have been developed and new data were produced for their validation. Spoilage models for fresh and MAP ground were developed based on pseudomonads and lactic acid bacteria growth respectively. Aiming at the evaluation of the variability in pathogens growth, the kinetic behavior of 60 isolates of the *Salmonella* was assessed at various environmental conditions while experiments on the variability of single cell behaviour are in progress. The above data and models were used for the exposure assessment of important pathogens on beef products.

### ***Exposure Assessments***

The above data and models were used for the exposure assessment of important pathogens on beef products. The work on exposure assessment of pathogens in beef has been divided in slaughterhouse/processing (UCD) retail and consumer modules ( AUTH) A complete chain exposure assessment model for *E. coli* O157 in ground beef has been drafted.

At the slaughter stage, a quantitative risk assessment of *Campylobacter* ssp. to the post carcass chill stage is being undertaken. The hide to carcass transfer prediction has been completed using a model for direct and indirect transfer. Work has also been completed on the evisceration step and the refrigeration step using input data from the baseline survey. Preliminary results using 350 animals as a slaughter run, give mean hide prevalence as 163 hides positive (90% CI 149 - 183), with a mean concentration of 0.844 Log<sub>10</sub> CFU/100cm<sup>2</sup> (90% CI 0.452 - 3.831 Log<sub>10</sub> CFU/100cm<sup>2</sup>). Carcass prevalence is 49 (90% CI 39 - 57) with mean carcass concentration of 0.476 Log<sub>10</sub> CFU/100cm<sup>2</sup> (90% CI -0.467 – 1.368 Log<sub>10</sub> CFU/100cm<sup>2</sup>).The model's outputs will be validated initially against surveillance data from Ireland. The model will also be able to use surveillance data from different countries as inputs to allow a risk assessment of that region to be undertaken. The model is being developed as a generic model which will allow it to be used for risk assessment of other pathogens. Separate work has been carried out on the effect of commercial chilling on the reduction in indicator organism (Enterobacteria, coliforms and TVC) prevalence and counts. The results show that there is no correlation at carcass level between number of indicator organisms before / after chilling and chilling conditions. However overall, the evidence is that refrigeration reduces the prevalence of indicator organisms.

At the retail to consumer stage, mathematical models on the behaviour of pathogens in beef, the storage conditions data and consumer handling data were combined and draft retail to consumption exposure assessment models for *L. monocytogenes*, *Salmonella* and verocytotoxigenic *E. coli* were developed. The data on strain variability and single cell behaviour were analyzed and approached were developed in order to incorporate this variability into exposure assessment. A stochastic approach for taking into account spoilage in exposure assessment was applied. The development of an excel spreadsheet for handling data from different countries and products in exposure assessment is in progress.

### **WP 1.4 Quantitative risk assessment of key known and emergent chemicals**

#### ***Development and validation of LC-MS/MS methods***

A robust and reliable method based on LC-MS/MS technology has been developed for the detection of 38 anthelmintic residues in bovine muscle. The limit of quantitation for the method is 10 µg/kg for 33 substances and 25 µg/kg for five flukicide residues (clorsulon, closantel, biothionol, nitroxinil and oxyclozanide). An SOP has been prepared and the method has been validated according to 2002/657/EC criteria.

This novel multi-residue method for anti-parasitic drugs was demonstrated at the EU Community Reference Laboratory Workshop in Berlin in May 2009 to representatives from the 25 Member States. Teagasc have evaluated the method through participating in inter-laboratory studies organised by the BRL, CRL and FAPAS. QUB and Teagasc have also produced incurred tissue from treated bovine animals. Some of this tissue has been donated to the Berlin, CRL and will be used to carry out further inter-laboratory trials in 2010. The tissue will also be used to investigate the stability of drug residues during cooking.

### ***Transfer of new residue technology to INCO country laboratories:***

A researcher from Microbioticos got additional training at IAEA during the period. Technology was transferred to Microbioticos laboratories in Brazil. A further transfer of the technology to 20 developing countries was organised and funded by the FAO/IAEA.

### ***Survey of beef for anti-parasitic residues***

The survey of EU beef commenced in October 2008. A total of 85 were taken per month from the period October to February, and the first phase of sampling finished March 2009. The first years survey of EU beef was completed successfully. The majority of the samples were found to be residues free (97.76%). The following residues were detected in samples closantel (1.22%), albendazole metabolites (0.20%), eprinomectin (0.20%), ivermectin (0.20%), levamisole (0.20%) and moxidectin (0.20%). In all cases the residue levels in samples were low and residue exposure was far below the ADI. One sample was found to be non-compliant for ivermectin, which is approved for bovine animals but does not have a maximum residue limit specified in muscle. The second year Europe sampling is underway and the survey of INCO beef is in progress.

### **IDENTIFY DEVIATIONS FROM THE PROJECT WORK PLAN, ISSUES ENCOUNTERED, THE REASON FOR THE DEVIATION AND CORRECTIVE ACTIONS TAKEN, INCLUDING NEW DEADLINES.**

Task 1.4: Delays in Task 1.4 occurred early in the project for a variety of reasons, including late start on project resulting in later than anticipated availability of the developed methods and a change in focus of survey activities from year long to risk based surveys (at set calendar months, when drugs administered to animals) and delays in transfer of technology to INCO country related to equipment problems. However excellent progress has now been made, all survey activities will now be finished in Month 42 and the risk assessment models will then be formulated.

### **PILLAR 2 Control and intervention strategies which can be implemented along the fork to farm chain to ensure safe beef**

#### **WP 2.1. Distribution and consumers strategies**

***Development of antimicrobial packaging:*** An active packaging system combining antimicrobial oregano essential oils (EO) volatiles (either in edible films or diffusing in atmosphere) and modification of the packaging atmosphere (MAP) has been developed by AUA. Growth of spoilage flora (e.g. Total Viable counts, pseudomonads, lactic acid bacteria, Enterobacteriaceae, yeasts and molds) was delayed and *L. monocytogenes* in (minced or pieces) beef at various storage temperatures were inhibited. EO had a synergistic effect to the MAP with respect to *Salmonella* and *Escherichia coli* O157:H7.

AUTH conducted studies water sorption, water vapor barrier properties and thermo-mechanical behavior and antimicrobial activity of a variety of films and agents: 1. Sorbitol-caseinate (SC) films, with incorporation of nisin, K-sorbate, Na-lactate. Nisin, followed by sorbate -containing SC films were most effective against *L. monocytogenes*. 2. Whey-protein-isolate films, incorporating Na-lactate,  $\epsilon$ -polylysine; oregano oil. Films reduced autochthonous bacterial growth of wrapped meat. 3. Na-caseinate films, with a *Lactobacillus sakei* protective culture. Sorbitol enhanced viability of the culture and the film inhibited *Listeria monocytogenes* inoculated onto beef samples significantly. 4. Studies on diffusion kinetics with K- sorbate in WPI films as a model have been conducted.

An oriented polyamide film releasing lactic acid (L-BOPA) has been developed by UVM. Thermal, mechanical properties and release kinetics (in aqueous solution) were studied. Vacuum-packaged beef cuts with a L-BOPA inlay were comparable in microbiological and sensory terms to beef cuts immersed in 2% lactic acid prior to vacuum-packaging. The active packaging system was able to reduce numbers of *Enterobacteriaceae* and *E. coli* O157:H7 by 1 to 1.5 log in artificially contaminated beef cuts, even under temperature abuse conditions. A questionnaire has been designed for distribution to meat processing and meat packaging industry to explore industry's opinion towards Active Packaging and current legislation on Active Packaging.

***Development and selection of TTI:*** AUA studied the microbial association (identification, characterization etc) of beef under a number of storage conditions (time/temperature/atmosphere), with respect to quantity and flora shift in order to provide data for the TTI validation (AUA).

AUA has developed a Time Temperature Indicator (TTI) system based on the growth and metabolic activity of a *Lactobacillus sakei* strain. An irreversible color change of a chemical chromatic indicator progressively occurs due to the pH decline as a result of microbial growth. The indicator was validated in a number of real beef storage scenarios, and from data generated by AUA with this task, models were developed to predict spoilage in stored ground meat. This indicator system can accompany beef during the entire chill chain and will indicate the end of shelf life by colour changes.

### **WP 2.2 Processing strategies**

***Use of biopreservation.*** During the first 36 months, a collection of lactic acid bacteria was screened for *Lactobacillus sakei* strains and strains were clustered to identify combinations of strains that could be used to be inoculated and quantified in meat. A protocol to extract DNA and to quantify each *L. sakei* strain by Q-RT-PCR was developed. Then 6 cocktails of 3 strains were challenged in ground meat to test their ability to compete *Escherichia coli*, *Brochothrix thermosphacta*, and *Salmonella enteritidis* under vacuum or modified atmosphere, at 8°C or 4°C. Two cocktails showed an activity toward *S. enteritidis* but difficult to reproduce due to the high natural contamination of ground meat.

***Marination*** Various types of marinades based on soy sauce, olive oil, or red wine and containing different ingredients such as spices (garlic or onion powder, black pepper), essential oils (oregano, thyme), chemicals (lactic acid, nisin, polylysine) or the bacteriocin nisin were tested. The target organisms were *Listeria monocytogenes*, *B. thermosphacta*, Pseudomonads, *E. coli*, *S. enteritidis*. Marinades were tested on beef slices or filets stored at 5°C or 15°C (abuse temperature), under vacuum or modified atmosphere packaging. The effect on the development of target organisms and on the sensory quality was monitored. Some of the combinations showed an effect against some target bacteria without affecting the sensory quality of meat

***Safety of newly developed products:*** The microbial population of beef meat (*Longitimus dorsi*) was determined. Survival of EHEC strains in beef was monitored after beef injection and it was observed that EHEC can survive during meat storage and that meat injection can thus be considered as a risk.

### **WP2.3 Slaughter strategies**

In Workpackage 2.3, the work was focused on development of novel microbial beef safety controls to be used during the slaughter phase of the beef chain. A three-step approach

comprised elimination or reduction of microbial (including pathogens) load on hides (Task 2.3.1), meat (Task 2.3.2) and surfaces (Task 2.3.3) using various decontamination treatments. Overall, the work in WP 2.3 was conducted in accordance with the original plan, it generated novel results, and with timely submitted deliverables.

**In Task 2.3.1**, a natural, food-grade resin (Shellac) was evaluated for use as a “microbial immobilisation” treatment of cattle hides, so to improve microbial status of carcass meat. Hide treatment by 23% Shellac-in-ethanol solution reduced recoveries (by sponge-swabbing) of general microflora (TVC) by a factor of 6.6 logs; and of faecal indicators (generic *E. coli* and Enterobacteriaceae) by factors of at least 2.9 and 4.8 logs, respectively. Furthermore, the Shellac treatment resulted in significant reduction of prevalence of pathogen *E. coli* O157 on hides. These reductions were superior to those achieved by a standard, sanitizer rinse-vacuum hide treatment. Furthermore, the validation studies under commercial abattoirs conditions indicated that Shellac treatment enables reduction of carcass meat microflora, so improves meat safety. In next period, the work will be focused on practical aspects of optimisation and maximisation of the Shellac treatment of hides. The work on Task 2.3.1 is continuing.

**In Task 2.3.2**, a novel treatment of meat using a “natural” product extracted from milk resulted in 5.0, 2.8, 3.7 and 2.2 log<sub>10</sub> CFU cm<sup>-2</sup> average reductions of *E. coli* O157:H7, *S. Typhimurium* DT104, *C. jejuni* and *L. monocytogenes*, respectively. The reductions compare favourably with all other currently available beef decontamination technologies. Furthermore, the dairy extract can be particularly attractive to the beef industry as this “natural” product does not require approval nor would its application necessitate a reduction in line speed. Treatment of the carcass with the dairy extract does leave a sour odour on the outside of the carcass, which dissipates over a 24 hour period in the chiller. Overall, the dairy extract is effective against a range of relevant bacterial pathogens and represents an innovative beef carcass and beef product decontamination/antimicrobial technology for application in assuring beef food safety. The work on Task 2.3.2 was completed in month 24.

**In Task 2.3.3**, the Biofilm Ring Test® device was proven as a very interesting tool for assessing the efficiency of decontamination products on the biofilm detachment or the biofilm formation after a curative or preventive treatment, respectively, of an abiotic surface. The screening of a great number of biocides and formulations can be performed simultaneously on several bacterial strains and species. The enumeration of viable bacteria after treatment allows completing the data on the efficiency of the products on bacterial mortality. Among the 4 decontamination solutions tested, the most effective are those containing QAC as a biocide, but adding a polyenzymatic complex to the product formulation significantly increases its efficiency in terms of bacterial detachment and mortality. The hydrogen peroxide and particularly peracetic acid appear ineffective on *L. monocytogenes* forming biofilm at the concentration recommended by the suppliers. The work on Task 2.3.3 was completed in month 30

#### **WP 2.4. On Farm Strategies**

**Task 2.4.1** 1 Epidemiology: Using a sampling plan and relevant microbiological methods beef farms in one partner country were selected and microbiologically screened (bovine faeces and/or manure/slurry samples) over the course of 1 year for the presence of *Salmonella*, and *Campylobacter*. A related study was undertaken for Verocytotoxigenic *E. coli* including *E. coli* O157. Isolates were serotyped/speciated and phage typed if appropriate. Molecular characterisation was performed using pulsed field gel electrophoresis (PFGE) and/or flaA-SVR typing and/or multilocus sequence typing (MLST) as appropriate to the isolate and

budgetary constraints. These studies will determine the relatedness of isolates and identify sources and distribution throughout the farm. Antibiotic resistance phenotypes and genotypes and/or other virulence factors are to be investigated (budgets permitting (Teagasc)). Virulence factors: All of the VTEC isolates from Task 2.4.1 will be PCR screened for the presence of the genes encoding a range of virulence factors (vt1, vt2, TIR, hlyA, eaeA, IpfA, katP, etpD, espF, espP, espB and espA). Their antibiotic phenotype will also be established. The expression of a selection of these genes will be investigated using reverse transcriptase PCR in rumen and human GI tract models.

**Task 2.4.2** Control: Candidate carbon sources were identified and checked by growth competition experiments in bovine digestive content by using wild-type EHEC and mutants altered in the corresponding metabolic pathways. Furthermore, the impact of probiotic yeast on expression of the metabolic, stress, and virulence pathways identified by microarray analysis were investigated by using original compartmented chambers for interaction experiments and real-time quantitative PCR. The role of lactic acid bacteria (LAB), yeasts as probiotics and plant secondary metabolites as anti-microbial agents to reduce the pathogen load in sheep and cattle digestive compartments have been carried out both in vivo and in vitro. During the first 30 months a study was conducted to assess the efficacy of yeasts and LAB probiotics and plant secondary metabolites as a means of feeding milk fermented with probiotic LAB as a means of controlling gut pathogens. This experiment is still on going.

#### **WP 2.5. Potential risks Associated with Strategies**

**Task 2.5.1.** Adaptive responses to interventions: The effect of acid, hot water decontamination treatments of beef on the ability of pathogens to survive during frozen storage, severe heat /acid stress simulating cooking/gastric secretions, is under investigation. For example a six-strain composite of *E. coli* O157:H7 rifampicin-resistant strains (From the derive data, it can be concluded that hot water alone and combinations of hot water and lactic acid are not very effective at inactivating pathogens on meat surfaces. However, it is important to remember that these treatments were applied by a static dipping process. Using a pressurized jet and / or wash solutions flowing over the meat surface, as is likely in an abattoir, may increase the efficacy of the treatments. The treatments did not significantly affect the ability of the pathogens to survive frozen storage. The pathogens tested survived better on storage at -26°C than at -13°C. Variation between the ability of the three pathogens to survive frozen storage was evident, but it is unclear whether this was due to differences between species or intra-species strain variation.

**Task 2.5.2** Adaptation in biofilms: AUA evaluated the ability of *Salmonella enterica* and *Listeria monocytogenes* to form biofilm before and after treatment with 3 different disinfectants (hydrochloric and lactic acids at pH 3 and sodium hydroxide at pH 11) using two different techniques (1) cells enumeration by agar plating, and (2) by automated conductance measurements (using Rapid Automated Bacterial Impedance Technique- RABIT). Satureja thymbra essential oil and hydrosol was also introduced as an alternative way of cleaning contaminated surfaces CSU is being evaluating biofilm formation by *Escherichia coli* O157:H7 in sterile tryptic soy broth (TSB) and in beef fluid (purge) containing natural flora, over a period of 14 days at 15°C.

Studies were focused on (i) acid and non-acid adapted cells of *Listeria monocytogenes* able to form biofilms. This scenario will be developed in the coming period to test the risk of food contamination due to the difficulty of cleaning them from surfaces;

The effect of cell-to-cell communication was also introduced in these studies (rate of biofilm formation). Differences in the proteomic profile from planktonic vs biofilm cells was also observed.

CSU is being evaluating biofilm formation by *Escherichia coli* O157:H7 in sterile tryptic soy broth (TSB) and in beef fluid (purge) containing natural flora, over a period of 14 days at 15°C. - Additional six-strain composite of *E. coli* O157:H7 rifampicin-resistant strains ATCC 51657, ATCC 51658, ATCC 43895, ATCC 43895 ISEH/GFP, F284 and F469) was used to inoculate (6 log CFU/ml or g) sterile tryptic soy broth (TSB), beef fat-lean (1:1) homogenate and ground beef. Stainless steel and high-density polyethylene (HDPE) surfaces (2×5 cm) were placed into each of the substrates, and samples were incubated under static conditions at 4 and 15°C for 168 h (7 days)

A six-strain composite of *E. coli* O157:H7 rifampicin-resistant strains (ATCC 51657, ATCC 51658, ATCC 43895, ATCC 43895 ISEH/GFP, F284 and F469) was used to inoculate (6 log CFU/cm<sup>2</sup>) beef fat surface. The fat surface was placed at 4°C for 24 h to simulate the chilling process of carcasses. Afterwards, dry sterile stainless steel, acetal, polypropylene and high-density polyethylene (HDPE) (2×5 cm) were placed between two pieces of inoculated fat for 30 min. The contaminated coupons were rinsed with 20 ml sterile distilled water and placed in empty sterile tubes or in tubes containing 20 ml beef fat-lean (1:1) homogenate. Coupons in empty tubes were stored statically, whereas, tubes containing coupons submerged in fat-lean homogenate were stored statically or under agitation (500 rpm). Samples were incubated at 15°C for up to 10 days.

**Task 2.5.3** Prediction of beef safety /quality: AUA collected data (factorial experiment: 3 packaging systems x 4 storage temperatures) for developing growth / survival mathematical model. Experiments on the effect of storage temperature on the growth spoilage microflora or inoculated pathogens (*Salmonella* Typhimurium, *E. coli* O157 or *Listeria monocytogenes*) on beef products stored under aerobic conditions are being performed by AUTH. The structure (mince or block pieces, agar surface were factors as well as the microbial association that have been considered in the application of models)

#### **DEVIATIONS FROM THE PROJECT WORK PLAN, ISSUES ENCOUNTERED, THE REASON FOR THE DEVIATION AND CORRECTIVE ACTIONS TAKEN, INCLUDING NEW DEADLINES**

In Task 2.4.2 The LAB probiotic study has been delayed by 12 months due to the poor health of the first batch of calves (low colostrum). This however will not interfere with the on time Completion of this task which is due Month 55.

### **PILLAR 3: Producing safe beef and beef products with enhanced nutritional and eating quality characteristics**

#### **Work Package: 3.1**

##### **Nutritionally-enhanced fresh beef**

Partners AbU, INRA, UGent and Teagasc have examined the impact of various dietary strategies on lipid metabolism in the rumen. Key studies at UGent and AbU demonstrated that long chain PUFA (EPA; DHA) inhibit biohydrogenation resulting in increased production of vaccenic acid and reduction in stearic acid in the rumen. Associated studies examining microorganisms involved in these changes in lipid metabolism have indicated that these are more complex than originally thought and revealed that as yet uncultured bacteria possibly play pivotal roles. Studies have investigated role of plant secondary compounds (polyphenol oxidase, saponins, catecholamines and tannins) in altering lipolysis and/or biohydrogenation. Polyphenol oxidase found in red clover resulted in beneficial reductions in lipolysis which

support the increases in PUFA in muscle lipids of animals fed diets containing this legume. Studies by UGent noted wide variation in the degree of “apparent” protection of dietary lipid in commercially available rumen-protected lipid products and noted that the most protected technology was formaldehyde treated whole oil seeds (i.e. linseed) or formaldehyde treated protein containing n-3 rich lipids.

Partners AbU, INRA, FBN and Teagasc have conducted a number of studies examining the effect of diet on fatty acid composition of beef lipids. All studies have progressed as planned and are at various stages of completion of chemical and sensory analysis of meat (see WP3.3). AbU and Teagasc have investigated the effect of “lifetime” nutritional strategies to enhance n-3 PUFA and/or CLA in beef lipids based on strategic feeding of n-3 PUFA from early life. INRA have conducted a study examining effect of breeds differing in genetic potential for fat deposition) (Angus, Limousin or Bonde d’Aquitaine breeds) and dietary lipids and plant polyphenolics on muscle fatty acids, colour shelf life and sensory. Partner FBN have completed two studies examining effect of dietary oils (i.e. rapeseed; linseed) of meat quality.

### **Work Package: 3.2**

#### **Development of novel and functional beef products**

Partner UB has focused on enrichment of n-3 long chain PUFA (LCPUFA) in either (1) whole muscle (upgrading low value muscles) or (2) beef burgers. All analysis is conducted under WP3.3. The whole muscle studies involved injection of LCPUFA directly into the muscle as encapsulates or emulsions. In year 2, after discussion with Pillar SMEs and Pillar 5 (consumer) less emphasis was placed on this work due to potential consumer concerns about altering a “natural” product – muscle relative to added nutraceuticals to “processed “minced” products. Studies by UB have demonstrated the ability to achieve large enhancement in long chain PUFA in beef muscle by injection and in beef burgers by use of encapsulated-DHA. In the burger studies and based on a 200g serving then they would supply 40, 68 and 96% of recommended daily allowance (based on 450 g/d LCPUFA). Shelf life of both muscle and burger LCPUFA-enhanced products was shorter than commercially required but additional of antioxidant to the meat matrix ameliorated this problem. This work is now in consideration by project SMEs for commercial adoption. Partner UB have also reviewed the EFSA guidelines in relation to nutritional labelling of foods (produced during 2009) and presented this to project partners. SME partner GFG produced corned beef sausages from the lean meat of experimental bulls produced by FBN under WP 3.1. Importantly beneficial enrichments in muscle lipids induced by dietary feeding were reflected in the fatty acid composition of the products.

### **Work Package: 3.3**

#### **Safety, sensory and nutritional assessment of beef and beef products**

NOFIMA have completed an assessment of the potential use of Raman spectroscopy as an on-line tool to measure fat and fatty acid composition (see D3.3.1). Muscle samples were provided to NOFIMA from Partners FBN and Teagasc and analysed by Raman Spectroscopy. Calibrations against the different fatty acid parameters were obtained. The results provide evidence that this methodology could be used to measure the levels of total saturated, monounsaturated and polyunsaturated fatty acids (see D3.3.1).

All partners have made good progress in relation to analysis of beef and beef products generated in WP3.1 and 3.2, respectively. Excellent progress has been made on analysis of meat produced under WP3.1 by all involved partners FBN, INRA, Teagasc and UB. Partner FBN have demonstrated the benefits of n-3 rich linseed in enhancing n-3 PUFA in beef lipids. The initial results from Partner INRA noted that linseed supplementation increased largely proportions of delta 10+11 trans 18:1 in total fatty acids (~40%) and of 18:3n-3 (~x3), EPA

(~x2) and DPA (~x1.5), and decreased 16:0 (~-12%). Partner UB has completed analysis of muscle lipids in animals produced by Partner AbU. Feeding the n-3 rich plant extract from lucerne (PX) relative to n-6 rich concentrate increased deposition of n-3 relative to n-6 PUFA in muscle lipids; including beneficial effects on n-3 LCPUFA. This study also confirmed the benefits of grass feedings on n-3 PUFA in muscle lipids and colour shelf life of meat. Partner UB has completed all sensory analysis on beef and beef products received from Partners FBN, INRA, Teagasc and GFG. FBN have also examined completed vitamin and trace metal analyses of the meat from their first production study involved bulls. No dietary effects were noted.

#### **Work Package: 3.4**

##### **Modelling, biochemical & molecular tools to improve prediction of beef quality**

Partner INRA have created a unique database to enable a European model of eating quality to be established. The model contains data on animal growth and diet, carcass composition and tissues properties and is currently based on 4704 animals from 20 different breeds of cattle (mainly Limousin, Charolais, Blond d'Aquitaine and Montbéliard). For muscle, the measurements concern fibre types (areas, contractile and metabolic properties), collagen (content and solubility), lipids (total, phospholipids and triglycerides), fatty acid composition, sensorial analysis, mechanical measurement such as Warner-Bratzler). This is a major advance in developing a predictive model of eating quality. The SME partner UNCEIA is very involved with this activity.

A number of molecular markers (SNPs by Teagasc and genes differentially expressed by INRA) were compiled. A subset of these markers was identified and genotyped in the Irish herd through a nationally funded project (see D3.4.1 and D3.4.2). INRA have confirmed that the expression of the adipocyte-fatty acid binding protein (A-FABP) can be considered as a good indicator of the ability of animals to deposit intramuscular fat.

In conjunction with Beef CRC, INRA and Teagasc the Meat Standard Australia (MSA) model of predicting and guaranteeing eating of beef to the consumer was studied to evaluate the suitability of this system in France & Ireland. Conclusion from the Teagasc activities was that the MSA model fitted Irish beef and Irish consumers at least as well as it does Australian beef and consumers. In general the model accounted well for all the factors. The Irish beef Industry is considering adaptation of the model to ensure that beef is marketed with consistent eating quality. A study to assess the MSA system in France (led by INRA in conjunction with Beef CRC and Teagasc) is in progress.

#### **Workshops**

Pillar 3 have organised and ran two one day workshops at INRA-Theix. The first, held December 2008, focused on the international development of an Animal Trait Ontology and featured a key note presentation by Dr James Reecy, Iowa State University and presentations by scientists involved in ProSafeBeef. The second workshop was held immediately after the International Symposium of Ruminant Physiology, Clermont September 2009, and focused on Animal agriculture in a climate changed world.

#### **IDENTIFY DEVIATIONS FROM THE PROJECT WORKPLAN, ISSUES ENCOUNTERED, THE REASON FOR THE DEVIATION AND CORRECTIVE ACTIONS TAKEN, INCLUDING A NEW DEADLINE**

No major deviations from the workplan have occurred. Some partners (UGent, Teagasc, INRA) did encounter some delays particularly in the first 24 months but this has had little effect on expected workplan delivery over the whole of the first 36 months.

## **PILLAR 4 Innovations in processing to develop nutritive convenient and added-value beef products**

### **WP4.1: Beef muscle profiling to select for muscles for use in added-value products**

During the first year of the project, a database containing spectroscopic information as well as chemical and physical properties from 10 different bovine muscles from 10 carcasses was established. This work has then been continued by focussing on fewer selected muscles with potential for value-adding. The work shows that for some muscles there is a large potential for added value if the today's cutting patterns are modified. Spectroscopic measurements on intact muscles have shown limited ability to estimate features such as tenderness and fat content. Inter pillar collaboration with WP3.4 on muscle profiling of LD muscles has commenced. Beef profiling as a concept was also subjected to consumer focus groups in the work of Pillar 5. All results so far are described in project reports and two scientific papers. A national network of beef cutting companies in Norway, organized by Pillar 6, has beef profiling as its main topic. The scientific findings and practical exercises have been of great interest to the companies, and improvement of their present practice is under planning.

The other research field of WP 4.1 is focussed on **new control methodologies and instrumentation for detection of desirable and undesirable carcass components.**

The effect of salt content and MAP conditions on a high frequency metal detector has been examined, and mathematical models have been made to model the impact of fat and salt on CT detector responses.

A conventional X-ray system made for metal detection (relatively high power X-ray) has been compared to a low dose X-ray system with respect to detection of unwanted fragments of cartilage. The low dose X-ray system gives significantly better contrast between various meat products and cartilage, and is promising with respect to new and more sensitive detection systems. A possible drawback is that the controlled samples must be rather thin (2 cm) or that the speed of scanning could be slow. All results are reported in project deliverables. The activity on foreign body detection was finalized in March 2009 with a demonstration activity in Copenhagen in collaboration with Pillar 6.

### **WP4.2 Innovations in marination processing for developing novel and convenient beef products**

This WP involves several different tasks and is connected to all WPs in Pillar 4, as well as to activities in all other Pillars.

In **Task 4.2.1** diffusion models for marinade compounds ( $H^+$ ,  $Na^+$  and  $Cl^-$ ) into beef is developed based on lab experiments. It started with 1D models, which are now expanded into 3D models for use on irregular shaped meat trimmings. An aim is that these model can predict the diffusion of marinades of different properties, and thereby be used as guidelines for practical marination processes with respect to time, pH, and process parameters.

The mechanical properties of collagen fibers as function of pH has been investigated and related to protein denaturation studied by FT-IR spectroscopy.

In **Task 4.2.2** Several experiments have been conducted to evaluate and better model the interactions between marination processes, marinade composition and type of muscles, and their impact on end product quality. Several experiments have been performed in order to study the impact of marinating processes on technological qualities of product (yield of cooking and total yield) and on the tenderness. Process steps such as tenderization, injection, and tumbling have been evaluated and compared.

The effect of marinating on technological properties of different beef muscles has been evaluated in collaboration with WP4.1 on beef profiling. Generally, the results reveal

significant differences between different muscles treated with the same marinade. This suggests that optimal quality can be obtained by tailor made marinades for certain muscle types. The results so far constitute a sufficient basis for some general guidelines for the industry.

Novel marinade ingredients such as blueberry juice and kiwi juice have been evaluated with varying success in terms of improved sensory properties. All results have been reported in project deliverables as well as in conferences.

There is an on-going collaboration between WP4.2 and WP4.3 on the potential inhibiting effect of marinade on the formation of heterocyclic aromatic amines. This work got delayed early in the project due to technical challenges, but are now on track.

There is an on-going collaboration with WP3.1? on novel ingredient for nutritional enhancement. The feasibility of incorporating ingredient suggested by Pillar 3 in marinades is being evaluated.

A study with Pillar 2 on the microbial side of different marinating processes has been conducted. Injection gives a good effect of the marinade on eating quality, however, this technique is also the most risky with regard to contamination. Studies of *E.coli* bacteria (some detected in Pillar 1) injected in beef indicate survival after many days.

### **WP4.3 Intelligent thermal process control models for beef and beef products**

The WP aims at optimising cooked meat qualities: juiciness, colour and tenderness and increasing meat safety by reducing heterocyclic amines (HAs, carcinogens) produced during roasting and grilling (connection with WP4.2). Work progression has been based: (1) on the development of thermal-process-control models, which combine heat-mass transfer models and quality modules, (2) on the ongoing studies of two specific cooking techniques: microwave and convective oven, and (3) on the development of specific thermal process control equipments and devices, applicable on different beef cuts, and which will be used to validate models and to interpret technical results.

Thorough results on formation of HAs as function of time and temperature have been obtained, and models has been made that can predict the formation of HA as function of time and temperature. High temperature results in high concentration of Has, while low temperatures does not generate the consumer desired crust on the beef. Preliminary results shows that crust formation can be obtained at low meat surface temperature by a strong initial drying preceding the heating stage. This process modification should reduced HAs formation and will be investigated.

Preliminary studies suggest that certain compounds added to marinade can inhibit formation of Has during heat treatment. This investigation will continue.

Several studies have been performed to investigate the effect of marination with subsequent heat treatment. The variation of the elastic modulus of collagen fibres under various conditions has been studied. Protein structural changes and water mobility/status have been studied as a function of different salt types and concentrations using marinating together with subsequent heat treatment. Preliminary analysis of the sensory results shows that combinations of KCl and NaCl have advantageous sensory properties and that KCl can - without any detrimental properties - be used to substitute parts of the NaCl in marinated cooked products. This work is a joined work with WP4.2.

A magnetic resonance imaging (MRI) system has been optimised to study the water mobility inside a piece of meat vacuum-packed and heated by circulating water. Water migration under protein contractions can then be observed dynamically during meat heating.

Microwave and air-convection treatments have been tested on marinated and non-marinated muscles to increase tenderness, juiciness and colour shelf life of cooked meat coming from lower value/underutilised beef muscles. All results are reported in project deliveries. Several scientific papers and presentations have been produced based on the work in Workpackage 4.3

#### **WP4.4 Innovative solutions in packaging of beef products**

**Task 4.4.1** A review of the scientific literature was carried out to examine past and current research in the area of high oxygen modified atmosphere packaging technology for use with fresh beef.

**Task 4.4.2** The effect of gas to meat ratio in MAP on the sensory properties of intact beef steaks has been studied through thorough experiments. Sensory analysis was performed. The results are to be reported soon.

**Task 4.4.4** Following recruitment problems in Teagasc due to a Government embargo on recruitment this task has been delayed but this has no knock-on effects for other tasks. These issues have now been resolved and a Research Officer has commence work. This Task will be completed by month 54.

**Task 4.4.5** on premature browning has been completed. Premature browning (PMB) is a condition at which beef appears fully cooked despite not having reached a safe internal temperature, with possible survival of pathogenic bacteria. The aim of the task was to examine food additives like antioxidants and reducing agents that can lower the extent of PMB in beef. Injected beef steaks stored under a high O<sub>2</sub> atmosphere were used to verify the effectiveness of candidate additives selected from a pre-experiment. PMB was slightly reduced by adding polyphosphates, due to higher pH of the treated meat. Other antioxidants and reducing agents as rosemary extract, ascorbic acid, lactate and lingonberry juice, did not decrease the extent of PMB. Selecting an anaerobic packaging method is the most efficient way of preventing PMB in beef.

#### **IDENTIFY DEVIATIONS FROM THE PROJECT WORK PLAN, ISSUES ENCOUNTERED, THE REASON FOR THE DEVIATION AND CORRECTIVE ACTIONS TAKEN, INCLUDING NEW DEADLINES.**

- **Deliverable 4.1.9** (Report on the effects of breed and feed on selected beef quality parameters) will be reported at month 36. However, some of the animals which should have been used in this study were slaughtered earlier than expected. We were not informed so no muscle samples were collected. Another set of animals was slaughtered in October 2009, and then samples were collected for analyses. The result is that the amount of data became less than planned.
- **Deliverable 4.2.10** has been delayed from Month 36 to Month 42 due to a delay in the receipt of input data from Pillar 3 on the functional ingredient to use for this research. This information will be received in Month 38 when research will be conducted in earnest.
- **Deliverable 4.2.11** has been delayed from Month 42 to Month 46. The models that were used to model the diffusion turned out to be too simplistic and inadequate for the purpose initially envisaged. A new modelling strategy –constituting another set of model equations will be applied to obtain more satisfactory results. This work is presently being conducted.
- **Deliverable 4.2.12** has been delayed from Month 36 to Month 48 due to a delay in the receipt of input data from Pillar 3 on the functional ingredient to use for this research. This information will be received in Month 38 when research will be conducted in earnest.
- **Deliverable 4.4.1 and Deliverable 4.4.2** were completed on Month 37 late due in part due to unforeseen technical problems encountered in Partner 20's UCC's laboratory equipment and also in part due to recruitment embargoes on Teagasc, Ashtown Food Research Centre, which have now been lifted. These delays have had no consequences for other tasks or work packages.

## **PILLAR 5 Consumer need for beef safety and health information and acceptability of novel processed beef products**

### **Qualitative consumer study**

The qualitative exploratory focus group discussions were conducted during May 2008 in the capital cities of four European countries (UK, France, Germany and Spain). In each country, two focus group discussions were conducted, one being composed by women and another one by men. In total, eight focus groups were conducted with 65 participants. For each of the four countries, the translated topic guide and an additional quantitative questionnaire were applied to the respondents on the respective languages of the countries. This topic guide has been included in Deliverable D5.1.1/D5.2.1. The additional questionnaire has been included as an annex in Deliverable D5.1.2/D5.2.2. The full transcripts of all focus group discussions were provided in Deliverable D5.1.2 / D5.2.2. These full transcripts were further used for data analysis.

In order to ensure consistency considering the complexity of the multi-language transcriptions, a selected group of researchers gathered in Ghent from June 25<sup>th</sup> to 27<sup>th</sup> for a training workshop. This activity allowed systematising and proceeding in a consistent manner with the coding work, necessary to qualitatively analyse and interpret the obtained results. In this regard, the group gratefully acknowledge the financial support of EU Commission throughout Pillar 6 (Training and Disseminating Activities) that allowed such a task to be successfully performed. The results of the content analysis were reported in Deliverable D5.1.3 and Deliverable D5.2.3. Furthermore, four journal papers drafts based on the results of this and additional content analyses were prepared for publication. Two of those have been published by now (Verbeke *et al.* in *Appetite*; Van Wezemael *et al.* in *Food Control*).

The summary outcome is that consumer demand in relation to food is shifting towards products that are safe, nutritious, and of good eating quality. Beef consumers are demanding for experience quality that matches their expectations, particularly with respect to beef tenderness. The development of a beef quality grading and guarantee system obtained through muscle profiling research, can allow the beef industry to meet these demands. A qualitative consumer study has been carried out with beef consumers in France, Spain, United Kingdom and Germany to assess their opinions about beef muscle profiling and their interest in a beef eating-quality guarantee. Findings indicate that both concepts are well accepted by European beef consumers, although not unconditional. Participants express some reserve related to the possible upgrading of lower value cuts, too much standardisation, and the fact that tenderness is to some extent subjective. They further require the system to be simple, sufficiently documented and independent-party controlled. This study indicates good opportunities for the development of a beef eating-quality guarantee system in Europe. As an increase in consumers' satisfaction could lead to higher consumption rates and industry profitability, the introduction of an eating-quality guarantee system can contribute to market development and improved competitiveness of the European beef industry.

In relation to safety, content analysis revealed that the focus group participants experienced difficulties in the assessment of the safety of beef and beef products and adopted diverging uncertainty reduction strategies. These include the use of colour, labels, brands and indications of origin as cues signalling beef safety. In general, consumer trust in beef safety was relatively high, despite distrust in particular actors.

## **2. First wave quantitative consumer study**

A quantitative (experimental) consumer study in five European countries (UK, France, Germany, Poland and Spain) has been prepared. The format and structure of the quantitative consumer study were discussed in pillar meetings in Girona (20-21/04/2009) and Copenhagen (20/08/2009), the latter being in conjunction with Q-PorkChains. While Pillar 5 met Pillar 1 and 2 during the Dublin conference (25-26/03/2009), the meeting in Girona was a joint pillar meeting with the pillars 3 and 4. Information from other pillars with respect to beef technologies was compiled during these joint pillar meetings, in order to draft the research protocol and the questionnaire for the quantitative consumer study. This material has been delivered as deliverables D5.1.4. and D5.2.4. The data collection takes place in March 2010. The data set from this experimental study will be delivered as deliverables D5.1.5 and D5.2.5 by April 2010.

### **IDENTIFY DEVIATIONS FROM THE PROJECT WORK PLAN, ISSUES ENCOUNTERED, THE REASON FOR THE DEVIATION AND CORRECTIVE ACTIONS TAKEN, INCLUDING NEW DEADLINES.**

The activities from Pillar 5 have deviated from the original timing due to the late recruitment of personnel (doctoral and post-doctoral researchers). As a consequence, the submission of the Deliverable D5.1.2./D5.2.2. was delayed till June 2008 (instead of April 2008, i.e. a delay of two months) and the submission of the Deliverables D5.1.3. and D5.2.3 was completed in September 2008 (instead of August 2008, i.e. a delay of one month). Because of this delay, the preparatory work for the first wave quantitative consumer (deliverable D5. 1.4 and D5.2.4) has been delayed.

The set-up of the experimental design required input from the other pillars. During the meetings with Pillar 1 & 2 (Dublin, March 2009) and with Pillar 3 & 4 (Girona, April 2009) several issues came up, demanding revisions of the design. These revisions have improved the quality of the design, however delaying the experimental study. The programming and pretesting of the experimental study has been more time-consuming than expected. Additional time was needed for the web-programming of the complex experimental design; especially the programming of the randomization process has asked particular attention. With respect to data collection, we opted to avoid fieldwork during the period December – January because this period is known for atypical eating habits. Because of these obstacles, the data collection will take place in March 2010. The deliverables containing the data set of the experimental study (deliverables D5.1.5 and 5.2.5) will be available by April 2010.

## **PILLAR 6 Training, Industry Networking and Dissemination of Results**

### **Workpackage 6.1 Demonstration activities**

The objective of this Workpackage is to organise demonstration actions on technologies developed in the framework of ProSafeBeef program. A demonstration action consists of a practical demonstration of a technology operating at the industrial or pilot plant scale in order to illustrate the technical viability for a further application in industry. A major part of the work in this Workpackage is to identify and select technologies developed within the ProSafeBeef research pillars (Pillar 1 to 4) which are suitable for demonstration purposes. A procedure for selecting technologies has been established based on the collaboration of Core Team members and researchers. Core Team members consisting of technologists with significant experience in technology transfer to industry liaise with researchers identifying topics/technologies for demonstrations emanating from the RTD research Pillars.

From the beginning of the project to now, 6 demonstrations have been performed:

1. *Low added value muscle profiling* at Nofima Mat (Norway) – Month 5
2. *Osmotic dehydration/restructuring* at ADIV (France) – Month 12
3. *Hot boning and restraining technique* at Nortura (Norway) – Month 16
4. *High hydrostatic pressure on beef meat products* at IRTA (Spain) – Month 18
5. *Detection of foreign bodies/unwanted carcass components* at DMRI (Denmark) – Month 24
6. *New method for anti-parasitic drug residue detection* at CRL (Germany) – Month 27

The average attendance at these demonstrations would be 20 people; an optimum number of participant for practical exhibitions. Nevertheless, in order to extend the impact of demonstrations to companies that couldn't attend the demonstration itself, a "demonstration kit" has been produced for each technology demonstrated. The kits include technical documentation, photos, PowerPoint presentations and a guideline leaflet detailing the technology and its advantages/disadvantages. These kits are available on the ProSafeBeef website to be downloaded by all interested stakeholders and to be used by Core Team members in their promotion activities.

## **Workpackage 6.2 Training of specialists and "training the trainers"**

This Workpackage is composed of two different tasks.

### *T6.2.1 Scientists and PhD students training period organization*

The objective of this task is to strengthen research quality and improve knowledge of ProSafeBeef research partners by granting PhD and early stage researchers exchanges between ProSafeBeef partners. The aim of the exchange is to train PhD or early stage researchers on advanced techniques, methods, methodologies and many more for application in their home laboratories.

A procedure was established during the first year of the project which details the terms of the call for proposal and the rules for application and reporting. A guide for applicants, an assessment form, an agreement model and a reporting model have been published on ProSafeBeef website and distributed to partners.

Two Calls for proposal were launched at month 14 and month 23. From which 8 exchanges have been successful. The calls for proposal have promoted exchanges between European, North American and INCO laboratories (exchanges with Canadian, American, Brazilian and New-Zealanders partners) and reinforced collaboration between older European/western countries and new and candidate state countries as well as INCO countries (exchanges with Polish(2) and Austrian partners). 6 women and 2 men were involved in those exchanges.

A third Call for proposal has been launched at month 36 and remains as an open ended call.

### *T6.2.2 The "Core team" of scientists and trainers for technology transfer to SMEs and industry*

The "Core Team" is a group of experienced meat technologists which are involved strongly in technology transfer and closed to industrial needs and concerns. This group is composed of 8 meat technologists : 3 from ADIV (France), 3 from Nofima Mat (Norway) and 2 from IRTA (Spain). Due to their skills and experiences the main role of the Core Team members is to be the act as a central medium of dissemination towards companies. To achieve this, their mission is to:

- identify topics for demonstration relevant for industry

- valorise the technologies demonstrated
- animate national networks dedicated to technology transfer
- organise and animate training sessions
- participate in the general diffusion of information

During the first 18<sup>th</sup> months the Core team members were engaged in training themselves in order to reinforce their knowledge of European beef meat companies, supplement their skills and knowledge in select technological topics and acquaint them with information on research work carried out within the RTD pillars. 5 Core team meetings have been organised during this period.

Apart from their work on identifying technologies for demonstration, Core team members have organised and ran, with the help of WP6.2 leader and national contact persons, 2 training sessions :

- *Beef meat technologies, tenderness development and valorisation factors* at Warsaw (Poland) – Month 29
- *Good practices in slaughterhouse* at Belgrade (Serbia) – Month 31

Bjorg Narum, Pierre Picouet and Sylvain Labayle are leading respectively the Norwegian, Spanish and French networks dedicated to technology transfer.

### **Workpackage 6.3 Technological transfer**

This Workpackage focus on setting up an effective technology transfer strategy directed towards industry and SMEs. The best methodology chosen to transfer technology was through the organisation of SME networks that bring together companies and technologists around a specific topic and area of interest on a national and at a European level.

At the national level, 3 networks have been set up in France, Norway and Spain. These networks bring together 5 to 10 companies on a dedicated technological topic. The concept of the networks is developed around a combination of common workshops and individual actions within companies to give them the opportunity to implement the “learning-by-doing” process.

#### *National network in France*

This network is led by ADIV. Discussions between ADIV and companies in the frame of training sessions and conferences in first years of the project have lead to the definition of a new concept for the French network which was launched at the end of 2009. The network is dedicated to bovine carcass contamination and 8 companies are enrolled. 2 plenary sessions (Month 37 and Month 46), 1 technical workshop (Month 40) are planned and individual actions to evaluate their own situation regarding the risk of contamination have to be designed.

#### *National network in Norway*

This network is led by Nofima Mat. 6 companies are enrolled in this network dedicated to muscle profiling and new techniques. Two workshops (Month 28 in France and Month 32) and individual works have been carried out. Two more workshops (Month 37) are planned to close the network and to evaluate the benefit for companies.

#### *National network in Spain*

The Spanish network will be organised by IRTA. There is a culture of privacy within industry in Spain leading to unforeseen problems and delays in the development of the network in Spain. The model used for the Spanish network has to be designed according to this SME

culture in Spain. The main concern of these companies is with actions which ensure cost reduction or increased income with minimum investment. The first workshop is planned for Month 39. The topic of the network is muscle profiling and cost savings actions.

At European level, the network is intended for CEOs and decision makers. The work carried out up to now consisted in building a database of contacts persons with a special emphasis on meat boards and organisations. All ProSafeBeef information is sent to this group which act as a relay to companies. Special events for this network will be designed by the end of the project.

#### **Workpackage 6.4 Dissemination**

The main objective of this Workpackage is to develop and implement a dissemination plan which ensures rapid, appropriate and effective dissemination of information to targeted audience on a wide scale.

To achieve this objective the main running tasks of this Workpackage are:

- *to build a database of key persons in public and private institutions, company representative bodies and NGO's relevant to the research*

The network of over 100 individuals, CEO's and leading researchers from over 37 countries has been set up in the first 36 month period. A database of some 3,000 plus members has been established and receives the bi-annual ProSafeBeef newsletter including thematic articles and regular updates on the ProSafeBeef events and research

- *to develop website as inter and intra Pillar communication and as central dissemination portal for ProSafeBeef*

The website with three sections for the General Public, Network associates and the Core Team and a consortium area has been set up and is running effectively with 5,000 to 10,000 visits a month. All areas continue to grow in complexity and substance. The ProSafeBeef website was fully revised and revitalised at the halfway stage in the project to reflect the changing needs of the project and stakeholders. This includes hosting a detailed list of all disseminated activities (Peer reviewed publications, poster presentations, technical publications, meetings with stakeholders, popular media), four newsletters (to date), the ProSafeBeef brochure and the demonstration toolkits, Executive Summaries and the most up to date information for the stakeholder. This process has been focused on all stakeholders, from scientific researchers to SME's to the general public with a key focal point on industry. The consortium area of the website has also been completely overhauled allowing easier and faster access to more detailed information regarding the evolution of the project for the consortium members.

- *to produce technical manuals, newsletters, brochure and toolkits*

A ProSafeBeef brochure describing the consortium and the objectives of ProSafeBeef has been released at month 4. BNF and TEAGASC have developed the bi-annual ProSafeBeef newsletter with four publications so far and three planned for 2010. Technical manuals, leaflets and toolkits surrounding technologies demonstrations have been produced and updated on website.

- *to organise conferences, workshops, seminars*

More than 200 dissemination actions have been carried by ProSafeBeef partners: technical and peer review publications, oral presentation and posters at conferences, popular media communication and meeting with stakeholders.

In addition to this ProSafeBeef held a Safety Conference in Dublin at Month 25 with more than 120 attendees and a ProSafeBeef seminar at ICOMST in Copenhagen at Month 30 with more than 70 attendees. Also 3 General Assembly have also been organised at Month 1 in Dublin (Ireland), Month 14 in Ghent (Belgium) and Month 32 in Athens (Greece).

**IDENTIFY DEVIATIONS FROM THE PROJECT WORK PLAN, ISSUES ENCOUNTERED, THE REASON FOR THE DEVIATION AND CORRECTIVE ACTIONS TAKEN, INCLUDING NEW DEADLINES.**

**Deliverable D6.1.7** has been postponed. From month 24 to the end of the project demonstration actions are based on the work emanating from the RTD Pillars within ProSafeBeef. One demonstration has been organised during the reporting period (D6.1.6). Research stemming from other RTD Pillars was not yet progressed enough to enable a second demonstration to take place. At the month 36 stage several topics have been identified yet there remains work to be completed at the research stage before these activities are available for practical demonstrations. A key strategy for this WP is to deliver technologies that could readily be applied at the industrial stage and not to approach industry with concept technologies. The development of the Spanish network of SMEs (D6.3.5) has also been subject to delays due to the aforementioned culture of privacy; active solutions have been advanced to overcome this issue as detailed above and the network is now functional.

## **MID TERM REVIEW OF TECHNOLOGIES WITH COMMERCIAL POTENTIAL**

Project management is highly cognisant of the importance at this stage of the project to nurture those technologies with most promise toward the commercialisation stage. Thus firstly a road map for product development and its commercial potential have been drawn up (see appendix 2). In addition a midterm audit of technologies emerging from *ProSafeBeef* is also being undertaken.

A template (see below) has been distributed to all partners to complete as a first step toward assessing the stage of development of the technologies and the development of a road map to bring those with most potential from the research stage towards commercialisation some of which may happen within and some outside of *Prosafebeef*.

**MIDWAY REVIEW OF TECHNOLOGIES WITH INDUSTRIAL / COMMERCIAL  
POTENTIAL**

Partner Name:

Pillar:

1) List Technologies/ Industrial Tools with commercial potential stemming from your research in ProSafeBeef:

- 
- 

**Technology title:** \_\_\_\_\_

- Which partner(s) are responsible

What stage of development is technology at? (*Please elaborate under one or more of the following headings*)

- Research: laboratory model
- In meat system
- Proof of concept
- Pilot scale trial
  
- Could it be demonstrated in its current form to industry
  
- If the answer is no –what are next steps to enable demonstration
  
- What are the next steps to be undertaken within or outside *Prosafebeef* to Progress development of the technology

2) For **each** above listed technologies: Complete the following

- Have any meat companies been informed about or expressed interest in the
  
- Is there Intellectual property associated with the technology

What are the likely barriers to commercialisation? (*Please elaborate under one or more of the following headings*)

- Technical
- Legislation
- Cost
- Consumer resistance
- Other
  
- Could the technology be the subject of an EU FP7 grant on “Competitiveness, industrial policy, innovation and entrepreneurship” or “Research for the benefit of SMEs”
  
- Have any meat companies been informed about or expressed interest in the technology ?
  
- Is there Intellectual property associated with the technology?
  
- Could the technology be the subject of an EU Industry commercialisation grant?

The collation of this data is ongoing but some of the most exciting technologies identified to date are:

**1) A novel method for anti-parasitic drugs**

A robust and reliable method based on LC-MS/MS technology has been developed for the detection of 38 anthelmintic residues in bovine muscle. An SOP has been prepared and the method has been validated according to 2002/657/EC criteria. This novel multi-residue method for anti-parasitic drugs was demonstrated at the EU Community Reference Laboratory Workshop in Berlin in May 2009 to representatives from the 25 Member States and is now in use in member state reference laboratories. IAEA have demonstrated the method to South America countries reference Labs including Argentina, Peru, Uruguay, Chile, Bolivia, Costa Rica, Haiti, Nicaragua, Venezuela

**2) Spectroscopic imaging method to detect faecal contamination on carcasses:**

A feed additive (PX) based on chlorophyll has been identified as potential additive to cattle feed in the preslaughter period and detectable on carcass by image analysis. A patent has been filed on the marker. The research work is now completed within *ProSafeBeef* but further funding was sought and obtained under a nationally funded project to continue refinement of the method. The technology is being investigated as a demonstration item in Pillar 6.

**3) Method for mobilisation of bacteria on hides**

A natural, food-grade resin (Shellac) has been shown to be an effective “microbial immobilisation” treatment of cattle hides, so to improve microbial status of carcass meat. Hide treatment by 23% Shellac-in-ethanol solution reduced recoveries of spoilage and pathogenic bacteria and has been validated under commercial abattoirs indicating that Shellac treatment enables reduction of carcass meat microflora, so improves meat safety. The study provided a “proof of concept” for a novel, “microbial immobilisation” approach to reducing transmission of micro-organisms from cattle hide onto the carcass meat. Efforts are focusing on how to bring to the next stage in terms of a system which is practical and economical.

**3) Raman spectroscopy** for determination of fatty acid profile in beef. The technique might be of interest for the beef industry, and could be of commercial interest for instrument vendors.

**4) Novel marinades** are in the process of development that have tenderizing effect on intact beef cuts. This can be an easy as well as healthy way to add value to less tender beef cuts.

**5) Bioprotective cultures** based on *Lactobacillus sakei* strains have been identified with the potential for commercial application in the guise of large scale production of protective cultures, meat inoculation process: for example spray, lyophilized powders and so on.

**6) Value added to muscles** Particular muscles in the carcass that have a clear potential for value adding. The cutting/deboning patterns must be slightly changed to exploit the findings. The information is openly available and in fact is in the process of being adopted by industry in Norway.

**7) Microbial Time Temperature Indicator (TTI)** for monitoring microbiological quality and safety of fresh meat, meat products and other chilled foods

**8) Edible films** for safety improvement: which containing different antimicrobial agents (oregano oil, sodium lactate,  $\epsilon$ -polylysine) for controlling quality and safety of fresh meat and meat products Edible films containing a *Lactobacillus* protective culture for the control of *Listeria monocytogenes* on fresh meat and meat products

**9) Antimicrobial marinades** based on wine and soy sauce

**10) Active Packaging/wrapping system** releasing lactic acid for meat (“antimicrobial packaging”/”active packaging”)

**11) Development of novel and functional beef products**

Studies have demonstrated the ability to achieve large enhancement in long chain PUFA in beef muscle by injection and in beef burgers by use of encapsulated-DHA. In the burger studies and based on a 200g serving then they would supply 40, 68 and 96% of recommended daily allowance (based on 450 g/d LCPUFA). Shelf life of both muscle and burger LCPUFA-enhanced products was shorter than commercially required but additional of antioxidant to the meat matrix ameliorated this problem. This work is now in consideration by project SMEs for commercial adoption.

**12) Predictive model of meat eating quality** in Europe based on biochemical & molecular tools

**Guidance Manual on Best practice in the beef industry**

It has also been decided to undertaken over the next two year the writting and publication of a guidance manual on best practice in beef production. This will be aimed at providing information directly for the beef industry on best practice in terms of safety, quality, technologies available etc. at each stage of the beef chain from primary production through to distribution. Writing will commence in 2010 and it is expected to be published in 2011.