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Specific targeted research project Poultryflorgut

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The objective is to provide a strong factual basis for the optimisation of the hygienic quality of the poultry products meant for human consumption, in conformation with the new European regulations concerning feed additives, and the changes occurring in poultry production. The project has been divided into five main work packages. The first work package is dedicated to the management of the project and to the dissemination of the scientific results. Work package 2 and 3 focus on the effects of the new European regulations and the new tendencies in breeding management on the food-borne pathogens (traditional and emerging) along the food chain: at the farms in work package 2 and at the slaughterhouse and processing plants in work package 3. Work package 4 aims to validate the utilisation of new molecular methodologies for the study of the whole intestinal flora of poultry as well as poultry meat and to determine the effect of dietary factors and rearing types, and interactions with the food-borne pathogens. Work package 4 will provide operational methodologies that will be also used in work packages 2 and 3. Work package 5 provides economic assessment of changes in poultry practice under the current pressure of the European regulatory and studies the effect of organizational arrangements in the poultry food chain affecting food safety.

Keywords: poultry; feed additives; intestinal flora; pathogens; molecular methods.

Introduction

Poultryflorgut is a Specific Targeted Research Project (STREP) supported by the European Commission for a three-year period initiated in the 6th Framework Program. This project started in March 2005. The project includes 15 partners (AFSSA, ITAVI, KVL, IRTA, INRA, VU, TiHO, UNIBO, IIVU, VFU, ASG-WUR, VLA, UNIPG, DVF, CreSA) from 10 European countries (France, Denmark, Spain, Austria, Germany, Italia, Lithuania, Czeck Republic, The Netherland, United Kingdom). The total estimated budget of this project is 3.1 M € and the European contribution is 2.6 M €.

Poultryflorgut aims to enhance control of the intestinal flora ecology in poultry for ensuring the products safety for human consumers. Indeed, European consumers are becoming increasingly concerned over the safety and nutritional quality of their food, which must be free from chemical and biological contaminants. At the same time, food production systems must meet the concerns of consumers for animal welfare, the environment and public health. This leads to, for instance, rapid changes in both poultry nutrition (e.g. banning of the use of in-feed antibiotics regulating the intestinal

micro-flora, use of 100%-vegetal diets) and rearing (e.g. reduction of the number of available coccidiostats, development of out-door breeding and organic farming) context. These drastic changes raised questions in the scientific community and the Agro-Food industry concerning an increasing risk of microbiologic contamination of poultry products (meat, eggs), and therefore demands a thorough and comprehensive investigation of all parts of the poultry industry.

The over-all objective of the project is to provide a strong factual basis for the optimisation of the hygienic quality of the poultry products meant for human consumption, in conformation with the new European regulations concerning feed additives, and the changes occurring in poultry production. A special focus is done on the control of the intestinal flora of the broilers and laying hens, including the food-borne pathogens. The main objectives are:

- Associate the occurrence of food-borne pathogens in poultry farms, in different European countries, with different production systems (in door, out-door) and to determine how to improve management in order to decrease the occurrence of food borne pathogens;
- Quantify the food-borne pathogens at the different steps of the food chain, from field to slaughterhouse, in different European countries;
- Create an European molecular database on traditional and emerging food-borne pathogenic strains/species in European Poultry production;
- Determine how to improve management in order to decrease the prevalence of food-borne pathogens along the food chain;
- Assess novel techniques for the study of the intestinal flora and of the relation-ship between the whole flora and the food-borne pathogens in poultry;
- Estimate the socio-economic consequences of the changes occurring in poultry production in response to the new European regulations and to the (public) European citizens concerns; Produce recommendations on future European policy.

Work plan of the project

The work plan has been devised into 5 work packages. The first work package concerns the management of the project, while the other work package cover almost all the poultry production chain, including the animal rearing, egg production, animal slaughtering, carcass and meat processing and packaging (see Figure 1).

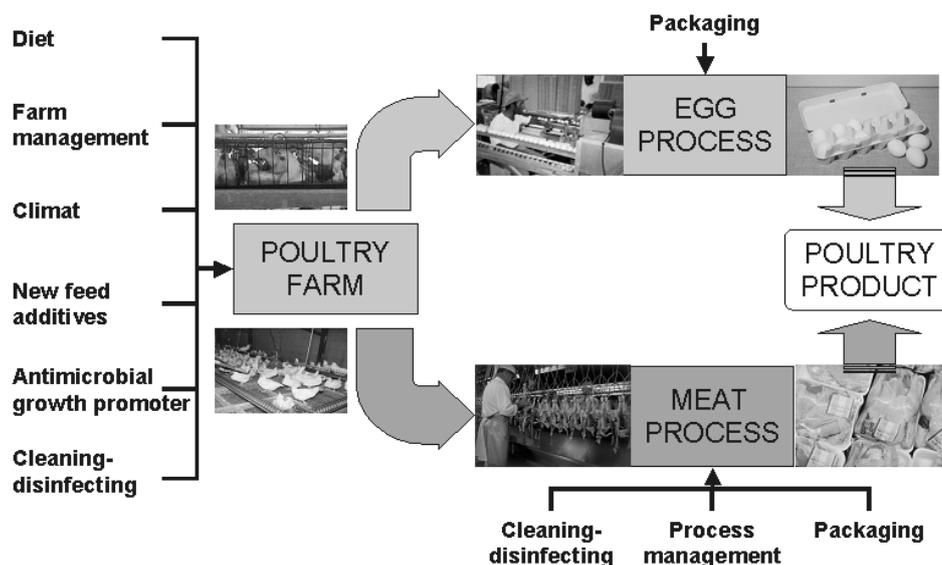


Figure 1: Total food chain approach

Work package 1:

The first work package concerns the scientific, technical, administrative and financial management of the whole project. It is of a great importance to stimulate and facilitate the exchanges and reach a good synergy between partners. It has also to ensure a clear and effective dissemination of results and transfer of knowledge and their applicability (valorisation) to all partners within the group, the international scientific community, agencies and local government, and industry.

Work packages 2 and 3:

The objective of work package 2 is to determine the effects of the new European regulations on poultry rearing on the prevalence and genetic diversity of *Campylobacter* in broilers, on the prevalence of *Salmonella* and *Listeria monocytogenes* at the farm level (broilers and laying hens), and on the emergence of new food-borne pathogens (*Helicobacter pullorum*, *Clostridium perfringens*). Work package 2 has also as objective to evaluate the effects of climatic, management factors, cleaning and disinfecting procedures on poultry intestinal bacterial pathogens in broilers. Finally, it has also as an objective improving the characterisation of the digestive troubles occurring in the field since the ban of in-feed antibiotics: specification of their prevalence and their incidence, determination of some pathogens possibly involved and of some causal factors. The objective of work package 3 is to monitor qualitatively and quantitatively the occurrence of traditional and emerging food-borne pathogens at the slaughterhouse and retail level, to identify possible intervention strategies to reduce contamination along the production line, and to evaluate the influence of different production and processing factors on the occurrence of the pathogens.

Regarding the methodology, the pathogens have been isolated quantitatively with an optimized Most Probable Number method. To complement the traditional methods using bacterial culture, molecular based techniques were used for:

- the quantification of microorganisms (Real Time PCR);

- the identification of microorganisms (biochemical tests, genotyping using pulsed field gel electrophoresis (PFGE) method, ribotyping, Restriction fragment length polymorphism analysis (RFLP-PCR/RFLP-), nucleic acid sequencing or identification based on rRNA sequences).

Work package 4:

Work package 4 has as its objective the validation of methodologies allowing the study of the whole intestinal flora of poultry as well as poultry meat and to determine the interactions with the food-borne pathogens. In fact this work package will provide novel molecular methodologies that will be also used in work packages 2 and 3. The idea is to go definitively beyond the experimental limitations related to the classical bacteriology techniques and to be able to characterize (qualitatively and quantitatively) the changes in the poultry intestinal microflora, in relation to external factors (alternative to in-feed antibiotics, free-range), but also in relation to the pathogenic zoonotic bacteria.

In a first part of this project, the methods used for the study of microbial diversity (DNA fingerprinting methods: Single Strand Conformational Polymorphism-SSCP-, Denaturing Gradient Gel Electrophoresis-DGGE-, Temporal Temperature Gradient Gel Electrophoresis-TTGE-, Terminal-Restriction Fragment Length Polymorphism-T-RFLP-) or for the quantification of microorganisms (Fluorescent *in situ* Hybridization-FISH-) were implemented, applied and compared in a second part of this project.

Work package 5:

The last work package (5) concerns the socio-economic aspects in European poultry production, related to the topics tackled in the other work packages and to the knowledge gained. This work package specifically addresses the economic assessment of changes in poultry practice and effect of organisational arrangements in the poultry food chain affecting food safety. The objectives within the analysis of organization arrangements are the description of managerial models adopted in selected production systems with respect to quality and safety; the determination changes required in the models under the current pressure of the European regulatory approach; and the assessment of the

related incentives to the food safety supply. Within the economic assessment of changes in poultry practice, the objectives are an investigation of the impacts of antibiotic growth promoters (AGPs) withdrawal on the costs and benefits relating to animal welfare, production levels, and consumer effects; to estimate the costs and benefits when increased biosecurity practices or other AGP alternative products are used; to make recommendations on where future EU research spending should be focused with regard to best practice, and to identify the knowledge gaps in this research area.

Conclusion

During the first step of the project, molecular-based methods (SSCP, DGGE, T-RFLP, FISH) have been adapted for the bacterial diversity study of chicken intestinal. Preliminary results showed some differences in the microbial count; in particular, the caecum of organic chicks compared to ileum, showed higher percentage of aerobic bacteria. The aerobic count in conventional birds had a opposite trend. The biochemical characterization of enteric flora did not identify large differences in organic and conventional chicks. The economic analysis of the costs and benefits associated with the withdrawal of antibiotic growth promoters and their replacements was also carried out.

The project is in progress until March 2008. Concerning the dissemination of results and the management of the project, four scientific and administrative meetings and two industry days have been held in France (2005), United Kingdom (2005), Italy (2006) and in Spain (2007). The final congress is planned in September 2008. Scientific results and management activities are presented in annual and periodical reports. The results and whole objectives of the project have been also presented in European and International congresses. Some results were published in international journals but the main of publications is still in progress. Finally, the project web site provides project information and results (<http://www.poultryflorgut.org/>).