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WORKSHOP III: 2006 EU BAN ON ANTIBIOTICS AS FEED ADDITIVES: CONSEQUENCES AND PERSPECTIVES

OBJECTIVE

This Workshop offers a forum to colleagues from academia and industry. It will present a critical re-appraisal on the rational of the EU ban, and the overall economic impact of this measure as well as regulatory attempts to stimulate licensing of new products. Additional contributions are devoted to current R&D activities within the industry, and new targets and strategies in product development will be presented and discussed.

WS13
Antibiotic ban in the European Union: a Pyrrhic victory?

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Major debates regarding the use of antibiotics as growth promoters (GP) have occurred during the last 35 years in the EU regarding the potential risk related to the antibiotic resistance in humans. From the Swann Committee report of 1969 concerns were raised on the potential problems due to the use of human antibiotics as GP in food producing animals and transference of resistance to humans. This correlation has never been clearly demonstrated or supported by evidence. The discussion concluded in 1974 with the elimination from the approved product list of all the antibacterials used in humans, such as tetracycline, lincosamides and others. A new wave of discussion started during the late 1990s, driven by the Nordic countries, on the risk of development of vancomycin resistant strains due to the use of avoparcin in animals. In spite of the fact that the Scientific Committee of Animal Nutrition (SCAN) did not support the evidence of this link, in 1997 the EU banned the use of this antibiotic in animal nutrition. The same process occurred later for four other antibiotics (zinc bacitracin, tylosin phosphate, virginiamycin and spiramycin). Only four antibiotics remained (avilamycin, flavomycin, monensin, salinomycin) each of which was not a member of any other class. Each possesses a narrow spectrum of activity with no cross resistance with products used in humans or for veterinary therapy. From January 1, 2006 the use of these is no longer permitted and some consequences have already been seen in the Nordic countries, where these products were already banned:

- increased diarrhoea in young piglets;
- evidence of more necrotic enteritis in poultry;
- increased coccidiosis and metabolic disorders in cattle.

In the meantime, the pattern development of resistance among human pathogens has not changed, being correlated more with antimicrobial use in human hospitals and general practice and to incorrect waste disposal than to veterinary use. The disease incidence in the veterinary field has been reduced by changing breeding management, together with increased medication using antibiotics with broad spectrum of activity, active also on food born pathogens like *Salmonella* spp and *Escherichia coli* and the same drugs are widely employed in human therapy. Have we made the best decision in the interests of consumers or, as can occur when decisions are based not on science but on emotion, have we produced an even greater risk? Is this a real or only a Pyrrhic victory?

WS14
The EU ban of antibiotics as feed additives (2006): alternatives and consumer safety

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INTRODUCTION

Antimicrobial feed additives have been used worldwide in animal production for many decades because of their favourable economic effects in livestock production. Added in low doses to the feed of farm animals, they improved growth and performance and hence were known as antimicrobial growth promoters (AGP). Due to the emergence of microbes resistant to antibiotics ('antimicrobial resistance') that are used to treat human and animal infections, the European Commission (EC) decided to phase out, and ultimately ban since 1 January 2006, the marketing and use of antibiotics as growth promoters in animal feed. Since then, the use of antibiotics is only allowed on veterinary prescription for direct applications or as medicated

feed. These restrictions are deemed necessary as antimicrobials may lead to the selection of resistant bacterial strains in animals that could be transferred to humans, by direct contact or via foodstuffs, and subsequently lead to an impairment of the efficacy of antibiotics used in therapy of human infectious diseases.

THE EUROPEAN UNION'S REGULATORY FRAMEWORK ON FEED ADDITIVES FOR USE IN ANIMAL NUTRITION

In 1997, the EC had already introduced the requirement to monitor the occurrence of resistance in animal bacteria associated with the use of antimicrobial feed additives and related substances (Commission Directive 97/6/EC of 30 January), suspending, at the same time, the use of avoparcin as a feed additive [1] in January 1997 and ardacin in January 1998. This obligation was reconfirmed by the Council Regulation 2821/98 of 17 December 1998 [2] suspending, in December 1998, the use of four other antibiotics (zinc bacitracin, virginiamycin, tylosin phosphate and spiramycin) that had been used as growth promoters in feed under the condition that their use should be re–examined [3]. Subsequently, the European Commission supported a surveillance programme, conducted by industry, to monitor antimicrobial resistance against feed additives in bacteria isolated from pigs and broiler chickens in the slaughterhouses in six European countries. However, as stated in the 'White Paper on Food Safety' (adopted January 2000), the European Commission at that time already considered the prohibition or phase–out of antibiotics used as growth promoters within the EU, as part of a broader strategy to control and combat antibiotic resistance. The new Regulation (EC) No. 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition [4] sets out the rules for the authorization, use, monitoring, labelling and packaging of feed additives. This Regulation replaces Council Directive 70/524/EEC of 23 November 1970 concerning additives in feedingstuffs [5] and completes the measures taken towards the total ban of antibiotics as growth promoters from 1 January 1 2006. On that date, the last four substances were removed from the EU Register of permitted feed additives.

FEED ADDITIVES FOR USE IN ANIMAL NUTRITION ACCORDING TO THE REGULATION (EC) NO 1831/2003

With the aim of avoiding economic losses, in particular in the production of pigs and broiler chickens, and to avoid a significant increase in the use of antimicrobials under veterinary prescriptions, the EC supported the view that the phasing out of AMG would be easier to pursue if other classes of growth promoting additives could be licensed. Subsequently, several microorganisms or probiotics, and an organic acid product were authorized for use as growth promoters. Moreover, enzymes preparations, such as glucanases, xylanases, proteases and phytases, as well as prebiotics (fructo–oligosaccharides and related compounds) were granted marketing authorization. In addition, general guidelines, establishing the prerequisites for marketing authorization of these alternative products, have been developed and implemented:

General prerequisites Article 5 of Regulation (EC) No. 1831/2003 states that a feed additive: (a) shall not have an adverse effect on animal health, human health or the environment, (b) shall not be presented in a manner which may mislead the user, and (c) shall not harm the consumer by impairing the distinctive features of animal products or mislead the consumer with regard to the distinctive features of an animal product.

Categories According to the Regulation (EC) No. 1831/2003 a feed additive shall be allocated to one or more of the following categories:

- Technological additives:
any substance added to feed for technological purposes;

- Sensory additives:
any substance where the addition of which to feed improves or changes the organoleptic properties of the feed and/or the visual characteristics of the food derived from the animals;

- Nutritional additives and zoothechnical additives:
any additive used to favourably affect the performance of animals in good health or the environment;
- Coccidiostats and histomonostats.

The *Zootechnical additives* include the following functional groups: (a) digestibility enhancers: substances which, when fed to animals, increase the digestibility of the diet through action on targeted feed components; (b) gastrointestinal flora stabilizers: microorganisms or other chemically defined substances, which, when fed to animals, have a positive effect on the gastrointestinal flora; (c) substances which favourably affect the environment; and (d) other zootechnical additives.

GUIDELINES CONCERNING THE APPLICATION FOR AUTHORIZATION OF FEED ADDITIVES FOR USE IN ANIMAL NUTRITION

Currently, a Commission Regulation laying down the rules and procedures regarding the application for authorization of feed additives for use in animal nutrition in accordance with Regulation (EC) No. 1831/2003 is in preparation; this new Regulation shall provide specific guidelines for the authorization of feed additives under the provisions of Article 7 of the Regulation. In preparation for this guideline, the FEEDAP Panel of EFSA has approved an Opinion defining the above mentioned categories, using as background Council Directive 87/153/EEC of 16 February 1987, describing the guidelines for the assessment of additives in animal nutrition [6] (amended by the Commission Directive 2001/79/EC of 17 September 2001 [7]. Three annexes of this Opinion cover the following aspects: models of applications forms referred to in Article 4(1)a and guidelines laying down the principles for the establishment of a dossier to be submitted with any application for authorization of additives for use in animal nutrition. Moreover, the animal categories for which products could be licensed have been defined. The common guidance for the establishment of a dossier consists of six sections: (I) summary of the dossier; (II) identity, characterization and conditions of use of the additive; methods of control; (III) studies concerning the safety of use of the additive to target animals; (IV) studies concerning the safety of the additive use for the human consumer; (V) studies concerning the safety of the additive for the user; (VI) studies concerning the safety of the additive for the environment. Specific guidelines have been prepared for additives that are already authorized for the use in food, in minor species, as nutritional additives, in pets

and other non food–producing animals as well as for sensory additives other than flavouring compounds, technological additives (silage additives), technological additives other than silage additives, zootechnical additives (enzymes, microorganisms), zootechnical additives other than enzymes and microorganisms, and coccidiostats and histomonostats.

SAFETY OF ADDITIVES FOR CONSUMERS

A variety of enzymes and microorganisms are used in feeds. Some of these have a long history of safe use and do not create any safety concerns (i.e. toxicity testing is not considered necessary) (see QPS). However, for viable microorganisms or active enzymes, for which there is no history of apparent safe use, certain toxicological tests, such as mutagenicity and genotoxicity studies and an oral subchronic toxicity study, are considered necessary to exclude the possibility of risks for the consumer.

ENZYMES:

Enzyme preparations are produced in varying degrees of purity from animal, plant and microbial sources, and may consist of whole cells homogenates, parts of cells, or cell–free extracts. The enzyme preparations may be formulated as liquid, semi–liquid or dry solid preparations. One of the important enzymes is phytase, which is added to feed to release phosphates from the plant material in the diet, hence reducing the need to add phosphates. In turn, phosphorus excretion is reduced, thus lowering the environmental burden. Other enzymes with a positive effect on the environmental burden of intensive animal husbandry are those reducing nitrogen excretion, methane production and off–flavours. Enzymes from a genetically modified source or genetically modified microorganisms (GMO) submitted for assessment have to comply with the requirements of Council and Parliament Directive 2001/18/EC of 12 March 2001 on the deliberate release into the environment of genetically modified organisms [8], and Council Directive 90/219/EEC on the use of genetically modified microorganisms [9,10]. The unique identifier for each GMO should be included as demanded in Commission Regulation (EC) No 65/2004 of 14 January 2004, establishing a system for the development and assignment of unique identifiers for genetically modified organisms [11].

PROBIOTICS:

Probiotics are defined as 'living micro–organism that, upon ingestion in certain numbers, exerts health benefits beyond inherent basic nutrition' [12]. Their use as powder or granulate formulations is linked to proven efficacy on the gastrointestinal microflora. This positive effect on the gastrointestinal flora results not only in an improved health status, especially in young animals, but also in improved animal performance, including growth rate and/or feed conversion efficiency. There are different categories of probiotic feed preparations: Additives containing about 10^{10} CFU g^{-1}, premixes containing 10^{8} CFU g^{-1}, and feeds, meals or pellets containing about 10^{6} CFU g^{-1} [13]. Microorganisms that are used in animal feeds in the EU are mainly Gram–positive bacteria belonging to the families *Bacillus* (*B. cereus var toyoi, B. licheniformis*; *B. subtilis*), *Enterococcoccus* (*E. faecium)*, *Lactobacillus* (*L. acidophilus, L. casei, L. farciminis, L. plantarum, L. rhamnosus*), *Pediococcus* (*P. acidilactici)*, *Streptococcus* (*S. infantarius*). Lactic acid–producing bacteria are used in a large variety of fermented feed applications. The consumption of feed with lactic acid-producing bacteria is not considered to be of major concern, but from the theoretical point of view, horizontal transfer of antibiotic resistance genes can take place in the feed, during production or in the animal's gastrointestinal tract, and constitute a risk for humans. Others probiotics are microscopic fungi or strains of yeast, such as *Saccharomyces cerevisae* or *Kluyveromyces* species [14]. While most of these species and genera are apparently safe, certain microorganisms may be problematic, particularly the enterococci, as these might harbour transmissible antibiotic resistance determinants. For example, some enterococcal strains have shown resistance to vancomycin and were able to transfer this type of resistance to other bacterial species. Bacilli, especially those belonging to the *B. cereus* group, are known to produce enterotoxins and an emetic toxin. Hence, the applicant is required to present studies that show the potential of each bacterial strain (but not yeasts) to carry resistance genes and to be able to transfer these to other microorganisms [14,15]. Even more importantly, microorganisms intended for use as probiotic should not be able to produce any antimicrobial substance that is used as an antibiotic in humans or animals.

THE QUALIFIED PRESUMPTION OF SAFETY (QPS) CONCEPT OF MICROORGANISMS IN FOOD AND FEED:

The QPS approach of microorganisms in food and feed [16] is a system, similar in concept and purpose to the GRAS (Generally Recognised as Safe) definition used in the USA, but modified taking onto account the different regulatory practices in Europe. QPS provides a mechanism for recognizing and giving weight to prior knowledge when assessing the safety of microorganisms in food and feed products. The QPS concept appears to be applicable to food, feed and microbial products. Prior to the implementation of the QPS concept, further harmonization of the existing guidelines is required. For example, at present microorganisms used for the fermentation of food are not subjected to any community regulation, with the exception of those encompassed by the Novel Food Regulation [17]. In contrast, microorganisms used as feed additives or plant protection products are comprehensively regulated. This has led to the illogical situation where individual bacterial strains, used freely in human food production, have been subject to stringent safety assessments when seeking EC approval as a feed additive. Nevertheless, the QPS approach represents a possible route to the harmonization of safety assessment of microorganisms used in feed and food production without introducing unnecessary precautionary measures in areas where there is no major concern for consumer safety. Therefore, QPS is proposed as an operating procedure within EFSA for risk assessment.

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WS15
EU promoted research towards zootechnical feed additives for the safe use in poultry production

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INTRODUCTION

The livestock industry in Europe is facing new feed additive regulations after the phasing out and banning of antimicrobial growth promoters in animal feed, which may cause drug resistance in microbes that afflict humans. In turn, alternatives for these antimicrobials need to be developed. In modern poultry husbandry, newly hatched broiler chickens do not come into contact with mother hens. This lack of contact is believed to result in delayed development of the intestinal microflora, and as a consequence broilers are particularly receptive to pathogen colonization at a very young age. In this respect, probiotics from the animal's gastrointestinal tract are of current interest, as they offer a biological alternative that should find acceptance from both producers and consumers. A multi–national project (C–EX QLK–CT–2002–71662) funded by the EU was initiated and brought together five industrial and three research partners in order to develop a safe microbial feed additive for poultry. The main scope was to establish a well-defined multi-component additive combining various effective strains, which complies in terms of identity, efficacy and safety with the current EU guidelines [1] for the evaluation of probiotics for use in feedstuffs.

MATERIAL AND METHODS

Bacterial strains were isolated from the fresh gastrointestinal contents from the crop, jejunum, ileum and caeca of healthy chickens. Standard culture techniques using different media were applied for isolating bacteria at 37 °C under aerobic, facultative anaerobic and strict anaerobic conditions. To classify the isolated strains, a polyphasic approach was carried out combining morphological, physiological and genotypic methods (e.g. morphology, SDS–PAGE of whole cell protein patterns, analysis of metabolic end products, 16S rRNA gene analysis). By using a co-cultivation assay, representative cultures were screened for any inhibitory activity against *Salmonella enteritidis* (Bio59), *S. choleraesuis* subsp *choleraesuis* (Bio554), *Escherichia coli* O147:H19 (CCUG 11447), *E. coli* O157:H7 (USDA71), *Campylobacter jejuni* (CCUG 25903) and *Clostridium perfringens* (CCUG 47895). Antibiotic susceptibility testing was done using the microdilution technique. Conjugative transfer of resistance to vancomycin and tetracycline was studied by direct plate colony mating using the enterococcal recipient strains DSM 13589 (*Enterococcus faecium*) and LMG 19456 (*Enterococcus faecalis*), respectively. PCR assays for detection of *vanA* were performed as described previously [2]. The presence of enterococcal virulence factors was examined by PCR as described previously [3]. Plasmid DNA was isolated with the Nucleo Spin Plasmid DNA Purification Kit (Machery–Nagel) according to the manufacturer's instructions, and additionally on a large scale using the alkaline lysis method followed by the separation in a caesium chloride gradient.

RESULTS

Numerous bacteria (n = 477) were isolated from the gastrointestinal tract of healthy chickens out of which 121 were selected as representative, based on differences in whole cell protein patterns and screening for antagonistic properties against common poultry pathogens. Ninety strains exhibited the ability to inhibit *S. enteritidis* and the most effective strains were able to inhibit several indicator pathogens such as *E. coli* (different serotypes), *S. choleraesuis*, *C. jejuni* and *C. perfringens.* On the basis of these first results, five well-defined strains belonging to the genera *Enterococcus*, *Pediococcus*, *Lactobacillus* and *Bifidobacterium* were selected for further evaluation according to the guidelines laid down in an Opinion of the Scientific Committee on Animal Nutrition [4]. These strains were sensitive to the majority of clinically effective antibiotics, although some of them showed single resistance to vancomycin or tetracycline. Based on the *in vitro* mating studies and molecular methods used to exclude the presence of easy transferable resistances or potential virulence traits, none of the vancomycin–resistant strains carried the enterococcal *vanA* gene. The strains contained no extrachromosomal DNA and were not able to transfer the resistance by means of conjugation. The enterococcal strain was demonstrated to lack the most concerning virulence markers, specific for the surface protein gene *esp*, the cytolysin activator *cylA* and the gelatinase *gelE* gene, the *cylB* gene involved in transport of cytolysin, the cell wall adhesion–encoding *efaAfs* and *efaAfm* genes, as well as sex pheromones (*cpd, ccf*).

DISCUSSION AND CONCLUSION

Five well-studied bacterial strains isolated from the ingesta of chickens were found to inhibit several indicator pathogens *in vitro*. Easy transferable resistances or potential virulence traits were excluded, mainly on basis of the absence of plasmids, and nontransferability by conjugation. This data encouraged us to proceed with a more detailed study of the strains intended for combined use as a natural feed additive in young chickens. Since the mode of protection is not exactly known, further work is in progress to evaluate their protective ability *in vivo* in feeding experiments.

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