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Simultaneous and rapid detection of five banned antibiotic growth promoters by immunoassay

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Abstract

As a result of increasing concerns over the transfer of resistance between different bacteria and between human and animals, a group of antimicrobial growth promoters including bacitracin, spiramycin, tylosin, virginiamycin and olaquindox have been banned in the EU since 1999. A rapid and sensitive enzyme-linked immunosorbent assay (ELISA) with simple extraction procedures was developed for simultaneous screening of the five banned feed additives in animal feeds. Banned substances were extracted from animal feeds with 70% methanol in water followed by clean-up on OASIS® HLB cartridges. Polyclonal (Pab) antibodies were generated and their specificities assessed by cross-reactivity studies with substantial numbers of antimicrobial agents that are administered to animals via feedingstuffs. The target minimum detectable concentrations (MDC) of 4 mg kg^{-1} for olaquindox and 1 mg kg^{-1} for bacitracin, spiramycin, tylosin and virginiamycin were set for the present study. Recoveries at the three levels of the target concentrations (4, 6 and 8 mg kg^{-1} for olaquindox, 1, 1.5 and 2.0 mg kg^{-1} for the other four compounds) ranged from 84 to 145% with coefficients of variation less than 18%. The detection capability for virginiamycin, bacitracin, spiramycin, tylosin and olaquindox were 0.2, 0.3, 0.6 and 1.5 mg kg^{-1}, respectively.

Keywords: Antibiotic; Simultaneous; Growth promoter; Feed additive; Animal feedingstuff; ELISA

1. Introduction

The intensive production of food animals has led to the extensive use of antibiotics for growth-promoting purposes besides disease treatment and prophylaxis. Approximately 42% of all veterinary antibiotics used world-wide were feed additives for performance enhancement [1] while 30% was reported in the European Union [2]. In the European Union, the performance-enhancing antimicrobial drugs were classified as zootechnical feed additives (Council Directive 70/524/EEC and Council Directive 96/51/EC) [3,4] and incorporated into feedingstuffs at small doses to improve feed conversion and reduce the formation of toxins. On the other hand, the use of antimicrobial feed additives can result in development of resistance to the antibiotics and related compounds, with the first evidence of tetracycline [5] and later vancomycin in the late 1980s [6] and most recently streptogramin resistances [7]. As a result of increasing concerns over the adverse effect of some antibiotics on human or animal health, and the transfer of resistance between different bacteria and between human and animals, several antibiotics have been banned as feed additives in the EU since 1999, including bacitracin, spiramycin, tylosin, virginiamycin (Council Regulation 2821/98) [8], and olaquindox (Commission Regulation 2788/98) [9]. The chemical structures of these compounds have been illustrated in Fig. 1.

Bacitracin, a cyclic polypeptide antibiotic produced by *Bacillus subtilis* and *Bacillus licheniformis* [10] with anti-Gram-positive activity, was one of the most common antimicrobial agents used world-wide as feed additive (at concentrations of 5–100 mg kg^{-1}) for growth promotion in

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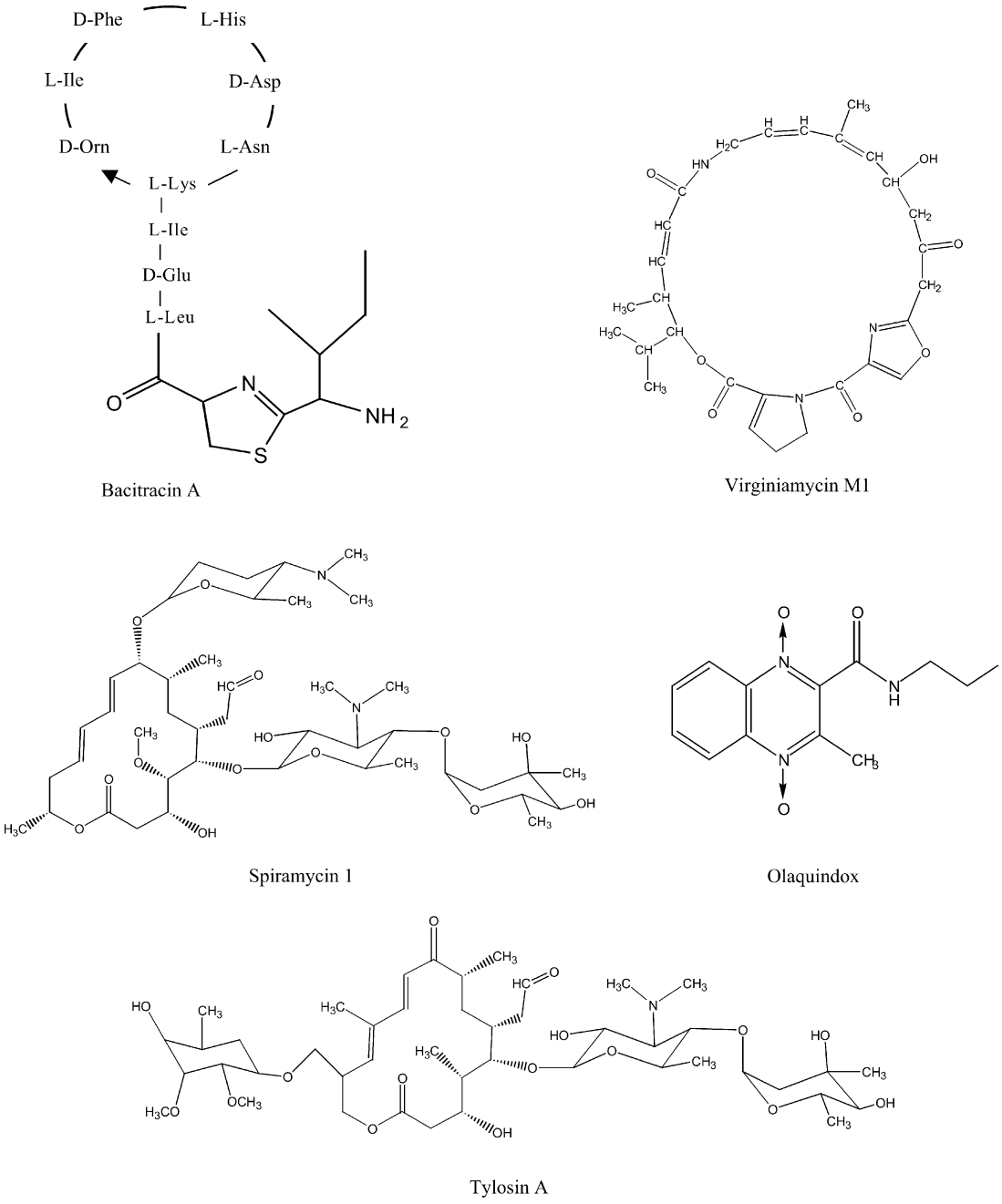

Fig. 1. Molecular structures of the antimicrobial reagents.

pig, poultry and cattle husbandry for more than 30 years [11,12]. Commercial preparation of zinc bacitracin is a mixture of several similar polypeptides with the main form of the peptide being bacitracin A [13]. The community method for determination of zinc bacitracin in animal feeds at lowest level of 10 mg kg^{-1} is a microbiological diffusion agar assay recommended by the Analytical Methods Committee in 1978 [14].

Spiramycin, produced by *Streptomyces* ambofacien, is a broad-spectrum macrolide antibiotic showing Gram-positive antibacterial activity. It has been extensively used for treatment of various bacterial infections such as respiratory disorders both in human and in animals, owing to its absence of toxicity at therapeutic doses. As a growth promoter, spiramycin was incorporated into animal feedingstuffs at concentrations ranging from 5 to 80 mg kg^{-1} depending on the age and species of animal. Commercial preparations of spiramycin are a mixture of three major components, differentiated in the substitute at 3-position, namely, I (3-OH), II (3-*O*-acetyl) and III (3-*O*-propionyl). Higgins et al. [15] reported a single-plate microbiological growth inhibition assay for screening of spiramycin at a concentration of 6 mg kg^{-1}