DEVELOPMENT OF A SINGLE PLATE ASSAY FOR SCREENING OF ANTIBACTERIAL SUBSTANCES IN ANIMAL FEEDS

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In spite of strict regulations controlling the quality of animal feeds in Sri Lanka, it is a common suspicion that therapeutic antimicrobial agents may be used at sub-therapeutic levels in management practices, in order to minimize animal deaths. Such malpractice is only economically viable if the drug is added to feed. The restrictions on irrational use of therapeutic antimicrobial agents in animal feeds are essential in order to avoid residues in food commodities and to minimize possibilities of emerging resistant bacteria which may contaminate the food chain. Danger of transmitting resistant bacteria through the food chain is remote due to proper cooking procedures adopted by Sri Lankans but is required to ensure good veterinary and farming practices. All these efforts will ensure a producer oriented food safety system. The objective of the present study is to establish a bioassay technique, which will screen the presence of antibacterial substances in animal feed of any type.

In this bioassay technique *Bacillus stearothermophillus* var. *calidolactis* is used as the indicator organism. The organism *Bacillus stearothermophillus* was maintained on Nutrient Agar slants and the test culture was prepared in Tryptone Dextrose Yeast Extract (TDYE) broth, every time testing is carried out. The routine assay was performed in Mueller Hinton Agar (MHA) by inoculating the broth containing indicator organism in predetermined quantities. Test sample of feed was extracted in acetone buffer. Bioassay was performed in petri dishes, which contained a standardized volume of MHA containing the indicator organism. The assay was based on the principle of agar gel diffusion. Therefore the test sample (100 µl) was added into wells of 8 mm diameter, cut in agar using a cork-borer. Every assay plate carried a commercial disk of antibiotics (Oxoid) at the center as the reference standard. Known negative feed samples were also extracted and placed on assay system to exclude false positive results.

The assay was validated using spiked sub-samples of a known negative feed sample, with a serial dilution of different antibiotics, which were suspected to be present in animal feeds. Accordingly, Minimum Detectable Concentrations (MDC) were established for different antimicrobials under the present laboratory conditions. The observed MDC for sulphadiazine and ciprofloxacin was 0.25 mg/kg, whilst zinc bacitracin, chlortetracycline, streptomycin, furazolidone showed MDC of 0.025 mg/kg. The best sensitivity (0.0025 mg/kg) was given with erythromycin while flavomycin was not detectable. Therefore it is concluded that the single plate assay procedure is suitable for detecting inhibitory substances in animal feed and provides a simple, rapid and multi-analytic screening test. Since the assay system is non-specific it was unable to identify or to quantify the inhibitory antimicrobial agent. It is suggested that the development of a secondary screening procedure such as Thin Layer Chromatography (TLC) method could be used to identify and quantify the inhibitory antimicrobial agents in the event of detecting positive samples.