

P018 Characterisation of the Antimicrobial and Antiprotease Activities of AWAPIV

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AWAP IV constitutes the C-terminal domain of a larger 81KDa protein called AWAK (Avian WAP domain and Kunitz domain containing). The protein is predicted to contain four WAP domains and one Kunitz domain. RT-PCR analyses revealed AWAP IV expression in the small intestinal and kidney tissues of 5-day old chicks. Time-kill assays using recombinant (r) AWAP IV indicated anti-microbial activity against gram-positive and gram-negative bacteria including Salmonella, Streptococcus and Staphylococcus spp. A rapid dose-dependent permeabilization of the outer membrane of Salmonella, as shown by the NPN dye assay, supported the ability of rAWAP IV to disrupt prokaryotic membranes. However at comparable concentrations rAWAP IV showed no significant toxicity against erythrocyte cell membranes.

WAP domains can function as inhibitors of serine protease activity. Activity of the microbial serine proteases subtilisin and proteinase K were inactivated by r AWAP IV. In contrast at comparable peptide concentrations no such inhibition of the mammalian serine protease elastase was observed. The combined broad spectrum anti-bacterial and anti-protease activities of AWAP IV suggest a novel role as part of the avian innate defence mechanisms against microbial infection.