

DIGESTIVE ENZYME ASSOCIATION IN CATTLE TICK RHIPICEPHALUS
(BOOPHILUS) MICROPLUS MIDGUT

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The cattle tick *Rhipicephalus (Boophilus) microplus* is responsible for severe damages and economical losses in cattle breeding. Detailed studies of the parasite physiology are crucial to identify potential targets for immunological control. The present work concerns on the isolation and characterization of digestive enzymatic activities relevant for tick development and for bloodmeal digestion. Previously, we identified a cysteine endopeptidase with vitellin degrading activity in *R. microplus* larvae. Here we describe the isolation of this activity from partially engorged females midgut by the same chromatographic protocol used for purification of the larval enzyme. Proteolysis reaction products (verified by SDS-PAGE) showed both activities are able to degrade vitellin and hemoglobin and also they are inhibited by E-64 (a cysteine endopeptidase inhibitor). Pepstatin, an aspartic protease inhibitor, also affected vitellin and hemoglobin degradation by both enzyme preparations. N-terminal aminoacid sequencing and western blotting analyses confirm the presence of aspartic protease in cysteine endopeptidase preparation. Size-exclusion chromatography in 1M NaCl was effective to separate these two enzymes. These results suggest both enzymes are associated, presenting digestive activity upon the two major proteins for nutrition of different stages of *R. microplus* life cycle. These findings increase our knowledge about the protein digestion process in this tick, enlarging the spectrum of possible targets for vaccines against this parasite.