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# Proteolytic digestion of blood meal in *Ornithodoros erraticus*, a vector of *Borrelia crocidurae* causing relapsing fever in Egypt.

**Nawal M. Shanbaky[1]; Nadia Helmy[1]; Hala M. Khater[2] and Ayat Yousery[1]**

**1 – Department of Entomology, Faculty of Science, Ain- Shams University.**
**2 – Research and Training Center on Vectors of Diseases, Ain- Shams University.**

## ABSTRACT

Proteolytic activity was demonstrated in each of the midgut lumen content (MLC) and midgut wall (MW) of the mated unfed and fed female and male *Ornithodoros erraticus*. In both midgut compartments proteinase activity levels increased after feeding to reach at their peaks about double times the levels in the unfed controls. The peak levels of the proteolytic activity were attained on 1 and 1 – 2 days after feeding (daf) in the MLC (48 and 31.10 – 32.63 µg alanine $\times 10^3$/ min/ mg gut content) and on 5 – 6 and 5 – 7 daf in the MW (196.33 – 200.33 and 156.80 – 161.23 µg alanine $\times 10^3$/ min/ mg tissue) in the female and male tick, respectively. However, the proteolytic activity in the MLC was much lower than that in the MW of all the corresponding physiological states studied, and represented about one fourth and one fifth of it at the peak activity levels in the female and male tick, respectively. Generally, proteolytic activity was higher in the female than male ticks in the unfed and up to the 2$^{nd}$ daf in the MLC and in all the corresponding examined states in the MW throughout the period of study (20 daf).

Changes in the level of proteolytic activity in each midgut compartment of the female and male ticks infected with *Borrelia crocidurae* followed almost the same pattern in the uninfected controls. However, the level was lower in the MW of *Borrelia* infected ticks than uninfected ones throughout most (2–20 daf) of the period of study.

The present results were discussed and conclusions conformed to the view of intracellular proteolytic digestion of blood meal in the MW, but did not exclude a possible role of extracellular proteolytic digestion in the MLC.

**Keywords:** *Ornithodoros erraticus, Borrelia crocidurae* , relapsing fever , Egypt

## INTRODUCTION

Ticks are haematophagous obligatory ectoparasites of vertebrates which affect human and animal health. Ticks have been incriminated as vectors for disease pathogens including viruses, rickettsiae, bacteria, spirochetes and protozoa (Balashov, 1972; Hoogstraal, 1985). The argasid ornithodorine ticks are vectors of several spirochetes of genus *Borrelia* which are pathogenic for man and mammals (Burgdorfer, 1976; Johnson, 1977; Schwan, 1996; Tarasov, 1999). The small race of *Ornithodoros (Pavlovskyella) erraticus* which had been recorded throughout Egypt was found to harbor *Borrelia crocidurae* in nature and was established as vector of the endemic North African relapsing fever (Davis and Hoogstraal, 1954).

The midgut with its internal milieu, enzymes and epithelial cell activities during digestion may represent a serious barrier along the spirochete route into the body cavity of the tick which may affect the vector potential. The ability of *Borrelia* to survive and multiply in the gut during blood meal digestion is an

important step in successful establishment of the pathogen colonization and infection inside the vector (Pal *et al*., 2000). Proteolytic enzymes are important in digestion of blood meal proteins to liberate the amino acids needed by the tick body. In ticks and insects a pathogen which is ingested with an infected blood meal is subjected to the proteolytic enzymes activity and might be influenced with it (Tsuji *et al*., 2008) or vice versa (Dillon and Lane, 1993; Daba *et al*., 1997b; Jahan *et al*., 1999). Generally, protease study in ticks may help to identify interesting target molecules toward which novel chemotherapy and immunoprophylaxis may be directed for controlling ticks and tick-borne diseases (Boldbaatar *et al*., 2006).

Physiological, biochemical (Tatchell, 1964; Tatchell *et al*., 1972; Balashov, 1972; Akov *et al*., 1976; Grandjean, 1984) and histological (Balashov, 1972; Grandjean and Aeschlimann, 1973; Balashov and Raikhel, 1977, 1978; Akov, 1982) studies of protease activity in argasid ticks have provided evidence that proteolytic digestion is a slow intracellular process within the lysosomes of the midgut epithelial cells. Intensive proteolytic digestion starts at the end of the initial phase of blood meal concentration and haemolysis of red blood cells in the midgut lumen, where little digestion and splitting of food components occur (Balashov, 1972; Akov, 1982). During the phase of intensive digestion, midgut cells are hypertrophied and project into midgut lumen accumulating hemoglobin and other liquid protein by pinocytosis and uptaking larger particles and components of the lyzed blood cells by phagocytosis (Balashov, 1972; Akov, 1982). In the digestive epithelial cells, hemoglobin and serum protein digestion and assimilation take place and elimination of haematin into the gut lumen occurs (Kitaoka and Fujisaki, 1976; Grandjean, 1984). Most of physiological and biochemical studies which assessed protease activity, measured changes in the amount of hemoglobin in the whole gut (Akov *et al*., 1976), whole tick (Tatchell *et al*., 1972) or gut content (Grandjean, 1984) using different methodology and physiological states of ticks which make it difficult to compare results of various studies. None of the previous studies measured proteolytic activity in each of the gut lumen and gut wall of the investigated ticks. Also, no one investigated the effect of borrelial infection on proteolytic activity in argasid ticks during blood meal digestion. Therefore, the present study aims to measure changes of proteolytic activity during digestion of blood meal in the midgut lumen content and midgut wall of female and male *O. erraticus* uninfected and infected with *B. crocidurae*.

## MATERIALS AND METHODS

**Ticks:**

The argasid tick, *Ornithodoros erraticus*, was collected from burrows of the Nile grass rat *Arvicanthis niloticus* in Monofeya, Egypt. Ticks were placed in rearing polyethylene tubes, sealed at one end by a mixture of gypsum. The tubes were covered with pieces of muslin cloth securely held by rubber bands. The rearing tubes were kept in desiccators containing saturated solutions of sodium chloride to obtain 75% relative humidity (RH). Desiccators were maintained at 28 ± 1° C. The hamster, *Mesocricetus auratus* was used as a laboratory host tick.

**Tick colonies:**

Samples of the collected ticks were examined for spirochetal infection with *Borrelia* using Fontana stain (Conn *et al*., 1960). Infected ticks were separated from uninfected ones. Uninfected F1 adults of field collected tick, showing no spirochetes in HL were used to start the

uninfected stock for the laboratory colonies.

Adults and nymphs of *O. erraticus* found to be naturally infected with *Borrelia crocidurae* were fed on hamsters. By 4-7 days later the hamsters developed spirochetemia were used as the infection source for ticks by feeding the uninfected ticks on them. Uninfected and infected tick colonies were carefully separated. Also hamsters infected with *Borrelia* and uninfected hamsters used for feeding ticks were kept in separate cages.

**Testing ticks and hosts for infections:**

To test ticks for infection, a drop of hemolymph (HL) was collected by cutting tarsus I and withdraw the exuding fluid on a glass slide the HL was smeared and stained with Fontana Stain. To test hamsters for infection, a drop of blood was collected by cutting off a claw. The blood was smeared on a glass slide and stained with Fontana stain.

The smears of tick HL or hamster blood were examined microscopically for the presence of spirochetes.

**Experimental groups and selection of ticks:**

Two groups of *O. erraticus* ticks were used in this study. The first group was uninfected ticks and the other group was infected with the spirochetes. Virgin females and newly emerged males from each group were obtained by keeping engorged 3$^{rd}$, 4$^{th}$ & 5$^{th}$ nymphal instars individually in separate rearing tubes until molting. Mating was allowed by placing pairs of males and females in the rearing tubes. The study was carried out on the midgut of mated newly emerged males and females before feeding and at various time intervals after feeding (immediately, 1-7 day and 20 day after feeding). To minimize the possible effects of various size of the meal (Tatchell *et al*, 1972 and Akov *et al*, 1976), ticks that had ingested approximately the same amount of blood were used to assess protease activity levels.

**Tick dissection and collection of samples:**

In order to obtain the midguts, ticks were dissected as described by Ribeiro (1988) with some modifications.. The tick was fixed in a small paraffin filled Petri dish and covered with phosphate buffer saline (PBS) and under a dissecting microscope the dorsum was removed by cutting around its edges with a microscalpel. The midgut was separated from other organs using fine forceps with care to keep the gut intact. The midgut lumen contents were carefully removed with a special care not to disrupt the gut epithelium. Lumen contents of 5 guts were collected in 1.25 ml PBS and centrifuged at 5000 rpm for 20 minutes at 4º C. The supernatant was stored at – 20° C until used.

The empty midguts were washed intensively with PBS to remove the host blood cells from them. The midgut walls were homogenized mechanically in chilled PBS (5 gut wall / 250 μL PBS) using a pellet pestle. The homogenized tissues were then centrifuged at 5000 rpm for 20 minutes at 4º C. The supernatant was recovered and stored at – 20° C until used.

Three replicates of each pool of midgut contents, midgut walls were used to assess proteolytic activity in each of the uninfected and infected female and male.

**Determination of proteolytic activity:**

Proteolytic activity was measured in tick midgut wall and lumen content as described by Tatchell *et al.* (1972), by measuring the increase in free amino acids split from substrate protein (Bovine serum albumin), during one hour incubation at 30° C.

The reaction mixture consisted of 150 μl gut homogenate or 20 μl gut content, 1 ml of a mixture of 0.01 N HCl and 0.75 % bovine serum albumin (substrate). The proportion was such that

the final pH was between 2.9 and 3.1. Volumes of 0.4 ml of the reaction mixture were incubated for one hour at 30º C or added directly to 0.5 ml 20 % trichloroacetic acid (TCA) for use as zero time standards. The reaction was stopped by adding 0.5 ml of 20% TCA. After standing for 20 minutes, the mixtures were centrifuged at 3000 rpm for 20 minutes, and the supernatant was used for measuring the quantity of the produced amino acids.

Amino acids were colorimetrically assayed by ninhydrin reagent according to the method described by Lee and Takahashi (1966). The reaction mixture consisted of 100 µl of the supernatant, 2.9 ml of 1% ninhydrin solution, 1.0 ml of 0.5 M citrate buffer (PH 5.5) .The mixture was heated in boiling water bath for 12 minutes and then cooled. The developed color was read at 570 nm against zero time standards.

Proteolytic activity measured by the amount of amino acids split from the substrate using alanine standard curve.

**Alanine standard curve:**

The standard curve of D, L alanine was constructed by preparing different concentrations of D, L alanine containing 100, 200, 400, 600, and 800 µg / ml. Hundred µl of each concentration were added to 2.9 ml of 1% ninhydrin solution, 1.0 ml of 0.5 M citrate buffer (pH 5.5) .The mixture was heated in boiling water bath for 12 minutes and then cooled. The developed color was read at 570 nm against blank reagent prepared from 100µl PBS instead of the alanine. The optical densities were plotted graphically against alanine concentrations. The resulting curve was used as standard and the amino acids were expressed as µg alanine $\times 10^3$/min/mg tissue or gut contents.

**Statistical Analysis:**

The obtained data were manipulated statistically with the help of STATISTICA version 5.0 for windows using multivariate ANOVA (ANOVA/MANOVA).

## RESULTS

**Proteolytic activity in the midgut lumen content of mated female and male *O. erraticus*, uninfected and infected with *B. crocidurae*, during blood meal digestion:**

In comparison with the unfed controls, proteolytic activity in the midgut lumen content (MLC) of each of the uninfected female (Fig. 1) and male (Fig. 2) greatly increased ($P < 0.001$) immediately after feeding (imm. a. f.) to reach a peak ($P < 0.001$) on the 1$^{st}$ daf and the 1$^{st}$ and 2$^{nd}$ daf (48 ± 0.72 and 32.63 ± 1.33 – 31.10 ± 0.62 µg alanine $\times 10^3$/ min/ mg gut content) before declining ($p < 0.001$, $p < 0.05$) on 2-3 and 3-4 daf in the female and male, respectively. On day 4-20 and 6-20, the enzymatic activity decreased ($P < 0.05$) to a more or less constant level approaching ($P > 0.05$) that in the unfed uninfected female and male, respectively (20.53 ± 1.52 and 14.93 ± 1.21 µg alanine $\times 10^3$/ min/ mg gut content). Similarly, the relative activity of proteinases increased immediately after blood meal ingestion and subsequent days up to the 3$^{rd}$ and 4$^{th}$ daf in the female and male, respectively. During these periods the relative activity increased 1.31-2.34 and 1.39-2.19 times that in the unfed uninfected female and male before declining to a level similar to that in the unfed uninfected control.

Changes in the proteolytic activity in the MLC of each of the infected female (Fig. 1) and male (Fig. 2) followed almost the same pattern of that in the uninfected female and male, respectively. Proteolytic activity greatly increased ($P<0.001$) imm.a.f. to reach a peak ($P < 0.001$) on 1 and 1-2 daf (45.73 ± 1.30 and 30.27 ± 1.41-31.17 ± 0.6 µg alanine $\times 10^3$/ min/ mg gut content) before declining ($P < 0.001$, $P < 0.05$) on 2-3 and 3-4 daf in the female and male, respectively. On 4-20 and 6-20 daf, the proteolytic activity decreased ($P < 0.05$) to reach a more or less constant level approaching ($P > 0.05$) that in the unfed

infected female and male, respectively (19.83 ± 0.81 and 13.93 ± 1.19 µg alanine $\times 10^3$/ min/ mg gut content).

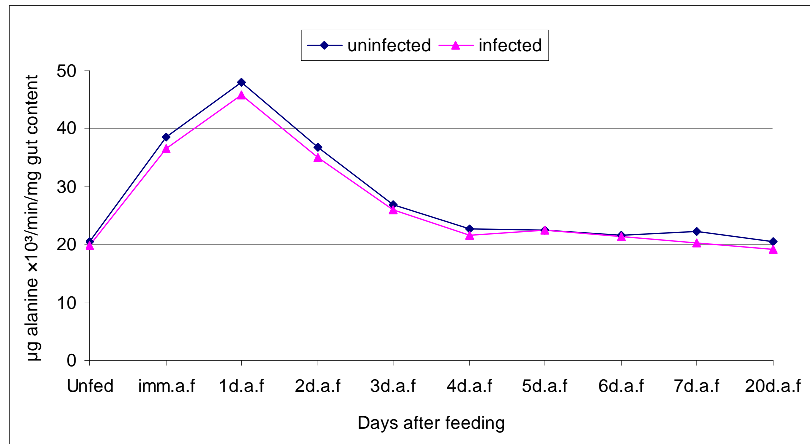


Fig. 1: Proteolytic activity in the midgut lumen content of female *O. erraticus* (uninfected and infected with *B. crocidurae*) at various time intervals after feeding.

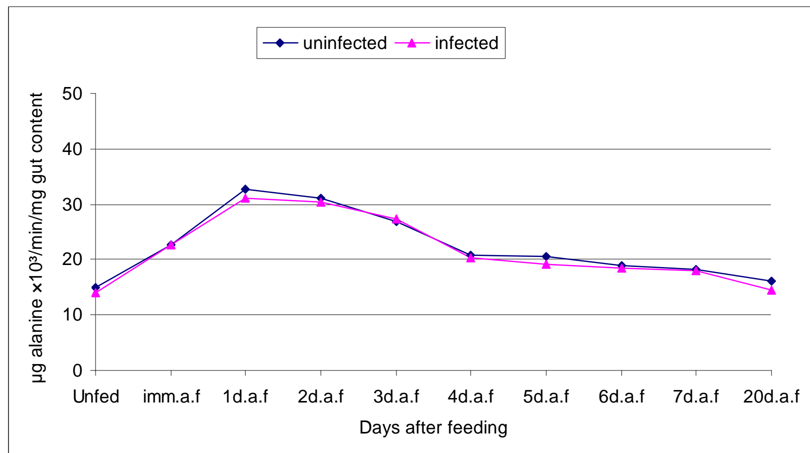


Fig. 2: Proteolytic activity in the midgut lumen content of male *O. erraticus* (uninfected and infected with *B. crocidurae*) at various time intervals after feeding.

Proteolytic activity levels in the MLC of the uninfected and infected ticks were similar ($P > 0.05$) in each of the female and male corresponding physiological states examined throughout the period of study (Fig. 3).

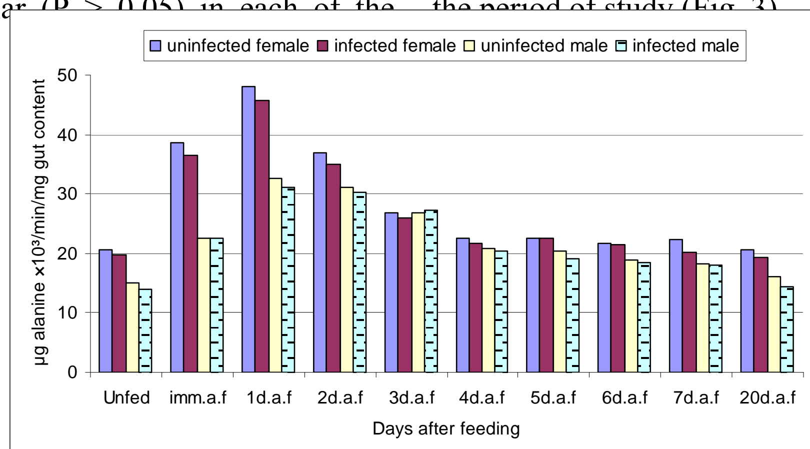

Fig. 3: Proteolytic activity in the midgut lumen content of female and male *O. erraticus* (uninfected and infected with *B. crocidurae*) at various time intervals after feeding.

Proteolytic activity level in the MLC in each of the uninfected and infected female was higher (P < 0.05) than in each of the uninfected and infected male in the unfed and up to the 2nd daf and was similar (P > 0.05) to that of the male on the 3rd to the 20th daf (Fig. 3).

**Proteolytic activity in the midgut wall of mated female and male *O. erraticus*, uninfected and infected with *B. crocidurae*, during blood meal digestion:**

The level of proteolytic activity in the midgut wall (MW) of each of the uninfected and infected mated female (Fig. 4) and male (Fig. 5) *O. erraticus* was much higher than that in the midgut lumen content (MLC) of each of the studied corresponding physiological states.

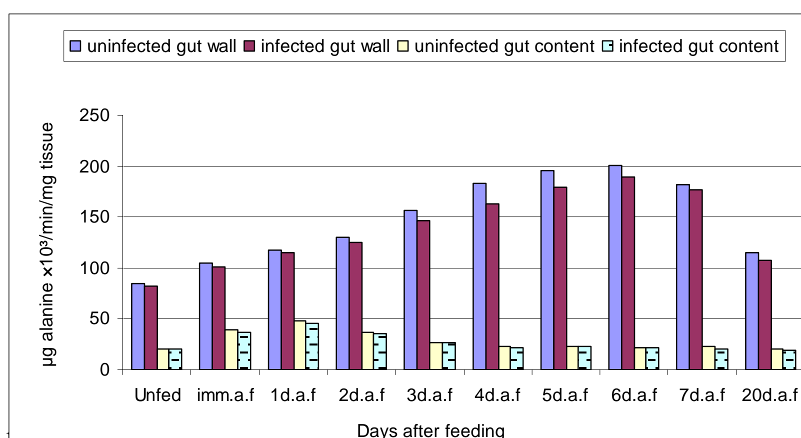


Fig. 4: Proteo[illegible] uninfected and infected with *B. crocidurae*) at various time intervals after feeding.

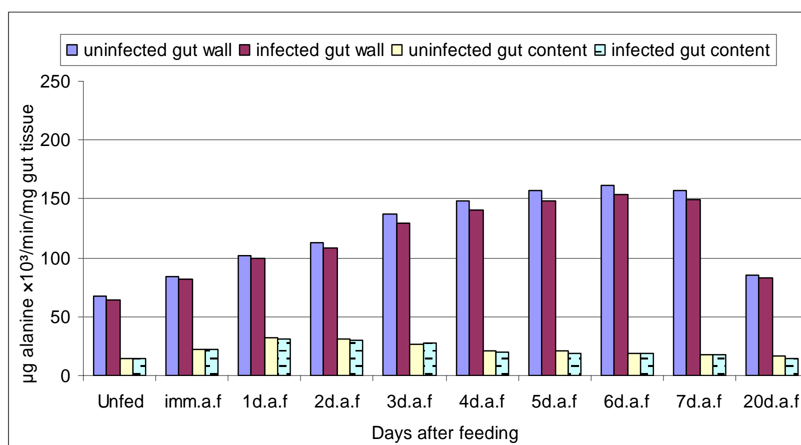


Fig. 5: Proteolytic activity in the midgut wall and lumen content of male *O. erraticus* (uninfected and infected with *B. crocidurae*) at various time intervals after feeding.

In comparison with the unfed controls, the proteolytic activity levels in the MW of uninfected female (Fig. 6) and male (Fig. 7) increased imm.a.f. and on subsequent daf ($P < 0.001$) to reach the highest levels on the 5$^{th}$ and 6$^{th}$ daf in the female 196.33±3.28 and 200±2.60 µg alanine ×10³/ min/ mg tissue) and the 5$^{th}$ to 7$^{th}$ daf in the male (156.80±1.91-161.23±0.79 µg alanine ×10³/ min/ mg tissue) tick. On the 7$^{th}$ daf the proteolytic activity in the female MW declined ($P < 0.001$) to approach ($P > 0.05$) its level on the 4$^{th}$ daf. Levels of the proteolytic activity decreased ($P < 0.001$) on 20 daf (114.63±3.73 and 84.77±1.62 µg alanine ×10³/ min/ mg tissue) to approach ($P > 0.05$) that on the 1$^{st}$ daf and imm.a.f. (117.07±1.75 and 83.80±2.10 µg alanine ×10³/ min/ mg tissue) in the MW of the female and male, respectively.

Changes in the proteolytic activity in the MW of each of *Borrelia* infected female (Fig. 6) and male (Fig. 7) followed similar patterns to those in the uninfected controls. The level of proteolytic activity increased ($P < 0.001$) imm.a.f. and on next daf to reach a peak on the 6$^{th}$ (
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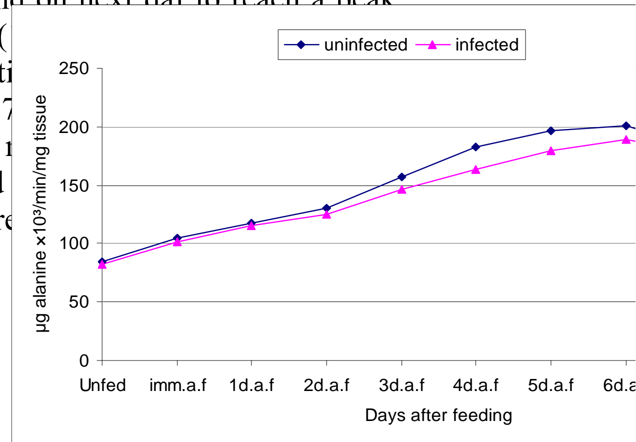


Fig. 6: Proteolytic activity in the midgut wall of female *O. erraticus* (uninfected and infected with *B. crocidurae*) at various time intervals after feeding.

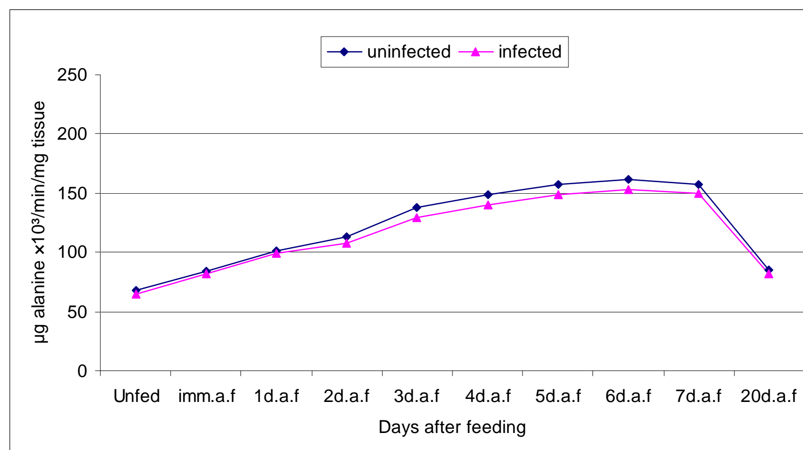


Fig. 7: Proteolytic activity in the midgut wall of male *O. erraticus* (uninfected and infected with *B. crocidurae*) at various time intervals after feeding.

Proteolytic activity levels in the MW of both infected and uninfected ticks (Fig. 8) were almost the same up to the 1st daf. However, on 2-6 and 2-7 daf the activity level in each of the infected female and male was lower ($P < 0.05$) than that in the uninfected control on each of the corresponding daf. Then the levels of activity of both infected and uninfected tick in each of the female and male approached each other on the 7th and 20th daf, respectively.

Generally, proteolytic activity in MW was higher ($P < 0.001$) in the female than male in all the corresponding states examined throughout the period of study (Fig.8).

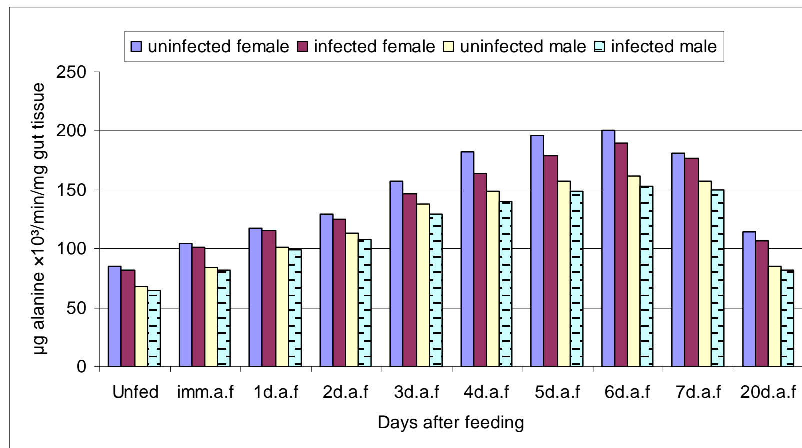


Fig. 8: Proteolytic activity in the midgut wall of female and male *O. erraticus* (uninfected and infected with *B. crocidurae*) at various time intervals after feeding.

## DISCUSSION

Digestion of 26.30% and 35.28% of protein amount in the ingested blood meal occurred in the whole gut of *O. erraticus* female and male, respectively during a period of 20 days after feeding (Yousery, 2011). In the midgut lumen content (MLC) the recorded decrease (Yousery, 2011) in the amount of protein (27.26%, 35.50% in female and male, respectively) in the ingested blood meal was attributed to either its absorption by epithelial cells in the midgut wall (MW) or proteolytic digestion in the MLC. Uptake of large serum molecules (Mendiola *et al*., 1996) and hemoglobin by pinocytosis and other blood large components including undamaged erythrocytes by phagocytosis has been recorded in some histological studies on argasid ticks (Sidorov, 1960; Tatchell, 1962, 1964; Balashov, 1972; Grandjean 1984). Based on histochemical visible changes, parietal digestion was assumed to occur first in the narrow peripheral zone of the concentrated blood meal mass, bordering midgut epithelial cells (Balashov, 1972). The presence of microvilli, esterases and leucine endopeptidase in the apical cytoplasm cell membrane bordering the blood mass (Tatchell, 1964; Balashov, 1972) supported this assumption.

The present study demonstrated proteolytic activity in the MLC and MW of mated unfed and fed female and male *O. erraticus.* The detection of proteolytic activity in both unfed and fed ticks rules out the possibility that the observed activity was derived from the host blood. In both midgut compartments, protease activity increased after feeding to reach at their peaks about double times the levels in the unfed controls. In *O. erraticus*, proteolytic activity reached its peak level on 1 and 1 – 2 daf in the MLC and 5-6 and 5-7 d.a.f. in the MW in female and male, respectively. An increase and upgrading of expression of protease activity by feeding have been reported in all studies on argasids (Tatchell *et al*., 1962, Tatchell, 1964, Akov *et al*., 1972) and ixodids (Abdul Alim *et al*., 2007; Motobu *et al*., 2007; Franta *et al*., 2010). However, the proteolytic activity in the MLC was much lower than that in the MW of the corresponding physiological states and represented about one fourth and one fifth of it at the peaks of activity in female and male, respectively. Furthermore, a noticeable proteolytic activity lasted for a shorter period (3 and 4 d.a.f. in female and male, respectively) in the MLC when compared with that (20 d.a.f. in female and male) in the MW. The early timing (Tatchell, 1964) and

short duration of the luminal proteolytic activity in comparison with that in the MW could minimize its role in digestion of blood meal protein (Yousery, 2011) which apparently extends for a relatively long period after feeding (> 20daf) in *O. erraticus*. Furthermore, the synchrony between the decrease in the amount of protein in the MLC and its increase in the MW (Yousery, 2011) and between the peaks of protein amount and the proteolytic activity in the MW of *O. erraticus* is consistent with the view of the uptake of protein from the MLC and its proteolytic digestion in the cells of the MW. These findings conform to the concept of intracellular digestion of blood meal proteins in the MW, but they do not exclude a probable occurrence of extracellular proteolytic digestion in midgut lumen even at a narrow scale. Evidence of extracellular proteolytic digestion have been provided by some investigations in ticks. Balashov (1972) suggested a combination of intracellular and lumen digestion and assumed a parietal digestion of blood meal involving esterases and endopeptidases as was aforementioned. Also, Balashov (1972) suggested that detachment of digestive and secretory cells into the midgut lumen greatly increases the active area of digestion and produces a more uniform enzymatic effect on the entire ingested blood mass in addition to the intermixing caused by gut peristalsis. On the other hand, Tatchell (1964) reported that the weak acid reaction (pH 6.3-6.5) of *Argas persicus* gut lumen and the weak alkaline reaction (pH 7.2-7.6) in *Ixodes ricinus* exclude the leucine endopeptidase from protein splitting in the lumen since the enzymatic activity is limited to pH 2-4.5. Tatchell (1964) concluded that digestion in ticks is exclusively intracellular. In a histochemical study on blood digestion in the ixodids *Boophilus microplus* and *Rhipicephalus appendiculatus*, Agyei *et al*. (1991) observed the detachment and disintegration of secretory cells with lysosomal hydrolase activity into the midgut lumen. The pattern of appearance of these cells paralleled the reported level of protease activity in the midgut lumen. The disintegrating cells were considered to function as holocrine secretory cells. Tatchell (1964) considered that luminal contents of the tick consisted of unchanged food material but Boctor and Araman (1971) studying the MLC of *Argas persicus* and *A. arboreus* found high levels of amino acids in the MLC and concluded that these resulted from the breakdown of blood meal protein in the lumen. The amino acid patterns in the gut contents and the vertebrate host (pigeon) serum were dissimilar (Boctor, 1972). Grandjean (1984) concluded that possible conversion to haemichrome crystals in the midgut lumen of *O. moubata* could indicate hemoglobin crystals to get enzymatically attacked and to loose their globin moieties. Furthermore, the pH optima have been determined for activity of some proteases which are involved in blood meal digestion in ticks. Hernandez *et al*. (2000) identified a neutral protease activity in the midgut of *Boophilus microplus*. The maximum activity was attained at pH 6. Also, a multifunctional cysteine protease with optimum pH of 5 was mainly expressed in the midgut epithelium and was specifically localized in the lysosomal vacuoles and possibly released into the gut lumen in *Haemaphysalis longicornis* (Tsuiji *et al*., 2008). On the other hand, proteolytic activity have been detected and proteinases have been identified in the midgut of fed ticks showed optimal activities at acidic pH range of about 3-5 in argasid (Tatchell, 1964; Tatchell *et al*., 1972; Akov *et al*., 1976; Akov, 1982; Grandjean, 1984) and ixodids (Bogin and Hadani, 1973; Vundla, 1992; Mendiola *et al*., 1996; Mulenga *et al*., 1999; Boldbaatar *et al*., 2006; Horn *et al*., 2009). Generally, previous studies

demonstrated that the main digestive proteinases are rather similar to mammalian cathepsin D which is an aspartic proteinase (Coons *et al*., 1986).

The present study showed that proteolytic activity in MLC of *O. erraticus* noticeably increased imm.a.f. and subsequent 3 and 4 daf with peak activity on 1 and 1-2 d.a.f. before declining to reach a level similar to that in the unfed female and male, respectively. The short period and early timing of the rapid increase of luminal proteases to peaks which preceded the noticeable gradual increment and peaks of proteolytic activity in MW on 5-6 and 5-7 daf in female and male tick, respectively, may suggest an integration between the function of proteases in the two midgut compartments. Also the previous results may point to a probable hemolytic function of the proteolytic activity in the MLC of *O. erraticus*. Generally, very little information is available about the hemolytic enzymes in the initial phase of digestion in ticks and especially in argasids. Agbede and Kemp (1985) found that certain secretory cells (s1) secrete a glycoprotein which may be a hemolysin in the gut caeca of *Boophilus microplus*. Tatchell (1964) also observed a saliva fast PAS positive material which was suggested to be hemolysin in the gut lumen of *Argas persicus*. In *Rhipicephalus appendiculatus,* Walker and Fletcher (1987) found an epithelial cells secreting a glycoprotein with acid phosphatase activity into the lumen of the gut caeca where it may act as a hemolysin. Recently (Miyoshi *et al*., 2004, 2007) a hemolytic serine proteinase, HlSP, was identified from *Haemaphysalis longicornis* midgut and its luminal contents indicating that HlSP is extracellularly secreted. This enzyme displayed optimal activity at pH 6.0 which is more close to the pH in the midgut (around pH 6) rather than the acidic pH in the lysosomes (Miyoshi *et al*., 2004).

By comparing proteolytic activity in each midgut compartment; it was found that changes in the level of the proteolytic activity in female and male *O. erraticus* followed the same pattern in each of the midgut lumen and wall throughout the period of the present study (20 daf). However, protease level was much higher in the female than male ticks in the unfed and up to 2 d.a.f. in MLC and in all the corresponding physiological states examined in the gut wall throughout the period of study. Also, the brief and shorter peak period and the earlier declining of the proteolytic activity in each of the MLC and MW in the female suggested a faster digestion and increasing demands of protein required for vitellogenesis in the female.

Infection of *O. erraticus* with *B. crocidurae* significantly reduced the proteolytic activity levels in the MW of most of the corresponding physiological states (and insignificantly in MLC). The MW proteolytic activity levels at the peaks were reduced to about 92.98% and 95.04% of the value in the uninfected female and male, respectively. Suppression of the proteolytic activity in the MW (and insignificantly in MLC) of *Borrelia* infected *O. erraticus* was associated with a reduction of protein digestion in the whole midgut and its two compartments (Yousery, 2011). Reduction of protease activity has been reported in the gut of sand flies infected with *Leishmania* (Schlein and Romano, 1986; Borovsky and Schlein, 1987; Dillon and Lane 1993; Daba, 1997b) and in mosquitoes infected with *Plasmodium* (Jahan *et al*., 1999). Daba *et al*. (1997a, b) found that protease activity and protein digestion were only suppressed in the arthropod vector (*Phlebotomus langeroni*) by the natural pathogen (*Leishmania infantum*) when feeding on blood of the likely vertebrate host

(human and dog). No previous work has been done on the effect of *Borrelia* or other pathogen on digestion and protease activity in the tick vector.

The uptake and engulfment of *B. crocidurae* into the MW cells of *O. erraticus* by phagocytosis to be enclosed within phagosomes and the intercellular and intracellular migration of the spirochete across the MW have been observed by Helmy *et al*. (1996). Also the presence and multiplication of *B. crocidurae* in the MW cells, rupture of phagosomes and the release of the spirochete (Helmy *et al*., 1996) might affect or interfere with some physiological and biochemical processes in the cells which lead to a suppression of the proteolytic activity and digestion in *O. erraticus*. Proteases are essential for tick survival because of their involvement in blood digestion and mediation of protein metabolism. Their suppression interferes with supplying the tick body with proteins, amino acids and energy required for different vital processes.

In conclusion, findings of the present study are consistent with the view of intracellular proteolytic digestion in the MW, but they do not exclude a possible occurrence of extracellular proteolytic digestion in the MLC. Further studies are needed to clarify the functions of the observed proteolytic activity in the MLC on plasma proteins, hemoglobin and blood cells and to assess changes in the pH and amino acids and the effect of *Borrelia* on these processes in *O. erraticus*.

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## ARABIC SUMMARY

**الهضم الإنزيمى المحلل لبروتينات وجبة الدم فى قراد *أورنيثودوروس إيراتيكاس* الناقل *للبوريليا كروسيديورى* المسببة للحمى الراجعة فى مصر.**

**نوال محمود شنبكى[١] – نادية حلمى أحمد[١] – هالة محمد خاطر[٢] – آيات يسرى محمد[١]**
١- قسم علم الحشرات - كلية العلوم - جامعة عين شمس.
٢- مركز الأبحاث والدراسات والتدريب لناقلات الأمراض - كلية العلوم - جامعة عين شمس.

تم إيضاح النشاط الإنزيمى المحلل للبروتين فى كل من محتوى تجويف المعى المتوسط وجدار المعى المتوسط لإناث وذكور *أورنيثودوروس إيراتيكاس* المخصبة غير المغذاة والمغذاة . وقد إرتفع مستوى هذا النشاط بعد التغذية حتى وصل عند أعلى مستوى إلى ضعف المستوى فى القراد غير المغذى. وتحققت أعلى مستويات النشاط الإنزيمى المحلل للبروتين فى اليوم الأول وفى اليوم الأول والثانى بعد التغذية فى محتوى تجويف المعى المتوسط (بمقدار ٤٨ ، ٣١.١٠ - ٣٢.٦٣ ميكروجرام ألانين × ١٠ ^٣ / دقيقة/ مجم من محتوى المعى المتوسط) وفى اليوم الخامس والسادس ومن اليوم الخامس حتى السابع فى جدار المعى المتوسط (بمقدار ١٩٦.٣٣- ٢٠٠.٣٣ ، ١٠٦.٨٠ - ١٦١.٢٣ ميكروجرام ألانين × ١٠ ^٣ / دقيقة/ مجم نسيج جدار المعى المتوسط) فى كل من الإناث والذكور على التوالى. وكان مستوى النشاط الإنزيمى فى محتوى تجويف المعى المتوسط أقل عنه فى جدار المعى المتوسط فى جميع الحالات الفسيولوجية المتماثلة التى تمت دراستها وقاربت قيمته الربع والخمس منه عند أعلى مستوى للنشاط الإنزيمى فى كل من الإناث والذكور على التوالى. وبوجه عام كان النشاط الإنزيمى المحلل للبروتين أعلى فى الإناث عنه فى الذكور وذلك قبل التغذية وحتى اليوم الثانى بعد التغذية فى محتوى تجويف المعى المتوسط كذلك فى جميع الحالات الفسيولوجية المتماثلة التى تمت دراستها فى جدار المعى المتوسط.

وتشابه نمط التغيرات فى مستوى النشاط الإنزيمى المحلل للبروتين فى كل من تجويف وجدار المعى المتوسط لكل من الإناث والذكور غير المصابة والمصابة *بالبوريليا كروسيديورى* ولكن كان مستوى النشاط فى جدار المعى المتوسط للقراد المصاب *بالبوريليا* أقل عنه فى غير المصاب وذلك طوال معظم فترة الدراسة (من اليوم الثانى حتى اليوم العشرين بعد التغذية).

تمت مناقشة النتائج المتحصل عليها وإتفقت دلالاتها مع الرأى بأن الهضم الإنزيمى المحلل لبروتينات وجبة الدم يتم داخل خلايا جدار المعى المتوسط للقراد ولكنها لم تستبعد وجود دور محتمل للهضم الإنزيمى المحلل للبروتين خارج الخلايا فى محتوى تجويف المعى المتوسط مما يتطلب مزيداً من البحث.