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Variations in protein banding patterns of salivary glands during feeding behavior of adult *Culex pipiens pipiens* (Diptera: Culicidae)

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Abstract
The saliva of hematophagous insects contains a variety of pharmacologically active substances that counteract the normal haemostatic response to injury in vertebrate hosts. In the present study, protein banding pattern (Native and SDS-PAGE) of salivary glands of adult *Culex pipiens* at sugar-fed, un-fed, starved and blood fed stages were investigated. Males and females of *Cx. pipiens* were dissected and their salivary glands were collected at 3, 12 and 24, 48, 72 h after sugar feeding, starved and un-fed stages. Female salivary glands were additionally collected at different stages of blood feeding; skin exploring time, 3, 12, 24, 48 and 72 h after blood meal and after oviposition. Results of native-PAGE from four different meals demonstrated that there were differences in the overall protein banding pattern in salivary glands of males and females of *Cx pipiens.* Results of SDS-PAGE clarified that the molecular weight of the separated proteins (in all stages) ranged from 205.13 to 10.47 KDa indicating that banding patterns differ from stage to stage. Interestingly, protein of 30 kDa was predominantly expressed in the female, but not observed in the male. In addition, 20 KDa protein was observed in starved female and the band intensity was higher in the case of sugar-fed female, but not in blood-fed females. Such finding revealed that this protein depleted after blood feeding, especially, at skin exploring time. Differences in the salivary protein's profile may be related to the function of salivary gland and the nature of food. In conclusion, better knowledge of the molecules synthesized in saliva and the salivary gland of mosquitoes as hematophagous insects could be of use for improving the control of pathogen transmission. Today, many salivary molecules have been identified and characterized as new targets to the development of future vaccines.

Keywords: Salivary glands, protein, Native-PAGE, SDS-PAGE, *Culex pipiens*

1. Introduction
Mosquito-borne diseases are a major health problem in both human and veterinary sectors. Diseases transmitted by mosquitoes include malaria, Japanese encephalitis, dengue, hemorrhagic fevers, yellow fever, etc. Culicine mosquitoes mainly *Culex pipiens* are subtropical species and relatively efficient vector of West Nile virus [1-5], vectors of filariasis [6, 7] and Rift valley fever virus [8-12]. Pathogens (viruses, bacteria, protozoa or filaria) take up residence in the mosquito salivary glands and then are transmitted to a new vertebrate host when female mosquito bites or takes a blood meal. Adult mosquito salivary glands are paired organs located on either side of thorax flanking the oesophagus [13-15]. There are some differences between the structure of male and female salivary glands. Female gland has three lobes, including two lateral lobes, with distinct proximal and distal portions, and a medial lobe. Another difference in feeding habit is female *Culex pipiens* mosquito has to take blood meal for egg development.
Studying mosquito salivary gland extract revealed that it contains α-glucosidases and α-amylases that initiate the digestion of carbohydrates. Other enzymes and peptides involved in blood feeding and ingestion such as anticoagulants, vasodilators, and platelet aggregation inhibitors were detected in mosquito's salivary glands, too[16]. In addition, the mosquito saliva may enhance or facilitate infectivity [17-19].
Most research investigating salivary glands of arthropod disease vectors has focused on identifying candidate proteins to be involved in the development of epidemiological techniques [20, 21], transmission blocking vaccines [22], or novel therapeutic agents [23, 24].

Salivary gland proteins of several mosquito species have been investigated [14, 24, 25-34].

The protein content of salivary glands and morphological differences have been observed in mosquito species and were related with the different feeding habits of males and females [14, 28, 32]. Deeper studies reported the accumulation of specific proteins in the different salivary gland regions for various mosquitoes [26]. In addition, the variability in salivary content among different groups of arthropods and individuals of the same species has been described [16]. For example, Soliman *et al.* reported that the total saliva of female *Cx. pipiens* was depleted by 64% after feeding on blood, within 24 h, but the protein level returned to the unfed value within the next 24-48 h[30]. Ribeiro studied the differences in protein expression of salivary gland after sugar and blood feeding in female *Culex pipiens quinquefasciatus* [17]. Furthermore, Wasin--piyamongkol *et al.* carried out similar study on female *Aedes aegypti* [33]. In contrast, Nascimento *et al*. showed that the major polypeptides found in the female salivary gland are secreted during blood feeding in the case of *Cx. Quinquefasciatus* [31]. The polypeptide pattern observed in mosquitoes from day 1 to day 7 was the same. This result concurs with the protein synthesis profile, which was unchanged throughout the first week of mosquito life after emergence [31].

The main objective of this study is to reveal differences in salivary gland protein profiles of male and female *Culex pipiens* at different feeding stages.

2. Materials and methods

2.1 Insect colonization

A laboratory colony of the mosquito, *Culex pipiens* was obtained from the Research Institute of Medical Entomology, Dokki, Giza, Egypt. This colony was maintained in the insectary of the Department of Entomology, Faculty of Science, Cairo University, under controlled conditions (27 ± 2 °C and 70 ± 5% RH and 14L: 10D photocycle) as described by Adham *et al.* [35].

2.2 Salivary gland dissection and preparation

Female mosquitoes were anaesthetized on ice before salivary glands dissection (30 pairs of salivary glands were used). Salivary glands of the mosquitoes were dissected in phosphate-buffered saline (PBS); 10mM Na2PO4145mM NaCl (pH7.2) (FertiProN-V, Belgium) using fine needles under stereoscopic microscope (Leica, Germany). The salivary glands were transferred to a microcentrifuge tube with a small volume of PBS and stored at -80 °C until use. Salivary glands of males and females were dissected at different time intervals; 3, 12, 24, 48 and 72 h after sugar feeding, at starved stage, unfed stage (males and females) and at blood-feeding stages which include skin exploration, at 3, 6, 12, 24, 48 and 72 h post-blood-feeding and after oviposition (only females).

2.3 Native polyacrylamide gel electrophoresis (Native-PAGE)

Native-PAGE was used to analyze protein content of salivary glands of males and females of *Cx. pipiens* at the above mentioned intervals. 15% polyacrylamide gels pH 4, using a discontinuous buffer system was used [36].

2.4 Sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was used to analyze protein content of salivary glands of males and females of *Cx. pipiens* at the above mentioned intervals. 15% polyacrylamide gels pH 8.8, in a discontinuous buffer system was employed [36]. Acrylamide/ bisacrylamide ratio was 50: 1. The gels contained no SDS before electrophoresis. Protein samples were pretreated with 1% SDS and 1% β-mercaptoethanol for 5- 10 min at 100 °C. The gels were run at 100 V until the tracker dye left the gel (approximately 4 h). All gels were fixed in 20% 5-sulfosalicylic acid, stained with Coomassie Brilliant Blue R_{250}, destained in 7% acetic acid and photographed using gel-documentation system with a 20-Mp camera (SonyCoRp, 6.8skD, Japan).

2.5 Data analyses

The data obtained from the scanning process of each gel were analyzed using Gel pro analyzer (Ver. 31 Media cybernetics, USA) and Alpha Ease FC stand alone for windows 2000/XP.

3. Results

Figs. (1- 5) and Tables (1- 2) show the results of protein banding patterns of salivary gland at different feeding stages of *Cx. pipiens*; at 3, 12, 24, 48, 72 h (male and female), starved, unfed, skin exploration, 3, 12, 24, 48,72 h and post-oviposition in blood-fed female only.

3.1 Native-Page

It is observed that the total number of protein bands resolved in 15% native gel was 5 bands at 3 h sugar-fed male compared to 6 bands in the case of female (Fig. 1). Four, 6, 5 and 7 bands were observed in the case of males at 12, 24, 48 and 72 h post-sugar-feeding, respectively (Fig. 1). Meanwhile, 9, 7, 6 and 5 bands were observed in the case of females at 12, 24, 48 and 72 h post-sugar-feeding, respectively (Fig. 1). Both unfed males and females showed 9 bands on native-PAGE (Fig. 2). In addition, 5 and 6 bands were observed in the cases of starved male and female, respectively (Figs. 1 and 2). Three, 6, 5, 4, 5, 6 and 9 bands were observed in the case of female at skin exploration stage, 3, 12, 24, 48 and 72 h post-blood feeding and post-oviposition, respectively (Fig. 2). Generally, native-PAGE demonstrated many changes in the total native protein content of male and female salivary glands of *Cx. pipiens* mosquitoes.

3.2 SDS-Page

Salivary gland proteins of male and female *Cx. pipiens* were electrophoretically separated by SDS-PAGE using 15% polyacrylamide gel (Figs. 3 and 4). Protein standard (marker) was used to estimate the molecular weights of the separated bands. Fig. (3) and Table (1) show changes in salivary gland proteins of male and female *Cx. pipiens* at 3, 12, 24, 48 and 72 h post-sugar-feeding. The total number of bands was 15 bands (molecular weight ranged from 191.67 to 11.35 KDa) at 3 h post-sugar-feeding in both males and females. Sixteen and 14 bands (molecular weight ranged from 199.51 to 11.35 KDa) were observed at 12 h post-sugar-feeding in the cases of females and males, respectively (Fig. 3 and Table 1). Twelve and 14 bands (molecular weight ranged from 181.86 to 10.47 KDa) were observed at 24 h post-sugar-feeding in the cases of females and males, respectively. Thirteen and 16 bands

(molecular weight ranged from 181.86 to 10.47 KDa) were observed at 48 h post-sugar-feeding in the cases of females and males, respectively. Fourteen and 15 bands (molecular weight ranged from 139.73 to 11.35 KDa) were observed at 72 h post-sugar-feeding in the cases of females and males, respectively (Fig. 3 and Table 1).

Fig. (4) and Table (2) show protein banding patterns of starved and unfed male and female *Cx. pipiens*. Protein banding patterns of females after blood feeding stages was investigated using 15% SDS gel (Fig. 4). Seventeen and 18 bands (molecular weight ranged from 205.13 to 10.47 KDa) were observed in the cases of female and male at starved stage. Fig (4) and Table (2) revealed that 14 bands were recorded in both unfed male and unfed female (molecular weight ranged from 205.13 to 10.47 KDa).

It was obvious that the females exhibited different numbers of protein bands at different intervals post-blood feeding. Thirteen bands (M. wt. ranged from 205.13 to 10.47 KDa), 8 bands (M. wt. ranged from 114.78 to 10.47 KDa), 11 bands (M. wt. ranged from 122.17 to 10.47 KDa), 6 bands (M. wt. ranged from 94.96 to 10.47 KDa), 7 bands (M. wt. ranged from 100.87 to 10.47 KDa), 12 bands (M. wt. ranged from 143.91 to 10.47 KDa) and 11 bands (M. wt. ranged from 137.83 to 10.47 KDa) were observed in the cases of skin exploration, 3, 12, 24. 48, 72 h post-blood feeding and post-oviposition, respectively (Fig. 4 and Table 2).

3.3 Polymorphism in protein patterns

Native-PAGE analyses showed that 10 monomorphic protein bands were observed at 3 h post-sugar-feeding in both male and female (Fig. 1, Lanes 1 and 2). Meanwhile, 12 monomorphic and 2 polymorphic bands were observed at 12 h post-sugar-feeding in the cases of male and female (Fig. 1, Lanes 3 and 4). Twelve monomorphic and 6 polymorphic bands were observed at 24 h post-sugar-feeding in the cases of male and female (Fig. 1, Lanes 5 and 6). At 48 and 72 h post-sugar-feeding, 13 and 12 monomorphic bands were observed, respectively, in both male and female (Fig. 1, Lanes 7, 8, 9 and 10). At starvation, 11 monomorphic bands were observed in both male and female (Fig. 1, Lane 11 and Fig. 2, Lane 1). In the case of unfed male and female, 14 monomorphic and 2 polymorphic bands were observed (Fig. 2, Lanes 2 and 3). Five monomorphic and 9 polymorphic bands were observed in blood-feeding females at different time intervals (Fig. 2, Lanes 4- 9). Fourteen monomorphic and one polymorphic bands (in relation to 72 h post-blood-feeding) were observed in female salivary glands after oviposition (Fig. 2, Lane 10).

SDS-PAGE analyses revealed that 19 monomorphic and 3 polymorphic bands were observed at 3 h post-sugar-feeding in the cases of male and female (Fig. 3, Lanes 1 and 2). Meanwhile, 13 monomorphic and 3 polymorphic bands were observed at 12 h post-sugar-feeding in the cases of male and female (Fig. 3, Lanes 3 and 4). Seventeen monomorphic and 2 polymorphic bands were observed at both 24 and 48 h post-sugar-feeding in the cases of male and female (Fig. 3, Lanes 5- 8). At 72 h post-sugar-feeding, 14 monomorphic and 2 polymorphic bands were observed in both male and female (Fig. 3, Lanes 9 and 10). At starvation, 17 monomorphic and 8 polymorphic bands were observed in both male and female (Fig. 4, Lanes 1 and 2). In the case of unfed male and female, 17 monomorphic and 2 polymorphic bands were observed (Fig. 4, Lanes 3 and 4). Six monomorphic and 13 polymorphic bands were observed in blood- feeding female at different time intervals (Fig. 4, Lanes 4- 9). Sixteen monomorphic and 3 polymorphic bands (in relation to 72 h post-blood-feeding) were observed in female salivary glands after oviposition (Fig. 4, Lane 10). Fig. (5) summarized the above mentioned results and confirms the polymorphic and monomorphic protein bands on the bases of SDS-PAGE.

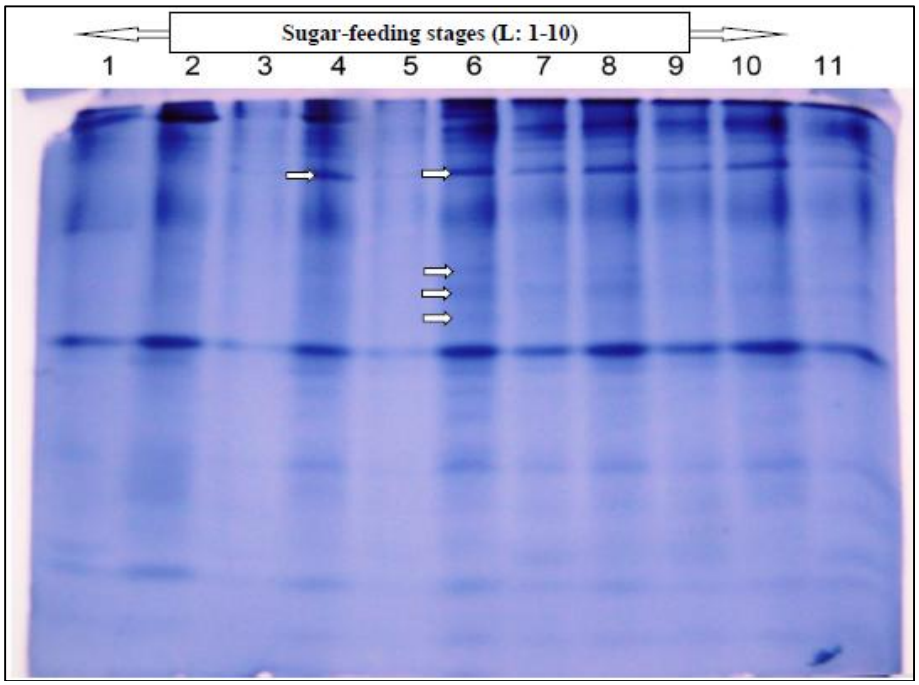

Fig 1: 15% native-PAGE (Stained with Coomassie Brilliant Blue) of salivary glands protein of *Culex pipiens* male and female at different times of feeding. L1: 3 h post-sugar-feeding male, L2: 3 h post-sugar-feeding female, L3: 12 h post-sugar-feeding male, L4: 12 h post-sugar-feeding female, L5: 24 h post-sugar-feeding male, L6: 24 h post-sugar-feeding female, L7: 48 h post-sugar-feeding male, L8: 48 h post-sugar-feeding female, L9: 72 h post-sugar-feeding male, L10: 72 h post-sugar-feeding female and L11: Starved male.

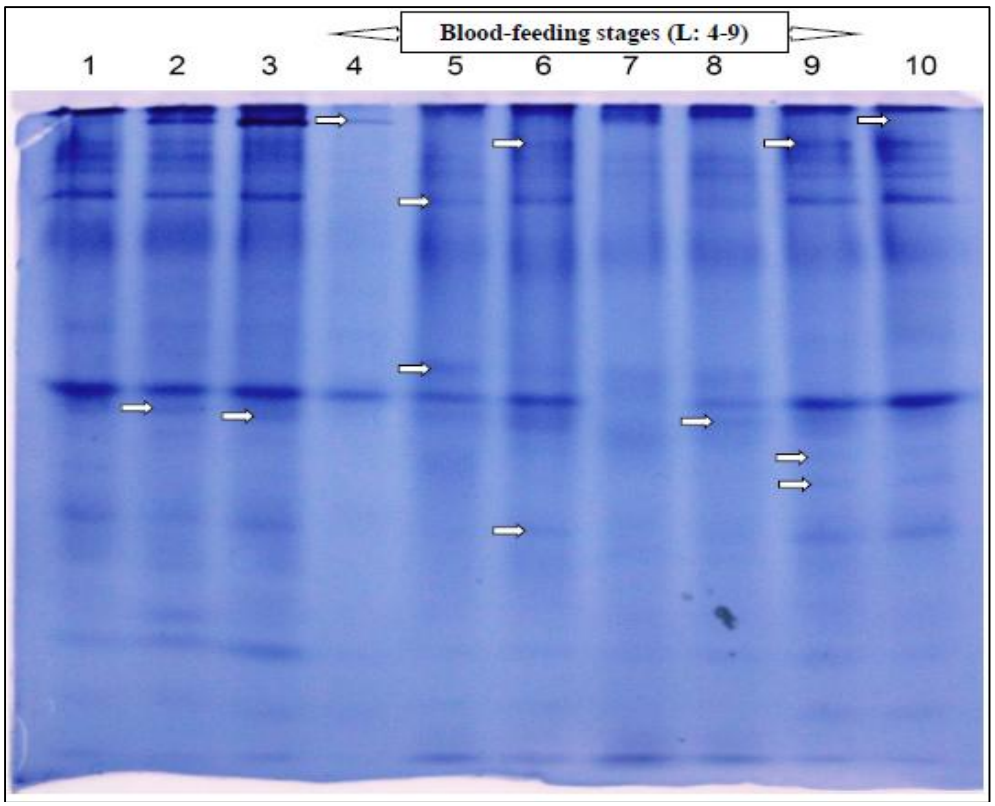

Fig 2: 15% native-PAGE (Stained with Coomassie Brilliant Blue) of salivary glands protein of *Culex pipiens* male and female at different times of feeding. L1: Starved female, L2: un-fed male, L3: un-fed female, L4: Skin exploring female, L5: 3 h post-blood-feeding female, L6: 12 h post-blood-feeding female, L7: 24 h post-blood-feeding female, L8: 48 h post-blood-feeding female, L9: 72 h post-blood-feeding female and L10: post-oviposition female.

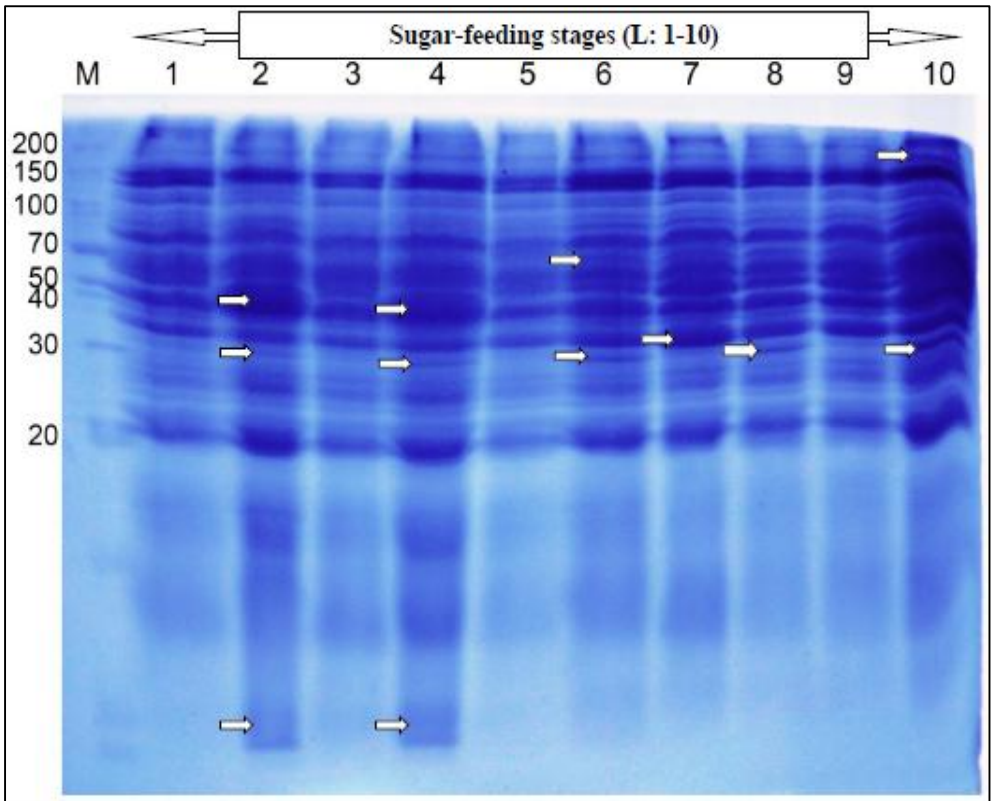

Fig 3: 15% SDS-PAGE (Stained with Coomassie Brilliant Blue) of salivary glands protein of *Culex pipiens* male and female at different times of feeding. L1: 3 h post-sugar-feeding male, L2: 3 h post-sugar-feeding female, L3: 12 h post-sugar-feeding male, L4:12 h post-sugar-feeding female, L5: 24 h post-sugar-feeding male, L6: 24 h post-sugar-feeding female, L7: 48 h post-sugar-feeding male, L8: 48 h post-sugar-feeding female, L9: 72 h post-sugar-feeding male, L10: 72 h post-sugar-feeding female and lane M: Size of molecular weight marker is indicated on the left.

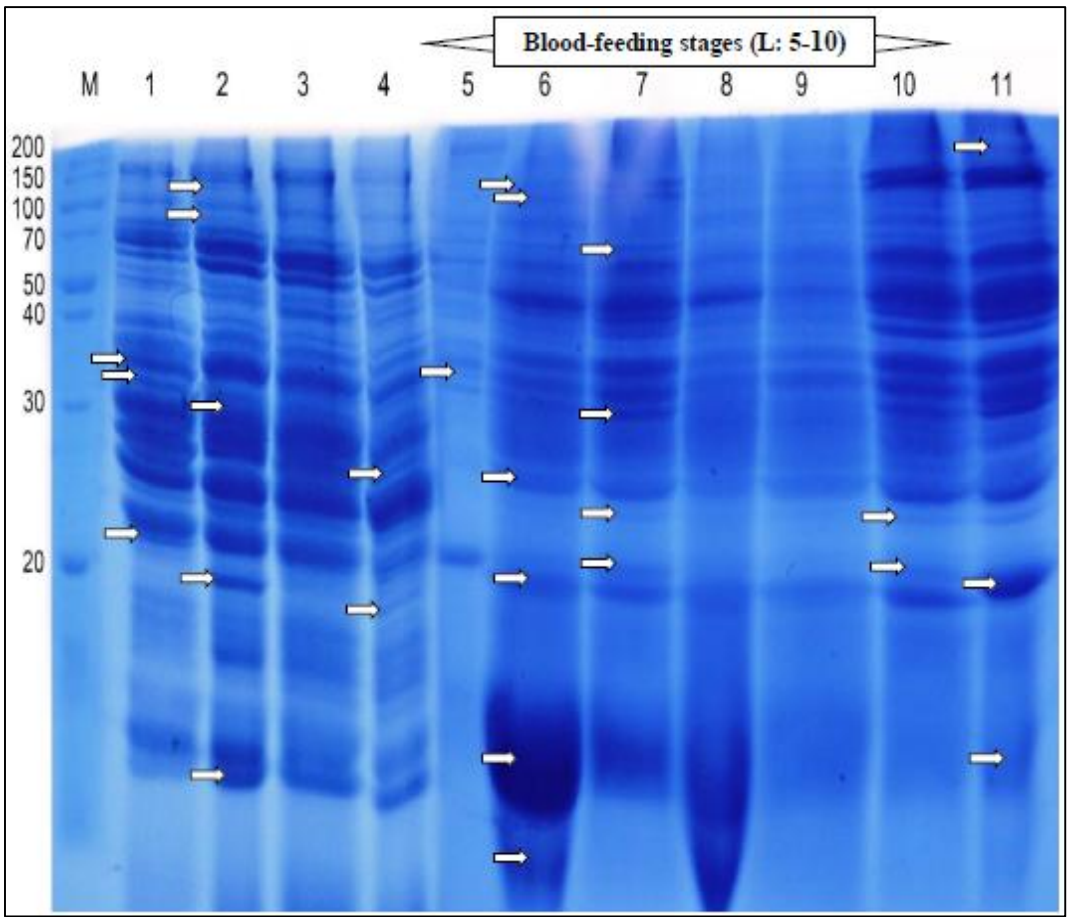

Fig 4: 15% SDS-PAGE (Stained with Coomassie Brilliant Blue) of salivary glands protein of *Culex pipiens* male and female at different times of feeding. L1: Starved male, L2: Starved female, L3: un-fed male, L4: un-fed female, L5: Skin exploring female, L6: 3 h post-blood-feeding female, L7: 12 h post-blood-feeding female, L8: 24 h post-blood-feeding female, L9: 48 h post-blood-feeding female, L10: 72 h post-blood-feeding, L11: post-oviposition female and lane M**:** Size of molecular weight marker is indicated on the left.

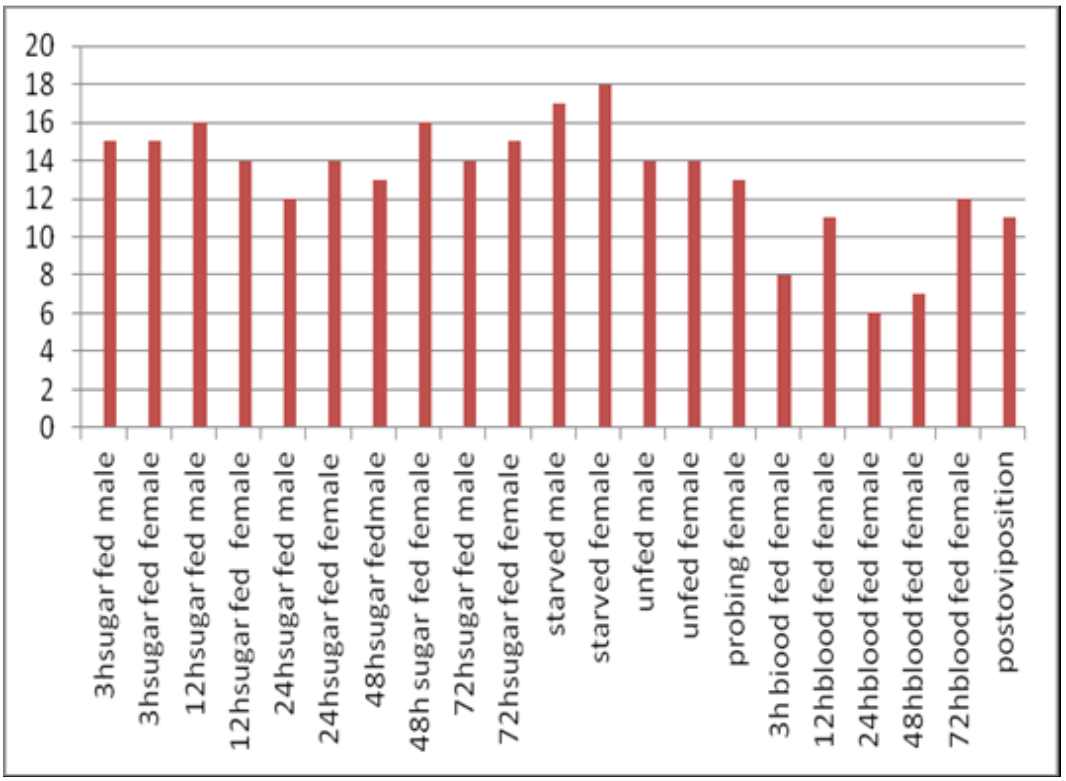

Fig 5: Number of protein bands generated by the SDS-PAGE in sugar-fed, starved, un-fed males and females, skin exploring, blood-fed and post-oviposition for female only.

Table 1: Molecular weight and percentage band intensity of protein salivary glands of male and female *Cx. pipiens* on the basis of SDS-PAGE for the individual lanes of Figure 3.

	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12	B13	B14	B15	B16
L1	191.6M 22.3%	151.4M 7.6%	139.7M 28.8%	115.1M 1.9%	87.81M 1.0%	83.94M 11.1%	66.0M 3.1%	52.8M 1.1%	46.6M 2.5%	37.7M 1.5%	37.76M 1.5%	24.5M 1.9%	21.6M 7.0%	20.5M 5.4%	12.4M 3.1%	
L2	191,6M 18.9%	142.4M 29.2%	93.03M 4.2%	88.97M 2.6%	66.29M 2.7%	63.71M 3.1%	54.2M 2.2%	46.6M 4.8%	43.3M 5.2%	34.5M 2.8%	24.51M 2.7%	20.5M 7.4%	19.6M 8.2%	12.4M 3.7%	11.3M 2.3%	
L3	199.5M 11.5%	181.8M 4.4%	162.7M 9.2%	133.0M 41.1%	11o.7M 3.2%	108,93M 3.2%	87.23M 0.9%	79.48M 6.4%	70.0M 1.1%	49.19M 2.1%	43.39M 3.3%	40.8M 3.8%	31.7M 2.2%	24.5M 1.3%	19.6M 5.7%	11.3M 0.6%
L4	188.7M 10.7%	142.4M 15.1%	127.2M 49.4%	102.6M 2.3%	70.0M 2.3%	63.71M 1.0%	46.6M 1.3%	43.3M 0.8%	40.8M 3.1%	31.7M 1.0%	30.17M 1.5%	24.5M 2.2%	19.6M 8.9%	11.3M 1.4%		
L5	174.0M 22.2%	139.7M 10.8%	127.6M 16.3%	72.90M 5.9%	63.71M 5.2%	50.86M 3.2%	46.6M 5.2%	43.3M 4.4%	40.8M 3.3%	30.1M 4.6%	19.64M 16.3%	11.3M 2.6%				
L6	181.8M 11.8%	133.0M 4.6%	123.6M 62.9%	73.87M 1.8%	63.71M 1.2%	49.19M 1.3%	46.6M 1.1%	37.7M 1.1%	30.1M 3.0%	24.5M 0.8%	20.57M 1.0%	19.6M 6.8%	11.3M 1.2%	1o.4M 1.4%		
L7	188.7M 4.0%	147.3M 32.1%	123.6M 17.8%	108.9M 13.2%	70.00M 3.2%	58.00M 2.1%	46.6M 1.8%	43.3M 2.0%	37.7M 1.2%	30.1M 7.4%	24.51M 1.2%	20.5M 5.4%	19.6M 8.6%			
L8	144.20M 7.6%	115.18M 61.9%	97.87M 0.8%	79.48M 1.1%	66.29M 4.4%	49.19M 2.5%	43.3M 1.7%	40.8M 2.5%	31.7M 4.4%	27.5M 2.8%	26.56M 2.8%	25.9M 0.7%	24.5M 1.3%	19.6M 4.5%	12.4M 1.1%	10.4M 1.1%
L9	139.73M 10.4%	110.71M 60.7%	95.94M 0.8%	72.90M 3.3%	68.00M 1.4%	54.29M 1.0%	52.8M 1.4%	46.6M 3.0%	43.3M 2.0%	37.7M 2.2%	30.17M 4.7%	24.5M 2.5%	19.6M 3.1%	11.3M 1.1%		
L10	139.73M 14.6%	123.66M 10.3%	105.36M 34.0%	93.03M 8.8%	87.81M 1.6%	73.87M 3.1%	63.7M 6.9%	52.8M 1.5%	49.1M 3.5%	40.8M 0.8%	31.72M 4.5%	27.5M 3.7%	24.5M 2.3%	19.6M 3.6%	12.48M 1.4%	

Table 2: Molecular weight and percentage band intensity of protein salivary glands of male and female *Cx. pipiens* on the basis of SDS-PAGE for the individual lanes of Figure 4.

	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12	B13	B14	B15	B16	B17	B18
L1	205.1M 0.0%	188.2M 0.9%	163.7M 1.0%	147.8M 0.0%	127.1M 15.1%	99.78M 14.4%	87.17M 0.4%	75.43M 0.1%	63.10M 0.0%	50.14M 0.1%	41.31M 0.0%	32.77M 0.4%	28.86M 28.5%	27.3M 15.7%	19.64M 10.8%	12.4M 2.0%	10.4M 10.6%	
L2	205.1M 0.0%	174.8M 0.0%	144.6M 2.6%	125.8M 4.1%	113.8M 17.9%	102.9M7.6%	89.13M9.1%	75.43M2.8%	63.10M0.6%	45.41M0.1%	38.77M0.0%	32.77M 2.5%	28.86M 8.7%	22.8M 0.0%	20.57M 25.5%	19.6M 0.0%	11.6M 0.0%	10.47M 18.5%
L3	205.1M 0.0%	168.2M 3.3%	142.5M 3.5%	127.1M 0.0%	102.9M 24.1%	91.52M9.9%	75.43M12.3%	56.90M0.0%	41.31M3.7%	28.86M2.2%	22.83M22.5%	19.64M 17.2%	11.64M 1.4%	10.4M 0.1%				
L4	205.13M0.0%	170.0M 6.2%	130.59M 0.0%	116.4M 2.0%	94.78M 9.7%	79.35M14.7%	65.92M2.9%	52.68M1.7%	41.31M0.2%	34.77M15.9%	28.86M1.2%	22.83M 20.7%	11.64M 24.7%	10.4M 0.1%				
L5	205.1M 0.0%	182.0M 0.6%	142.5M 0.0%	130.5M 1.4%	109.3M 8.1%	91.52M16.1%	81.09M3.6%	69.30M1.6%	48.36M0.2%	38.77M15.2%	28.86M24.4%	22.83M 11.6%	10.47M 17.2%					
L6	114.78M0.0%	74.35M 10.0%	55.22M 0.0%	41.75M 0.0%	37.19M 12.5%	30.51M20.8%	26.58M47,7%	10.47M0.0%										
L7	122.17M1.9%	73.66M 12.1%	52.26M 0.0%	46.73M 10.2%	41.75M 2.2%	38.20M3.2%	34.61M0.0%	26.58M17.2%	23.52M53.2%	12.48M0.0%	10.47M0.0%							
L8	94.96M 3.8%	74.35M 0.0%	52.26M 0.0%	40.88M 42.9%	26.58M 50.6%	10.47M2.7%												
L9	143.91M1.2%	93.82M 6.9%	75.50M 2.1%	53.48M 5.1%	46.43M 11.3%	40.88M6.3%	37.19M2.8%	34.61M5.7%	25.69M3.5%	23.52M41.8%	12.48M36.1%	10.47M 3.9%						
L10	143.91M1.2%	93.82M 6.9%	75.50M 2.1%	53.48M 5.1%	46.43M 11.3%	40.88M6.3%	37.19M2.8%	34.61M5.7%	25.69M3.5%	23.52M14.8%	12.48M36.1%	10.47M 3.9%						
L11	137.83M0.0%	73.66M 0.0%	58.00M 0.0%	46.43M 27.4%	40.88M 1.9%	38.20M11.7%	34.61M0.9%	29.74M13.3%	25.69M0.0%	22.60M44.7%	10.47M0.1%							

4. Discussion

Results obtained from native and SDS-PAGE analyses of *Cx. pipiens* salivary gland proteins at different time intervals demonstrated many changes in both native and denatured proteins. The banding patterns differ from stage to stage during feeding behavior of both male and female.

Our results revealed that sugar feeding stages showed less polymorphism (in salivary gland proteins) than blood feeding stages. Starvation of males and females induced more polymorphic bands in salivary glands than in the case of unfed stage after emergence from pupa. The higher degree of polymorphism (in salivary gland proteins) is displayed in the case of blood feeding females. These findings support the hypothesis that salivary glands are triggered to produce specific proteins in relation to mosquito's feeding behavior and to nature of food (nectar or blood), as well.

Interestingly, a protein of 30 kDa was predominantly expressed in female, but not observed in the male. In addition, a protein of 20 KDa was observed in starved female. Comparing the effects of the blood meal on salivary gland (SG) protein expression revealed that protein band density among sugar-fed (SF) mosquitoes was different than that in blood-fed (BF) mosquitoes. The higher band intensity of salivary protein in the case of SF than in the case of BF revealed that protein depleted after blood feeding, especially, at skin exploration time. Agreeable results were presented by Wasinpiyamongkol *et al.* [33] and Ribeiro [17] who studied differences in SG protein expression in the cases of SF and BF female *Aedes aegypti* and *Culex pipiens quinquefasciatus*, respectively. Similar results were recorded on the SG proteins of SF and BF *Anopheles gambiae* mosquitoes. Salivary glands of BF *An. gambiae* showed high and low molecular mass proteins 1 h post-feeding. The most notable difference was expression of 100 and 29 kDa proteins in response to a blood meal when compared to SF mosquitoes [37]. In addition, SG protein profiles of *Ae. aegypti*, *Armigeres subalbatus* and *Cx. quinquefasciatus* mosquitoes were studied by Siriyasatien *et al.* [38, 39]. SDS-PAGE analysis demonstrated 8 major polypeptides of 20, 35, 37, 42, 45, 47, 70 and > 118 kDa in female *Ae. aegypti* pre-blood feeding. After blood meal, depletion of some major peptides of 35, 37, 45, 47, 70 kDa and > 118 kDa was observed. In the case of *Cx. quinquefasciatus*, 9 major polypeptides were observed with molecular weights of 20, 25, 36, 38, 45, 47, 49 and two bands of > 118 kDa. The bands of 20, 26, 36 and 38 kDa were depleted after blood feeding [39]. Similar results were observed in other blood sucking insects e.g. *Thyrsopelma guianense*, the vector of onchocerciasis in Brazil. The SG of this species starts synthesizing proteins soon after adult emergence, similar to the protein expression observed in mosquitoes. The levels of soluble proteins present in the salivary secretions are comparable to other anthropophilic species such as *Simulium metallicum* and *Simulium ochraceum* [40, 41]. Protein level reflects the size of SG secretions at each feeding stage. Depletion of some bands may be associated with the fact that efficient blood feeding occurs mainly during maximum salivary secretions, in which insect regurgitate saliva into the host's skin. These results are agreeable with Soliman *et al.* [30] who reported that after *Cx. pipiens* had blood-fed, the total saliva was depleted by 64% within 24 h, but the protein level returned to the unfed value within the next 24- 48 h. ORR *et al.* [42] observed a change in the salivary gland cells 24 h after female *Ae. aegypti* had taken blood meals. The nucleoli of the median and lateral acini of SG became greatly enlarged and there was a concomitant increase in RNA around the nuclei. They concluded that blood feeding may deplete female *Ae. aegypti* SG. This depletion would lead to re-synthesis of secretory products within 24 hours. It has been previously described that variability in the salivary content can occur among different groups of arthropods and individuals of the same species [16, 43]. In contrast to our results, Nascimento *et al.* [31] showed that the major polypeptides found in the female SG are secreted during blood feeding in the case of *Cx. quinquefasciatus*. The polypeptide pattern observed in mosquitoes from day 1 to day 7 was the same. This result concurs with the protein synthesis profile, which was unchanged throughout the first week of mosquito life after emergence. These results are in accordance with the data obtained for *Ae. aegypti* in which, SG protein synthesis pattern is constant during adult life [44]. These morphological and protein content differences have been observed in other mosquito species and were related with the different feeding habits of males and females [14, 28, 32]. The accumulation of specific proteins in the different salivary gland regions has been described for various mosquitoes [26].

5. Conclusion

Conclusively, our results revealed that sugar feeding stages showed less polymorphism (in salivary gland proteins) than blood feeding stages. Starvation of males and females induced more polymorphic bands in salivary glands than in the case of unfed stage after emergence from pupa. The higher degree of polymorphism (in salivary gland proteins) is displayed in the case of blood feeding females. These findings support the hypothesis that salivary glands are triggered to produce specific proteins in relation to mosquito's feeding behavior and to nature of food (nectar or blood), as well. A better understanding of the role of saliva in host's defense, including hemostasis and immune response, leads to its potential use as a biological marker of exposure to arthropod bites or vaccine candidates which could improve host protection against vector-borne diseases.

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7. References

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