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RESEARCH ARTICLE

THE EFFECT OF ABAMECTIN ON SOME BIOCHEMICAL COMPONENTS IN *ARGAS (PERSICARGAS) ARBOREUS* EGGS DURING EMBRYOGENESIS

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ABSTRACT

Abamectin is one of the avermectins that are known for their efficacy in controlling the mites and ticks. Understanding the biochemical effects of abamectin on ticks is crucial for identifying their possible mode of action and optimizing its application to manage tick populations. This study aimed to investigate the biochemical effects of abamectin on *Argas (Persicargas) arboreus* eggs during embryogenesis. The topical application of 5 ppm abamectin (LC_{50}) on newly deposited *Argas (P.) arboreus* eggs quantitively changed the total protein and DNA content, causing fluctuation and decrease of their concentration, respectively, during embryogenesis. Also, such treatment disturbed the normal electrophoretic patterns of the separated protein fractions of the developing embryos on 0, 3, 5, and 8 days post-treatment and post-oviposition (POP). Comparison among the protein fractions patterns (molecular weight, number, percent of the amount, and appearance or disappearance of bands) in each of the untreated controls and abamectin-treated eggs, and between them showed noticeable changes of the patterns during normal embryogenesis, and disturbance from the normal as a result of such treatment. The changes and disturbances of the total protein concentration and fractions were most prominent on 5-8 days post-treatment and POP during the periods of gastrulation and organogenesis. These findings demonstrate the significant impact of abamectin on biochemical components and highlight the disruptions caused by the abamectin treatment during embryonic development in *A. (P.) arboreus* eggs.

INTRODUCTION

Ticks are considered significant vectors of a wide range of pathogens, including *Babesia*, *Theileria*, *Rickettsia*, and *Borrelia*, which can be transmitted to both humans and animals, ranking second after mosquitoes in disease transmission[1,2]. Furthermore, ticks can inflict direct harm to their hosts through attachment and feeding[1,3]. The argasid tick, *Argas (Persicargas) arboreus* is a hemato-phagous obligatory ectoparasite that infests the medium size wading birds in their heronries in Africa[4]. In Egypt, it commonly inhabits the rookeries of the agriculturally beneficial bird Abu-Qerdan or the buff-backed egret, *Bubulcus ibis* (L.) in the Nile

Valley, Delta, and nearby oases[5]. It seriously harms the bird causing severe blood loss by feeding, paralysis by secreting toxins, and disease by transmitting a wide variety of pathogens including rickettsial, spirochetal, and other bacterial and viral microorganisms, which may cause death of the bird[6]. Occasionally, dozens or even hundreds of moribund nestlings of the buff-backed egret were found under heronries infested with *Argas (P.) arboreus* during the breeding season[6].

Abamectin is one of the avermectins (AVMS), which are fermentation products of the soil fungus *Streptomyces avermitilis*[7]. These are relatively newly developed anti-parasitic compounds, which proved potent against many parasitic nematodes and arthropods[8]. The efficacy of AVMS and their analogues against insects and acarine pests of animals and man has been reported by several authors[9-12]. AVMS caused mortality, paralysis, inhibited feeding, reduced digestion, delayed oviposition, and prevented growth and development in the treated arthropods as in the mosquitoes *Aedes aegypti*[13], *Anopheles arbiensis*[14], and the argasid tick *Argas persicus*[15,16]. In *Argas (P.) arboreus*, abamectin application decreased egg production and hatching, interfered with nymphal molting, and produced malformations and abnormalities during development[17]. Furthermore, AVMS were found to inhibit chitin synthesis in the brine shrimp[18], disturb immunity, decrease protease activity in *Culex pipiens*[19], and interfere with DNA synthesis in some other organisms (fungi)[20].

The mode of action of AVMS in ticks is still unknown, but it has been demonstrated that the selective toxicity of AVMS and their analogues against arthropods[21,22] over mammals and probably other vertebrates is attributed to the action of AVMS on the highly sensitive glutamate-activated chloride ion channels expressed at the neuromuscular and neuronal synapses in the arthropods, but not in mammals, which suggested them as promising safe antiparasitic to be used in mammals[23] and probably other vertebrates. In spite of the wide variety of the aforementioned studied biological and physiological effects of AVMS on blood-sucking insects and acarine pests, relatively few studies were concerned with the biochemical effects of AVMS on these arthropods[13,24,25]. DNA and proteins are essential components for all known forms of life. The alteration of one or both is expected to affect the different physiological and biological processes in the arthropod, especially during the active developmental stages as embryogenesis. The present study investigated the effects of the topical treatment of the freshly deposited eggs of *A. (P.) arboreus* with the LC_{50} of abamectin (causing 50% unhatching) on DNA and total protein levels and on the patterns of the electrophoretically-separated protein fractions during embryogenesis of this tick species.

MATERIAL AND METHODS

Tick rearing

A. (P.) arboreus was collected from trees supporting the rookeries of *Bubulcus ibis ibis* at Al-Mansoureya Canal, Giza governorate, Egypt. Ticks were held in the laboratory at 28.0±1.0ºC and 75% relative humidity. The ticks were placed in plastic vials, with a bottom sealed with gypsum and a top securely screened with muslin cloth and a piece of filter paper inside serving as a foothold and for absorbing tick secretions[26]. Ticks were fed on domestic pigeons *Columbia livia domestica* as described by Kaiser[27].

Preparation and application of the avermectin material

Abamectin is a macrocyclic lactone mixture containing a minimum of 80% avermectin B1a (i): 5-Odemethylavermectin B1a, and a maximum of 20% avermectin B1b (ii): 5-O-demethyl-25-de(1-methylpropyl)-25-(1-methylethyl)avermectin B1b. The commercial product of abamectin (Biomectin 5% EC) was kindly supplied by the Ministry of Agriculture, Giza, Egypt. It was dissolved in distilled water to prepare the concentration used in the present study. Mated, engorged females were placed individually in glass

vials under laboratory condition of rearing the tick and the freshly deposited eggs of untreated and topically treated with 1.0 µL of 5 ppm abamectin, which is the LC_{50} causing 50% unhatching[17] when applied per egg batch (60 eggs). The treated eggs were collected on different days post-treatment and post-oviposition (0, 3, 5 and 8 days POP).

Determination of proteins

The total protein concentrations of the control and abamectin-treated eggs were measured on different days of the egg incubation period (0, 3, 5, and 8 days POP) using the method of Bradford[28]. The protein concentration was measured photometrically at 595 nm and compared to a standard of bovine serum albumin. The protein concentration was expressed as mg protein/ 100 mg eggs.

The fractions of egg protein were separated by sodium dodecyl sulphate (SDS)-polyacrylamide gel electrophoresis (PAGE) according to Smith[29]. Before electrophoresis, the total protein of each sample was adjusted to 1.0 mg protein/mL. Molecular weight (MWt) standard of 30-270 kDa was prepared in the solubilization buffer. The gels were photographed and scanned using a Gel Pro-Analyzer (version 3.1; Media Cybernetics, L.P., Rockville, M, DUSA) for protein analysis of tested samples.

Determination of DNA level

The eggs of *A.(P.) arboreus* were subjected to DNA extraction using the DNeasy Blood & Tissue extraction mini kit (cat. no. 69504; Qiagen, Hilden, Germany), according to the manufacturer's protocol. DNA Levels in both control and abamectin-treated eggs were measured at the different days 0, 3, 5, and 8 POP using a UV spectrophotometer at 260 nm[30].

Statistical Analysis

The obtained data were manipulated statistically with SPSS version 16 and Minitab 18.1, while probabilities (p) were carried out using repeated measure ANOVA (significant: $P<0.05$).

RESULTS

Total protein concentration in control and abamectin-treated eggs during embryogenesis

Data illustrated in Figure "1" showed that the total protein concentration of the eggs of the control group on day 0 POP was 30.17±0.55 mg/100 mg eggs. In the next intervals (days 3-8 POP) of embryonic development, the total protein concentration decreased ($P<0.05$) to reach more or less constant levels on the 3rd, 5th, and 8th days POP. However, in the LC_{50} abamectin-treated eggs, the total protein concentration showed a fluctuating pattern during the same period. In comparison with the control group, the total protein concentration of abamectin-treated eggs was higher ($P<0.05$, 36.42± 0.92 mg/100 mg eggs) on day 0 POP, then decreased significantly ($P<0.05$) to reach a similar level ($P>0.05$) to that of the untreated control eggs on the 3rd day POP. This was followed by a sharp increase ($P<0.05$) to reach 27.133±0.63 mg/100 mg eggs on day 5 POP, then dropped to a low level similar to that on the 3rd day POP, but lower than control ($P<0.05$) on the 8th day POP.

Protein fractions in control and abamectin-treated eggs during embryogenesis

The electrophoretic analysis and comparisons among the protein fraction patterns in each of untreated control and abamectin-treated eggs and between them during days of their embryonic development (days 0-8 POP) in *A. (P) arboreus* eggs showed noticeable changes of fraction patterns during normal embryogenesis and disturbances from normal as a result of the treatment. On the day of oviposition (day 0 POP), the untreated control eggs showed a small total number of protein fractions (10 fractions), which slightly increased (11 fractions) in abamectin-treated eggs on the same day. The number and percent

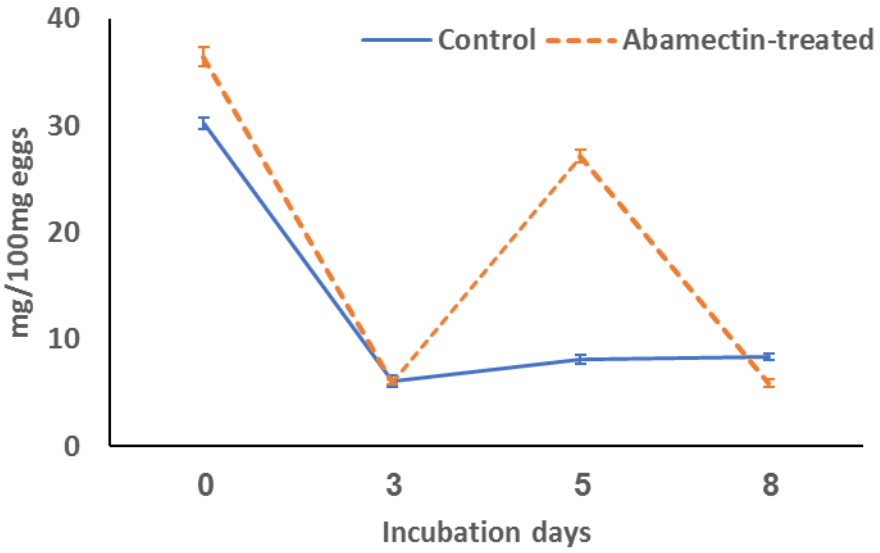

Figure 1: Changes in total protein concentration in untreated control eggs and LC_{50} abamectin-treated eggs of *Argas (P.) arboreus* on different days post-treatment and embryogenesis ($P<0.05$, repeated measure ANOVA).

amount of the fractions with relatively low electrophoretic mobilities (MWt ≥ 100 kDa) were more (7 and 7 bands with 84.42% and 69.78%) than those of the fractions with the higher mobilities (MWt < 100 kDa), which were represented by 3 and 4 bands with relatively small percentages (15.42% and 30.22%) in the untreated control and abamectin-treated eggs, respectively, on day 0 POP (Table 1). In the untreated controls on the 3rd and 5th days POP, the total number of the protein fractions and the number and percent amount of the fractions with low MWt were increased to 14 and 14 total protein fractions and 8 and 8 low MWt fractions, which represented 59.59% and 51.21% of the total protein amount, respectively (Tables 2 and 3). On the 8th day POP, the total number of the protein fractions and the number and percent amount of the fractions with low MWt of the control group increased to reach 16 and 11 bands, and 74.32% of the total protein amount, respectively (Table 4). These increments were associated with the appearance of several new bands with low MWt and the disappearance of some bands with high MWt (352, 304.5, 260, 155.3, 122.4, and 112.9 kDa) that have large percentages of the amount (11.75-20.45%) and were replaced by a few others of high MWt proteins, but mostly with less percentages of the amount. This has led to a decrease in the number and percent amount of the fractions with high MWt to 5 bands and 25.70% of the total protein amount on the 8th day POP.

Similar to the control group, but not identical, rearrangement of bands occurred in the abamectin-treated eggs to reach a total number of fractions of 14 bands and the number and percent amount of the low and high MWt fractions of 8 and 6 bands, and 59.08% and 40.76% of the total protein amount, respectively, on the 3rd day POP (Table 2). However, abamectin-treatment disturbance of normal patterns of protein fractions during embryo development of *A. (P.) arboreus* was more prominent on the 5th and 8th days POP (Table 3 and 4). Instead of the normal increments of total and low MWt protein fractions, the highest decrease in the total number of protein fractions (8 bands) and of those with low and to less extent high MWt fractions (4 bands each) was manifested on the 5th day post-treatment and POP (Table 3). This was associated with temporary disappearance of almost all fractions in abamectin group on day 3 POP (except three low MWt fractions)

Table (1): Molecular weight and amount of egg protein fractions (%) of *Argas (P.) arboreus* eggs post-treatment with abamectin in comparison with control on day 0 post-oviposition.

Band number	Molecular weight (kDa)	Control group Amount (%)	Abamectin group Amount (%)
1	352.0	12.54	12.83
2	304.5	11.80	11.17
3	260.0	14.23	13.93
4	155.3	12.07	10.64
5	122.4	20.45	18.57
6	112.9	11.75	-
7	110.8	-	1.80
8	104.7	1.58	0.84
9	92.5	2.78	-
10	90.8	-	2.67
11	21.7	11.45	12.57
12	19.5	1.19	4.92
13	14.4	-	10.09
Total number of bands		10	11
High MWt ≥ 100kDa: Number of bands/Amount (%)		7 / 84.42%	7 / 69.78%
Low MWt < 100kDa: Number of bands/Amount (%)		3 / 15.42%	4 / 30.22%

Table (2): Molecular weight and amount of egg protein fractions (%) of *Argas (P.) arboreus* eggs post-treatment with abamectin in comparison with control on day 3 post-oviposition.

Band number	Molecular weight (kDa)	Control group Amount (%)	Abamectin group Amount (%)
1	308.8	5.30	-
2	304.5	-	10.16
3	215.5	-	3.24
4	211.2	18.38	-
5	155.3	-	10.20
6	152.2	10.21	-
7	123.5	-	13.07
8	122.4	2.91	-
9	112.9	-	2.08
10	111.8	2.39	-
11	104.7	1.14	-
12	102.7	-	2.14
13	90.0	-	3.12
14	89.0	4.64	-
15	72.0	7.13	-
16	71.0	-	7.14
17	51.6	11.12	19.80
18	44.0	9.03	0.30
19	33.7	11.53	13.48
20	23.6	1.04	1.77
21	21.0	12.65	11.16
22	19.0	2.45	2.31
Total number of bands		14	14
High MWt ≥ 100kDa: Number of bands/Amount (%)		6 / 40.33%	6 / 40.76%
Low MWt < 100kDa: Number of bands/Amount (%)		8 / 59.59%	8 / 59.08%

Table (3): Molecular weight and amount of egg protein fractions (%) of *Argas (P.) arboreus* eggs post-treatment with abamectin in comparison with control on day 5 post-oviposition.

Band number	Molecular weight (kDa)	Control group Amount (%)	Abamectin group Amount (%)
1	308.8	8.70	-
2	224.3	-	23.1
3	215.5	12.60	-
4	165.0	-	17.16
5	158.5	10.59	-
6	125.6	-	16.25
7	124.6	12.79	-
8	113.9	-	2.80
9	111.8	2.43	-
10	103.7	1.64	-
11	92.5	2.50	-
12	44.1	8.24	-
13	36.5	17.00	21.89
14	33.6	6.69	13.66
15	30.8	10.33	-
16	23.4	0.61	4.56
17	20.8	3.06	0.55
18	18.9	2.78	-
Total number of bands		14	8
High MWt ≥ 100kDa: Number of bands/Amount (%)		6 / 48.75%	4 / 59.31%
Low MWt < 100kDa: Number of bands/Amount (%)		8 / 51.21%	4 / 40.66%

that replaced by new fractions mostly with high MWt of 224.3, 165.0, 125.6, and one with low MWt of 36.5 kDa, and large percentages of the amount (16.25-23.1% with a total of 78.4%). Almost all the new fractions on the 5th day POP disappeared on the 8th day POP (total number of 14 fractions: 8 with high MWt and 6 fractions with low MWt and 70.21% and 29.73% amount, respectively; Table 4).

Results in Tables "1-4" showed that some protein fractions with certain MWt were common fractions and were detected in both untreated control and abamectin-treated eggs, while other fractions were specific and were only detected in one of the two groups. Some of the fractions that were only detected in abamectin-treated eggs on some days POP were completely absent throughout normal embryogenesis of the control group from day 0 to day 8 POP such as fractions with MWt of 110.8, 123.5, 165, and 274.3 kDa found in abamectin-treated eggs on days 0, 3, 5, and 8, respectively.

DNA concentration in control and abamectin-treated eggs during embryogenesis

Quantitative changes in DNA level during embryogenesis of untreated control and LC_{50} abamectin-treated eggs of *A. (P.) arboreous* are illustrated in Figure "2". On the day 0 POP, the DNA concentration in the untreated and abamectin-treated eggs were 0.657±0.011 µg/mg eggs and 0.525±0.014 µg/mg eggs, respectively. These concentrations showed a gradual increase (P<0.05) on the 3rd and 5th day POP to reach the highest level (P<0.05) of 1.204±0.018 and 0.913±0.014 µg/mg eggs, respectively, on the 8th day POP. However, the DNA levels of the abamectin-treated eggs showed a significant decrease (P<0.05) as compared with the control on each of the studied days of embryonic development on 0, 3, 5, and 8 days POP.

Table (4): Molecular weight and amount of egg protein fractions (%) of *Argas (P.) arboreus* eggs post-treatment with abamectin in comparison with control on day 8 post-oviposition.

Band number	Molecular weight (kDa)	Control group Amount (%)	Abamectin group Amount (%)
1	347.7	-	3.66
2	308.8	-	2.28
3	274.3	-	22.24
4	224.3	14.35	12.56
5	171.6	0.25	20.68
6	127.8	-	4.85
7	126.7	7.18	-
8	112.9	-	1.62
9	111.8	0.52	-
10	104.7	3.4	-
11	102.7	-	2.32
12	92.5	6.98	2.83
13	74.1	9.02	5.58
14	52.8	1.02	11.53
15	43.7	8.03	-
16	40.9	4.43	-
17	37.1	-	3.60
18	36.8	16.06	-
19	33.9	0.29	3.93
20	30.6	18.43	-
21	23.9	4.21	-
22	21.1	1.92	-
23	20.9	-	2.26
24	18.9	3.93	-
Total number of bands		16	14
High MWt ≥ 100kDa: Number of bands/Amount (%)		5 / 25.7%	8 / 70.21%
Low MWt < 100kDa: Number of bands/Amount (%)		11 / 74.32%	6 / 29.73%

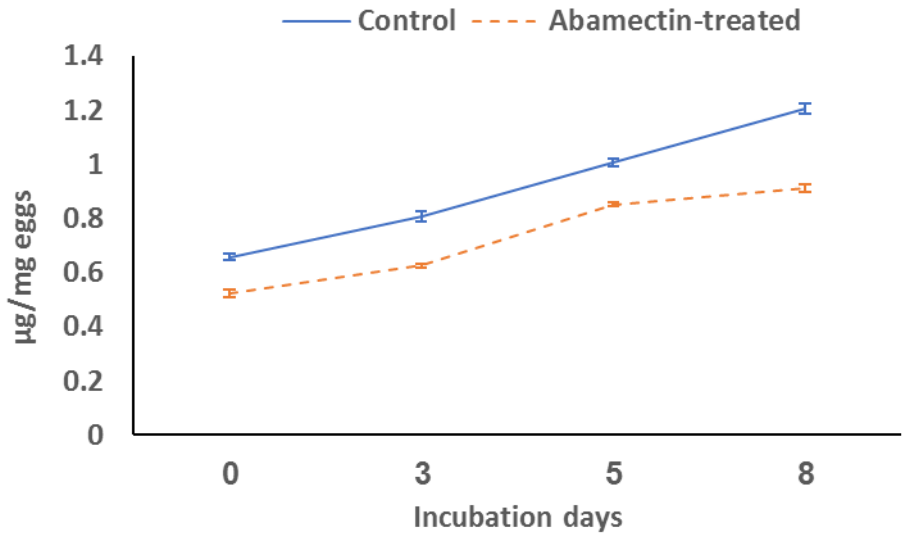

Figure 2: Changes in DNA level in untreated control eggs and LC_{50} abamectin-treated eggs of *Argas (P.) arboreus* on different days post-treatment and embryogenesis ($P<0.05$, repeated measure ANOVA).

DISCUSSION

The topical application of the LC_{50} abamectin causing 50% unhatching[17] on the newly deposited eggs on the day of oviposition (day 0 POP) was found to change the concentrations of total protein and DNA and disturbed the patterns of electrophoretically separated protein fractions during embryogenesis in *A. (P.) arboreus* (days 0-8 POP) in the present study. The total protein concentration of normal *A. (P.) arboreus* eggs was sharply decreased during the early period of embryogenesis (days 0-3 POP) to reach more or less constant levels on the 3rd day POP and the next studied period of embryonic development (days 5-8 POP). The period of the initial decrease of the total protein corresponded to the period of cleavage up to blastoderm and the period of constant protein levels (3-8 days POP) corresponded to the period of cell layer formation (gastrulation) and tissue differentiation (organogenesis), in *A. (P.) arboreus* (unpublished data) and the closely related species *A. (P.) persicus*[31]. In the abamectin-treated eggs on the day 0 POP, the concentration of total protein was higher than that in the untreated eggs. As normal, the amount of the total protein in the treated eggs was decreased to a similar level to that of the control eggs on the 3rd day POP. However, the reduction of total proteins in the treated eggs was followed by an evident increase on the 5th day POP to drop again on the 8th day POP below the normal. The initial decrease of total protein during early embryogenesis (0-3 days) in untreated control and abamectin-treated eggs of *A. (P.) arboreus* might be attributed to the breakdown and hydrolysis into amino acids[32,33], as well as a consumption of yolk proteins stored in the egg to synthesize the developing embryo proteins. However, the constant level of total protein during the next interval of embryogenesis (days 3-8 POP) in the control eggs of *A. (P.) arboreus* may point to a balance between synthesis and catabolism of proteins during this active interval of embryogenesis in building new tissues and organs. The fluctuations in the concentration of total protein during embryogenesis in abamectin-treated eggs reflected a disturbance of the normal pattern of the total protein change and of the normal balance between synthesis and catabolism of protein during the relatively late embryogenesis (days 5-8 POP). The high 3.34-fold increase of the amount of total protein in the abamectin-treated eggs relative to control eggs on the 5th day POP suggested a temporary accumulation of egg proteins probably as a result of a decrease of their breakdown and consumption/or synthesis of additional proteins. The decrease in the amount of total protein in early embryogenesis is in accordance with the findings of Gadallah *et al*.[34] in the eggs of *A. (P.) arboreus*, which was extended up to the 6th day POP. However, the total protein levels were fluctuated with a net increase during embryogenesis (20 days POP) in eggs of the control and 20-hydroxyecdyson-treated females of *Hyalomma dromedarii*[35]. Eggs deposited by the 20-hydroxyecdyson treated females exhibited a high increase in the total protein concentration during embryogenesis of *Hyalomma dromedarii* when compared with the control group[35]. Gadallah *et al*.[35], suggested that the greatest increase in protein synthesis could have happened due to an increase in a free form of ecdosteriods (EDs) with a subsequent influence of increased protein synthesis. EDs were found to accumulate in ovaries and newly laid eggs of many hard and soft ticks[36,37]. A probable effect of abamectin on EDs metabolism[38] in *A. (P.) arboreus* eggs could have caused the observed temporary increase, fluctuation, and disturbance of normal balance in protein levels in the present study. This suggestion needs further investigation to be verified and understood. In *Dermacentor andersoni*, *Hyalomma dromedarii*, and *Rhipicephalus (Boophilus) microplus*, the total protein concentration remained unchanged from oviposition to hatching[30,33,39]. Kamel *et al*.[30] suggested that the constancy of the protein may result of the balance in the synthesis of new proteins and the degradation of the yolk protein.

In the embryogenesis of ticks and insects, proteins are synthesized through the metabolism of amino acids[32,33], incorporation and aggregation of peptides[40,41], and breakdown (proteolysis) and catabolism of larger proteins already existing in the egg yolk[30,34,35]. Embryonic development has been reported as a sequential and complex process controlled by genes[42]. The existence of proteins expressed in specific stages of embryo development suggests that different stages need specific proteins to proceed correctly. In the present study, comparison among the parameters used to analyze and evaluate changes of the protein fraction pattern (MWt, number, percent of the amount, appearance and disappearance of bands) during embryogenesis in each of the untreated controls and abamectin-treated eggs showed noticeable changes of the fractions patterns during normal embryogenesis and disturbance from normal as a result of the treatment especially during the period corresponding to that of cell layer formation and tissue differentiation at gastrulation and organogenesis, respectively, on days 5-8 POP. In the untreated normal embryogenesis, the total number of fractions gradually increased from 10 to 16 bands during day 0-8 POP of *A. (P.) arboreus* eggs. This increment was associated with a gradual increase in the number (from 3 to 11 bands), percent of the amount (from 15.42% to 74.32%), and appearance of new small protein fractions with low MWt less than 100 kDa. The concomitant decrease of the number (from 7 to 5), percent of the amount (from 84.42% on day 0 to 25.70% on day 8), and the disappearance of some big protein fractions with high MWt equal or more than 100 kDa during 3-8 day POP reflected a probable breakdown of them into smaller proteins. Also, the occasional replacement of some disappeared large proteins by others even in smaller amount suggested an apparent interconversion of protein fractions and balance between synthesis of new proteins and degradation of the yolk proteins during embryogenesis of *A. (P.) arboreus*, which seemed to be similar to ways of exchange between protein fractions that were observed during embryogenesis of *Hyalomma dromedarii*[30]. Generally, the appearance of new bands and disappearance of others and changes in the number, MWt, amounts of protein fractions reflected high activities among the protein fractions including synthesis, proteolysis, hydrolysis, catabolism, and consumption of the fractions during embryogenesis in the untreated normal tick.

Similar to control, but not identical, rearrangement of bands occurred in the abamectin-treated eggs of *A. (P.) arboreus* up to day 3 POP, where the total number of protein fractions increased from 11 bands (day 0) into 14 bands (day 3), and the number of low MWt fractions from 4 bands with 30.22% amount on the day of oviposition and treatment (day 0) to 8 bands with 59.08% amount on day 3 POP. However, this was followed by a considerable disturbance on the 5^{th} and 8^{th} days POP including a great reduction of the total number of fractions from 14 bands on the 3^{rd} day to 8 bands on the 5^{th} day. This was associated with a noticeable decrease in number and amount of the low MWt fractions, which mostly disappeared with no replacement by new bands (except one) and a concomitant increase of percent of the amount of new 4 bands mostly with high MWt on the 5^{th} day. The last findings may point to a probable accumulation of fractions with the high MWt and a decrease in the synthesis of new low MWt fractions in abamectin-treated eggs on the 5^{th} day as compared with the control group. Also, the pattern of change of protein fractions in abamectin-treated eggs on the 8^{th} day was much different from that in the control eggs, with larger number and percent amount of the fractions with high MWt than lower MWt, and the disappearance of a relatively large number of bands in (10 bands). Generally, the disappeared fractions might have been hydrolyzed to amino acids[33], broken into small peptides, consumed in catabolism, and synthesis of new protein fractions or non-protein compounds[34].

In the present study, some protein fractions were specific, detected only in the abamectin-treated eggs and were completely absent throughout the period of studied embryogenesis in untreated control eggs of *A. (P.) arboreus*. Similarly, Kelly and Huebner[43] found 3 protein bands, which are normally absent, were distinct in fenoxycarb-treated embryos of *Rhodnius prolixus*. These proteins were suggested to help the synthesis of detoxifying enzymes in the treated insect. The observed phenomenon was reported in ticks[34,35] and insects[44-46] treated with hormones, chemosterilants, chitin synthesis inhibitors (CSI), and rice bran extract. In the present study, disturbance from normal patterns of protein fractions during the egg incubation period and embryogenesis of abamectin-treated eggs could have been reflected in the observed abnormalities of development and hatching of the treated eggs of *A. (P.) arboreus*. Generally, changes in numbers, MWt, percent of the amount, appearance and disappearance of electrophoretically-separated protein fractions were confirmed by the finding of Gadallah *et al*.[34], Ahmed *et al*.[31], and Kamel *et al*.[30] during normal embryogenesis of *A. (P.) arboreus*, *A. persicus*, and *Hyalomma dromedarii*, respectively. Also, present results are in accordance with normal embryogenesis in some insects as *Musca domestica*[45] and *Schisocerca gregaria*[46], where an increase in the total number of protein fractions and/or appearance and disappearance of protein bands were recorded during normal embryogenesis. Disturbance from the normal pattern of change of protein fraction was found in eggs directly treated or resulted from females treated with hormones as juvenile and 20-hydroxyecdyson in *A. (P.) arboreus* and *Hyalomma dromedarii*, respectively[34,35], chemosterilants as aziridinyl in *M. domestica*[44], CSI as lufenuron and rice bran extract in *Musca domestica* and *S. gregaria*, respectively[45,46].

In the present study, determination of the DNA concentration showed that the amount of DNA gradually increased throughout the embryonic development of *A. (P.) arboreus* in normal and LC_{50} abamectin-treated newly deposited eggs (day 0 POP). However, there were significantly lower levels of DNA in the abamectin-treated eggs than in the normal control on the corresponding egg incubation days POP. The amounts of DNA reached the highest levels of 1.204± 0.018 µg/mg and 0.913±0.014 µg/mg with 1.82 and 1.72 fold increase on the 8th day POP in normal and abamectin-treated eggs, respectively. The aforementioned results suggested that abamectin treatment of newly deposited eggs interfered with and significantly decreased DNA synthesis in developing embryos of *A. (P.) arboreus*. However, the observed gradual increase of DNA amount in both normal and treated eggs during embryonic development could be attributed to the increasing demands of DNA for nuclear multiplication and cell mitotic divisions during cleavage till blastoderm formation (0-3 days POP), germ layers and tissue differentiation at gastrulation and organogenesis, respectively (3-8 days POP) in normal *A. (P.) arboreus*. Results of the present work conformed that of Kamel *et al*.[30] and Gadallah *et al*.[34,35] on embryogenesis of normal *Hyalomma dromedarii* and *A. (P.) arboreus* and in eggs resulted from the female tick treated with juvenile hormone or 20-hydroxyecdyson, where the amounts of DNA gradually increased during embryogenesis. In contrast to the present study, these amounts were not significantly changed from the control on corresponding days of egg incubation. However, in consistence with the present results, Kilgore and Painter[47], Gadallah *et al*.[44] and Guneidy *et al*.[45] found that the amounts of DNA in the house fly, *Musca domestica*, increased gradually during the embryonic development of normal and to less extent in eggs of the females chemosterilized by a relatively low concentration of apholate[47]. This was detected in spite of the noticeable inhibition of DNA synthesis to slightly measurable amounts. Also, the amounts of DNA were decreased significantly in the house fly eggs treated with CSI and rice bran extract in comparison

with control throughout embryogenesis[45]. However, Gadallah *et al*.[44] showed a great inhibition of DNA, where no measurable DNA amount was found in eggs of the house fly female chemosterilized with aziridinyl. Furthermore, some CSIs as benzyol phenyl urea were reported as potential genotoxic agents to *Drosophila melanogaster*[48]. These compounds were suggested to have the ability to form ducts with DNA through its nucleophilic sites[48]. Abamectin as other AVMs are considered as CSIs inhibiting chitin synthesis in arthropods[18], having lethal effects and interfering with DNA synthesis in some other organisms[20]. Therefore, abamectin may probably has genotoxic agent[48]. In the present study, abamectin treatments of *A. (P.) arboreus* eggs reduced the amount of DNA to about 75-84% of that in normal. Also, Aboutaleb *et al*.[17] reported that the treatments with abamectin induced concentration-dependent ovicidal effects, and caused distortions of the eggshell and abnormality of hatching, which were attributed to probable defects in chitinization of the eggshell and fully developed embryos. In conclusion, this study provides valuable insights into the bio-chemical impact of abamectin on tick eggs and its potential role as an effective tick control measure.

ETHICAL APPROVAL

The experimental design of the current study was approved by the Research Ethics Committee of Faculty of Science, Ain Shams University (approval number: ASU-SCI/ENTO/2023/5/2).

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

[1] De la Fuente, J.; Estrada-Peña, A; Venzal J. M. *et al*. (2008). Overview: ticks as vectors of pathogens that cause disease in humans and animals. Front Biosci, 13: 6938-6946.

[2] Orkun, Ö.; Karaer, Z.; Çakmak, A. *et al*. (2014). Spotted fever group rickettsiae in ticks in Turkey. Ticks Tick Borne Dis, 5(2): 213-218.

[3] Pagel Van Zee, J.; Geraci, N. S.; Guerrero, F. D. *et al* (2007). Tick genomics: the Ixodes genome project and beyond. Int J Parasitol, 37(12): 1297-1305.

[4] Khalil, G. M.; Hoogstraal, H. and Oliver, Jr., J. H. (1980). Biological evaluation of the systematic validity of the African *Argas (Persicargas) arboreus* and the Asian-Australian *A. (P.) robertsi* (Ixodoidea, Argasidae). Int J Parasitol, 10(4): 253-259.

[5] Guirgis, S. S. (1971). The subgenus *Persicargas* (Ixodoidea, Argasidae, Argas). II. Ecology and seasonal dynamics of *A (P.) arboreus* Kaiser, Hoogstraal & Kohls in Egypt. J Med Entomol, 8: 407-414.

[6] Hoogstraal, H. (1985). Argasids and nuttaliellid ticks as parasites and vectors. Adv Parasitol, 24: 135-238.

[7] Crump, A. and Ōmura, S. (2011). Ivermectin, 'wonder drug' from Japan: the human use perspective. Proc Jpn Acad Ser B Phys Biol Sci, 87(2): 13-28.

[8] Campbell, W. C.; Fisher, M. H.; Stapley, E. O. *et al*. (1983). Ivermectin: a potent new antiparasitic agent. Science, 221(4613): 823-828.

[9] Ash, L. S. and Oliver, Jr., J. H. (1989). Susceptibility of *Ornithodoros parkeri* (Cooley) (Acari: Argasidae) and *Dermanyssus gallinae* (DeGeer) (Acari: Dermanyssidae) to ivermectin. J Med Entomol, 26(3): 133-139.

[10] Tesh, R. B. and Guzman, H. (1990). Mortality and infertility in adult mosquitoes after the ingestion of blood containing ivermectin. Am J Trop Med Hyg, 43(3): 229-233.

[11] Davey, R. B.; Pound, J. M.; Miller, J. A., *et al*. (2010). Therapeutic and persistent efficacy of a long-acting (LA) formulation of ivermectin against *Rhipicephalus (Boophilus) microplus (*Acari: Ixodidae) and sera concentration through time in treated cattle. Vet Parasitol, 169(1-2), 149-156

[12] Doan, H. T. T.; Noh, J. H.; Kim, Y. H. *et al*. (2013). The efficacy of avermectins (ivermectin, doramectin and abamectin) as treatments for infestation with the tick *Haemaphysalis longicornis* on rabbits in Korea. Vet Parasitol, 198(3-4), 406-409.

[13] Mahmood, F.; Walters, L. L.; Guzman, H. *et al*. (1991). Effect of Ivermectin on ovarian development. of *Aedes.aegypti* (Diptera:Culicidae). J Med Entomol, 28(5): 701-707.

[14] Lyimo, I. N.; Kessy, S. T.; Mbina, K. F. *et al*. (2017). Ivermectin-treated cattle reduces blood digestion, egg production and survival of a free-living population of *Anopheles arabiensis* under semi-field condition in south-eastern Tanzania. Mala J, 16(1): 239 (DOI: 10.1186/s12936-017-1885-x).

[15] Swelim, H. H.; Marzouk, A. S. and Montasser, A. A. M. (2003). Ultrastructural and histological changes induced by ivermectin in the ovary of *Argas persicus* after feeding. Egypt J Hosp Med, 10: 154-172.

[16] Montasser, A. A.; Marzouk, A. S.; El-Alfy, S. H. *et al*. (2011). Efficacy of abamectin against the fowl tick, *Argas (Persicargas) persicus* (Oken, 1818) (Ixodoidea: Argasidae*)*. Parasitol Res, 109(4), 1113-1123.

[17] Aboutaleb, K. S.; Shanbaky, N. M.; Ahmed, S. S. *et al*. (2019). Effect of Abamectin on Reproduction and Development of an Avian Tick, *Argas (Persicargas) arboreus* (Ixodoidea: Argasidae). Egypt Acad J Biolog Sci, A: Entomology, 12(6): 35-51.

[18] Mayer, R. T.; Cunningham, G. N. and Gupton J. T. (1990). Insecticides Based on Differences in Metabolic Pathways. In: Safer Insecticides: Development and Use (Hodgson , E. and Kuhr, R. J., eds), pp. 209-255. Marcel Dekker, New York, NY, USA.

[19] Abdeltawab, M. S. A.; Rifaie, S. A.; Shoeib, E. Y. *et al*. (2019). Insights into the impact of ivermectin on some protein aspects linked to *Culex pipiens* digestion and immunity. Parasitol Res, 119: 55-62.

[20] Calcott, P. H. and Fatig, 3rd, R. O. (1984). Inhibition of chitin metabolism by avermectin in susceptible organisms. J Antibiot (Tokyo), 37(3): 253-259.

[21] Kane, N. S.; Hirschberg, B.; Qian, S. *et al*. (2000). Drug-resistant *Drosophila* indicate glutamate-gated chloride channel are targets for the antiparasitics nodulisporic acid and ivermectin. Proc Natl Acad Sci USA, 97(25): 13949-13954.

[22] Eguchi, Y.; Ihara, M.; Ochi, E. *et al*. (2006). Functional characterization of *Musca* glutamate- and GABA-gated chloride channels expressed independently and coexpressed in *Xenopus* oocytes. Insect Mol Biol, 15(6): 773-783.

[23] Narahashi T.; Zhao X.; Ikeda T. *et al*. (2010). Glutamate-activated chloride channels: unique fipronil targets present in insects but not in mammals. Pestic Biochem Physiol, 97(2): 149-152.

[24] Lunke, M. D. and Kaufman, W. R. (1992). Effects of the avermectin analogue MK-243 on vitellogenesis and reproduction in the ixodid tick, *Amblyomma hebraeum*. Exp Appl Acarol, 13(4): 249-259.

[25] Saldivar, L.; Guerrero, F. D.; Miller, R. J. *et al*. (2008). Microarray analysis of acaricide-inducible gene expression in the southern cattle tick, *Rhipicephalus (Boophilus) microplus*. Insect Mol Biol, 17(6): 597-606.

[26] Hefnawy, T. (1972). Biochemical and physiological studies of certain

ticks (Ixodoidea). Hemolymph volume determined by isotope and dye dilution during the gonotrophic cycle of *Argas (Persicargas) persicus* (Oken) and *A. (P.) arboreus* Kaiser, Hoogstraal, and Kohls (Argasidae). J Parasitol, 58(2): 358-364.

[27] Kaiser, M. N. (1966). The subgenus Persicargas (Ixodidea: Argasidae: *Argas*). 3. The life cycle of *A. (P.) arboreus* and standardized rearing method for argasid ticks. Ann Entomol Soc Am, 59(3): 496-502.

[28] Bradford M. M. (1976). A rapid sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-Dye Binding. Anal Biochem, 72: 248-254.

[29] Smith, I. (1975). Chromatographic and Electrophoretic Techniques: Zone electrophoresis. Wiley, Hoboken, NJ, USA.

[30] Kamel, M. Y.; Shalaby, F. Y. and Ghazy, A. M. (1982). Biochemical studies of tick embryogenesis DNA, RNA, haemoprotein, guanosine and guanine in developing eggs of *Hyalomma dromedarii*. Insect Biochem, 12: 15-23.

[31] Ahmed, N. H.; Radwan, W. A.; Gueindy, N. A. *et al*. (2009). Effect of the juvenile hormone analogue (admiral) on embryogenesis of the soft tick *Argas persicus* (Oken). Egypt Acad J Biolog Sci, 2: 165-176.

[32] Chen, P. S. and Briegel H. (1965). Studies on the protein metabolism of *Culex pipiens* L. 5. Changes in the free amino acids and peptides during embryonic development. Comp Biochem Physiol, 14: 463-473.

[33] Boctor, F. N. and Kamel, M. Y. (1977). Purification and characterization of two lipovitellins from eggs of the tick *Dermacentor andersoni*. Insect Biochem, 6: 233-240.

[34] Gadallah, A. L; Khalil, G. M.; Dees, W. H. *et al*. (1989). Biochemical effects of juvenile hormone III on the tick, *Argas (Persicargas) arboreus* (Acari: Argasidae) during embryogenesis. J Med Entomol, 26(4): 360-367.

[35] Gadallah, A. I.; Khalil, G. M.; Dees, W. H. *et al*. (1990). Biochemical changes in *Hyalomma (Hyalomma) dromedarii* (Acari: Ixodidae) embryos and effect of 20-hydroxyecdysone applied to the mother. J Med Entomol, 27(5): 763-772.

[36] Wigglesworth, K. P.; Lewis, D. and Rees H. H. (1985). Ecdysteroid titre and metabolism to novel apolar derivatives in the adult female *Boophilus microplus* (Ixodidae). Arch Insect Biochem Physiol, 2: 39-54.

[37] Connat, J.-L. and Datson, E. M. (1988). Comparative investigation of the egg ecdysteroids of ticks using radioimmunoassay and metabolic studies. J Insect Physiol, 34(7): 639-645.

[38] Isaac, R. E.; Sweeney, F. P. and Rees, H. H. (1983): Enzymic hydrolysis of ecdysteroid phosphate during embryogenesis in desert locust (*Schistocerca gregaria*). Biochem Soc Trans, 11(4): 379-380.

[39] Santos, V. T.; Ribeiro, L.; Fraga. A. *et al*. (2013). The embryogenesis of the tick *Rhipicephalus (Boophilus) microplus*: the establishment of a new chelicerate model system. Genesis, 51(12): 803-818.

[40] Taylor, D. and Chinzei, Y. (2001). Vitellogenin and its Synthesis in the Soft Ticks. In: Acarology: Proceeding of the 10th International Congress, Conterra, Australia (Holliday, R. B.; Walter, D. E; Proctor, H. C. *et al*. eds), pp. 622-627. CSIRO Publishing, Collingwood, Australia.

[41] Bakr, R. F. A.; Helmy, N.; Nawwr, G. A. *et al*. (2010). Changes in protein content of *Culex pipiens* mosquito treated with two agriculture waste extracts. Egypt Acad J Biolog Sci, A: Entomology, 3(1): 95-103.

[42] Fang, Y. and Li, J.-K. (2010). Analysis of developmental proteome at egg stage of drone honeybees *(A. m. ligustica*). Agr Sci China, 9(3): 392-400.
[43] Kelly, G. M. and Huebner, E. (1987). Juvenoid effects on *Rhodnius prolixus* embryogenesis. Insect biochem, 17(7): 1079-1083.
[44] Gadallah, A. I.; Kilgore, W. W. and Painter, R. R. (1970): Metabolism of nucleic acid and protein of normal and chemosterilized house flies during oögenesis and embryogenesis. J Econ Entomol, 63(6): 1777-1781.
[45] Guneidy, N. A.; Salem, D. A. M.; Helmy, N. *et al*. (2011). Effect of a chitin synthesis inhibitor and a waste product on embryogenesis of *Musca domestica*. J Am sci, 7(12): 704-712.
[46] Mahdy, N. M.; Mohammed, M. I.; Abdou, M. A. *et al*. (2019). Toxicological and biochemical effects of lufenuron and rice bran on desert locust, *Schistocerca gregaria* (Forskal) (Orthoptera: Acrididae). Egypt Acad J Biolog Sci, F: Toxicology and Pest Control, 11(2): 37-58.
[47] Kilgore, W. W. and Painter, R. R. (1964). Effect of the chemosterilant apholate on the synthesis of cellular components in developing housefly eggs. Biochem J, 92(2): 353-357.
[48] Eid, J. I.; Awad, A. A.; Basal, W. T. *et al.* (2017). Evaluation of genotoxicity of lufenuron and chlorfluazuron insecticides in *Drosophila melanogaster* using a germ-line cell aneuploidy and chromosomal aberrations test. Int J Adv Agric Environ Eng, 4: 93-101.

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تأثير الأبامكتين على بعض المكونات البيوكيميائية في بيض القراد "*Argas (Persicargas) arboreus*" في أثناء التكوين الجنيني

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الأبامكتين هو أحد مجموعة الآفرمكتين المعروفة بفعاليتها في مكافحة الحَلَم والقراد. يُعد فهم التأثيرات البيوكيميائية للأبامكتين على القراد أمرًا بالغ الأهمية لتحديد طريقة عمله الممكنة وتحسين تطبيقاته لإدارة تجمعات القراد. تهدف هذه الدراسة إلى التعرف على التأثيرات البيوكيميائية للأبامكتين على بيض القراد "*Argas (Persicargas) arboreus*" في أثناء التكوين الجنيني. أدي التطبيق الموضعي للأباميكتين على البيض الذي تم وضعه حديثًا للقراد بتركيز 5 جزء في المليون المانع لفقس 50% من البيض إلى تغير المحتوى الكُلي للبروتين والحمض النووي، حيث تسبب في تذبذب وانخفاض تركيزهما على التوالي خلال مرحلة التطور الجنيني. كما أثرت المعاملة بالأبامكتين على الأنماط الطبيعية لشرائط البروتين المنفصل بالحمل الكهربي لبروتينات البيض في أثناء مراحل تكوينها الجنيني في الأيام 0 و 3 و 5 و 8 بعد وضع البيض وتطبيق الأبامكتين. أظهرت المقارنة بين أنماط شرائط البروتين من حيث الوزن الجزيئى، والعدد، والنسبة المئوية، وظهور واختفاء الشرائط فى كل من البيض غير المعامل والمعامل باالأبامكتين تغيرات ملحوظة في أنماطها أثناء تكونها الجنيني واضطراب عن النمط الطبيعي نتيجة المعاملة بالأبامكتين. كانت التغيرات والاضطرابات في تركيز البروتين الكُلي والحمل الكهربي للبروتينات أكثر وضوحًا في الأيام 5-8 بعد الوضع خلال فترة تكون الجسترولة وتكون الأعضاء في الجنين. توضح هذه النتائج التأثير الكبير للأبامكتين على المكونات البيوكيميائية، وتسلط الضوء على الاضطرابات التي تسببها المعاملة بالأبامكتين في أثناء التكون الجنيني في بيض القراد.