# QUANTITATIVE STUDIES ON THE PROTEINS, FREE FATTY ACIDS AND GLYCOGEN CONTENTS OF EGGS OF *AMBLYOMMA VARIEGATUM* (FABR) AND *BOOPHILUS DECOLORATUS* (KOCH) (TICKS, IXODOIDEA : IXODIDAE)

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TICKS
OVIPOSITION
EGGS
COMPONENTS
DEPLETION

ABSTRACT : The proteins, free fatty acids and glycogen contents of freshly laid eggs of two ixodid tick species *Amblyomma variegatum* and *Boophilus decoloratus* were estimated after the methods of LOWRY et al. (1951), ITAYA and UI (1965) and ROE (1955) respectively, using units of eggs deposited sequentially over the full span of oviposition of the respective ticks.

Mean protein contents decreased initially from over 500 ug/mg egg (*A. variegatum*) and 180 ug/mg egg (*B. decoloratus*) in the early egg batches, to less than 250 ug/mg egg (*A. variegatum*) and 125 ug/mg egg (*B. decoloratus*) in the later batches ; but subsequently rose to over 400 ug/mg egg (*A. variegatum*) and 230 ug/mg egg (*B. decoloratus*) in the terminal egg batches.

Mean fatty acid components of eggs of both species also showed a rapid but unaltered depletion sequence from over 310 ug/mg egg (*A. variegatum*) and 380 ug/mg egg (*B. decoloratus*) in early egg batches to less than 75 ug/mg egg (*A. variegatum*) and 90 ug/mg egg (*B. decoloratus*) in the terminal batches.

Estimated glycogen contents of the eggs showed a trend basically similar to that of the fatty acids. Starting from initial levels of 1.78-1.8 ug. glycogen/mg egg (*A. variegatum*) and 0.94-1.03 ug. glycogen/mg egg (*B. decoloratus*) the glycogen content per mg egg diminished to practically a zero level in the terminal batches of eggs produced by ticks of both species.

Factors responsible for the differential depletion rates/patterns of the various egg components are considered, and their possible implications in terms of the development and hatch rates of young ticks generally are discussed.

ZECKEN
OVIPOSITION
EIERN
KOMPONENTEN
VERRINGERUNGSTENDENZ

ABSTRACKTUM : Proteine, freie Fett-säuren und Glycogen Gehälte von frisch gelegten Eiern von zwei ixodiden Zekenarten, *Amblyomma variegatum* und *Boophilus decoloratus*, wurden nach methoden von LOWRY et al. (1951), ITAYA und UI (1965) und ROE (1955) bestimmt. Die Bestimmung wurde mit reihenmässig gelegten inzel Eiern über den Zeit — Raum der oviposition der infrage Commenden Zeken vorgenommen.

Der Durchschnitts protemegehalt verringerten sich von 500 ug/mg Ei (*A. variegatum*) und 180 ug/mg Ei (*B. decoloratus*) inden früheren Eiern auf weniger als

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250 ug/mg Ei (*A. variegatum*) und 125 ug/mg Ei (*B. decoloratus*) in späteren Eiern. Danach stiegen die Werte auf über 400 ug/mg Ei (*A. variegatum*) und 230 ug/mg Ei (*B. decoloratus*) aus Ende.

Die Fett-säure Komponenten der Eiern beider Arten zeigen in Durchschmittswerten rapide aber stetige Verringerungstendenz von 310 ug/mg Ei (*A. variegatum*) und 380 ug/mg Ei (*B. decoloratus*) in frühen Eiern zu weniger als 75 ug/mg Ei (*A. variegatum*) und 90 ug/mg Ei (*B. decoloratus*) in den letzten stadien. Die gefundem werte für die Glycogen Gehalt der Eiern zeigten grundsatzlich änliche Tendeuz wie bei den Fettsäuren Die Werte varrieben von 1.78 bis 1.8 ug Glycogen/mg Ei. (*A. variegatum*) und 0.94 bis 1.03 Glycogen/mg Ei (*B. decoloratus*) und verringeten auf null Niveau in den letzen Eiern.

Die Factoren die verentwortlich für die differentiale verringerungsgeschwindigkeiten/verlaufsweisen der verschiedenem Eikomponenten sind berücksichtig und ihre möglichen Einflüsse in Bezug auf Entwicklung und Ausschlupfgeschwindigkeinhen der jungen zekem werden allgemein diskutiert.

## Introduction

Oviposition rates in various ixodid ticks have been known to conform to some parabolic pattern, with peak egg output during the first few days, followed by a gradual but steady decline in subsequent days (Hitchcock, 1955 ; Drummond and Whetstone, 1970 ; Iwuala and Okpala, 1977). Weights and hatch rates of these eggs have also been found to show definite decline with the sequence of their deposition (Drummond et al., 1971 ; Iwuala and Okpala, 1977). This has been attributed in part to possible sequential depreciation in quantities of fats, proteins, nucleic acids and other vital components of later batches of eggs, as against perhaps the situation in early egg-batches which presumably possess richer stocks of nutrients.

There is however hardly enough information on the detailed nutritional composition of eggs of ixodid ticks. Jaskoshi and Molinari (1974) using thin-layer chromatography method had revealed presence of wax esters, cholesterol esters, unsaturated hydrocarbons and lecithin in the egg-shell of *Rhipicephalus sanguineus*. Cherry (1976), also reported cholesterol and its esters from the eggs and tissues of *Boophilus microplus*. In other studies Hussein and Kamal (1977) have attempted a classification of the phospholipid components of eggs, larvae and the haemolymph of *Argas* (*Persicargas*) *arboreus* (Argasidae) and *Dermacentor andersoni* (Ixodidae), while Iwuala, Umezurike and Nwadiogbu (1980) have noted presence of a range of other lipid components in the eggs of *Boophilus decoloratus*. The latter studies compliment a series of biochemical and physiological investigations earlier reported by Boctor and Kamal (1976).

Nevertheless, information on the quantitative nutritional composition of eggs of ticks is still very lacking ; and in any case is required for a proper understanding of the factors influencing hatch rates of the eggs and perhaps the subsequent development and viability of the larvae.

The present study is concerned with quantitative estimation of the over-all amounts of proteins, free fatty acids and glycogen present in eggs of *Amblyomma variegatum* and *Boophilus decoloratus*, and an assessment of the changes in the concentration of the various substances in egg batches deposited sequentially in the course of oviposition.

## Materials and Methods

Ticks used for this study comprised adult female *Amblyomma variegatum* (Fab) and *Boophilus decoloratus* (Koch) harvested from herds of Ndama trade cattle in Enugu Nigeria. The engorged females were specifically selected (after Iwuala and Okpala, 1977 *b*) and kept individually in separate filter-paper-floored petridishes (6 cm diameter each) in the laboratory at 25 ± 2°c and

75 ± 5 % R. H. The ticks were left to oviposit on the filter paper, and eggs of the same ' age ' (i.e. in terms of sequence of deposition of the respective female ticks viz : Day 1, 2, 3, ... etc.) were collected into batches and used for quantitative studies. In this way at least 30 batches of successive egg batches of *A. variegatum*, and 22 batches of eggs of *B. decoloratus* were used for investigation of the quantitative presence of each type of nutrient material as follows :

PROTEINS : Quantitative determination of proteins in the eggs was carried out by the colorimetric method of LOWRY et al. (1951) with bovine serum albumin as standard. Specific weights of eggs (0.1 gm-0.5 gm) were homogenised in 1.5 ml of phosphate buffer (pH 7.1) and further diluted in the ratio of 0.1 ml of homogenate to 1.0 ml buffer, and aliquots of the solutions were used for protein determination.

FATTY ACIDS : Free fatty acids were quantified by the colorimetric method of ITAYA and UI (1965). Units of 0.1 to 0.5 gm of eggs were homogenised in 5 ml diethyl ether-ethanol (v/v 1 : 3) mixture, washed repeatedly with equivalent volumes of the solutions, and the homogenate later filtered through Whatman filter paper into a 100 ml conical flask and evaporated to dryness. 6 ml of chloroform and 2 ml of 0.1 m phosphate buffer (pH 7.1) were added to the flask and the mixture was thoroughly shaken and transferred to a test tube.

Spectrophotometric readings of the mixture were then taken at 440 nm, using a Unican sp 500 spectrophotometer. A calibration curve for interpretation of the readings was prepared, with known weights of palmitic acid as standard.

GLYCOGEN : Glycogen was isolated from known weights of eggs (0.1 g to 0.5 g) by digestion of the eggs with hot 30 % (W/V) KOH. It was then purified by repeated precipitation from KOH, water and 2.5 m $H_2SO_4$ — treated samples following addition of 2 volumes of 95 % ethanol. Portions of the final neutralised solutions were used for reducing sugar determination by the FOLIN-WU method (FOLIN and WU, 1920) and glycogen concentration in the eggs was calculated after the method of ROE (1955).

## RESULTS

Mean protein contents in eggs of the two tick species at the onset of oviposition (i.e. first 3-5 days) were 510-570 Ug of protein per mg of eggs in the case of *A. variegatum* and 170-230 Ug. of protein per mg of eggs of *B. decoloratus*. This protein component showed rapid and progressive depletion in eggs of both species during the first 10-18 days of oviposition, reaching the lowest

TABLE 1a : Quantitative Protein Analysis of Eggs of *Amblyomma variegatum*.

| DAYS OF OVIPOSITION (÷ 2) | WEIGHT OF EGGS (g) EXAMINED | ug PROTEIN IN TOTAL EGG SAMPLE | ug PROTEIN PER mg EGG |
|---|---|---|---|
| 1 | .013 | 6875 | 528.85 |
| 2 | .012 | 4675 | 388.89 |
| 3 | .013 | 4500 | 346.15 |
| 4 | .015 | 5500 | 366.67 |
| 5 | .015 | 4625 | 308.33 |
| 6 | .015 | 5625 | 375.00 |
| 7 | .015 | 4250 | 283.30 |
| 8 | .015 | 4600 | 306.67 |
| 9 | .014 | 4000 | 285.71 |
| 10 | .015 | 3600 | 239.67 |
| 11 | .013 | 3925 | 301.92 |
| 12 | .015 | 4875 | 325.00 |
| 13 | .015 | 5625 | 375.00 |
| 14 | .019 | 3750 | 416.67 |
| 15 | .013 | 5250 | 403.85 |

TABLE 1b : Quantitative Protein Analysis of Eggs of *Boophilus decoloratus*.

| DAYS OF OVIPOSITION (÷ 2) | WEIGHT OF EGGS (g) EXAMINED | ug PROTEIN IN TOTAL EGG SAMPLE | ug PROTEIN PER mg EGG |
|---|---|---|---|
| 1 | .024 | 4500 | 187.50 |
| 2 | .051 | 6750 | 132.35 |
| 3 | .044 | 6600 | 150.00 |
| 4 | .040 | 6150 | 153.75 |
| 5 | .050 | 8100 | 162.00 |
| 6 | .055 | 7050 | 128.18 |
| 7 | .045 | 8100 | 180.00 |
| 8 | .041 | 7200 | 175.61 |
| 9 | .030 | 5850 | 195.00 |
| 10 | .015 | 3450 | 230.00 |

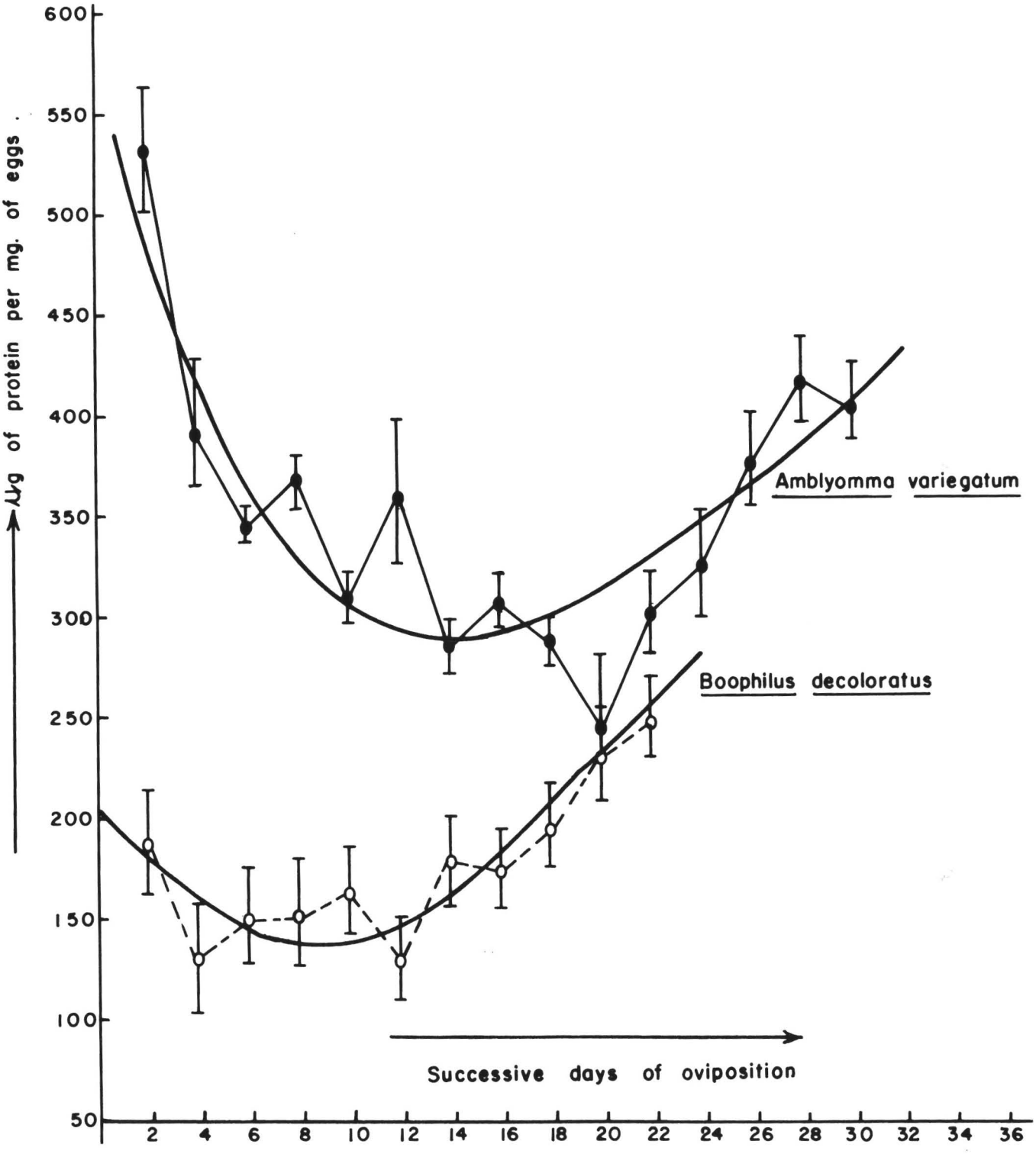


FIG. 1. — Mean quantitative distribution of proteins in sequential egg batches of *Amblyomma variegatum* and *Boophilus decoloratus*.

levels of 230-250 ug protein/mg egg (*A. variegatum*), and 120-140 ug protein/mg egg (*B. decoloratus*) by the 20th and 12th days respectively (Fig. 1). This stage was followed by a rather sharp rise in the protein contents of subsequent egg batches, reaching a level of 380-420 ug protein/mg egg in the case of eggs deposited by females of *A. variegatum* and 220-260 ug protein/mg egg in those of *B. decoloratus*. These terminal egg batches were those deposited during the 26th to 30th day of oviposition in the case of *A. variegatum* and between the 18th and 22nd day of oviposition of *B. decoloratus* females.

Graphical correlation of the mean protein contents of all the egg batches deposited by the respective tick species gave in each case a definite arc-like curve (Fig. 1), with the lowest levels spanning the 12th to 16th days of *A. variegatum* oviposition, and the 6th to 10th days of *B. decoloratus* oviposition.

As in the case of the proteins, the relative quantities of free fatty acids in eggs of both tick species were relatively high at the onset of oviposition. Mean scores recorded from several replicate batches of eggs deposited in the first 3-5 days of oviposition were 302.6 to 320.5 ug of free fatty acids per mg of eggs of *A. variegatum* and 374.4 to 387.2 ug free fatty acids per mg of eggs of *B. decoloratus*. Progressive decrease of these fatty acid contents were noted in subsequent egg batches, onwards till the end of oviposition (Fig. 2). Hence the terminal batches of *A. variegatum* eggs (deposited about 30 days from the commencement of oviposition) had mean free fatty acid contents of 74.4 to 94.9 ug per mg of eggs. In both cases however some measure of stabilisation was noted (in the mean fatty acid contents per unit weight of eggs) at some stage in the oviposition sequences. This featured in egg batches deposited between the 18th and 22nd day in the case of *A. variegatum*, and in those of *B. decoloratus* deposited between the 12th and 14th days (Fig. 2). The former contained between 161.6 ug and 169 ug free fatty acids/mg of eggs, while the latter had between 207.7 and 212.8 ug free fatty acids/mg of eggs.

Estimated glycogen contents of the eggs showed a trend similar in general outline to those of the proteins and fatty acids. Starting from mean scores of 1.73 to 1.8 ug glycogen/mg of egg (*A. variegatum*) and 0.94 to 1.03 ug glycogen/mg of eggs (*B. decoloratus*) in egg batches deposited during the first 1-3 days of oviposition, the mean glycogen content per mg of egg diminished very rapidly and steadily as oviposition progressed till a stage where practically no glycogen was detectable in the eggs (Fig. 3). This featured in eggs of *A. variegatum* deposited as from the 28th day following commencement of oviposition, and in those of *B. decoloratus* deposited as from the 22nd day of oviposition (Fig. 3).

TABLE 2b : Colorimetric Determination of Free Fatty Acids in Eggs of *Boophilus decoloratus*.

| DAYS OF OVIPOSITION (÷ 2) | WEIGHT OF EGGS (g) EXAMINED | u MOLES FREE FATTY ACID (PALMITIC EQUIVALENT) | u MOLES FREE FATTY ACID/mg. EGG | Ug FREE FATTY ACID PER mg EGG |
|---|---|---|---|---|
| 1 | .051 | 76.5 | 1.50 | 384.7 |
| 2 | .062 | 73 5 | 1.19 | 305.2 |
| 3 | .069 | 73 5 | 1.07 | 274.4 |
| 4 | .050 | 49 | .98 | 251.3 |
| 5 | .057 | 55.5 | .97 | 248.7 |
| 6 | .054 | 45 | .83 | 212.8 |
| 7 | .033 | 35 | .81 | 207.7 |
| 8 | .020 | 13 | .65 | 166.7 |
| 9 | .032 | 24.5 | .77 | 197.5 |
| 10 | .017 | 10 | .59 | 151.3 |
| 11 | .010 | 3.5 | .35 | 89.8 |

TABLE 2a : Colorimetric Determinaton of Free Fatty Acids in Eggs of *Amblyomma variegatum*.

| DAYS OF OVIPOSITION (÷ 2) | WEIGHT OF EGGS (g) EXAMINED | u MOLES FREE FATTY ACID (PALMITIC EQUIVALENT) | u MOLES FREE FATTY ACID/mg. EGG | Ug FREE FATTY ACID PER mg EGG |
|---|---|---|---|---|
| 1 | .034 | 41.7 | 1.231 | 315.4 |
| 2 | .056 | 59 | 1.05 | 269.3 |
| 3 | .057 | 57 | 1.99 | 253.9 |
| 4 | .065 | 62.5 | .96 | 246.2 |
| 5 | .047 | 42 | .89 | 228.2 |
| 6 | .045 | 35 | .78 | 200.0 |
| 7 | .055 | 42 | .76 | 194.9 |
| 8 | .047 | 35 | .74 | 189.8 |
| 9 | .048 | 31.5 | .66 | 169.8 |
| 10 | .053 | 34 | .63 | 161.6 |
| 11 | .043 | 28 | .65 | 166.7 |
| 12 | .042 | 20.5 | .48 | 123.1 |
| 13 | .048 | 21 | .43 | 110.3 |
| 14 | .035 | 11.2 | .32 | 82.1 |

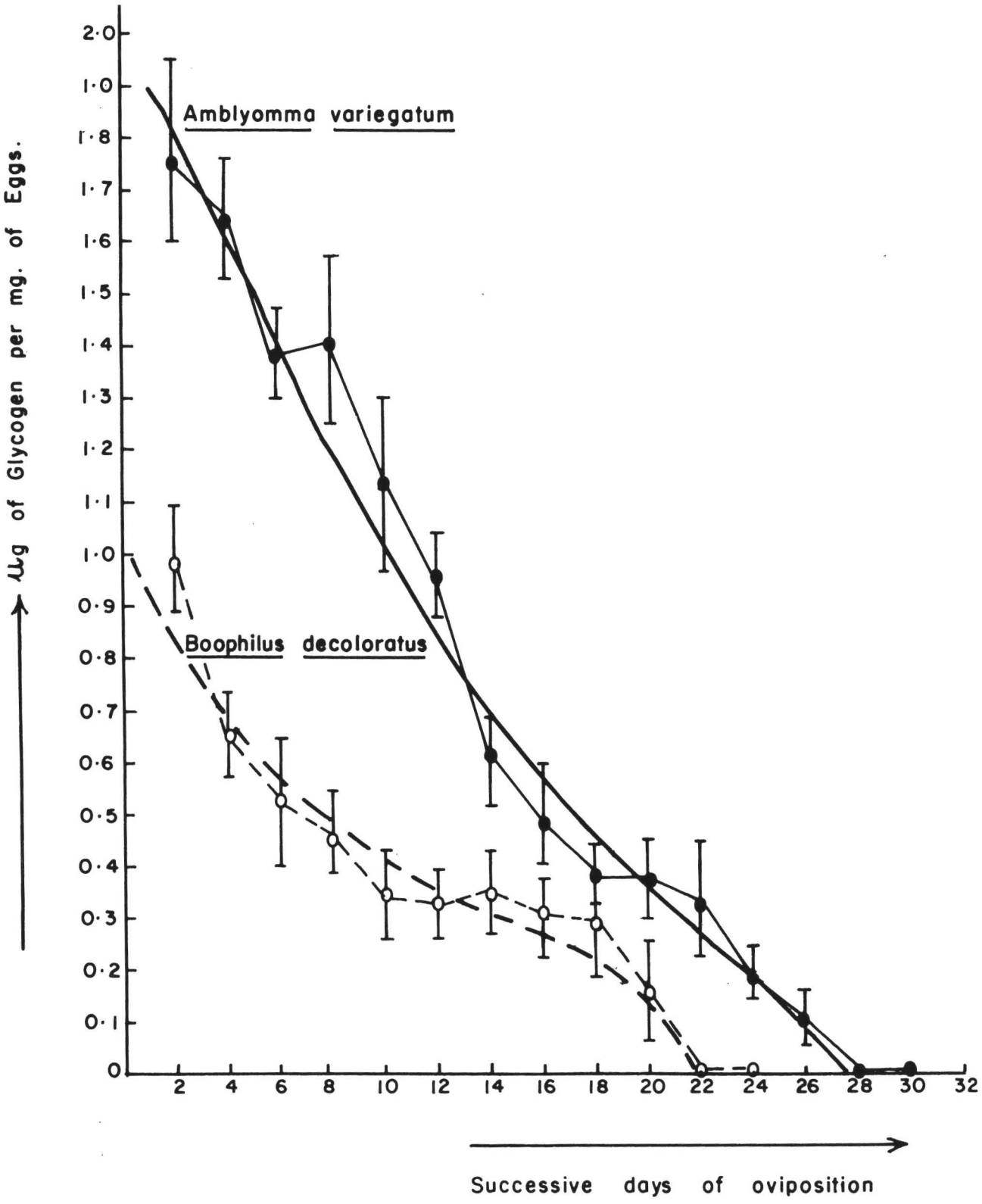


FIG. 2. — Mean quantitative distribution of free fatty acids in sequential egg batches of *Amblyomma variegatum* and *Boophilus decoloratus*.

TABLE 3a : Glycogen Estimation of Eggs of *Amblyomma variegatum*.

| DAYS OF OVIPOSITION (÷ 2) | WEIGHT OF EGGS (g) EXAMINED | ug GLUCOSE IN EGG SAMPLE | ug GLYCOGEN (× 0.9) | ug GLYCOGEN PER mg. EGG | % GLYCOGEN CONTENT OF EGGS |
|---|---|---|---|---|---|
| 1 | .023 | 45.0 | 40.5 | 1.76 | .176 |
| 2 | .020 | 36.5 | 32.85 | 1.6425 | .164 |
| 3 | .026 | 40.0 | 36.0 | 1.385 | .139 |
| 4 | .032 | 50.0 | 45.0 | 1.406 | .141 |
| 5 | .029 | 31.0 | 27.9 | 0.962 | .114 |
| 6 | .030 | — | — | — | — |
| 7 | .029 | 20.0 | 18.0 | 0.621 | .062 |
| 8 | .028 | 15.0 | 13.0 | 0.482 | .048 |
| 9 | .018 | 7.5 | 6.75 | 0.375 | .038 |
| 10 | .018 | 7.5 | 6.75 | 0.375 | .038 |
| 11 | .020 | 7.5 | 6.75 | 0.338 | .034 |
| 12 | .015 | 0 | 0 | 0 | 0 |
| 13 | .015 | 0 | 0 | 0 | 0 |
| 14 | .017 | 0 | 0 | 0 | 0 |
| 15 | .016 | 0 | 0 | 0 | 0 |
| 16 | .013 | 0 | 0 | 0 | 0 |
| 17 | .010 | 0 | 0 | 0 | 0 |

TABLE 3b : Glycogen Estimation of Eggs of *Boophilus decoloratus*.

| DAYS OF OVIPOSITION (÷ 2) | WEIGHT OF EGGS (g) EXAMINED | ug GLUCOSE IN EGG SAMPLE | ug GLYCOGEN (× 0.9) | ug GLYCOGEN PER mg. EGG | % GLYCOGEN CONTENT OF EGGS |
|---|---|---|---|---|---|
| 1 | .059 | 65.0 | 58.5 | .992 | .099 |
| 2 | .068 | 50.0 | 45.0 | .662 | .066 |
| 3 | .068 | 40.0 | 36.0 | .529 | .053 |
| 4 | .069 | 35.0 | 31.5 | .456 | .046 |
| 5 | .074 | 29.0 | 26.1 | .353 | .035 |
| 6 | .066 | 25.0 | 22.5 | .341 | .034 |
| 7 | .061 | 20.0 | 22.2 | .360 | .036 |
| 8 | .020 | 7.5 | 6.75 | .332 | .033 |
| 9 | .011 | 3.75 | 3.37 | .307 | .031 |
| 10 | .020 | 3.75 | 3.37 | .169 | .017 |
| 11 | .014 | 0 | 0 | 0 | 0 |
| 12 | .011 | 0 | 0 | 0 | 0 |

Unlike the situation with protein contents of the eggs, the glycogen component, like the fatty acids, did not show a rise at any stage. Some limited and rather transitory degree of stabilisation was however noticeable in the glycogen quantities of eggs of *A. variegatum* deposited between the 18th and 22nd days of oviposition, and in eggs deposited between the 10th and 18th days of *B. decoloratus* oviposition.

## DISCUSSION

Eggs of most animals normally contain sizeable quantities of food reserves, — commonly in form of carbohydrates (especially glycogen), lipids and proteins, — needed to support the developing embryo. Beyond certain threshold limits, differences in amounts of these food reserves in various eggs may affect the development and viability of the embryo, and subsequently the hatchability of the eggs (BALINSKY, 1965). Indeed WILLIAMS (1967) had noted that embryonic development requires raw materials and free energy which in most cases are provided by foodstores laid down in the oocyte during oogenesis. For instance among arthropods generally KOZHANTSHIKOV (1938) had shown that eggs deposited by starved females of the noctuid lepidopteran *Agrotis segetum* will not normally complete embryonic development, while MONROE (1960) working with houseflies *Musca domestica* noted that whereas eggs laid by flies fed on cholesterol-deficient diet showed only about 21 % hatch rates, those laid by flies fed on cholesterol-rich diet gave more than 90 % hatch rates. With specific reference to the ticks, there is at present hardly any information on the nature or extent of influence of egg nutritional reserves on their embryo/larval development, nor indeed on hatchability of the eggs.

It is noteworthy that the protein contents of the earliest laid batches of eggs were comparatively very high, while those of subsequent batches showed progressive fall onwards till some stage about mid-way in the span of the oviposition period, subsequent to which featured a " rise phase " in the whole protein contents of the later batches of eggs. The reason for this arclike pattern is not known, although interestingly it was consistently discernible from analyses of the eggs of both *A. variegatum* and *B. decoloratus*.

Possible explanations may lie perhaps in some kind of phased break down and utilisation of host blood proteins (BOCTOR and KAMAL, 1976), followed by gradual synthesis of specific egg pro-

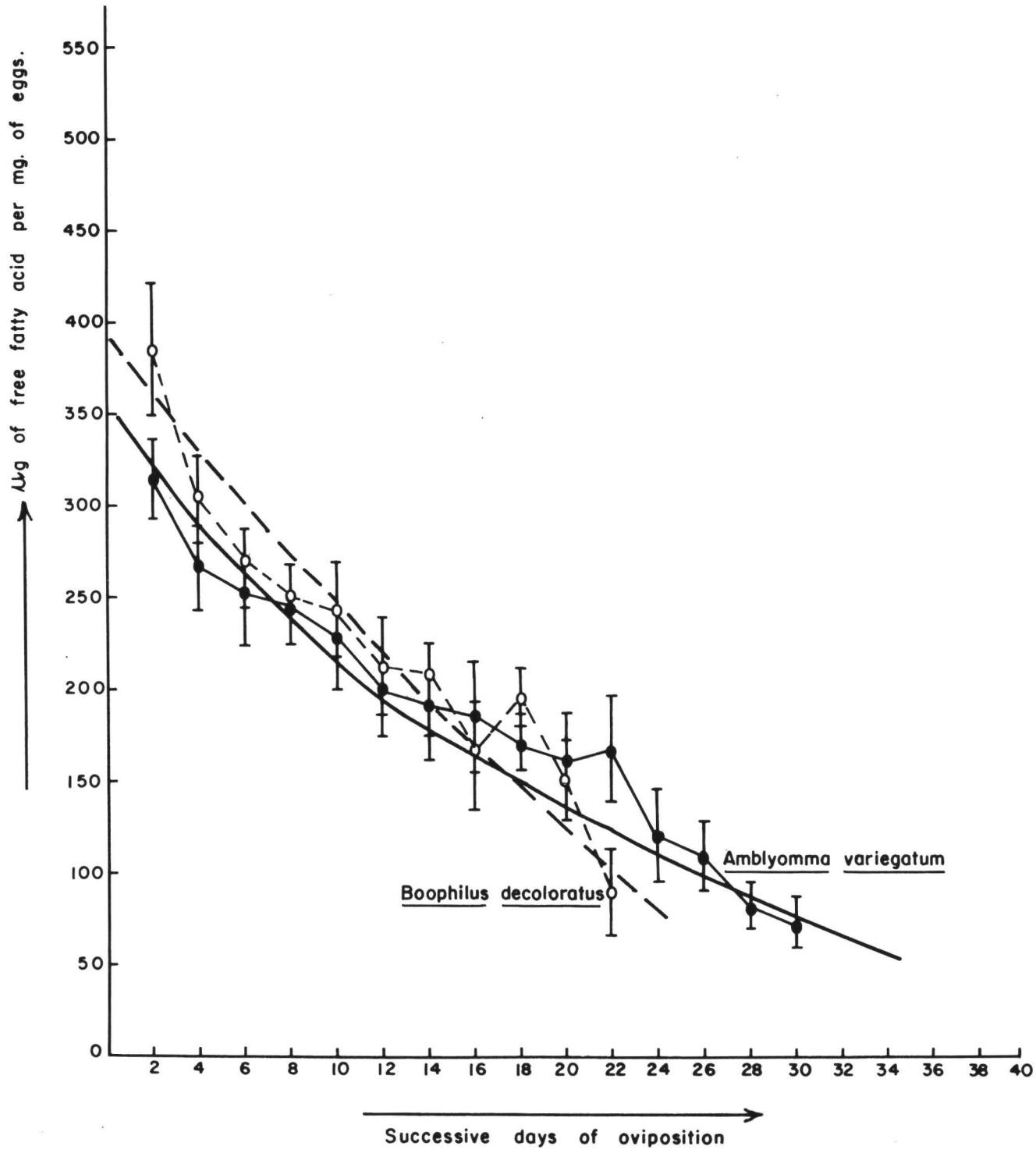


FIG. 3. — Mean quantitative distribution of glycogen in sequential egg batches of *Amblyomma variegatum* and *Boophilus decoloratus*.

teins (primarily complex glycolipoproteins) during ovogenesis in the engorged female tick. On the other hand, if the eggs were uniformly endowed at the onset with reasonably equal protein components, the observed progressive reduction of the latter following commencement of oviposition may be due to an initial breakdown of the proteins to various amino acids, some of which may have continued up to a point when, with the onset of organogenesis and larval formation, anabolic processes in the formative embryo outstrip the catabolic processes, thereby accounting perhaps for the observed protein build-up in egg batches deposited later in the oviposition cycle. But CHAPUT and LILES (1969) in a study of the changes in free and peptide — bound amino acids during larval-pupal transformation of the mosquito *Aedes aegypti* had noted no specific correlation of amino acid changes with development in these insects, adults of which, like the ticks, normally require host blood proteins for maturation of their eggs.

Furthermore it may be argued that although proteins play a vital role in the formation of body structures, they are usually not one of the primary sources of the energy used up in the early embryogenesis of most animals (BARTH and BARTH, 1954). Hence the reason for the peculiar protein distribution in the various tick egg batches remains uncertain. Perhaps the answer may be found in some of the mechanisms underlying yolk utilization in various other animals as reviewed by WILLIAMS (1967).

Lipids, although they also enter importantly into formation of body structures have the primary function of enegry storage in eggs, commonly in form of triglycerides and to some extent as fatty acids and lipoproteins. The energy reserves are of course required for both oxidative and phosphorylative processes, and also for embryonic synthesis and egg-hatching. Although triglycerides and other lipids were not measured in this study, the progressive decrease in fatty acid stores of the successive egg batches studied suggests gradual burning-up of their lipid contents with time and progress of embryonic development within the eggs. This would presuppose that the bulk of the eggs were proliferated in the ovary at about the same time only to be sequentially released thereafter.

This is in line with earlier observations by SLIFER (1930), who in studies of eggs of the grasshopper *Melanoplus differentialis* and the silkworm, *Bombyx mori* had noted that the total amount of lipids in each case falls by more that half between the time of laying of the eggs and their hatching ; and that the lipids constitute the main source of energy for the developing insects. In like manner therefore, it is to be expected that unless a reasonable amount of stored energy is available in eggs of the ticks to drive through the processes of embryonic synthesis, egghatching, and subsequent moulting, these processes may not freely occur. (MAROUN, 1972 ; MAROUN, and KAMAL, 1973).

Furthermore, the stepwise decrease in hatch rates of tick eggs laid on successive days of oviposition as hitherto reported by IWUALA and OKPALA (1977) as well as the earlier records of progressive decrease in weights of such eggs (BENNET, 1974) would appear to be related partly to the quantities of free fatty acids present in these eggs. Perhaps some correlation is found for this in the work of HUSSEIN and KAMAL (1977), who in a study of the phospholipid classes present in eggs of *Argas* (*Persicargas*) *arboreus* and *Dermacentor andersoni* have reported a decrease of up to 10 % in the phosphatidylcholine, (the principal phosphatide component of the eggs), in batches of the former, within the first three days of their oviposition, although percentage presence of some other less important egg phospholipids showed reasonable increase during the same period.

However it should be noted that meaningful speculation on the reasons for decreasing hatchability of the later egg batches must also reckon with such other factors as triglyceride, cholesterol and water contents of the eggs. The latter will of course depend on the proper functioning of the Gene's organ in the respective ticks.

Results of the glycogen assays are of special interest too in the light of the sharp trend observed in the depletion of this compound in successive egg batches deposited by ticks of both

species. Normally glycogen like lipids, serves as a reserve energy source in animal cells, and its carbon component is usually utilised for many biosynthetic purposes (KREBS and PREISS, 1975), many of which would ordinarily suffer in situations of acute glycogen deficiency. Also it would be expected that decrease in glycogen contents may account for reduction in weights and hatchability of eggs laid during the later phase of the ticks' oviposition, particularly as the energy sources are very much needed in the early embryogenesis and larval formation. Indeed WILLIAMS (1967) had noted that glycogen accounts for up to two-thirds of the components of the unfertilised eggs of various animals, and tends to be utilised right from the onset of cleavage in the developing embryo.

Another interesting aspect of the results is the fact that while the glycogen contents of the eggs showed a decline downwards to zero percent in the terminal egg batches, some amount of free fatty acids was still observed in the egg samples upwards till the last day of oviposition. This can be explained by the fact that lipids are better energy-conserving compounds than glycogen, and it is common knowledge that single molecules of the former, when fully oxidised, yield more energy than those of the latter. It may therefore be suggested that the most persistent and perhaps principal energy storage compounds in eggs of ixodid ticks are the lipids. Glycogen, although of special importance too, tends to be exhausted with progress of oviposition and in consonance with the ageing of the eggs. This, per se, may be largely responsible for the non-hatchability of most of the terminal batches of eggs deposited by the respective tick species (IWUALA and OKPALA, 1977).

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