GROWTH AND MOLECULAR RESPONSES OF *Culex quinquefasciatus* MOSQUITO (DIPTERA: CULICIDAE) TO QUALITY AND QUANTITY OF LARVAL DIETS

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ABSTRACT

With the decreased efficacies of present mosquito control strategies, in reducing the burden of mosquito borne diseases, attention has been shifted to larval stage of these vectors. However, no effective control protocol can be developed without sound knowledge of critical aspects of the bio-ecophysiology of a vector and interplays, which bring about success of the species. This study, thus, aims at bringing into clearer perspective, the influence of quality and quantities of mosquito larval diets on rates of larval growth and accumulation of teneral reserves. To this end, conventional mosquito feeds, namely, fish feed, yeast and biscuit were analysed for proximate composition, and categorised into high (HP), moderate (MP) and low proteinous (LP) diets, respectively, using standard methods, with a 'Control' devoid of feed. Six (6) quantity of HP feed regimens, ranging from 0.16 through to 1.60 mg/ 100 larvae, was adopted. In both studies, approximately Day-old larvae were fed *ad libitum* with these diet types and quantities, and reared following standard protocols. The rates of larval growth (LGR) and teneral accumulation (LTA) were determined according to standard methods. Analyses revealed a significant effect of quantities and quality of larval diets on these entomological indices. Mosquitoes raised on HP feed had significantly higher LGR than those reared on MP and LP feeds (range = 0.0116 ± 0.0224 to 0.0710 ± 0.0067 mg/ day) and had significantly higher LTA for all teneral components analysed. Further, mosquitoes raised on 0.32 mg/ 100 larvae had the highest LGR, while those raised on 1.60 mg/ 100 larvae had the least, range = 0.0433 ± 0.00329 to 0.0678 ± 0.00304 mg/day. Similar trend was observed for LTA in these quantities of diet regimen. The results of this study revealed significant effects of quantities and quality of feed on growth and accumulation of teneral reserves in mosquitoes. This information is important, as baseline in the development of effective integrated mosquito management protocol.

Keywords: Teneral Reserve, Environmental Manipulation, bioaccumulation, Proximate

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INTRODUCTION

Mosquitoes constitute a formidable enemy of man, especially, in their incrimination as vectors of important public health diseases such as malaria, yellow fever, and lymphatic filariasis (Olayemi *et al.,* 2016). The decreased efficacy of most currently used control strategies for adult life stage, due to increased insecticide resistance (Njan-

Nloga *et al.,* 2007), unacceptability of control strategies (Olayemi *et al.,* 2012), non-sustainability (WHO, 2000) and reports of ever increasing mortality and morbidity (Addiss and Brady, 2007), has necessitated studies targeted at the mosquitoes' vulnerable life stage, i.e., the larvae.

This vulnerable life stage, thrive in a microenvironment regulated by an array of factors (biotic and abiotic) which determine its suitability for growth and hence its success (Bentley and Day, 1989). Modern control strategies, therefore, now focus on the manipulation of some of these factors, in a bid to produce, vectorially less-fit adult mosquitoes that are inefficient in disease transmission (Randell *et al.,* 2010).

The reproductive success of mosquitoes as vectors is dependent on the effectiveness of the adult female mosquito to develop and mature eggs through larval-derived teneral reserve (Akoh *et al.,* 1992), or acquired blood meal from man (Naksathit *et al.,* 1999a; 1999b). This capability is, further enhanced by the availability of favourable pre-imaginal breeding conditions, especially qualitative and quantitative diets, which support growth and development of immature stages, which in turn, increase rates of pre-imaginal survivorship (Lillie and Nakasone, 1982), duration of development (Van-Handel, 1986) and adult fecundity (Lang, 1978).

The importance of protein, a component of teneral reserve, in the diet of mosquitoes can never be over-emphasised, owing to the vital role it plays in the metabolism of insects, having structural (Wolfersberger, 2000) and functional (Murray *et al.,* 2000) roles that determine fitness (Gillott, 2005). The protein required for development of mosquito eggs is derived basically from that stored during the larval stage especially at the last larval instar (Hurd, 1998), while that required for egg maturation is derived from acquired blood meal (Kerkut and Gilbert, 1985). More so, its rate of accumulation has been used as an index of larval growth (Day and Van-Handel, 1986; Van-Handel, 1985a; b).

Growth, generally, in mosquitoes is determined, partly, by larval-derived nourishment provided by the quality and quantity of available diet (Van-Handel and Day, 1988). Further, in mosquito ecology, the rates of growth and biomass accumulation are strong indices of pre-imaginal stress conditions (Mpho, 2000; Mpho *et al.,* 2002), success in mosquito species (Timmermann and Briegel, 1999) and quality of adult life (Lang, 1978). These are, however, dependent on the quantity of teneral reserve amassed (Timmermann and Briegel, 1993). The teneral reserve accumulation, especially, at the last foraging stage (fourth larval instar), is dependent on the ability of this stage to forage on the available feed and its quality (Nasci and Mitchell, 1994).

Several physiological studies on mosquitoes have attempted to determine the role of this important factor on the aspects of development (Lang, 1978; Lillie and Nakasone, 1982; Farkas *et al.,*1985; Van-Handel, 1986; Nasci *et al.,* 1994; Canyon *et al.,* 1999; Briegel *et al.,* 2002; Briegel, 2003; Joy *et al.,* 2010; Damiens *et al.,* 2012; Khan *et al.,* 2013), none has reported its role or influence on larval growth rates and rate of accumulation of

teneral reserve, vis-à-vis, its role in disease transmission. The present study attempts to fill the gap in knowledge.

Furthermore, based on the foregoing, a sound knowledge of how the quality and quantity of feed affect rates of growth and accumulation of teneral components may be vital in predicting adult success rates. Such information could also serve as baseline in the development of a robust cost-effective, environmental-friendly and sustainable mosquito management protocol and, thus, curb the menace they presently pose.

MATERIALS AND METHODS

Source and Maintenance of Mosquito for the Study

The Mosquitoes werve obtained from an established colony of wild strain of *Culex quinquefasciatus.* The insectary was set up as described by Olayemi and Ande (2009). Newly laid eggs were collected from ovitraps and incubated in plastic bowls (5 cm height and 30 cm diameter) for 24 to 72 hours for hatching in a plastic tray (30 cm x 25 cm x 5 cm) for rearing till pupation and emergence. The larvae were fed with feed materials sprinkled on the water surface. Every alternate day, the water from the culture tray was changed carefully to avoid formation of scum and accumulation of debris.

Determination of Quality of Larval Feed

Routine mosquito larval feed types were used in the study. Three categories of feed qualities was adopted after subjecting the larval diets to proximate analyses using standard procedures (AOAC, 2005). The analyses provided a basis for categorisation into a High proteinous, HP, (fish feed), moderately proteinous, MP, (Yeast) and Low proteinous, LP (Biscuit) diets. However, a fourth regimen (control) involving rearing the larvae in a medium devoid of feed material was set-up to mimic an environment devoid of any of the above (three) quality categories.

Effect of Quality of diets on Rates of Growth and Teneral Reserve Accumulation

Approximately Day-old larvae were put in well-labelled plastic troughs at the rate of 25 larvae per bowl and fed *ad libitum* with the above (3) larval feed material types. This was done as described by Amalraj *et al.* (2005). The feed quality types and the Control (set-up devoid of any form of feed) were in four replicates and equal quantities (0.32 mg/ 100 larvae) of the feed were used. Larvae were reared as described by Ukubuiwe *et al.* (2013).

Effects of Quantities of Feed on Rates of Growth and Teneral reserve Accumulation

This was done according to the methods described by Burger and Promislow (2004), with slight modification. Briefly, six (6) different levels of the HP feed regimen were used; these include, concentrations of 0.16, 0.32 (Control), 0.64, 0.96, 1.28 and 1.60 mg/ 100 larvae in 200 ml of water. Each experimental feed regimen had four replicates, containing 100 larvae each. The larvae were reared according to the methods of Ukubuiwe *et al.* (2013).

Determination of Larval Growth Rate

Growth rate of individual mosquitoes was estimated as weight at emergence divided by the age at pupation, and indicates the average increase of weight per day

throughout the larval period (Lyimo *et al.*, 1992).

Determination of Larval Rate of Teneral Accumulation

Teneral reserve (Lipid, Glycogen, Carbohydrate, and Protein) were determined according to the method described by Bradford (1976), Van-Handel (1985a; 1985b), Van-Handel and Day (1988), and Kaufmann and Brown (2008) for single mosquito. The rate of accumulation of each component of the teneral reserve was determined by dividing the quantity of the component at fourth larval stage by the age at pupation. This gives an indication of the average quantity of teneral component accumulated during the larval period (Timmerman and Briegel, 1999).

Data Analysis

The Differences among entomologic variables (larval growth rates and accumulation rates) were compared for significant differences using one-way Analysis of Variance (ANOVA). All decisions on statistical comparison of means were taken at $p<0.05$ level of significance and values were expressed as mean $\pm$ standard deviation. The means were separated using Duncan Multiple Range Test (DMRT).

RESULTS

Effect of Quality of diets on Rates of Growth and Accumulation of teneral reserve

Analyses revealed significant ($p<0.05$) effects of the qualities of feed on the larval growth rate and accumulation rates of teneral components. High proteinous diets (HP) produced mosquitoes with faster growth rates, than other feed types, while the Control (devoid of feed) had the least larval growth rate (LGR). The values of LGR ranged from 0.0116 ± 0.0224 (in control) to 0.0710 ± 0.0067 mg/ day (in HP). Similar trend was observed for rate of teneral reserve accumulation as occasioned by quality of feed. The rate of accumulation for Lipid ranged from 0.45 ± 0.03 to 3.70 ± 0.33 µg lipid/ mosquito/ day; for protein, 0.79 ± 0.19 to 6.54 ± 0.58 µg protein/ mosquito/ day; glucose, 0.30 ± 0.02 to 2.66 ± 0.28 µg glucose/ mosquito/ day and glycogen, 1.33 ± 0.09 to 7.89 ± 0.71 µg glycogen/ mosquito/ day (Table 1).

Effects of Quantities of Feed on Rates of Growth and Teneral reserve Accumulation

The effects of quantities of larval feed on rates of growth and accumulation of teneral reserves of *Cx. quinquefasciatus* is shown in Table 2. Analyses revealed significant ($p<0.05$) effects of the quantities of feed on these important entomological variables. There was an initial increase in the LGR as the quantities of feed increased progressively, however, from 0.64 mg/ 100 larvae of feed, it reduced significantly ($p<0.05$), having the lowest at 1.60 mg/ 100 larvae. The values ranged from 0.0433 ± 0.00329 mg/day (at 1.60 mg/ 100 larvae) to 0.0678 ± 0.00304 mg/day (at 0.32 mg/ 100 larvae) (Table 2). The lowest rates of accumulation of teneral components were observed at the lowest (0.16 mg/ 100 larvae) and highest (1.60 mg/ 100 larvae) feed quantities tested, while the highest rates of accumulation was at 0.32 mg/ 100 larvae. These rates were also high at 0.64 mg/ 100 larvae feed regimen. The rate of accumulation for Lipid ranged from 1.31 ± 0.06 to 3.77 ± 0.22 µg lipid/ mosquito/ day; for protein, 1.99 ± 0.11 to 5.84 ± 0.31 µg protein/ mosquito/ day;

glucose, 0.83±0.04 to 2.25±0.15 µg glucose/ mosquito/ day and glycogen, 3.86±0.19 to 9.64±0.56 µg glycogen/ mosquito/ day (Table 2).

Table 1: Effect of **Quality** of Larval Feed on Growth Rate and Metabolic Reserve Accumulation Rate of Larvae of *Culex quinquefasciatus*

*Values followed by same superscript alphabet in a column are not significantly different at $p<0.05$

Quality of Larval Feed	Larval Growth Rate (mg/day)	Larval Rate of Accumulation (µg nutrient/ mosquito/ day) Lipid	Protein	Glucose
		Glycogen		
High Protein Feed	0.0710±0.0067^{c}	3.70±0.33^{d}	6.54±0.58^{d}	2.66±0.28^{d}	7.89±0.71^{d}
Moderate Protein Feed	0.0644±0.0034^{bc}	2.79±0.24^{c}	4.96±0.09^{c}	1.68±0.09^{c}	6.14±0.11^{c}
Low Protein Feed	0.0601±0.0059^{b}	2.15±0.21^{b}	4.10±0.23^{b}	1.41±0.09^{b}	5.06±0.37^{b}
No Feed	0.0116±0.0224^{a}	0.45±0.03^{a}	0.79±0.19^{a}	0.30±0.02^{a}	1.33±0.09^{a}

All values are expressed as Mean±SD of Mean

Table 2: Effect of Quantity of Larval Feed on Growth Rate and Metabolic Reserve Accumulation Rate of Larvae of *Culex quinquefasciatus*

Quantity of Larval Feed (mg/ 100 larvae)	Larval Growth Rate (mg/day)	Larval Rate of Accumulation (µg nutrient/ mosquito/ day) Lipid	Protein	Glucose
		Glycogen		
0.16	0.0442±0.0019^{a}	1.31±0.06^{a}	2.07±0.11^{a}	0.93±0.08^{a}	3.86±0.19^{a}
0.32	0.0678±0.00304^{e}	3.77±0.22^{e}	5.84±0.31^{e}	2.25±0.15^{e}	9.64±0.56^{e}
0.64	0.0605±0.00331^{d}	3.44±0.23^{d}	5.17±0.35^{d}	1.78±0.12^{d}	8.18±0.58^{d}
0.96	0.0525±0.00240^{c}	2.60±0.08^{c}	3.79±0.15^{c}	1.41±0.06^{c}	6.29±0.23^{c}
1.28	0.0470±0.01890^{b}	2.17±0.13^{b}	3.07±0.22^{b}	1.16±0.07^{b}	4.89±0.21^{b}
1.60	0.0433±0.00329^{a}	1.32±0.08^{a}	1.99±0.11^{a}	0.83±0.04^{a}	4.11±0.23^{a}

*Values followed by same superscript alphabet in a column are not significantly different at $p<0.05$
All values are expressed as Mean±SD of Mean

DISCUSSION

The quality and quantity of diet available to the larval stages of an insect is a very important abiotic factor, which determines the quality of adult life of the species (Akoh *et al.,* 1992). More important, is the rate at which these feed materials are ingested and teneral reserve accumulated; since it determines the rate of growth and/or amount of energy available for adult life history traits (Van-Handel, 1986; Amalraj *et al.,* 2005). Although, the nutritional conditions of female mosquitoes are determined by larval-derived reserves, sugar meals and blood meals (Aparna *et al.* 2006), larval-derived teneral reserves have primarily dictated the capability of mosquitoes to self-reproduce (in autogenous mosquito), and strongly influenced female fitness-related traits such as body size, teneral metabolic reserves and fecundity of mosquitoes (Briegel, 1990a; b).

In the present study, although, the feed used in the categorisation of protein-contents were routine laboratory (improvised) diets, statistical differences were found on their influence on rates of larval growth and accumulation of teneral reserves. The regimen devoid of feed, which mimics habitats, devoid of debris and detritus, especially, in peridomestic containers, or large flowing water bodies, produced larvae with significantly reduced larval growth and accumulation rates. This information is vital in integrated mosquito control approaches, in the face of dwindling economic resources, as it will assist in making well-informed decisions as regards habitats and their productivity. Mosquitoes, from such habitat as above, though capable of growth, had their growth rates reduced by one-fifth times growth rates in low proteinous diet, as shown in this study. This implies a significant increase in duration of development; with such mosquitoes becoming grossly over-spent, by the time they reach adulthood and may not be competent in disease transmission. Moreso, female mosquitoes from environment devoid of feed require more than one blood meal to complete ovarian development (Chena, 2003). Although, such condition increases the mosquito's foraging frequency and, perhaps, host-vector contacts, it also increases the risk associated in acquiring such blood meals, and greater exposure to other control strategies. This condition may increase histolysis of organs for gametogenesis by these mosquitoes (Vinogradova and Shaikevich, 2007), which further depletes its energy store, and the quantity available for important adult life activities. Further, Larval-derived teneral reserves affect important female reproductive processes, such as utilization of reserves, fecundity, longevity and blood meal consumption and utilization (Briegel, 1990a; 1990b; Zhou *et al.*, 2004).

In the present study, higher quantity regimen of feed significantly reduced larval growth rates and accumulation rates. This is contrary to hypothesis made as seen in previous reports (Hwang *et al.*, 1974; Telang and Wells, 2004). The reduced rates could have been due to scum formation, which induced stress (Asahina, 1964), reducing consumption rate, and, ultimately, accumulation of metabolic reserve. Whichever the case, an optimum quantity of diet, which can enhance normal metabolism and reduce larval mortality is imperative for the production of very fit mosquitoes, below or above this may produce nutritional stress, which affects development.

In the present study, the rates of growth and accumulation of metabolic reserves (lipid, carbohydrate, protein, and glucose) were, significantly, influenced by the quantity of larval feed available during development. Contrary to earlier reports, which correlated increased teneral reserve accumulation with higher larval feed (Akoh *et al.* 1992; Couret *et al.* 2014). The present study revealed that although, there was initial rise in the teneral reserve of the species, as feed increased, this was reduced as the feed quantity exceeded the threshold. These observations may also be a reflection of inefficiency of the larvae to optimize teneral reserves as feed quantity increased, probably due to stress (Asahina, 1964), and may have effects on the quality of adult mosquitoes produced.

It, however, seems that feed quantities of 0.32 to 0.64 mg/ 100 larvae is the

optimum range of values for feed that best support growth and development of the species. This is important in laboratory procedures, where large-sized and better-fit mosquitoes are required for scientific studies. Field studies have revealed that most habitats devoid of larval feed, especially, water storage tanks for drinking water at home, does not support the growth of mosquitoes, whereas those laden with organic matter, especially, human and animal excrement support the breeding of mosquitoes. It, thus, means that efforts should be focused on the later habitats than the former, as the former may pose less threat epidemiologically in mosquito population explosion.

CONCLUSION

The result of the present study, revealed significant effects of quality and quantities of larval diet on growth and teneral reserve accumulation. Though subject to field investigations, this information forms a baseline for the development of an integrated approach in the control of mosquitoes.

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