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# The use of a chemically defined artificial diet as a tool to study *Aedes aegypti* physiology

Octávio A.C. Talyuli [a,b], Vanessa Bottino-Rojas [a], Mabel L. Taracena [a], Ana Luiza Macedo Soares [a,b], José Henrique M. Oliveira [a], Pedro L. Oliveira [a,c,*]

[a] *Programa de Biologia Molecular e Biotecnologia, Instituto de Bioquímica Médica Leopoldo de Meis, Centro de Ciências da Saúde, Universidade Federal do Rio de Janeiro, Rio de Janeiro 21941-590, Brazil*

[b] *Instituto Biomédico, Universidade Federal do Estado do Rio de Janeiro, Brazil*

[c] *Instituto Nacional de Ciência e Tecnologia em Entomologia Molecular (INCT-EM), Brazil*

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ABSTRACT

*Aedes aegypti* mosquitoes obtain from vertebrate blood nutrients that are essential to oogenesis, such as proteins and lipids. As with all insects, mosquitoes do not synthesize cholesterol but take it from the diet. Here, we used a chemically defined artificial diet, hereafter referred to as Substitute Blood Meal (SBM), that was supplemented with cholesterol to test the nutritional role of cholesterol. SBM-fed and blood-fed mosquitoes were compared regarding several aspects of the insect physiology that are influenced by a blood meal, including egg laying, peritrophic matrix formation, gut microbiota proliferation, generation of reactive oxygen species (ROS) and expression of antioxidant genes, such as catalase and ferritin. Our results show that SBM induced a physiological response that was very similar to a regular blood meal. Depending on the nutritional life history of the mosquito since the larval stage, the presence of cholesterol in the diet increased egg development, suggesting that the teneral reserves of cholesterol in the newly hatched female are determinant of reproductive performance. We propose here the use of SBM as a tool to study other aspects of the physiology of mosquitoes, including their interaction with microbiota and pathogens.



## 1. Introduction

*Aedes aegypti* females, being an anautogenous mosquito, need to feed on a protein-rich diet to be capable of producing eggs and need to obtain, from a vertebrate blood meal, the nutrients that are essential for oogenesis. In addition to proteins, a blood meal also provides lipids, in particular cholesterol, a molecule that insects are unable to synthesize and must acquire from the diet (Canavoso et al., 2001; Clark and Block, 1959). Cholesterol is an essential component of biological membranes and is a precursor of the ecdysteroid hormones that regulate yolk synthesis and egg maturation and are indispensable for the success of oogenesis (Clifton and Noriega, 2012). Moreover, cholesterol is an essential regulator of insect development that must be present in the diet for the completion of their life cycle (Canavoso et al., 2001).

Chemically defined media have been extensively used to characterize the nutritional needs of microorganisms to increase the metabolic pathway or synthesis of virulence factors (Donnellan et al., 1964; Kim et al., 2012), but chemically defined artificial meals that permit the direct testing of the physiological role of specific molecules are not available for most animal species. Synthetic diets have been described for a few insect species to study many aspects of insect physiology and nutritional dependence. Noland and Baumann (1949) reported that choline is essential for *Blattella germanica* to grow and avoid death in early stages. Monroe (1959, 1960) used a synthetic diet to show that dietary cholesterol is important to support the viability of eggs in the house fly *Musca vicina*. Piper et al. (2014) published a complete chemically defined media that was capable of supporting development in *Drosophila melanogaster* and compared diets differing in the contents of cholesterol, vitamins or amino acids. The flies that were fed a cholesterol-free holidic media had reduced lifespan and oogenesis. Pitts (2014) showed that, in *Aedes albopictus*, a synthetic diet containing just albumin was enough for success in oogenesis, similar to blood-fed insects. *Culex quinquefasciatus* females were maintained for 50 generations with synthetic diets; however, during the first generations, this diet did not reproduce the blood effect on egg raft size, which was reversed after some gonadotropic cycles (Griffith and Turner, 1996). In the present study, we used an

* Corresponding author at: Programa de Biologia Molecular e Biotecnologia, Instituto de Bioquímica Médica Leopoldo de Meis, Centro de Ciências da Saúde, Universidade Federal do Rio de Janeiro, Rio de Janeiro 21941-590, Brazil. Tel.: 55 21 3938 6751; fax: 55 21 2270 8647.

*E-mail address:* pedro@bioqmed.ufrj.br (P.L. Oliveira).

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artificial diet that was based on previously reported protocols (Cosgrove and Wood, 1996; Kogan, 1990) and supplemented with cholesterol, herein named Substitute Blood Meal (SBM), to compare several physiological parameters of SBM-fed and blood-fed adult females of *A. aegypti*. In addition, we used SBM to investigate the role of cholesterol in egg development in mosquitoes that were exposed to different nutritional regimens during larval development.

## 2. Materials and methods

### 2.1. Ethics statement

All of the animal care and experimental protocols were conducted following the guidelines of the institutional care and use committee (Comissão de Ética no Uso de Animais, CEUA-UFRJ) and the NIH Guide for the Care and Use of Laboratory Animals (ISBN 0-309-05377-3). The protocols were approved under the registry # CEUA-UFRJ 155/13. The technicians of the animal facility at the Institute of Medical Biochemistry (UFRJ) carried out all aspects related to rabbit husbandry under strict guidelines to ensure the careful and consistent handling of the animals.

### 2.2. Mosquitoes

*A. aegypti* (Red Eye strain) were raised in an insectary at the Federal University of Rio de Janeiro, Brazil, under a 12-h light/dark cycle at 28 °C and 70–80% relative humidity. The larvae were fed dog chow (Pedigree Co, Brazil) that had the following nutritional composition, as declared by the manufacturer: Moisture (max) 12%, Crude Protein (min) 25%, Crude Large (min) 8%, Crude Fiber (max) 3%, Mineral content (max) 11%, Calcium (min) 1%, Calcium (max) 2%, Phosphorus (min) 1%, Phosphorus (max) 1.8%, Sodium (min) 3100 mg/kg, Potassium (min) 6200 mg/kg, Zinc (min) 240 mg/kg, Linoleic Acid (min) 24 g/kg, Vitamin E (min) 120 UI/kg, and Selenium (min) 0.31 mg/kg. The adults were maintained in a cage and given a solution of 10% sucrose *ad libitum*. For the development of the "large" mosquito, the larvae were raised in polypropylene trays (8 cm × 28 cm × 44 cm) with 1.5 g of dog chow in 1 L of water per tray, while for the "small" mosquito, only 0.7 g of dog chow was added. On average, 250 larvae were raised per tray for both diets. Five- to ten-day-old females were used in the experiments. Wing length was used as a body size parameter, which individual female wing was dissected and measured in a microscope containing a micrometer ocular Heparinized rabbit (New Zealand) blood was obtained from ear vein and used in artificial feeding with water-jacketed artificial feeders that were maintained at 37 °C sealed with Parafilm membranes.

To measure oviposition, fully engorged females were transferred to individual cages (10 cm diameter × 15 cm height), and eggs laid onto a wet piece of filter paper were counted 7 days after the meal. Adult insects were maintained with 10% sucrose solution *ad libitum*.

The SBM basic composition has the purified bovine proteins solubilized in Tyrode's buffer, which one is composed by 8 g of NaCl; 0.2 g of $CaCl_2$; 0.1 g of $MgCl_2$; 0.2 g of KCl; 0.05 g of $NaH_2PO_4$ and 1 g of glucose per liter of water (pH 7.4).

### 2.3. Lipids

Small and large females, at 1 and 7 days after emergence, were used to measure the lipid content in the whole body. For glycerol, pools of 14–20 females were homogenized in phosphate-buffered saline (PBS; 10 mM Na-phosphate buffer, pH 7.2, 0.15 M NaCl), and the neutral lipid content was measured using a commercial kit assay that evaluated the glycerol that was produced upon triacylglycerol hydrolysis (Doles) according to the manufacturer's instructions. For cholesterol, the samples were analyzed by the ferric chloride method (Zlatkis et al., 1953).

### 2.4. Lipoprotein purification and cholesterol micelles

The human Low Density Lipoprotein (LDL) that was used here was kindly provided by Dr. Nuccia De Cicco using a KBr ultracentrifugation gradient protocol that was described in De Cicco et al. (2012) and LDL protein concentration was estimated by the Lowry method (Lowry et al., 1951). To produce cholesterol-containing micelles, chloroform solutions of cholesterol (Sigma) 2 mg/mL (5 mL) and phosphatidylcholine (asolectin from Soybean; Sigma) 70 mg/mL were mixed and dried as a film under a stream of $N_2$ in a round-bottom flask. The lipid film that was obtained was resuspended in Tyrode's physiological solution by vortexing and sonication until complete emulsification. Phospholipid micelles without cholesterol were obtained using the same protocol. For the experiments evaluating Reactive Oxygen Species (ROS), a different source of L-α-phosphatidylcholine (*Sigma*, #61755) with a higher degree of purity (60%) was used to prepare micelles instead of the asolectin, which produced a high autofluorescence background under the microscope.

### 2.5. Reactive Oxygen Species (ROS) in the midgut

Two hours after feeding, the midguts were dissected in PBS and were incubated in Schneider's *Drosophila* medium with L-glutamine (Gibco) supplemented with 10% Fetal Bovine Serum (FBS; Cultilab) containing 50 μM Dihydroethidium (hydroethidine; DHE; *Invitrogen*) for 30 min at room temperature in the dark. The midguts were then washed in dye-free medium, and the fluorescence of the oxidized DHE was recorded using an Olympus MVX10 epifluorescence stereomicroscope with a DP-72 camera (Benov et al., 1998; Kalyanaraman et al., 2012; Oliveira et al., 2011).

### 2.6. RNA extraction and qPCR analyses

At least two pools of 10 midguts for each replicate were dissected in PBS, and RNA was extracted using TRIzol (Invitrogen) according to the manufacturer's instructions. RNA was treated with DNase, and cDNA was synthesized with the High-Capacity cDNA Reverse transcription kit (Applied Biosystems). Quantitative PCR (qPCR) was performed with a StepOnePlus Real Time PCR System (Applied Biosystems) using Power SYBR-green PCR master MIX (Applied Biosystems). The Comparative Ct Method was used to compare changes in the gene expression levels (Pfaffl, 2001). The *A. aegypti* ribosomal protein 49 (RP-49, AAEL003396-RA) gene was used as an endogenous control (Gentile et al., 2005) and the bacterial load was measured, using the same cDNA samples above mentioned, through analyses of 16S expression, a eubacterial ribosomal gene. The primer sequences that were used in this study were RP49_F: GCTATGACAAGCTTGCCCCCA, RP49_R: TCATCAGCACCTCCAGCT; Catalase (AAEL013407-RB) Cat_F: CAATGAACTGCACCGACAAC, Cat_R: AGCCTCATCCAGAACACGAC; Ferritin (AAEL007385-RA) Fer_F: GGCAGCAATGACTTCCACTT, Fer_R: TTTAAGCGTGGCGATCTTCT and 16S_F: TCCTACGGGAGGCAGCAGT, 16S_R: GGACTACCAGGGTATCTAATCCTGTT.

### 2.7. Peritrophic matrix staining

Midguts from insects that were fed SBM, blood or sugar were dissected 24 h after feeding and fixed in 4% paraformaldehyde for 3 h. All of the midguts were kept on PBS-15% of sucrose for 12 h and then in 30% sucrose for 30 h. After a 24-h infiltration in OCT, serial microtome 14-μm-thick transverse sections were obtained

and collected on slides that were subsequently labeled with the lectin WGA (Wheat Germ Agglutinin; a lectin that is highly specific for N-acetylglucosamine polymers) coupled to fluorescein isothiocyanate (FITC). The slides were washed 3 times in PBS buffer containing 2 mg/mL BSA (PBSB). The samples were then incubated in 50 mM NH4Cl/PBS for 30 min; in 3% BSA, 0.3% Triton X-100 PBS for 1 h; and in PBSB solution with 100 μg/mL WGA-FITC (EY Laboratories) for 40 min. The slides were then washed three times with PBSB and mounted with Vectashield with DAPI mounting medium (Vector laboratories). The sections were examined under a fluorescence microscope (Zeiss Observer.Z1 with Zeiss Axio Cam MrM) (Farnesi et al., 2012; Rudin and Hecker, 1989).

### 2.8. Statistical analysis

Statistical analyses were performed using GraphPad Prism Software, and the experiments were repeated at least twice. For Fig. 1, the mosquito's weight, wing length and oviposition were evaluated by an unpaired *T* test. Comparisons between mosquitoes that were fed phospholipids and phospholipids + cholesterol (SBM) were performed by an unpaired *T* test (Fig. 2). All of the other experiments were analyzed using ANOVA followed by Tukey's or Dunnett's Multiple Comparison Test. The relative expressions of the qPCR results were expressed as mean ΔΔCt values, but the SEM bars and statistical analysis were based on ΔCt values.

## 3. Results

### 3.1. Small and large mosquitoes

Mosquito larvae were raised under two distinct feeding regimens, as described in Section 2, which differed in the amount of food that was offered, resulting in mosquitoes with significantly body size difference and almost doubling of the wet weight of adult female mosquitoes (Fig. 1A and B). The small mosquitoes laid 25% fewer eggs than did the large mosquitoes after feeding on blood (Fig. 1C). Moreover, on the 1st day of life, small female mosquitoes had almost half of the cholesterol content compared to that of large females. On the 7th day after emergence, cholesterol reserves in the small mosquitoes were reduced by 50% compared to those on the 1st day, while the large mosquitoes maintained a similar cholesterol content (Fig. 1D). In contrast, when the triacylglycerol (TG) content was evaluated, the small females contained approximately 70% less TG than did the large females. However, on the 7th day of life, there was no significant difference between the TG content of both small and large mosquitoes (Fig. 1E). These results indicate that the nutritional status of the larval stage is directly related to success in oogenesis and impacts on lipid reserves.

### 3.2. Substitute of blood meal favors oogenesis

The SBM composition is essentially a mixture of proteins and cholesterol diluted in physiological saline (Table 1) and is based on the diet described by Kogan (1990) and modified by Cosgrove and Wood (Cosgrove and Wood, 1996), which was a chemically defined artificial diet that allowed oviposition in adult females of *A. aegypti*. Feeding small mosquitoes with a synthetic diet without lipid supplementation allowed oviposition, although the number of eggs that were laid was less than half that of the blood-fed insects (Fig. 2A). This differed from the original report of Kogan (1990), who obtained a similar number of eggs to that produced by females who were fed blood. All of the mosquitoes that were fed the lipid-supplemented diet had the three proteins solubilized in

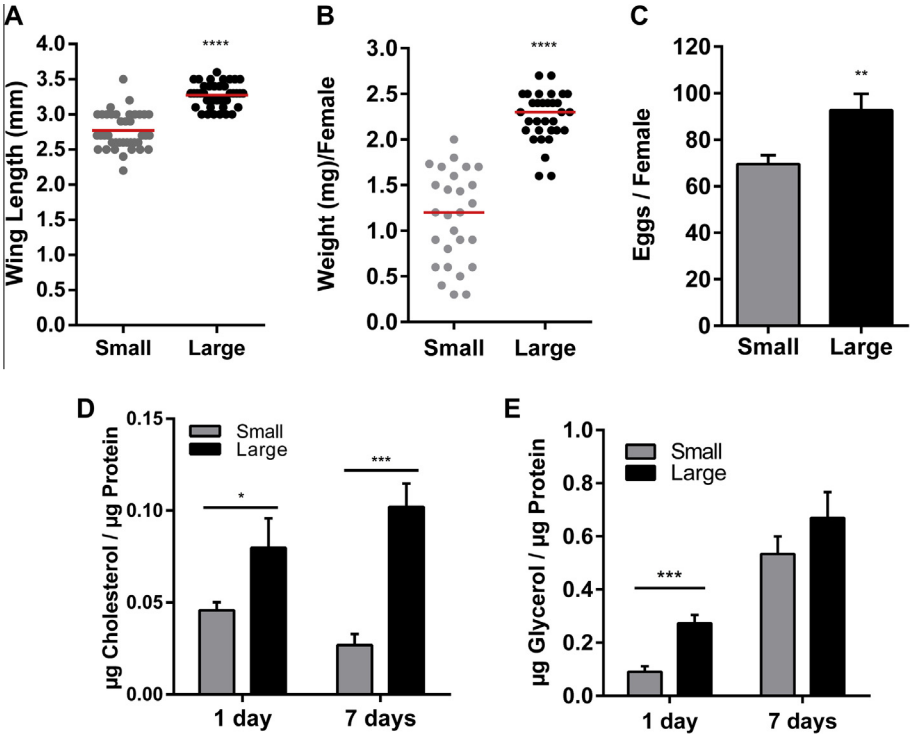


**Fig. 1.** *Female wing length, weight and oviposition after blood meal* – (A) wing length of individual female mosquitoes that emerged from distinct larval feeding protocols ($n = 80$); (B) weight of individual females ($n = 59$); (C) cholesterol content of small and large females on days 1 and 7 after the emergence of adults expressed as the ratio of cholesterol (μg) to total protein (μg) content ($n = 6$); (D) glycerol content of small and large females on days 1 and 7 after the emergence of adults ($n = 6$); (E) eggs laid by blood-fed small or large females ($n = 37$). Small and large females were obtained using larval rearing protocols that offered different amounts of food to the larvae, as described in Section 2. The data are the mean ± SEM (A, C, D and E) or median (B). The asterisks are $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ and $^{***}P < 0.0001$.

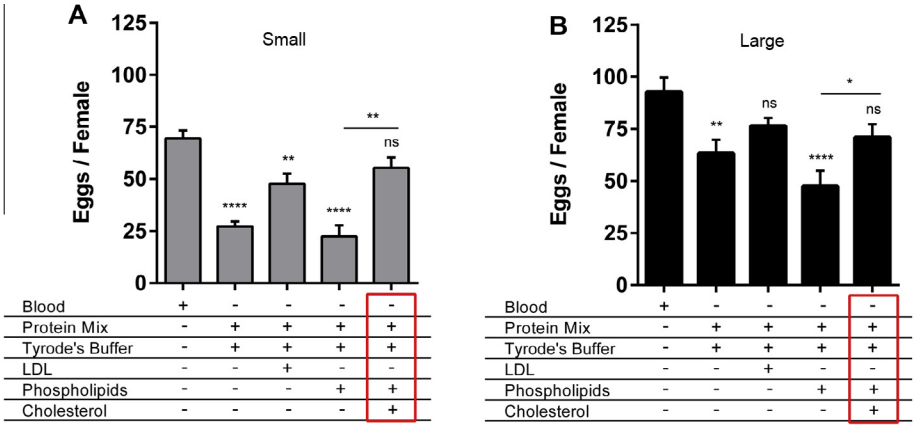


**Fig. 2.** *Egg laying after feeding different diets* – (A) eggs laid by small female mosquitoes ($n = 77$ females); (B) eggs laid by large female mosquitoes ($n = 69$ females). The composition of each diet is described below the graph. The data are the mean ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ and $^{***}P < 0.0001$.

**Table 1**
Substitute Blood Meal (SBM) composition.

| Basic Components | Concentration |
|---|---|
| Hemoglobin | 8 mg/mL |
| Albumin | 100 mg/mL |
| Y-globulin | 30 mg/mL |
| Tyrode's | 1× |
| Supplementary components | |
| Cholesterol* | 2 mg/mL |
| Asolectin or phosphatidylcholine* | 70 mg/mL |
| Low Density Lipoprotein (LDL) * | 0.8 mg/mL |

* Added as mentioned in the figure legends.

Tyrode's buffer as the basis, while varying only the lipid composition. Feeding with phospholipid micelles without cholesterol did not change the number of eggs that were laid by the insects. However, feeding small mosquitoes with the diet that was supplemented with either LDL or phospholipid micelles loaded with cholesterol (SBM) increased oviposition to close to 80% that of the blood-fed females, showing that the cholesterol in fact is an important molecule to allow the oogenesis. When the large mosquitoes were fed diet with LDL or cholesterol, the positive effect of cholesterol supplementation was also observed, but the increase in egg laying was less pronounced than the result obtained with small females (Fig. 2B). Taken together, the best diet composition for mosquito's physiology is the one supplemented with cholesterol and we will adopt this synthetic diet as Substitute of Blood Meal (SBM).

### 3.3. Mosquitoes that were fed SBM show decreased ROS levels and increased antioxidant defenses

Oliveira et al. (2011) showed that after a blood meal, ROS generation by the midgut epithelium decreased. This downregulation of the ROS level is triggered by heme from the blood meal. Thus, we evaluated the ROS levels in SBM-fed mosquitoes using an oxidant-sensitive probe, dihydroethidium (hydroethidine – DHE). Similar to the previous report (Oliveira et al., 2011), compared to mosquitoes fed sugar, the ROS levels decreased when the insects were fed blood, and the same profile was observed in the mosquitoes that ingested SBM (Fig. 3A–D)or other diet combination without cholesterol supplementation. Two antioxidant genes, catalase and ferritin, have increased expression in the *A. aegypti* midgut after a blood meal (Sanders et al., 2003), and a similar profile was observed after feeding with SBM, (Fig. 3E and F). Compared to the levels following a blood meal, both catalase and ferritin showed similar expression levels in the midguts of females fed SBM.

### 3.4. The SBM permits peritrophic matrix development

After a blood meal, the mosquito midgut secretes an extracellular layer that surrounds the intestinal bolus. This layer, the peritrophic matrix (PM), is formed by the association of chitin and proteins and represents a major event of blood digestion in mosquitoes, performing a protective role (Pascoa et al., 2002; Villalon et al., 2003). To determine whether the SBM can induce normal PM formation, females were fed sugar, blood or SBM, and the PM was observed by WGA lectin conjugated to FITC (a lectin that binds N-acetyl-D-glucosamine, the unit forming chitin) 24 h after feeding (Fig. 4). Notably, the SBM induces the formation of a PM in the intestinal lumen with a similar appearance to that formed by blood-fed females.

### 3.5. Substitute of blood meal permits the proliferation of midgut microbiota

In response to the incoming blood meal, the midgut microbiota proliferate explosively, increasing 100–1000-fold in magnitude (Oliveira et al., 2011). The intestinal microbiota were evaluated by qPCR for the 16S ribosomal bacterial gene (Fig. 5), revealing similar bacterial growth to that in the gut of the insects that were fed blood or SBM.

## 4. Discussion

In the *A. aegypti* life cycle, blood feeding provides, in only a couple of minutes, all of the raw materials that are essential to oogenesis, and this large flood of nutrients triggers an array of signaling cascades that promote a concerted regulation of digestion, absorption of nutrients, and oogenesis and fuels the metabolic demands of activities such as flight and the immune response to pathogens. Given these facts, it is surprising that almost no study of mosquito physiology has been reported using the diet that was originally proposed by Kogan (1990). Here, we have improved this artificial diet by supplementation with cholesterol, as insects are not able to perform *de novo* synthesis (Clark and Block, 1959). Our results show that, in spite of the mosquito auxotrophy for cholesterol, the positive effect of dietary cholesterol on egg development is highly dependent on the nutritional status of the newly hatched adult, which in turn is a product of its nutritional regimen during larval development. Telang et al. (2006) also showed that

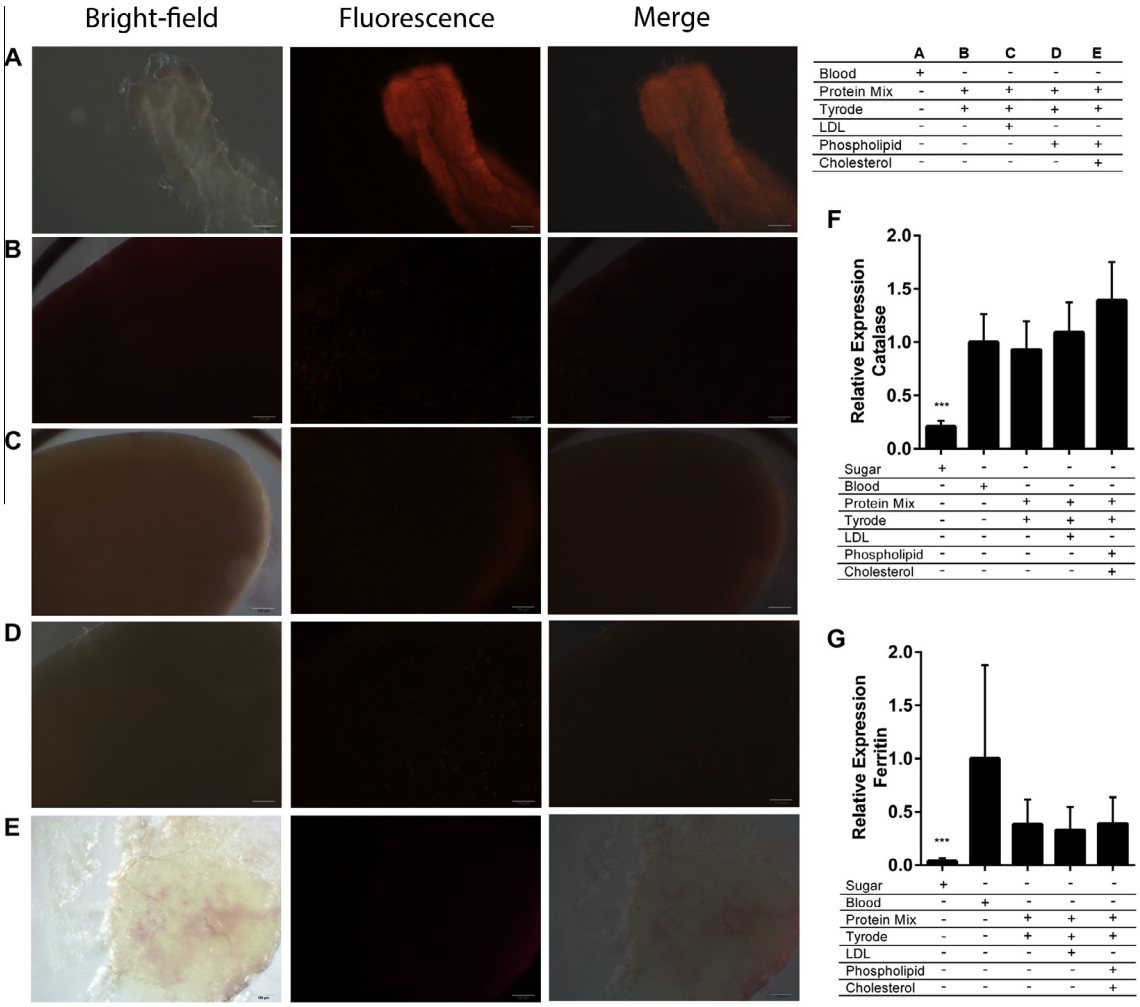


**Fig. 3.** *Levels of ROS in the midgut after SBM feeding and the expression of antioxidant genes* – (A–E) the midguts were dissected 2 h after a meal with the indicated diets and were incubated with 50 μM DHE for 30 min. DHE oxidation was observed under a fluorescence microscope. The scale bar is 100 μM. Bright field images are shown as insets. (F and G) Gene expression was analyzed in the mosquito midgut 24 h after feeding. (F) Catalase. (G) Ferritin. The relative expression of genes was analyzed through q-PCR and RP-49 was used as an endogenous control. The gene expression in blood-fed mosquitoes was used as a reference ($n = 6$). The data are the mean ± SEM. $^{***}P < 0.001$.

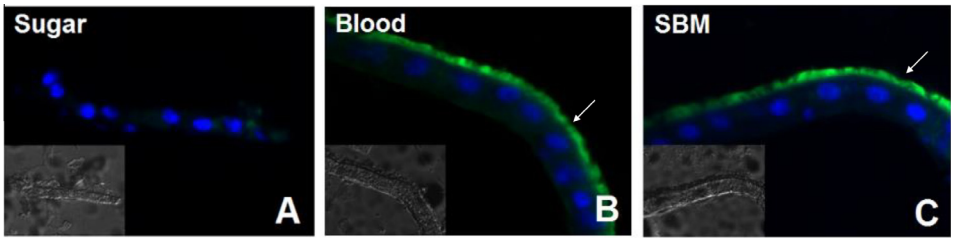


**Fig. 4.** *Peritrophic matrix formation after a meal* – the midguts of females that were fed sugar or 24 h after blood or SBM feeding were dissected and fixed, and sections were stained with WGA-FITC (green) and DAPI (blue). (A) Sugar-fed; (B) Blood-fed; (C) SBM-fed. The peritrophic matrix is indicated by white arrows in panels B and C. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

nutritional availability during larval instar regulates the body size, lipid reserves and oogenesis in *A. aegypti* adults. Here, we obtained similar results showing that the larval nutritional status directly affects the body size, wet weight, oviposition of adult mosquitoes (Fig. 1A–C) and the size of lipid reserves (Fig. 1D and E). The use of SBM or another diet combination without cholesterol revealed that dietary cholesterol in the blood meal becomes an important factor if the larvae are reared under suboptimal food availability. In this way, the cholesterol of teneral reserves were crucial for egg development because small females, which had smaller cholesterol reserves (Fig. 1C), showed an increased number of eggs after feeding with SBM supplemented with this lipid. However, the large females had more cholesterol reserves and did not show the same dependence on additional dietary cholesterol as did the small mosquitoes, revealing an important aspect of mosquito biology, as under field conditions, food is rarely available *ad libitum*. It is interesting to note that large variations in the cholesterol plasma concentration exist between different species of mammals and among different human populations, which led us to speculate on whether cholesterol is a dietary component whose availability

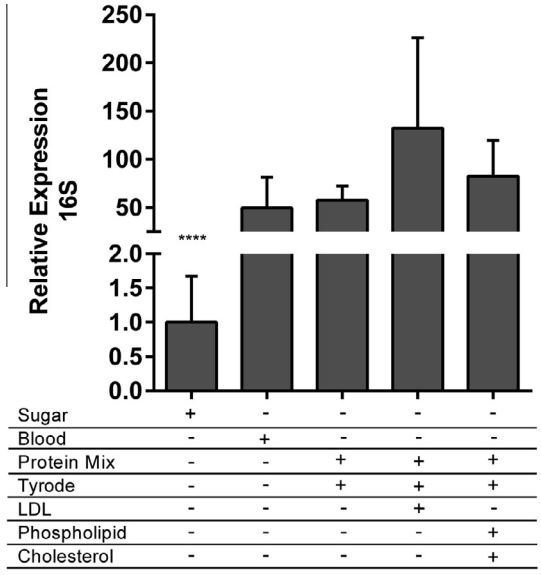


**Fig. 5.** *Midgut microbiota proliferation induced by SBM* – The eubacterial 16S abundance was analyzed through q-PCR 24 h after the meal; RP-49 was used as an endogenous control, and 16S in sugar-fed mosquitoes was used as a reference. The data are the mean ± SEM $n = 6$. $^{****}P < 0.0001$.

may be of critical relevance for establishing the population dynamics of mosquitoes.

The diet described in the original reports of Kogan (1990) and Cosgrove and Wood (1996), and used in other studies, such as the recent communication of Gonzales et al. (2015), only oviposition was followed as a readout for the physiological performance of the mosquitoes that were fed the artificial meal. Here, we investigated several aspects of the mosquito physiology beyond egg development that could be studied using this approach and found that the gut physiology of the SBM-fed mosquitoes closely resembled that in mosquitoes fed a regular blood meal.

Free radicals have been implicated as relevant players in the biology of the insect midgut and as mediators of the relation with the indigenous microbiota (Ha et al., 2009) and regarding insect vectors of diseases, redox metabolism has been put in the center of the vectorial competence, defining an important role in the relationship with pathogens (Molina-Cruz et al., 2008; Oliveira Gde et al., 2012; Pan et al., 2012). In the *A. aegypti* midgut, a consequence of blood intake is a dramatic change in redox equilibrium, which down-regulates the production of ROS, and this response is also triggered by the SBM meal (Fig. 3). Lowering ROS production seems to be one of the factors that permits microbial growth in the gut and was suggested to be an antioxidant mechanism to compensate for the intake of large amounts of heme, a pro-oxidant molecule.

A counterpart of the free-radical chemistry that takes place after a blood meal is the essential role that is performed by genes with antioxidant roles. These genes are essential for the gut tissue to withstand the oxidative challenge that is elicited by the large amounts of incoming heme, a pro-oxidant molecule, as well as by oxygen and nitrogen reactive species that control microbial growth and pathogen transmission (Bahia et al., 2013; Ha et al., 2005). In agreement with this concept, several genes with antioxidant function are upregulated after a blood meal, including catalase – a hydrogen peroxide scavenging enzyme – and ferritin – which limits the occurrence of free iron that otherwise would generate hydroxyl radicals through the Fenton reaction. Both of these genes were upregulated in insects that were fed SBM when compared to sugar-fed insects, as occurs with a regular blood meal (Sanders et al., 2003). Catalase expression in SBM-fed mosquitoes increases to levels that are indistinguishable from those that are found in blood-fed insects. Ferritin also increased expression after SBM feeding but did not reach the same mean level as that of the blood-fed mosquitoes (Fig. 3).

A hallmark of blood meal digestion in mosquitoes is the formation of the peritrophic matrix (PM), which involves the blood bolus, separating it from the epithelia. The PM has been suggested to work as a barrier that parasites (as shown with *Plasmodium* sp. and *Trypanosoma* sp. parasites) must have for transposition to infect the midgut epithelia (Billingsley and Rudin, 1992; Shahabuddin et al., 1996; Weiss et al., 2014). *A. aegypti* PM plays a role in the deposition of heme aggregates that constitute a heme detoxification mechanism in mosquitoes (Devenport et al., 2006; Pascoa et al., 2002). Recently, Cázares-Raga et al. (2014) reported that a very incipient small PM is formed upon feeding with an artificial meal that is based on salts, glucose and latex beads. In contrast, when mosquitoes were fed SBM, a chitin-containing PM was secreted with a thickness similar to that observed in blood-fed mosquitoes (Fig. 5).

One of the most rapidly evolving areas in gut biology is the study of the intestinal microbiome, and insects are not an exception to this trend. Once termed the commensal microbiota, the intestinal bacterial community is a major player in gut homeostasis, exerting its influence in the regulation of metabolism and development at the systemic level, much beyond the gut epithelium (Buchon et al., 2013; Lee et al., 2013; Shin et al., 2011). In insect vectors, several studies have approached a relationship between microbiota diversity and vectorial competence (Castro et al., 2012; Cirimotich et al., 2011; Xi et al., 2008). We also show here that, despite its simple composition, SBM permits the proliferation of midgut microbiota to levels that are similar to those found after a blood meal (Fig. 5), suggesting the use of artificial diets as an important tool for studying the interaction between mosquitoes and their indigenous microbiome. Additionally, in a currently article, we report that SBM allows dengue proliferation. Moreover, in this report, we used a hemoglobin-free SBM as a tool to investigate the effect of heme on viral replication in the midgut and showed that heme from the diet is a modulator of the mosquito immune response (Bottino-Rojas et al., 2015).

Taken together, our data show that the synthetic diet SBM closely reproduces the physiological changes that take place in *A. aegypti* after a blood meal, and we propose its use as a tool for studying mosquito physiology and its interaction with intestinal microbiota and pathogens. In the course of the validation of SBM use as a proxy for a blood meal, we showed that dietary cholesterol is an important factor in the onset of oogenesis in *A. aegypti* depending on the nutritional history of larval development.

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