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PACKED RED BLOOD CELLS AND BOVINE SERUM ALBUMIN AS A BLOOD MEAL SOURCE FOR *ANOPHELES FARAUTI*

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ABSTRACT. Packed human red blood cells, diluted 1:1 with phosphate-buffered saline (PBS) containing 0–21% bovine serum albumin was used as a blood meal source for *Anopheles farauti*. Egg and larval production linearly increased with bovine serum albumin concentration in PBS. When the saline contained 9–21% bovine serum albumin, egg production was equivalent to that obtained when mosquitoes were fed directly on guinea pigs.

Feeding mosquitoes on laboratory animals has long been accepted as the simplest and often most efficient method of maintaining a mosquito colony. However, economic, logistic, and animal welfare concerns increasingly dictate the use of alternate blood meal sources, usually blood furnished through an artificial or natural membrane (Bailey et al. 1978, Hagen and Grunewald 1990). The need to establish a mosquito colony 5,000 km from our main laboratory and laboratory animal facility in Jakarta prompted efforts to adapt *Anopheles farauti* Laveran to membrane feeding. In order to meet stringent human use requirements, because the progeny of reared mosquitoes would be fed directly on human volunteers positive for *Plasmodium vivax* gametocytes, blood had to be obtained from laboratory-reared animals or, in the case of membrane feeding, from a United States approved blood bank. Logistically, this procedure was complicated because we lack laboratory animals that can tolerate repeated 200- to 400-ml blood draws and the local blood bank facilities in Jakarta are not certified by an outside agency.

Packed red blood cells (RBCs) obtained from the United States Navy Hospital Okinawa Blood Donor Center met our requirements and could be obtained at minimal cost. Packed RBCs must be resuspended in an equal volume of fluid prior to mosquito feeding. Ideally RBCs would be resuspended with equal volumes of plasma; however, plasma that has a longer shelf life and is more commonly used than RBCs was not available. Glass mosquito membrane-feeders similar to those detailed by Rutledge et al. (1964) were obtained from Perpetual Systems Corporation (Rockville, MD) or were made locally. Each membrane-feeder was 10 or 40 mm in diameter and was filled with 1 or 4 ml of blood, respectively. Water, circulated from a water bath through the membrane-feeder's water jacket, maintained the blood at 37–38°C. Mouse skins from laboratory-reared mice, sacrificed for other purposes, were frozen fresh (−40°C, 1–8 weeks) and served as membranes. After *An. farauti* mosquitoes were fully adapted to membrane feeding, a bovine intestine preparation (*baudruche* membranes, Joseph Long, Inc., Belleville, NJ), functioned equally well. The *An. farauti* colony used in this experiment was established from female mosquitoes collected at Sorong, Indonesia during 1981. These mosquitoes were maintained on guinea pigs.

Initial trials using packed RBCs diluted 1:1 with phosphate-buffered saline (PBS) resulted in low egg production (one or two per female) and no larvae. The addition of bovine serum albumin (BSA) (Sigma Chemical Company, Saint Louis, MO) resulted in a significant increase in egg and larva yield. To quantify this improvement, 10 female 3–5-day-old *An. farauti* mosquitoes were placed in 8-oz. paper cups and presented with packed RBCs diluted 1:1 with PBS containing 0.03–0.21 g/ml BSA. Mosquitoes fed on guinea pigs served as controls. The number of eggs was counted 3 days postbloodfeed and the number of larvae was counted 2 days after egg laying. Mosquitoes resulting from the larvae were returned to the adult pool and subsequently used in later experiments. This experiment was repeated 4 times with 3 replications per BSA concentration.

Mosquitoes fed readily through the mouse skin with 100% of the mosquitoes feeding to repletion within 30 minutes. Figure 1 demonstrates the linear relationship between mean egg and larva yield and g/ml BSA, $r = 0.94$ and $r = 0.95$, respectively. Percent hatch and egg yield was analyzed by 2-way analyses of variance followed by a Bonferroni t-test to compare treatments with control (SigmaStat for DOS version 1.01, 1992). The percentage of larvae hatching was not dependent on BSA concentration and there was no difference between treatment and control groups ($P > 0.05$). When BSA concentration was ≥9% in PBS there was no difference in egg yield between membrane-fed and guinea-pig-fed mosquitoes (Table 1). However, because of the linear relationship between mean egg yield and BSA concentration, it is thought that high variability among replicates may have masked significant differences between control and treatment effects at the higher BSA concentrations.

This study demonstrated the feasibility of feeding *An. farauti* on packed RBCs diluted with PBS containing BSA and that egg and larvae yield from

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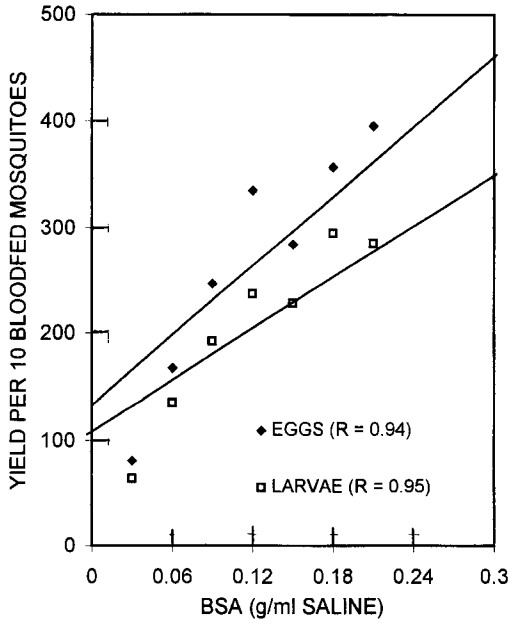

Fig. 1. Effect of various concentrations of bovine serum albumin (BSA) on egg and larvae yield in *Anopheles farauti*. Each point is the mean of 4 trials with 3 replicates per trial.

mosquitoes fed packed RBCs diluted with PBS containing ≥9% BSA was equivalent to that obtained from mosquitoes fed on guinea pigs. Preliminary trials with other mosquito species suggest that this approach may work equally well with *Aedes aegypti* (Linn.) and *Culex quinquefasciatus* Say.

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Table 1. Effect of bovine serum albumin concentration on egg yield, percent hatch, and larval yield.

Bovine saline albumin (g/ml saline)	x̄ egg yield (SD)	x̄ % hatch (SD)	x̄ larval yield (SD)
0.03	80.6 (75.8)	56.1 (45.3)	63.8 (63.5)
0.06	167.7 (121.5)	75.1 (18.9)	135.8 (112.7)
0.09	246.7 (154.5)	71.8 (25.0)	192.3 (147.8)
0.12	335.7 (159.8)	71.1 (19.3)	236.7 (123.5)
0.15	284.1 (122.8)	73.2 (18.1)	228.1 (133.1)
0.18	357.1 (171.4)	77.3 (21.0)	294.6 (192.9)
0.21	395.7 (154.3)	68.8 (14.0)	285.3 (148.0)
Control	373.7 (210.1)	59.2 (22.1)	231.8 (144.8)