

From Blood to SkitoSnack:

Artificial blood diet shapes *Anopheles stephensi* fitness and malaria parasite development.

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Abstract

Ethical and scalable mosquito research may require moving away from a reliance on vertebrate blood. While artificial blood replacements (ABRs) diets like SkitoSnack offer a promising alternative, their biological impact on mosquito fitness and *Plasmodium* development across multiple generations is not well understood. We investigated these effects by transitioning a colony of *Anopheles stephensi* from mouse blood (MB) to SkitoSnack (SS) and monitoring them for 10 generations. Our results show that the initial transition to an artificial diet carries significant biological costs. In the founder generation (F_0), SkitoSnack reduced the proportion of females laying eggs by 37% and decreased average clutch sizes by 33%. These maternal effects extended to the next generation, with F_1 offspring showing significantly lower pupation success. However, these fitness hurdles were transient. By the sixth generation (F_6), the mosquitoes showed clear signs of physiological adaptation where survival began to recover, and feeding rates were comparable to blood-fed controls. Crucially, long-term maintenance on SkitoSnack did not impair the mosquitoes' ability to support malaria parasites. When F_6 mosquitoes were infected with *Plasmodium chabaudi*, oocyst prevalence and burden, size, and sporozoite prevalence and burden were robust and comparable to those maintained on MB. By the tenth generation (F_{10}), reproductive traits and pupation success showed further recovery. Our study shows that while switching to an artificial diet causes initial fitness bottlenecks, mosquitoes can adapt within a few generations. This makes SkitoSnack a practical and ethical tool for investigating mosquito biology and parasite-vector interactions.