

# Implementation of the Artificial Feeders in Hematophagous Arthropod Research Cooperates to the Vertebrate Animal Use Replacement, Reduction and Refinement (3Rs) Principle

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## Abstract

Vector-borne diseases are transmitted to humans by hematophagous arthropods and these blood-sucking organisms are target to researches worldwide. The laboratory colonization of these species is an important factor in the development of innovative strategies to control these vectors. However, this maintenance requires blood to make these invertebrates able to complete their life cycle. Although live vertebrate animals are frequently used for this feeding procedure, artificial feeders are available as potential alternatives to replace the use of live animals in some situations, especially in vector colony maintenance. The aim of this commentary is to discuss the use of artificial feeding methods concerning the 3Rs principle application. The scientific community focused on vector-borne diseases studies needs to strongly consider these artificial feeding options as a bioethical alternative to maintain blood-feeding arthropods in laboratory.

**Keywords:** Artificial feeder; Blood-sucking arthropods; Blood-feeding; Three Rs principle; Use of laboratory animals

## Introduction

Many species of blood-sucking arthropods transmit different human pathogens during the blood-feeding behavior. Consequently, these organisms are targets for different types of basic and applied research worldwide in an attempt by the scientific community to develop innovative strategies for controlling disease transmission by vectors [1]. Studies benefit tremendously when these species can be colonized in laboratory insectary facilities but in this case, to complete the life cycle, these anautogenous organisms necessarily require a blood meal to produce their eggs and small vertebrate laboratory animals like mice, rabbits, chickens and guinea pigs are frequently used as blood suppliers [2].

Obviously, the use of live animals for this purpose is only authorized after bioethical certification by animal care committees under frequently revised protocols. They describe in detail the animal anesthetization or immobilization procedures before the invertebrate feeding process as well as the method for euthanasia when an experiment is completed. It is recommended that protocol evaluations by bioethical committees take into account the 3Rs principle (replacement, reduction, refinement) related to animal welfare [3,4]. The application of the 3Rs guiding principle strongly contributes to ethical use of animals [5,6], and the invertebrate's engorgement in laboratory conditions involving live animals it is not the only option, given the published literature in this field.

## Artificial Feeders

Based on the pioneering apparatus, a Rutledge feeder [7], many articles have addressed this issue in the previous decades and a large proportion of them developed creative adaptations to promote the construction of different feeders for blood-sucking invertebrate organisms with easily accessible materials [8-20]. Most of these feeders are composed of a heating element (for blood warming) and a feeding part containing a blood reservoir and an artificial membrane surface simulating a vertebrate skin. Although natural membranes can also

be applied, there are artificial options to replace the use of animal skin [2]. The large number of different feeders available for mosquito species is notable and the availability of artificial systems for arachnids, phlebotomines and triatomines is also considerable [8,12,13,19,21].

These devices reveal the replacement to the use of live animals is feasible with respect to blood delivery for invertebrate vectors. Much of the published data involving artificial feeders' application is focused on vector *in vitro*/artificial infection establishment. There is a dependency on the use of artificial infection procedures in the laboratory, to closely mimic the host pathogen relationship, in pursuance of more accurate study vector-borne diseases. In these cases, infected blood is prepared by mixing the pathogenic agent with defibrinated/anti-coagulated blood and the mixture is offered to blood-sucking vectors via artificial feeders.

Nowadays commercial blood from sheep, horse and bovine are good sources for defibrinated blood due to lower cost compared to small vertebrate animals, also larger amounts of blood can be acquired without harming or having to perform euthanasia of donor animals representing a valuable refinement of laboratory animal's usage.

## Application in Colony Maintenance

When the purpose is not to obtain experimental infection but to

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maintain colonies in laboratory facilities, the use of the artificial feeders is not prioritized as can be observed in the methods section of a large number of published articles. This led us to ask an important question: how viable these apparatuses can be in relation to maintenance of species' life cycles in the laboratory? It is a crucial point to be addressed since the argument here is to promote an ethical conscience based on 3Rs principle to increase the artificial feeder application in substitution for live animal use to blood-feed invertebrate vectors. In fact, although some articles reported negative effects on fecundity caused by artificial blood-feeding for *Rhodnius prolixus* and *Simulium damnosum* [22,23], there are many works showing positive examples in which colonies maintained by artificial feeders are comparable to live animal-fed counterparts for different species [9,11,16,18,21,24-26].

In addition to laboratory studies, mass rearing facilities for vector control programs through population suppression, such as SIT (Sterile Insect Technique) or RIDL (Release of Insects containing a Dominant Lethal) programs [27,28] are under development. To achieve a mass production of insects, a great amount of blood is periodically necessary to maintain egg production without compromising production or releases. A realistic estimative can be performed as an example: a single insect can ingest 3.5  $\mu$ L of blood, considering the mean of a mouse blood volume is 1.5 mL, 560 mice would be necessary per week to supply a mass production of 240,000 mosquito females. For those large facilities artificial blood systems are welcome and recommended [29]. Liters of blood can be easily obtained daily from abattoirs or bought from other suppliers.

These results argue favorably for the replacement of live animal use by artificial feeders, generating a lower demand of small vertebrate animals, nonetheless it is important to highlight that a comparative evaluation in relation to colony fitness consequences is necessary in each specific case. In the same way, variation in the reproductive fitness of a colony maintained by an artificial feeding process can also be attributed to the blood source as examples have demonstrated for some mosquito species [30-32]. Since defibrinated blood originating from different vertebrate species is commercially available, multiple sources can be tested in order to achieve the best artificial feeding performance. Moreover, one recent article demonstrated that out-of-date human blood from blood banks can be used [25] as a viable alternative to artificially feed two mosquito species, although the age of out-of-date blood (5 to 25 days post-expiration date) shows a negative effect on mosquito fecundity. The authors suggested that out-of-date human blood can be used for this purpose since expired blood bags are discarded as biological waste. Obviously, bioethical concerns, local/institutional ethics committee's evaluation, certifications and blood bank authorization need to be obtained before this blood source can be applied as a laboratory option to artificially feed colonized vectors, and appropriate blood tests need to be adopted to prevent accidental infections.

## Conclusion

In summary, the relevant aspects of artificial feeders usage described here strongly support the reduction, replacement and refinement of live animals for maintenance and experimentation of blood-sucking insects' colonies. Those aspects are fully supported by scientific research in this field.

This positioning has the intention to bring awareness for the feasibility of artificial feeders utilization in entomology laboratories and finally the research community could start to change the way they perform their experiments over time. Although this is the purpose of

the current commentary, artificial feeders are not only suggested as laboratory alternatives to hematophagous arthropods. A recent article proposed the use of these devices as agents for controlling mosquito-borne diseases by satiating mosquitoes with food from the artificial feeders therefore reducing the number of human mosquito meals [33]. The proposal in the present work is much less ambitious and innovative but is totally founded in considerations of animal welfare and application of the 3Rs principle.

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