Optimisation of *in vitro* feeding: steps towards long-term storage and colony maintenance of the hematophagous mite *Dermanyssus gallinae*.

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Poultry red mites (PRM) are blood feeding ectoparasites that live off-host, only seeking a bird to rapidly engorge every few days. Three of the five lifestages are hematophagous and infestations can rapidly increase. PRM are small and highly mobile making the parasite difficult to contain in a controlled experimental environment. *In vitro* feeding techniques have been previously been devised to overcome containment issues (e.g. McDevitt *et al*., 2006; Bartley *et al*., 2015) for the preliminary screening of PRM vaccines. Mite feeding rates can be highly variable after storage and here we describe steps towards minimising that variability, optimising egg laying and evaluating feeding rates of mites stored longer term.

Recently we described utilising Baudruche membrane in an *in vitro* device to feed adult females (Nunn *et al* 2020) using goose blood as a food source, which led to improved and reproducible feeding rates and fewer animal procedures due to the increased blood volume per procedure. We then evaluated the device to feed the hematophagous nymph stages of PRM and demonstrated significant correlation between the feeding rates of deutonymph stages and adult females (Spearman $r_{s.}$ = 0.54, p = 0.0008, n = 48) and protonymphs and adult females (Spearman $r_{s.}$ = 0.60, p = 0.00001, n = 48). A highly significant correlation was demonstrated between the proportion of the two nymph stages (Spearman $r_{s.}$ = 0.919, p = >0.00001, n = 48) fed adults with no significant feeding of nymph stages in the absence of adult females.

To obtain reasonable *in vitro* feeding rates, mites are generally starved (conditioned) to allow digestion of their last blood meal, moulting and egg laying. Traditionally, mites are conditioned at room temperature (RT) before being stored at 4-8 ^{O}C, being briefly brought to RT before a feeding assay. Mite feeding can be variable under these conditions and are generally only useful for 2-4 weeks post conditioning at RT. Based on a study by Wang *et al* (2020) we performed a study to establish feeding rates of mites kept over longer periods of time after two different conditioning regimes. Three collections of mites (1-3) were each divided into two, with one cohort stored at 5^{O}C until conditioning at RT for one week prior to feeding (1a-3a) and with the other cohort (1b-3b) conditioned at RT for one week followed by storing at 5^{O}C until feeding. The percentage feeding rate of adult females in cohorts 1a-3a, fell by less than 10% over the course of the study (10 weeks), demonstrating feeding rates of 2.6, 1.26 and 1.4 times that of cohorts 1b-3b at 6 weeks and 6.75, 2.1 and 4.75 respectively at 8 weeks.

To see if fecundity of mites fed *in vitro* was improved by being kept in groups of fed females, we compared numbers of offspring per fed mite on two occasions by incubating fed mites individually (n = 60, n = 41 respectively) and then 5 replicate groups of 5, 15 and 30 mites and counting offspring after incubation for 7 days. No difference in offspring/fed mite was demonstrated across the different experimental groups.

Repeated feeding of adult female mites was then performed, with 6 flasks of 100 adult females fed on four consecutive days and 6 flasks of 100 adult females fed only once. Flasks were incubated for 5 further days to allow eggs to hatch and for larvae to moult into protonymphs. They were then counted and progeny per adult female was calculated. Using an unpaired t-test with Welch's correction, a significant increase (P = 0.02; t=2.844, df=6) in progeny/mite was demonstrated in those mites fed on four consecutive days (range 0.49-1.9, mean 1.115, SEM 0.21) compared to those fed once (range 0.23-0.49, mean 0.485, SEM 0.08).

In summary, PRM feeding rates are stable (+/- 10%) up to ten weeks post collection if kept at 5^{O}C prior to conditioning at RT before *in vitro* feeding. Nymph feeding is greatly enhanced by feeding all hematophagous life stages together and fecundity was improved by repeated feeding of females but not by housing groups of fed females together.

References:

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