

# Detection of Anemia in Female CF1 Mice After Feeding *Aedes aegypti*

Robert Schmidt<sup>1</sup>, Jeri Rippetto<sup>1</sup>, Jeffrey Shryock<sup>1</sup>, Joan Massat<sup>1</sup>, Matthew Myles<sup>1</sup>

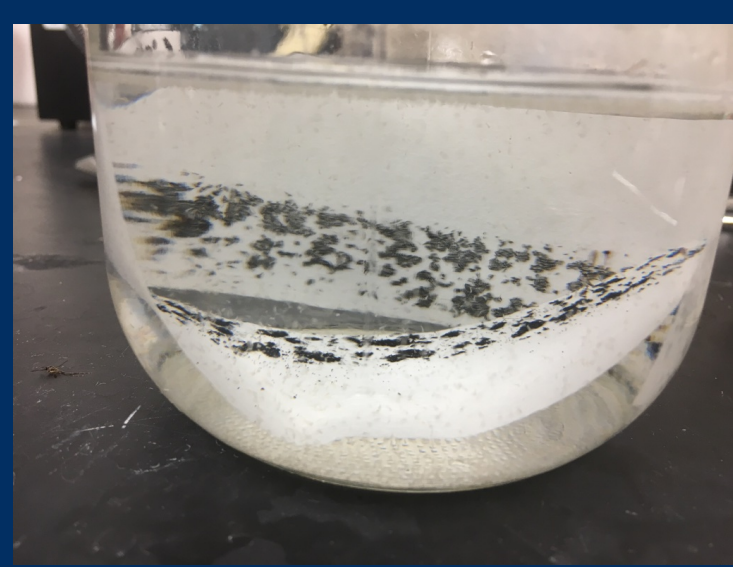
<sup>1</sup>Boehringer Ingelheim Animal Health, Missouri Research Center



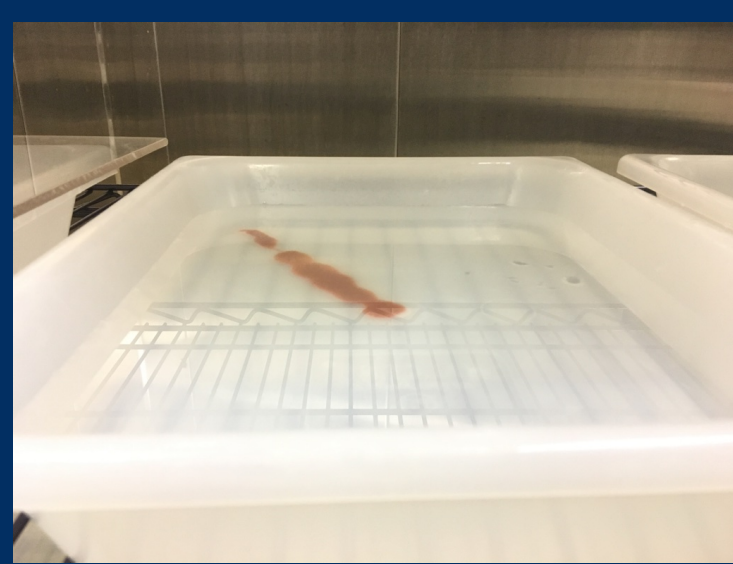
## Background

Mosquito-borne diseases, including Malaria, Zika, and West Nile virus, are responsible for millions of deaths across the globe, each year. The study of the pathogens that cause these illnesses often requires maintenance of a mosquito colony. Because female mosquitoes must consume a blood meal before laying eggs, propagation of mosquitoes requires a constant supply of blood. Currently at the Missouri Research Center (MRC) a system consisting of restrained mice is utilized in order to live feed the mosquitoes. The purpose of this study was to investigate the presence and duration of anemia in mice after they are utilized in this process in order to ensure that mice are being used in a responsible manner that minimizes individual discomfort and total number of mice required.

## Materials and Methods



*Aedes aegypti* eggs were hatched in deoxygenated water at room temperature for two hours.



The concentration of larvae was measured and 450 larvae were aliquoted into 40 separate Nalgene pans. Larvae were fed a commercial fish food slurry for eight days.



After eight days, mosquitoes were separated into populations of 1000, mixed sex pupae.



At 13 days post hatching, adult mosquitoes were ready to take a blood meal.



Female CF1 mice, weighing between 20 and 30 grams, were separated into four groups of four. On Day 0 of the study, one mouse from each group was euthanized as a control. The remaining three mice from each group were utilized to blood feed the mosquitoes. One mouse per group was euthanized immediately following the feeding procedure. Blood was collected via cardiocentesis in order to run a CBC.



One week following feeding, an additional mouse per group was euthanized and blood collected.



Two weeks after feeding, the final mouse in each group was euthanized and blood collected.

## Results

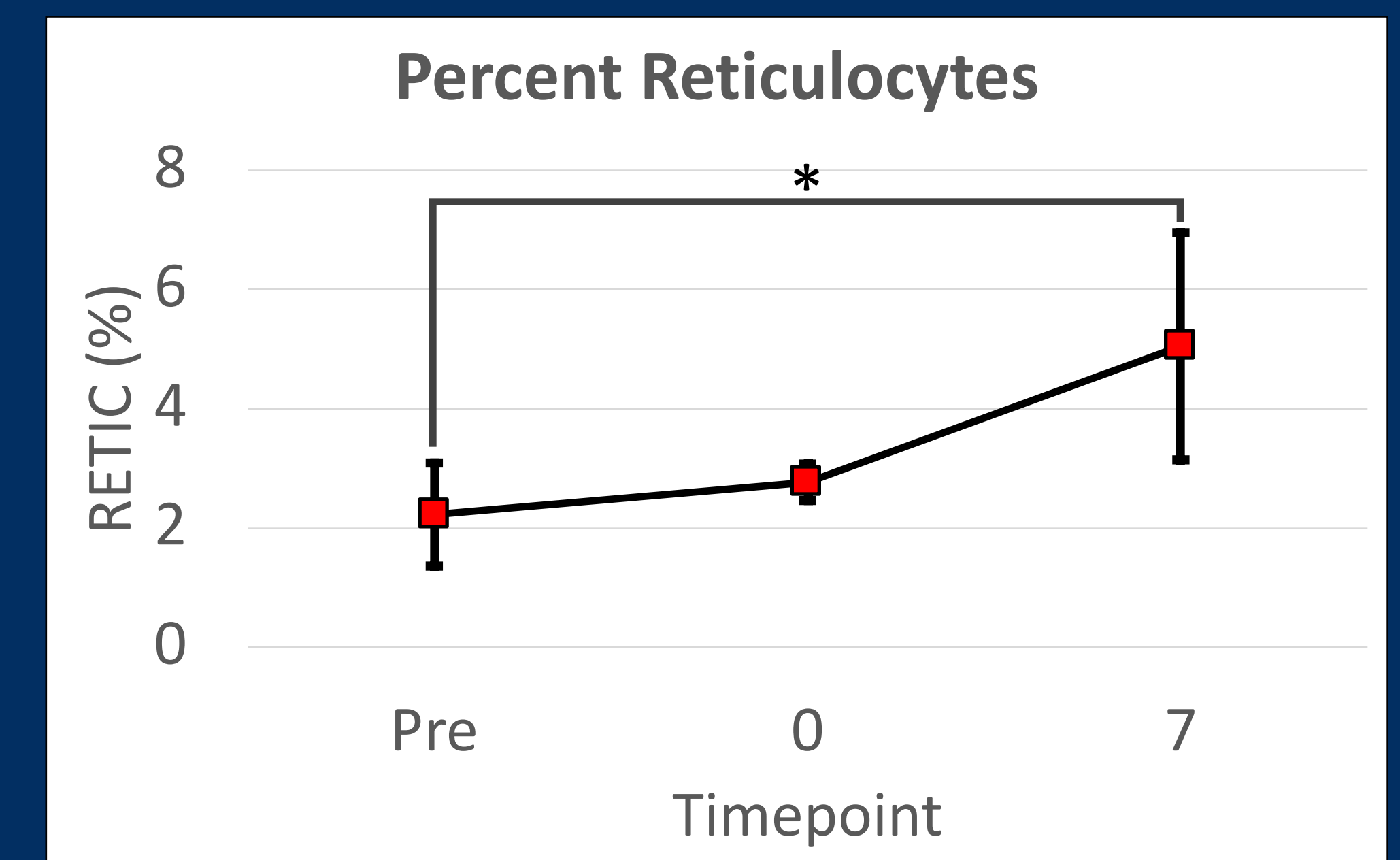
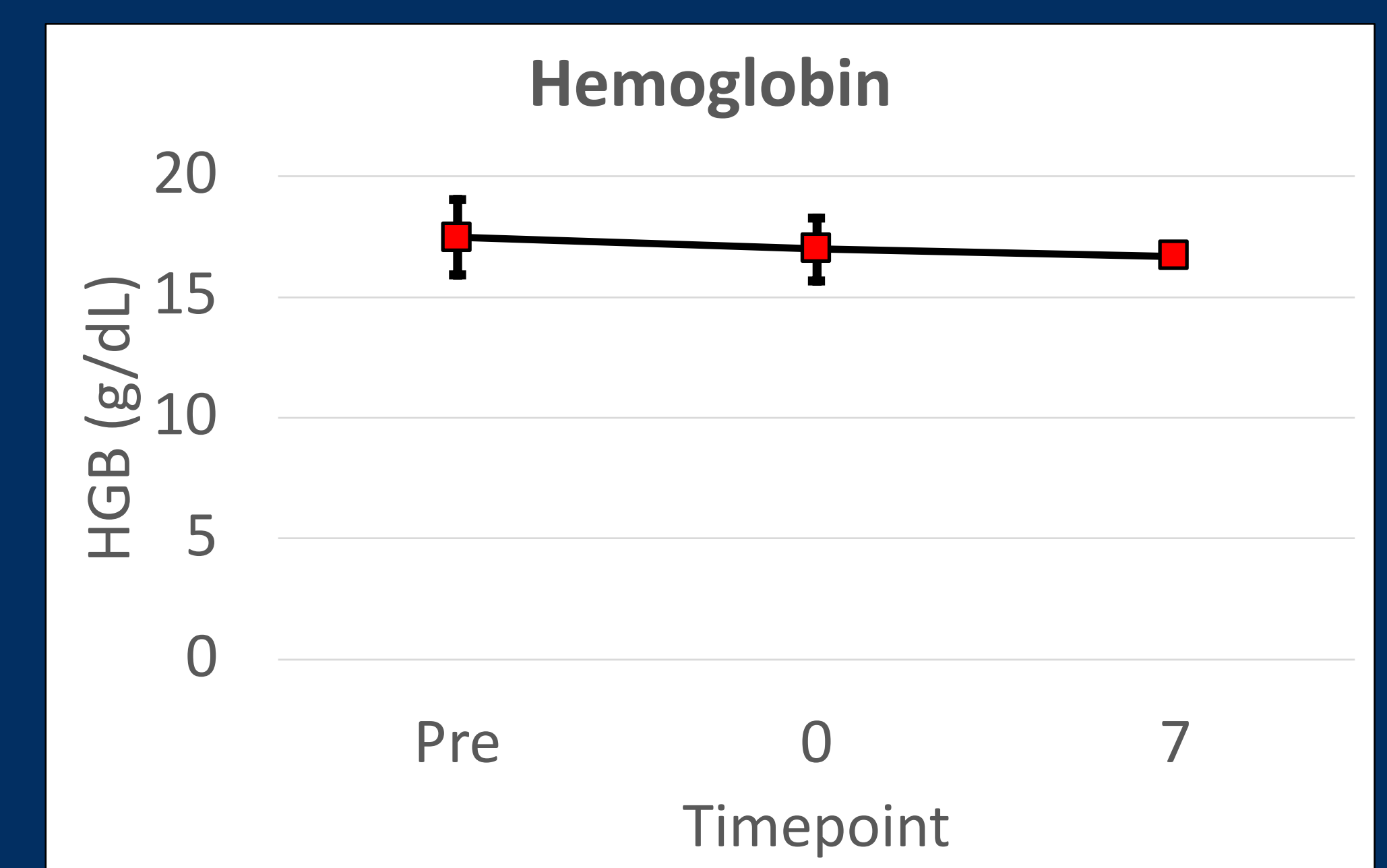
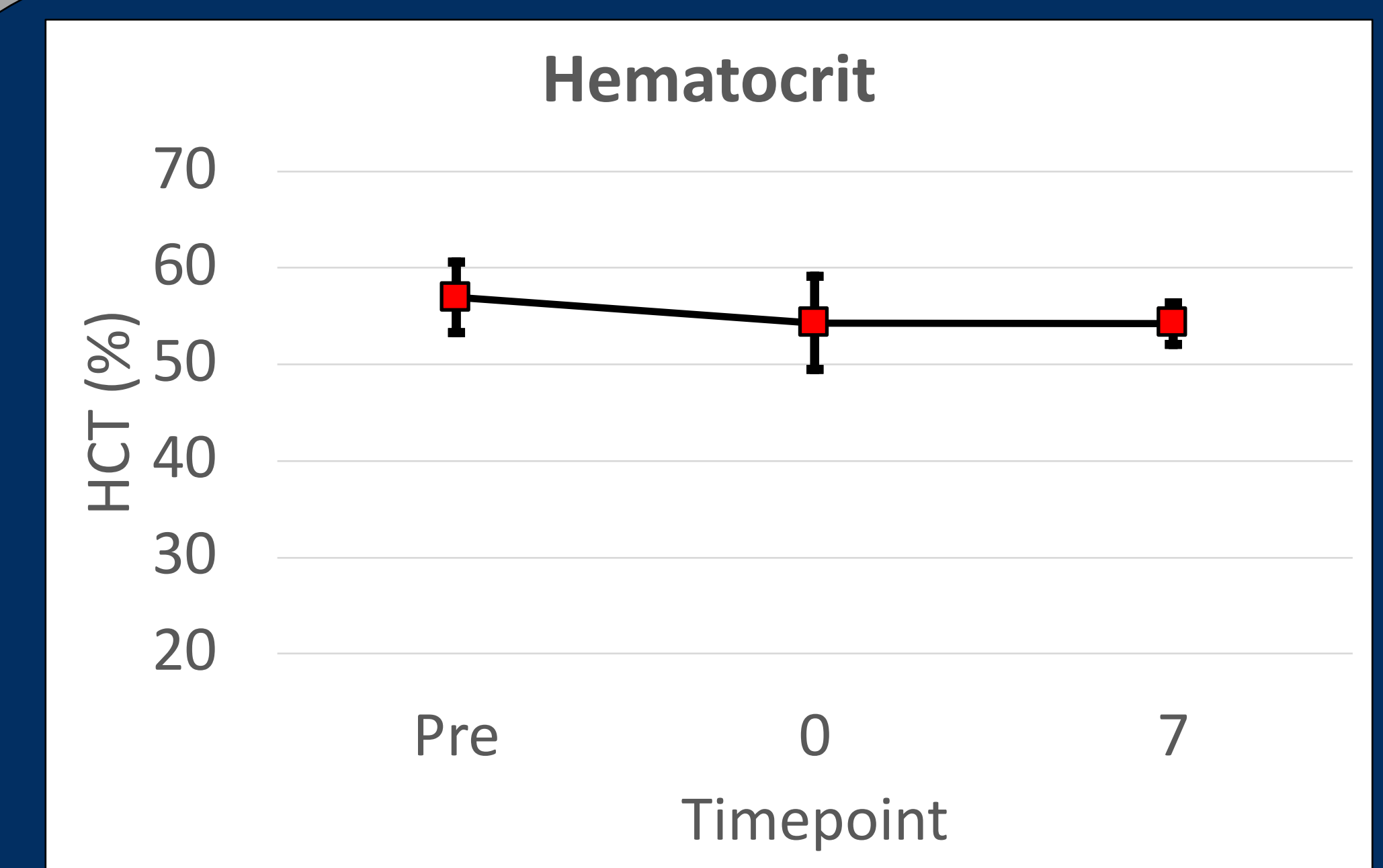
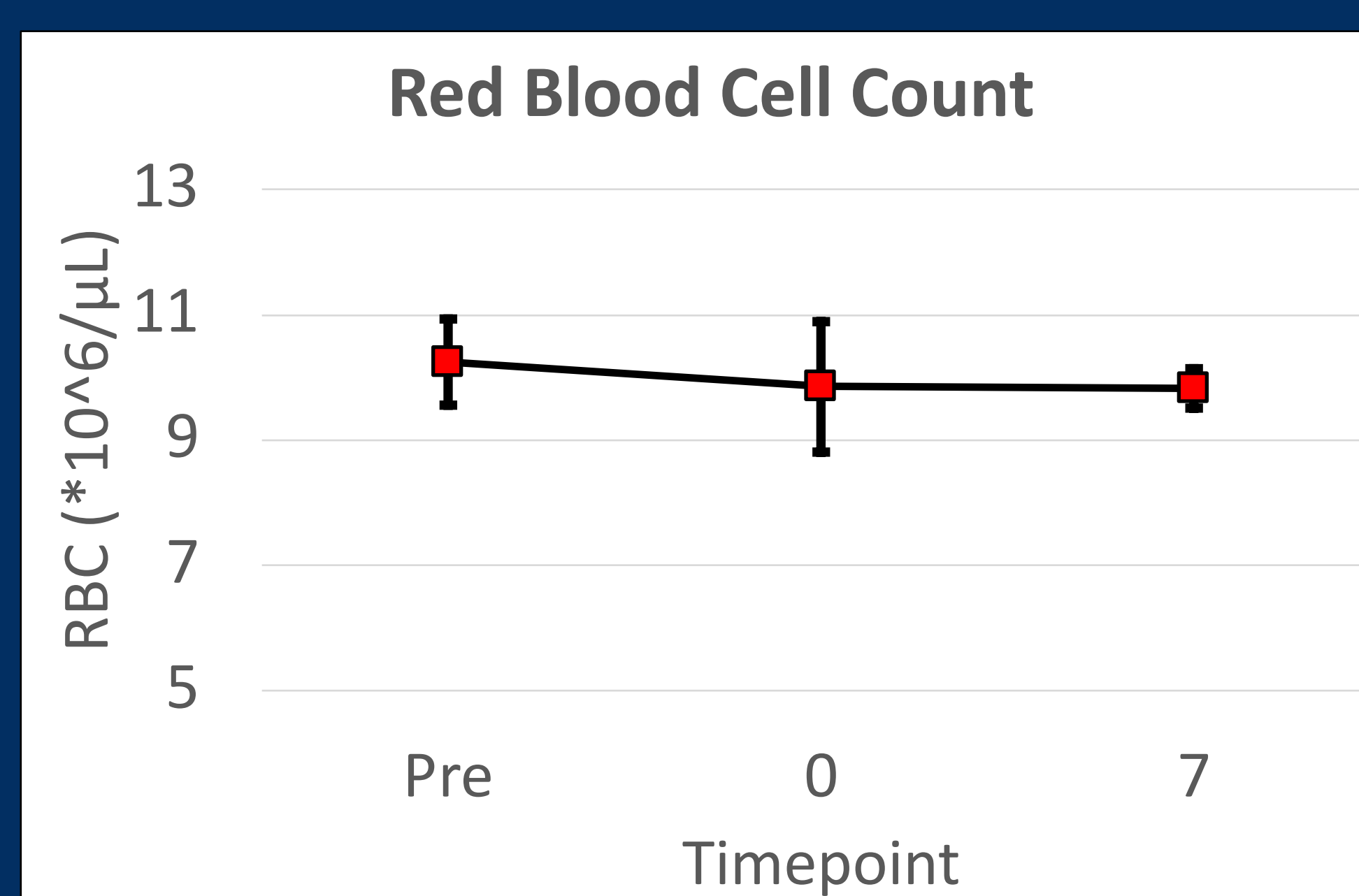
At this time, 12 blood samples have been collected from mice before feeding (n=4), immediately following (n=4), and one week after (n=4). One sample that was collected immediately following feeding was too coagulated in order to be analyzed. A CBC was performed on each sample and the white blood cell, red blood cell (RBC), hematocrit (HCT), hemoglobin (HGB), mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, red cell distribution, mean platelet volume, platelet count, and percent reticulocytes (%RETIC) values were measured (Table 1). Four more samples will be collected at two weeks after feeding.

**Table 1.** RBC, HCT, HGB, %RETIC values for each individual mouse and average at each time point.

Group Number	Before Feeding			
	RBC	HCT	HGB	%RETIC
1	11.14	61.5	19.7	1.6
2	10.01	55.5	16.4	1.8
3	10.32	57.9	17.4	3.5
4	9.50	52.9	16.4	2.0
<b>Avg +/- SD</b>	10.25+/- 0.69	57.0+/- 3.7	17.5+/- 1.6	2.2+/-0.9
Group Number	Immediately Following			
	RBC	HCT	HGB	%RETIC
1	8.83	49.2	15.7	3.1
2	10.91	58.8	18.3	2.7
3	9.80	54.9	16.9	2.5
<b>Avg +/- SD</b>	9.85+/- 1.04	54.3+/- 4.8	17.0+/- 1.3	2.8+/-0.2
Group Number	One Week After			
	RBC	HCT	HGB	%RETIC
1	10.07	55.4	16.7	5.5
2	9.46	51.0	16.2	3.1
3	10.10	55.4	17.0	4.1
4	9.66	55.1	16.8	7.5
<b>Avg +/- SD</b>	9.82+/- 0.31	54.2+/- 2.2	16.7+/- 0.3	5.1+/-1.9

Physiological values associated with anemia, including hematocrit, hemoglobin, and red blood cell count, did not show significantly different changes between the different timepoints. The percentage of cells present that are reticulocytes did not initially increase, but one week after feeding had increased significantly from the control levels (Figure 1).

**Figure 1.** Average RBC, HCT, HGB, and %RETIC values before (Pre), immediately after (0), and one week following (7) being used in mosquito feeding procedure.



## Conclusion

Physiological indicators of anemia, including red blood cell count, hematocrit and hemoglobin levels were not significantly different between mice that were utilized to feed mosquitoes and those that were not. This suggests that mice do not suffer severe anemia due to blood lost in the mosquito feeding process. That the percentage of reticulocytes in the blood is increased one week after feeding indicates that the mice are working to replenish levels of mature red blood cells. These results are not incongruous. Most likely, after the feeding process, stored red blood cells are released from the spleen in order to compensate for lost blood. In turn, the production of red blood cells is increased in order restore these reserves. Depending on the two week data, these results suggest that fewer mice could be used overall without causing undue individual distress. This would be consistent with the principles of replacement, reduction, and refinement.

## Acknowledgements

Robert Schmidt's VRSP stipend was funded by IDEXX-BioAnalytics and his research support was provided by Boehringer Ingelheim Animal Health. This study was approved by the Boehringer Ingelheim Institutional Animal Care and Use Committee.