

# Effect of EDTA on *CalModulin 1 (CAM1)* Gene Expression and Cell Viability in *Tetrahymena thermophila*

## Introduction

- *Tetrahymena thermophila* (*T. thermophila*) is a unicellular, eukaryotic ciliate that is utilized as a popular model organism.
- Ethylenediaminetetraacetic acid (EDTA) is a metal ion chelator present in many body cleansing products. Calcium ( $\text{Ca}^{2+}$ ), a metal ion present throughout the body, would be sequestered by EDTA.
- Calmodulin, short for calcium-modulated protein, is an intracellular target activated by the secondary messenger  $\text{Ca}^{2+}$ .
- The calmodulin-1 gene (*CAM1*) codes for the CAM1 protein needed for many pivotal biomolecular pathways.
- **Hypothesis:** If *T. thermophila* are exposed to EDTA, then *CAM1* gene expression will increase, and cell viability along with number of feeding vacuoles will decrease.

## Methods

- **Primer synthesis** for *CAM1* was completed through the software Oligoanalyzer.
- **Culturing** *T. thermophila* was initiated in NEFF media. Upon experimentation, all cultures were transferred into SPP media and experimental cultures were exposed to a single 24-hour dose of 5.0 mM EDTA.
- **RNA extraction** was completed with Qiagen's RNeasy Mini Kit.
- **RevertAid Reverse transcription** was used to synthesize cDNA.
- **Quantitative PCR** was performed using PowerUp SYBR Master Mix. *BTU1* gene expression was used as positive control.
- **Cell Counts** were performed with hemocytometers.
- **Feeding Assays** were performed with India Ink.

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## Results

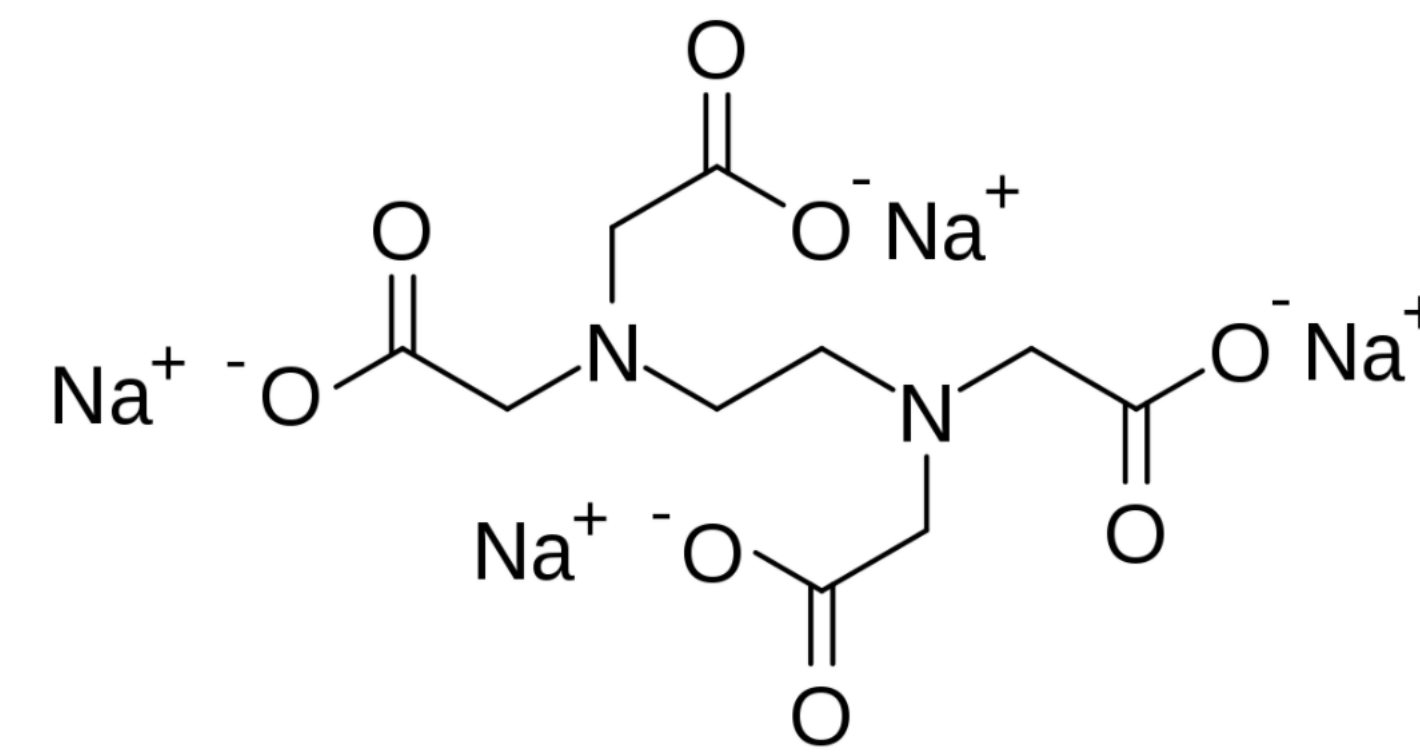


Figure 1: Chemical structure of tetrasodium EDTA



Figure 2: Image of *T. thermophila* with dyed feeding vacuoles

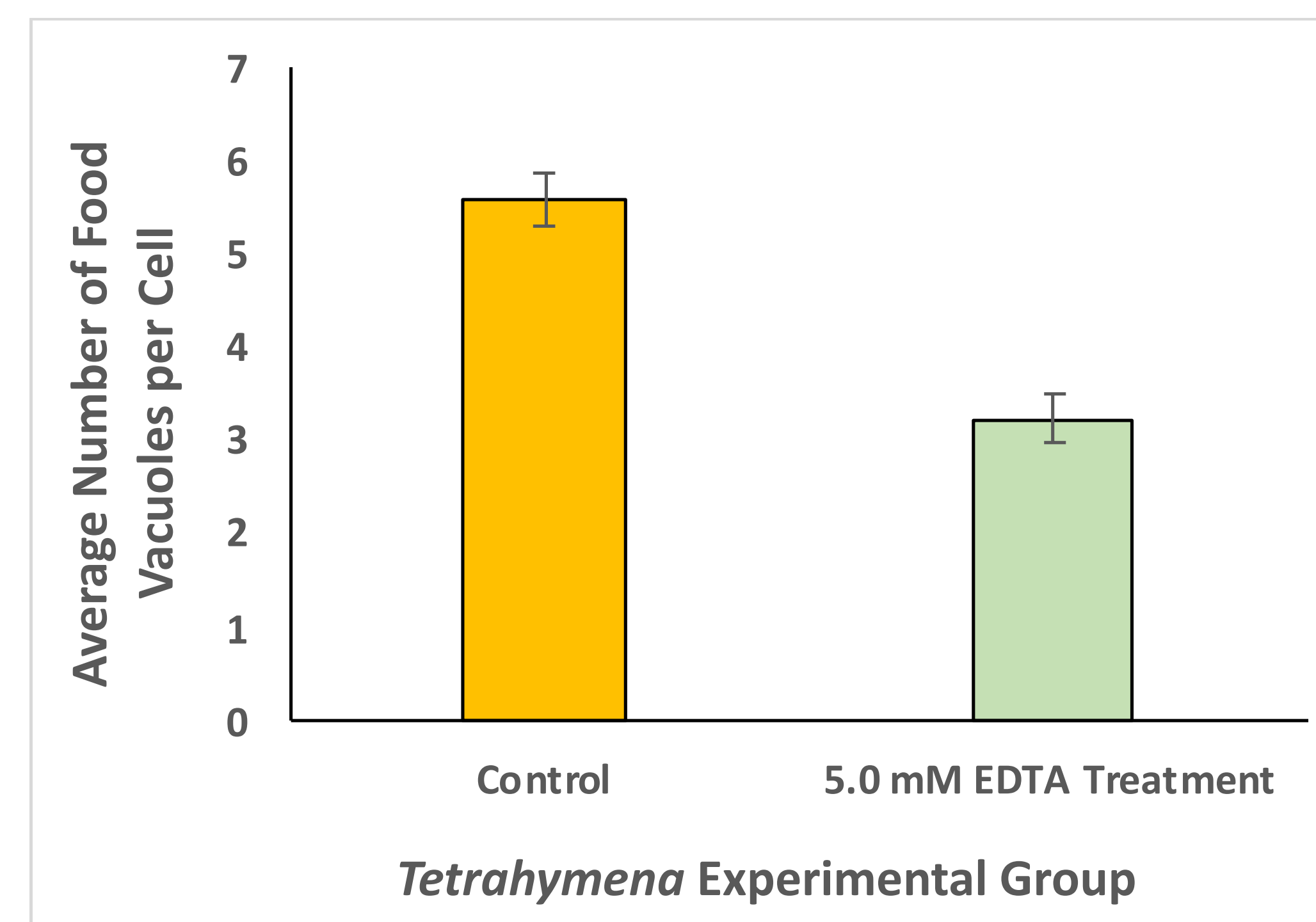


Figure 3: Average number of feeding vacuoles ( $p=1.27 \times 10^{-7}$ ;  $n=3$  for each group)

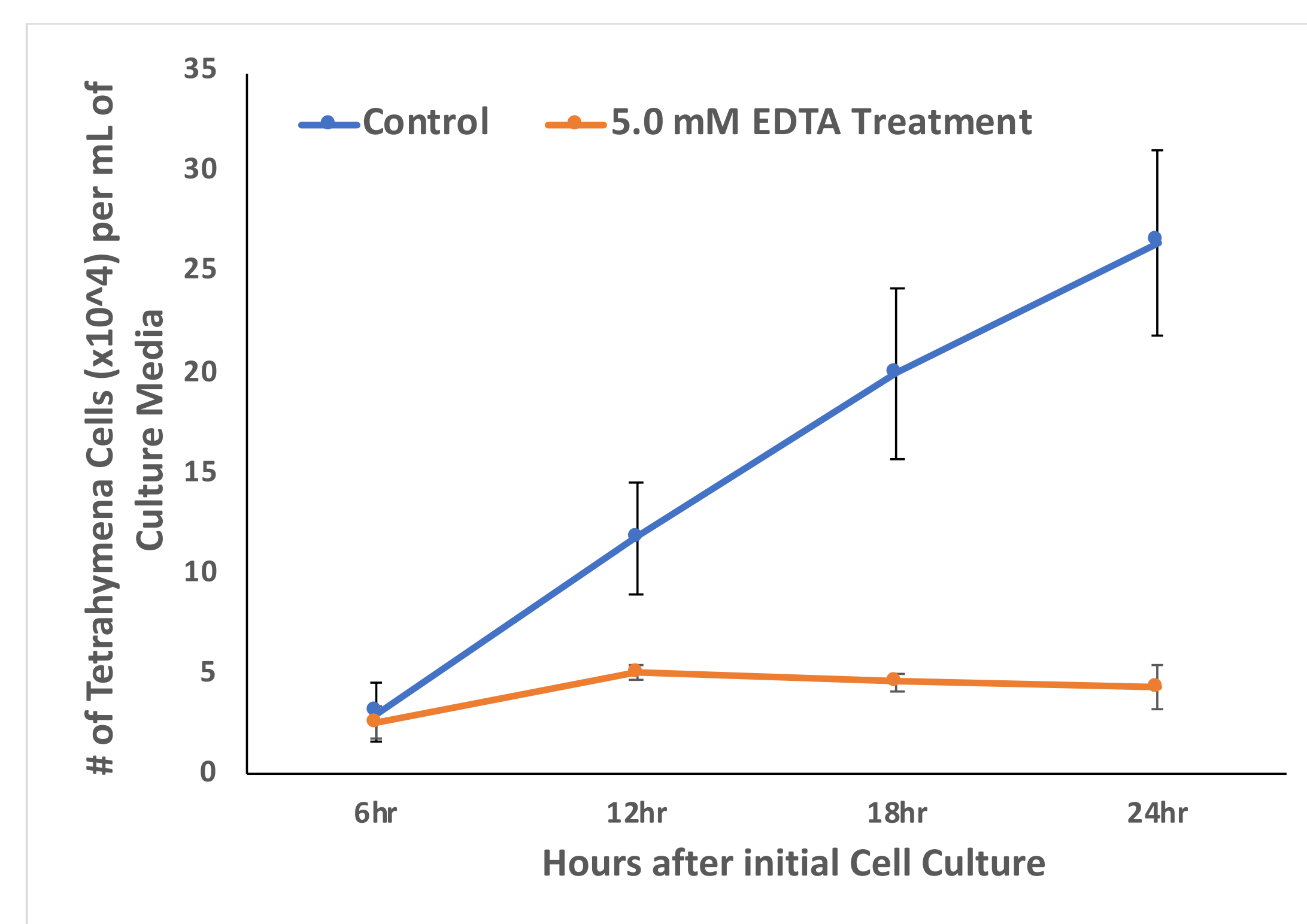


Figure 4: Average number of cells per milliliter of media (Hour 6:  $p=0.781$ ; Hour 12:  $p=0.0979$ ; Hour 18:  $p=0.0365$ , Hour 24:  $p=0.0188$ ,  $n=4$  for each group)

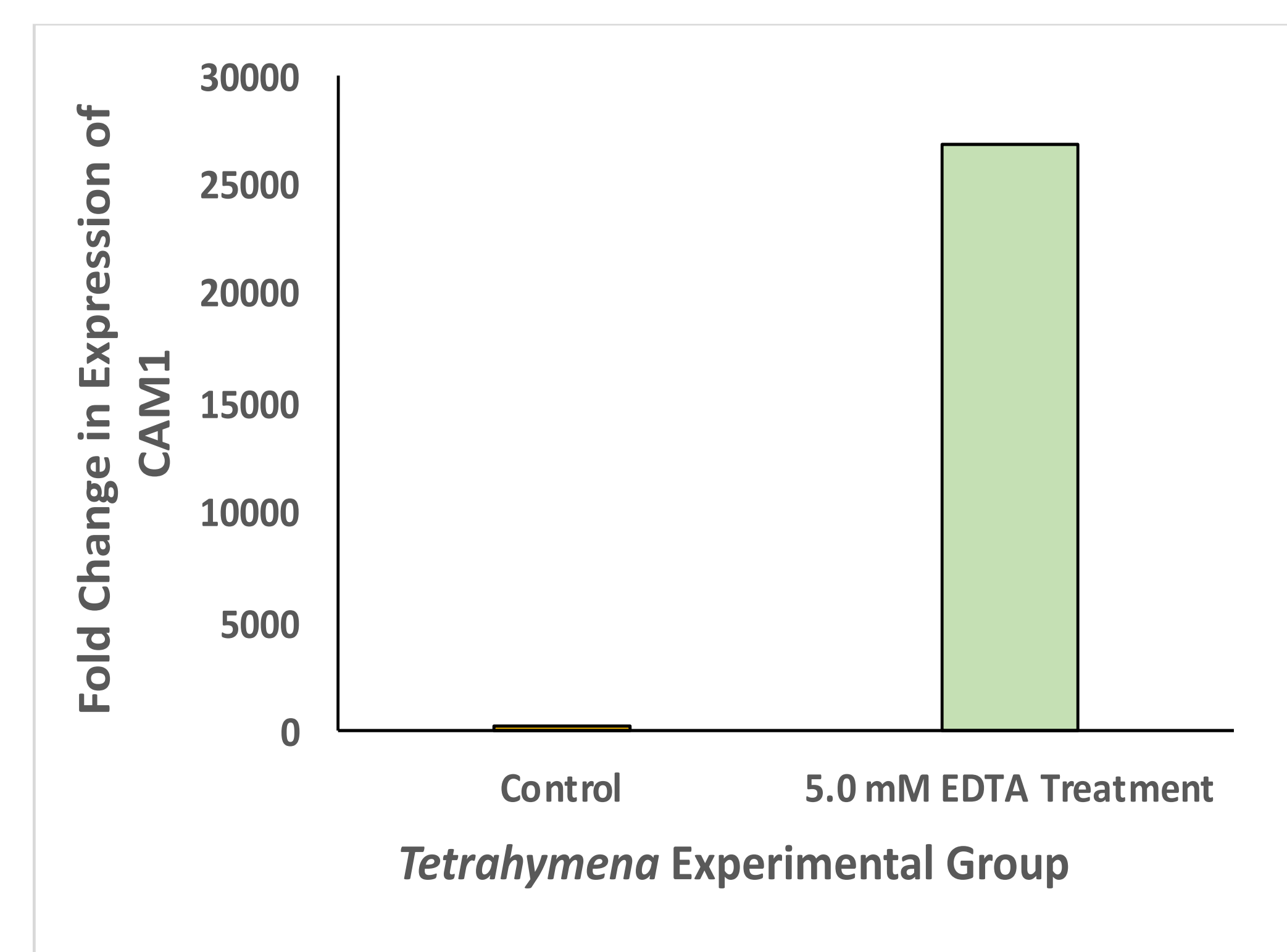


Figure 5: Fold change in expression of *CAM1* ( $n=2$  for each group)

## Conclusion

- The number of feeding vacuoles was significantly increased in the control treated *T. thermophila*.
- Cell counts showed significant differences in the 18 and 24-hour mark where *T. thermophila* were more abundant in the control.
- The single round of qPCR could not be statistically analyzed, but it did show promising results with an upregulation in *CAM1* expression in the EDTA treated cells.
- The results agree with our hypothesis regarding the number of feeding vacuoles and cell growth. In addition, the appearance of increased gene expression further supports our hypothesis.

## References

- Esposito, E., Knauth, T., Ohnstad, A., Effect of EGTA on SIT1 SIT1 Scramblase Gene Expression, Cell Viability, and Cell Growth in *Tetrahymena thermophila*. Carroll College Student Undergraduate Research Festival. Helena, MT.
- Edta: Uses, Side Effects, Interactions, Dosage, and Warning. (n.d.). Retrieved from <https://www.webmd.com/vitamins/ai/ingredientmono-1032/edta>
- Jennifer K. NYBORG, Olve B. PEERSEN. 1 August 2004. That zinging feeling: the effects of EDTA on the behaviour of zinc-binding transcriptional regulators. *Biochem Journal*. 381 (3):e3-e4.
- Shen, Y., Lee, Y. S., Soelaiman, S., Bergson, P., Lu, D., Chen, A., ... Tang, W. J. 2002. Physiological calcium concentrations regulate calmodulin binding and catalysis of adenylyl cyclase exotoxins. *The EMBO Journal*. 21(24):1-2.

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