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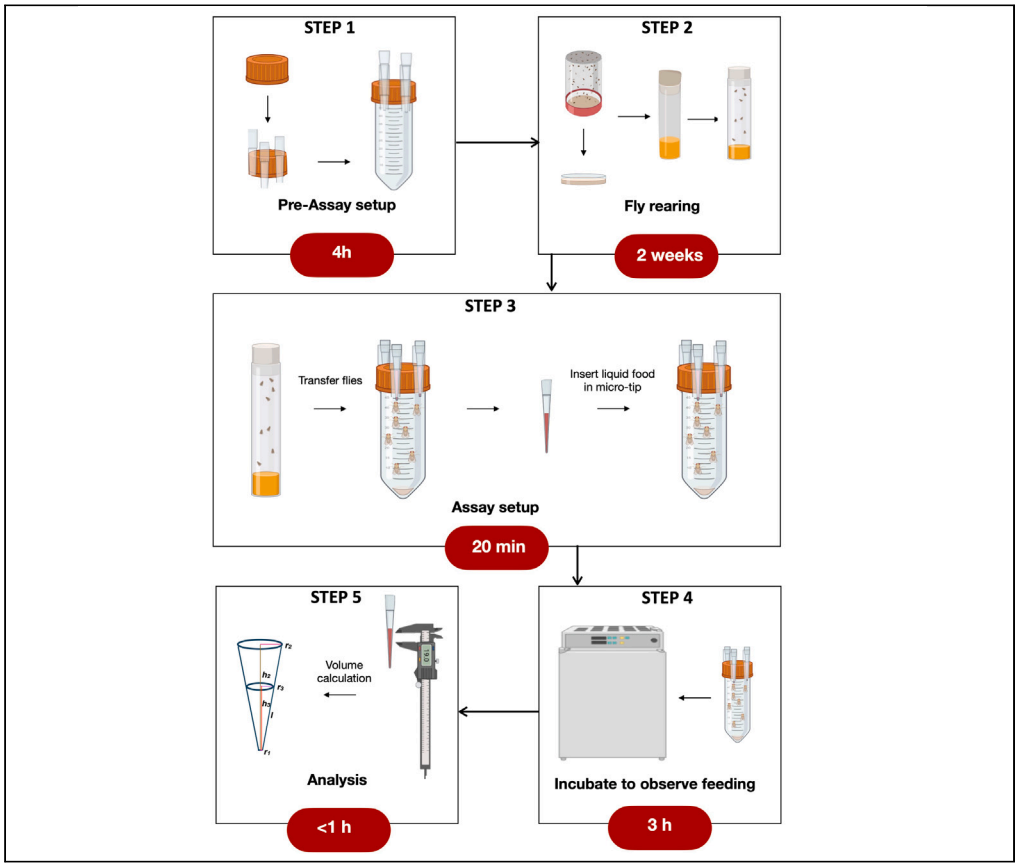
Protocol

Modified Capillary Feeder assay using micro-tips to measure real-time feeding in *Drosophila*



Capillary Feeder assay (CAFE) is a real-time feeding assay used in *Drosophila* that employs micro-capillaries, which are costly. Here, we present a modified version of the assay by replacing micro-capillaries with micro-tips, hence ensuring the same principle with cost reduction by 500 times. We developed a mathematical approach to measure volume for the conical shaped micro-tips. In this protocol, we describe step-by-step procedures of pre-assay setup along with fly rearing; assay setup included with detailed analysis for volume calculations.

Publisher's note: Undertaking any experimental protocol requires adherence to local institutional guidelines for laboratory safety and ethics.

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Highlights

Quick and efficient technique for real-time feeding

A cost-effective capillary-action-based feeding assay using micro-tips

Employed with equation of line for volume calculation

Simplified for use with greater reproducibility

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Modified Capillary Feeder assay using micro-tips to measure real-time feeding in *Drosophila*

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SUMMARY

Capillary Feeder assay (CAFE) is a real-time feeding assay used in *Drosophila* that employs micro-capillaries, which are costly. Here, we present a modified version of the assay by replacing micro-capillaries with micro-tips, hence ensuring the same principle with cost reduction by 500 times. We developed a mathematical approach to measure volume for the conical shaped micro-tips. In this protocol, we describe step-by-step procedures of pre-assay setup along with fly rearing; assay setup included with detailed analysis for volume calculations.

For further verification and use of this protocol, please refer to Segu and Kannan.[1]

BEFORE YOU BEGIN

Feeding is an essential behavior involved in foraging, adaptation, evolution, metabolism, and energy homeostasis of an organism.[2,3] It has also been shown that multiple sensory modalities contributing to food intake in *Drosophila* are conserved to those in mammals.[4] Flies extend their proboscis to ingest food. This is termed the proboscis extension response (PER).[5] To understand the neural states associated with feeding and food choices, researchers perform PER assays where a single fly is immobilized and the extension of the proboscis based on the presentation of the food is tracked. However, PER assays cannot quantify the amount of food ingested. More so, as the flies are immobilized, feeding responses associated with locomotion such as foraging cannot be studied using PER. To study feeding, spectrophotometric assays based on food traced with colors are used (dye tracer methods).[6] In those studies, food intake is quantitatively measured. These dye tracers can be used on both solid and liquid food labelled with dye.[6–8]

Although dye tracer methods efficiently measure feeding it has disadvantages. These assays used starved flies to initiate feeding because satiated flies do not feed immediately. If feeding is not induced, the food labelled with dye will not be visible for quantification as it is excreted out. As hunger can initiate multiple downstream signaling processes based on nutritional conditions like sensory perception etc., inducing hunger may change the signaling process involved in feeding.[9,10] The result of such an experiment is always bound to be a hunger induced feeding assay. Further, the flies start defecating ingested food post 30 min of feeding and thus this assay cannot be used to understand long term feeding behaviors.

To understand long-term feeding and also feeding rhythms radio-labelled food was used.[11] In this assay feeding was measured using a scintillation counter across different time points in the day.

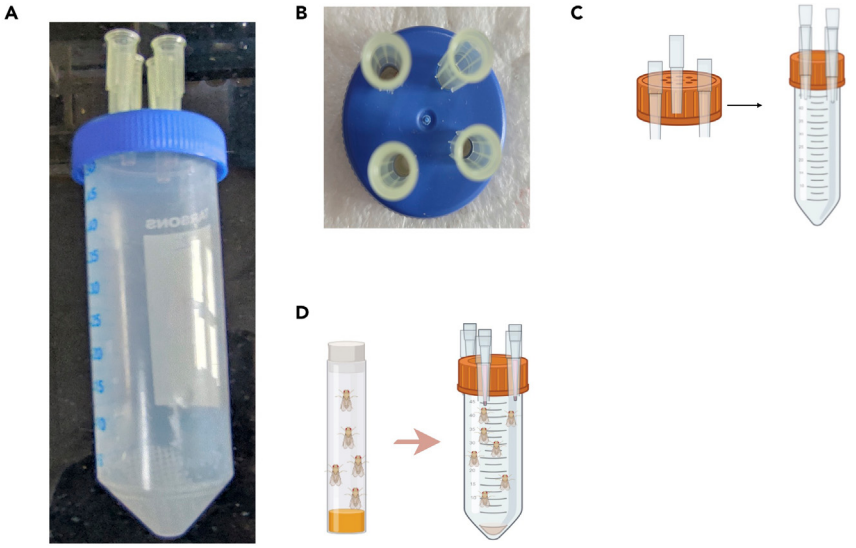

Figure 1. Modified CAFE assay setup

(A) Photograph of the lid used for the setup, (B) containing 4–200μL micro-tips fitted onto the falcon lid. These tips are cut from the bottom at 2mm Photograph of the assay setup, C) Schematic representation of the CAFE assay setup, (D) Schematic representation of the modified CAFE assay (made using *BioRender*).

However, this assay is not accurate as it does not quantify undigested and defecated food. To overcome this, a more inclusive feeding assay called Capillary Feeder assay (CAFE) was developed by Seymour Benzer's group.[12] This assay helps us measure food intake in short as well as long durations. Here, liquid food is presented to flies and the decline in the food present in the capillary is measured as the food intake.[7] CAFE measures food intake in flies without involving any starvation prior to the assay and allows us to measure food intake in real-time. Previous reports have also used CAFE with starved flies as well.[13] Apart from this, these flies can also be used in quantification using spectrophotometric absorbance methods. In this way, CAFE became one of the widely accepted protocols for understanding feeding behavior in flies.

Modified CAFE assay setup using micro-tips

Micro-capillary tubes used in classical CAFE assay are very costly (50 cents) and thus limiting the usage of the assay. Micro-tips function similarly to capillary tubes as they can hold the volume of liquid food without dispensing it under gravitational force. Simultaneously, the capillary action also replaces consumed food. In our assay, we replaced capillary tubes with 10-μL XL micro-tips costing only 0.096 cents (procured from Tarsons products limited). The length of the 10-μL tips need to be long (XL range tips) as the short tips are not long enough to protrude out of the setup and thus flies cannot access the food. The protocol below explains the CAFE assay using micro-tips for a 3 h feeding protocol modified from a previously reported CAFE assay with micro-capillary tubes.[7] The modified protocol is validated for two wild type strains *iso*^{31} *and Canton S (CS)*. Furthermore, this protocol can be used for measuring feeding rhythm and for 2-choice feeding assay to assess food choice in flies.

Preparation of CAFE setup

Timing: 2 h

1. Punch two to four holes on the lids of 50 mL conical bottom centrifuge tubes using a sharp object that can fit a 200 μL micro-tip (Figures 1A and 1B). The other empty holes are to allow air exchange in the centrifuge tube. A schematic is shown in Figure 1C.
2. Cut around 0.5 cm of 200 μL micro-tips from the tip-end.

3. Fix these pre-cut 200 μL micro-tips into the holes of the 50 mL conical bottom, centrifuge tube lid. (These cut tips are fixed onto the lid permanently and should not be disturbed).
4. Make sure that the tip fits tightly into the hole (in case it is loose you could use any non-smelling adhesive to glue the tip).
5. Introduce new, uncut micro-tips (200 μL) onto these holes to block flies from escaping.
6. These tubes are called CAFE assay tubes henceforth.

Preparation of CAFE assay tube

Timing: 4 h

7. Wash and autoclave the CAFE assay tube before the start of the experiment.
8. Close the tubes and the lids for each set of flies separately.
9. Dispense 1 g of agar-agar powder in 100 mL of MilliQ Type-II water.
10. Bring the mixture to boil, until a clear solution is obtained. This is 1% agar solution.
11. Pour 2 mL of the 1% agar-agar solution into the tubes to avoid desiccation stress during the experiment.

Preparation of liquid food

Timing: 3 h

12. Preparation 10 mL of 1M sucrose solution as a stock.
    a. Measure 3.432 g of sucrose.
    b. Autoclave Type-II MilliQ water along with empty measuring cylinders, conical flask, beaker and magnetic beads.
    c. Dissolve 3.432 *g* of sucrose with sterile water in a Laminar Air Flow (LAF) chamber. (Sucrose is a hydrated sugar. Hence, slowly dissolve the sucrose by adding water gradually. Make up the volume once all the sucrose is dissolved. Alternatively, the solution can be filter sterilized as well.).
    d. Store this sucrose solution at −20°C. The solution can be stored for up to 6 months.
13. Prepare 0.25 v/v food color solution (red) for the experiment. (we got a commercially available stock of 2.5 v/v color solution (10X), which we have diluted 10 times for all experiments) (The concentration and the color of the liquid food color is not important for the protocol, it is only used for identification purposes).
14. Prior to the experiment, freshly prepare 0.1M sucrose solution from the 1M stock solution. It is always advised to prepare fresh solution of the liquid food. The solution could be used for 2–3 days.

Fly rearing and breeding

Timing: 2 weeks–1 month

15. *Iso*[31] and *Canton S* (*CS*) flies in the ratio of 2:1 (female: male) are used to set up the *Drosophila* cage.

Note: Double the number of female flies are used to avoid increased mating, which could lead to female death.

16. Change the 60-mm petri-plate containing apple-juice agar every 24 h for the time course of the larval collection. (Alternatively, transfer males and females to fresh media bottles every two days once and incubate them).
    Apple-juice agar preparation:
    a. Dispense 12 *g* of sucrose in 350 mL of 100% Apple juice.
    b. Dispense 24 *g* of agar-agar powder in 700 mL of Type-II MilliQ water.

c. Melt the agar solution in a microwave until agar melts completely.
d. Mix the sucrose solution in the agar solution to obtain apple-juice agar media. (The sucrose solution is not heated above 70°C to avoid charring of the sugars and thus they are mixed separately. The plates should be stored at 4°C and can be used for up to a week.
e. Pour the media into 60 mm petri plates for larval collection.

17. Collect 50 first instar larvae into each of the *Drosophila* vial containing fly media. Five such vials are collected for each experiment.
18. Incubate these vials containing 1st instar larvae in an incubator maintained with 25°C temperature, 60% relative humidity and 12 h light : 12 h dark condition where light came on at Zeitgeber Time (ZT 00) and went off at ZT 12 .
19. Post nine days freshly emerged flies are separated based on sex by anaesthetizing the flies using CO_2.
20. Collect 16 male flies post eclosion (female flies and mixed sex flies can be used for the assay) in each *Drosophila* food vial until the day of the experiment. This is done to avoid overcrowding and malnutrition.
21. 2-day-old flies are used in the following experiments. (The age of the fly can also be determined based on the experiment).

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Chemicals, peptides, and recombinant proteins		
Agar agar type 1	Himedia	GRM666
Sucrose	Himedia	MB025-500G
Yellow cornmeal	Quaker	N/A
Synthetic liquid food color red and green	Amazon.in	https://www.amazon.in/dp/B07XLL7JG7?ref_=cm_sw_r_mwn_dp_E3FNHBJG09M1YQP8GV1J
Dextrose anhydrous	Nice Chemicals	G10229
Dry active yeast	Star Yeast	N/A
Experimental models: Organisms/strains		
iso31	Gift from Dr. Sheeba Vasu, JNCASR	
CantonS	Gift from Dr. Sheeba Vasu, JNCASR	
Software and algorithms		
GraphPad Prism 9	GraphPad	https://www.graphpad.com/features
Other		
Conical bottom centrifuge tubes (falcon tubes - 50 mL)	Tarsons (Others)	546041
200-µL micro-tips	Tarsons (Others)	521014
10-µL XL micro-tips (XL range only)	Tarsons (Others)	521050
Micro capillary tubes, calibrated (1–5µL)	Sigma-Aldrich	P0549-1PAK
Vernier calipers	Amazon.in	
Drosophila incubators	Panasonic	Mir-154

MATERIALS AND EQUIPMENT

0.1M Sucrose solution:

Reagent	Final concentration	Amount
Sucrose solution (1M)	0.1M	100 µL
Liquid food color (2.5 v/v) (10 X)	0.25 v/v (1X)	100 µL
ddH_2O / Sterile MilliQ H_2O	N/A	800 µL
Total	**N/A**	**1000 µL**

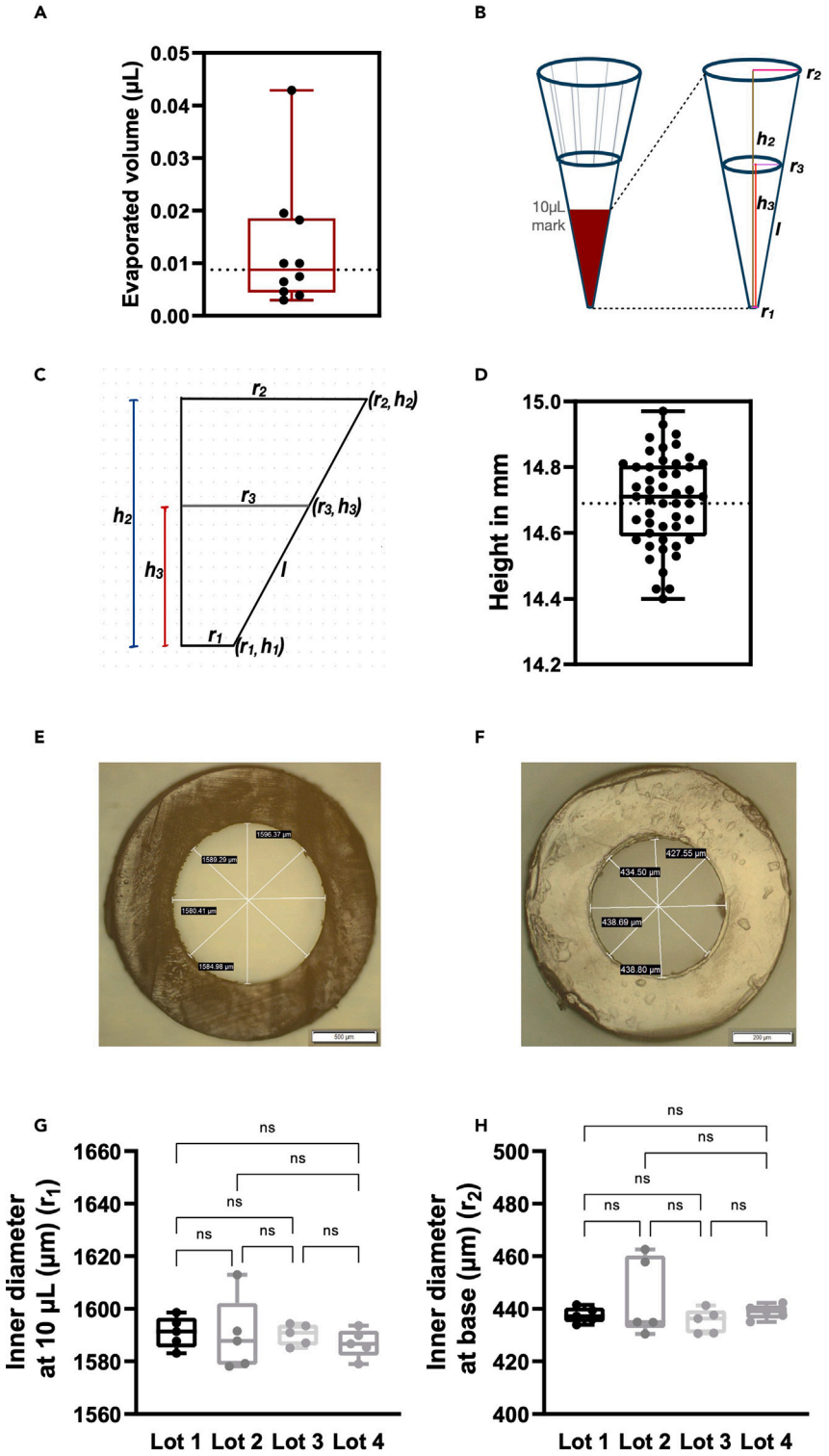

Figure 2. Volume calculations for the protocol

(A) Evaporated volume after 3h feeding assay, (B) Schematic representation of the micro-tip showing the radii and height used for calculations, (C) 2-Dimensional diagram of the cone showing calculations used to calculate the volume where r_1 is radius at the base; r_2 is the radius at the 10µL mark; h_1 is the height of the tip at base; h_2 is the height of the tip from base to 10µL mark; r_3 is the new radius after feeding; h_3 is the new height after feeding from the base and l is

Figure 2. *Continued*
the line showing the length of the tip, (D) Average heights measured at 10μL mark (h_1) using vernier calliper for 60 different micro-tips, (E) Representative image of the inner diameter of the tip at 10μL mark, (F) Representative image of the inner diameter at the bottom of the tip, (G) Average diameter of the tip at 10μL mark from different lots, (H) Average diameter at the bottom of the tip from different lots.

STEP-BY-STEP METHOD DETAILS

3 h CAFE assay setup

Timing: 4 h; setup, 20 min; assay time, 3 h

CAFE assay measures real time feeding. This feeding assay method does not involve flies to undergo starvation to measure feeding. Our method utilizes micro-tips to measure feeding unlike the classical capillary tubes. The assay is performed at ZT 01, i.e., 1 h post lights on condition. Flies feed the highest post lights on condition and thus this time was used.

1. Transfer age matched flies into the CAFE assay tubes without anaesthetizing them (as shown in Figure 1D).

   Note: We used 16, two-day old male flies in each assay tube, this is to avoid overcrowding of the assay tube. The number, sex and age of the fly can be adjusted according to the experimental needs.

2. Ensure to cover all the holes of the assay tube with uncut tips as described (This is to ensure no fly escapes from the tube).
3. Set up 3–5 technical replicates for each feeding assay.
4. Once all the tubes are setup with flies, remove one of the uncut 200 μL tip and introduce 10 μL (XL) tip containing 10 μL of liquid food with 0.1M sucrose solution.
5. Set up an empty tube with only food as the evaporation control for each experiment.

   Note: The evaporated volume is subtracted from the fed volume. The evaporation loss was calculated and is given in Figure 2A. Volume dissipated was accounted to be on an average $\geq$ 0.008 μL, with an SD of 0.012).

6. Carefully place the assay tubes inside a 25°C incubator for 3 h under 60% relative humidity for the feeding to be recorded.
7. After 3h (at ZT 04) using a Vernier caliper measure the height of the remaining food from the base.

   Note: Ensure the tips are held straight while measuring the height.

8. Measure the height for all the tubes.
9. Calculate the volume consumed by using the height measured as described below.
10. Freeze the tubes at −20°C for 1 h to count the number of flies.
11. Count the number of flies in each tube and note it along with the height corresponding to that particular assay tube.

    Note: As the flies are transferred without anaesthetizing at the start of the assay and thus one or two flies could escape at times and step 8–9 are followed to account for this error.

12. Divide the volume calculated by the number of flies to obtain average volume fed by each fly.
13. The flies post the assay can also be used to measure feeding through dye tracer methods[8] as mentioned in the introduction, by homogenizing the flies. Thus, this assay provides us volume as well as color intensity.

⚠ CRITICAL: The accuracy of the results is critically based on the volume introduced. Therefore, it is important to pipette out exact volumes. It is advisable to have an internal evaporation control for all experiments.

Volume calculation using deduction method

Micro-tip is an open-ended cone unlike the cylindrical capillary tube. Volume of an open-ended cone is given by the formula

$$V = \left(\frac{1}{3}\right)\pi\left\{(r_1)^2 + (r_1 r_2) + (r_2)^2\right\} \times h$$

where r_1 and r_2 are the two radii of the cone and h is the height of the cone.

To calculate the volume from an open-ended cone, one requires both the radii as described above. That meant cutting the tips post each experiment to measure the volume. This is a very tedious process and introduces large errors. To overcome this we deduced a method using 2-point line equation to calculate the unknown radius.

14. Calculating the slope of the line (l).

Note: The 2-point line equation is given by the formula $y - y_1 = m(x - x_1)$ *where* $m = \frac{y_2 - y_1}{x_2 - x_1}$ *and x and y are the co − ordinates*

a. According to the equation the x, y coordinates are the radius and the height respectively (Figures 2B and 2C).
b. To calculate the radius, measure the inner diameter (id) at h_1 (y_1) and h_2 (y_2) using an optical microscope (Figures 2E and 2F).
c. Measure the variance using multiple tips and further to account for the manufacturer's variability measure the radius from different lots/bags (Figures 2G and 2H).
d. Measure the height h_2 (at 10 μL mark) of multiple tips using Vernier caliper (Figure 2D).

Note: Height h_1, being at the base of the tip is always going to be zero.

e. Calculate the mean radii and height to be used for slope calculations.
f. With the available information, we are now able to calculate the slope of the equation, which is given by

$$m = \frac{h_2}{r_2 - r_1}$$

The mean values of radii and height from our calculations is given below

Attributes	Calculations
r_1	0.21744 mm
r_2	0.703881 mm
h_2	14.69 m

g. Calculate the slope of the line (*l*) using the calculated radii and height. Using the above values, we calculated the slope of the line (*l*) to be 30.1989.

Note: We used this value in all our experiments.

h. Using this slope of the line, any point on the line (*l*) can be calculated from the 2-point line equation.

i. Calculate the unknown radius (r_3) after feeding by using the height measured at the end of the experiment.

⚠ CRITICAL: The above slope calculations used throughout the assay are specific to the Tarsons company Ltd. micro-tips used by us. Alternatively tip from any brand can be used after determining the radii and the height.

15. Calculating the new radius of the tip post feeding:
    a. The equation of the line (l) with radius r_1 and r_3 and h_1 and h_3 with slope m, is represented as follows

$$h_3 - h_1 = m\,(r_3 - r_1), where$$

$$h_3 = height\ of\ the\ tip\ after\ feeding,$$

$$h_1 = height\ at\ the\ base,$$

$$r_3 = radius\ at\ the\ point\ of\ volume\ remaining\ in\ the\ tip$$

$$r_1 = radius\ at\ the\ base$$

    b. We know that $h_1 = 0$, and the unknown radius (r_3) is thus

$$r_3 = \left(\frac{h_3}{m}\right) + r_1$$

    c. Calculate radius r_3 using the above equation.
    d. Calculate the volume of the new cone (leftover food) using the given formula.

$$V_1 = \left(\frac{1}{3}\right)\pi\left\{(r_1)^2 + (r_1 r_3) + (r_3)^2\right\} \times h$$

    e. To calculate the fed volume (V_2) subtract V_1 from the initial volume i.e., 10 μL and to account for evaporation, subtract the evaporated volume from $V2$.

$$V_2 = Total\ Volume - V_1 - Evaporated\ volume$$

    f. To calculate the average volume fed by each fly V_3 divide the fed volume by total number of flies $V_3 = \frac{V_2}{Total\ number\ of\ flies}$

Note: From the radii and height obtained from our method, we back calculated the volume of the cone at the 10μL mark. This was found to be 10.70 μL. The difference is assumed to be experimental error caused from variance in calculations. The total volume introduced at the start of the assay is thus taken as 10.70 μL and not 10 μL in our experiments.

CAFE assay setup for feeding rhythm

Timing: setup, 20 min; assay time, 12 h

16. Culture flies in corn-meal dextrose medium before the start of the experiment.
17. Follow steps 1–10 from 3h feeding setup starting from ZT 01.

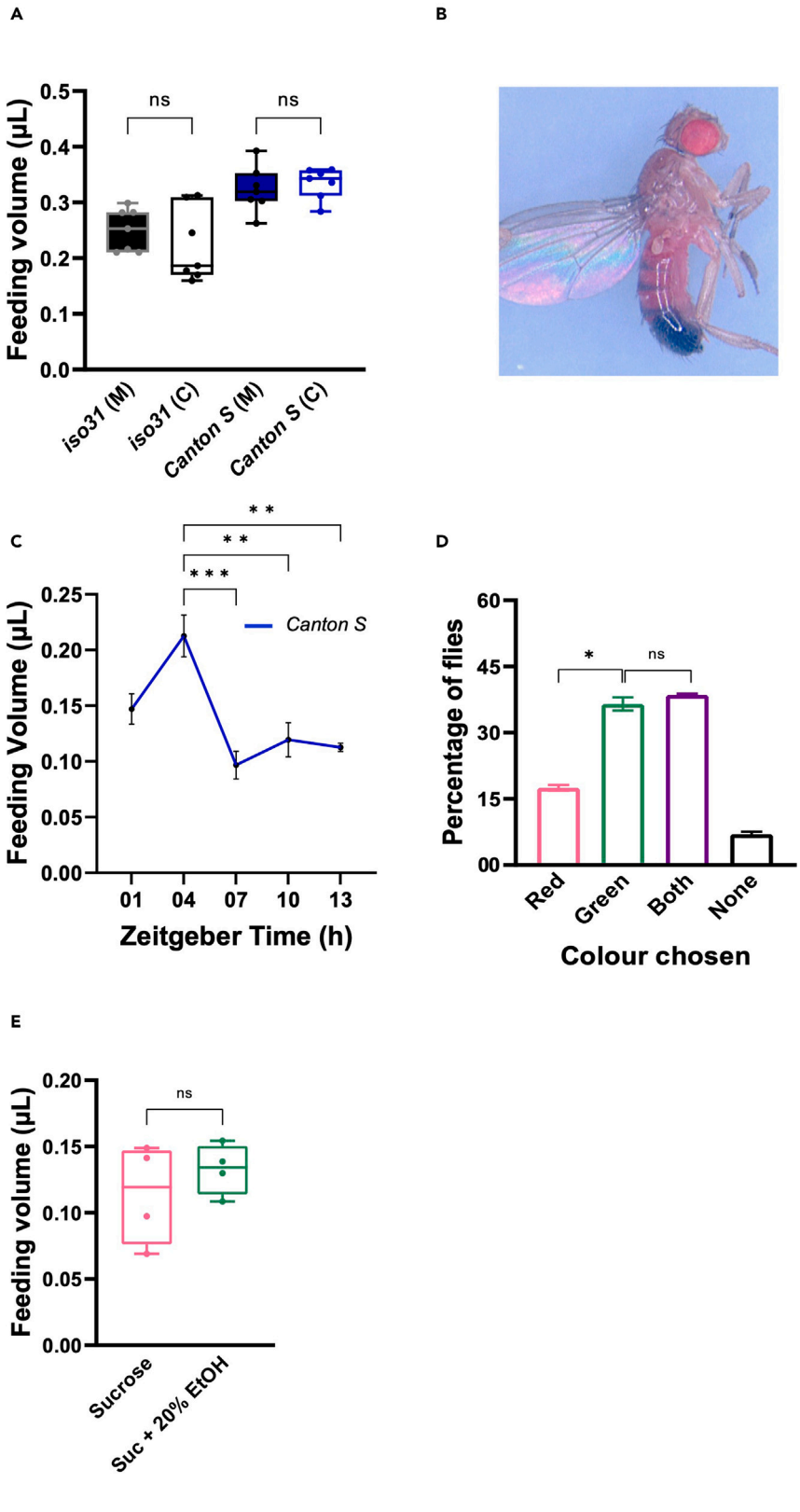
A
Feeding volume (µL)
0.5
0.4
0.3
0.2
0.1
0.0
ns
ns
iso31 (M)
iso31 (C)
Canton S (M)
Canton S (C)
B
C
* *
* *
* * *
Canton S
Feeding Volume (µL)
0.25
0.20
0.15
0.10
0.05
0.00
01
04
07
10
13
Zeitgeber Time (h)
D
Percentage of flies
60
45
30
15
00
*
ns
Red
Green
Both
None
Colour chosen
E
Feeding volume (µL)
0.20
0.15
0.10
0.05
0.00
ns
Sucrose
Suc + 20% EtOH

Figure 3. Validation of the assay

(A) 3h feeding compared between capillary tubes and micro-tips in *Iso*^{31} and *Canton S (CS)* (Student's unpaired t-test *Iso*^{31} (M) v/s *Iso*^{31}(C) $p > 0.05$; *CS* (M) v/s *CS* (C) $p > 0.05$), (B) Representative image of the fly after 3h feeding in CAFE setup using micro-tips, (C) Feeding rhythm of CS flies in the 12h light phase under LD with 3hr intervals (One way ANOVA followed by Sidak's multiple comparisons ZT 04 v/s ZT 07 $p < 0.001$; ZT 04 v/s ZT 10 $p < 0.01$; ZT 04 v/s ZT 13 $p < 0.01$), (D) Percentage of the flies with red and green color abdomen after the 2-choice feeding assay, where red indicates percentage of flies which chose red colored food (0.1M sucrose solution), green indicates percentage of flies which chose green colored food (0.1M sucrose solution along with 20% ethanol), both indicate the percentage of flies which choose both the food and none indicate the percentage of flies which had no color present in their abdomen (One way ANOVA followed by Sidak's multiple comparisons red v/s green $p < 0.05$); (E) 3h feeding volume of *Canton S* after 2-choice feeding assay where red food color was associated with 0.1M sucrose solution and green color was associated with 0.1M sucrose along with 20% Ethanol (Suc + 20% EtOH).

18. To assess the feeding rhythm, measure food intake at every 3-h intervals. We recommend using a new set of flies for every 3h time interval as the food available during the experiment is only sucrose and overtime this could affect the metabolism and thus food intake.

CAFE assay for 2-choice feeding:

Timing: setup, 20 min; assay time, 12 h

2-choice feeding assay is conducted to determine the innate preference for food in flies. In the previous studies, 2-choice feeding assays were conducted on pre-starved flies.[14,15] In our method, flies without any pre-conditioning are used. To test 2-choice feeding assay we used alcohol laced food along with sucrose. Although, alcohol was used here, the assay is malleable for other choices of food based on experimental needs.

19. Introduce two different sources of 10μL of liquid food associated with different colors (red and green into the CAFE assay tubes.
20. Follow steps 1–10 from 3h feeding protocol to calculate the fed volume.
21. Post measuring the volume, further quantify the choice made by analyzing the abdomen color of the flies under the microscope. Based on the percentage of flies chosen under each color the choice preference is calculated.

EXPECTED OUTCOMES

Validation of the assay

To validate our assay, we assayed the amount of food intake for 3h in micro-tips (M) as well as in the capillary tubes (C) simultaneously in two different wild type strains namely *Iso*^{31} and *Canton S (CS)*. We measured the fed volume in both the conditions and we did not find any significant difference in food intake between both cases (Figure 3A). In our recent study, we have also validated the modified CAFE setup with w^{1118} wild type strain.[1] This indicates that our modified protocol can be used to measure food intake via CAFE method. To further confirm if the flies were indeed feeding we observed their abdomen for the presence of red color that was incorporated in the food (Figure 3B).

Feeding rhythm

Feeding rhythm is assayed to check the impact of circadian clock on feeding. It has been previously shown that feeding is significantly higher during the morning hours.[13] Results of our modified protocol also showed that the feeding was highest in the morning post lights on at ZT 04 (Figure 3C). Feeding decreased as the day progressed with the least feeding in the mid-afternoon. This result showed a rhythm in food intake with a peak during the morning. The feeding rhythm for the night phase was not recorded.

2-Choice feeding assay

2-choice feeding assays are performed to understand the food preference in flies. It is previously shown that flies prefer alcohol-laced food.[9] To test 2-choice feeding we used *Canton S* flies tested

for 20% alcohol along with 0.1M sucrose solution (green color solution) and 0.1M sucrose solution without alcohol (red color solution). As we are measuring food preferences, some flies may choose both the available choices and to recognize this we need to use colors such that the secondary color may be easily visualized. Hence, we have used red and green color in our assay.

Like previous reports, we quantified the number of flies fed with alcohol-laced food from food presented without alcohol by using the abdomen color. We observed a significant increase in the percentage of flies fed with green colored food when compared to the red color. A significant percentage of flies did indeed choose both the food components resulting in dark- blue colored abdomen (Figure 3D). However, when we measured the fed volume we did not find any significant difference between the red and green colored food intake (Figure 3E). This indicated the flies indeed preferred alcohol laced food and the initial preference of food could be influencing the volume of food consumed.

TROUBLESHOOTING

Problem 1

Conditions where feeding is not recorded.

This occurs in two cases:

- When the 10μL tips used in the assay is not projected out into the assay tube.
- Secondly, when the 200μL tip is not cut properly.

Potential solution

To avoid this ensure the tip is projected out before the start of the experiment.

Problem 2

Using mean height length for the assay.

This creates two issues due to pipetting errors:

- Obtaining negative volume post the assay (Over-pipetting): A negative value indicates that the mean initial height value is smaller than the height measured post the assay. To avoid this one could eliminate the outliers and repeat the experiment.
- Overestimation due to under-pipetting: When under-pipetting occurs we overestimate the feeding and this cannot be estimated as one does not observe a negative value.

Potential solution

To avoid these issues ensure strict pipetting practices. We advise using the same pipette every time. Alternatively, to ensure efficient feeding the initial height can be measured before the start of the experiment.

Measuring the initial height means also measuring the initial radius at the mark for every tip. This is tedious and to overcome this one could check the range for the initial height and ensure each initial height belongs to the range and use the slope accordingly. In this case, over-pipetting and under-pipetting scenarios are avoided.

LIMITATIONS

This modified CAFE assay is validated and reproducible with micro-tips obtained from Tarsons limited. Nonetheless, the method is adaptable for other sources accordingly. As mentioned earlier, from the radii and height obtained from our method we calculated the volume of the cone at the 10 μL mark. This was found to be in 10.70 μL. The difference is assumed to be experimental error caused due to experimental variance and the usage of mean values for slope calculations. The total volume introduced at the start of the assay is thus taken as 10.70 μL and not 10 μL in our experiments.

To assess the feeding rhythm in every 3h interval in 24h, we flipped the flies into CAFE assay tubes at night. This disturbance lead to increased feeding in the night and thus we were unable to accurately measure the food intake during the night phase for the feeding rhythm assay.

In the 2-choice feeding assay, the alcohol evaporation was not accounted for and thus the assay sensitivity is comprised. The assay was conducted to demonstrate 2-choice feeding in flies and the assay is not mandatorily based on alcohol as a choice to test. It can be modified based on the experimental condition (For example, quinine and sugar, or two different concentrations of sugar etc., can also be used). Furthermore, the influence of different colors on the output have not been tested.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Dr. Nisha N. Kannan, nishankannan@iisertvm.ac.in.

Materials availability

No new materials were generated during the course of the protocol. The 10μL tips used in this assay are available from Tarsons Limited.

Data and code availability

The protocol includes all datasets generated or analysed during this study. Raw data will be provided on request.

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AUTHOR CONTRIBUTIONS

Conceptualization, A.S., D.R.; data curation, A.S.; formal analysis, A.S.; methodology, A.S., D.R.; writing, A.S., N.N.K.; validation; A.S., D.R.; funding acquisition, N.N.K.; investigation, N.N.K.; project administration, N.N.K.; supervision, N.N.K.; resources, N.N.K.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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