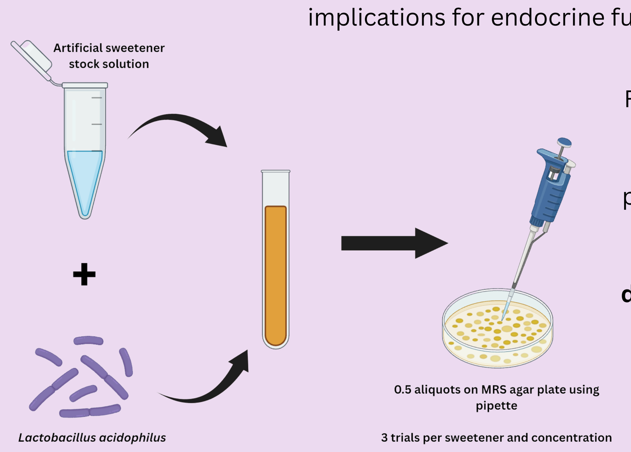
The Effect of Artificial Sweeteners on *Lactobacillus acidophilus* and Its Potential Impacts on the Female Endocrine System

How does the consumption of artificial sweeteners affect the growth of *Lactobacillus acidophilus*, and what are the potential implications for female endocrine and reproductive health?

Aiza Farooq | Signature School

Overview

Artificial sweeteners are widely used as **sugar substitutes, especially by women seeking to reduce calorie intake and maintain body image in response to societal and cultural pressures.** While these products are often marketed as healthier alternatives, growing research suggests they may disrupt the gut microbiome. Because the **gut microbiome is closely connected to the female reproductive system through the gut-vaginal axis,** these disruptions may have broader implications for endocrine function and fertility.



Results showed that **higher concentrations significantly altered colony growth patterns**, suggesting microbial stress and potential dysbiosis. These findings indicate that artificial sweeteners **may disrupt beneficial bacteria in a concentration-dependent manner,** providing insight into a possible biological mechanism **linking dietary habits to gut health and female reproductive outcomes.**

Image created using BioRender

Introduction / Background

Implications of Artificial Sweeteners

- Nearly half of American women are actively dieting daily (UAMS). Sugar-free diets are popular due to caloric reduction goals, but may involve high consumption of non-nutritive sweeteners (NNS)
- Common sweeteners: Aspartame (Equal), Sucralose (Splenda), Saccharin, and Ace-K are widely used in snacks, desserts, and beverages
- While they may reduce sugar intake, chronic consumption is associated with gut microbiome disruptions, hormonal imbalance, and reproductive system dysfunction

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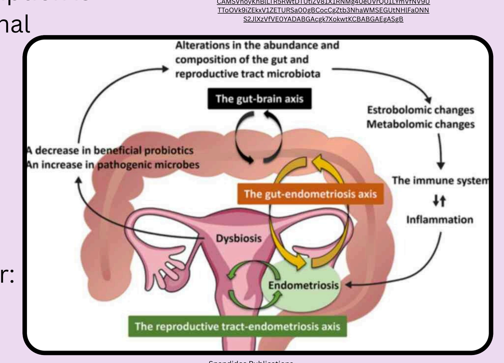
Gut Microbiome and Female Health

- The gut microbiome is an ecosystem of trillions of microorganisms involved in digestion, vitamin production, immune modulation, and hormone metabolism
- *Lactobacillus acidophilus* is especially important for:
  - Producing lactic acid to maintain pH
  - Inhibiting pathogens
  - Modulating hormone signaling pathways
- The gut-vaginal axis links intestinal microbiota with vaginal health. A healthy Lactobacillus abundance supports fertility, embryo implantation, and sperm survival (de Souza et al. 2023)



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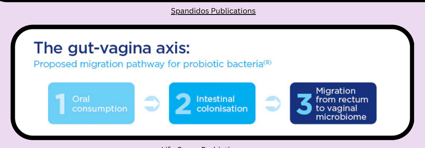
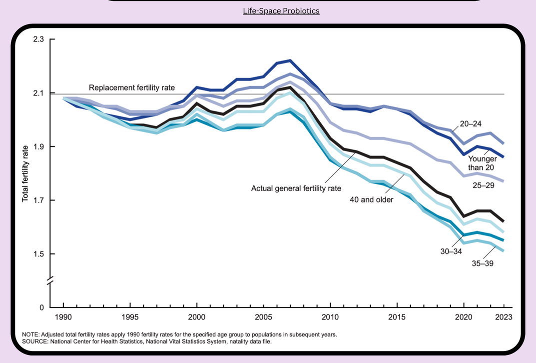


Life-Space Probiotics

Trend Analysis

- Fertility rates in the U.S. have declined:
  - Women under 30: 14% decline in total births (1990-2023)
  - Women under 20-44: 44% decline; women 25-29 23% decline (Driscoll et al, 2025)
- These declines correlate with the increased consumption of NNS, especially in women of reproductive age (Sylvetsky et al, 2017)



Hypothesis

Increasing concentrations of artificial sweeteners (Aspartame and Sucralose) will **inhibit/slow the growth of *Lactobacillus acidophilus***. Reduced growth in vitro **may suggest** a mechanism through which NNS disrupts gut microbiome balance, **potentially contributing to female endocrine and reproductive dysfunction.**

Rationale: Literature/past studies show that *Lactobacillus* abundance is **linked** to hormonal regulation and fertility, & **artificial sweeteners have bacteriostatic effects** in vitro and vivo.

Research Objectives

1.) Quantify the effects of aspartame and sucralose on L. acidophilus growth at multiple concentrations (mg/mL)

2.) Determine whether the sweetener type has an effect

3.) Explore potential implications of microbiome disruption on female infertility/endocrine health

Significance

This research is significant because it **contributes to a growing need to better understand health issues that specifically affect women, both socially and biologically.** Many women regularly consume artificial sweeteners in **response to societal pressures related to body image and diet culture, yet the long-term effects of these choices on female physiology remain underexplored.** Historically, much of biomedical research and drug development has been **centered on male physiology, creating gaps in knowledge** about how substances uniquely impact women's bodies, particularly in areas like hormonal regulation and reproductive health.

Additionally, as artificial sweeteners become increasingly **prevalent in global dietary patterns**—especially in the management of conditions such as **obesity and diabetes**—it is critical to evaluate their broader biological effects. This research provides insight into how these substances may influence the gut microbiome, which plays a key role in overall and reproductive health. These findings may help inform future research, **guide healthcare professionals in making more comprehensive dietary recommendations, and encourage more inclusive scientific investigation into women's health.**

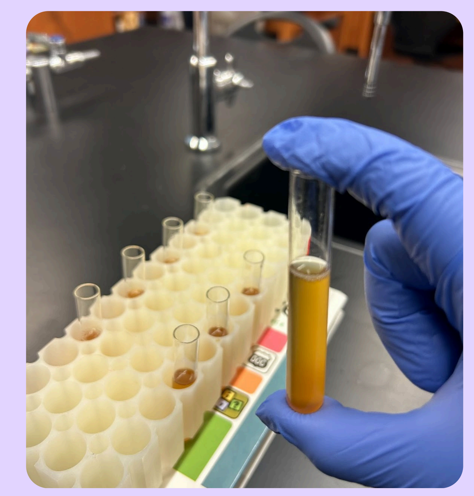
Methodology

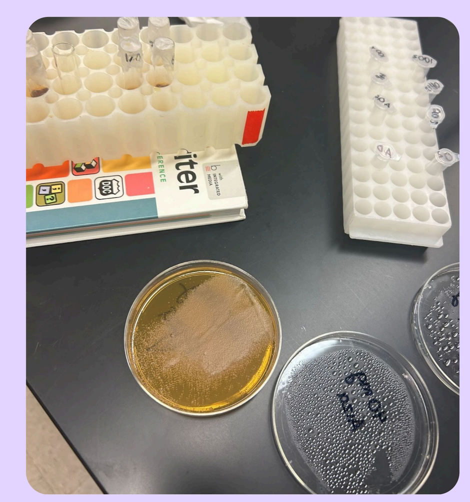
Experimental Design

The experiment employed a controlled in vitro design to investigate the effects of different concentrations of two commonly consumed artificial sweeteners, aspartame and Sucralose, on the growth of *Lactobacillus acidophilus*. *L. acidophilus* was selected due to its well-documented role in maintaining gut and vaginal microbiome balance, regulating pH, inhibiting pathogenic bacteria, and contributing to metabolic and hormonal pathways.

Procedure

Freeze-dried cultures of *L. acidophilus* were rehydrated in a 1% peptone solution to achieve a homogeneous bacterial suspension with a consistent initial optical density (OD) of approximately 2.50. Artificial sweetener stock solutions (mg/mL) were prepared at four concentrations (0, 20, 40, and 100 mg/mL) to reflect realistic human intestinal exposure levels. The 3 mL of bacterial culture was then combined with each sweetener solution, while control cultures received distilled water instead of sweeteners. Each treatment condition was replicated three times to increase the reliability of measurements. After thorough mixing, 0.5 mL aliquots of the bacterial-sweetener mixtures were plated onto MRS agar and evenly spread using sterile L-shaped spreaders to ensure consistent surface coverage. Plates were sealed and incubated at 37°C for 24 hours to provide optimal growth conditions. Post-incubation, bacterial growth was quantified using colony morphology analysis quantified by the fractal dimension (Db) to evaluate colony pattern complexity. All procedures were conducted under strict aseptic conditions, with controlled variables including agar type, incubation temperature and time, initial bacterial density, and sweetener volume to minimize confounding effects.





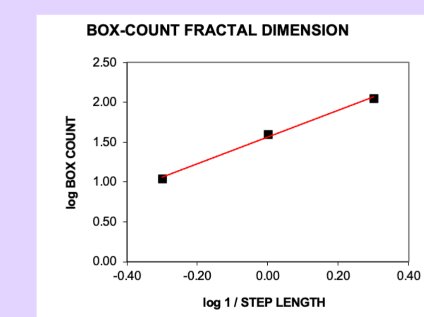
Data Analysis

BOX-COUNT FRACTAL DIMENSION (D_B)

Enter data into blue cells:

S (cm)	N	log 1/S	log BC
0.5	112	0.30	2.05
1.0	40	0.00	1.60
2.0	11	-0.30	1.04

D_B = 1.67

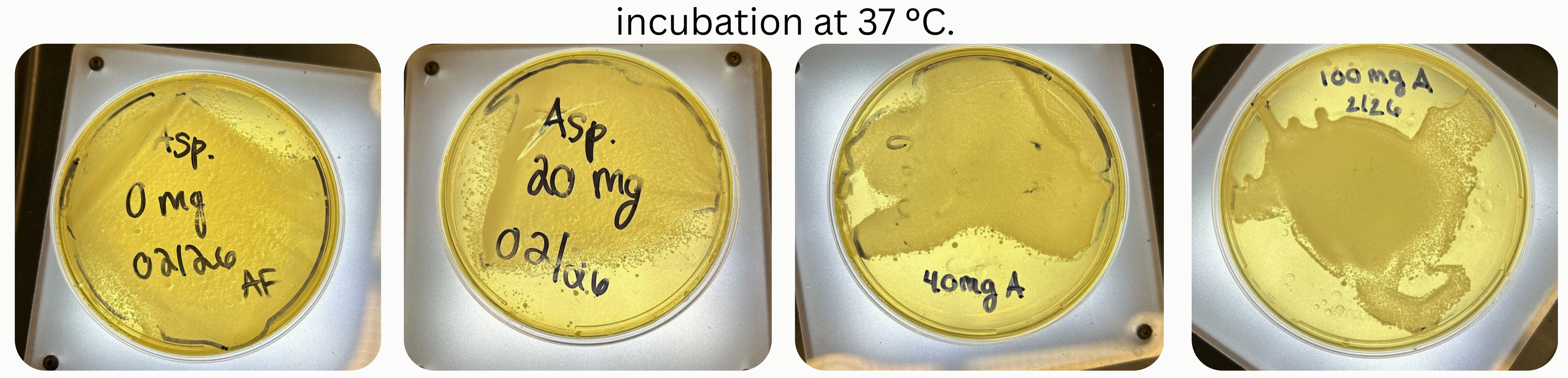


Statistical analysis was performed using ANOVA to determine whether sweetener type, concentration, or their interaction significantly influenced *L. acidophilus* growth. This allowed for precise evaluation of artificial sweetener effects on bacterial growth, providing a biologically meaningful model to infer potential implications for female gut microbiome integrity and reproductive health.

Fractal dimension (Db) was calculated using the box-counting method to quantify the spatial complexity of bacterial colony growth. Images of each agar plate were overlaid with grids of three box sizes (0.5 cm, 1 cm, and 2 cm), and the number of boxes containing *Lactobacillus acidophilus* colonies was counted for each grid size. These values were then entered into a fractal dimension calculator, which determines Db using the logarithmic relationship between box size and the number of occupied boxes.

Results

Figure 1: Representative growth of *Lactobacillus acidophilus* colonies on MRS agar at increasing aspartame concentrations (0, 20, 40, and 100 mg/mL) after 24 hours of incubation at 37 °C.



Images taken by Me

- Increasing aspartame concentration produced visible changes in Lactobacillus acidophilus colony growth patterns. Higher concentrations showed more irregular and dispersed colony structures compared to the control, consistent with the observed increase in fractal dimension (Db) values.

Table 2: Two-factor ANOVA analysis

Source	p-value
Sweetener Type	0.0635
Concentration	0.0079
Interaction	0.279

$F(3,16) = 5.635$

Figure 2: Mean Db of Aspartame & Sucralose

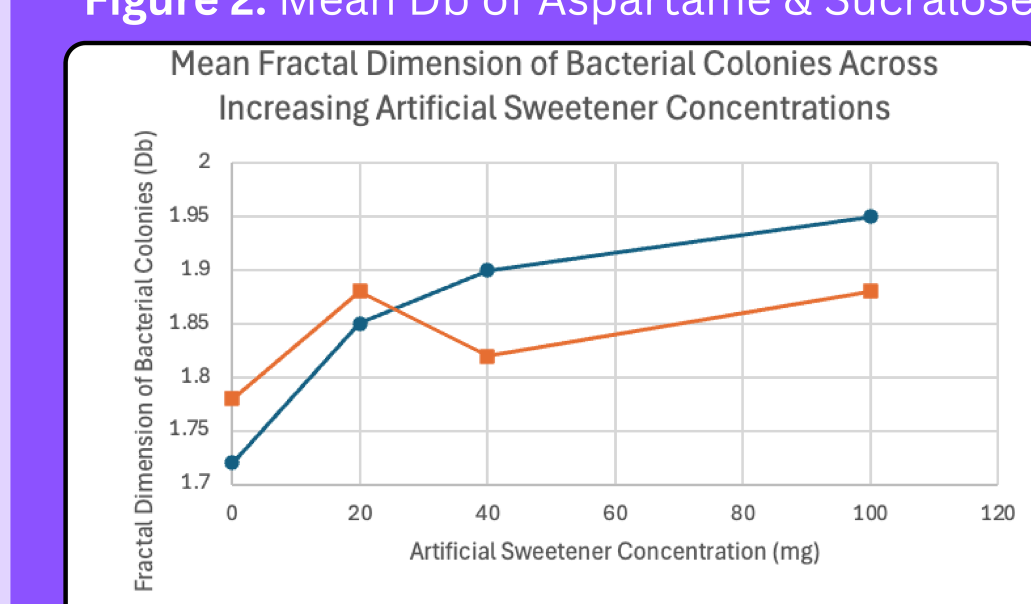


Table 1: Mean fractal dimension (Db) values for *Lactobacillus acidophilus* colonies exposed to varying concentrations of artificial sweeteners.

Sweetener	Concentration (mg/mL)	Mean Db	Standard Deviation
Aspartame	0	1.72	0.0611
Aspartame	20	1.85	0.0680
Aspartame	40	1.90	0.0832
Aspartame	100	1.95	0.070
Sucralose	0	1.78	0.0360
Sucralose	20	1.88	0.0520
Sucralose	40	1.82	0.0873
Sucralose	100	1.88	0.0960

Conclusions

- The concentration of artificial sweeteners had a **statistically significant effect** on *Lactobacillus acidophilus* growth, as indicated by **ANOVA results (p=0.0079).** Higher sweetener concentrations **corresponded with increasing colony complexity**, suggesting altered growth patterns that may reflect microbial dysbiosis.
- In contrast, **sweetener type didn't significantly affect growth (p = 0.635)**, which indicates that the overall impact on *L. acidophilus* is **more dependent on concentration rather than the specific sweetener** (aspartame vs. sucralose) used.
- The interaction between sweetener type and concentration was not significant (p=0.279), suggesting that the **effect of increasing sweetener concentration is consistent across both** aspartame and sucralose.
- These results **support previous research** showing that artificial sweeteners can disrupt beneficial gut bacteria, potentially affecting female endocrine and reproductive health by altering the gut microbiome
- The **observed increases in colony complexity (Db) at higher sweetener concentrations** may serve as an agent for dysbiosis, reflecting **irregular growth patterns that could correspond to imbalances in microbial populations**

Discussion

High concentrations of NNS in the gut may **contribute to microbial dysbiosis**, which can have downstream effects on the female endocrine system and reproductive health:

- Alter hormonal regulation, including pathways related to estrogen and other reproductive hormones
- **Reduce fertility** by **disrupting the gut-vaginal axis**, affecting *Lactobacillus* and vaginal health
- Increase risk of ovarian dysfunction, as suggested by histological studies in mice exposed to sweeteners

- Findings **align** with the literature demonstrating that:
  - *Lactobacillus*-rich microbiomes **correlate with higher pregnancy success and improved reproductive outcomes** (Alley et al.,2025)
  - NNS reduces *Lactobacillus* populations, which may lead to metabolic dysbiosis, hormonal changes, and oxidative stress, contributing to fertility issues
- The increase in fractal dimension of colonies at higher sweetener concentrations may indicate **changes in colony density**, **reflecting stress on bacterial growth** and potential dysbiosis

Limitations

- Since it's an in vitro study, this experiment **can't fully replicate the complex interactions of the human gut microbiome**, like the immune system, hormonal feedback, and nutrient absorption
- Only two NNS types (Aspartame and Sucralose) were tested; other artificial sweeteners, such as saccharin, stevia, ace-K, **may have different biological effects**
- Fractal dimension **doesn't capture full microbial density**, interactions, or population dynamics
- The sweetener concentrations **may not perfectly reflect cumulative exposure in humans**
- The gut microbiome contains a complex network of microbial species, so even though *Lactobacillus acidophilus* is a representative model, **other species may respond differently** to NNS exposure

Implications & Future Work

- The findings demonstrate that **moderate consumption of NNS may minimize disruption** to gut microbial balance and support reproductive and endocrine health
- Future work could include:
  - Testing additional artificial sweeteners, such as Saccharin, Stevia, and Ace-K, to compare effects across more NNS types
  - Conducting long-term exposure studies/in vivo experiments to test cumulative effects in animal or human systems
  - Using microbiome sequencing and hormonal assays to track changes in microbial composition, diversity, and associated endocrine function
  - Downstream effects on ovarian function and fertility markers
  - Combinatorial effects of multiple NNS in mixed diets to mimic human consumption patterns
  - Measuring the modulation of hormone-related genes in host cells co-incubated w/ *Lactobacillus* (qPCR/RNA-Seq)

References

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