

# Effect of Various Antibiotics on Digestive Enzyme Activity

## Question

Do penicillin, erythromycin, ampicillin, and neomycin affect the activity of enzymes involved in the digestion of carbohydrate, lipids and proteins?

## Hypothesis

- One or all the antibiotics will reduce digestive enzyme activity.
- The expected outcomes are that the addition of antibiotics will reduce the amounts of proteins, lipids, and carbohydrates digested by the enzyme pepsin, pancreatin, and amylase respectively.

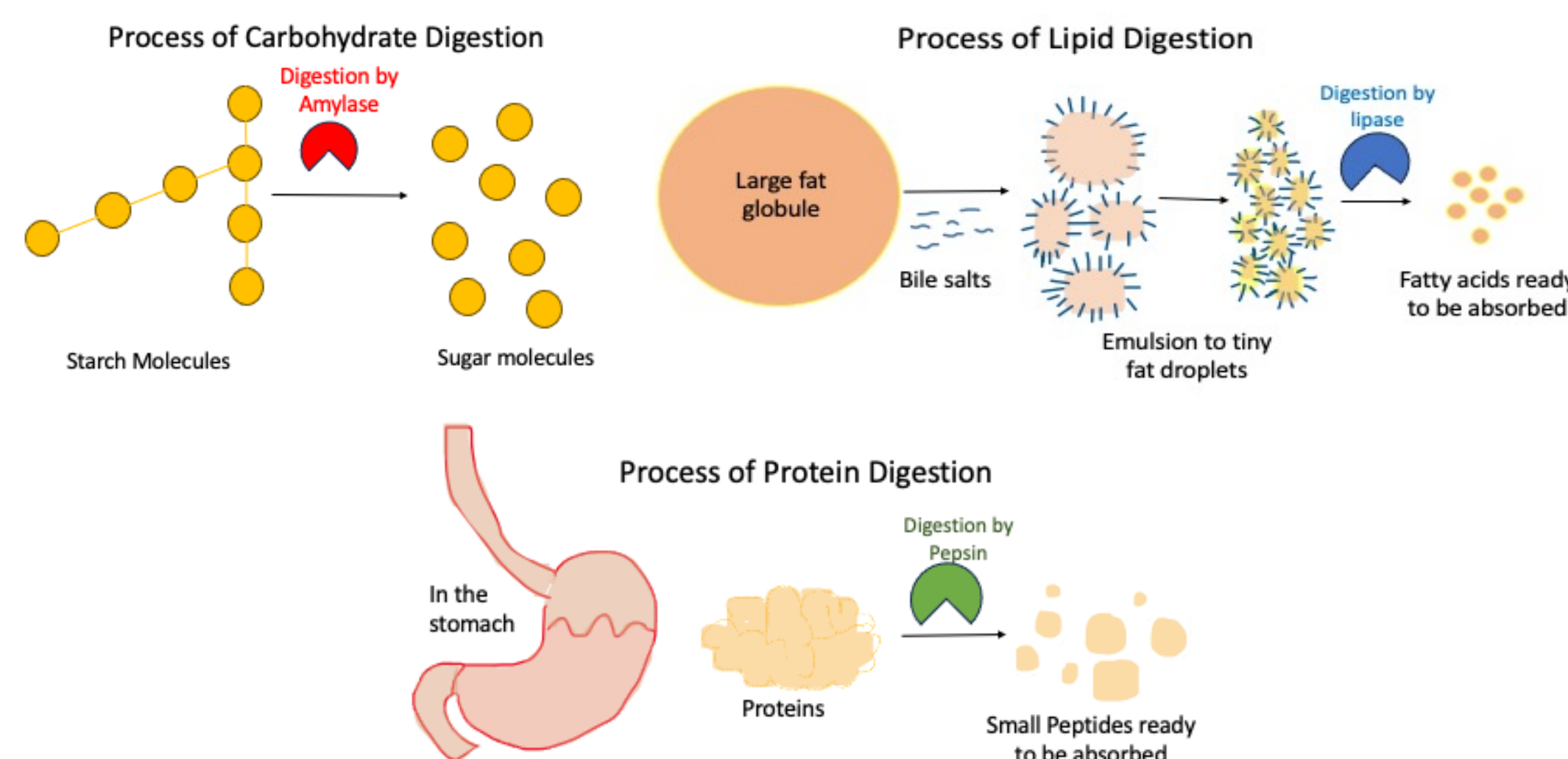
## Background Research

Bacterial infections are usually treated with antibiotics. The antibiotics can be administered orally, via injection, or topically. Antibiotics can either be bactericidal (kill the bacteria), or bacteriostatic (inhibit growth or reproduction). In addition to specific effect against pathogenic bacteria, antibiotics can also have non-specific effects on the human body.

These effects can include gastrointestinal (GI) problems such as nausea, vomiting, abdominal pain, and diarrhea. The main function of GI tract is to digest food and provide nutrients for the body to function and grow. Various digestive enzymes help in this process.

In addition to the antibiotics non-specifically killing the good bacteria in the gut, they could also affect nutrient digestion and absorption. My research project is to investigate how different antibiotics affect various digestive enzyme activity, and whether this would be one of the reasons for the side effects on our GI tract.

Knowing this information will help doctors and patients to prevent these side effects while taking antibiotics to overcome infection.



## Materials

- Antibiotics** (purchased from Home Science Tools):
  - Neomycin discs (30 mcg/disc; catalog # N30-2203)
  - Penicillin discs (10 units/disc; catalog # P10-2303)
  - Erythromycin discs (15 mcg/disc; catalog # E15-2118)
  - Ampicillin discs (10mcg/disc; catalog # AMP10-2214)
- Reagents** (purchased as a kit Catalog # IS3009-SGL from Home Science Tools):
  - Starch solution (1g/100 mL)
  - Iodine/potassium iodide/Lugol's solution (10mL)
  - Albumin solution (1g/100 mL)
  - Phenolphthalein (10 mL)
  - Biuret reagent (30 mL)
  - Olive oil (10 mL)
  - 0.1 M Sodium Hydroxide (10 mL)
  - 0.1 M Hydrochloric acid (20 mL)
- Enzymes** (purchased as a kit Catalog # IS3009-SGL from Home Science Tools):
  - Bile salt/pancreatin solution (2.05g/100mL)
  - Pepsin (1.75g/100mL)
  - Amylase (1g/100mL)
- Other materials:**
  - Tape
  - Sharpie or black marker
  - Plastic graduated pipettes
  - Graduated cylinder
  - Distilled water
  - Syringe
  - Glass test tubes with cork stopper
  - Hotplate
  - Test tube holder
  - Thermometer
  - Glass beaker
  - Sterile discs (Home Science Tools catalog #SD02301)
  - Pyrex container
- Lab safety:**
  - Safety goggles
  - Gloves
  - Lab apron

## Methods

### I. Carbohydrate digestion: Assay Setup

Tube Label	Number of trials	Materials Added
Positive Control	3	Starch + Amylase
Negative Control	3	Starch Only
Test 1	3	Neomycin + Starch + Amylase
Test 2	3	Erythromycin + Starch + Amylase
Test 3	3	Penicillin + Starch + Amylase
Test 4	3	Ampicillin + Starch + Amylase
Disc Control	3	Sterile Disc + Starch + Amylase

### Methods:

- Preparation of Starch solution:
  - Add 100mL of Distilled water to a beaker.
  - Add 1g of starch to the water. Stir.
  - Heat solution until completely dissolved.
  - Label as 'Starch Solution'.
  - Cool to room temperature.
- Preparation of Amylase solution:
  - Add 10 mL of distilled water to 100 mg of amylase.
  - Shake well to dissolve.
- Preparation of tubes:
  - Add 4mL of starch solution to each tube.
  - Add antibiotic discs to tubes 1-4 and a sterile disc into the Disc Control tube.
  - Add 5 drops of Amylase to all tubes except the negative control tube.
  - Incubate at 37 °C for 30 minutes.
  - Add 3 drops of Iodine/ Potassium iodide solution.
  - Tap bottoms thoroughly to mix well.
  - Take a picture and write down the color of the solutions.
  - Repeat the experiment two more times and call them Trial 2 and Trial 3.

### II. Lipid Digestion Assay Setup

Tube Label	Number of trials	Materials Added
Positive Control	3	Olive oil + Pancreatin/Bile salt
Negative Control	3	Olive oil only
Test 1	3	Neomycin + Olive oil + Pancreatin/Bile Salt
Test 2	3	Erythromycin + Olive oil + Pancreatin/Bile Salt
Test 3	3	Penicillin + Olive oil + Pancreatin/Bile Salt
Test 4	3	Ampicillin + Olive oil + Pancreatin/Bile Salt
Disc Control	3	Sterile disc + Olive oil + Pancreatin/Bile Salt

### Methods:

- Preparation of Bile salt/ Pancreatin Solution:
  - Mix 85 mL of water with the bottle containing Pancreatin/Bile salt.
  - Replace cap and shake thoroughly until dissolved.
- Preparation of tubes
  - Add 4 mL of Bile Salt/Pancreatin solution to the all the tubes except the Negative Control
  - Add 4 mL of Distilled water to the Negative Control.
  - Add 5 drops of 0.1M Sodium Hydroxide to all tubes.
  - Add the antibiotic discs to tubes 1-4 and a sterile disc into the Disc Control tube.
  - Add 5 drops of Phenolphthalein solution to all test tubes. Shake well.
  - Add 15 drops of olive oil to all test tubes. Shake well.
  - Incubate the tubes at 37°C for 60 mins.
  - Take picture and note down the color change.
  - Repeat the experiment two more times and call them Trial 2 and Trial 3.

### III. Protein Digestion: Assay Setup:

Tube Label	Number of trials	Materials Added
Positive Control	3	Albumin + HCl + Pepsin
Negative Control	3	Albumin + HCl
Test 1	3	Neomycin + Albumin + HCl + Pepsin
Test 2	3	Erythromycin + Albumin + HCl + Pepsin
Test 3	3	Penicillin + Albumin + HCl + Pepsin
Test 4	3	Ampicillin + Albumin + HCl + Pepsin
Disc Control	3	Sterile Disc + Albumin + HCl + Pepsin

### Methods:

- Preparation of Albumin
  - Mix 100mL distilled water into the bottle containing 1g Albumin.
  - Replace cap and shake well.
- Preparation of Pepsin
  - Add 100mL water to the 1.75 g of Pepsin.
  - Mix well.
- Preparation of tubes
  - Add 1mL albumin to all test tubes.
  - Add antibiotic discs to tubes 1-4 and a sterile discs into the Disc Control tube.
  - Add 5 drops of 0.1M HCl to all tubes.
  - Add 0.2mL pepsin solution to all tubes except Negative Control.
  - To the Negative Control, add 0.2mL of distilled water to make the volume equal.
  - Shake gently and incubate tubes at 37°C for 60 mins.
  - Remove the tubes and add 4 drops of biuret reagent to all tubes. Mix well.
  - Observe color of solution and record the data. Take a picture.
  - Repeat the experiment two more times and call them Trial 2 and Trial 3.

## Variables

### Controlled variables

- Temperature of the reaction
- Time of digestion
- The amount carbohydrate, protein or lipid used in the reaction

### Independent variable

- Four different antibiotics were tested to see if they affect enzyme activity

### Dependent variable

- The amount of nutrient being digested
- Measured by a colored reaction

## Photos



Fig. 1: Set up of experimental materials



Fig. 2A and B: Preparation of enzyme and substrate solutions



Fig. 3: Incubation at 37°C



Fig. 4: Reaction set up



Fig. 5: Addition of antibiotic discs



Fig. 6: Incubation at 37°C

## Data

Digestion	Test Used	Independent Variable	Dependent Variable (Color assessment estimates % digestion)	100% Digestion	0% Digestion
Carbohydrate Digestion	Iodine/KI (Lugol's) test	Neomycin, Erythromycin, Penicillin, Ampicillin, or sterile disc	100 (yellow), 75 (orange), 50 (red), 25 (purple), 0 (dark blue)	Starch + Amylase + Iodine/KI	Starch + Iodine/KI
Lipid Digestion	Phenolphthalein as indicator	Neomycin, Erythromycin, Penicillin, Ampicillin, or sterile disc	100 (pink), 75 (red), 50 (purple), 25 (dark purple), 0 (dark blue)	Olive oil + Bile Salt + Pancreatin + Phenolphthalein	Olive oil + Phenolphthalein
Protein Digestion	Biuret test	Neomycin, Erythromycin, Penicillin, Ampicillin, or sterile disc	100 (purple), 75 (dark purple), 50 (black), 25 (grey), 0 (white)	Albumin + HCl + Pepsin + Biuret reagent	Albumin + HCl + Biuret reagent

Fig. 7A: Results from Carbohydrate Digestion Experiment

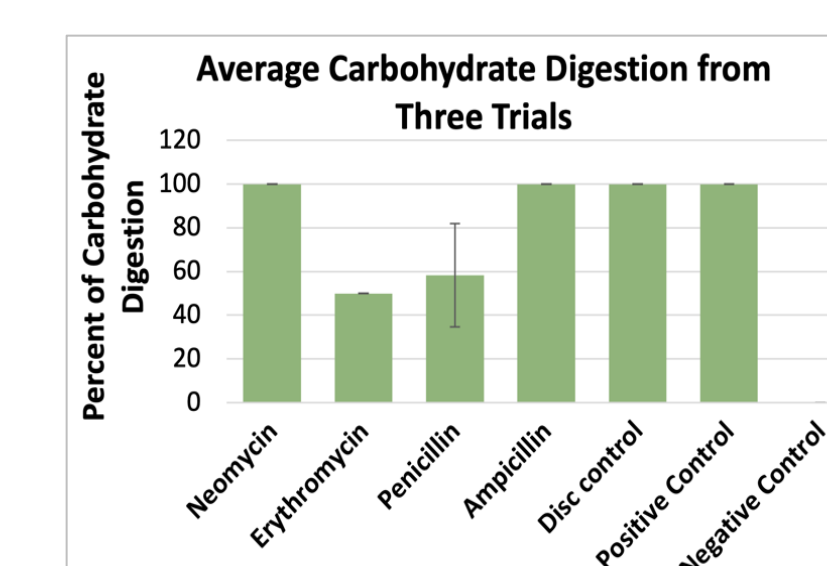


Fig. 7B: Graph of the results from Carbohydrate Digestion

Fig. 8A: Results from Lipid Digestion Experiment

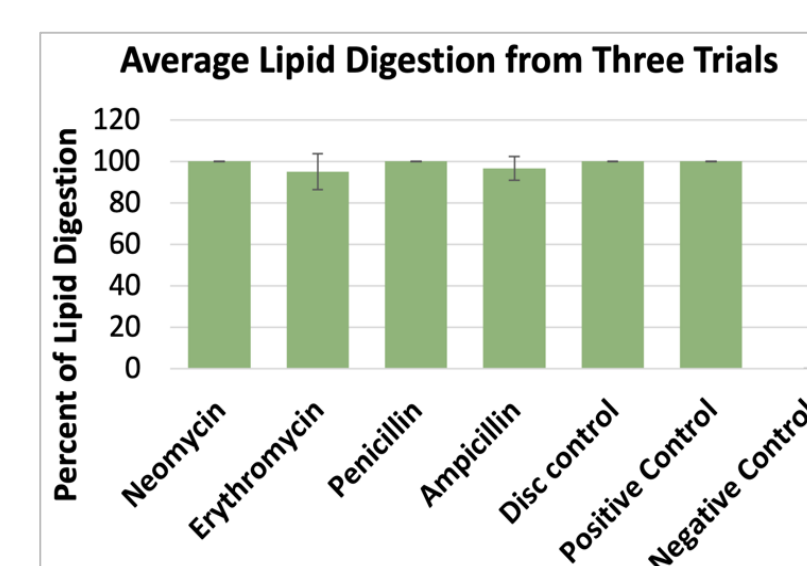
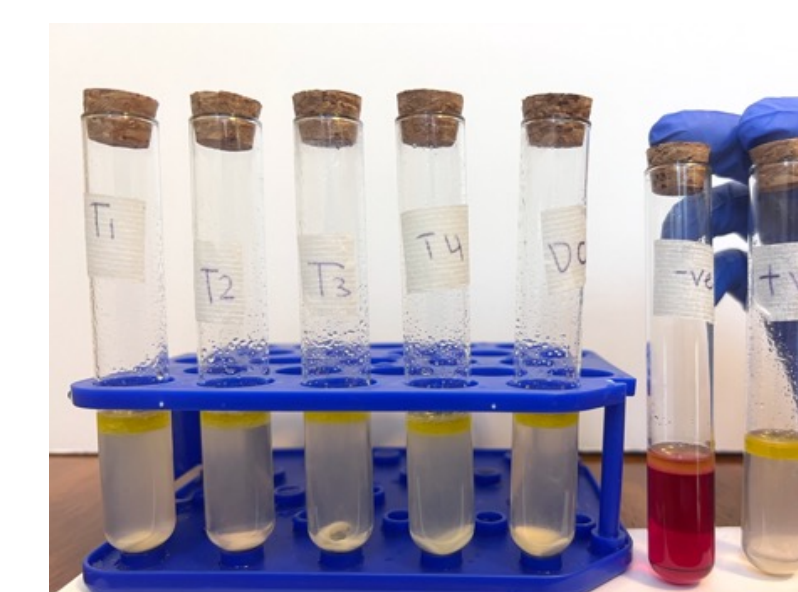


Fig. 8B: Graph of the results from Lipid Digestion

Fig. 9A: Results from Protein Digestion Experiment

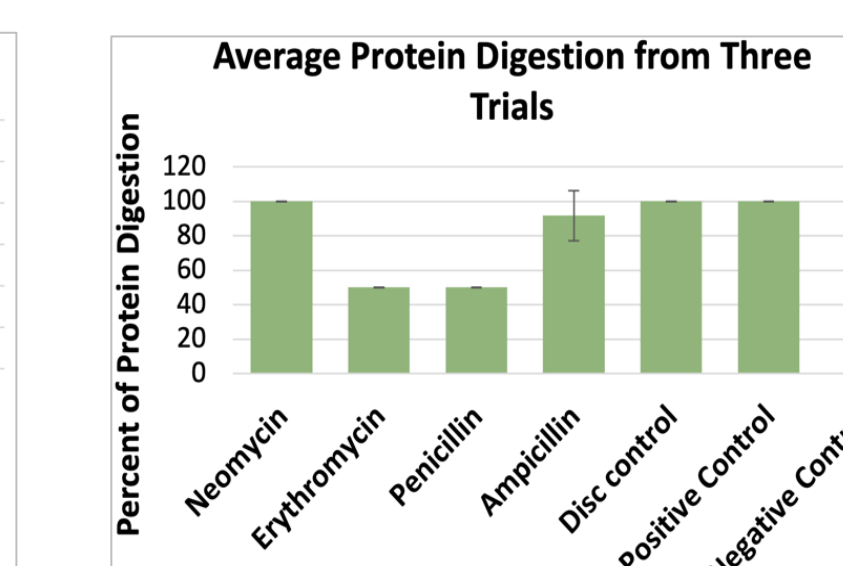
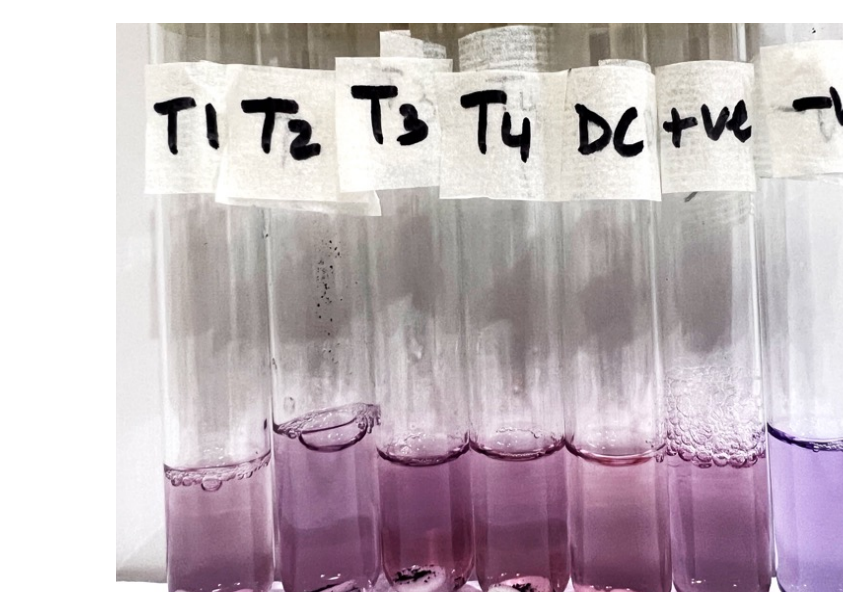


Fig. 9B: Graph of the results from Protein Digestion

## Results

- The results from this study showed that the antibiotics erythromycin and penicillin reduced the activity of the enzyme amylase by 50% and 40% respectively.
- Antibiotics erythromycin and penicillin reduced the activity of the enzyme pepsin by 50%.
- Ampicillin reduced the activity of pepsin by ~10%.
- Erythromycin and Ampicillin reduced the activity of pancreatin by only 5%.
- Neomycin was the least inhibitory, having no effect on the activity of any of the three enzymes.

Antibiotic	Enzyme	Activity Inhibition
Erythromycin	Amylase	50%
	Pepsin	50%
	Pancreatin	5%
Penicillin	Amylase	40%
	Pepsin	50%
	Pancreatin	0%
Ampicillin	Amylase	0%
	Pepsin	10%
	Pancreatin	5%
Neomycin	Amylase	0%
	Pepsin	0%
	Pancreatin	0%

## Conclusion

- Based on my results, my conclusion is that antibiotics can affect your ability to digest food by inhibiting digestive enzyme activity. Therefore, my hypothesis was proven correct.
- Of the four antibiotics tested, Erythromycin and Penicillin had the greatest effect on the digestion of carbohydrates and proteins.
- The digestion of lipids was the least affected by all four antibiotics.
- Neomycin had the least effect on the digestion of all three nutrients.

## References

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