**SRI VENKATESWARA INTERNSHIP PROGRAM FOR RESEARCH IN ACADEMICS (SRI-VIPRA)**

**SRI-VIPRA**

# Project Report of 2024: SVP-2443

"Effect of Different Dietary Composition on *Drosophila melanogaster*"

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# SRI-VIPRA PROJECT 2024

**Title:** Effect of Different Dietary Composition on *Drosophila melanogaster.*

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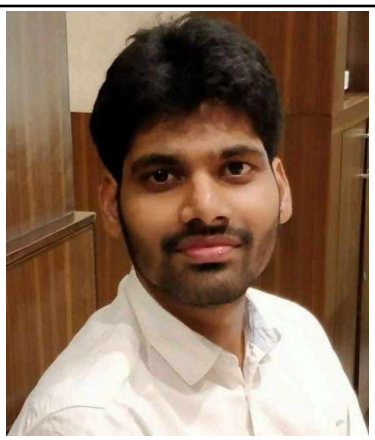
| **Name of Mentor:** Dr. Sidhartha Taritla<br>**Name of Department:** Biochemistry<br>**Designation:** Assistant Professor |  |

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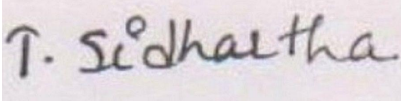

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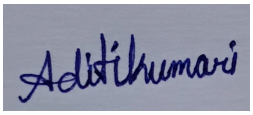
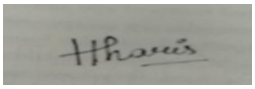
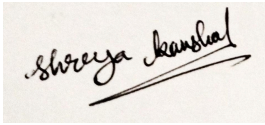
| 1 |  | Aditi Kumari | 1223075 | B.Sc. (Hons) Biochemistry |  |
| 2 |  | Hiba Haris | 1222028 | B.Sc. (Hons) Biochemistry |  |
| 3 |  | Shreya Kaushal | 1222011 | B.Sc. (Hons) Biochemistry |  |
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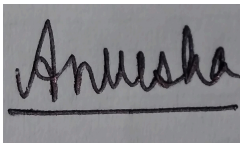
| 5 |  | Anvesha Yadav | 1222035 | B.Sc. (Hons) Biochemistry |  |

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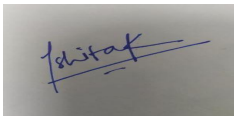
| 6 |  | Ishita Kayathwal | 1222009 | B.Sc. (Hons) Biochemistry |  |
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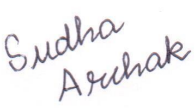
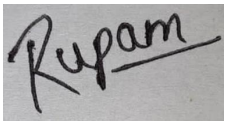
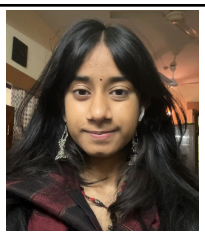
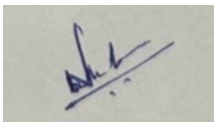
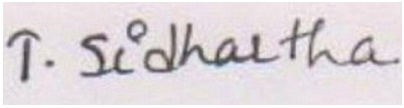
| 8 |  | Sudha Archak | 1322002 | B.Sc. (Hons) Biological Sciences |  |
| 9 |  | Rupam Singh | 1422011 | B.Sc. (Hons) Botany |  |
| 10 |  | Nandita B Krishnakumar | 1223060 | B.Sc (Hons) Biochemistry |  |

# CERTIFICATE OF ORIGINALITY

This is to certify that the aforementioned students from Sri Venkateswara College have participated in the summer project SVP-2443 titled "**Effect of Different Dietary Composition on *Drosophila melanogaster*"**. The participants have carried out the research project work under our guidance and supervision from 1st July 2024 to 30th September 2024. The work carried out is original and has been carried out in an offline mode.

**Signature of Mentor**
(Dr. Nandita Narayanasamy)



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# ACKNOWLEDGEMENTS

We express sincere gratitude to our college for introducing the Sri Venkateswara Internship Program for Research in Academics (SRI-VIPRA). Our sincere thanks to **Prof. Vajala Ravi**, Principal, and **Prof. Vartika Mathur**, IQAC Coordinator, for initiating the program this year. We would also like to thank the two conveners of SRI-VIPRA 2024, **Dr. P. Jayaraj** and **Dr. Haokam Vaiphei** for their support during this internship.

We are deeply indebted to our mentors **Dr. Sidhartha Tartila** and **Dr. Nandita Narayanasamy** for providing us with the opportunity to work on this project under their expert guidance. Their enthusiasm, wisdom, and remarkable patience have been a continual source of inspiration for all of us. We would also like to acknowledge **Dr. Vandana Malhotra** and **Mr. Abhishek Garg** for their constant support.

Last but not least, we extend our heartfelt thanks to all the lab staff. Their cooperation has been essential for the success of this project, and we truly appreciate their support.

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# ABSTRACT

*Drosophila melanogaster* is a key model organism in genetics and behavioral studies, known for its short life cycle, ease of maintenance, and well-studied genome. Its small genome, large offspring numbers, and visible mutations make it ideal for studying inheritance, gene function, and development. Drosophila research has contributed to understanding development, behavior, and human disease, ensuring its continued relevance in modern genetics and molecular biology. Current research focuses on the use of *Drosophila melanogaster* in studying lifestyle disorders. Our study aims to investigate the effect of different dietary composition on growth patterns and storage biomolecules. The larval protein, lipid, and carbohydrate profile was determined. Low-calorie, high-protein diet shows a better growth profile and survival as compared to a high-calorie high-fat diet. Metabolic stores in the high-protein, low-calorie diet are primarily protein and glycogen while in the high-calorie and fat diet, it is mainly TAG. There is a difference in the SDS PAGE profiles of particularly low molecular weight between a high-calorie high-fat diet and a high-protein, low-calorie diet. Metabolic profiling using computational tools can be used to identify the metabolic networks that could sustain growth in the two diets.

# INTRODUCTION

*Drosophila Melanogaster*, commonly and widely known as the fruit fly, belongs to the family *Drosophilidae* and is characterized by its distinctive red eyes and yellowish-brown body.
These flies have a short life cycle of about 10-14 days, making them an ideal model organism to be used for studying genetics and developmental biology. They reproduce quickly, producing a large number of offspring while going through metamorphosis during these 14 days, which enables scientists to observe genetic variation and inheritance patterns easily. Thomas Hunt Morgan, an American geneticist who conducted pioneering research using *Drosophila melanogaster* in the early 20th century, is credited with providing conclusive proof for the chromosomal theory of inheritance and deducing the first genetic map of the Drosophila X chromosome.

Various features make *Drosophila melanogaster an* ideal organism for genetics and behavioral studies. These include:

- **Short life cycle:** A generation can be completed in about 10-14 days at 25°C.
- **Simple genetics:** With only four pairs of chromosomes, its genome is relatively small (about 132.9 million base pairs), yet it shares many genes with humans.
- The giant polytene chromosomes from Drosophila third instar larval salivary glands provide an important model system for studying the architectural changes in chromatin morphology associated with the process of transcription initiation and elongation[1].
- **Sexual dimorphism:** Females are generally larger than males, with a lighter, striped abdomen with more distinct segments, while males have a darker, more rounded abdomen and a "sex comb" which is a small tuft of bristles on their front legs. In addition, males have a rounded genital region, whereas females have a more pointed ovipositor, used for laying eggs. This enables easy sex identification, which is vital for controlled breeding and genetic studies, allowing for the investigation of sex-specific traits, behaviors, and reproductive strategies, aiding in the understanding of sexual selection and evolutionary biology. It facilitates the study of sex-linked traits and inheritance patterns while enabling researchers to explore sex-based differences in behavior, physiology, and reproductive outcomes [2]
- **High fecundity:** Oviposition by the female starts as early as the second day after it emerges from its pupal case. It increases for about a week until a female adult may be laying 50-75 eggs per day for a total of approximately 400-500 eggs in 10 days. A single female can lay many eggs, providing a large sample size for experiments [3].
- **Conserved pathways:** Many of its developmental and cellular pathways are conserved across species, including humans.[3]

The **life cycle** of *Drosophila melanogaster* spans about 10-12 days at 25°C and consists of four stages: egg, larva, pupa, and adult is a complete life cycle or holometabolous life cycle. Females lay eggs that hatch into larvae within 24 hours. The larval stage lasts about 4-5 days, during which the larvae undergo

three molts, growing through three instar stages while feeding continuously. After reaching the third instar, the larva forms a pupal case and undergoes metamorphosis for 4-6 days, during which its body reorganizes into the adult form. Once the adult fly emerges, it reaches sexual maturity within a few hours (males 12 hrs, females 18 hrs), ready to begin the cycle again.

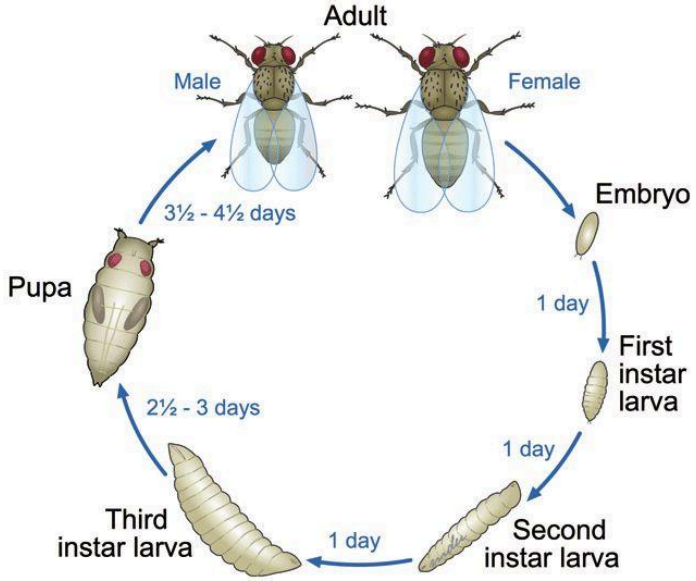



**Figure 1:** Life cycle of *Drosophila melanogaster*[4]

**Overview of *D. melanogaster* Metabolism**

Metabolic regulation and physiological feedback systems are essential in balancing energy needs with dietary input thereby contributing to sexual maturation, influencing adult fertility, and acting as a key determinant of aging. Metabolic dysfunction is associated with major human diseases such as Diabetes, cardiovascular diseases, and some forms of cancer. *Drosophila Melanogaster* shares most of the same basic metabolic functions found in vertebrates. The fly maintains appropriate circulating sugar levels that act as a backup in stressful conditions and stores excess energy in the form of glycogen and lipids. The prevalence of analogous organ systems that control nutrient uptake, storage, and metabolism in *Drosophila* makes it an important model for studies of various disorders[5,6,7].

## Carbohydrate Metabolism

Glucose is an important resource for energy production and is used by many organisms to synthesize and retain sugar storage molecules such as glycogen, and trehalose[6].

In *D. melanogaster*, glucose is stored in two different forms: the disaccharide trehalose and the branched polymer glycogen. The circulating sugar taken up from the diet produces energy via glycolysis. Excess glucose in circulation is used for glycogenesis. Glycogen is stored mainly in body flight muscles and fat body (the mammalian equivalent of liver and adipose tissue) in adult flies whereas it is only present in body wall muscles in the larva during their development.

*Drosophila* possesses functional homologs for mammalian hormones such as glucagon and insulin called Adipokinetic hormone (AKH) and Drosophila insulin-like peptides (DILP) respectively. However, AKH has been shown to not affect glycogen metabolism. Dilps, on the other hand, result in the stimulation of glycogenesis[8].

Glycogen metabolism is regulated by two enzymes, glycogen synthase and glycogen phosphorylase. The enzyme, phosphorylase kinase, responsible for activating the phosphorylase by transferring a phosphoryl group to its Ser residue is in turn activated by the circulating sugar levels. This results in a conformational change and hence the activation of the enzyme causing the breakdown of glycogen. Further, an increase in the concentration of adenosine monophosphate (AMP) allosterically activates the enzyme.

Like glycogen phosphorylase, glycogen synthase can also exist in phosphorylated and dephosphorylated forms. Its active form, glycogen synthase a, is unphosphorylated. Phosphorylation of the hydroxyl side chains of several Ser residues of both subunits converts glycogen synthase a to glycogen synthase b, which is inactive unless its allosteric activator, glucose 6-phosphate, is present. The activity of glycogen synthase is phosphorylated by two kinases: Glycogen synthase kinase 3 (GSK3) and casein kinase II (CKII). AMP-activated protein kinase (AMPK) also phosphorylates glycogen synthase, inhibiting glycogen synthesis during periods of metabolic stress[9].

In addition to glycogen, *Drosophila Melanogaster* maintains a high concentration of the non-reducing disaccharide trehalose in circulating hemolymph. Trehalose is synthesized from glucose by trehalose-6-phosphate synthase (Tps1) in the fat body and degraded to glucose by trehalase (Treh). Tps1 has two functionally distinct catalytic domains. The N-terminus trehalose-6-phosphate synthase (TPS) domain produces Trehalose-6-Phosphate (Tre6P) from glucose-6-phosphate (Glu-6P) and UDP-glucose. The C-terminus Tre6P phosphatase (TPP) domain de-phosphorylates Tre6P to generate trehalose. The Treh enzyme causes the breakdown of Trehalose to glucose which enters the glycolytic pathway[10].

Under fasting conditions, glycogen metabolism is regulated in a tissue-specific manner. The fat body glycogen along with the circulating levels of trehalose acts as a backup source to maintain glucose levels.

**Lipid Metabolism**

Lipids are known for providing energy and playing an important role in growth and development. They serve as precursors of hormones and vitamins in mammals and humans.

Compared to vertebrates*, Drosophila Melanogaster,* fruit flies show relative homology in organ anatomy and cell types. This is supported by the presence of adipose-like tissues; the fly's fat body acts as a glycogen and lipid reserve. Moreover, the oenocyte cells accumulate lipids during starvation, akin to hepatocyte-like cells involved in lipid processing in mammals.[11]

In *Drosophila,* the lipid stores are present in the form of triacylglycerols (TAG) and cholesterol esters, accumulated together in the form of lipid droplets (LDs), within a fat body[12]. These fat bodies are regulated organelles and are present throughout the life cycle of the flies. Their genome encodes for eight

insulin-like peptide (dilp) genes that regulate the lifespan and also influence appetite in Drosophila. These eight dilp known, are involved in the accumulation of triglycerides, glycogen, glucose, and trehalose which act as storage metabolites in fruit flies. In *Drosophila*, lipid metabolism is also regulated by glucagon-like adipokinetic hormone (AKH) secreted by *corpora cardiaca* cells located in the larval ring gland which behave like pancreatic alpha-cells[11,12].

Lipid Metabolism in *Drosophila* can be divided into two processes: the formation of lipids in the form of TAG, called **lipogenesis,** and the mobilization of TAG from lipid droplets, called **lipolysis.** Furthermore, *Drosophila* has lipogenic enzymes, which are involved in *de novo* biosynthesis of TAG from fatty acids, including glycerol-3-phosphate acyltransferase, acylglycerol phosphate acyltransferase, phosphatidate phosphatase (LIPIN), and diacylglycerol acyltransferase (DGAT)[11,13].

*Drosophila* is a cholesterol auxotroph, it can't synthesize cholesterol *de novo*, hence, cholesterol uptake is dependent on diet. In mammals, SREBP is a transcription factor that promotes fatty acids and cholesterol synthesis is negatively regulated by cholesterol while in *Drosophila*, phosphatidylethanolamine controls the release of sterol regulatory element-binding protein (SREBP) from Drosophila cell membranes, exerting feedback control on the synthesis of fatty acids and phospholipids[13,14].

These lipid stores play an important role in providing energy during spermatogenesis, and oogenesis. During embryogenesis, these lipid droplets move throughout the embryo and ultimately participate in the emergence of an adult fly after a pupal period. Post-embryonic development of *Drosophila* does not progress from the first instar larva to the second instar larva in the absence of Cholesterol or a low-cholesterol diet, reflecting on the uptake of Cholesterol from the diet[15].

### ***D. melanogaster*** **as a Model to Study Lifestyle Disorders**

While *Drosophila melanogaster* has been extensively used in genetic research, its relevance extends beyond genetics, offering profound insights into human lifestyle, behavior, and health.[16] Due to the conservation of genetic pathways between flies and humans[17], *Drosophila* serves as a powerful model for studying how lifestyle factors—such as diet, sleep, stress, and aging—affect overall health and disease susceptibility.

One of the most significant parallels between humans and *Drosophila* is in behavioral research. Flies exhibit fundamental behaviors, such as eating, mating, sleeping, and social interactions, all of which are vital to human life. These behaviors are regulated by conserved molecular pathways, allowing researchers to study the genetic basis of lifestyle-related behaviors in *Drosophila* and apply their findings to human contexts. For instance, sleep studies in *Drosophil*a have identified genes that influence circadian rhythms[17]. Which are critical for maintaining healthy sleep patterns in humans. Disruptions to these rhythms in humans can lead to sleep disorders, mood disturbances, and metabolic imbalances—conditions that are also observable in flies.

Dietary research using *Drosophila* has been particularly impactful in understanding human metabolic processes. Similar to humans, *Drosophila* responds to variations in diet, and its metabolic pathways closely mirror those found in humans. Studies on nutrient intake, caloric restriction, and high-fat diets in flies have provided valuable insights into obesity, diabetes, and aging. For instance, caloric restriction in *Drosophila* has been shown to extend lifespan and improve metabolic health, findings that parallel observations in human and mammalian studies[18]. These investigations underscore the potential for dietary interventions to mitigate age-related diseases and improve life expectancy in humans.

Stress responses, both psychological and physiological, can also be modeled in *Drosophila*. Flies experience environmental stressors such as changes in temperature, oxidative stress, and social stress—situations that are comparable to human experiences[19]. The genetic and biochemical responses in flies to these stressors involve pathways that are conserved in humans, such as the stress-activated protein kinase pathways. By studying stress in *Drosophila*, researchers can better understand the long-term effects of chronic stress on health, providing critical insights into stress-related conditions like anxiety, depression, and cardiovascular diseases in humans[19].

Another area where *Drosophila* is invaluable is in aging research. The fly's short lifespan allows for rapid, large-scale studies on the effects of aging and interventions to extend lifespan and promote healthy aging. Many of the molecular mechanisms that regulate aging, such as those involved in cellular senescence, mitochondrial function, and nutrient-sensing pathways, are shared between *Drosophila* and humans. Research on aging in flies has led to the identification of genetic and environmental factors that influence lifespan, offering potential therapeutic targets for age-related diseases like Alzheimer’s, Parkinson’s, and cardiovascular disorders in humans[20].

Furthermore, *Drosophila* is an essential model for studying how genetic factors interact with environmental influences, shedding light on lifestyle diseases such as obesity, diabetes, and cardiovascular conditions. The fly’s genetic makeup allows researchers to manipulate specific genes and observe how they interact with lifestyle factors like diet and stress. These studies are crucial for understanding complex human conditions where both genetic predisposition and lifestyle choices play a role in disease progression.

Lifestyle disorders, particularly metabolic diseases like obesity, diabetes, and cardiovascular conditions have seen a dramatic rise worldwide. Factors such as excessive calorie intake, sedentary lifestyles, and genetic predisposition contribute to these issues[21]. Given that the main metabolic pathways in *Drosophila melanogaster* are highly conserved with those of humans, researchers are increasingly utilizing it to better understand the molecular and genetic mechanisms underlying these disorders. The striking similarity of genes involved in lipid metabolism and insulin signaling between humans and flies, combined with the ability to easily manipulate the *Drosophila* genome, its short lifespan, and low maintenance cost, makes it an ideal organism for studying these complex conditions[21,22].

Researchers can manipulate the *Drosophila* genome using tools like transgenic lines and RNA interference, allowing for precise studies of specific genes related to metabolic disorders. The fruit fly has organs analogous to those in humans, such as the fat body, which functions similarly to human adipose tissue, and oenocytes, resembling liver cells[22]. Feeding *Drosophila* high-fat or high-sugar diets induces obesity-like conditions, leading to hyperglycemia, insulin resistance, and cardiovascular dysfunction, closely mirroring metabolic syndrome in humans. Flies accumulate triacylglycerols in their fat body, resulting in metabolic dysfunction akin to human obesity[22].

*Drosophila* has also been instrumental in modeling Type 2 diabetes. Just like in humans, insulin-like peptides (ILPs) in flies help regulate sugar metabolism, and disruptions in insulin production or signaling can result in diabetic-like conditions. When the insulin-producing cells of the fly are ablated or mutated, the flies exhibit elevated sugar levels, insulin resistance, and reduced growth, mirroring the physiological effects seen in diabetic patients[23].

Thus, it is justified to use *Drosophila melanogaster* as a model organism to understand the molecular and genetic underpinnings of human lifestyle disorders and identify potential treatments.

## OBJECTIVES

The objective of our study is to investigate the effect of different dietary composition with varying ratios of carbohydrate, protein, and lipid, on:

(a) growth patterns of *D. melanogaster*, and
(b) its storage biomolecules (protein, carbohydrate, and lipid stores).

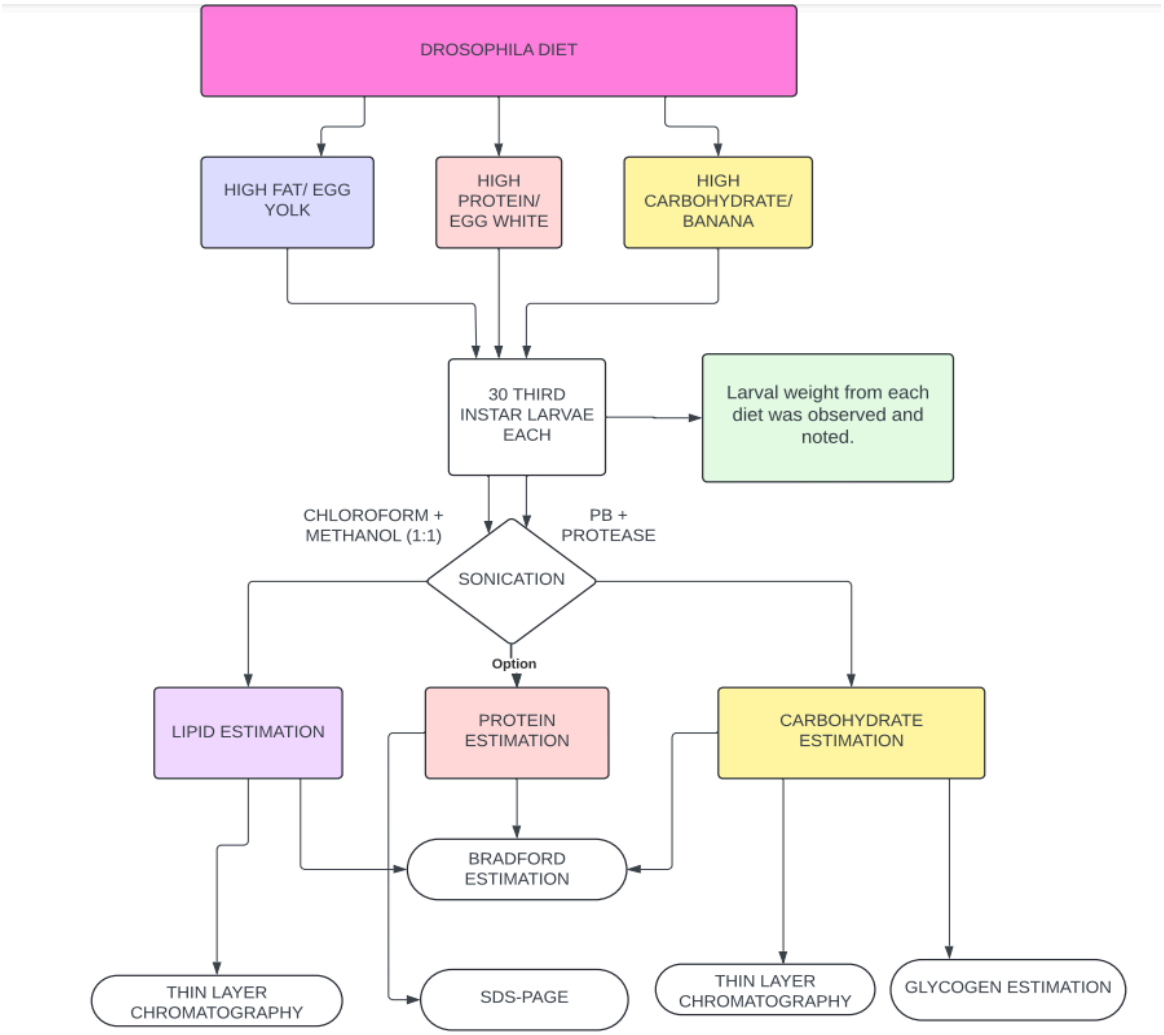
## RELEVANCE OF OUR STUDY

*Drosophila melanogaster* was selected for this project due to its well-established relevance in studying complex biological processes, including metabolism and nutritional physiology. Its genetic similarity to humans, short life cycle, and ease of maintenance make it an ideal model organism for investigating how dietary changes affect physiological responses.

Our literature review revealed that most existing studies on *Drosophila* have primarily focused on the effects of carbohydrate-rich diets. However, research on the impact of high-protein diets and high-fat diets on *Drosophila* per se remains limited. We also observed that *Drosophila* have predominantly been fed plant-based diets, and there has been almost no exploration of the effects of animal-based diets. In our study, we aimed to fill this gap by modifying the dietary composition to include high-protein and high-fat **animal-based** diets and investigate how varying macronutrient levels affect *Drosophila*'s physiological responses, behavior, and overall fitness. This approach allowed us to improve our understanding of the effects of various macronutrient profiles on *D. melanogaster*'s physiology and fecundity.

## MATERIALS AND METHODS:

### Experimental Design




| HIGH PROTEIN DIET | HIGH FAT DIET | HIGH CARBOHYDRATE DIET |
|---|---|---|
| 4 EGG WHITE<br>YEAST : 4.7 Grams<br>PROPIONIC ACID : 1.5mL<br>BENZOATE : 0.5 Grams<br>SUGAR : 1.88 Grams | 4 EGG YOLK<br>YEAST : 4.7 Grams<br>PROPIONIC ACID : 1.5mL<br>BENZOATE : 0.5 Grams<br>SUGAR : 1,88 Grams | 1 BANANA<br>YEAST : 4.7 Grams<br>PROPIONIC ACID : 1.5 mL<br>BENZOATE : 0.5 GRAMS<br>SUGAR : 1.88 Grams |

Agar-Agar 12.9 grams in 100mL was Boiled. and added in the flasks (2-3 mL)

**Figure 2(a):** Experimental Design; **2(b):** Modified Diet Preparation

| High fat diet (EY) | High protein diet (EW) | High carbohydrate diet (B) |
|---|---|---|
| Total Calories in 4 egg yolk : 220 cal<br><br>preconsisted amount of :<br>total fat : 18 grams<br>cholesterol : 736 miligrams<br>total carbohydrates : 2.4 grams<br>protein : 10.8 grams<br><br>additional amount of calories added<br><br>1.88 grams of sugar : 7.52 calories<br>4.7 grams of yeast : 15 calories<br>12 grams of agar agar (2-3 ml added) : 0.24 cal<br><br>total calories in high fat diet (egg yolk,100ml) : 242.76 calories.<br>calorie per ml of media : 2.42 calories/ml | Total Calories in 4 egg white : 68 cal<br><br>preconsisted amount of :<br>total fat : 0.2 grams<br>cholesterol : 0 miligrams<br>total carbohydrates : 1 grams<br>protein : 14.4 grams<br><br>additional amount of calories added<br><br>1.88 grams of sugar : 7.52 calories<br>4.7 grams of yeast : 15 calories<br>12 grams of agar agar (2-3 ml added) : 0.24 cal<br><br>total calories in high protein diet (egg white,100ml) : 90.76 calories<br>calorie per ml of media : 0.90 calories/ml | Total Calories in 1 banana : 105 cal<br><br>preconsisted amount of :<br>total fat : 0.4 grams<br>cholesterol : 0 miligrams<br>total carbohydrates : 27 grams<br>protein : 1.3 grams<br><br>additional amount of calories added<br><br>1.88 grams of sugar : 7.52 calories<br>4.7 grams of yeast : 15 calories<br>12 grams of agar agar (2-3ml added) : 0.24 cal<br><br>total calories in high carbohydrate diet (banana,100ml) : 127.76 calories<br>calorie per ml of media : 1.27 calories/ml |
| fat:carbohydrate:protein | | |
| 15:2:9 | 1:5:72 | 4:270:13 |

**Figure 3:** Calorie Content & F: C:P[1] ratios of Modified Diets.

## Life Cycle and Growth in *Drosophila melanogaster*

To investigate the impact of dietary composition on the life cycle and growth of *Drosophila melanogaster*, we designed an experiment utilizing three distinct diets: banana, egg yolk, and egg white.

- Three types of media- carbohydrate-rich (with banana), protein-rich (with egg white), and fat-rich (with egg yolk ) were prepared under sterile conditions and allowed to solidify.
- Adult *Drosophila melanogaster* were transferred on 23/09/2024 in a conical flask with the following diets egg yolk with 3 males and 3 females, egg white with 3 males and 4 females, banana with 3 females and 6 males, by inverting the stock tube and shaking the flies into a new vial, utilizing a funnel to guide them into the new container without escaping.
- The vials were maintained at 25°C with a 12-hour light/dark cycle.
- The flies were regularly monitored after being introduced to the media and the progression through all stages of the *Drosophila melanogaster* life cycle was observed and recorded

  → time from egg laying to hatching

  →first instar, second instar, third instar, and the time spent in each stage

  → day of transition to pupal stage and the duration of it

[1] F:C:P stands for Fat:Protein:Carbohydrate

→ day at which the adult flies emerged from the pupae.

After recording all observations, the data was analyzed and compared across all three diets to study the effect on the life cycle and survival of *Drosophila melanogaster* across the different diets.

## Measurement of Average Weight of Drosophila Larvae and Flies

- Preparation:
  - Calibrate the balance with the empty container.
  - Gather 12 larvae and 12 flies.
- Weighing the Larvae:
  - Place all 12 larvae on the balance and record the total weight.
  - Remove 2 larvae, weigh the remaining 10 larvae, and record the weight.
  - Repeat this process until only 2 larvae remain.
- Calculating Average Weight for Larvae:
  - Divide each recorded weight by the number of larvae weighed.
  - Average all group weights and divide by 6 to get the average weight of a single larva.

-Average Weight = Total weight/No of larvae weighed

- Weighing the Flies:

- Follow the same process for the 12 flies as described for the larvae.

- Calculating Average Weight for Flies:
  - Divide each weight by the number of flies, average all weights, and divide by 6 for the average weight of a single fly

## Protein Extraction

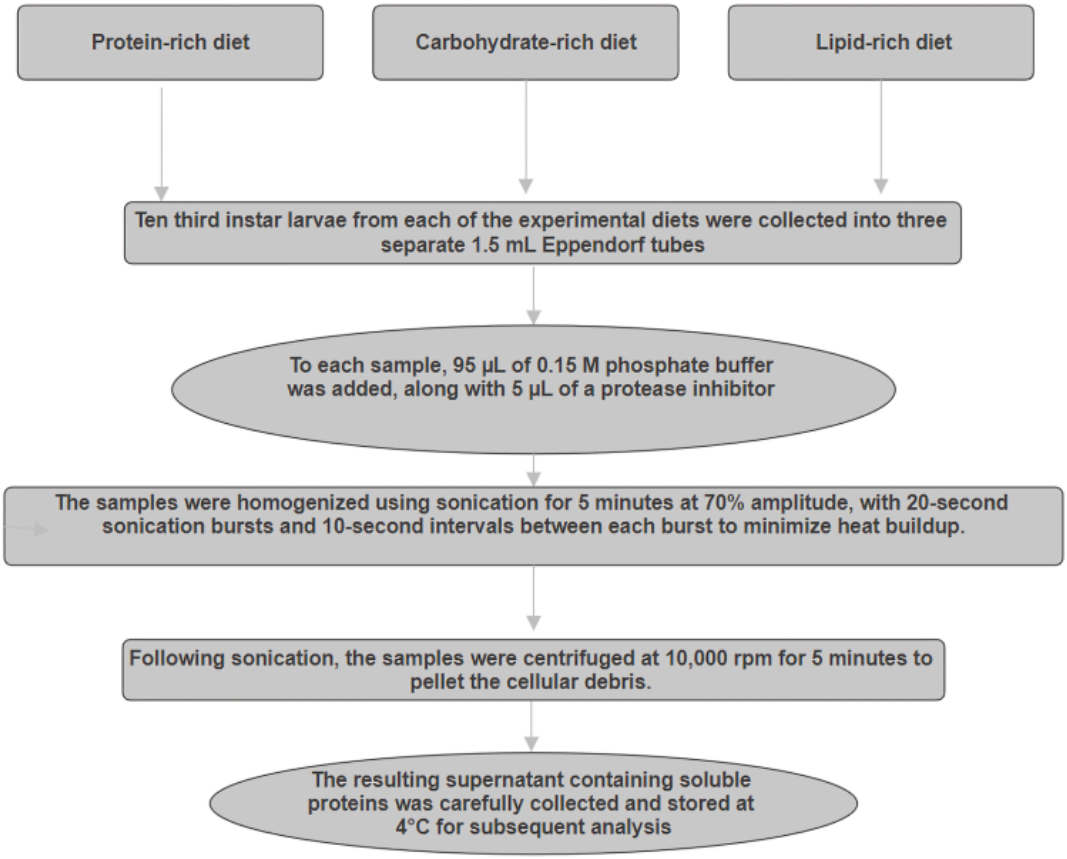



**Figure 4:** Flowchart for Protein Extraction.

## Bradford Estimation [24]

- A standard curve was generated using bovine serum albumin (BSA) at known concentrations of 1 mg/ml diluted with distilled water, following the volumes indicated in the accompanying table.
- After preparing the standards, 100 µL of Bradford reagent was added to each sample, and absorbance readings were recorded.
- A standard curve was then plotted based on the absorbance values versus the BSA concentrations.
- For the experimental protein samples, 1 µL of each sample (in duplicates) was mixed with 4 µL of 0.15 M phosphate buffer.
- Subsequently, 100 µL of Bradford reagent was added to each mixture, and the absorbance was measured.
- The protein concentration in each sample was calculated by referencing the standard curve, and **back-calculation** was performed to estimate the total protein content per larva.

## Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) [25]

- 25 µg of protein was loaded and separated on a 10% resolving gel was prepared using the specified concentrations of the components.
- Following this, a stacking gel was similarly prepared, and a comb was inserted to create wells for sample loading.
- The samples were prepared based on the protein concentration in each lysate, and the appropriate volume of loading dye was added. The samples were denatured by boiling at 70°C for 10 minutes.
- The denatured protein samples, along with a molecular weight marker (ladder), were loaded into the wells and subjected to SDS-PAGE.
- After electrophoresis, the gel was transferred to a staining solution for 1–2 hours to visualize the protein bands.
- It was then placed in a destaining solution and left overnight to remove excess stain.
- The gel was subsequently visualized to observe the resolved protein bands.

## Lipid Extraction[26]

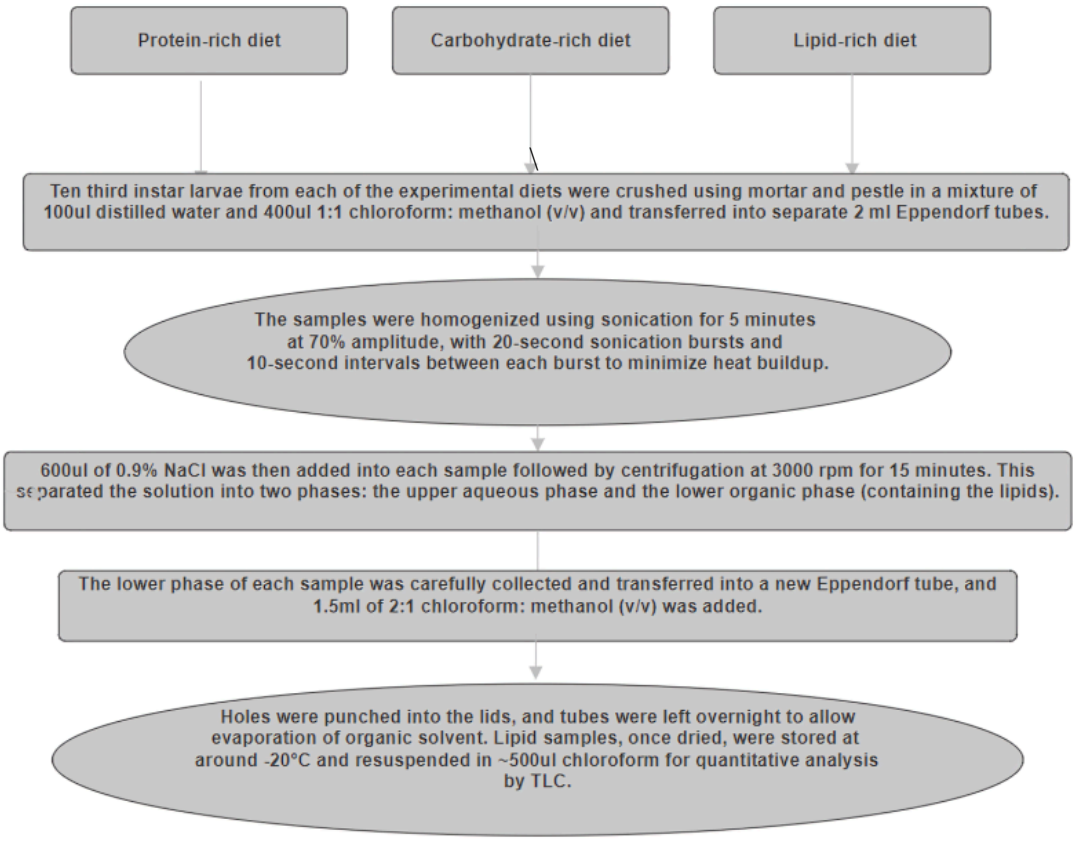



**Figure 5:** Flowchart for Lipid Extraction.

## Thin Layer Chromatography of Lipids

- For standard 1g of triolein (solid at 4°C and liquid at room temperature) was taken.
- The solvent system was made with petroleum ether: diethyl ether: glacial acetic acid in a ratio of 90:10:1.
- 5 µl of triolein (around 5 mg of triolein was taken) was added in 95 µl of hexane [100 µl of hexane and diethyl ether = 90 µl of hexane + 10 µl diethyl ether][ ] Hence 0.50 µg of triolein/100 µl was prepared.
- The solvent system was prepared by dissolving 90 ml of petroleum ether, 10 ml of diethyl ether, and 1 ml of glacial acetic acid. It was prepared and kept in the beaker for saturation using Whatman paper for an hour.
- For visualization, iodine fumes were used.
- The lower layer of lipid extraction was obtained from each growth medium and was run against standard triolein.

## Glycogen Estimation

- 50 microlitres and 100 microlitres of sample were made up to a volume of 200 microlitres using 0.1 M Phosphate buffer.
- Samples were then subjected to acid hydrolysis using 3N HCl at 100 C for 3 hours.

**o-toluidine method[27]:**

- 40, 80, 120, 160, and 200 micrograms of glucose were used to plot the standard curve.
- After the incubation, o-toluidine reagent was added to the samples and incubated for 10 minutes at 80 C.
- The absorbance values were measured at 630 nm and were used to determine the concentration of reducing equivalents in the sample.

**Glucose-Peroxidase method[28]:**

- 10, 20,40, 60,80, and 100 micrograms of glucose were used to plot the standard curve.
- Post incubation, 2N NaOH was used to make the solution's pH = 7.
- 100 microlitres of Glucose-Oxidase Peroxidase kit reagent was added and the solutions were allowed to incubate at room temperature (27 C) for 10 mins.
- Absorbance values were taken at 505 nm and used to determine the concentration of glucose units in the sample.

## RESULTS :

To investigate the impact of dietary composition on the life cycle and growth of *Drosophila melanogaster*, the growth patterns w.r.t appearance of larval, pupal, and adult flies were monitored daily for wild-type flies cultured in three distinct diets: banana, egg yolk, and egg white.

| **Different developmental stages of *D. melanogaster*** | **Date of Developmental Stages** | |
|---|---|---|
| | **Egg Yolk** | **Egg White** |
| Egg laid | 26/09/2024 | 23/09/2024 |
| First instar larvae | 27/09/2024 | 24/09/2024 |
| Second instar larvae | 28/09/2024 | 25/09/2024 |
| Third instar larvae | 29/09/2024 | 26/09/2024 |
| Pupa | 30/09/2024 | 28/09/2024 |
| Flies | | 01/10//2024 |

**Table 1:** Life cycle of *Drosophila melanogaster* grown on EW and EY diets

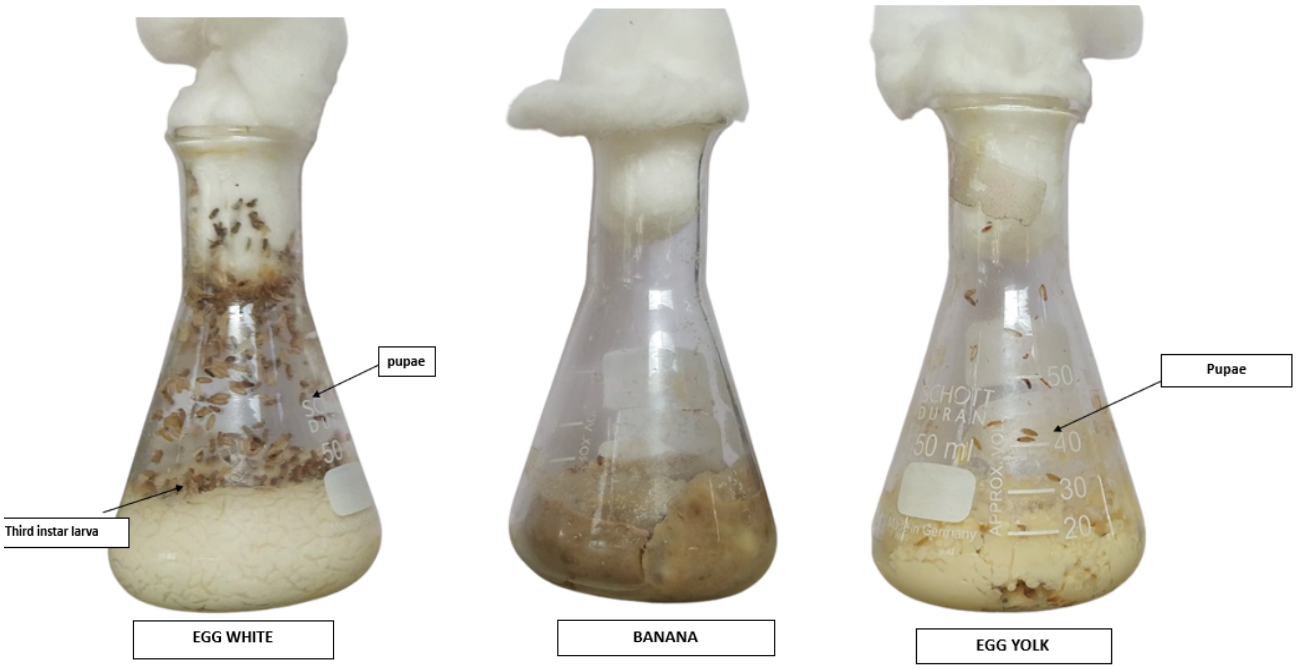



**Figure 6:** Culture vials depicting different stages of *Drosophila* life cycle in different diets

**Observations:**

**Egg yolk Diet:** It was observed that *Drosophila melanogaster* larvae fed with the egg yolk diet exhibited normal growth patterns initially. However, during the second instar larval stage, a significant mortality rate was noted, and only a few larvae progressed to the third instar. Those that survived were able to pupate at the expected developmental time.

**Egg white Diet**: It was observed that *Drosophila melanogaster* larvae fed with an egg white diet exhibit accelerated growth and increased activity levels.

**Banana Diet:** Banana-based diet resulted in a failure to sustain the growth of the *Drosophila melanogaster*. Despite maintaining consistent environmental and experimental conditions, very few third-instar larvae were obtained. Progression to pupal and adult flies was also minimal.

| **S.No.** | **Drosophila larvae from diet composition of** | **Average weight per larvae ( in mg)** | **Number of larvae weighed (x)** |
|---|---|---|---|
| 1 | Banana | 1.776 ± 0.02 | 12 |
| 2 | Egg White | 1.682 ± 0.018 | 12 |
| 3 | Egg Yolk | 1.237 ± 0.015 | 8 |

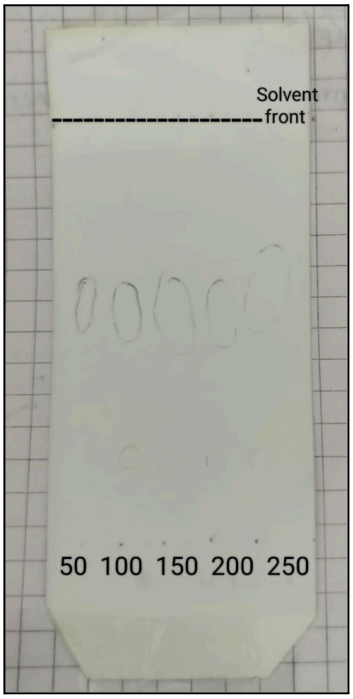
**Table 2**: Larval weight of *D. melanogaster* fed on EW, EY, and B diets

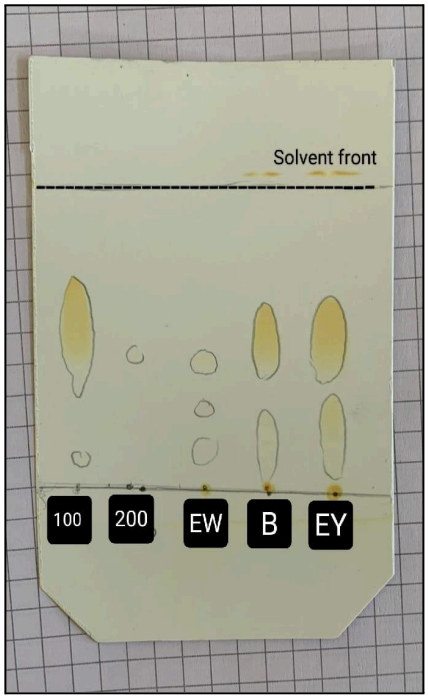
| S.No. | Drosophila flies from the diet composition of | Average weight per fly ( in mg) | Number of flies weighed (x) |
|---|---|---|---|
| 1 | Banana | 1 ± 0 | 4 |
| 2 | Egg White | 0.992 ± 0.02 | 12 |
| 3 | Egg Yolk | 0.778 ± 0.018 | 12 |

**Table 3:** Weight of adult flies of *D. melanogaster* fed diets EW, EY, and B.

**Observation:** It was observed that *Drosophila Melanogaster* larvae grown on Banana and Egg white exhibited no difference in the larval and adult flyweights. However, despite being calorie-dense, *Drosophila* cultured on the Egg yolk diet showed lower larval as well as adult flies.

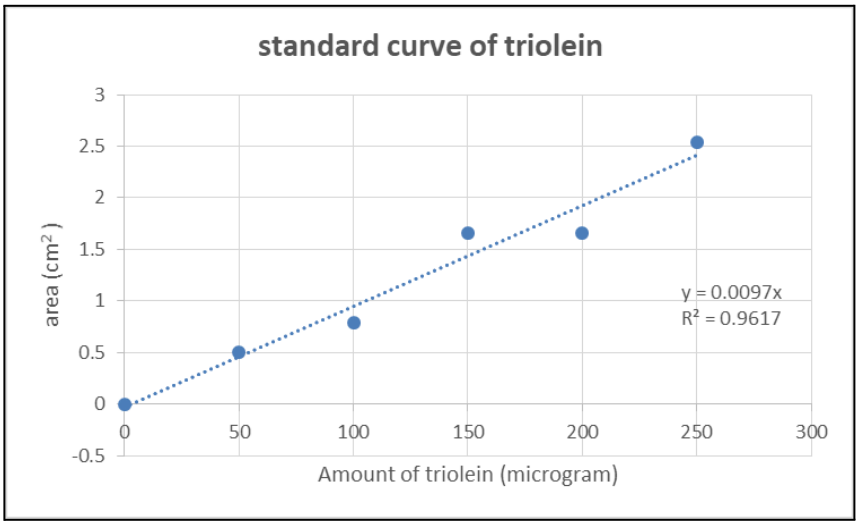
**Lipid Estimation**




**a)** **b)**

**a)Figure 7:** Standard concentration of triolein separated on TLC.
**b)Figure 8:** TLC for TAG estimation in various diets. RF value of triolein came out to be




| S. No. | Amount of Triolein (µg) | Area of spot ($cm^2$) |
|---|---|---|
| 1 | 0 | 0 |
| 2 | 50 | 0.50 |
| 3 | 100 | 0.79 |
| 4 | 150 | 1.66 |
| 5 | 200 | 1.66 |
| 6 | 250 | 2.54 |

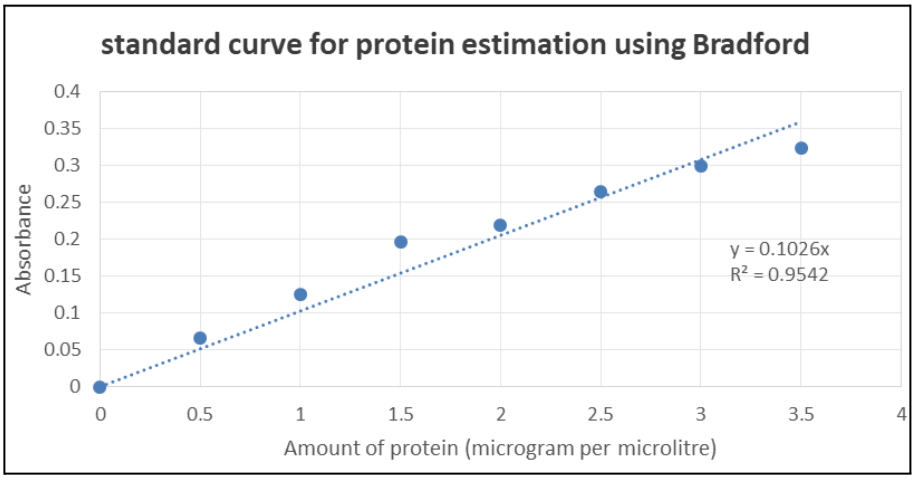
**Table 4:** The standard plot of concentration of triolein vs area of a spot on TLC

| Diet | Spot 1 area ($cm^2$) | Spot 2 area ($cm^2$) | Concentration of spot 1 (µg) | Concentration of spot 2 (µg) | Rf of spot 1 (cm) | Rf of spot 2 (cm) |
|---|---|---|---|---|---|---|
| Egg white | 0.13 | 0.03 | 12.95 | 3.23 | 0.13 | 0.43 |
| Egg yolk | 1.54 | 1.77 | 158.61 | 182.09 | 0.188 | 0.53 |
| Banana | 0.79 | 1.13 | 80.93 | 116.53 | 0.15 | 0.51 |

**Table 5:** TAG concentrations of larvae grown on EW, EY, and B diets

**Observation:** The concentration of triacylglycerol (TAG) in a high-fat diet derived from egg yolk was found to be the highest, as determined by comparison to the standard curve. This was followed by the TAG concentration in the larvae grown on the Banana diet, with the lowest concentration observed in egg white. The triacylglycerol (TAG) storage is minimal in the egg-white diet compared to other diets. However, the turnover of TAG—referring to the rate at which TAG is metabolized—is higher in egg white than in banana and egg yolk. This suggests that, despite its low-fat content, TAG in egg white undergoes a more rapid breakdown.

**Protein Estimation**




| Amt of BSA (µg) | $A_{595nm}$ |
|---|---|
| 0 | 0 |
| 0.5 | 0.066 |
| 1 | 0.125 |
| 1.5 | 0.197 |
| 2 | 0.22 |
| 2.5 | 0.265 |
| 3 | 0.299 |
| 3.5 | 0.324 |

**Table 6:** Standard curve for protein estimation by Bradford method

| Sample | $A_{595nm}$ | $A_{595nm}$ | Amount 1 (µg) | Amount 2 (µg) | Average amount of protein (µg) | S.d |
|---|---|---|---|---|---|---|
| Banana | 0.263 | 0.268 | 2.56 | 2.61 | 2.585 | 0.025 |
| Egg white | 0.3239 | 0.3101 | 3.51 | 3.022 | 3.086 | 0.064 |
| Egg Yolk | 0.2963 | 0.2801 | 2.88 | 2.73 | 2.805 | 0.075 |

**Table 7:** Protein concentrations of larvae grown on EW, EY, and B diets

**Observation:**

The protein concentration per larvae is higher in the egg white diet than in the banana and egg yolk diet. Larvae grown in the Egg White diet were found to contain greater protein reserves, followed by egg yolk and banana as estimated by Bradford.

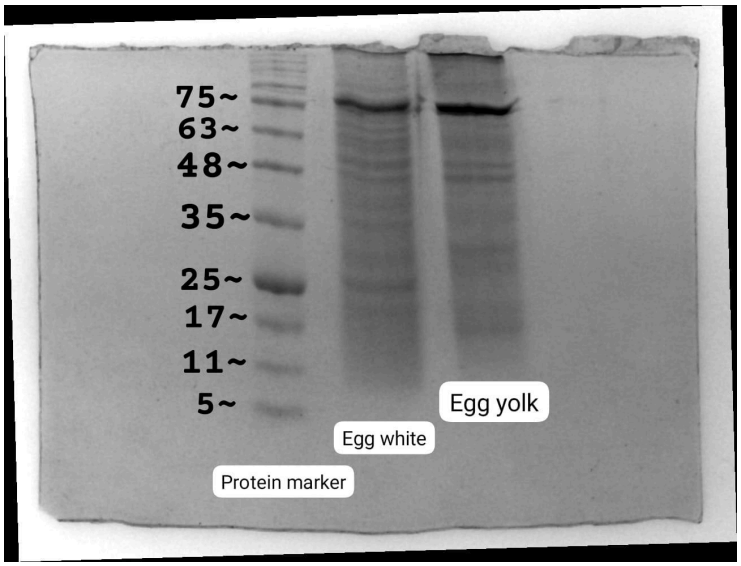
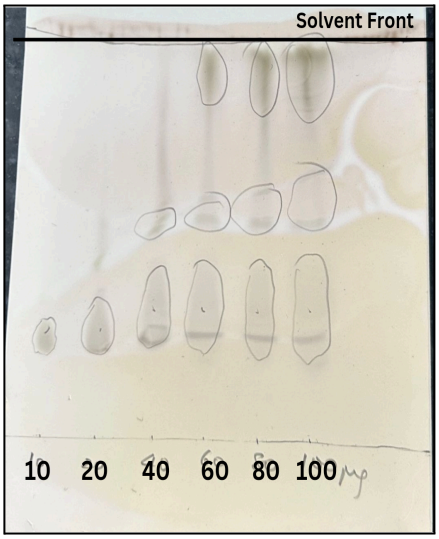



**Figure 8:** SDS-PAGE Gel Picture.

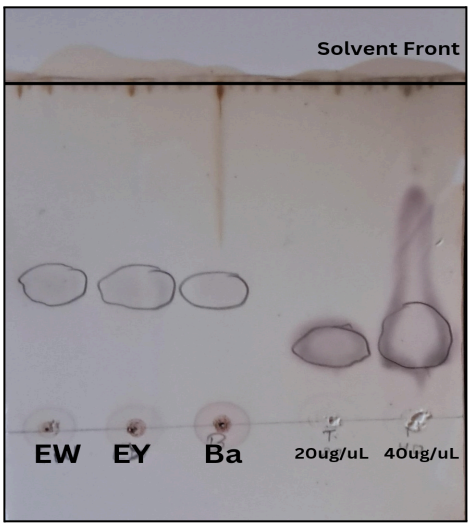
**Observation:**

The protein content in egg white-grown larvae was observed to be higher than that of egg yolk-grown larvae. Additionally, the resolution of protein banding patterns in egg white cell extracts, in SDS-PAGE, is more distinct and clearer compared with egg yolk extracts. Moreover, low molecular weight proteins have been identified in egg white extracts but further analysis is required to detect their identity and function.

**Glucose and Trehalose Estimation**



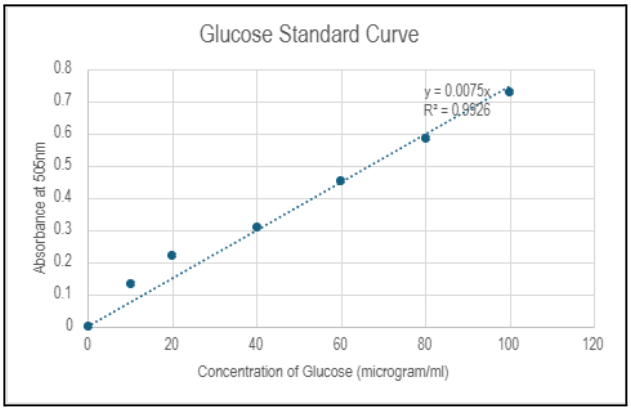

a)



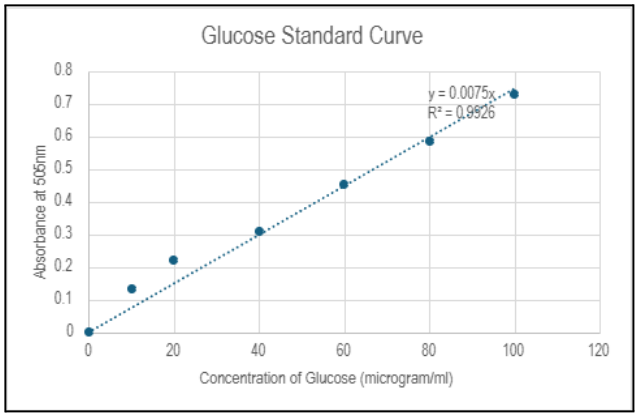

b)

a) **Figure 9a:** TLC for different Trehalose Concentrations (µg/µL);
b) **Figure 9b:** TLC to visualize Trehalose in third-instar larvae fed on different diets.

**Observation:** In the standard for different Trehalose concentrations, a spot is observed near the solvent front at higher concentrations of trehalose, which needs to be identified. The RF values observed for EW, EY, and B are higher as compared to the values of the Trehalose standard that were loaded. This probably indicates that the spot observed on the TLC is not trehalose but is a product of its hydrolysis, which could be glucose but the identification was not carried out.




a)




b)

a) **Figure 10a:** Glucose Standard Curve using Glucose Oxidase and Peroxidase method.
b) **Figure 10b:** Glucose Standard Curve using the o-toluidine method.

| **Diet** | **The volume of the Sample (µL)** | **$A_{505nm}$** | **Concentration (µg/µL)** | **Avg Conc of Glucose for different diets*(µg/µL)** | **Avg Trehalose Conc* (µg/µL)** |
|---|---|---|---|---|---|
| EW | 50 | 0.065 | 8.67 | 16.53 | 8.266 |
| | 50 | 0.053 | 7.07 | | |
| | 100 | 0.137 | 18.26 | | |
| | 100 | 0.123 | 16.4 | | |
| EY | 50 | 0.099 | 13.2 | 27.55 | 13.77 |
| | 50 | 0.094 | 12.53 | | |
| | 50 | 0.084 | 11.2 | | |
| | 100 | 0.194 | 25.86 | | |
| | 100 | 0.301 | 40.13 | | |
| | 100 | 0.191 | 25.46 | | |

| | | | | | |
|---|---|---|---|---|---|
| B | 50 | 0.112 | 14.93 | 32.36 | 16.18 |
| | 50 | 0.138 | 18.4 | | |
| | 100 | 0.192 | 25.6 | | |
| | 100 | 0.279 | 37.2 | | |

**Table 8**: Estimation of Reducing Equivalents and Trehalose using Glucose Oxidase Peroxidase method

| Diet | Volume of Sample (µL) | $A_{630nm}$ | Concentration (µg/µL) | Avg Glucose conc*(µg/µL) | Avg conc. of reducing equivalents*(µg/µL) |
|---|---|---|---|---|---|
| EW | 50 | 0.083 | 48.35 | | |
| | 50 | 0.134 | 73.85 | 61.1 | |
| | 100 | 0.245 | 129.35 | | |
| | 100 | 0.256 | 134.85 | 132.1 | 127.15 |
| EY | 50 | 0.08 | 46.85 | | |
| | 50 | 0.085 | 49.35 | | |
| | 50 | 0.087 | 50.35 | 48.85 | |
| | 100 | 0.213 | 113.35 | | |
| | 100 | 0.257 | 135.35 | | |
| | 100 | 0.184 | 98.85 | 115.85 | 106.775 |
| B | 50 | 0.128 | 70.85 | | |
| | 50 | 0.144 | 78.85 | | |
| | 50 | 0.199 | 106.35 | 85.35 | |
| | 100 | 0.4 | 206.85 | | |
| | 100 | 0.222 | 117.85 | | |
| | 100 | 0.299 | 156.35 | 160.35 | 165.525 |

**Table 9:** Estimation of Reducing Equivalents and Trehalose using the o-toluidine method.

| Diet | Concentration of reducing equivalents per larva (OT method) | Concentration of glucose per larva (GOD POD method) | Concentration of Trehalose per larva |
|---|---|---|---|
| EW | 18.825 ±2.171 | 1.653±0.1772 | 0.82667±0.1772 |
| EY | 15.5625 ± 1.546 | 2.7556 ± 0.6317 | 1.37778 ± 0.6317 |
| B | 25.0875 ± 3.72 | 3.23667 ±0.5629 | 1.618333 ±0.5629 |

**Table 10:**Average concentration of Trehalose and Reducing Equivalents per larva for different diets.

**Observations:**
**Egg white:** Larvae grown on egg white showed the lowest concentration of glucose units as determined by the Glucose-Oxidase Peroxidase (GOD POD) method and subsequently, lower trehalose concentrations. However, the concentration of reducing equivalents is relatively higher than that of larvae grown on egg yolk. This could be due to the presence of other potential aldohexoses that may be present in the larvae.
**Egg yolk:** Larvae grown on egg yolk had lower concentrations of reducing aldohexose equivalents as compared to Egg white but higher concentrations of glucose and trehalose stored.
**Banana:** Larvae grown on a banana diet had high amounts of both glycogen and trehalose stores.

## CONCLUSIONS

1. *Drosophila* grown on the EW diet which was calorie deficit but high protein diet showed a very good growth profile with flies appearing within one week, Larval weight and fly weight were also comparable to high carbohydrate and high-fat diet.
2. *Drosophila* grown on EY, which had a caloric excess and high-fat failed to grow efficiently with very few flies maturing even after 10-15 days. The larval mobility and survival was poor.
3. As was expected the larval TAG stores were seen in both egg yolk and banana diets and significantly lower levels were observed in larvae grown on a high protein diet (EW).
4. Again, as was expected, the larva protein content was higher in the EW diet as compared to the banana and EY diet. The comparison of the SDS profile of the egg yolk diet as compared with egg white showed a different profile in low molecular weight proteins which needs to be further investigated.
5. The trehalose stores in all three diets were significant but were lower in the EW diet as compared to the EY and Banana diets. The TLC profile showed that the trehalose stores are undergoing rapid hydrolysis signifying a higher turnover. The lower trehalose stores in EW seem to be compensated by a higher glycogen content. As was expected, the high carbohydrate diet showed significant levels of glycogen and trehalose.

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