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GUT MICROBIAL DIVERSITY IN CARBOHYDRATE AND PROTEIN RICH DIET IN MALE FLIES OF *Drosophila melanogaster*

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Objective: How the host organism and its gut bacteria interact to modulate host physiology. The goal of this study is to learn more about the impact of food composition on gut microbiota in *D. melanogaster*. Material and methods; Male flies of *Drosophila melanogaster* was raised in three different medium nomal diet (wheat cream agar medium), carbohydrate rich diet (20% of sucrose), protein rich diet (60% of casein). Twenty male flies midguts were isolated from each media and subjected to microbial diversity analysis. Result and conclusion**;** It was noticed in *D. melanogaster* that gutmicrobial vary with diet. The investigation revealed that gut microbial flora in relation to host diet corresponds to *Acetobacters* species and *Lactobacillus* species. *A. pomorum*, *A. tropical, L. brevis, L. fructivorans,* and *L. plantarum.* The relative abundance of each of the species varies in relation to host diet, Male flies of *D. melanogaster* fed on a carbohydrate-rich diet shows the highest density of *L. plantarum* and *L. fructivorans* bacteria. *L. brevis,* on the other hand, had the maximum density shown in male flies fed on protein-rich medium. Furthermore, males raised on a protein-rich diet shows the highest density of *A. pomorum* and *A.tropicalis*. Male flies raised on a normal diet had the lowest density of all five microbes from two distinct species. Thus these studies clearly explain that host physiology changes with diet, inturn, it has significant influence on resident gut microbial diversity in male flies of *Drosophila melanogaster*. As a result, our findings show that the diet of host has substantial impact on the density of gut microbial in *D. melanogaster.*

Keywords: Diversity; Gut microbe; Pyrosequencing; Host diet; Nutrition; 16s rRNA primer.

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1. INTRODUCTION

Food is broken down for nutritional and energy extraction, synthesis of vital vitamins, and defence against pathogen colonisation are all key functions of microrganism communities identified in the gut of mammals [1]. Microbiome composition and stability, on the other hand, can change based on both intrinsic and external factors in the host, such as age, sex, and genotype. Effects of Nutritional arising on or after variety in foods form accessibility are the most straightforward method that environmental difference be able to control body state and fecundity. Dietary impacts can be categorised as quantitative (availability of food) or qualitative (quality of food) (i.e. food composition). As a outcome, there is a optimistic relationship among food accessibility and body situation or fecundity under natural range. Food provides energy and nutrition to animals, hence nutrition can be regarded a major factor that influences many aspects of their lives [2,3]. The study of how organisms modify their energy allocation has been aided by experimental changes to animal diets [4,5]. Disease susceptibility, fertility, reproduction, longevity, and stress tolerance are all affected by the amount and quality of nutrients consumed by organisms. The physically and morphological reactions of persons exposed to varying quality and amounts of nutrients are frequently assessed in studies concerned with the effects of nutrition.

Aside from cline impacts, the availability of food in that specific place is also a significant influence in variation of stress resistance. With this in vision, the purpose of this research was to measure the physiological adaptations and life-history impacts of different feeding regimes on energy allocation. Thus have investigate relative relevance of two key macronutrients on desiccation, hunger, thermal tolerance (hot and cold), life-history variables such as egg to adult viability, egg production, and morphometric traits such as ovariole quantity.

Nutrition is the most important environmental component that influences an organism's ability to reach its genetically encoded development potential [6]. The amount [7], quality [8], and bioavailability [9] of various nutrients in the diet define the nutritional characteristics. In animal nutrition, the resident gut bacteria play a critical role [10,11]. By changing the animal host's nutrition-sensing and -signaling pathways, they can vary feeding and nutrient absorption rates, as well as resource allocation behaviours [12-15].

Several survey have found that resident microbes usually increase animal nutrition, although the nutritional impact varies depending on diet, microbiota composition, and animal genotype [16-19]. In comparison to humans, *D. melanogaster* has a simpler gut microorganism, consisting mostly of yeast and a few bacterial families, mostly from the *Acetobacteraceae* and *Lactobacillales* taxa [20]. The decreased complex of these microbial relationships in *Drosophila's* gut has benefited in the study and hypothesis testing of microbiota–host connections on the host's nutritional phenotype [21].

Nonetheless, different conditions might affect bacterial communities, which in turn form microbiota–host interactions [22]. Diet plays an important effect in the changing of *Drosophila* bacterial groups, according to several studies [23-25]. Furthermore, host-genotype specific variables have been reported to influence *D. melanogaster* gut microbiota [26,27]. *Drosophila* has previously proven to be a useful model for studying how the microbiome affects the nutrition and metabolism of the host. Drosophila is affected by the gut microbiota, which is subject by members of the *Acetobacteraceae* and *Lactobacillales* families [24,28,29]. The gut microbiota, which is controlled by members of the *Acetobacteraceae* and *Lactobacillales* families, has a significant impact on the reaction of *Drosophila melanogasters* to food in this study, which would be ideally suitable for large research design including extensive dietary perturbations and where the gut microbiota, which is controlled by *Acetobacteraceae* and *Lactobacillales* members, has significant impacts on the gut microbiota [30,31]. The major goal of this study is to look at how the gut microbiota of *D.melanogasters* fed on carbohydrate and protein-rich diets changes in composition and diversity.

2. MATERIALS AND METHODS

In this experiment, Oregon-K (*Drosophila melanogaster*) flies were obtained from the *Drosophila* stock centre at Mysore University's Zoology Department. Twenty males and females flies were placed in a *Drosophila* culture bottle with wheat cream agar medium and incubated at 22ºC±1ºC with a relative humidity of 70% using a 12h:12h light:dark cycle. The eggs from the aforesaid flies were placed in culture bottles containing a control diet (wheat cream agar medium), a protein enrich diet (wheat cream agar medium + 60% casein), and a carbohydrate enrich diet (wheat cream agar medium + 20% sucrose). Wheat cream agar was mixed with either sucrose or casein in the following proportions to provide a protein and carbohydrate-rich diet. Before adding water, Wheat cream agar and Sucrose were combined in a 4:1 ratio for a carbohydrate-rich diet. Similarly, Casein and wheat cream agar media were combined in a 3:2 ratio for a protein-rich diet. Each culture vial was filled with 7mL of the

appropriate medium and pasted with dried yeast solution.

2.1 Dietary Effects on Gut Microbial Diversity

Unmated males flies of *D. melanogaster* which is reared on different diet (normal media/carbohydrate rich media/protein rich media) are collected above were used to study composition and diversity of gut bacteria using pyrosequencing.

2.2 Collection of Gut Microbe and Isolation of DNA

Seventy percent ethanol and the QIA ampDNA micro kit (Qiagen,51304) were used to extract DNA from thirty male *Drosophila* midguts, one for each diet (Normal diet, Carbohydrate heavy diet, and Protein rich diet). With an electric pestle, these midguts were externally sterilised with 70% ethanol and homogenised in 180l ATL buffer with a 0.5 percent DX reagent for foam minimization (Kimble TM Kontes TM pellet Pestle, 749540-0000). For further testing, 20 µL proteinase K SOLUTION was added to samples and incubated for 30 min at 56°C with shaking at 650 rpm. Again the sample was homogenized by adding glass beads (425-600µm, sigma Aldrich, G8772-100G) in a fast prep FP120 machine (Bio 101 Savant) and later the sample was incubated for 60 min at 56ºC. RNase A was added to digest RNA(Qiagen, 19101) and then the sample was incubated for 2 min at room temperature, later cool it, Ethanol was added of about 200µl and these sample was shifted to spin column as per production directions, along with washing and elution step were carried out. By adding sodium acetate precipitation the sample are made concentrated.

2.3 Pyro Sequencing of 16s rRNA for Identification of Bacterial Species

Axon-specific 16S rRNA gene primers was used to identify main gut microbial diversity of *Drosophila melanogaster*, identified microbial was *A. pomorum, A. tropicalis*, *L. brevis, L. fructivorans* and *L. plantarum* using software called primer3 and exceptional regions identified from alignments of full 16S rRNA gene sequences. Initially want to check whether this experiment confirms that the primers generated to detectable cross- amplification between species. If the experiment confirms next step is to carry out PCRs were performed as above with 65ºC annealing temperature and 35 cycles. PCR products were separated by gel electrophoresis using 1% agarose gel and visualized with SYBR (Invitrogen), and later the identities were confirmed by Sanger sequencing.

Measurement of Bacterial Loads Quantification of gut microbe found in gut of male flies of *D. melanogaster* which was reared in three different diets (normal media/protein rich media/carbohydrate rich media). MRS agar was used for quantifying all microbes except for the strains, which were *Acetobacter pomorum* quantified on mannitol plates. To measure microbe growth, guts were placed on either MRS or mannitol agar plates. Possible bacterial load were calculated on the source of colony forming units (CFU's) a colony –forming unit is a unit/mi (used to calculate approximately the number of viable bacteria in a sample). Viable is defined as the capability to multiply via binary fission under the controlled state. To count the colony forming unit's microbes were cultured and only the viable cells were counted in contrast with microscopic examination. Abundance is calculated using colony forming units' which were expressed using logarithmic.

2.4 Statistical Analysis

In all of the gut microbial species found, one way ANOVA followed by Tukeys post hoc test revealed substantial difference in gut microbial diversity in male flies raised on varied diets (normal media/protein rich media/carbohydrate rich media).

3. RESULTS

The host diet is one of the most critical factors that influence an organism's resident microbiota. In the present investigation host diet- related changes in gut microbial diversity has been studied in *D. melanogaster*. Prior to experiment as these flies have been standardized by rearing under three different medium (normal medium, carbohydrate rich medium and protein rich medium). These flies was utilised for analysis and subjected to gut microbial diversity to better understand how changes in host physiology and nutrition affect resident microbiota.

In the present experiment, five microbial species were identified using diagnostic primers listed in Table 1 [32], and furthure each of the identified species were quantified through CFU's. It was noticed from Fig.1 that gut microbial flora in relation to hostage correspond to *Acetobacter* and *Lactobacillus* species. *A. pomorum, A. tropicalis, L. brevis, L. fructivorans, L. plantarum*. The relative abundance of each of the above species varies in relation to host diet. The density of *L. brevis, L. fructivorans, L. plantarum. A. pomorum, A. tropicalis* was found to be lowest in male flies reared on normal medium. The density of *L. brevis* was found high in male fed on protein rich medium when compare to carbohydrate rich diet. The density of *L. fructivorans*, *L. plantarum* was found high in male flies reared on carbohydrate rich medium

when compare to protein rich medium. The density of *A. pomorum*, *A. tropicalis* found high in protein rich medium when compare to carbohydrate rich medium. This shows clearly that host physiology changes with diet which had significant influence on resident gut microbial diversity. In the present studies flies used were of same age and other conditions for maintenance and culture where same, only difference was the quantity of nutrients such as protein and carbohydrate therefore observed variation in gut microbial diversity resulted in physiological changes noticed with host diet.

The importance of the resident microbiota in animal nutrition has long been recognised [10,11]. The microbiota involved in the acquisition and distribution of animal nutrients has a significant impact on an animal's nutritional condition. These microorganisms influence feeding and nutrient absorption rates by either consuming ingested nutrients or providing additional nutrients to the host.

The reads acquired by pyrosequencing each sample were allocated to their corresponding OUTs and then analysed for microbiota richness and evenness using theirrespectiveindices,whichshowedthemicrobialcommunities varied along with diet as shown in Table 2.

One way ANOVA followed by Tukey's hoc test revealed significant change in these species among diets. Tukey's post hoc test showed that male flies of *D. melanogaster* raised on protein rich media had significantly greater number of CFU's of L. bervis, A. pomorum and A. tropicals when compare to normal and carbohydrate rich diet. One way ANOVA followed by Tukey's hoc test showed that male flies of *D. melanogaster* reared on carbohydrate rich diet had significantly greater number of CFU's of L. Plantarum and L. fructivorans when compare to normal and protein rich diet.

Table 2.Richness and evenness estimation of gut microbiota in flies developed from each of the diets(Normal diet/Carbohydrate rich diet and Protein rich diet) of *D. melanogaster*. Diversity estimations were obtained following normalization of OUT'S.

4. DISCUSSION

The link between animals and their gut microbiota is well understood to entail numerous interactions that change depending on the microbiota's composition, host genotype, and environmental variables, including food [17,33]. This study is focused on the whether the concentration/ density of the microbiota differ, when the diet alters in *Drosophila melanogaster*. In animals including *Drosophila* host diet is the most important factor which is known to affects the resident microbiota. In present study effects of host diet such as carbohydrate and protein rich diets related changes in gut microbial diversity and their abundance has been studied in *D.melanogsater*. It was noticed from fig1 that gut microbial diversity in relation to carbohydrate and protein rich diets of the host consisting of species belongs to *Lactobacillus* and *Acetobacter* genus. The relative abundance of each

Table 1. Diagnostic primers used for identification of bacteria [32]

Bacterial species	End point PCR		QRT-PCR	
	Forward	**Reverse**	**Forward**	**Reverse**
Acetobacter pomorum	5'-TGGGTGGGGGATAACACTGGGA-3'	5'-AGAGGTCCCTTGCGGGAAACA-3'	5'-TGTTTCCCGCAAGGGACCTCT -3'	5'-AGAGTGCCCAGCCCAACCTGA-3'
Acetobacter tropicalis	5'-AGGGCTTGTATGGGTAGGCT-3'	5'-CAGAGTGCAATCCGAACTGA-3'	5'-TAGCTAACGCGATAAGCACA -3'	5'-ACAGCCTACCCATACAAGCC-3'
Lactobacillus brevis	5'-ACGTAGCCGACCTGAGAGGGT-3'	5'-AGCTTAGCCTCACGACTTCGCA-3'		
Lactobacillus fructivorans	5'-TGGATCCGCGGCGCATTAGC-3'	5'GCCCCCGAAGGGGACACCTA-3'	5'-AACCTGCCCAGAAGAAGGGGA -3'	5'-GCGCCGCGGATCCATCCAA-3'
Lactobacillus plantarum	5'-TCCATGTCCCCGAAGGGAACG-3'	5'-TGGATGGTCCCGCGGCGTAT-3'	5'-TGTCTCAGTCCCAATGTGGCCG -3'	5'-GGCTATCACTTTTGGATGGTCCCGC-3'

Table 2. Diversity estimations were obtained following normalization of OUT's

Diet	OTUs	Chao1	Shannon	Evenness
NM	55	68	2.16	0.75
CR	64	65	3.41	0.78
PR	51	70	3.56	0.81

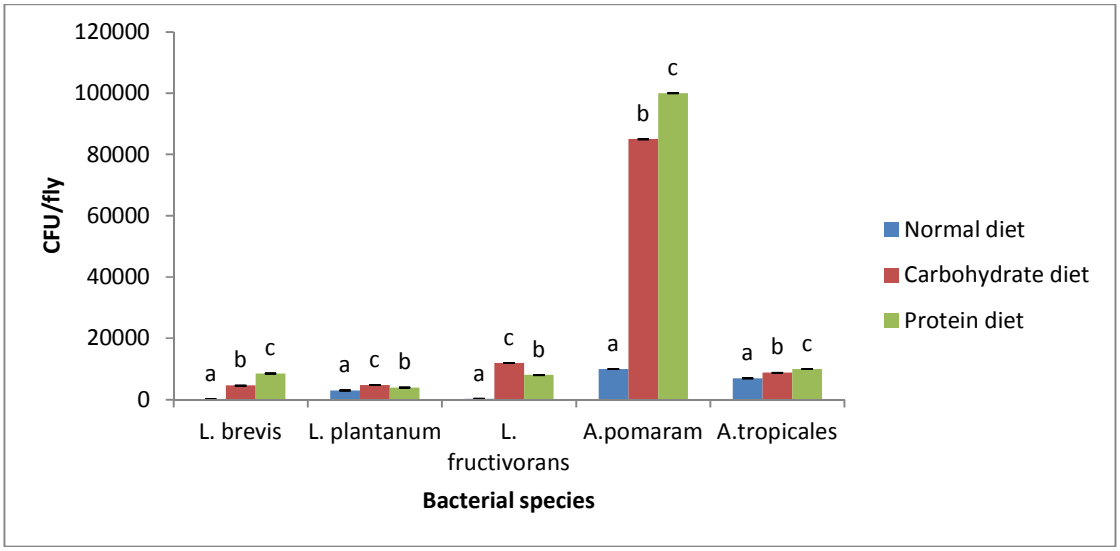

Fig. 1. Effect of male diet on five major gut microbial species of *D. melanogaster* under three different medium (normal medium, carbohydrate rich medium and protein rich medium)

L. brevis; f=53639544.09, df=2,57, p≤0.05: *L. plantarum*; f=1340733.179, df= 2,57, p≤0.05: *L. fructivorans*; f= 707310401.3, df= 2,57, p≤0.05: *A. pomaram*; f=28350090002, df=2,57, p≤0.05: *A. tropicales*; f=8591143.112, df= 2,57, p≤0.05

species, however, changes depending on the host food. This implies that the host's food has a big impact on gut bacteria diversity. In the present studies flies used were of same age and other conditions for maintenance only difference was the different nutrients supplement such as protein rich media and carbohydrate rich media.

As a result, the observed change in gut microbial diversity in the current study, which was an experiment, was attributable to the impact of the host diet in *D. melanogaster*. Our findings with previous research that showed that resident microorganisms typically support animal diet, and that the advantages of nutrition vary depending on food, microbiota makeup, and animal genotype [16-19]. The concentration of *L. bervis* was found higher in gut of male flies reared on Protein rich diet and the least concentration was found in gut of male flies reared in normal diet. In *L. plantarum,* the higher concentration was observed in gut of male flies fed in Carbohydrate rich media (CR) and least concentration was found in gut of male flies reared on Normal diet (NM), were in *L. fructivorans* bacteria was found higher in gut of male flies reared on Carbohydrate rich media(CR) when compare to male flies reared on NM/PR and least concentration of *L. fructivorans* was observed in gut of male flies reared in Normal media in *Drosophila melanogaster*. In *Acetobacter* species such as *Acetobacter pomorum* and *Acetobacter tropicales* microbes which was found in gut of male flies reared on different diet shows higher density in male flies reared on protein rich media when compare to normal diet and carbohydrate rich diet and the least concentration of *Acetobacter pomorum* and *Acetobacter tropicales* was found in normal diet. The variation of the concentration among two different bacteria species found in gut of male flies reared on different diet was due to the different food composition which was in taken by flies.

This is due to the fact that the occupant gut microbiota has a significant impact on organism diet [10,11]. These microbes interact with organism achievement, nutrient distribution, both fundamental processes that determine an animal's nutrition, in a variety of ways. They can absorb nutrients eaten by the host or offer more nutrients; they can change feeding and nutrient

absorption rates; and also can change the host's resource allocation patterns by regulating the nutrient-sensing and -signaling pathways according to [12-15]. This clearly shows that the host diet has a significant impact on *D. melanogaster* gut bacteria diversity. Shin et al., Storelli et al., Yamada et al., Keebaugh et al., [34-37] propose that connections Fly food has a big impact on the relationship between the host and the microbiome. Gut bacteria, in particular, reduce development time and lengthen life span in the absence of nutrients. Excess dietary protein, according to Keebaughet [38] is considered to diminish the influence of the microbiome (particularly bacteria) on development and lifespan. This clearly indicates that host diet has major effect on gut microbial diversity in *D.melanogaster*. Furthermore, in the current study, the reads obtained by pyro sequencing were assigned to their respective OTUs and then analysed for microbiota richness and evenness using their respective indices, implying that gut microbial diversity varied with host 50 Gut Microbial Diversity in male *Drosophila melanogaster* reared on different diets (normal media/carbohydrate rich media/protein rich media) Table 2: Tukey's PostHoc test revealed a significant difference in gut microbial diversity of *Lactobacillus* and *Acetobacter* species in male *Drosophila melanogaster* flies reared on different diets (normal media/carbohydrate rich media/protein rich media).

Microbes that help in larval development and also linked to the capability to increase on fly culture medium indicate that microbial abundance is a significant predictor of impacts on physiology and lifespan under nutrition and reveal an unexpected variety of microbial species that support fly growth and life durability. The gut microbiota was either helpful or benign (encourage host routine) or benign (no detectable influence on host performance), but not harmful to *Drosophila* flies raised on various diets (normal media, carbohydrate rich media, protein rich media). There are two parts to the ending. First, neither the host nor the microbiota compete for dietary nutrients as evidenced by the host's higher-quality performance on certain diets. As a result, it appears that a range of dietary-derived nutrients are either not utilised by both the host and the microbiota, or are plentiful enough that microbiota intake has little influence on host function.

Second, *Drosophila* does not require microbiota for normal physiological functions, as evidenced by the higher effects of typical *Drosophila* on all diets. Instead, the microbiota mostly improved *Drosophila* performance on diets with poor or imbalanced nutrient content, suggesting that the link is nutritional in nature. The deficiency of metabolic activity as of the gut bacteria may possibly be responsible for the lack of high protein and carbohydrate supplementation in *Drosophila* flies. Because *Drosophila's* gut bacteria (*Acetobacteraceae* and *Lactobacillales*) may use nutrition, it's possible that they lower the nutrition concentration of the food swallowed by female flies, and therefore calorie intake per unit food consumed, when compared to male flies on the identical diet. It was also shown that the pathways influencing microbiota-host metabolic interaction are likely diverse and interacting. It was also suggested that male and female host signalling pathways regulating metabolism could react in a different way to microbial products and their absence, and that numerous metabolic and other physiological differences among the sexes, particularly females' nutritional demands for egg production, may influence the metabolic traits of the microbiota. *Drosophila* protein consumption is aided by bacteria and yeasts in the gut microbiome, notably in females and on low-protein diets. This relationship helps *Drosophila* raised on diets lacking in particular critical amino acids live longer [35,36,37] and produce more eggs. Dietary supplements can also influence the gut microbiome. Prebiotics are dietary supplements that, once consumed, serve as food or substrate for the host microbiota. Depending on the substrate on which they eat, *Drosophila* can potentially affect the makeup of microorganisms [39,40]. The influence of a high-protein, high-carbohydrate diet on gut microbial variety in male *Drosophila melanogaster*. As a result of these research, it appears that feeding male *Drosophila* flies a food supplement has a substantial impact on gut microbial diversity in *D. melanogaster* flies. In addition, the host's nutrition and gut bacteria abundance have a substantial impact on the host's physiology, metabolism, and fitness.

5. CONCLUSION

Based on the finding of this research, It indicates that providing a food supplement to male Drosophila flies has a significant influence on gut microbial diversity in *D. melanogaster* flies. Furthermore, the host's physiology, metabolism, and fitness are all influenced by the host's diet and gut bacteria abundance.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Rajilic-Stojanovic M, De Vos WM. The first 1000 cultured species of the human gastrointestinal microbiota. FEMS Microbiol Rev. 2014;38:996–1047.
2. Sterner RW, Schulz KL. Zooplankton nutrition: Recent progress and a reality check. Aquatic Ecology.1998;32:261–279
3. Taylor EN, Malawy MA, Browning DM, Lemar SV, DeNardo DF. Effects of food supplementation on the physiological ecology of female Western diamond-backed rattlesnakes (*Crotalus atrox*). Oecologia. 2005; 144:206–213.
4. Chown SL, Nicolson SW. Insect Physiological Ecology. Oxford University Press, New York; 2004.
5. Cruz-Neto AP, Bozinovic F. The relationship between diet quality and basal metabolic rate in endotherms: insights from intraspecific analysis. Physiol. Biochem. Zool. 2004;77: 877–889
6. Lifshitz F. Nutrition and growth. J Clin Res Pediatr Endocrinol. 2009;1:157–163
7. Shanley DP, Kirkwood TBL. Calorie restriction and aging: a life-history analysis. Evol. 2000;54:740–750.
8. Chipponi JX, Bleier JC, Santi MT, Rudman D. Deficiencies of essential and conditionally essential nutrients. Am J Clin Nutr. 1982;35: 1112–1116.
9. Fairweather-Tait SJ, Southon S. Bioavailability of nutrients. In: Caballero B, editor. Encyclopedia of Food Sciences and Nutrition (Second Edition). Oxford: Academic Press. 2003;478–484.
10. Flint HJ, Scott KP, Louis P, Duncan SH. The role of the gut microbiota in nutrition and health. Nature reviews. Gastroenterol. Hepatol. 2012;9:577-589
11. Karasov WH, Douglas AE. Comparative digestive physiology. Comprehensive Physiology 2013;3:741-783.
12. Bäckhed F, Ding H, Wang T, Hooper LV, Koh GY, Nagy A, Semenkovich CF, Gordon JI. The gut microbiota as an environmental factor that regulates fat storage. Proc. Natl. Acad. Sci. USA. 2004;101:15718-15723.
13. Caricilli AM, Saad MJ. The role of gut microbiota on insulin resistance. Nutrients. 2013;5:829-851.
14. Goodman AL, McNulty NP, Zhao Y, Leip D, Mitra RD, Lozupone CA, Knight R, Gordon JI. Identifying genetic determinants needed to establish a human gut symbiont in its habitat. Cell Host Microbe. 2009;6:279-289.
15. Vijay-Kumar M, Aitken JD, Carvalho FA, Cullender TC, Mwangi S, Srinivasan S, et al. Metabolic syndrome and altered gut microbiota in mice lacking Toll-like receptor 5. Science. 2010;328:228-231.
16. Benson AK. Kelly SA, Legge R, Ma F, Low SJ, Kim J, Zhang M, Oh PL, Nehrenberg D, Hua K. Individuality in gut microbiota composition is a complex polygenic trait shaped by multiple environmental and host genetic factors. Proc. Natl. Acad. Sci. USA. 2010;107:18933-18938.
17. Kau AL, Ahern PP, Griffin NW, Goodman AL, Gordon JI. Human nutrition, the gut microbiome and the immune system. Nature. 2011;474:327-336.
18. Parks BW, Nam E, Org E, Kostem E, Norheim F, Hui ST, Pan C, Civelek M, Rau C D, Bennett BJ. Genetic control of obesity and gut microbiota composition in response to high-fat, high-sucrose diet in mice. Cell Metab. 2013;17:141-152.
19. Smith MI, Yatsunenko T, Manary MJ, Trehan I, Mkakosya R, Cheng J, et al. Gut microbiomes of Malawian twin pairs discordant for kwashiorkor. Science. 2013;339:548-55
20. Jehrke L, Stewart FA, Droste A, Beller M. The impact of genome variation and diet on the metabolic phenotype and microbiome composition of *Drosophila melanogaster*. Sci. Rep. 2018;8:6215.
21. Wong AC, Vanhove AS, Watnick PI. The interplay between intestinal bacteria and host metabolism in health and disease: Lessons from *Drosophila melanogaster*. Dis. Model. Mech. 2016;9:271–281.

22. Broderick NA, Buchon N, Lemaitre B. Microbiota-induced changes in *Drosophila melanogaster* host gene expression and gut morphology. MBio. 2014;5:e01117-14.
23. Broderick NA, Lemaitre B. Gut-associated microbes of *Drosophila melanogaster*. Gut Microbes. 2012; 3:307–321
24. Chandler JA, Lang JM, Bhatnagar S, Eisen JA, Kopp A. Bacterial communities of diverse Drosophila species: Ecological context of a host–microbe model system. PLoS Genet. 2011;7:e1002272.
25. Staubach F, Baines JF, Künzel S, Bik EM, Petrov DA. Host species and environmental effects on bacterial communities associated with Drosophila in the laboratory and in the natural environment. PLoS ONE. 2013;8:e70749.
26. Early AM, Shanmugarajah N, Buchon N, Clark AG. Drosophila genotype influences commensal bacterial levels. PLoS ONE. 2017;12:e0170332
27. Leftwich PT, Clarke NV, Hutchings MI, Chapman T. Gut microbiomes and reproductive isolation in Drosophila. Proc. Natl. Acad. Sci. USA. 2017;114:12767–12772.
28. Wong AC, Chaston JM, Douglas AE. The inconstant gut microbiota of Drosophila species revealed by 16S rRNA gene analysis. ISME J. 2013;7:1922–1932.
29. Wong CN, & Douglas AE. Low-diversity bacterial community in the gut of the fruitfly *Drosophila melanogaster*. Environ. Microbiol. 2011;13:1889–1900.
30. Broderick NA, Lemaitre B. Gut-associated microbes of *Drosophila melanogaster*. Gut Microbes. 2012;3:307–321.
31. Erin S, keebaugh, Ryiuchi Yamada, Benjamin obadia, William B. Ludington Microbial Quantity Impacts Drosophila Nutrition, Development, and Lifespan. Science. 2018;29(4):247–259.
32. Harshavardhana HR, MS. Krishna. High sugar induced changes in gut microflora in *Drosophila melanogaster*: Protective role of Gymnema sylvestre. Asian J. Pharm. Sci. 2019;5(6):1097- 1103.
33. Turnbaugh PJ. A core gut microbiome in obese and lean twins. Nature. 2009;457:480–484.
34. Erkosar B, Storelli G, Defaye A, Leulier F. Host-intestinal microbiota mutualism: Learning on the fly Cell Host Microbe. 2013;13:8–14.
35. Shin SC, Kim SH, You H, B. Kim, AC. Kim, KA. Lee, Yoon, JH, Ryu WJ. Lee Drosophila microbiome modulates host developmental and metabolic homeostasis via insulin signalling Science. 2011;334670-674
36. Storelli G, Defaye A, Erkosar B, Hols J, Royet F Leulier. Lactobacillus plantarum promotes Drosophila systemic growth by modulating hormonal signals through TOR-dependent nutrient sensing. Cell Metab. 2011;14:403-414.
37. Wong AC, Dobson AJ, Douglas AE. Gut microbiota dictates the metabolic response of Drosophila to diet. Exp. Biol. 2014;217:1894–1901.
38. Yamada SA, Deshpande KD, Bruce EM, Mak WW. Microbes promote amino acid harvest to rescue under nutrition in Drosophila Cell Rep. 2015;10:865-872
39. Keebaugh ES, Yamada R, Ja WW. The nutritional environment influences the impact of microbes on *Drosophila melanogaster* life span. mBio. 2019;10:e0885-19.
40. Storelli G. Drosophila perpetuates nutritional mutualism by promoting the fitness of its intestinal symbiont Lactobacillus plantarum. Cell Metab. 2018;27:362–377.