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The Effects of Traditional Asian Diet on Metabolism, Gut Microbiota, and Liver Tissue in NASH Rats

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Abstract

Traditional Asian Diets (AD) in rural areas have a significant risk of mortality due to cirrhosis and hepatocellular carcinoma as nonalcoholic fatty liver disease (NAFLD). This study aims to determine the relationship between AD and liver cancer cases using rat experimental animals Rattus norvegicus strain Wistar. The measured variables include metabolic parameters, gut microbiota profile, and liver histology. This study used 14 rats in two groups: Chow Diet (7 rats to CD) and AD (7 rats to AD), and were given the respective diets for 12 weeks. Enzyme-linked immunosorbent assay (ELISA) methods are used to analyze liver enzymes, lipid profiles, and blood sugar levels. The analysis of gut microbiota used variable region-specific 16S rRNA gene and V3-V4. Biopsy stained with Hematoxylin Eosin was used to study the histology of the liver. Moreover, it was analyzed utilizing NAS (NAFLD Activity Score). The result of this study indicated that reduce body weight the rats treated with AD significant different than treated with CD. Firmicutes, Lactobacillus reuteri, Prevotellaceae bacterium, Romboutsia ilealis, and Bacteroidota in AD

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greater than CD. Alzheimer's disease had notably higher levels of alkaline phosphatase compared to those diagnosed with Crohn's disease on individual diagnosis. Differences in total bilirubin, alanine transaminase, aspartate transaminase, blood sugar, total cholesterol, high-density lipoprotein, low-density lipoprotein, and triglycerides were not significant. The NAS analysis indicated that the two groups comprised rats lacking non-alcoholic fatty liver disease. Despite the high caloric content of the Asian diet, it did not lead to significant changes in metabolic parameters and liver histology related to non-alcoholic steatohepatitis. This behavior can be ascribed to the advantageous influence of the gut microbiota.

Keywords

Asian, diet, Non-alcoholic, hepatitis, liver histology.

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Introduction

Non-alcoholic fatty liver disease (NAFLD) is characterized by the abnormal deposition of fat in the liver among individuals who do not consume large quantities of alcohol. It is commonly associated with metabolic syndrome (Neuschwander-Tetri, 2017; Golabi et al., 2018). This disease presents significant challenges due to its extensive prevalence, complex diagnosis, convoluted progression, and lack of standardized treatment (Alamer et al., 2023). NAFLD encompasses various conditions, including non-alcoholic fatty liver and non-alcoholic steatohepatitis (NASH). These conditions can progress to fibrosis when there is a minimum of 5% accumulation of lipids in liver cells (hepatocytes) and inflammation, characterized by an increase in the size of liver cells (hepatocellular enlargement). This syndrome also carries a significant risk of developing cirrhosis and hepatocellular carcinoma (HCC) (Stefan et al., 2019; Ramakrishnan et al., 2019).

NAFLD is highly prevalent worldwide and across all continents. Based on a meta-analysis, the regions with the highest occurrence rates are South America (31%) and the Middle East (32%), followed by Asia (27%), the United States (24%), and Europe (23%). Conversely, Africa exhibits the most minimal prevalence rate, at 14% (Loomba & Sanyal, 2013). Further studies suggest the prevalence of NAFLD and NASH is expected to rise further, resulting in substantial medical and economic challenges and worse clinical outcomes for patients (Younossi et al., 2019; Cicero et al., 2020).

Multiple factors contribute to the metabolic condition, increasing the risk of progressing NASH. Asia data suggests that certain persons with NASH do not fulfill the requirements for obesity, also referred to as non-obese (Younossi et al., 2019). Research shows the occurrence non-obese, non-alcoholic fatty liver disease linked to a propensity to live in rural regions (Venugopal, 2023). Conversely, individuals with NAFDL disease who live in metropolitan areas have a similar level of risk as NAFLD patients in Western countries. This leads to the suspicion that the risk profile variation is due to the noticeably slim lifestyle disparities between urban and rural regions. Urban communities commonly embrace "Westernized" dietary habits and lifestyles, leading to a comparable risk profile for NAFDL disease patients in these places as in Western regions.

In addition to adopting a Westernized diet with reduced food consumption, other variables that may lead to the development of NAFDL disease in Asia include a sedentary lifestyle, as evidenced by the study of a cross-sectional in South Korea (Ryu et al., 2015). Moreover, the higher prevalence of NAFLD in Asian populations in comparison to Western countries, despite differences in metabolic profiles, may be potentially ascribed to genetic polymorphisms (Fan et al., 2017). Genome-wide research has established a correlation between NAFDL disease and a particular gene called patatin-like phospholipase domain-containing protein 3 in Asian cultures. This illustrates the progress of urbanization and the adoption of Western eating habits, such as monitoring calorie consumption, among the Asian population. The proximity of additional risk factors contributes to the growing prevalence of non-alcoholic fatty liver disease in Asia.

The Asian slim-down is distinguished by a significant reliance on carbohydrates, vegetables, and legumes while minimizing the intake of animal fats. The numbers are and (Buzzetti et al., 2016; Ko et al., 2014). Recent studies have shown that both traditional and modified Asian diets have beneficial effects on certain metabolic illnesses and cardiovascular disorders in Asia (Oya et al., 2010). This is frequently caused by the excessive intake of saturated fat from meat, high fiber from vegetables, and high intake of n-3 polyunsaturated fatty acids from fish (Ko et al., 2014).

There is a lack of research exploring the correlation between reduced food intake among Asians and metabolic outcomes and the occurrence of NAFLD. However, it has been seen that certain interventions such as dietary n-3 supplementation, a diet rich in vegetables and grains, and a low-fat diet high in fiber, nuts, and vitamin K have been effective in reducing the risk of developing non-alcoholic fatty liver disease (NAFLD) among Asian populations. 12 to 15 Several clinical trials have demonstrated that administering 1.0-2.7 g/day for 6-12 months to individuals with n-3 NAFLD can effectively reduce hepatic steatosis, inflammation, and fibrosis (Capanni et al., 2006; Tanaka et al., 2008).

Hsu et al., (2014) compared the organization of the Conventional Asian Eat Less (TAD) with the Ordinary Western Slim Down (TWD) regarding metabolic reactions in patients at risk of creating T2DM. The ponder appeared that the organization of TAD made strides against resistance and diminished body weight and body fat (Hsu et al., 2014). The findings above suggest that factors outside weight can influence non-obese NAFLD in Asia, and a negative relationship exists between Asian cuisine and NAFLD. However, it is imperative to research and evaluate the impact of Asian cuisine on the prevalence of NAFLD, as there is a need to understand the relationship between these two conditions.

The human gastrointestinal system harbors a varied and dynamic population known as the intestinal microbiota, including around 10 (Han et al., 2014) to 10 (Chan et al., 2015) microbial cells. The main phyla present in the gut microbiota are Firmicutes, Bacteroidetes, and Actinobacteria, classified explicitly (Thursby & Juge, 2017; Kho & Lal, 2018). Individuals exhibit variation in the composition of their gut flora. The individual's characteristics, such as sex, race, and age, as well as environmental factors like food habits, hygiene, antibiotic use, and delivery methods, impact it. Counting calories is a significant factor affecting the intestinal microbiota's composition (Shin et al., 2019). Following a low plant fiber, low animal fat, and low animal protein diet can promote metabolic health for the intestinal microbiota by increasing energy expenditure, regulating glucose metabolism, and promoting the secretion of certain substances (Ramakrishnan et al., 2019; Bobir et al., 2024). This study was performed on Wistar strain rats, especially the Rattus norvegicus species, and involves introducing an Asian food intervention. The study sought to investigate the impact of an Asian diet on the advancement of NASH, related metabolic factors, and the makeup of the gut flora (Miller et al., 2002).

Material and Methods

Plan Inquire: Typically, an authentic assessment involves using a posttest as a comprehensive approach. This study is a live experiment conducted on rats of the Rattus norvegicus Wistar breed. The trial aimed to promote reduced food consumption among Asian persons in the mediation group and among atypical individuals in the control group.

Investigate Test: This study included 14 tests involving male Wistar strain rats of the species Rattus norvegicus. The rats were 8-12 weeks old, weighed 150-200 grams, and exhibited normal behavior. Seven rats were selected from the Asian eat-less group and seven rats from the control group. These rats had shiny white hide. This selection process is a common practice when conducting weight loss experiments. Before the commencement of the treatment, the rats had a period of acclimatization lasting for seven days. The rats were fed a regular diet and placed in cages cleaned daily.

Experimental Diets: The rats were subjected to a caloric restriction for 12 weeks, with a daily intake of 40 grams per rodent. A standardized weight loss protocol for rats was devised by combining the PARS chemical with water and adding wheat to produce 25.81t, 42.87 carbs, 5% fiber, and 31.32% protein. During the interim, a weight reduction program was implemented for rats from Asia by mixing 5% PARS flour with water to create a solid consistency. The PARS flour is combined with 5% PARS fat, resulting in a fat content of 20 grams per 100 grams. Egg yolks, which have a fat content of 12 grams per egg, are included to enhance the total fat level in the cuisine. The fat component of these yolks is 10%, comparable to 4 grams of fat. This dish has a total fat level of 15%, and each serving contains 6 grams of fat. The recipe consists of 24 grams of wheat flour containing 70 grams of carbohydrates, 15% protein powder, and 10 grams of fiber powder. Ultimately, the process concludes with the addition of flavoring. During the dietary intervention, the additional food was consistently weighed at the same time every day, and the body weight was measured once per week.

Surgical Strategy: Sometime recently, surgery, rats to begin with euthanized with ten ml xylazine 10%. At that point, rats were settled and dismembered from the midriff utilizing bowed scissors. After that, the liver organs were taken and washed utilizing refined water and 0.9% NaCl over and over and carefully. Afterward, the organs were depleted on channel paper, set in a dry petri dish, and weighed. The weight of each organ was recorded, and after that, the organs were put in a pot containing 10% formalin and buffered formalin.

Histopathological Liver Tissue Planning Procedure: The liver was washed employing an arrangement of Phosphate Buffered Saline (PBS), and after that, tissue was taken from the back portion of the correct projection of the liver to be arranged. The liver tissue that has been taken is placed in tissue cassettes. The liver tissue was dried out steadily utilizing an ethanol solution with a concentration of 50%, 70%, 80%, 90%, and 100% to remove the water substance within the liver tissue. Another is the tissue, which is put in a tissue processor and dried using a vacuum. Blocked clan by utilizing fluid paraffin, and the piece was cut into 3-5 um sizes using a rotatory microtome machine. At that point, each piece of the clan was connected to the question glass and recolored utilizing Hematoxylin Eosin (HE). Recoloring comes about when watched employing a light magnifying instrument with amplifications of 40x, 100x, and 400x.

Data Collecting Procedure for Liver Enzyme, Lipid Profile, and Blood Sugar: Blood taken from rats for 15 minutes was centrifuged at 3000 rpm to be used in plasma to calculate liver enzyme levels, namely bilirubin, AST, ALT, and ALP, and lipid profiles, namely HDL, LDL, triglycerides (TG), cholesterol, and blood sugar.

Analysis of Sequence Processing and Microbiota Profiles: The obtained DNA will undergo sequencing utilizing the Next-Generation Sequencing (NGS) platform. The DNA tests were saved on a plate reader and underwent four stages: segmentation, enhancement, and sequencing using the Illumina HiSeq 2500. This approach can be used to identify the phylum and classify and define the species of microbiota present in fecal samples obtained from experimental organisms. PCR analysis can calculate the Bacteroidetes to Firmicutes ratio (B/F ratio).

Statistical Analysis: The normality test Shapiro-Wilk data due to the sample size (n) being less than 50 was employed to assess the numerical. In addition, a T-test will be performed to compare the data if it follows a normal distribution and is homogeneous. The Mann-Whitney test is employed when the data does not follow a normal distribution or exhibit homogeneity. A Chi-Square test was used to ascertain if there was a statistically significant discrepancy between the Asian diet treatment group and the control group in terms of liver histology markers, which serve as indicators of the presence of NASH. The Firmicutes to Bacteroidetes ratio was determined using the Kruskal-Wallis Test ($p<0.01$). Alpha diversity is quantified using the Shannon Entropy and Faith Phylogenetic Diversity (Faith PD) index, whereas beta diversity is assessed using the Weighted Unifrac and Bray Curtis index. The distribution and clustering patterns can be easily visualized by employing Principal Coordinates Analysis (PcoA) and Emperor plots.

Results and Discussion

Table 1 presents the normality test results of the rats' body weight, blood sugar, lipid profile, and liver enzymes. The study involved measuring the rats' body weight weekly for 12 weeks. The results indicated that the average body weight of rats on the Asian diet was consistently lower than that of rats on the standard diet. Nevertheless, the rats subjected to the Asian food intervention exhibited a greater weight increase than the rats on a conventional diet Table 2.

Table 1. Normality test results for body weight, blood sugar, lipid profile, and liver enzymes

Observation	Group	P Value	Conclusion
Weight	Normal Diet asian diet	0.035 (abnormal) 0.086 (normal)	Non parametric comparative test
Blood sugar	Normal Diet Asian Diet	0.747 (normal) 0.138 (normal)	Homogeneity & parametric comparative test
Total cholesterol	Normal Diet Asian Diet	0.691 (normal) 0.980 (normal)	Homogeneity & parametric comparative test
HDL	Normal Diet Asian Diet	0.027 (abnormal) 0.014 (abnormal)	Non parametric comparative test
LDL	Normal Diet Asian Diet	0.176 (normal) 0.640 (normal)	Homogeneity & parametric comparative test
Triglycerides	Normal Diet Asian Diet	0.008 (abnormal) 0.411 (normal)	Non parametric comparative test
Total Bilirubin	Normal Diet Asian Diet	0.020 (abnormal) 0.554 (normal)	Non parametric comparative test
ALP	Normal Diet Asian Diet	0.049 (abnormal) 0.929 (normal)	Non parametric comparative test
ALT	Normal Diet Asian Diet	0.400 (normal) 0.902 (normal)	Homogeneity & parametric comparative test
AST	Normal Diet Asian Diet	0.699 (normal) 0.733 (normal)	Homogeneity & parametric comparative test

The Shapiro-Wilk normality test was carried out on the body weight data of rats on a regular diet and an Asian diet. Average diet data showed a p-value <0.05, so the distribution was abnormal. Meanwhile, in the Asian diet, the p-value is more than 0.05, indicating that the data distribution followed a Gaussian distribution. Furthermore, the Mann-Whitney non-parametric comparison test was conducted to evaluate the significance of the difference in body weight between the two groups. The test results showed a statistically significant reduction in body weight in rats that underwent the Asian diet intervention compared to those on the conventional diet (p=0.001). Comparative analysis of rat body weight shown in table 3.

Table 2. Results of mouse weight measurements per week

Observation	**Normal Diet (gr)**	**Asian Diet (gr)**
Week 1	247.71 ± 22.31	202.83 ± 18.72
Week 2	243.71 ± 17.20	212.17 ± 15.79
Week 3	258.00 ± 16.78	220.33 ± 15.77
Week 4	271.29 ± 15.18	227.67 ± 16.27
Week 5	277.29 ± 13.54	236.33 ± 17.41
Week 6	285.71 ± 14.61	244.33 ± 17.22
Week 7	283.29 ± 34.36	232.00 ± 16.55
Week 8	288.00 ± 12.54	247.50 ± 25.05
Week 9	281.00 ± 16.49	241.50 ± 17.95
Week 10	286.57 ± 14.67	261.33 ± 19.33
Week 11	293.57 ± 9.93	267.67 ± 21.45
Week 12	293.86 ± 9.92	264.88 ± 24.13
Weight Gain (gr)	51,15	62.05

Table 3. Comparative analysis of rat body weight

Observation (gr)	**Normal Diet**	**Asian Diet**	**P Value**
Final Weight	293.86 ± 9.92	264.88 ± 24.13	0.001

Meanwhile, the blood glucose measurements of the rats indicated that the average blood glucose levels of the rats on the Asian diet were not significantly lower than those on the normal diet. Specifically, the average blood glucose level for the Asian diet rats was 254.83 gr/dL, while it was 280.86 gr/dL for the rats on the usual diet (p> 0.05). Comparative analysis of rats' blood sugar shown in table 4.

Table 4. Comparative analysis of rats' blood sugar

Observation (gr/dL)	**Normal Diet**	**Asian Diet**	**P Value**
Average Blood Sugar	280.86 ± 90.14	254.83 ± 46.76	0.538

Table 5 shows the average lipid profile of the rats, including total cholesterol, HDL, LDL, and TG of the Asian and normal diet rats, respectively. The following data shows that the average total cholesterol, HLD, LDL, and TG on the Asian diet tends to be lower than the normal diet.

Table 5. Comparative analysis of mouse lipid profiles

Observation (gr/dL)	**Group**		**P Value**
	Normal Diet	**Asian Diet**	
Average Total Cholesterol	57.57 ± 7.50	55.33 ± 9.29	0.640*
HDL average	35.01 ± 18.68	30.75 ± 4.67	0.475**
Average LDL	31.33 ± 10.10	23.90 ± 2.73	0.104***
Average Triglycerides	79.71 ± 30.11	77.67 ± 33.91	0.775**

*P value obtained from independent T-Test with homogeneous data

**P value is obtained from the Mann Whitney Test

***P value obtained from Independent T-Test with non-homogeneous data

Table 6 displays the results of a comparative study on liver function profiles in two groups of rats. The results suggest that the average levels of total bilirubin and ALT in the Asian diet group were significantly higher than in the conventional diet group ($p > 0.05$). Furthermore, the mean AST levels in the Asian diet group were shown to be lower compared to the traditional group diet, with a p-value greater than 0.05. The average alkaline phosphatase (ALP) level of rats who were given the Asian diet was significantly higher than that of rats who were given the normal diet ($p < 0.05$).

Table 6. Comparative analysis of rat liver enzymes profiles

Observation (gr/dL)	**Group**		**P Value**
	Normal Diet	**Asian Diet**	
Average Total Bilirubin	0.44 ± 0.79	0.57 ± 0.14	0.069**
ALP average	57.57 ± 10.55	189.00 ± 59.06	0.003**
ALT average	48.00 ± 12.97	69.33 ± 28.05	0.098*
AST average	113.29 ± 27.23	109.17 ± 22.18	0.773*

*P value obtained from independent T-Test with homogeneous data

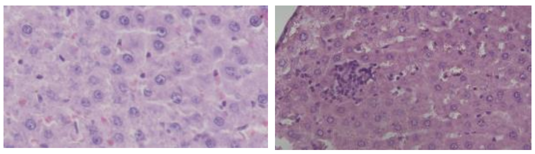
**P value is obtained from the Mann Whitney Test

The findings of histopathological the liver in both groups are depicted in Figure 1. Hepatocytes in rats fed a typical diet have normal characteristics. Concurrently, the rats subjected to the Asian diet exhibited lobular inflammation. Standard or Asian diet intervention rats did not exhibit cell ballooning or steatosis. Meanwhile, rats who underwent the Asian diet intervention were able to identify one of the key indicators of nonalcoholic steatohepatitis (NASH), specifically lobular inflammation (Younossi et al., 2016). The mean NAS score is displayed in Table 7, 8. The rats on the conventional diet intervention had an average NAS score of 0, but the rats on the Asian diet intervention had an average NAS score of 1.67. A Chi-Square test was performed on this score, which indicated that the lowest predicted frequency was below 5, hence failing to meet the conditions for the Chi-Square test. The value is determined by applying Fisher's Exact Test, resulting in a statistically significant p-value of less than 0.05 ($p = 0.021$). A p-value less than 0.05 signifies the rejection of the null hypothesis (H0) and the acceptance of the alternative hypothesis (Ha).

Table 7. Fisher's exact test results for nas score rats' liver tissue

Observation	**Normal Diet**	**Asian Diet**	**P Value**
NAS score	0	1.67 ± 0.52	0.021

A comparison analysis was performed on the intestinal microbiota composition of seven mice that were exposed to an Asian diet and seven mice that were adhering to a regular diet. The research results indicate that the predominant phyla in both groups were Firmicutes and Bacteroidetes, as shown in Table 9. Bacteroidota is the predominant taxonomic category of organisms present in a normal diet, while Firmicutes is the dominant phylum in an Asian diet.



(A) (B)

Figure 1. Histopathological Anatomical Observation Results of Rat Livers in Both Groups with 400x magnification. (A) images of hepatocyte cells in rats with normal diet intervention. (b) Images of lobular inflammation in rats with the Asian diet intervention

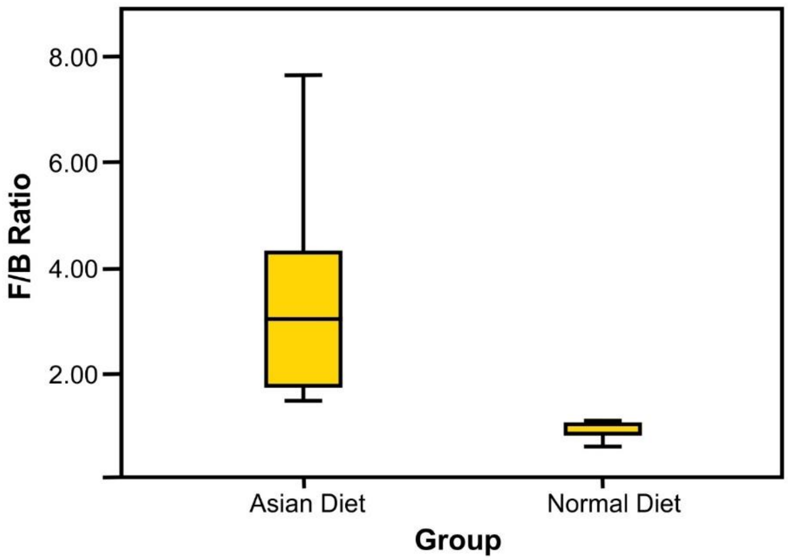


Figure 2. Ratio of firmicutes to bacteroidetes

Significant Differences between Groups Were Calculated Using Kruskal-Wallis Test (P< 0.01)

The phylum Proteobacteria was the dominant group in both the conventional diet and the Asian diet. The prevalence of Desulfobacterota was greater in the usual diet compared to the Asian diet. There was notable heterogeneity in the number of Bacteroidota, Firmicutes, Proteobacteria, and Desulfobacterota among the different categories. The Firmicutes to Bacteroidetes (F/B) ratio can be utilized to characterize dysbiosis, which indicates an imbalanced makeup of the gut microbiota. Figure 2 illustrates that the F/B ratio was greater in the Asian diet group compared to the normal diet group.

Table 8. The most common bacterial phyla in the normal diet and the asian diet

Phylum	Normal Diet	Asian Diet	P value
Bacteroidota	42.34 ± 5.54	22.53 ± 10.09	0.001*
Firmicutes	39.06 ± 4.79	60.23 ± 11.25	0.0005*
Proteobacteria	10.537 ± 2.02	7.71 ± 14.15	0.026*
Desulfobacterota	2.18 ± 0.538	0.740± 0.525	0.002*
Actinobacteriota	1.64 ± 0.395	1.707 ± 1.21	0.62
Spirochaetota	1.501± 2.86	4.594 ± 6.09	0.80
Campilobacterota	0.93 ± 0.87	0.48 ± 0.417	0.317

Note: Data are presented as the mean ± SD.

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* p value is obtained from Wilcoxon rank-sum test

The gut microbiota composition at the genus level was determined by selecting the ten most common genera (Table 9). The identified genera consisted of five from the Bacteroidota phylum, three from the Firmicutes phylum, and one each from the Proteobacteria and Spirochaetota phyla. Several taxonomic groups, including as Prevotella, Muribaculaceae, Alloprevotella, Lactobacillus, Lachnospiraceae_NK4A136_group, and Anaerobiospirillum, exhibit significant variation in relative abundance. Prevotella was the most prevalent genus of microbiota in the normal diet, whereas Lactobacillus was the most prevalent genus of microbiota in the Asian diet (Fan & Pedersen, 2021).

In addition to the phylum and genus levels, the composition of gut microbiota was evaluated at the species level Table 10. The study found notable differences between rats who consumed an Asian diet and those that followed a conventional diet, especially in terms of the abundance of Lactobacillus reuteri, Romboutsia ilealis, and Prevotellaceae bacterium.

Table 9. The most common bacterial genus in the normal diet and the asian diet

Phylum	Genus	Normal Diet	Asian Diet	p
Bacteroidota	Prevotella	16.22 ± 4.07	8.47 ± 6.92	0.038*
	Muribaculaceae	5.886 ± 1.26	2.26 ± 1.18	0.0005*
	Prevotellaceae_UCG-003	2.409 ± 0.819	2.23 ± 1.878	0.259
	Alloprevotella	4.23± 1.83	1.87 ± 1.54	0.011*
	Bacteroides	2.010 ± 0.327	1.98± 1.70	0.535
Firmicutes	Lactobacillus	9.49 ± 3.22	34.73 ± 12.80	0.004*
	Blautia	3.51 ± 0.98	3.44 ± 3.26	0.535
	Lachnospiraceae_NK4A 136_group	5.35 ± 2.63	1.51± 1.84	0.0069*
Proteobacteria	Anaerobiospirillum	7.83 ± 1.50	0.91± 0.94	0.0005*
Spirochaetota	Treponema	1.77 ± 3.57	5.32 ± 7.07	0.71

Note: Data are presented as the mean ± SD.

Standard deviation (SD). The p-value is determined from the Wilcoxon rank-sum test.

Table 10. Gut microbiota composition at the species level in Asian diet and normal diet

Species	Normal Diet n(7)	Asian Diet n(7)	P value
Lactobacillus murinus	3.028 ± 2.84	7.37 ± 7.64	0.804
Lactobacillus reuteri	0.767± 0.44	6.05 ± 3.24	0.00058 *
Lactobacillus johnsonii	2.65 ± 1.57	5.10 ± 2.94	0.053
Weissella cibaria	0.44 ± 0.26	4.26 ± 5.069	0.053
Romboutsia ilealis	0.73 ± 0.23	2.28 ± 1.61	0.026 *
Prevotellaceae bacterium	0.42 ± 0.13	1.01 ± 2.39	0.026 *
Helicobacter rodentium	0.33 ± 0.35	0.35 ± 0.345	0.080
Anaerobiospirillum succiniciproducens	0.22 ± 0.20	0.22 ± 0.48	0.128
Eubacterium hallii	0.167 ± 0.15	0.179 ± 0.195	1
Collinsella tanakaei	0.042 ± 0.007	0.36 ± 0.72	0.128

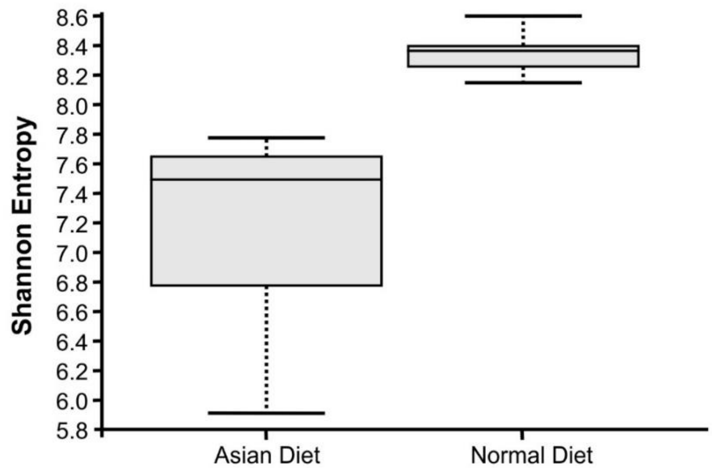
Data are shown as means ± standard deviation (%).

The data are presented as means plus or minus the standard deviation expressed as a percentage.

Notable disparities.

Alpha diversity quantifies the size of diversity within a specific community or habitat. The diversity index quantifies the range of elements inside a community, whether in a specific region or group. Shannon Entropy and Faith Phylogenetic Diversity (Faith PD) are two indices used to quantify alpha diversity. A more excellent value for each measure of diversity indicates a higher level of variety within the community. Shannon Entropy and Faith PD consistently demonstrate that the normal diet group exhibits greater bacterial variety than the Asian diet group Figure 3. Beta diversity is concerned with quantifying variations in species composition between two or more locations or environments. Two indices commonly used to measure beta diversity are Weighted Unifrac and Bray Curtis. One can efficiently see the distribution and clustering patterns by using Principal Coordinates Analysis (PcoA) using Emperor plots. Weighted UniFrac measures the degree of evolutionary difference between microbial communities by considering their phylogenetic links.

Meanwhile, Bray-Curtis evaluates variations in the composition of species abundance. The average diet group had a more distinct clustering pattern than the Asian diet group, indicating that the community structure of the average diet is significantly dissimilar to that of the Asian diet. Based on the Bray-Curtis index, the conventional diet group exhibits comparable diversity, whereas the Asian diet group has more dispersed data Figure 4. The Jaccard's measure of diversity likewise yielded comparable outcomes, indicating that the average diet group data exhibit proximity to one another, signifying a higher level of character diversity. Conversely, the findings from the Asian diet group are more widely dispersed Figure 5. The findings revealed a substantial disparity in the microbial community structure between the Asian diet and the usual diet, with a minimum difference level of 10-27%.



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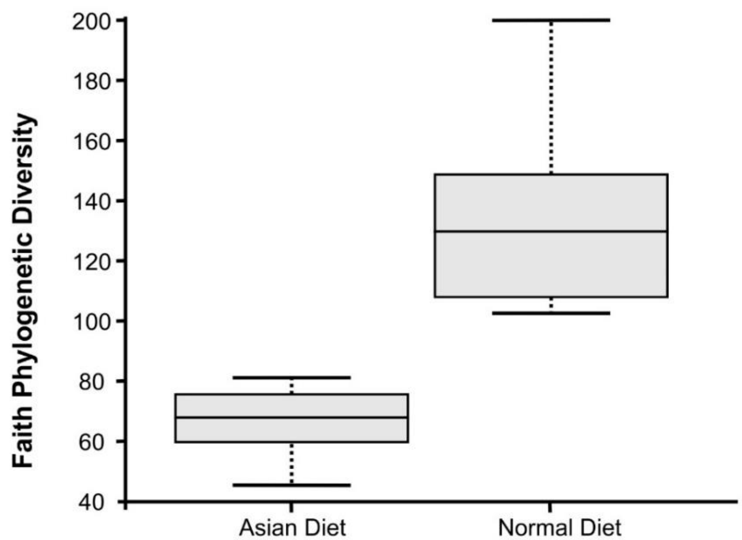

B

Figure 3. The bacterial diversity in the asian diet group and normal diet group was assessed using the shannon entropy diversity index (A) and faith PD diversity index (B)

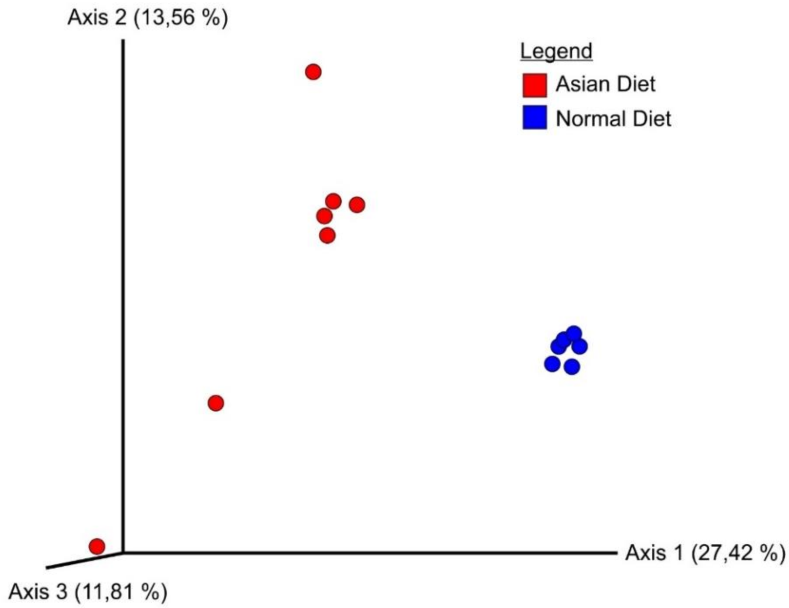

Figure 4. The data distribution of Asian diet and normal diet is plotted in an emperor plot using the bray-curtis index

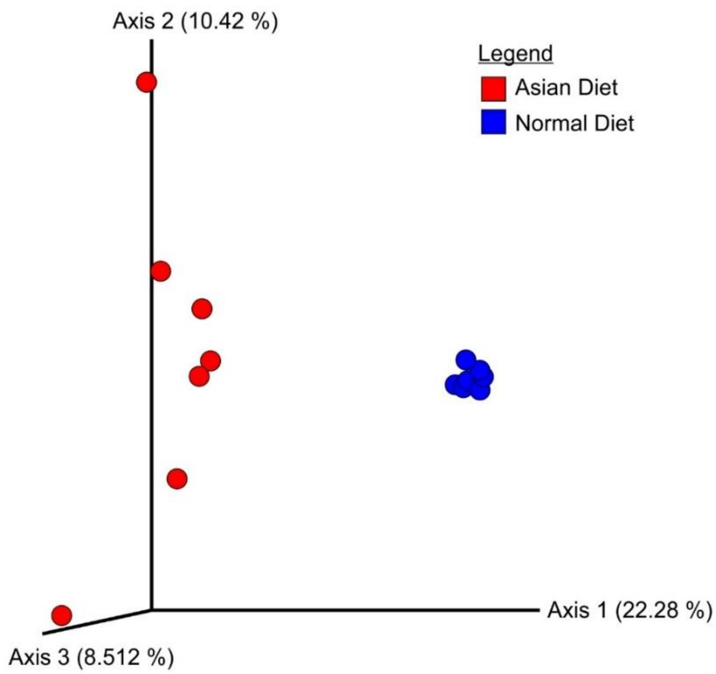

Figure 5. Data distribution of Asian diet and regular diet in Emperor plot based on jaccard index

DISCUSSION

Differences in Final Weight and Weight Gain of Rats between Groups

The weekly measurements of the body weight of each rat diet group revealed that the Asian diet group rats experienced a slightly higher body weight increase than the average diet group rats. Specifically, the Asian diet group rats gained 62.05 grams, while the normal diet group rats gained 51.15 grams. While the Asian diet rats had somewhat higher average feed intake and body weight gain than the typical diet rats, the ultimate body weight of the Asian diet group rats was considerably lower than the average diet rats ($p = 0.001$). The Asian diet rats had a final body weight of 264.88 ± 24.13 grams, whereas the average diet rats had a final body weight of 293.86 ± 9.92 grams.

The disparity in nutritional composition, namely the elevated fiber content and reduced fat content in the Asian diet in contrast to the conventional diet, affects the body weight of the rats. Ingesting a meal abundant in fiber can reduce the rate of glucose absorption in the body.

Furthermore, reduced fat levels can enhance the body's response to insulin, leading to improved management of insulin and blood sugar levels (Hsu et al., 2014). Prior investigations. Also corroborate this finding. According to the study, feeding rats a traditional Asian diet (TAD) with a similar composition was strongly linked to reduced body weight compared to the comparison group. The Asian diet, which is fiber-rich, is related to low body weight and can decrease glucose absorption (Hsu et al., 2014).

Comparison of Rat Blood Sugar between Groups

The blood sugar measurements revealed that the average blood sugar levels in the rats on the Asian diet were not substantially lower than those in the rats on the usual diet (254.83 gr/dL and 280.86 gr/dL, respectively, $p > 0.05$). Research has demonstrated that the increased fiber content in the rats following the Asian diet group leads to improved insulin sensitivity. Glucose absorption, enhancing insulin response, and improving glycemic control were promoted (Weickert & Pfeiffer, 2008). Twenty-two In addition, the reduced lipid content of the Asian diet rats, in comparison to the standard diet, promotes insulin sensitivity by lowering levels of free fatty acids (FFA), thus preventing insulin resistance (Tierney et al., 2011). Twenty-three Hsu et al. conducted a study that showed that administering Traditional Asian Diet (TAD) to individuals can improve insulin sensitivity and glucose metabolism in comparison to the Typical Western Diet (TWD) (Hsu et al., 2014).

Comparison of Mouse Lipid Profiles between Groups

In general, the Asian diet rats had a reduced lipid profile, although the difference from the regular diet rats was not statistically significant (Table 6). This can be attributed to a combination of Asian diet rats, which possess a low-fat composition and high fiber content yet have elevated carb levels. Consuming meals that are low in fat and high in fiber can effectively lower both total cholesterol and LDL levels. Nevertheless, the intake of low-fat foods containing monounsaturated fatty acids and polyunsaturated fatty acids may decrease HDL cholesterol levels. The numerical value is 10.24. Furthermore, a diet abundant in fiber can slow down the process of gastric emptying and impede the transportation of triglycerides and cholesterol within the intestines. However, the Asian diet's high consumption of carbohydrates stimulates the production of new lipids in the liver, resulting in increased levels of free fatty acids (FFA). Elevated hepatic levels of free fatty acids (FFAs) might lead to an increase in triglyceride levels (Semiane et al., 2017).

Comparison of Rat Liver Enzymes Profiles between Groups

Elevated levels of ALT compared to AST often suggest the existence of liver injury (Kwo et al., 2017). Nevertheless, this study found that the alanine aminotransferase (ALT) level in rats following an Asian diet was lower compared to the AST (aspartate aminotransferase) level. Prior research has demonstrated no established association between ALT levels and the seriousness of liver histopathology (Schumacher-Petersen et al., 2019). Furthermore, AST levels are not exclusive to the liver and can be seen in other organs (Kwo et al., 2017). Thus, considering the findings of this study and past research, it can be inferred that ALT and AST values should not be solely relied upon as the primary criteria for diagnosing NAFLD.

The alkaline phosphatase (ALP) levels in rats on the Asian diet were considerably greater than those on the usual diet. Research conducted Okazaki & Katayama, (2019) demonstrated that oligosaccharides can enhance intestinal ALP activity, hence protecting against inflammatory disorders (Okazaki & Katayama, 2019). This finding may account for the observed elevation in ALP levels in Asian diets, which are often rich in carbohydrates and fiber. Nevertheless, the nonspecific marker results in various potential causes of elevated ALP levels (Kwo et al., 2017). Consequently, ALP levels cannot serve as a reliable indicator for diagnosing NAFLD, necessitating the utilization of further tests to confirm the diagnosis.

The bilirubin concentration in the meal consumed by Asian rats showed a modest increase, although this difference did not reach statistical significance. In addition, the rats on the Asian diet had a total bilirubin level that was insufficient for distinguishing between conjugated and unconjugated bilirubin, as the bilirubin level was too low to be detected by the absorbent.

Hence, the precise reason behind the minimal rise in bilirubin levels in rats on an Asian diet remains unknown. Several variables influence bilirubin levels. Conditions that occur outside the liver (extrahepatic), inside the liver (intrahepatic), or that cause blockage or disruption of bile flow (obstructive or cholestatic) can have an impact on the overall amount of bilirubin in the blood (Kwo et al., 2017).

Comparative Histopathological Analysis of Liver Tissue in Rats Across different Groups

The evaluation results of the NAS score revealed that the Asian diet group had an average NAS score of 1.67 points higher than the normal diet group. Despite the Asian diet group having a higher score compared to the conventional diet group, the average suggests that the rats in the Asian diet group did not show any symptoms of NASH (non-alcoholic steatohepatitis). The Asian diet group obtained a score of 1.67, indicating a higher likelihood of having non-alcoholic fatty liver (NAFL) as opposed to non-alcoholic steatohepatitis (NASH). NAFL, in contrast to NASH, is characterized by the presence of at least 5% steatosis without any hepatocyte damage, such as cell ballooning or fibrosis (Kleiner & Brunt, 2012). However, upon analyzing the liver anatomy of rats on an Asian diet, it was noted that there was an absence of steatosis and only the presence of lobular inflammation. Specifically, a maximum of three inflammatory sites were found in 20 fields of view. Therefore, it may be deduced that the Asian diet does not affect the occurrence of NASH in Rattus norvegicus Strain Wistar rats.

Dysbiosis is an imbalanced state of the gut microbiota, marked by a decrease in beneficial microorganisms or an excessive growth of harmful microorganisms, resulting in reduced microbiota diversity.[31] This condition can be characterized by analyzing the ratio of two distinct kinds of bacteria: Firmicutes and Bacteroidetes (F/B). The Asian diet group demonstrated a higher F/B ratio compared to the usual diet group. The results align with a study conducted by Shin et al., which examined the disparity in gut microbiota composition between a typical Korean diet (60-65% carbohydrates and 20-25% fats) and a Western diet (50-55% carbohydrates and 30-35% fats). The study found that consuming the Korean diet resulted in a significant increase in Firmicutes and a decrease in Bacteroidetes, which led to an improved F/B ratio (Shin et al., 2019).

Among the microbiota, the Asian cuisine exhibits the greatest prevalence of Lactobacillus at the genus level. Lactobacillus is a widely recognized genus commonly employed in the production of commercial probiotic goods. Probiotics possess beneficial functional attributes, such as lowering plasma cholesterol levels, preventing and managing diarrhea, and altering the immune system (Hsu et al., 2014). There were significant differences in the species-level composition of gut microbiota between rats that followed an Asian diet and those that followed a standard diet. Specifically, there were considerable variations in the presence of

Lactobacillus reuteri, Romboutsia ilealis, and Prevotellaceae bacterium. Sinkiewicz et al. conducted a study that found a higher prevalence of reuteri colonization in Japanese women compared to women from other nations. This could be attributed to their diet rich in high-fiber and low-fat meals (Li et al., 2015).

Limitations of the Study

Limitations found in this study included one rat that was excluded because it died during the study period. In addition, Asian diet research has different compositional variations that affect variations in research results. Then, this study was conducted for 12 weeks so that the long-term effects of giving the Asian diet to rats had not been obtained.

Conclusion

The administration of the Asian diet had no impact on the incidence of NASH in Wistar strain Rattus norvegicus rats. The histological results of liver tissue, lipid profile, bilirubin, and aminotransferases showed no notable disparity between the two groups of rats. However, the Asian diet employed in this study successfully reduced the body weight of the rats that adhered to it. However, it did not significantly impact reducing the blood sugar levels of the rats on the Asian diet. Additionally, the liver histology of the rats on the Asian diet revealed signs of lobular inflammation. To assess the lasting effects of the Asian diet on the development of NASH, conducting a study with a longer intervention duration is crucial. To address the inconsistency in findings across several research, it would be necessary to standardize the composition of the Asian diet. The Asian diet is thought to modify the composition of gut microbiota by increasing the abundance of helpful bacteria and decreasing the amount of non-beneficial bacteria, as evidenced by a higher ratio of Firmicutes to Bacteroidetes (F/B) in the Asian diet group compared to the normal diet group.

Author Contributions

All Authors contributed equally.

Conflict of Interest

The authors declared that no conflict of interest.

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