

Effect of Different Types of Oil Intake on the Blood Index and the Intestinal Flora of Rats

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Abstract

The effect of different oil-based diets on the blood index and the gut microbiota of rats was investigated. Rats in sample treatment groups were fed with stewed lard, refined lard, fish oil and soybean oil at a normal dose of 16.9% for 6 weeks. Compared with refined lard group, stewed lard treatment group showed reduced fasting blood sugar and blood lipid levels and improved nutrient absorption capacity of the intestine. The blood indexes of glucose (Glu), total cholesterol (TC) and total triglyceride (TG) in fish oil treatment group were relatively low. The abundance of *Bacteroidetes* in the fat-free group decreased, and the abundance of *Firmicutes* increased. The *Firmicutes/Bacteroidetes* ratio of the fish oil group was relatively low, and the *Firmicutes/Bacteroidetes* ratio of the stewed lard group and the soybean oil group was lower than that of the refined lard group. The abundance of *Bacteroidaceae* in the stewed pork fat group was increased. Research results show that fat-free diets will increase the risk of obesity to a certain extent; compared with refined lard, stewed lard, soybean oil and fish oil can reduce the risk of obesity to a certain extent; the addition and types of dietary fat will affect the abundance and diversity of rat intestinal flora.

Introduction

Dietary fat is an important part of human diet, and its nutritional value depends to a large extent on the composition of fatty acids, which is closely related to human health (Roche et al., 2014). At present, epidemiological studies mainly focus on the side effects of saturated fat and the positive effects of unsaturated fatty acids. Studies have shown that the consumption of saturated fat is positively correlated with the mortality of coronary heart disease, and other studies believe that the consumption of fish oil is negatively correlated with the mortality of coronary heart disease (Krisetherton et al., 2002). However, the existing studies have not taken into account the body's real needs for fat, and most of them are short-term studies based on high fat or disease models (Gauze-Gnagne et al., 2020; Adil et al., 2018). Lard and soybean oil are commonly used edible oils for Chinese residents, and fish oil is the most studied health oil. Stewed lard is obtained by simmering pork belly in low heat for a long time to extract fat. Compared with refined lard, the saturated fatty acid (SFA) of stewed lard decreased, and the monounsaturated fatty acid (MUFA) increased, and the content of other fatty acids also changed accordingly. The fatty acid composition of stewed lard is different from refined lard, fish oil, and soybean oil: refined lard has a high content of SFA and MUFA, fish oil is rich in n-3 PUFA such as EPA and DHA, and soybean oil is rich in n-6 PUFA.

Dietary fat can provide the body with essential fatty acids and is a carrier of fat-soluble vitamins. At the same time, it plays an important role in the body's fat metabolism, sugar metabolism and lipid peroxidation. Different types of fats have different effects on the body's growth performance and intestinal microbes. At present, there are relatively few studies on the effect of dietary fat on the intestinal microbes of the body, and most studies have shown that high-fat diets can disrupt the intestinal microflora of the body (Zhang et al., 2012; Ghosh S et al., 2013). It was found that diets with different fat content can regulate the function of gut microbes in rats before and after weaning (Z Š and Bujňáková, 2017). Compared with a low-fat and high-carbohydrate diet, a high-fat and low-carbohydrate diet significantly reduces the concentration of short-chain fatty acids and the number of *bifidobacteria* in the body's feces (Brinkworth et al., 2009). Studies have shown that diets with different fat sources will have a certain impact on the microflora of the cecal contents of piglets (Li et al., 2017). Different effects of high-fat diets rich in different oils on lipid metabolism, oxidative stress was investigated, gut microbiota and the high-fat diet exerted detrimental effects on the specific alterations in the gut microbiota, which increased the *Actinobacteria* and *Enterococcaceae* abundance, also decreased *Bacteroidetes*, *Proteobacteria*, *Lactobacillales* and *microbiota* diversity (Liu et al., 2021).

At present, most of the research on intestinal microbes uses high-throughput technology, which has obvious advantages in throughput and high resolution compared with traditional molecular detection technology.

Materials And Methods

Animals and diets

All experiments were carried out in compliance with the relevant guidelines and regulations of the Ethical Committee of Experimental Animal Center of Yangzhou University. A total of 48 4-week-old female Wistar rats were housed in a specific pathogen-free animal center (SCXK (Jiangsu) 2017-0007, Yangzhou, Jiangsu, China). The temperature ($24.0 \pm 0.5^{\circ}\text{C}$) and relative humidity ($60 \pm 10\%$) were kept constant during the experiment, with a 12-h light cycle. Rats were fed a standard chow diet during a 1-week acclimation period. Then, animals were assigned to one of five diet groups (eight rats in each group), that is, fat-free (WZ), stewed lard (SY), refined lard (JL), fish oil (YY) and soybean oil (DY) groups. Refined lard, fish oil and soybean oil were commercially obtained. Stewed lard is made by simmering pork belly for 120 min, removing the broth, chopped, freeze-dried, and then extracted according to the method of Folch (Folch et al., 1957). The stewed lard was placed in a reduced-pressure vacuum drying oven at 40°C for 48 hours to completely remove the remaining organic solvents, and vacuum packaged at -20°C until use. The composition of fatty acids in different oil were shown in Table 1. The diets were prepared according to the AIN-93G diet formulation and the diet formulation was shown in Table 2. During the experiment, the feed of each rat was refreshed every day to prevent oxidative deterioration of fat in the feed.

Observe the rat's diet rule to ensure that the daily diet is basically free of excess food, and provide a certain basis for the feeding amount of each experimental group during the formal experiment. Body weight and feed intake of rats were routinely recorded for calculating the average daily gain.

Sample collection

Fresh feces were collected after rats were fed for 14 days. Normally, the animals excrete feces when they are hung by their tails. The fecal samples were immediately frozen in liquid nitrogen and then stored at -80°C until further analyses. At 6 weeks of feeding, the blood of rats were collected without fasting, the tubes stood at room temperature for 30 min and then were centrifuged at $3,000\text{ g}$ for 30 min to pellet the blood cells. Serum samples were collected and stored at -80°C . At 6 weeks, all the rats were euthanized by cervical dislocation. The liver, kidney, heart and spleen were taken and weighed. Relative weights of the liver, kidney, heart and spleen tissues were calculated according to body weight. The duodenum and jejunum tissues of rats were soaked in 4% paraformaldehyde solution for fixation.

Serum biochemical indicators

Serum biochemical indicators containing glucose (GLU), triglycerides (TG), total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), and low density lipoprotein cholesterol (LDL-C) were determined using the commercially available kits from Nanjing Jiancheng Bioengineering Institute (Nanjing, China).

16S rRNA gene sequencing

Total genomic DNA in fecal samples was extracted using the QIAamp DNA Stool Mini Kit (Qiagen 51 504, Dusseldorf, Nordrhein-westfalen, Germany) according to the manufacturer's instructions. The DNA was quantified by a Nanodrop® spectrophotometer (Nanodrop 2000, Thermo Fisher Scientific, Waltham, MA). Purified DNA was used

to amplify the V4 region of 16S rRNA, which is associated with the lowest taxonomic assignment error rate. All DNA samples were kept at -20°C until sequencing. The V4-V5 hypervariable region of the 16S ribosomal RNA gene was selected for amplification from DNA samples. Polymerase chain reaction (PCR) was performed in triplicate. Amplicons were extracted from 2% agarose gels and purified using the AxyPrep DNA Gel extraction kit (Axygen Biosciences, Union City, CA) according to the manufacturer's instructions and quantified using QuantiFluor™ -ST (Promega, Madison, WI). The pooled DNA product was used to construct Illumina Pair-End library following Illumina's genomic DNA library preparation procedure. Then the amplicon library was double-end sequenced ($2 \times 300\text{bp}$) on an Illumina MiSeq platform (San Diego, CA) according to the standard protocols.

Bioinformatics analysis

Raw fastq files were trimmed and chimeric sequences were identified and removed from all samples to reduce noise, and operational taxonomic units (OTUs) were clustered with $\geq 97\%$ similarity cutoff using QIIME. Then community richness estimator (Chao and ACE), diversity indices (Shannon and Simpson), and Good's coverage were calculated (Schloss et al., 2009). Duncan's multiple comparison was applied to compare averages between any two groups. Differences were considered significant if p values were < 0.05 . Principal coordinate analysis (PCoA) and clustering analysis were applied on the basis of the OTUs to offer an overview of the fecal microbial composition (Lozupone et al., 2011). Multivariate analysis of variance (MANOVA) was conducted to further confirm the observed differences. Linear discriminant analysis effect size (LEfSe) analysis (<http://huttenhower.sph.harvard.edu/galaxy/>) was carried out to discover biomarkers for fecal bacteria and to distinguish between biological conditions among different groups (Segata et al., 2011). The R (pheatmap package) and Cytoscape were applied to visualize the relationships through correlation heatmap and network diagrams respectively.

Statistics analysis

All of the data were generated using SPSS 16.0 software and are represented here as mean \pm standard deviation (SD). Differences between the mean values were evaluated using oneway analysis of variance and Tukey's multiple comparisons test (if applicable). A P value < 0.05 was considered to be statistically significant.

Results

Physical Characteristics

During the experimental period, the rats in each group grew well, and there was no significant difference in initial body weight. It can be seen from Figure 1 that the body weight of the rats has increased, and there is no significant difference between the groups at each time point ($P > 0.05$). The experimental results show that under the condition of intake of nutritional energy, the addition and type of dietary fat has no significant effect on the body mass of rats ($P > 0.05$). Under the conditions of intake and other nutritional energy, the addition and types of dietary fat have no significant effect on the body mass of rats.

It can be seen from table 3 that dietary fat has no significant effect on the heart index, spleen index and kidney index of rats in each group, indicating that the addition and type of dietary fat has no effect on rat kidney, heart and spleen. The liver index of JL group was significantly higher than that of rats in WZ, YY and DY groups ($P < 0.05$).

Blood biochemical indicators

As is shown in Table 4, compared with the JL group, the serum GLU content of rats in the SY group and the DY group was relatively low, but the difference was not significant. The fasting blood glucose value (GLU) of rats in the YY group was lower than that of the WZ group, and significantly lower than other dietary fat mixed diet groups ($P < 0.05$). Compared with the JL group, the serum total cholesterol (TC) content of the SY group was relatively lower. The serum TC content of rats in the YY group was significantly lower than that in the WZ group and other dietary fat mixed diet groups ($P < 0.05$). Serum triglyceride (TG) levels in WZ and YY groups were significantly lower than in SY, JL and DY groups ($P < 0.05$). Compared with refined lard, stewed lard can reduce the content of TC and TG in rat serum to a certain extent. The high-density lipoprotein (HDL-C) of WZ and DY groups was significantly higher than that of SY, JL and YY groups ($P < 0.05$). The HDL-C content of YY group was lower than that of other groups. The level of low-density lipoprotein (LDL-C) in the JL group was significantly higher than that in the YY and DY groups ($P < 0.05$). The serum HDL-C level of the SY group was higher than that of the JL lard group and the LDL-C level was lower than that of the JL group, but the difference was not significant ($P > 0.05$).

Morphology of rat intestine

As is shown in Fig.2 and Table 5, the villus height of the homozygous diet supplemented with dietary fat was significantly higher than that of the fat-free group ($P < 0.05$). The increase in villus height helps to improve the intestinal health of rats, thereby increasing their digestion and absorption of nutrients ability. The depth of the crypt reflects the rate of intestinal villi forming epithelial cells, and the shallower crypt indicates that the maturation rate of intestinal epithelial cells has increased. The villi and crypts in the intestinal tract of rats in the homozygous diet supplemented with dietary fat grew better, and their digestive physiological functions were better than those in the WZ group, which helped the growth of animals. The intestinal wall thickness of the WZ group was significantly lower than that of other homozygous diets supplemented with dietary fat ($P < 0.05$). The ratio of villus length/crypt depth in the WZ group was significantly lower than that of other homozygous diets supplemented with dietary fat ($P < 0.05$). The ratio of villus height/crypt depth in the SY group was higher than that of the other groups, it shows that the intake of a certain amount of stewed lard can increase the villus height and crypt depth of the duodenum and jejunum, and enhance the digestion and absorption function of the intestine.

Cholesterol and triglyceride content in rat liver

As is shown in Fig. 3, it is found that the cholesterol (TC) and triglyceride (TG) levels in the liver of the fish oil group were significantly lower than those of the other homozygous diets supplemented with dietary fat ($P < 0.05$). Fish oil not only significantly reduced the blood lipid content in rat serum, but also significantly reduced the cholesterol and triglyceride content in the liver.

Richness and diversity analyses

The community richness estimators (Chao and ACE), and diversity indices (Shannon and Simpson) were calculated in order to evaluate the alpha diversity (Table 6). The Chao1 and ACE indexes of the SY group were higher than other experimental groups. Compared with other dietary fat mixed diet groups, the Chao1 and ACE indexes of the WZ group were relatively low, and the Shannon index was relatively low. Experimental results show that stewed lard can increase the abundance of intestinal flora to a certain extent, while fat-free diet will reduce the abundance and diversity of intestinal flora.

Rat Fecal Microbe Composition

At the phylum level, *Bacteroidetes* and *Firmicutes* were predominant in all samples. In the WZ group, *Bacteroidetes* was the most abundant phylum, accounting for 48.00%, followed by *Firmicutes* and *Proteobacteria* accounting for 44.11% and 3.37% respectively. In the SY group, *Bacteroidetes* was the most abundant phylum, accounting for 54.50%, followed by *Firmicutes* and *Proteobacteria* accounting for 42.47% and 1.22%, respectively. In the JL group, *Bacteroidetes* was the most abundant phylum, accounting for 51.20%, followed by *Firmicutes* and *Proteobacteria* accounting for 43.76% and 2.11%, respectively. In the YY group, *Bacteroidetes* was the most abundant phylum, accounting for 67.74%, followed by *Firmicutes* and *Proteobacteria*, accounting for 29.09% and 1.74%, respectively. In the DY group, *Bacteroidetes* was the most abundant phylum, accounting for 54.93%, followed by *Firmicutes* and *Proteobacteria* accounting for 37.65% and 2.11%, respectively. It can be seen from Fig. 4 that the dominant bacteria attached to the WZ group, SY group, JL group, YY group and DY group are basically the same at the phylum level, and there are significant individual differences among all samples. As is shown in Table 7, the proportion of *Bacteroidetes* in each sample ranges from 41.52% to 77.38%, and the proportion of *Firmicutes* in each sample ranges from 20.90% to 48.52%. The proportion of *Proteobacteria* in each sample ranges from 0.71% to 6.20%. It can be seen from Table 7 that the ratio of *Firmicutes/Bacteroidetes* (F/B) in the SY group is 0.79 lower than that of the JL group. Compared with the fat-added diet group, the abundance of *Bacteroidetes* in the WZ group decreased, and the abundance of *Firmicutes* increased. The F/B ratio of the WZ group was the highest at 0.92. In addition, the abundance of *Bacteroidetes* in the YY group was significantly higher than that in the fat-free group ($P < 0.05$), the abundance of *Firmicutes* in the YY group was significantly lower than that in the SY, JL, and WZ groups ($P < 0.05$), and the lowest F/B ratio in the YY group is 0.44.

As is shown in Fig. 5 and Table 8, the abundance of Bacteroidaceae in the SY group was 29.95% higher than the other groups, and was significantly higher than that in the JL group ($P < 0.05$). There was no significant difference in the abundance of Bacteroidaceae in the WZ, YY and DY groups. The abundance of S24-7 in the WZ group was lower than that of other dietary fat mixed diet groups, and significantly lower than that of the JL group ($P < 0.05$). The abundance of S24-7 in SY group was not significantly different from WZ, YY and DY groups. The highest abundance of Ruminococcaceae in the WZ group was 19.82%, and the lowest abundance of Ruminococcaceae in the YY group was 12.60%.

Discussion

The weight of rats in the fat-free group was lower than that in the homozygous diet group with added dietary fat, and the experimental results were similar to the results of previous studies. The liver is a key organ for the metabolism of various nutrients. Lipid metabolism is inseparable from the liver. The liver is the main place for the metabolism of intermediate products. Once lipid metabolism disorder occurs, excess lipids are easy to accumulate in the liver and cause fatty liver disease (Kang et al., 2021). The liver index of SY group was not significantly different from that of WZ and DY groups ($P > 0.05$). Our present results showed that compared with refined lard, stewed lard has a lower probability of liver metabolism disorders or liver function damage.

Dietary fat has different effects on the serum indexes of rats. Compared with refined lard, stewed lard can reduce the content of GLU, TC, TG, LDL-C in the serum to a certain extent, and increase the content of HDL-C. Fish oil is rich in n-3 PUFAs, especially EPA and DHA, which can improve the body's metabolic parameters (such as blood sugar, cholesterol and triglycerides) and reduce the accumulation of lipids in the carcass (Gondim et al., 2018). The atherosclerosis index of the refined lard group was significantly higher than that of the other groups, and the liver index was also higher than that of the other groups (Tung et al., 2019). The decrease in HDL-C levels in the YY group may be due to the richness of EPA and DHA in fish oil. As PUFA has no selective lipid-lowering effect, it reduces the

level of HDL-C while reducing the TC level (Breetha et al., 2018). The serum TC content of rats fed fish oil was significantly lower than that of fat-free and other fat groups, which is consistent with related literature reports (Gondim et al., 2018; Harari et al., 2020). In contrast, refined lard tends to increase the level of LDL-C. Tung (Tung et al., 2019) found that excessive intake of refined lard rich in SFA will increase serum TC, TG and LDL-C levels and reduce HDL-C levels, while intake of tea seed oil rich in MUFA will reduce blood lipid levels.

The gut is a complex and dynamic ecosystem. The temporal pattern of microbial survival is the key for finding out core members responding to environmental changes. Many studies have shown that the composition of fecal microbiota is highly correlated with the colonic lumen and mucosa and moderately correlated with the distal small intestine (Yasuda et al., 2015). The small intestine is the main part for animals to digest and absorb nutrients, and the function of the digestive tract is closely related to the growth state of the animal (Xu et al., 1994). Small intestinal villi is the main tissue for nutrient absorption, and its height can reflect the mitotic activity of intestinal crypt cells. Studies have shown that changes in villi height can affect the surface area of intestinal villi, thereby affecting the absorption of nutrients (Czech et al., 2020; Caspary et al., 1992). Nutritional physiology studies have shown that the ratio of villi length/crypt depth can comprehensively reflect the physiological functions of the intestine. An increase in the ratio indicates an increase in the digestion and absorption function of the intestine, and a decrease in the ratio indicates a decline in the digestion and absorption function (Rehman et al., 2018). The villus height of the homozygous diet supplemented with dietary fat was significantly higher than that of the fat-free group, and the ratio of villus height/crypt depth in the experimental group was higher than that of the other groups of rats, indicating that the consumption of stewed pork fat can enhance intestinal digestion and energy absorption. It can be seen that a fat-free diet can reduce fasting blood sugar and blood lipid levels to a certain extent, but it also reduces the intestinal nutrient absorption capacity. Compared with the refined lard group, stewed lard can reduce the fasting blood sugar and blood lipid levels of the body to a certain extent, and can improve the intestinal nutrient absorption capacity.

Fish oil can significantly down-regulate the expression of fatty acid synthesis-related enzymes G6PDH, ME, FAS and other mRNA, and can increase the mRNA expression of fatty acid decomposing enzymes CPT-1 and CPT-2 in the rat liver, resulting in a decrease in liver and serum lipid concentrations (Godea et al., 2020). The content of TC and TG in the liver of the refined lard group was higher than that of the fat-free group and other homozygous diet groups with added dietary fat. Wang xiaoke (Wang et al., 2013) compared the occurrence of obesity induced by high-fat diet with different oil components and found that the liver lipid content of the lard group was significantly higher than that of the soybean oil group. It is speculated that the type of fat is responsible for inducing lipid production and promoting lipid breakdown. The expression of related factors is related to the different utility. The results of this study show that compared with refined lard, stewed lard has lower levels of TC and TG in the liver.

Firmicutes and Bacteroides are the most dominant bacterial phyla in the body, which can affect the energy extraction efficiency of the host and are related to the host's excessive obesity. Obese animals or humans have a higher abundance of Firmicutes in the gut flora (Kallus et al., 2012). In addition, many studies have found that obesity induced by high-fat diet will increase the ratio of Firmicutes to Bacteroides, which affects the metabolic function of intestinal microbes and increases their energy intake (Turnbaugh et al., 2006; Power et al., 2014; Smits et al., 2013). From the changes in the abundance of Bacteroidetes and Firmicutes, it can be proved that fish oil has a certain inhibitory effect on obesity and the beneficial effects of eating dietary fat on the intestinal flora, refined lard is more likely to cause obesity than stewed lard.

The composition of the intestinal microbial colonies of five groups of rat fecal samples and the distribution and abundance of bacterial species were analyzed. The abundance of Bacteroidaceae in the SY group was higher than the other groups. The experimental results show that the stewed lard can significantly increase the abundance of Bacteroidaceae in the body. The abundance of Bacteroides in the intestinal flora of obese rats induced by high-fat diet was lower than that in the normal group, green tea polyphenols can significantly increase the relative abundance of Bacteroides and Proteus after 3 weeks of intervention in rats, thereby reducing obesity in rats induced by high-fat diet (Guo et al., 2017).

S24-7 under the phylum Bacteroides has not been isolated and cultured in vitro, and its biological characteristics are not yet clear. Ormerod (Ormerod et al., 2016) found that S24-7 is the main member of the gut microbiota and is highly present in the gastrointestinal tract of warm-blooded animals. They named it "Candidatus Homeothermaceae" and speculated that it belongs to Gram-negative anaerobes that can ferment carbohydrates extensively. Partial hepatectomy resulted in a significant increase in S24-7 of the intestinal flora Bacteroides phylum and a significant decrease in Firmicutes, Lachnospiraceae and Ruminococcaceae (Liu et al., 2016). It is also suggested that liver metabolism and immune function are closely related to the abundance of Lachnospiraceae, Ruminococcaceae and S24-7. The results show that fat-free diet will reduce the abundance of S24-7 in the body to a certain extent. Rumen cocci mainly exist in the rumen, cecum and large intestine of mammals. Studies have shown that the rumen cocci in the intestine of high-fat rats will significantly decrease (Daniel et al., 2014), and the intake and types of dietary fat will have a certain impact on the relative abundance of Ruminococcaceae in the body. The abundance of Lachnospiraceae, Desulfovibrionaceae, Acidaminococcaceae, Coriobacteriaceae and Bilophila is positively correlated with obesity (Zhao et al., 2017). The experimental results showed that compared with the dietary fat group, the abundance of Lachnospiraceae in the WZ group was relatively higher, and the abundance of Lachnospiraceae in the SY group was lower than that in the JL group. It shows that fat-free diet may also lead to obesity, refined lard is more likely to produce obesity than stewed lard.

Our study revealed that fat-free diet can reduce fasting blood sugar and blood lipid levels to a certain extent, but it also reduces the intestinal nutrient absorption capacity. Compared with the refined lard group, stewed lard can reduce the body's fasting blood sugar and blood lipid levels to a certain extent, and can improve the intestinal nutrient absorption capacity. Compared with other dietary fat groups, stewed pork fat will increase the abundance of *Bacteroidaceae* in rats, and fat-free diet will decrease the abundance of S24-7 in rats. It shows that the addition and types of dietary fat will affect the distribution of rat intestinal flora to a certain extent. Therefore, we can try to choose some fats with higher unsaturated fatty acid content which similar to fish oil, and at the same time properly intake stewed lard to improve the abundance of intestinal flora.

Abbreviations

WZ: fat-free ; SY: stewed lard; JL: refined lard; YY: fish oil ; DY: soybean oil ; Glu : glucose; TC: total cholesterol; TG: total triglyceride; SFA : saturated fatty acid; MUFA monounsaturated fatty acid ; HDL-C: high-density lipoprotein ; LDL-C: low-density lipoprotein; F/B: the ratio of Firmicutes/Bacteroidetes

Declarations

Ethics approval and consent to participate

All animal procedures were performed in accordance with the Guidelines for Care and Use of Laboratory Animals of Yangzhou University.

Consent for publication

All authors approved the final version of the manuscript and consent for publication

Availability of data and materials

All data generated or analysed during this study are included in this published article.

Competing interests

The authors declare that they have no competing interests

Authors' contributions

YX, as the lead contributor, contributed in designing and conducting the experiments, collecting the data and summarizing the literatures. WZ assisted the lead contributor to conduct the experiments and revise the manuscript. QG and XZ provided technical guidance and modified the manuscript. XZ, as the corresponding author, provided financial support and contributed in organizing, writing, editing and revising the manuscript. All authors reviewed the manuscript. All authors read and approved the manuscript.

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Tables

Table 1 Fatty acid composition of experimental oils [g/100g oil]

Fatty acid	SY	JL	DY	YY
C12:0	0.08	0.11	0.00	0.00
C14:0	1.55	1.76	0.07	1.13
C15:0	0.02	0.00	0.00	0.41
C16:0	25.85	24.79	10.89	20.64
C17:0	0.11	0.25	0.05	1.15
C18:0	11.34	15.25	4.86	6.72
C19:0	0.01	0.00	0.00	0.00
C20:0	0.22	0.22	0.41	1.26
C14:1	0.03	0.04	0.01	0.02
C16:1	3.51	2.55	2.11	0.12
C17:1	0.15	0.15	0.03	0.36
C18:1	44.65	37.81	22.44	27.78
C19:1	0.06	0.00	0.00	0.15
C20:1	1.04	0.81	0.17	2.97
C16:2	0.02	0.00	0.00	0.18
C18:2	10.64	15.65	52.61	17.48
C20:2	0.41	0.61	0.00	0.25
C18:3	0.01	0.00	6.35	0.30
C20:3	0.09	0.00	0.00	2.61
C20:4	0.13	0.00	0.00	0.11
C20:5	0.03	0.00	0.00	15.79
C22:4	0.05	0.00	0.00	0.57
C22:6	0.00	0.00	0.00	12.68
SFA	39.18	42.38	16.28	31.31
MUFA	49.44	41.36	24.76	31.40
PUFA	11.38	16.26	58.96	37.29
UFA	60.82	57.62	83.72	68.69
UFA/SFA	1.55	1.36	5.14	2.19

Table 2 Formula and calculated nutrient composition of experimental diet

Component (g/kg)	WZ	SY	JL	YY	DY
Casein	180.35	200.0	200.0	200.0	200.0
Corn starch	519.82	397.49	397.49	397.49	397.49
Dextrin	119.03	132.0	132.0	132.0	132.0
Sucrose	90.17	100.0	100.0	100.0	100.0
Stewed lard	-	70.0	-	-	-
Refined lard	-	-	70.0	-	-
Fish oil	-	-	-	70.0	-
Soybean oil	-	-	-	-	70.0
Cellulose	45.09	50.0	50.0	50.0	50.0
Minerals	31.56	35.0	35.0	35.0	35.0
Vitamins	9.02	10.0	10.0	10.0	10.0
L-cystine	2.71	3.0	3.0	3.0	3.0
Choline Chloride	2.25	2.5	2.5	2.5	2.5
Tert-butyl hydrogen	0.01	0.01	0.01	0.01	0.01
Nutritional level					
Total energy [Kcal/g]	3.4	3.7	3.7	3.7	3.7
Protein [%]	19.4	19.4	19.4	19.4	19.4
Carbohydrates [%]	80.6	63.6	63.6	63.6	63.6
Fat [%]	0	16.9	16.9	16.9	16.9

Table 3 Effects of dietary fat on rat organ index

Project	heart	liver	spleen	kidney
WZ	0.37±0.03 ^a	2.53±0.09 ^{bc}	0.20±0.01 ^a	0.63±0.03 ^a
SY	0.35±0.03 ^a	2.58±0.06 ^{ab}	0.21±0.02 ^a	0.64±0.06 ^a
JL	0.38±0.04 ^a	2.68±0.12 ^a	0.20±0.01 ^a	0.67±0.04 ^a
YY	0.35±0.03 ^a	2.45±0.09 ^c	0.21±0.01 ^a	0.65±0.05 ^a
DY	0.37±0.05 ^a	2.57±0.12 ^{bc}	0.21±0.02 ^a	0.65±0.02 ^a

Different letters on the shoulder in the same column indicate significant difference in organ index at the 0.05 significant level

Table 4 Effects of dietary fat on blood biochemical indices (mmol/L, n=8, x±s)

Project	GLU	TC	TG	HDL-C	LDL-C
WZ	4.44±0.60 ^{bc}	2.48±0.27 ^b	1.04±0.08 ^b	0.85±0.18 ^a	0.73±0.08 ^{ab}
SY	5.06±0.51 ^{ab}	2.81±0.35 ^{ab}	1.50±0.26 ^a	0.66±0.09 ^b	0.71±0.17 ^{abc}
JL	5.25±0.43 ^a	3.11±0.39 ^a	1.52±0.29 ^a	0.61±0.12 ^b	0.75±0.09 ^a
YY	4.07±0.52 ^c	1.97±0.31 ^c	1.21±0.24 ^b	0.59±0.16 ^b	0.60±0.15 ^{bc}
DY	4.77±0.65 ^{ab}	2.56±0.34 ^b	1.48±0.30 ^a	0.88±0.18 ^a	0.59±0.07 ^c

Different letters on the shoulder in the same column indicate significant difference in blood biochemical indices at the 0.05 significant level

Table 5 Effect of dietary fat on morphological structure of jejunum in rats

Project	WZ	SY	JL	YY	DY
villus height(μm)	821.76±76.88 ^d	1078.80±43.50 ^{ab}	1101.18±55.01 ^a	990.67±103.25 ^{bc}	925.78±60.66 ^c
crypt depth(μm)	254.44±13.68 ^d	293.48±10.12 ^{ab}	303.94±10.71 ^a	271.42±21.71 ^{cd}	279.48±11.40 ^{bc}
wall thickness(μm)	202.80±15.71 ^c	277.36±19.71 ^a	279.55±28.56 ^a	242.86±27.96 ^b	256.15±26.55 ^{ab}
villus height / crypt depth	3.23±0.26 ^c	3.68±0.23 ^a	3.62±0.15 ^{ab}	3.66±0.36 ^a	3.31±0.18 ^{bc}

Different letters on the shoulder in the same column indicate significant difference in morphological structure of jejunum at the 0.05 significant level

Table 6 Diversity index

Sample Name	Chao1	ACE	Shannon	Simpson
WZ	1799.84±407.96 ^a	1867.58±193.98 ^a	8.32±0.39 ^a	0.98±0.00 ^a
SY	2051.61±463.54 ^a	2159.56±735.43 ^a	8.34±0.31 ^a	0.98±0.01 ^a
JL	1706.45±156.80 ^a	1858.77±401.48 ^a	8.45±0.27 ^a	0.99±0.00 ^a
YY	1937.37±523.08 ^a	2090.81±432.75 ^a	8.41±0.30 ^a	0.98±0.01 ^a
DY	1991.97±590.71 ^a	1940.17±521.48 ^a	8.18±0.13 ^a	0.98±0.00 ^a

Different letters on the shoulder in the same column indicate significant difference in diversity index at the 0.05 significant level

Table7 Differences in gut microbiota at the level of phylum in each group

Sample Name	<i>Bacteroidetes</i> %	<i>Firmicutes</i> %	<i>Firmicutes</i> / <i>Bacteroidetes</i>
WZ	48.00±3.09 ^b	44.11±4.99 ^a	0.92±0.14 ^a
SY	54.50±4.31 ^{ab}	42.48±5.59 ^a	0.79±0.16 ^{ab}
JL	51.20±8.42 ^{ab}	43.76±4.14 ^a	0.88±0.24 ^a
YY	67.74±10.29 ^a	29.09±8.44 ^b	0.44±0.19 ^b
DY	54.93±12.66 ^{ab}	37.65±7.74 ^{ab}	0.73±0.27 ^{ab}

Different letters on the shoulder in the same column indicate significant difference in the level of phylum at the 0.05 significant level

Table 8 Differences in gut microbiota at the level of family in each group

Sample Name	<i>Bacteroidaceae</i> %	<i>S24-7</i> %	<i>Ruminococcaceae</i> %	<i>Lachnospiraceae</i> %
WZ	26.67±2.55 ^{ab}	16.06±1.54 ^c	19.82±0.47 ^a	4.85±0.47 ^a
SY	29.95±7.34 ^a	20.49±6.58 ^{bc}	17.79±5.45 ^a	3.77±0.74 ^a
JL	14.14±6.90 ^b	28.72±4.74 ^a	19.15±6.91 ^a	3.93±1.50 ^a
YY	24.57±7.21 ^{ab}	25.98±3.13 ^{ab}	12.60±3.89 ^a	4.42±0.98 ^a
DY	27.05±11.59 ^{ab}	24.50±1.78 ^{ab}	16.43±6.99 ^a	4.65±0.27 ^a

Different letters on the shoulder in the same column indicate significant difference in the level of family at the 0.05 significant level

Figures

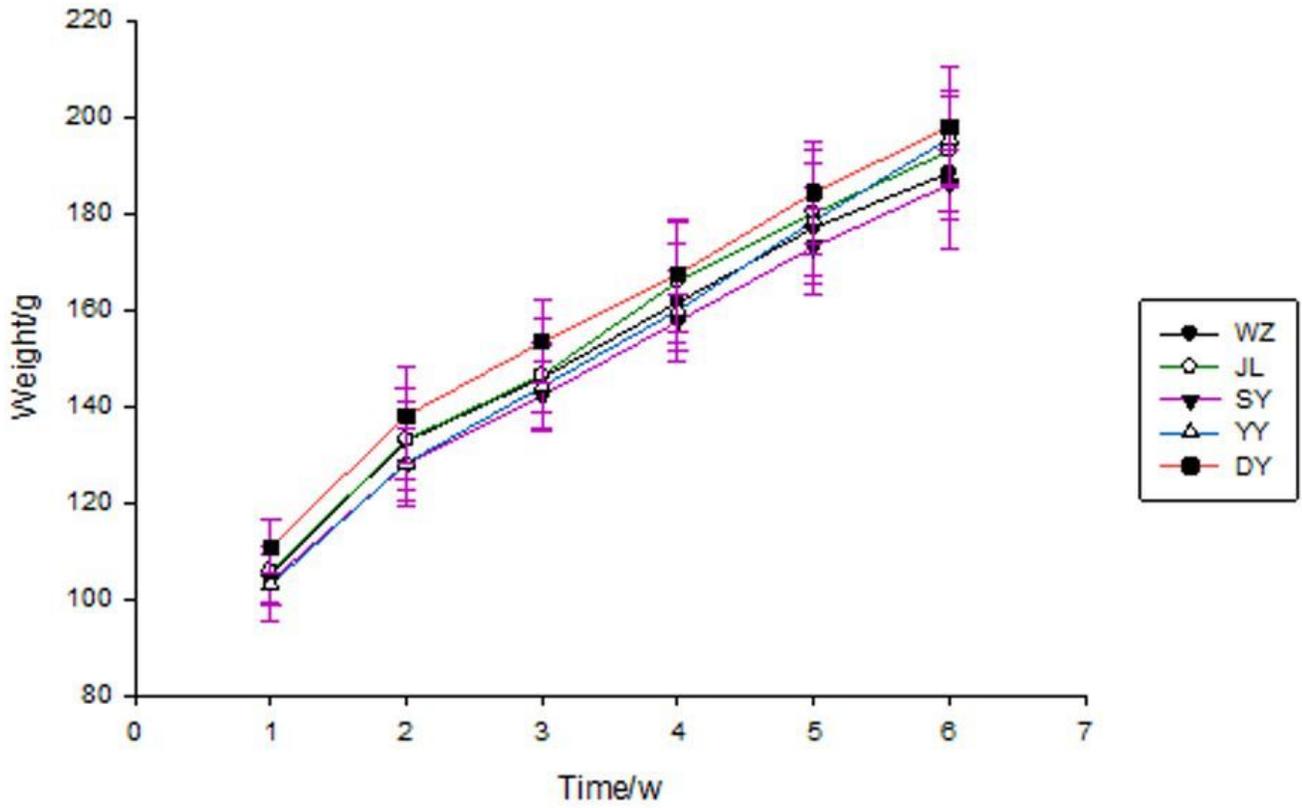


Figure 1

Effect of dietary fat on body weight in rats

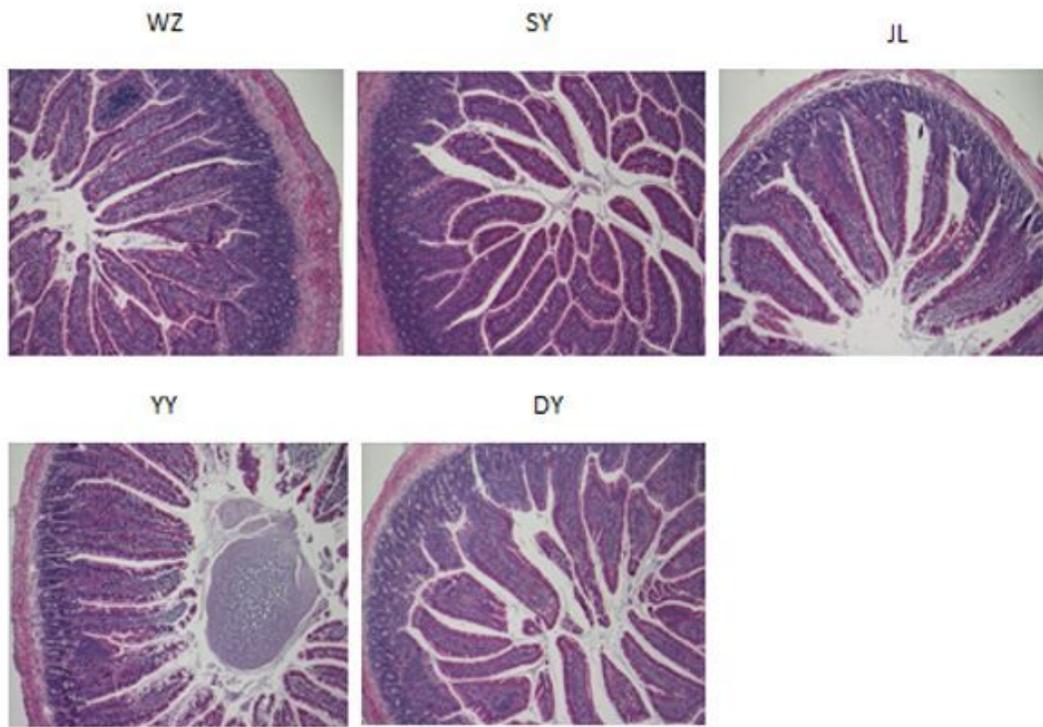


Figure 2

H&E-stained of dietary fat intake for 6 weeks on the morphological structure of jejunum in rats

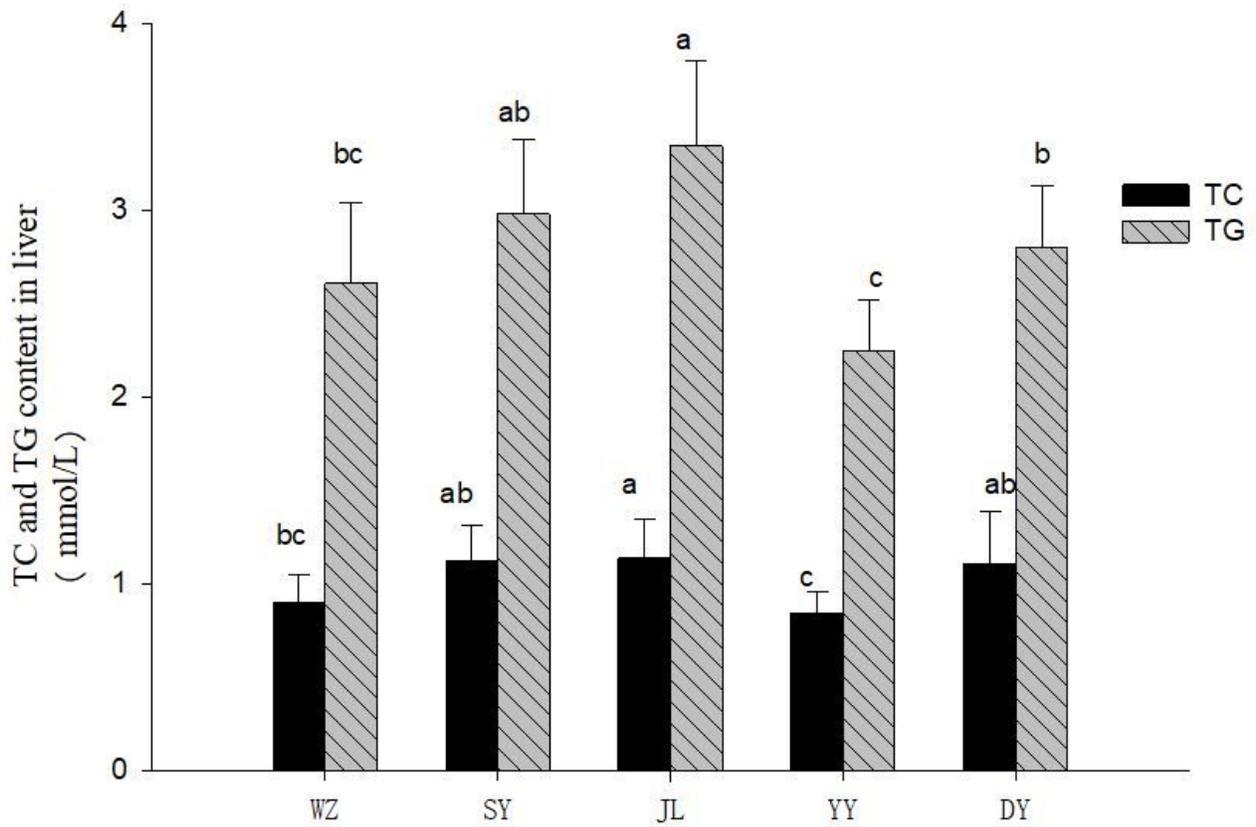


Figure 3

Content of cholesterol and triglyceride in rat liver Different superscript letters indicate that the contents of TC and TG in liver are significantly different at 0.05 significant level

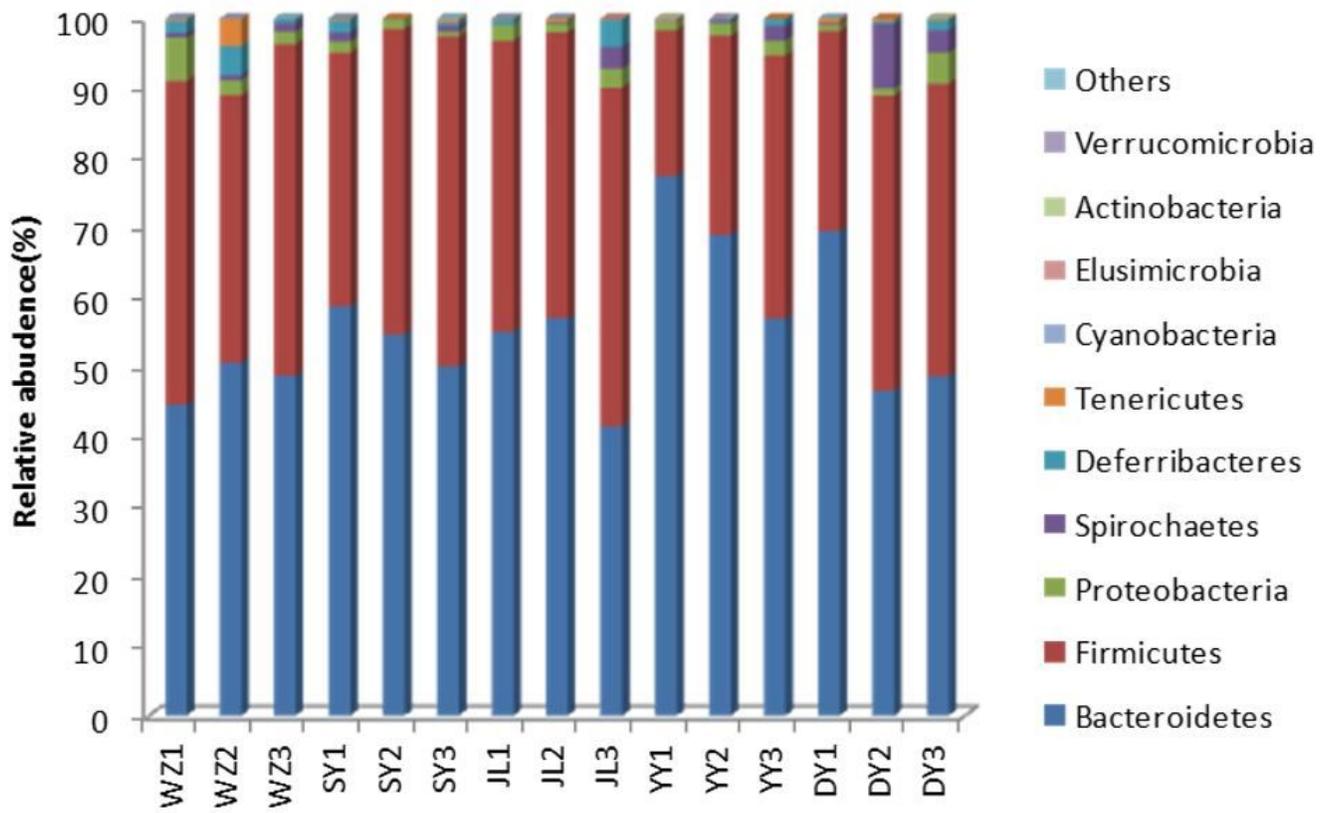


Figure 4

Effect of dietary fat on the phylum level of intestinal flora in rats

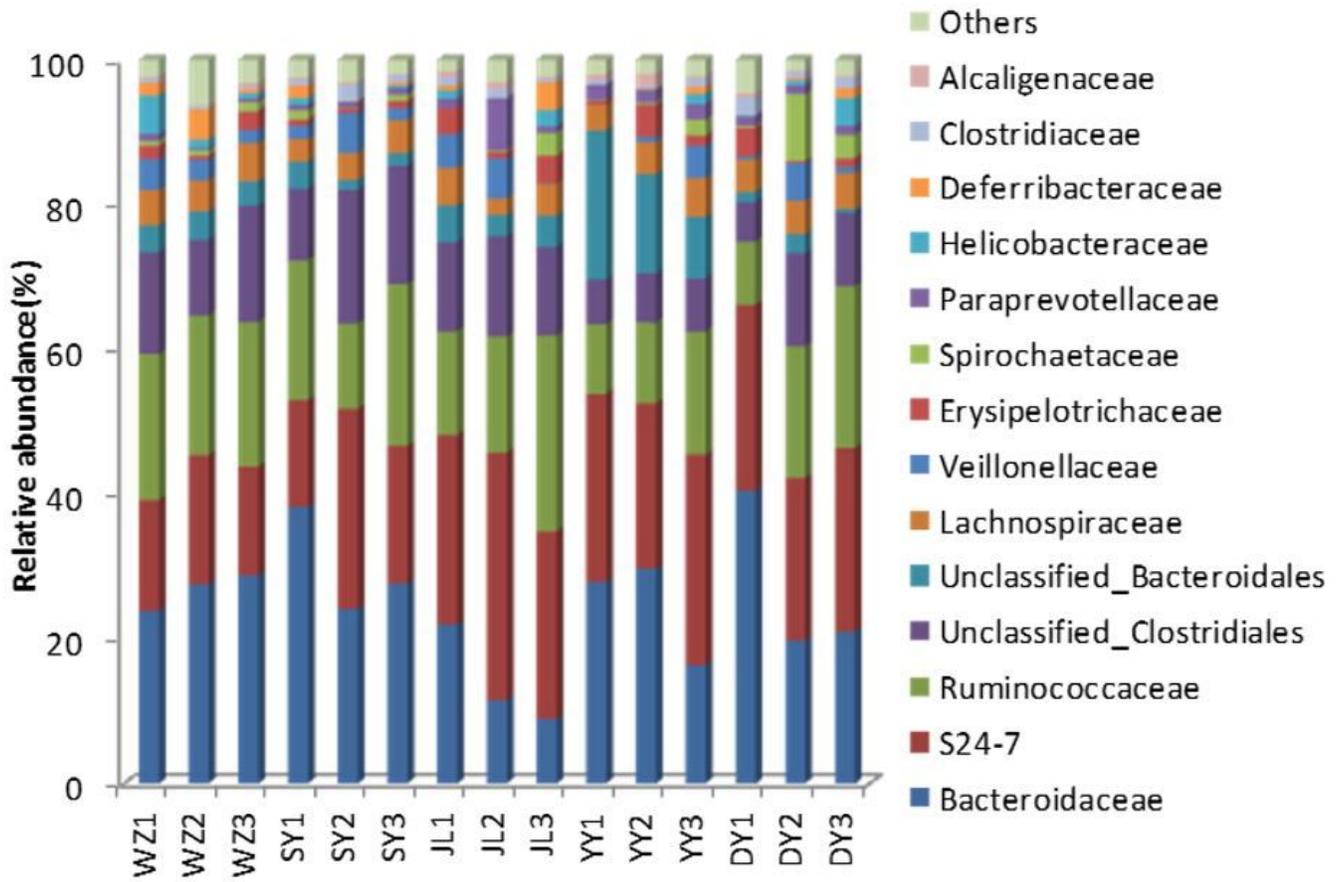


Figure 5

Effect of dietary fat on the family level of intestinal flora in rats