Fish Oil Can Significantly Reshape Diet-Based Gut Microbiota in Mice

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Abstract

Background

Studies have demonstrated the influence of diet on the gut microbiota, and recent evidence has revealed the beneficial effects of fish oil supplements on the gut microbiota. The goal of the present study was to investigate the influence of fish oil on diet-based gut microbiota changes in mice.

Results

AIN-93M significantly decreased the gut microbial diversity of mice, increasing the abundances of *Bacteroides* and *Parabacteroides* and decreasing the abundance of *Odoribacter*. In contrast, gut microbial diversity was maintained in mice fed a fish oil-intensive diet, where the Firmicutes: Bacteroidetes ratio was increased, the abundance of *Parabacteroides* was increased and that of *Odoribacter* was decreased. In contrast, the VSL#3 intervention had little influence on gut microbiota diversity, decreasing the abundance of Firmicutes.

Conclusions

AIN-93M can decrease gut microbiota diversity, which may be associated with a potential proinflammatory effect. Fish oil may have anti-inflammatory effects by restoring and maintaining microbial diversity.

Background

The gut microbiota plays important roles in metabolism and immunity.[1] This microbial community is shaped from infancy and can be greatly influenced by several factors[2], among which diet is the most important, with many studies having shown that changes in macronutrients can lead to dramatic changes in the gut microbiota.[3]

Fish oil is regarded as a beneficial food supplement, with documented benefits on cardiac vascular health. The primary active ingredient of fish oil is ω -3 PUFAs, including EPA [20:5(n = 3)] and DHA, [22:6(n-3)]. Studies have also demonstrated beneficial immunomodulatory effects of ω -3 PUFAs, and the ratio of ω -3 and ω -6 PUFAs was shown to play an important role in inflammation by influencing eicosanoid metabolism.[4] ω -6 PUFAs are metabolized into several proinflammatory mediators, while ω -3 PUFAs function as competitive inhibitors of enzymes and can be metabolized into several anti-inflammatory mediators.[4]

Fish oil may have an indirect influence on inflammation by modifying the gut microbiota. However, limited studies have investigated the influence of fish oil on the gut microbiota, and the results are controversial, especially the observed changes in abundance of different bacteria. Multiple studies have

demonstrated that ω -3 PUFAs can increase the abundances of *Lactobacillus* and *Bifidobacteria*, [5–9] which are thought to have a beneficial influence on health. Although some studies have shown that ω -3 PUFAs can increase the abundance of Bacteroidetes and decrease the Firmicutes: Bacteroidetes ratio, [8, 10, 11] the results of another study supported the opposite conclusion. [12] The change in the abundance of *Desulfovibrio* induced by ω -3 PUFAs remains debated. One study [13] showed a decrease in *Bilophila*, while another [14] observed an increase in Proteobacteria, although further investigation is needed to validate these results.

In the present study, we performed dietary interventions in mice to assess the influence of fish oil on gut microbiota changes due to diet and determine its potential influence on health.

Results

1. Fecal microbiota changes associated with the standardized AIN-93M diet

Whole-microbiome significance testing using Anosim showed significant differences after AIN-93M intervention ($R=1$, $P=0.003$, CA vs CB) (Fig. 2A). The alpha diversity test using the Shannon index with the Wilcoxon signed-rank test showed that the AIN-93M intervention significantly decreased the microbial diversity of the fecal microbiota ($P=0.0082$, CA vs CB) (Fig. 2B).

The MetaStat analysis results showed no significant differences in the abundances of Firmicutes and Bacteroidetes, which were the most abundant phyla (abundance >0.1) (Fig. 2C 2E). At the genus level, *Bacteroides*, *Parabacteroides*, *Alistipes* and *Odoribacter* were the most abundant genera (Abundance >0.01). AIN-93M significantly increased the abundances of *Bacteroides*, *Parabacteroides* and decreased that of *Odoribacter* (Fig. 2D).

2. Fecal microbiota changes associated with the fish oil-intensive diet

Whole-microbiome significance testing results showed no significant difference between the fish oil and control groups before intervention ($R=0.1426$, $P=0.081$, FB vs CB). The fish oil-intensive diet had a significant influence on the microbiota ($R=0.9185$, $P=0.001$, FA vs FB), and there was a significant difference in the whole microbiome between these two groups after intervention ($R=0.8926$, $P=0.002$, FA vs CA) (Fig. 2A). No difference in microbial diversity was observed between the fish oil and control groups before intervention ($P=0.1989$, FB vs CB). Interestingly, the fish oil-intensive diet significantly increased the microbial diversity ($P=0.7024$, FA vs FB) (Fig. 2B).

At the phylum level, the fish oil-intensive diet intervention significantly reduced the abundance of Bacteroidetes and increased that of Firmicutes (Fig. 2C 2E). At the genus level, the fish oil-intensive diet intervention increased the abundance of *Parabacteroides* and decrease that of *Odoribacter* but had no influence on the abundance of *Bacteroides* (Fig. 2D).

3. Fecal microbiota changes associated with VSL#3 intervention

There was a significant difference in the fecal microbiota between the VSL#3 and control groups before intervention ($R=0.2148$, $P=0.034$, VB vs CB). However, no significant difference was observed after intervention ($R=0.1315$, $P=0.083$, VA vs CA). The intervention had significant influence on the fecal microbiota ($R=0.8778$, $P=0.002$, VA vs VB). There was no significant difference between the VSL#3 and control groups on microbial diversity ($P=0.0729$, VB vs CB, $P=0.7296$, VA vs CA). Similar to the AIN-93M intervention, the AIN-93M plus VSL#3 intervention significantly reduced the diversity of the fecal microbiota ($P=0$, VA vs VB) (Fig. 2B).

At the phylum level, Firmicutes, Bacteroidetes and Proteobacteria were the most abundant phyla (abundance>0.1). The AIN-93M and VSL#3 intervention significantly reduced the abundance of Firmicutes and increased that of Proteobacteria but had no influence on Bacteroidetes. (Fig. 2C 2E) At the genus level, *Bacteroides*, *Parabacteroides*, *Alistipes*, *Bilophila*, *Odoribacter*, *Parasutterella* and *Muribaculum* were the most abundant genera influenced in all the groups (Abundance>0.1). The AIN-93M and AIN-93M plus VSL#3 interventions showed a similar influence over the fecal microbiota at the genus level, exhibiting increased abundances of *Bacteroides*, *Parabacteroides*, *Bilophila* and *Parasutterella* and decreased abundances of *Alistipes*, *Odoribacter* and *Muribaculum* (Fig. 2D).

Discussion

The Western-style diet may contribute to the pathogenesis of chronic intestinal diseases, such as inflammatory bowel disease, but the associated mechanisms remain unelucidated. Diet may influence host health by directly affecting nutrient digestion and absorption or by influencing the gut microbiota. The relative deficiency of ω -3 PUFAs in the Western diet has been hypothesized to be one of the potential contributors to its adverse effects.[15] With growing evidence showing that the gut microbiota has an essential impact on human health[1], it is possible that a disrupted gut microbiota may promote nutrition deficiency with respect to host health. In this study, we observed that diet plays an essential role on the gut microbiota, having a large influence on the structure and diversity of the gut microbial community. By supplementing the diets of mice with ω -3 PUFAs, the diversity of the microbiota was restored, and its structure was shifted toward an increased Firmicutes: Bacteroidetes ratio.

The results of our study showed that compared to a standard diet, AIN-93M can greatly influence the gut microbiota. AIN-93[16] was developed up by the American Institute of Nutrition Rodent Diets in 1993. Compared with the natural diets of mice, the composition of AIN-93 is precise, making it a good tool to study the effects of specific diet compositions and their influence on the gut microbiota. However, the results of the present study showed that AIN-93 significantly influences the gut microbiota of mice by reducing the species diversity. Feeding mice AIN-93M can increase the abundance of *Bacteroides*, which was shown to be significantly more abundant in IBD patients than in healthy controls.[17] Thus, a better chow with an exact composition and little influence on the normal microbiota of mice is needed for studies on the interaction between specific diet ingredients and the microbiota.

As previously reported, the influence of fish oil on the gut microbiota has been debated and requires further investigation. In our study, mice fed a fish oil-intensive diet were observed to have a gut microbiota with greater species diversity, an increased Firmicutes abundance and decreased Bacteroidetes abundance, thereby increasing the Firmicutes: Bacteroidetes ratio. Several studies[18] have shown that the relative proportion of Bacteroidetes is decreased in obese individuals in comparison to those who are lean and that this proportion increases with weight loss. Thus, ω -3 PUFAs have been proposed to regulate fat metabolism by modifying the gut microbiota. Furthermore, several studies[5–9, 13, 14] have shown ω -3 PUFAs can influence specific probiotic bacteria and pathogens, thereby affecting inflammation of the gut. In our study, we showed that ω -3 PUFAs can reverse the increase in the abundance of *Bacteroides*, which is thought to be a pro-inflammatory genus. ω -3 PUFAs may also function as an anti-inflammatory component by modifying the structure of the gut microbiota.

VSL#3 is a probiotic mixture indicated for managing IBS, UC and ileal pouch. VSL#3 contains eight strains of probiotic bacteria. Several studies[19–24] have shown that VSL#3 can modify the microbiota in the lower digestive tract and suppress inflammation. In the present study, we used VSL#3 to study its effect compared to that of fish oil. In contrast to the results of a previous study, the results of our study showed that VSL#3 had little influence on the gut microbiota of mice. This difference may be related to the lower dosage used drinking water instead of the lavage used in other studies. Another possible explanation is that VSL#3 is designed for humans, and the probiotics it contains failed to function similarly in mice.

There are several limitations of this study. The sample size was limited, and more mice in each group may provide better understanding of the differences between the groups. Furthermore, we analyzed the fecal microbiota of mice, which can be obtained easily and repeatedly, but may not be representative of the microbiota in the host.[25] Further study to assess the microbiota directly from the intestine may allow for a better understanding of the interaction between the host and microbiota. Our study focused on the influence of fish oil on the microbiota in mice, and additional studies focusing on the associated metabolic and inflammatory pathways would be of great interest.

Conclusions

In summary, in the present study, we showed that AIN-93M had a large influence on the fecal microbiota of mice, which is believed to be the result of proinflammatory effects. The fish oil-intensive AIN-93M diet could reverse this effect and maintain the healthy microbiota, whereas the VSL3# probiotics had little influence on the microbial changes caused by the AIN-93M diet. To better understand specific components of diet and its influence over the gut microbiota, a standard diet with defined components and little influence over the gut microbiota is needed for further studies. The results indicated that the fish oil-intensive AIN-93M diet would be a better choice for further study than AIN-93M. Furthermore, fish oil has the potential to be a prebiotic, maintaining the microbial diversity and inhibiting pro-inflammatory microbiota.

Methods

1. Study objectives

Male C57BL/6N mice (eight weeks old) were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. Mice were maintained in the Institute of Laboratory Animal Science, CAMS & PUMC under SPF conditions. The mice were maintained on a 12 h light-12 h dark cycle at 23 ± 3°C, with four mice per cage.

2. Diets

Two different types of diets were formulated and supplied by Beijing Keao Xieli Feed Co. Ltd. AIN-93M[16] is a standardized diet with a precise composition that was developed by the American Institute of Nutrition Rodent Diets in 1993. The fish oil-intensive diet was customized based on the AIN-93M diet, with 40 g/kg soybean oil being replaced by 30 g/kg of soybean oil and 10 g/kg fish oil. VSL#3, a mixture of 8 bacterial individual strains, including *L. acidophilus*, *L. plantarum*, *L. paracasei*, *L. helveticus*, *S. thermophilus*, *B. longum*, *B. breve*, and *B. infantis*, was manufactured by AlfaSigma USA, Inc. The detailed compositions of the diets are shown in Table 1.

Table 1
Detailed compositions of the intervention diets

Material(g/kg)	AIN-93M	Customized diet
Casein	140.00	140.00
L-Cystine	1.80	1.80
Corn Starch	465.69	465.69
Maltodextrin	155.00	155.00
Sucrose	100.00	100.00
Cellulose	50.00	50.00
Soybean Oil	40.00	30.00
Fish Oil*	0.00	10.00
Choline Bitartrate	2.50	2.50
AIN Mineral Mix	35.00	35.00
AIN Vitamin Mix	10.00	10.00
BHT	0.01	0.01
*Fish oil was obtained from Chengdu Aowei Health Tech Co. Ltd. Every 100 g of fish oil contained 18.2 g of EPA and 10.3 g of DHA.		

3. Experimental design

C57BL/6N male mice were randomly assigned into three groups: the control (C), fish oil (F) and VSL#3 (V) groups. All mice were fed regular diets (provided by Beijing Vital River Laboratory Animal Technology Co., Ltd.) and purified water after birth until diet intervention. The control group was fed the AIN-93M diet and purified water, the fish oil group was fed the customized diet and purified water, and the VSL#3 group was fed the AIN-93M diet and purified water mixed with VSL#3 (10^8 CFU/ml). The intervention duration was 2 weeks. Feces were collected immediately after they were produced and stored at -80°C . The feces of each mouse were collected before and at the end of the intervention (Fig. 1). The mice were euthanized by intraperitoneal injection of pentobarbital sodium ($10\mu\text{L/g}$) followed by neck breaking method after experiment.

4. Microbiota diversity analysis

The collected feces of each group were labeled as control before intervention (CB), control after intervention (CA), fish oil before intervention (FB), fish oil after intervention (FA), VSL#3 before intervention (VB), and VSL#3 after intervention (VA). Total genomic DNA was extracted using the SDS method. The V4 region of the 16S rRNA gene was PCR amplified using the primers 515F and 806R with Phusion® High-Fidelity PCR Master Mix (New England Biolabs). Subsequently, the PCR products were purified with a Qiagen Gel Extraction Kit (Qiagen, Germany), and sequencing libraries were generated using a TruSeq® DNA PCR-Free Sample Preparation kit (Illumina, USA). The library quality was assessed using a Qubit® 2.0 Fluorometer (Thermo Scientific) and an Agilent Bioanalyzer 2100 system and then sequenced on an Illumina NovaSeq platform to generate 250 bp paired-end reads.

5. Bioinformatics analysis

Paired-end reads were merged using FLASH (V 1.2.7). The data were then filtered to obtain clean, high-quality tags according to the QIIME (V 1.9.1) quality-controlled process. The tags were then compared with the reference database (Silva database) using the UCHIME algorithm to remove chimeric sequences. OTU grouping was performed using Uparse (V 7.0.1001), and the Silva Database was used based on the Mothur algorithm to annotate taxonomic information. Alpha diversity was calculated with QIIME (V 1.7.0) and displayed with the R software environment (V 2.15.3). Beta diversity was calculated using QIIME (V 1.9.1), and principal component analysis (PCA) was performed using the FactoMineR and ggplot2 packages in the R software environment (V 2.15.3).

Abbreviations

PUFAs

Polyunsaturated fatty acids
EPA
eicosapentaenoic acid
DHA
docosahexaenoic acid
IBS
irritable bowel syndrome
UC
ulcerative colitis
CAMS
Chinese Academy of Medical Science
PUMC
Peking Union Medical College
SPF
specific pathogen free
SDS
sodium dodecyl sulfate
OTU
operational taxonomic unit
LEfSe
LDA effect size

Declarations

- Ethics approval and consent to participate

Animal protocols were approved by the Peking Union Medical College Hospital Animal Welfare Ethics Committee.

- Consent for publication

Not applicable

- Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

- Competing interests

The authors declare that they have no competing interests

- Funding

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- Authors' contributions

HS performed the animal experiment and was one of the major contributors in writing the manuscript. DC and BT helped modified the manuscript. All authors read and approved the final manuscript.

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References

1. Gilbert JA, Blaser MJ, Caporaso JG, Jansson JK, Lynch SV, Knight R. Current understanding of the human microbiome. *Nat Med*. 2018;24(4):392–400. doi:10.1038/nm.4517.
2. Yatsunenko T, Rey FE, Manary MJ, Trehan I, Dominguez-Bello MG, Contreras M, et al. Human gut microbiome viewed across age and geography. *Nature*. 2012;486(7402):222–7. doi:10.1038/nature11053.
3. David LA, Maurice CF, Carmody RN, Gootenberg DB, Button JE, Wolfe BE, et al. Diet rapidly and reproducibly alters the human gut microbiome. *Nature*. 2014;505(7484):559–63. doi:10.1038/nature12820.
4. Esser-von Bieren J. Immune-regulation and -functions of eicosanoid lipid mediators. *Biol Chem*. 2017;398(11):1177–91. doi:10.1515/hsz-2017-0146.
5. Ghosh S, DeCoffe D, Brown K, Rajendiran E, Estaki M, Dai C, et al. Fish oil attenuates omega-6 polyunsaturated fatty acid-induced dysbiosis and infectious colitis but impairs LPS dephosphorylation activity causing sepsis. *PLoS One*. 2013;8(2):e55468. doi:10.1371/journal.pone.0055468.
6. Watson H, Mitra S, Croden FC, Taylor M, Wood HM, Perry SL, et al. A randomised trial of the effect of omega-3 polyunsaturated fatty acid supplements on the human intestinal microbiota. *Gut*. 2018;67(11):1974–83. doi:10.1136/gutjnl-2017-314968.
7. Piazzzi G, D'Argenio G, Prossomariti A, Lembo V, Mazzone G, Candela M, et al. Eicosapentaenoic acid free fatty acid prevents and suppresses colonic neoplasia in colitis-associated colorectal cancer acting on Notch signaling and gut microbiota. *Int J Cancer*. 2014;135(9):2004–13. doi:10.1002/ijc.28853.
8. Lu C, Sun T, Li Y, Zhang D, Zhou J, Su X. Microbial diversity and composition in different gut locations of hyperlipidemic mice receiving krill oil. *Appl Microbiol Biotechnol*. 2018;102(1):355–66. doi:10.1007/s00253-017-8601-1.

9. Robertson RC, Seira Oriach C, Murphy K, Moloney GM, Cryan JF, Dinan TG, et al. Omega-3 polyunsaturated fatty acids critically regulate behaviour and gut microbiota development in adolescence and adulthood. *Brain Behav Immun*. 2017;59:21–37. doi:10.1016/j.bbi.2016.07.145.
10. Robertson RC, Seira Oriach C, Murphy K, Moloney GM, Cryan JF, Dinan TG, et al. Deficiency of essential dietary n-3 PUFA disrupts the caecal microbiome and metabolome in mice. *Br J Nutr*. 2017;118(11):959–70. doi:10.1017/S0007114517002999.
11. Cui C, Li Y, Gao H, Zhang H, Han J, Zhang D, et al. Modulation of the gut microbiota by the mixture of fish oil and krill oil in high-fat diet-induced obesity mice. *PLoS One*. 2017;12(10):e0186216. doi:10.1371/journal.pone.0186216.
12. Yu HN, Zhu J, Pan WS, Shen SR, Shan WG, Das UN. Effects of fish oil with a high content of n-3 polyunsaturated fatty acids on mouse gut microbiota. *Arch Med Res*. 2014;45(3):195–202. doi:10.1016/j.arcmed.2014.03.008.
13. Lam YY, Ha CW, Hoffmann JM, Oscarsson J, Dinudom A, Mather TJ, et al. Effects of dietary fat profile on gut permeability and microbiota and their relationships with metabolic changes in mice. *Obesity (Silver Spring)*. 2015;23(7):1429–39. doi:10.1002/oby.21122.
14. Li H, Zhu Y, Zhao F, Song S, Li Y, Xu X, et al. Fish oil, lard and soybean oil differentially shape gut microbiota of middle-aged rats. *Sci Rep*. 2017;7(1):826. doi:10.1038/s41598-017-00969-0.
15. Simopoulos AP. The Importance of the Omega-6/Omega-3 Fatty Acid Ratio in Cardiovascular Disease and Other Chronic Diseases. *Experimental Biology Medicine*. 2008;233(6):674–88. doi:10.3181/0711-MR-311. doi.
16. Reeves PG, Nielsen FH, Fahey GC Jr. AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. In.: Oxford University Press; 1993.
17. Swidsinski A, Weber J, Loening-Baucke V, Hale LP, Lochs H. Spatial organization and composition of the mucosal flora in patients with inflammatory bowel disease. *J Clin Microbiol*. 2005;43(7):3380–9. doi:10.1128/JCM.43.7.3380-3389.2005.
18. Jandhyala SM, Talukdar R, Subramanyam C, Vuyyuru H, Sasikala M, Nageshwar Reddy D. Role of the normal gut microbiota. *World J Gastroenterol*. 2015;21(29):8787–803. doi:10.3748/wjg.v21.i29.8787.
19. Uronis JM, Arthur JC, Keku T, Fodor A, Carroll IM, Cruz ML, et al. Gut microbial diversity is reduced by the probiotic VSL#3 and correlates with decreased TNBS-induced colitis. *Inflamm Bowel Dis*. 2011;17(1):289–97. doi:10.1002/ibd.21366.
20. Sanchez E, Nieto JC, Boullosa A, Vidal S, Sancho FJ, Rossi G, et al. VSL#3 probiotic treatment decreases bacterial translocation in rats with carbon tetrachloride-induced cirrhosis. *Liver Int*. 2015;35(3):735–45. doi:10.1111/liv.12566.
21. Meng XC, Wang YN, Yan PG, Li YH, Wang HY, Qian JM, et al. [Effect of VSL#3 and *S.Boulardii* on intestinal microbiota in mice with acute colitis]. *Zhonghua Yi Xue Za Zhi*. 2019;99(22):1735–42. doi:10.3760/cma.j.issn.0376-2491.2019.22.011.

22. Liu XJ, Yu R, Zou KF. Probiotic Mixture VSL#3 Alleviates Dextran Sulfate Sodium-induced Colitis in Mice by Downregulating T Follicular Helper Cells. *Curr Med Sci.* 2019;39(3):371–8. doi:10.1007/s11596-019-2045-z.
23. Kuhbacher T, Ott SJ, Helwig U, Mimura T, Rizzello F, Kleessen B, et al. Bacterial and fungal microbiota in relation to probiotic therapy (VSL#3) in pouchitis. *Gut.* 2006;55(6):833–41. doi:10.1136/gut.2005.078303.
24. Arthur JC, Gharaibeh RZ, Uronis JM, Perez-Chanona E, Sha W, Tomkovich S, et al. VSL#3 probiotic modifies mucosal microbial composition but does not reduce colitis-associated colorectal cancer. *Sci Rep.* 2013;3:2868. doi:10.1038/srep02868.
25. Tropini C, Earle KA, Huang KC, Sonnenburg JL. The gut microbiome: connecting spatial organization to function. *Cell Host Microbe.* 2017;21(4):433–42.

Figures

Figure 1

Flow chart of the experiment. Eighteen male C57/BL6N mice fed a regular diet and purified water after birth were randomly assigned to 3 groups: the control (C), fish oil (F) and VSL#3 (V) groups. Mice in the control group were provided the AIN-93M diet and purified water; mice in the fish oil group were provided a fish oil-intensive diet and purified water; and mice in the VSL#3 group were provided the AIN-93M diet and purified water mixed with VSL#3 (108 CFU/ml). Mice were fed with dietary intervention for two weeks, and the feces of each mouse were collected before and after the intervention.

Figure 2

Influence of AIN-93M and fish oil on the gut microbiota of mice. (A) Principal component analysis. Both the AIN-93M and fish oil-intensive diets could alter the gut microbiota, but in different directions. (B) Shannon index with Wilcoxon signed-rank test. The CA group had a significantly lower Shannon index value compared to the other 3 groups, which had similar index values. (C) MetaStat analysis at the phylum level. The FA group had a higher abundance of Firmicutes and a lower abundance Bacteroidetes comparing compared to the other 3 groups, which had similar abundances. (D) MetaStat analysis at the genus level. The CA group had a significantly higher abundance of Bacteroides compared to that observed in the other three groups. (E) Cladogram of LefSe analysis. Firmicutes played a dominant role after the fish oil-intensive diet intervention, while Bacteroidetes and Proteobacteria played a dominant role after AIN-93M intervention.

Figure 3

Influence of AIN-93M and VSL#3 on the gut microbiota of mice. (A) Principal component analysis. There were some differences in microbial structure before intervention. Both AIN-93M alone and AIN-93M plus VSL#3 treatments altered the microbiota structure of mice in a similar direction. (B) Shannon index with Wilcoxon signed-rank test. Both the AIN-93M alone and AIN-93M plus VSL#3 treatments decreased the Shannon index value of microbiota. (C) MetaStat analysis at the phylum level. AIN-93M plus VSL#3 dramatically decreased the abundance of Firmicutes. (D) MetaStat analysis at the genus level. The AIN-93M alone and AIN-93M plus VSL#3 treatments showed similar impacts on the microbiota at the genus level. (E) Cladogram of the LDA effect size (LEfSe) analysis. Bacteroidetes was dominant in the CA group, Firmicutes was dominant in the VB group and Proteobacteria was dominant in the VA group.