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Soybean oil induces neuroinflammatory response through brain-gut axis under high-fat diet

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ABSTRACT

Neuroinflammation is considered the principal pathogenic mechanism underlying neurodegenerative diseases, and the incidence of brain disorders is closely linked to dietary fat consumption and intestinal health. To investigate this relationship, 60 8-week-old C57BL/6J mice were subjected to a 20-week dietary intervention, wherein they were fed lard and soybean oil, each at 15% and 35% fat energy. At a dietary fat energy level of 35%, inflammation was observed in both the soybean oil and lard groups. Nevertheless, inflammation was more pronounced in the mice that were administered soybean oil. The process by which nerve cell structure is compromised, inflammatory factors are upregulated, brain antioxidant capacity is diminished, and the TLR4/MyD88/NF-κB p65 inflammatory pathway is activated resulting in damage to the brain-gut barrier. This, in turn, leads to a reduction in the abundance of *Akkermansia* and *unclassified_f_Lachnospiraceae*, as well as an increase in *Dubosiella* abundance, ultimately resulting in brain inflammation and damage. These results suggested that soybean oil induces more severe neuroinflammation compared to lard. Our study demonstrated that, at a dietary fat energy level of 35%, compared to soybean oil, lard could be the healthier option, the outcomes would help provide a reference basis for the selection of residents' daily dietary oil.

1. Introduction

Diet is closely related to human health and plays an important role in influencing factors of brain function, and changes in dietary composition are considered risk factors for developing brain diseases,[1–3] especially lipids play a structural and functional role in neurons.

Oil is an essential nutrient and an important component of the human body. For a considerable duration, vegetable oils have been touted as healthier alternatives to lard.[4] However, many earlier lard-related studies utilized 60% fat energy supply to demonstrate that lard is harmful to health,[5] which is very dissimilar from the diet of Asian nations, including China.[6] Humans have consumed lard for thousands of years.[7] As a traditional dietary oil of Chinese residents, lard was widely used in the treatment of diseases recorded in many classical works because of its diuresis, blood clearance, activating blood circulation, and detoxification.[8]

Soybean oil, the most widely consumed edible oil worldwide,[9] accounting for approximately 30%.[10] The predominant fatty acid in soybean oil is linoleic acid (LA), an essential n-6 polyunsaturated fatty acid (PUFA), which accounts for approximately 49–53% of the total fat content.[4,11] Because soybean oil is cheap and advertised as healthy, the increase in the intake of soybean oil has inadvertently tripled the content of n-6 in the diet in the past few decades,[12] yet very little data are available on the exact risk of daily intake on human health. Studies have shown that the replacement of saturated fatty acids (SFA) with soybean oil improves circulating lipid and lipoprotein levels, without increasing

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inflammatory markers.[13–15] However, some studies have also found that excessive consumption of LA-rich oils will increase the brain's vulnerability to inflammation, which may play a role through its oxidative metabolites.[16]

Neuroinflammation is the innate immune response of the central nervous system and is the main pathogenesis of a variety of neurological diseases.[17–19] The main manifestation of neuroinflammation is the activation of glial cells[20] and the increased release of proinflammatory factors and chemokines, leading to the inflammatory response. Microglia are resident macrophages in the central nervous system, close to the neural structure, and are closely related to brain homeostasis, playing a vital role in innate immune response and tissue injury repair.[21] Neurotoxicity mediated by the overactivation of microglia is an important pathological mechanism of neurodegenerative diseases mediated by neuroinflammatory reactions.[22] However, the difference in the role of lard and soybean oil in neuroinflammation caused by a high-fat diet is not yet known.

According to the recommendations of the World Health Organization, dietary fat should provide 15%–30% of daily energy intake.[23] With the improvement in people's living standards, the daily intake of fat exceeds 40% of total energy.[24] PUFA and SFA in the brain come mainly from the diet. However, long-term eating habits can affect the diversity of intestinal bacteria and cause changes in hypothalamic energy homeostasis.[25] Recent research shows that diet can greatly affect the composition of gut microbiota, thus significantly affecting the health of the host.[26] Dietary changes account for 57% of gut microbiota changes, whereas genes account for only 12%.[27] Therefore, the occurrence of brain diseases is closely related to dietary fat intake and intestinal health.

Our study focused on lard and soybean oil, the most consumed vegetable oil at present, to investigate the effects of low- (15% fat energy supply, energy supply level of mice control diet fat) and high-fat (35% fat energy supply, the average energy supply level of dietary fat in Chinese residents) energy supplies on the growth performance of mice, damage to brain tissue, expression of genes and proteins related to brain inflammatory factors, and gut microbiota to explore the relationship between the two oils and neuroinflammation and provide a reference basis for the selection of residents' daily dietary oil.

2. Materials and methods

2.1. Experimental procedure

Sixty 8-week-old male C57BL/6J mice (purchased from Hunan SJA Laboratory Animal Co., Ltd (Hunan, China) (SCXK (Xiang) 2019-0004)) were randomly divided into four groups, and all experimental animals were kept in a special pathogen free environment and fed a regular diet for one week. Lard and soybean oil were used as experimental oils, and the mice were fed with purified diets containing 15% (soybean oil, S15; lard, L15), and 35% (soybean oil, S35; and lard, L35) fat energy, respectively, for 20 weeks. Growth performance data were collected throughout the experiment. The mice were housed at a humidity of 50%–60%, temperature of 24–26 °C, and 12 h light/12 h dark cycle. At the end of 20 weeks, all mice were fasted without water for 6 h and euthanized. The brains and intestines were collected and frozen. All experimental procedures were approved by the Animal Welfare and Use Guidelines of China and the Animal Welfare Committee of Hunan Agricultural University (No.43321820). Purified diets diet formulas are shown in Tables S1–S2.

Brain index = Brian weight (g) / body weight (g)*100 %

2.2. Histological evaluation of the brain

The histopathological detection of brain was performed as described previously.[28] The histopathological changes of brain were observed using hematoxylin and eosin (H&E) staining. The protein expression of Iba-1 and NF-κB p65 in the brain tissue of mice in each group was detected by immunohistochemistry.

2.3. Measurement of antioxidative capacity

The activities of superoxide dismutase (SOD), malondialdehyde (MDA), catalase (CAT), and glutathione peroxidase (GSH-Px) were determined using assay kits purchased from Nanjing Jiancheng Bioengineering Institute Co., Ltd, Nanjing, China.

2.4. Determination of inducible nitric oxide synthase (iNOS) in brain tissue

The expression of iNOS in the brain was determined by enzyme-linked immunosorbent assay (ELISA), according to the manufacturer's guidelines (MultiSciences (Lianke) Biotechnology Co., Ltd, Hangzhou, China).

2.5. Real-time quantitative PCR (RT-qPCR)

Total RNA was extracted separately from the brain using TRIzol (Hunan Akerui Bioengineering Co., Ltd., Changsha, China), and 1 μg of RNA was reverse-transcribed using a PrimeScript RT reagent kit with gDNA Eraser (Hunan Akerui Bioengineering Co., Ltd., Changsha, China). According to the mRNA sequences of mouse *IL-6, IL-10, IL-1β, TNFα, Gfap, Dcx, Tau, Ampk, Bdnf, Myd88, NF-κB, Tlr4, iNOS,* and *β-actin* in GenBank, quantitative PCR (qPCR) was performed with a real-time PCR system (StepOne, Foster, CA, USA). RT-qPCR analysis was performed as described previously.[11] The primers used in this study are listed in Table S3.

2.6. Western blotting analysis

The protein expression levels of ZO-1, CLAUDIN-1, OCCLUDIN, TLR4, MyD88, NF-κB P65, and p-NF-κB P65 were detected by western blotting in the four groups. Western blot analysis was performed as described previously.[8] Equal amounts of protein (50 μg) from each colon sample were electrophoresed on SDS polyacrylamide gels with pre-stained protein markers. The results were quantitatively analyzed using ImageJ software.

2.7. 16S rRNA gut microbiota analysis

Total microbial DNA was extracted from colonic content samples (n = 5) using HiPure Stool DNA kits (Magen, Shanghai, China), according to the manufacturer's instructions. The DNA concentration was measured using a NanoDrop2000 instrument (NanoDrop Technologies Inc., USA). The V3-V4 region of the 16S rRNA gene was amplified using the universal primer 338F (5′-ACTCCTACGGGAGGCAGCAG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′). The PCR was performed in a total volume of 20 μl. The PCR program included a 3-min incubation at 95 °C, followed by 27 cycles of denaturation at 95 °C for 30 s, and annealing and extension at 55 °C for 30 s and at 72 °C for 45 s. The amplified PCR product was clearly identified using 1.2 % agarose gels. Amplicons were then extracted from the agarose gels and purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) according to the manufacturer's instructions and quantified using Quantus™ Fluorometer (Promega, USA). Purified amplicons were pooled in equimolar amounts and paired-end sequenced on an Illumina MiSeq PE300 platform/NovaSeq PE250 platform (Illumina, Sand Diego, CA, USA) according to the standard protocols. The instructions for the

platform and manufacturer were from a commercial service provider (Majorbio, Shanghai, China).

The quality control of the original sequencing sequences was performed using FASTP software, and splicing was performed using FLASH software: The sequences were subjected to OTU clustering using UPARSE software according to the similarity of ninety-seven percent, and each sequence was annotated by species classification using RDP Classic, and the Silva 16S rRNA database (v138) was compared, with the comparison threshold set at 70%. Through the analysis of OTU, the effects of dietary fats on the gut microbiota of mice were compared by analyzing Alpha diversity, beta diversity, and species composition analysis.

2.8. Statistical analysis

SPSS version 25.0 was used to process the data, and the data were expressed as mean standard error (X±SEM). An independent samples T-test was used to test the data according to the homogeneity of variance. If the variances were not neat, the nonparametric rank sum test was used, and the test type was Mann-Whitney U (M). For all analyses, values

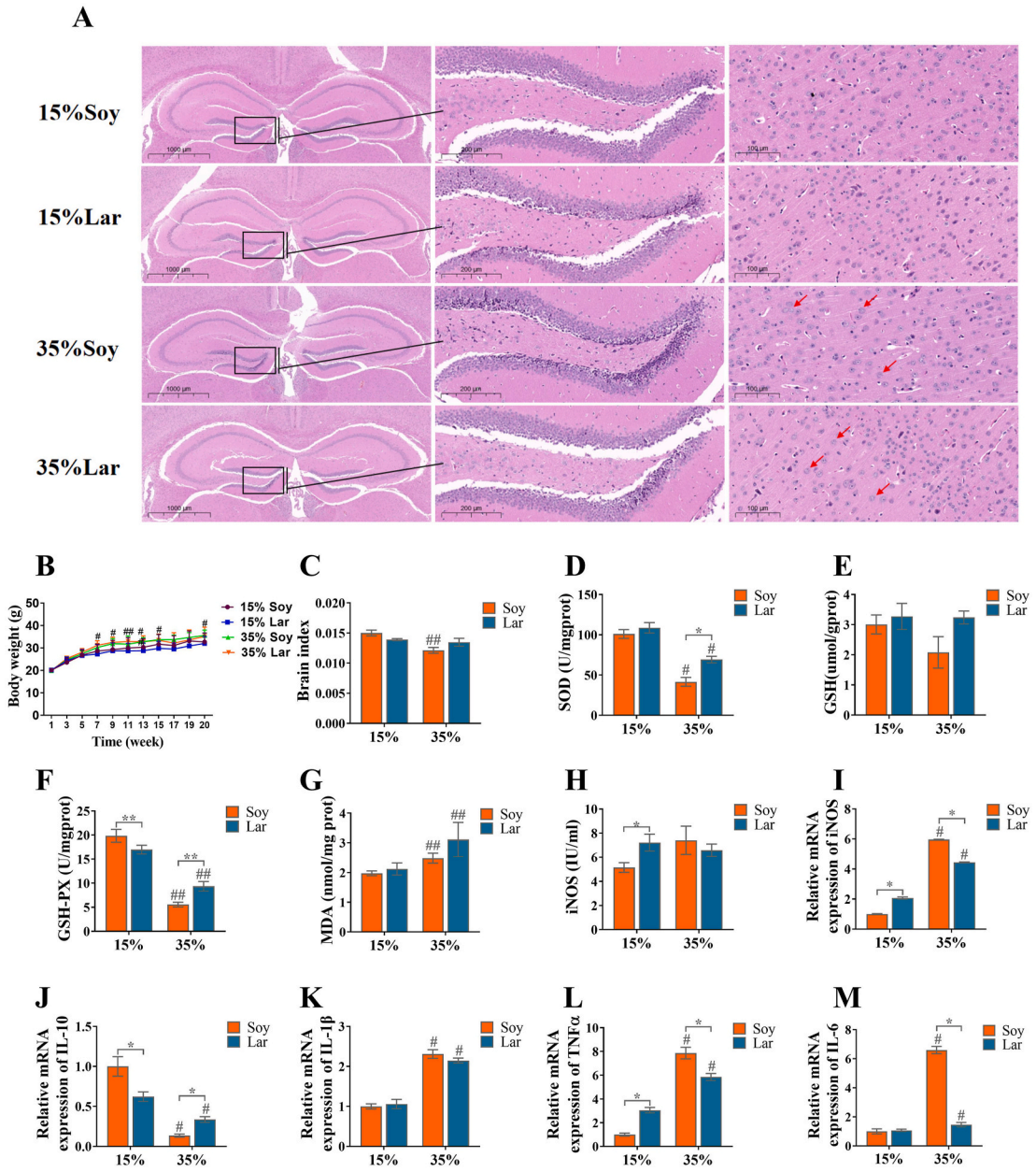

Fig. 1. Expression of antioxidant factors and inflammation genes in mice with different dietary fats (soybean oil and lard) on brain tissue
A: Hematoxylin and eosin (HE) staining of the hippocampus (1 ×); HE staining of the hippocampal dentate gyrus in the brain (5 ×); HE staining of the cerebral cortex (10 ×); B: Bodyweight change curve of mice; C: Brain index; D: Superoxide dismutase (SOD) content; E: Glutathione (GSH) content; F: glutathione peroxidase (GSH-Px) content; G: malondialdehyde (MDA) content; H: iNOS expression; I: Relative mRNA expression of *iNOS*; G: Relative expression of *IL*-10 mRNA; G: Relative expression of *IL-1β* mRNA; K: Relative expression of *TNFα* mRNA; L: Relative expression of *IL*-6 mRNA. * indicates $p < 0.05$ (Soy versus Lars), * * indicates $p < 0.01$ (Soy versus Lard), # indicates $p < 0.05$ (15% versus 35%), # # indicates $p < 0.05$ (15% versus 35%).

of $p < 0.05$ were considered significant, and values of $p < 0.01$ were considered extremely significant. GraphPad Prism 9 software was used to process the graphs.

3. Results

3.1. Effects of dietary fats (soybean oil and lard) on oxidative damage and inflammatory reaction in mice

Hippocampal tissues of mice were H&E stained to more intuitively reflect the effect of dietary fats, as presented in Fig. 1A. With higher fat energy supply levels, the body weight of mice in both the soybean oil and lard groups significantly increased ($p < 0.01$, Fig. 1B). In the mice fed soybean oil, the brain index of the S35 group decreased significantly relative to that of the S15 group ($p < 0.01$, Fig. 1C). At 15% fat energy group, the nerve cells were arranged neatly and round. The structure was intact, the cell membrane and nucleus were clear. The nerve cells at 35% fat energy group were decreased and showed a damaged morphology and a blurred cell structure. With the increase in the energy supply level, oxidative damage occurred in the brains of mice. Likewise, in comparing soybean oil with lard, the expression levels of antioxidant SOD and GSH-Px in L35 were significantly higher than those in S35 ($p < 0.05$, Fig. 1D and F). iNOS is not usually present in the brain of young people, but can be detected in the brain after inflammation, infection, or ischemic injury, as well as in normally aged brains.[29] The expression of iNOS and the relative mRNA expression of *iNOS* in the brain tissue of the L15 group were significantly higher than those in the S15 group ($p < 0.05$, Fig. 1H and I). Similarly, the relative mRNA expression of *iNOS* in the S35 group was significantly higher than that in the L35 group ($p < 0.05$, Fig. 1I). Moreover, with the increase in the energy supply level, the MDA and the relative mRNA expression of *iNOS*, *IL-6*, *IL-1β*, and *TNFα* was significantly increased in both the soybean oil and lard groups ($p < 0.05$ Fig. 1G, I, 1K-M), the relative mRNA expression levels of *IL-10* were significantly decreased ($p < 0.05$, Fig. 1G). In addition, the expression of *IL-10* in the S35 group was significantly lower than that in the L35 group, However, the expression of inflammatory factors in the L35 group was significantly lower than that in the S35 group ($p < 0.05$; Fig. 1L and M).

3.2. Effects of dietary fats (soybean oil and lard) on genes and proteins related to brain injury in mice

As shown in Fig. 2A, compared with the S15 group, the relative mRNA expression levels of *Bdnf* were significantly decreased in the L15 and S35 groups ($p < 0.05$). Among the same levels, at 15% fat energy, the relative mRNA expression levels of *Gfap*, *Dcx*, *Myd88*, *Ampk*, and *NF-κB p65* in the lard group were significantly higher than those in the soybean oil group ($p < 0.05$). The relative mRNA expression levels of *Dcx*, *Tlr4*, and *NF-κ B p65* in the L35 group were significantly lower than those in the S35 group ($p < 0.05$). The relative mRNA expressions of *Gfap*, *Dcx*, *Myd88*, *NF-κB p65*, *Tau,* and *Ampk* at 35% fat energy were significantly higher than those in the 15% fat energy group ($p < 0.05$). In addition, with the increase in energy supply, the expression levels of TLR4, MyD88, and NF-κB p65 in the S35 and L35 groups were significantly higher than those in the S15 and L15 groups ($p < 0.05$, Fig. 2B–F). In addition, the expression levels of TLR4 and MyD88 were higher in the S35 group than in the L35 group ($p < 0.05$, Fig. 2C and F).

Neuroinflammation is closely related to microglial activation.[30] Studies have shown that Iba-1 is specifically expressed in microglia, and its expression is significantly increased during microglial activation.[31] In addition, studies have found that NF-κB p65 can participate in the activation of proinflammatory genes, thereby triggering and amplifying neuroinflammation.[32] At the same level, the relative mRNA expression of *NF-κ B p65* and protein expression of p-NF-κB p65 at 35% fat energy groups was significantly higher than those in the 15% soybean oil and lard groups ($p < 0.05$, Fig. 2A and E). As shown in Fig. 2G-N. The protein expression levels of Iba-1 and NF-κB P65 in L15 were significantly higher than those in S15 ($p < 0.01$, $p < 0.05$), while the protein expression levels of Iba-1 and NF-κB P65 in L35 were significantly lower than those in S35 ($p < 0.01$, $p < 0.05$). With the increase in energy supply, the expression levels of Iba-1 and NF-κB P65 in the S35 and L35 groups were significantly higher than those in the S15 and L15 groups ($p < 0.05$, $p < 0.01$).

3.3. Effects of dietary fats (soybean oil and lard) on brain barrier and intestinal barrier in mice

The blood-brain barrier (BBB) is a tight barrier that is essential for preventing the entry of pathogens and small molecules into the brain.[33] The destruction of the BBB is a potential feature of neuroinflammation leading to neurodegenerative diseases.[34] ZO-1, CLAUDIN-1, and OCCLIDIN are important in maintaining the pores and leak paths of BBB permeability. As shown in Fig. 3, with the increase in the energy supply, the expression levels of ZO-1, CLAUDIN-1, and OCCLIDIN at 35% fat energy group were significantly lower than those in the 15% fat energy group ($p < 0.05$, Fig. 3A–E). In the colon, the expression levels of CLAUDIN-1, OCCLIDIN, and ZO-1 in the S35 group were significantly lower than those in the S15 group ($p < 0.05$, Fig. 3G and H), and the expression levels of CLAUDIN-1 and OCCLIDIN in the S35 group were significantly lower than those in the L35 group ($p < 0.05$, Fig. 3F–J).

3.4. Effects of dietary fats (soybean oil and lard) on the diversity of gut microbiota in mice

We analyzed fecal microbiota using 16SrRNA amplicon high-throughput sequencing. Alpha diversity analysis showed that the Sobs, Chao, and Shannon indices were lower in the S15 group than in the other three groups (Fig. 4A–C). The Venn diagram shows 188 OTUs common to all four groups, with 320, 390, 325, and 312 unique OTUs in the S15, L15, S35, and L35 groups, respectively (Fig. 4D). PCoA showed significantly different gut microbiota among the groups (Fig. 4E, $p = 0.001$ by PERMANOVA). The results of the species hierarchical clustering tree showed obvious differences in the gut microbiota structure between the 15% and 35% fat energy groups. At 15% fat energy, the composition of gut microbiota was similar in two groups, with some overlap between samples; at 35% fat energy, the difference between soybean oil and lard groups was obvious (Fig. 4F).

3.5. Effects of dietary fats (soybean oil and lard) on species composition of gut microbiota in mice

The species composition and differences in gut microbiota in each group at the phylum and genus levels were analyzed, as shown in Fig. 5. At the phylum level, the top five species in each group were *Firmicutes*, *Actinobacteriota*, *Verrucomicrobiota*, *Desulfobacterota,* and *Bacteroidota*. The analysis of species differences among different groups is shown in Fig. 5B–G. Although the abundance of *Firmicutes* in each group was the highest, there were no significant differences between the groups ($p > 0.05$, Fig. 5C). The abundance of *Verrucomicrobiota* in the L35 group was significantly higher than that in the S35 group ($p < 0.01$, Fig. 5E). Among the different levels of the same oil, *Actinobacteriota* was significantly higher in the L35 group than in the L15 group ($p < 0.05$. Fig. 5D), and *Desulfobacterota* was significantly lower than in the L15 group ($p < 0.05$, Fig. 5E). At the genus level, six species with significant differences between the groups were *Faecalibaculum*, *norank_f_Eubacteriaceae*, *Dubosiella*, *Lactobacillus*, and *Akkermansia* (Fig. I-N). The abundance of *Faecalibaculum, unclassified_f_Lachnospiraceae*, and *Akkermansia* was significantly higher in the L35 group than in the S35 group ($p < 0.05$, $p < 0.01$, Fig. 5I, M, and 5 N), whereas *Dubosiella* was significantly lower in the L35 than in the S35 group ($p < 0.05$, Fig. 5K). Among the different levels of the same oil, *Dubosiella,* and *Lactobacillus* in the S35 group were significantly higher than in the S15 group ($p < 0.05$, Fig. 5K and L),

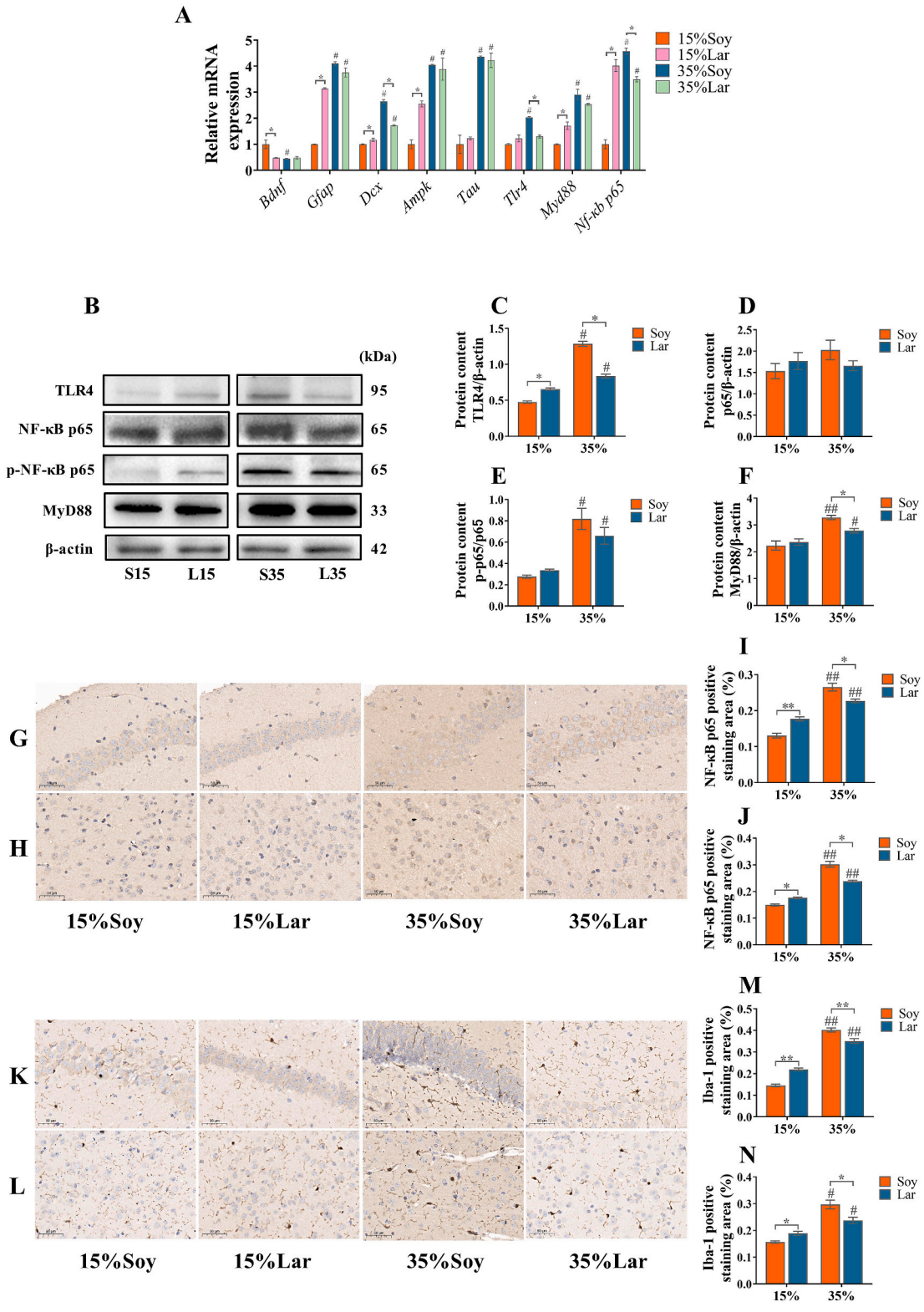

Fig. 2. Expression of mRNA and protein related to neuroinflammatory response in mice
A: Relative mRNA expression; B: Western blot protein bands in the brain; C: Protein content of TLR4 in the brain; D: Protein content of NF-κB p65 in the brain; E: Protein content of p-NF-κB p65 in the brain; F: Protein content of MyD88 in the brain; G and H: Expression of NF-κB p65 protein in the hippocampus and cerebral cortex detected by immunohistochemistry (20 ×); K and L: Expression of Iba-1 protein in the hippocampus and cerebral cortex detected by immunohistochemistry (20 ×); I and M: statistics of hippocampal immunohistochemical results; J and N: statistics of cerebral cortex immunohistochemical results. * indicates $p < 0.05$ (Soy versus Lard), * * indicates $p < 0.01$ (Soy versus Lard), # indicates $p < 0.05$ (15% versus 35%), # # indicates $p < 0.05$ (15% versus 35%).

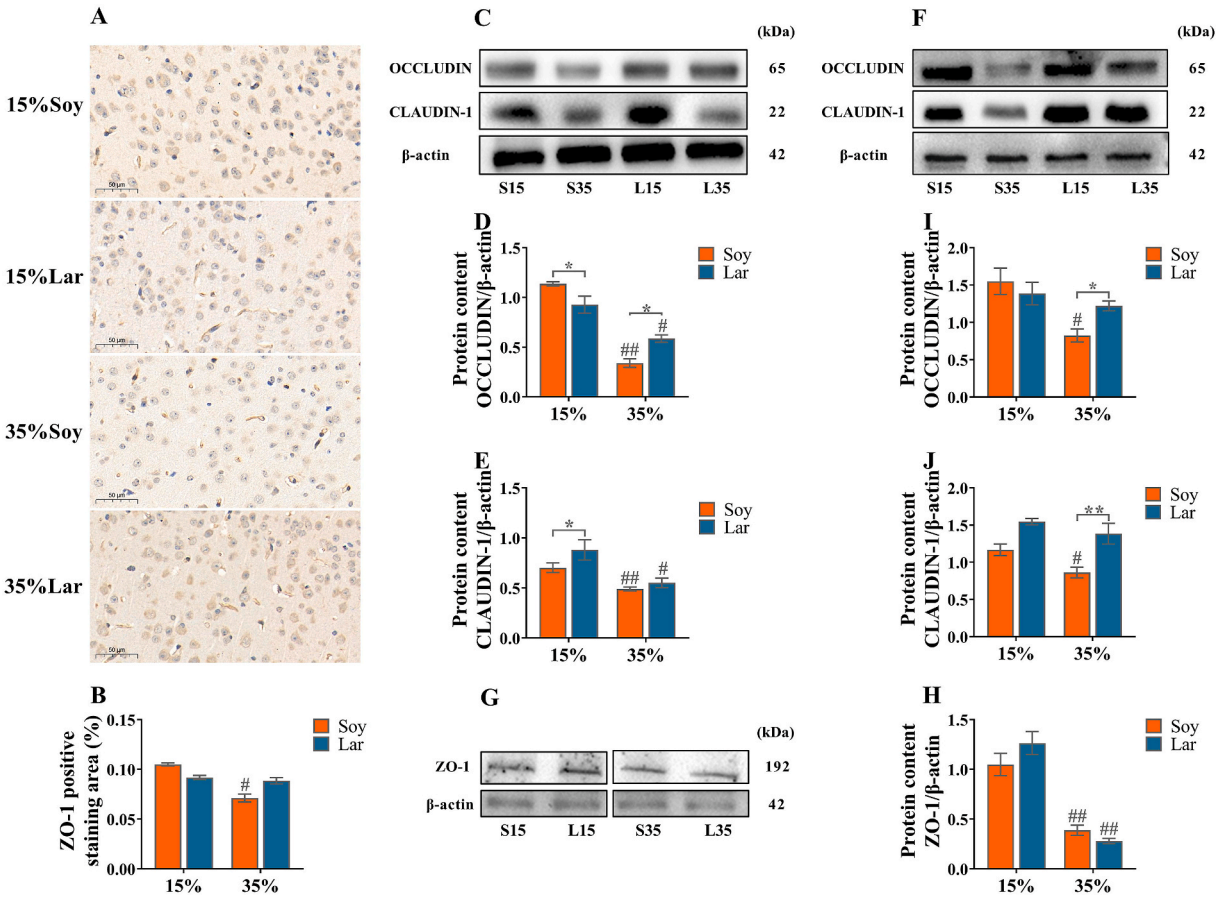

Fig. 3. Expression of mRNA and protein related to the brain barrier and intestinal barrier in mice
A: The expression of ZO-1 protein in the cerebral cortex was detected by immunohistochemistry (20 ×); B: statistics of cerebral cortex immunohistochemical results; C: western blot protein bands of the brain; D: Protein content of OCCLUDIN in the brain; E: Protein content of CLAUDIN-1 in the brain; F–G: western blot protein bands of the colon; H: Protein content of ZO-1 in the intestine; I: Protein content of OCCLUDIN in the intestine; J: Protein content of CLAUDIN-1 in the intestine. * indicates $p < 0.05$ (Soy versus Lard), * * indicates $p < 0.01$ (Soy versus Lard), # indicates $p < 0.05$ (15% versus 35%), # # indicates $p < 0.05$ (15% versus 35%).

whereas *norank_f_Eubacteriaceae* and *Akkermansia* were significantly lower than in the S15 group ($p < 0.05$, Fig. 5J and N). *Lactobacillus*, *unclassified_f_Lachnospiraceae*, and *Bifidobacterium* in the L35 group were significantly higher than in the L15 group ($p < 0.05$, Fig. 5L, M, and 5O).

3.6. Effects of dietary fats (soybean oil and lard) on correlation analysis between species and phenotypic indicators in mice

A multistage species discrimination alnalysis based on linear discriminant analysis with effect size measurement revealed that the abundance of *norank_f_Eubacteriaceae* was highest in the S15 group and that of *norank_f_Lachnospiraceae* was highest in the L15 group, whereas that of *Dubosiella* was highest in the S35 group and that of *unclassified_f_ Lachnospiraceae* was highest in the L35 group (Fig. 6A and B). The BugBase platform was used to analyze and predict phenotypes in the gut microbiota at the genus level. Through the analysis of the different levels of the different fats at the genus level, the top 10 forms-biofilms bacterial species in each group were *Akkermansia, Desulfovibrio, Bifidobacterium, Enterorhabdus, Coriobacteriaceae_UCG-002, Adlercreutzia, Parvibacter, norank_f_Desulfovibrionaceae, Burkholderia-Caballeronia-Paraburkholderia,* and *Escherichia-Shigella* (Fig. 6C). Among them, the S15 group had significantly more *Akkermansia, Bifidobacterium,* and *Coriobacteriaceae_UCG-002* than the L15 and S35 groups ($p < 0.05$, Fig. 6F). The top 10 Gram-negative bacterial species in each group were *Akkermansia, Desulfovibrio, norank_f_Muribaculaceae, Helicobacter, Candidatus_Saccharimonas, Rikenella, Bacteroides, norank_f_Desulfovibrionaceae, Alloprevotella,* and *Mucispirillum* (Fig. 6D). The abundance of microbiota in the S35 group was decreased, but there was no significant difference among the groups ($p > 0.05$, Fig. 6G), and the bacteria in S35 were mainly *Akkermansia, Desulfovibrio,* and *norank_f_Muribaculaceae*. The top 10 stress-tolerant bacterial species in each group were *unclassified_f_Lachnospiraceae, Faecalibaculum, Dubosiella, Romboutsia, Akkermansia, Lactobacillus, Blautia, Bifidobacterium, Enterorhabdus,* and *Coriobacteriaceae_UCG-002* (Fig. 6E). Among them, the L35 group was significantly higher in *unclassified_f_Lachnospiraceae, Dubosiella, Romboutsia*, and *Akkermansia* than the L15 and S35 groups ($p < 0.05$, Fig. 6H).

3.7. Analyze and predict phenotypes of the gut microbiota in mice

We analyzed the relationship between body weight, body weight gain, brain weight, brain index, inflammatory factor, TLR4/MyD88/NF-κB p65 inflammatory pathway, and gut microbial diversity through correlation analysis, and the species composition and difference in gut microbiota; the results are shown in Fig. 7. Distance-based redundancy analysis was conducted on the phenotypic indicators and samples at the OTU level. The results are shown in Fig. 7A and B. Samples from the level 35% group (especially S35) were positively correlated with body weight, body weight gain, *IL-6*, *IL-1β*, *TNF-α*, and iNOS, but negatively correlated with brain weight, brain index, and *IL-10*. In contrast, samples from the S15 and L15 groups positively correlated with brain weight, brain index, and *IL-10*, but negatively correlated with body weight, body weight gain, *IL-6*, *IL-1β*, *TNF-α*, and *iNOS*. We analyzed the Spearman correlation between the top five species at the phylum level

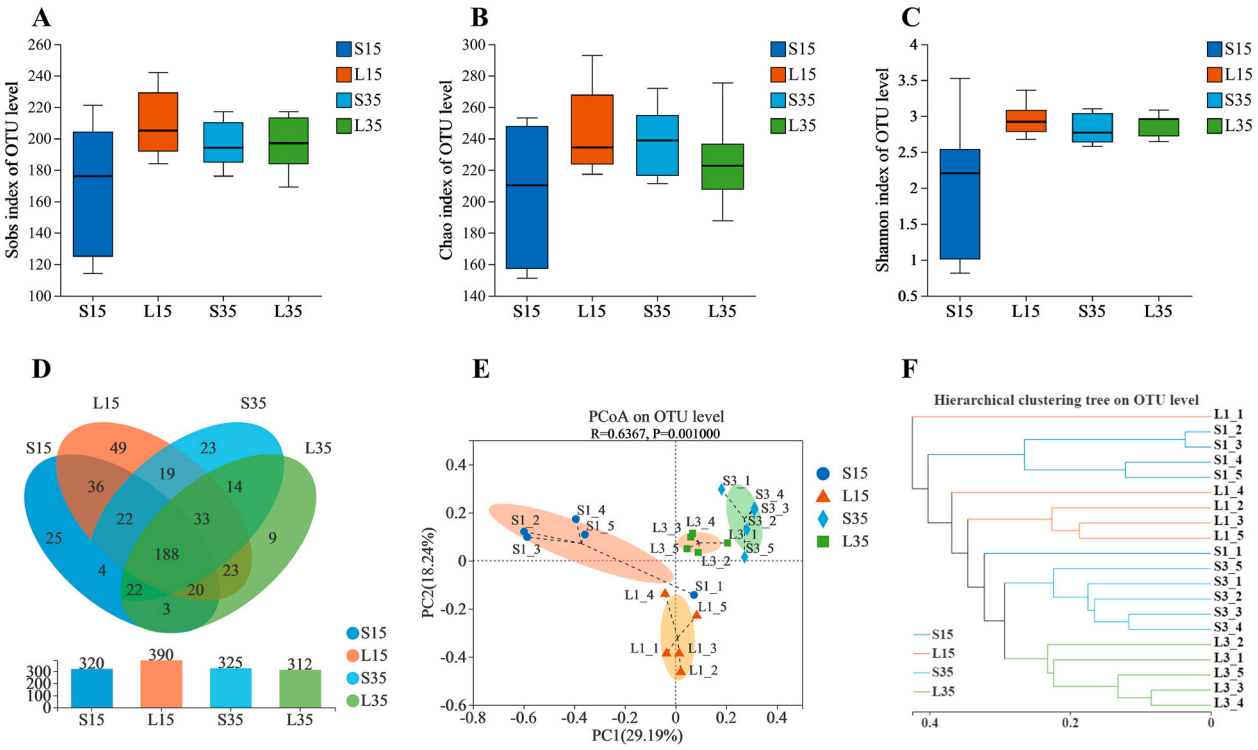

Fig. 4. Species annotation and evaluation of gut microbiota
A: Sobs index; B: Chao index curve; C: Shannon index; D: Venn diagram of OTU level; E: principal coordinates analysis; F: Hierarchical clustering analysis of species.

and the phenotypic indexes in Fig. 7C. *Verrucomicrobiota* was positively correlated with brain weight ($p < 0.01$), while *Actinobacteriota* was negatively correlated with brain index ($p < 0.01$), and positively correlated with body weight and body weight gain ($p < 0.01$). The results of the correlation analysis between genus-level species and phenotypic indicators are shown in Fig. 7D. Considering the large difference in the dietary background among different oil groups, we once again analyzed the Spearman correlation between species and phenotype in the S35 and L35 groups. Combining the six species with significant differences between groups at the genus level mentioned in Fig. 5, as shown in Fig. 7E and F, we found a significant positive correlation with brain weight in *Akkermansia* ($p < 0.01$). Moreover, the genus was negatively correlated with *IL-6, TNF-α*, and the TLR4/MyD88/NF-κB p65 inflammatory pathway ($p < 0.05$, $p < 0.001$). *Dubosiella* was negatively correlated with brain index and *IL-10* ($p < 0.05$) and generally positively correlated with body weight, body weight gain, *iNOS*, *IL-6*, and *Tlr4* ($p < 0.05$). In addition, *Faecalibaculum* was negatively correlated with *iNOS* and TLR4 ($p < 0.05$, $p < 0.01$), and *unclassified_f_Lachnospiraceae* was negatively correlated with *iNOS, TNF-α, Dcx*, and the TLR4/MyD88/NF-κB p65 inflammatory pathway ($p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively).

4. Discussion

Adequate dietary intake and nutritional status have important effects on brain function and brain health, such as cognitive processes, emotion, behavior, neuroendocrine function, and synaptic plasticity.[35] Lipids play structural and functional roles in neurons.[36] However, current studies have focused on modeling with a high SFA diet with an energy supply level of 40%–60% to explore the damaging effects on the brain. Based on the current situation of dietary fat intake of approximately 32.9% fat in Chinese residents, our study compared mice fed with 15% and 35% fats energy for 20w, and found a significant difference in body weight with the increase in the energy level, and there was a significant difference in brain index between the soybean oil group. But no significant difference in the body weight of mice fed different oils at the same energy level. The results indicated that high-fat consumption of only lard and only soybean oil would both lead to weight gain under high-fat diet, but their effects on the brain might be different.

First, we consider the oxidative damage. Most studies indicate that high-fat diet can promote oxidative stress.[37,38] Oxidative stress is known to be linked to a variety of diseases and is involved in the progress of several neurodegenerative disorders. Oxidative stress in the brain can cause brain tissue damage.[39] The results of the SOD, GSH, and MDA antioxidant assays showed no significant difference between the S15 and L15 groups. Under the high-fat (35%) conditions, the expression levels of SOD and GSH-Px in the lard group were significantly higher than those in the soybean oil group. These results indicate that soybean oil causes more oxidative damage to the brain than lard in a long-term high-fat diet. In addition, several studies reported that high body adiposity may induce the production of ROS, accompanied by trigger proinflammatory signaling and activate NF-κB transcriptional factor, and thus inducing NF-κB dependent proinflammatory molecules, such as interferon-γ (IFN-γ), TNF-α, and inducible nitric oxide synthase (iNOS).[40,41] Interestingly, our results showed significantly increased expression of glial hyperplasia markers GFAP, Iba-1, proinflammatory mediators (NF-κB, iNOS, IL-6 and TNFα), and inflammatory pathway-related proteins (TLR4, MyD88, and p-NF-κB p65) in the L15 group compared with those in the S15 group. However, under the high-fat (35%) conditions, the expression in the S35 group was significantly higher than that in the L35 group. Soybean oil was a vegetable oil that is rich in omega-6 PUFA. It was thought that a high dietary omega-6: omega-3 ratio led to a proinflammatory state.[42] Besides, it was commonly believed that increasing dietary intake of the omega-6 fatty acids or its precursor LA will increase inflammation.[43] Inflammation was thought to result from the proinflammatory potential of arachidonic acid (AA), a metabolic product of LA. Clinical results have shown that excess dietary LA increases the brain's vulnerability to inflammation, possibly through its oxidative metabolites.[44,45] One study reported that a high maternal breast milk LA percent composition (>9.7% of fatty

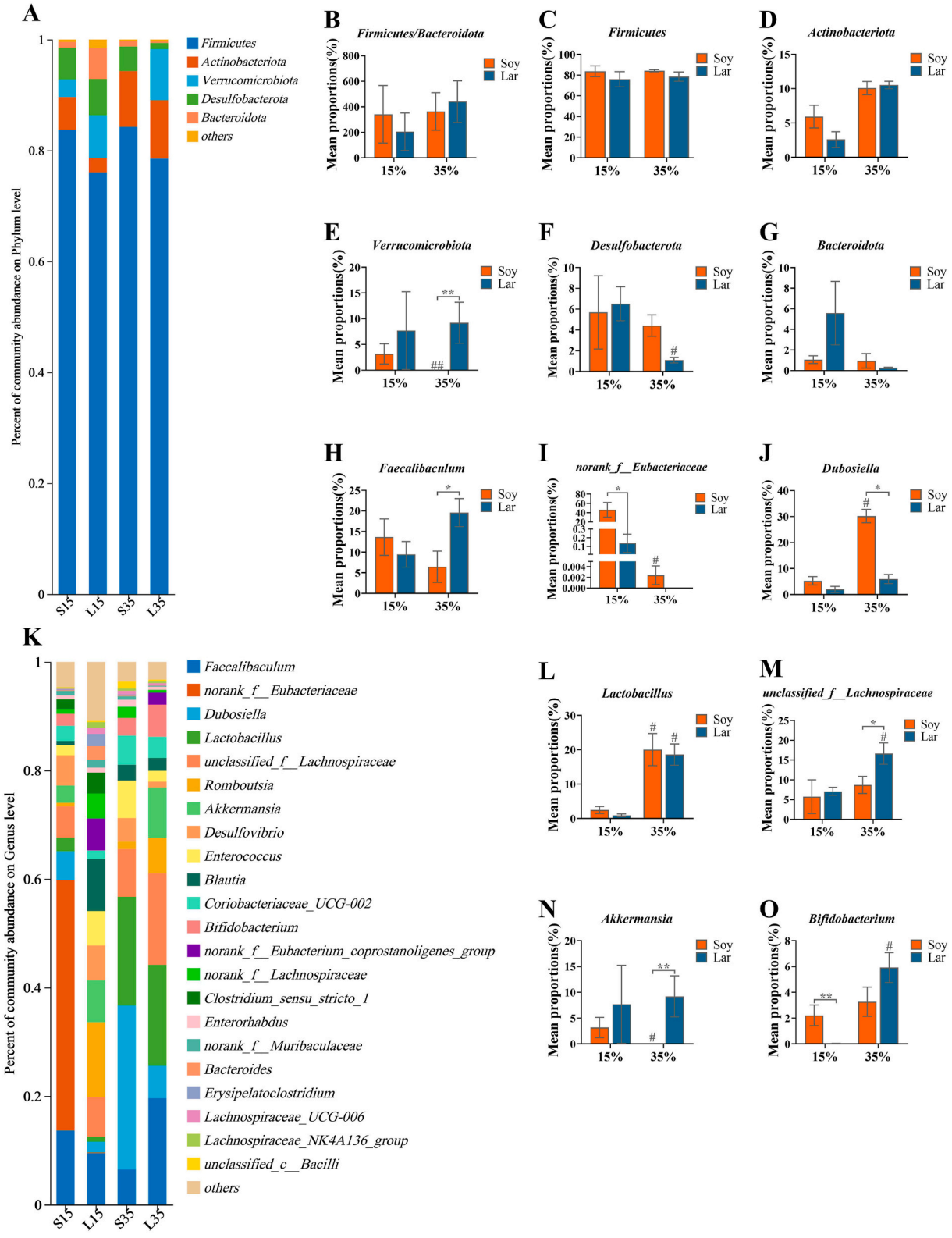

Fig. 5. Species composition and difference analysis at phylum and genus level
A: Species composition at the phylum level; B–G: Difference analysis at the phylum level. K: Species composition at genus level; H-O: Difference analysis at genus level. For all groups, n = 5. * indicates $p < 0.05$ (Soy versus Lard), * * indicates $p < 0.01$ (Soy versus Lard), # indicates $p < 0.05$ (15% versus 35%), # # indicates $p < 0.05$ (15% versus 35%).

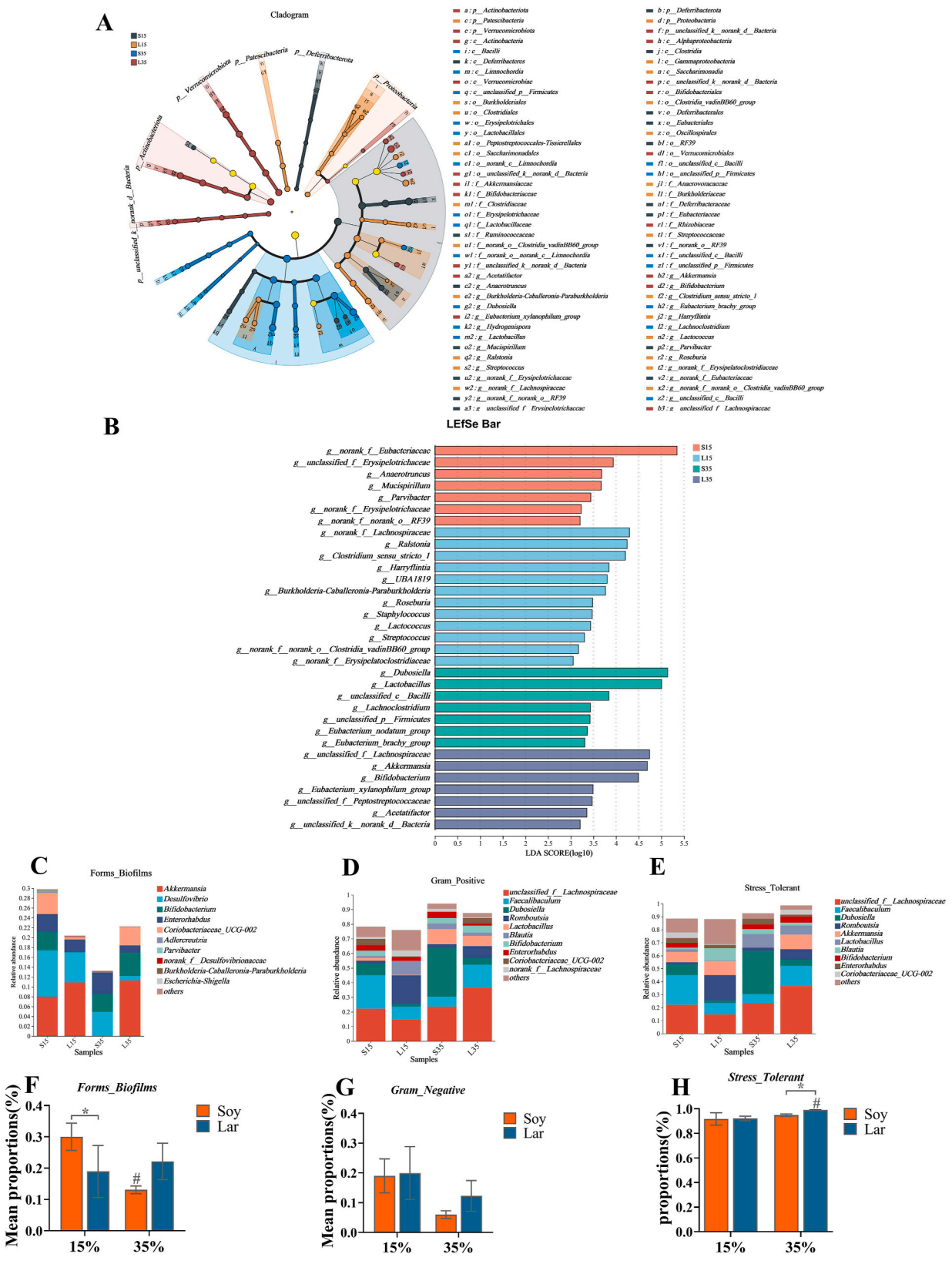

Fig. 6. Species contribution and differences in the abundance of forms-biofilms, Gram-negative, and stress-tolerant at the genus level
A and B: Linear discriminant analysis with effect size measurement for estimating multi-level species differences; C and F: Species contribution of forms-biofilms bacteria and differences in their abundance at the genus level; D and G: Species contribution of Gram-negative bacteria and differences in their abundance at the genus level; E and I: Species contribution of stress-tolerant bacteria and differences in their abundance at the genus level. For all groups, n = 5. * indicates $p < 0.05$ (Soy versus Lard); # indicates $p < 0.05$ (15% versus 35%).

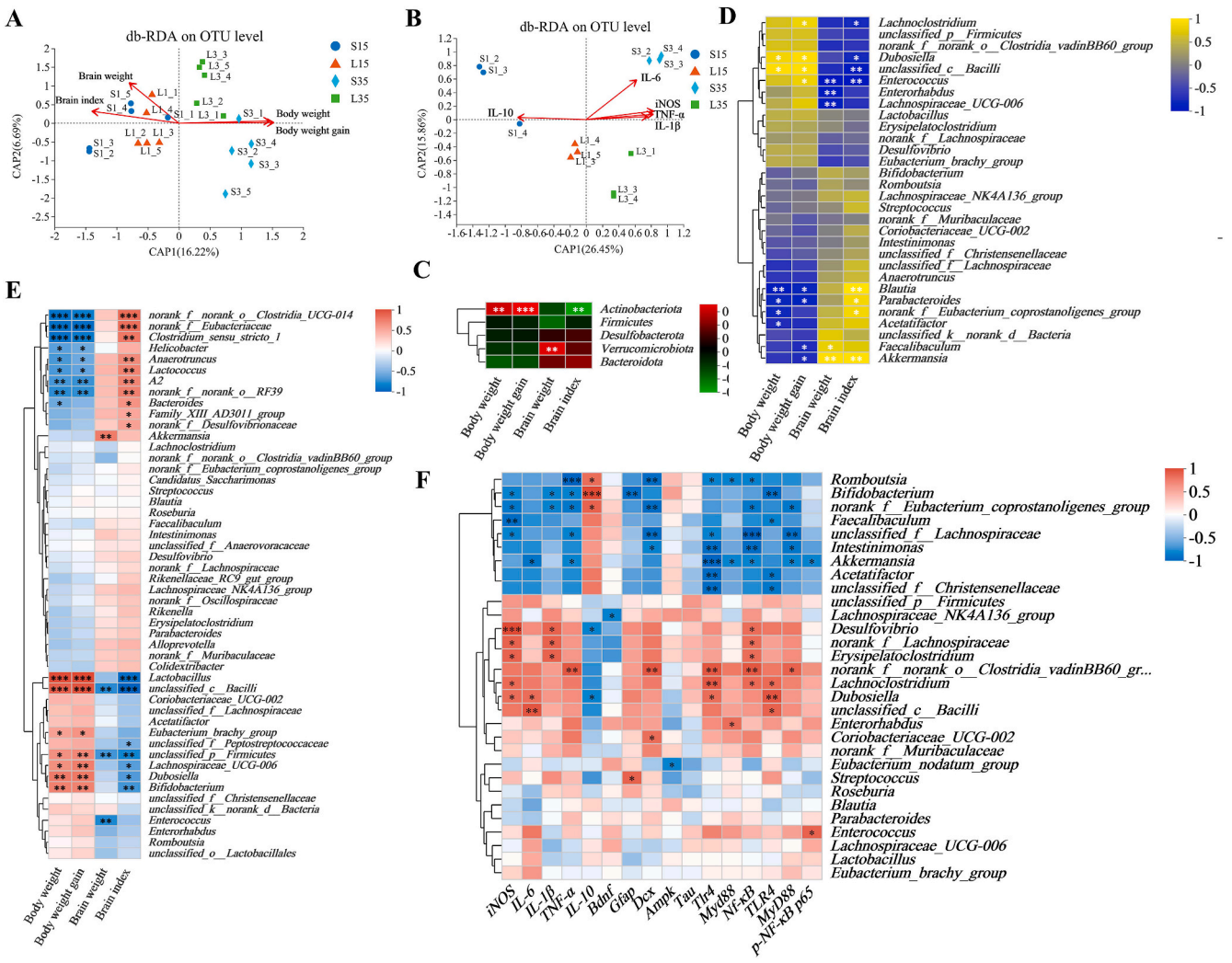

Fig. 7. Correlation analysis between species and phenotypes
A and B: Distance-based redundancy analysis at OTU level; C: Spearman correlation analysis at phylum level among all samples; D: Spearman correlation analysis at genus level among all samples; D and E: Spearman correlation analysis at genus level among 35% groups. Fig. A, C-E, n = 5; Fig. B and F, n = 3. * indicates $p < 0.05$ (Soy versus Lard), * * indicates $p < 0.01$ (Soy versus Lard), *** indicates $p < 0.001$ (Soy versus Lard).

acids) was associated with reduced motor and cognitive scores in 2- to 3-year-old infants.[46] Many of pre-clinical and clinical studies eliminated previous hypothesis that LA was a benign fatty acid in the brain. On the contrary, when present in excess and chronically, it promotes neuro-inflammation in rats[16] and was linked to abnormal neurodevelopment in humans.[47] Therefore, under the high-fat (35%) conditions, with the cumulative increase of LA content, it might be the reason why the oxidative damage and neuroinflammation of soybean oil group are more serious than that of lard group.

The neurotrophic factor BDNF is a marker related to the integrity of the hippocampus.[48] It plays a key role in the survival, maintenance, and growth of many types of neurons.[49,50] However, several studies have shown that rats fed a diet rich in SFA and refined carbohydrates for consecutive months had reduced BDNF levels in the hippocampus.[51,52] Under the 15% fat energy condition, the relative mRNA expression of *Bdnf* levels of SFA-rich lard was significantly lower than that of soybean oil, whereas under high-level conditions, BDNF levels were decreased in both the soybean oil and lard groups. This might be the reason why the lard group was inferior to the soybean oil group in the low-fat (15%) condition.

Interestingly, intestinal diseases often appear in patients with neurodevelopmental disorders.[53,54] In our results, with the increase in energy supply level, the expression of inflammatory factors in the brain increased significantly, whereas the expression of intestinal barrier function-related proteins ZO-1, CLAUDIN, and OCCLIDIN decreased continuously. In addition, evidence has shown that dietary components significantly affect the gut microbiota.[55] Dietary fat is one of the main energy-supplying substances, and its intake level and type cause changes in the structure of the gut microbiota.[56] As our results show, the forms-biofilms were significantly lower in the S35 group than in the S15 group, and the stress-tolerant bacteria were significantly lower in S35 than in the L35 group. Studies have shown that bacterial biofilms are communities of microorganisms attached to a surface that provide additional protection against environmental, host, and antimicrobial assaults.[57] Research indicates that increasing the stress-tolerant condition would help hosts profit from having an adapted microbiome.[58] Therefore, it is possible that the S35 group had a damaged intestinal barrier. In our results, the PCoA analysis results showed that the gut microbiota structures of mice in four different groups were significantly different, and the hierarchical clustering analysis of species showed that the difference between the lard and the soybean oil groups at 35% fat energy was greater than that at 15% fat energy. A large body of evidence has shown a significantly reduced abundance of *Akkermansia* in neurological diseases such as Alzheimer's disease and Parkinson's disease in patients or animal models.[59,60] In addition, in mouse models, the abundance of *Dubosiella* was positively correlated with indicators such

as body weight, serum inflammatory factors, and intestinal permeability,[61] and the reduction of *Dubosiella* might mediate protection from metabolic syndrome. In this study, the composition of the dominant species was significantly different between S15 and L15. However, in the L35 group, *Faecalibaculum*, *unclassified_f_Lachnospiraceae,* and *Akkermansia* were significantly higher than those in the S35 group, and *Dubosiella* was significantly lower than that in the S35 group. Among them, phenotypic correlation analysis results from the whole sample showed that *Akkermansia* had a significant positive correlation with brain weight and brain index, and a negative correlation with body weight gain. *Dubosiella* was significantly negatively correlated with brain index and significantly positively correlated with body weight and body weight gain. In phenotypic correlation analysis at 35% fat energy, *Akkermansia* presented a significant positive correlation with brain weight and negatively correlation with *IL-6*, *TNF-α*, and the TLR4/MyD88/NF-κB p65 inflammatory pathway. *Dubosiella* was positively correlated with body weight, body weight gain, *iNOS*, *IL-6*, and *Tlr4* and negatively correlated with brain index and *IL-10*. These results suggest that lard intake, compared with soybean oil, lard might help slow body weight gain and reduce brain injury by increasing *Akkermansia* abundance and decreasing *Dubosiella* abundance at 35% fat energy. In addition, a study showed that the abundance of *unclassified_f_Lachnospiraceae* in animals was positively correlated with the level of intestinal inflammation.[62] In this study, the abundance of *unclassified_f_Lachnospiraceae* in L35 was significantly higher than that in S35.

Our trial has several limitations. In our results, the *Akkermansia* was not further explored, which will be explored and verified in our later experiments. In addition, the present study focuses on soybean oil and lard, and did not involve vegetable oil rich in monounsaturated acids. In the future, we can further broaden the level and enrich the types of oils, and provide a more comprehensive horizontal comparison for the selection of oils.

5. Conclusion

This study is the first time to explore the effect of isocaloric diets enriched with lard or soybean oil on markers of Neuroinflammation. At a dietary fat energy level of 35%, inflammation was observed in both the soybean oil and lard groups. Nevertheless, inflammation was more pronounced in the mice that were administered soybean oil. The process by which nerve cell structure is compromised, inflammatory factors are upregulated, brain antioxidant capacity was diminished, and the TLR4/MyD88/NF-κB p65 inflammatory pathway was activated resulting in damage to the brain-gut barrier. This, in turn, leads to a reduction in the abundance of *Akkermansia* and *unclassified_f_Lachnospiraceae*, as well as an increase in *Dubosiella* abundance, ultimately resulting in brain inflammation and damage. In conclusion, our research suggested that in the pattern of dietary fat levels of contemporary residents, eating lard is less likely to produce inflammation than soybean oil, and provided a reference for our daily dietary fat intake.

Author contributions

Xiangyan Liu: Methodology, Writing – original draft. Ran Tao: Data curation, Formal Analysis, Writing – original draft. Fangrui Guo: Visualization, Writing – original draft. Linyu Zhang: Software, Writing – review & editing. Jianyu Qu: Data curation, Writing – review & editing. Mengyao Li: Investigation, Writing – original draft. Xiaoran Wu: Validation, Writing – original draft. Xianglin Wang: Methodology, Writing – original draft. Yuanyuan Zhu: Methodology, Writing – review & editing. Lixin Wen: Writing – review & editing, Funding acquisition. Ji Wang: Conceptualization, Writing – review & editing.

Declaration of competing interest

The authors declare no competing interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jtcme.2024.02.002.

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