Piper sarmentosum extract alleviates inflammatory responses and improves barrier function in porcine intestinal epithelial (IPEC-J2) cells induced by lipopolysaccharide (LPS)

Dingfa Wang
Tropical Crops Genetic Resources Institute, Chinese Academy of Tropical Agricultural Sciences
Luli Zhou
Tropical Crops Genetic Resources Institute, Chinese Academy of Tropical Agricultural Sciences
zhou Hanlin (zhouhanlin8@163.com)
Tropical Crops Genetic Resources Institute, Chinese Academy of Tropical Agricultural Sciences
https://orcid.org/0000-0002-6990-8792
Guanyu Hou
Tropical Crops Genetic Resources Institute, Chinese Academy of Tropical Agricultural Sciences

Research

Keywords: Piper sarmentosum extract, Inflammatory responses, Barrier function, IPEC-J2 cells, NF-κB, Metabolomics

Posted Date: March 19th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-17917/v1

Abstract

Background: In this study, we investigated the anti-inflammatory effect of *Piper sarmentosum* extract (PSE) in the IPEC-J2 cells induced by lipopolysaccharide (LPS). Meanwhile, we also tested the metabolomics profile of cells treated by LPS and PSE.

Method: IPEC-J2 cells (6×10^5 cell/well) were seeded on 6-well plates, and cells were divided into three treatments (control, LPS treatment and LPS + PSE-NB treatment). Each treatment was conducted in five replicates. After incubation for 24 h, cells in LPS + PSE-NB treatment were treated by media containing PSE-NB at 10 ug/ml (cells in control and LPS treatments were treated by complete medium). Cells were culture for 24 h, and cells in LPS treatment and LPS + PSE-NB treatment then were treated by media contain 1 μg/ml of LPS (cells in control was treated by complete medium) for another 24 h. After treatment, cells were used for gene expression assays, protein expression assays and metabolomics analysis.

Results: We demonstrated that LPS stimulation significantly up-regulated the mRNA expression of IL-1, IL-6 and TNF-α ($P < 0.05$) compared with the control in the IPEC-J2 cells. *Piper sarmentosum* extract with n-butanol (PSE-NB) pre-treatment with 10 ug/mL before LPS stimulation significantly decreased the expression of IL-1, IL-6 and TNF-α compared with LPS treatment ($P < 0.05$). We found that PSE-NB improved the expression of intestinal tight junction proteins (ZO1 and Occludin) and NHE3 that were reduced by LPS stimulation ($P < 0.05$). Moreover, PSE-NB alleviated LPS-induced protein expression of p65 and p-p65 ($P < 0.05$) and inhibited the NF-κB signaling pathway. Metabolic pathway analysis indicated that PSE-NB exert anti-inflammatory activity mainly through affecting tryptophan metabolism. Its metabolic product, melatonin, has anti-inflammatory properties by inhibition of NF-κB activation, which consistent with our results regarding to anti-inflammatory activity of PSE-NB on inflammatory signaling pathway.

Conclusion: These results suggested that PSE-NB might attenuate LPS-induced inflammatory responses in the IPEC-J2 cells by regulating inflammatory NF-κB signaling pathway and intracellular metabolic pattern.

Background

Intestinal epithelial cells (IECs) play important roles in the animal's physical defense, which is the first line of defense against the invasion of various pathogens and antigens in the external environment of gut lumen [1]. Impaired intestinal epithelium tends to be associated with a series of both intestinal and systemic diseases, including intestinal inflammation and diarrhea [2]. Although gut inflammation may not cause the full-blown clinical symptoms in weaned piglets, it could lead to increased mucosal permeability and reduced growth performance [3]. So, it is necessary to prevent gut inflammation and maintain normal barrier function of IECs in animals.

Piper sarmentosum, is a terrestrial herb. This plant is distributed mainly in tropical and subtropical region of Asia. It is used as a folk medicine in certain countries of Southeast Asia for the treatment of various ailments, and the leaves of Piper sarmentosum are also consumed as a popular spice [4, 5]. Piper sarmentosum contains several bioactive compounds, such as alkaloids, amides, pyrones, dihydrochalcones, flavonoids, phenylpropanoids, lignans, and neolignans [6]. Previous reports have elucidated that Piper sarmentosum extract (PSE) have anti-inflammatory properties [7, 8] and have beneficial effects to piglet intestinal health [9]. However, little is known about the anti-inflammatory molecular mechanism of PSE on porcine intestinal epithelial cells during inflammation. Understanding the mechanisms underlying the anti-inflammatory activities of PSE is critical to its effective application in improving gut health and function in swine.

Metabolomics could provide holistic information on endogenous metabolites, and it is an important tool for biological research because of the ability of revealing the unique chemical fingerprints that involved in multiple biochemical processes of living organs [10]. Thus, metabolomics can provide insights to discover novel biomarkers and to understand the mechanistic pathways underlying a pathophysiological state [11].

Thus, in this study, we investigated the anti-inflammatory molecular mechanism of PSE in porcine intestinal epithelial cells. Our results suggest that PSE-NB might attenuate LPS-induced inflammatory responses in the IPEC-J2 cells by regulating inflammatory signaling pathways and intracellular metabolic pattern.

Material And Methods

Materials

The intestinal porcine epithelial cell line (IPEC-J2) was purchased from CCTCC (Wuhan, China). Dimethyl sulfoxide (DMSO) and lipopolysaccharide (LPS) (Escherichia coli 055:B5) were purchased from Sigma-Aldrich (Oakville, Ontario, Canada). Fetal bovine serum (FBS) and DMEM/F12 medium were obtained from Gibco (Grand Island, NY). ELISA kits for IL-1, IL-6 and TNF-α were obtained from R&D (Minneapolis, MN, USA). Antibodies against p65 and p-p65 were purchased from Cell Signaling Technology Inc. (Shanghai, China). Antibody against β-actin and the secondary antibody of goat anti-rabbit IgG were obtained from Boster Biological Technology Inc.(Wuhan, China). Pyrrolidinedithiocarbamic acid (PDTC, inhibitor of NF-κB activation) were purchased from Beyotime Biotechnology Inc. (Shanghai, China). Methanol (MS1922-801, HPLC grade) and water (WS2211-001, HPLC grade) were purchased from TEDIA (Fairfield, USA); Acetonitrile (ACN) (1.00030.4000, HPLC grade) was purchased from MERCK (Darmstadt, Germany); Formic acid (94318-250 mL-F, MS grade) was purchased from SIGMA (Deisenhofen, Germany); Ammonium formate (65929-0025, LCMS grade) was purchased from ROE SCIENTIFIC INC (Newark, US); Phenylalanine-2,3,4,5,6-d5 (D-5466, 99.7 atom % D) was purchased from CDN isotopes (Canada).

Preparation of Piper sarmentosum extract (PSE)

Fresh Piper sarmentosum stem leaves were collected from the Tropical Botanical Garden of the Tropical Crops Genetic Resources Institute (Danzhou, Hainan, China). Fresh stems and leaves were oven-dried at 60 °C for 24 h and pulverized with a plant grinder. The stem leaf powder was extracted by volume fraction of 95% ethanol and recovered by rotary evaporation to obtain crude ethanol extract with a yield of 5.96%. Crude ethanol extract of Piper sarmentosum stem leaves was extracted with petroleum ether (PE), ethyl acetate (EA) and n-butanol (NB), respectively, and then were concentrated under reduced pressure to obtain three extracts with different polarities (Piper sarmentosum extract with PE, PSE-PE; Piper sarmentosum extract with EA, PSE-EA; Piper sarmentosum extract with NB, PSE-NB). Three extracts were dissolved in dimethyl sulfoxide (DMSO) to stock solution with 2 mg/ml, and then stored at 4 °C.

Cell culture

IPEC-J2 cells were grown in DMEM/F12 supplemented with 10% FBS, 100 IU/ml penicillin, 100 μg/ml streptomycin, and maintained in an atmosphere of 5% CO_2 at 37 °C for cultures and assays. Culture medium was replaced every 2–3 d.

Cytokine measurement by ELISA

Preliminary anti-inflammatory effects of three extracts (PSE-PE, PSE-EA and PSE-NB) on IPEC-J2 cells induced by LPS were determined and screened. Stock solutions of three extracts were diluted in complete medium at 10 μg/mL. LPS were diluted in complete medium with 1 ug/mL. IPEC-J2 cells cultured into a 12-well plate for 24 h, and washed with plain medium and then were treated by three extracts (control and LPS treatment were treated by complete medium) for 24 h. At last, IPEC-J2 cells were washed with plain medium and then treated with 1 μg/mL of LPS (control was treated by complete medium) for 24 h. The IL-1, IL-6 and TNF-α concentration of culture supernatants were measured by ELISA kits, following the manufacturer's instructions. We selected the one kind of PSE from three extracts, which have the lower levels of IL-1, IL-6 and TNF-α for subsequent experiment.

Determination of inflammatory responses and barrier function by RT-PCR

In the previous experiments, PSE-NB significantly reduced the concentrations of IL-1, IL-6 and TNF-α in the supernatant of IPEC-J2 cells induced by LPS. We choose PSE-NB to study the inflammatory responses in the current research.

IPEC-J2 cells cultured into a 6-well plate for 24 h were first washed with plain medium, and were treated with 10 ug/mL PSE-NB (control and LPS treatment were treated by complete medium) for 24 h. Then, IPEC-J2 cells were washed with plain medium and then treated with 1 μg/mL of LPS (control was treated by complete medium) for 24 h. After treatment, cells were used for RNA extraction and gene expression assays. Meanwhile, cells were also used for protein extraction and protein expression assays.

Total RNA from IPEC-J2 cells was extracted using Trizol reagents (Invitrogen, Carlsbad, CA, USA) according the manufacturer's instructions. Quality and quantity of RNA were measured by the RNA 6,000 Nano LabChip kit using the Bioanalyzer (Agilent Technologies, Shanghai, China). Two μg of total RNA was used in a 20 μL reverse transcription reaction volume (Bioteke Corporation, China) to reverse transcribed into cDNA following the manufacturer's protocol. Quantitative RT-PCR was performed using SYBR green real-time PCR master mix (Fermentas, Burlington, Canada). Primers sequences are listed in Table 1. The GAPDH gene was used as an internal control. Each reaction was completed with a melting curve analysis to ascertain that only the expected amplification products had been generated. PCR data were analyzed using the $2^{-\Delta\Delta CT}$ method [12] to calculate the relative expression levels of target gene, using GAPDH as the reference gene.

Table 1
Primer sequences used in the current study

Gene	Primer sequences	Product size (bp)
GAPDH	TCGGAGTGAACGGATTTGGC	189
	TGACAAGCTTCCCGTTCTCC	
IL-1	CCAGCCAGTCTTCATTGTTCAG	227
	GCCTGATGCTCTTGTTCCAG	
IL-6	TCGAGGCTGTGCAGATTAGT	280
	ACAGGTTTCTGACCAGAGGAG	
TNF-α	TTATCGGCCCCCAGAAGGAA	288
	GGAAAACGTTGGTGGAAGGG	
ZO-1	AAGGTCTGCCGAGACAACAG	137
	TCACAGTGTGGTAAGCGCAG	
Claudin-1	TCTTAGTTGCCACAGCATGG	207
	GTTTTGGATAGGGCCTTGGT	
Occludin	AGGTGCACCCTCCAGATTG	208
	GGCTGAGAAAGCATTGGTCG	
NHE3	TCTGCTGCCAGAAGTACGTG	200
	GACCCGGTACACGGAGATAA	

Abbreviations: GAPDH, glyceraldehyde-3-phosphate dehydrogenase; IL-1, Interleukin 1; IL-6, Interleukin 6; TNF-α, Tumor necrosis factor α; ZO-1, Zonula occludens-1; NHE3, Na^+-H^+ exchanger 3.

Inflammatory signaling pathway was measured by Western blotting and RT-PCR

For protein extraction, IPEC-J2 cells which were seeded into 6-well plates were harvested and lysed in 100 μL ice-cold lysis buffer. The supernatants of lysates were collected, and then were sonicated and centrifuged. The protein concentrations were measured using the Bradford dye-binding procedure [13]. Protein lysate samples (50 μg) from each treatment groups were used in Western blotting. The primary antibodies of rabbit polyclonal antibodies against NF-κB p65 and NF-κB p-p65 were employed. The following secondary antibody of goat anti-rabbit IgG was employed. The results were normalized to the β-actin standard and analyzed using the image analysis software, AlphaEase FC 4.0 (Alpha Innotech, Co., USA).

Pyrrolidinedithiocarbamic acid (PDTC, inhibitor of NF-κB activation, 25 μg/ml) were used to verify the inflammatory signaling pathway of NF-κB in IPEC-J2 cells induced by LPS. IPEC-J2 cells cultured into a 6-well plate and were treated with PDTC (control, LPS and LPS + PSE-NB treatments were treated by complete medium) for 30 min. Then, cells in LPS + PSE-NB treatment were washed with plain medium and then were treated with 10 μg/mL PSE-NB (other treatments were treated by complete medium) for 24 h. At last, cells were washed with plain medium and then treated with 1 μg/mL of LPS (control was treated by complete medium) for 24 h. After treatment, cells were used for RNA extraction and gene expression assays for IL-1, IL-6 and TNF-α.

Metabolomics analysis

Preparation of cell samples

IPEC-J2 cells (6×10^5 cell/well) were seeded on 6-well plates in a 2 ml of plain medium. Cells were divided into three treatments (control, LPS treatment and LPS + PSE-NB treatment). Each treatment was conducted in five replicates. After incubation for 24 h, cells in LPS + PSE-NB treatment were treated by media containing PSE-NB at 10 ug/ml (cells in control and LPS treatments were treated by complete medium). Cells were culture for 24 h, and cells in LPS treatment and LPS + PSE-NB treatment then were treated by media contain 1 μg/ml of LPS (cells in control was treated by complete medium) for another 24 h. Then, culture supernatants were transferred to centrifuge tubes and centrifuged at 1,500 rpm and 4 °C for 5 min, and supernatant was discarded. Remaining cells were digested with trypsin and harvested, and were transferred to centrifuge tubes and centrifuged at 1,500 g and 4 °C for 5 min, and supernatant was discarded. Precipitated cells were washed with PBS and combined with the pecipitated cells in the previous tube. Then, cells in PBS were centrifuged at 1,500 g and 4 °C for 5 min, and supernatant was discarded. Precipitated cells were added 1 ml cold extraction solvent methanol (80%, v/v) rapidly to quech cellular metabolism. The quenched cells were ultrasonicated in an ice bath for 3 min, and subsequently centrifuged at 4 °C for 15 min at 10,000 g and. The supernatant were collected and filtered by microporous membrane (0.22 μm) for metabolomics analysis.

UPLC-QTOF-MS method for metabolic profiling

UPLC-QTOF-MS analyses were conducted on a TripleTOFTM 5600 plus mass spectrometer (Sciex, USA, Concord, ON.) equipped with Turbo V sources and a Turbolon Spray interface, which was coupled to a LC-30AD series ultra performance liquid chromatography system (NexeraTM, Shimadzu, Japan). Data acquisition for metabolomics analysis was obtained in positive mode. Five microliter of each sample was injected onto a ZIC-pHILIC 2.1 × 150 mm (5 μm particle size) column (EMD Millipore). Mobile phase A was 20 mM ammonium carbonate, 0.1% ammonium hydroxide; Mobile phase B was acetonitrile. The chromatographic gradient was run at a flow rate of 0.25 ml/min as follows: 0–24 min: linear gradient from 80–20% B; 24-24.5 min: linear gradient from 20–80% B; 24.5–33 min: hold at 80% B. The column oven was set to 30 °C.

The mass range scanned was m/z 70 − 1,000 in full data storage mode. Drying gas temperature was set at 550 °C, the ion spray voltage at 5,500 V. Atomization gas pressure, auxiliary heating gas pressure and curtain gas pressure in both ionization mode were set at 55, 55 and 35 psi, respectively. The instrument was mass calibrated by automatic calibration infusing the Sciex Positive Calibration Solution (part no. 4460131, Sciex, Foster City, CA) after every six-sample injections. One QC sample and one blank vial were run after each 10 cell samples.

Statistical analysis

Statistical analysis of cytokine concentration, gene expression, and protein content among the groups were evaluated by one-way ANOVA using SAS software (SAS Institute Inc., Cary, NC, USA). Data were presented as mean ± standard deviations. Significant differences were evaluated by Duncan's multiple comparisons test at $P < 0.05$.

The instrument software MarkerView (version 1.2.1.1, Sciex, USA) is used to perform peak extraction, peak matching, peak alignment and normalization preprocessing on the acquired data. The main parameters are set as follows: the retention time range is 1 ~ 33 min; the retention time and m/z tolerances are 0.1 min and 10 ppm respectively; the response threshold is 100 counts; the isotope peak is removed. After Pareto-scaling (Par transformation), principal components analysis (PCA) and partial least-squares discrimination analysis (PLS-DA) models were carried out to visualize the metabolic difference among LPS vs control and LPS + PSE-NB vs LPS treatments. The quality of the models is described by the R^2X (PCA) or R^2Y (PLS-DA) values. To avoid model over-fitting, 999 cross-validations in SIMCA-P 13.0 was performed throughout to determine the optimal number of principal components. R^2X, R^2Y and Q^2Y values of models are nearly 1.0, indicating that these models have good ability of explaining and predicting the variations in the X and Y matrix.

Differences between data sets were analyzed by t-test, and $p < 0.05$ was considered to be statistically significant. Fold change was calculated as the average normalized peak intensity ratio between two groups. The structural identification of differential metabolites was performed by matching the mass spectra with an in-house standard library including accuracy mass, retention time and MS/MS spectra and online databases: Metlin (http://www.metlin.scripps.edu) and HMDB (http://www.hmdb.ca).

The impact of LPS and PSE-NB on metabolic pathways was evaluated based on a tool for metabolomics data analysis platform, which is available online (https://www.metaboanalyst.ca). The Pathway Analysis module combines results from powerful pathway enrichment analysis with the pathway topology analysis to help researchers identify the most relevant pathways involved in the conditions under study. By uploading the discriminatory compounds that were significantly influenced by LPS and LPS + PSE-NB treatments, the built-in predicted metabolite library for pathway analysis and hypergeometric test for over-representation analysis were employed. A results report was then presented graphically as well as in a detailed table. Potential drug efficacy biomarkers were identified based on the identified metabolic pathways and the statistics.

Results

Effect of three extracts (PSE-PE, PSE-EA and PSE-NB) on cytokines level in IPEC-J2 cells

To determine and screen anti-inflammatory activity of three PSEs, we measured the level of cytokines in IPEC-J2 cells induced by LPS. As shown in Table 2, LPS significantly increased concentration of IL-6 and TNF-α in culture supernatants of IPEC-J2 cells ($P < 0.05$). Three PSEs all reduced the concentration of IL-1, IL-6 and TNF-α in culture supernatants of IPEC-J2 cells induced by LPS. Among three PSEs, PSE-NB could significantly decrease the levels of IL-1, IL-6 and TNF-α in culture supernatants of IPEC-J2 cells induced by LPS. Therefore, PSE-NB has the best anti-inflammatory activity among three PSEs in the current study, which means that the anti-inflammatory active ingredients of PSE were mainly presented in the PSE-NB. So, we selected the PSE-NB for subsequent experiment.

Table 2
Concentrations of IL-1, IL-6 and TNF-α in culture supernatants of IPEC-J2 cells

Items	IL-1(ng/L)	IL-6 (ng/L)	TNF-α (ng/L)
Control	108.03 ± 8.97^{a}	15.91 ± 2.75^{c}	100.59 ± 7.02^{b}
LPS treatment (μg/mL)	115.42 ± 10.69^{a}	48.47 ± 8.97^{a}	122.73 ± 9.59^{a}
PSE-EA treatments (μg/mL)	113.92 ± 7.53^{a}	29.93 ± 7.13^{b}	100.89 ± 6.15^{b}
PSE-PE treatments(μg/mL)	76.09 ± 6.22^{b}	43.47 ± 7.46^{a}	77.38 ± 7.57^{c}
PSE-NB treatments(μg/mL)	40.59 ± 9.04^{c}	24.04 ± 5.93^{b}	48.92 ± 5.66^{d}
Abbreviations: LPS, lipopolysaccharide; PSE-EA, Piper sarmentosum extract with ethyl acetate; PSE-PE, Piper sarmentosum extract with petroleum ether; PSE-NB, Piper sarmentosum extract with n-butanol.			
The data were presented as mean ± SD, n = 5. Different superscript letters represent a significant difference ($P < 0.05$).			

Effect of PSE-NB on cytokine gene expression in IPEC-J2 cells

As shown in Fig. 1, LPS stimulation significantly up-regulated the mRNA expression of IL-1, IL-6 and TNF-α ($P < 0.05$) compared with the control. PSE-NB pre-treatment with 10 ug/mL before LPS stimulation significantly decreased the expression of IL-1, IL-6 and TNF-α compared with LPS treatment ($P < 0.05$).

Effect of PSE-NB on relative gene expression of tight junction proteins and NHE-3 in IPEC-J2 cells

Compared with control, LPS reduced the mRNA expression of Claudin-1, Occludin, ZO-1 and NHE-3 significantly ($P < 0.05$) (Fig. 2). PSE-NB pre-treatment with 10 ug/mL before LPS stimulation improved the mRNA expression of ZO-1, Occludin and NHE-3 in IPEC-J2 cells induced by LPS ($P < 0.05$).

Effect of PSE-NB on inflammatory signaling pathway in IPEC-J2 cells induced by LPS

NF-κB signaling pathways are involved in the pathogenesis of intestinal inflammation. Therefore, we determined the expression levels of related proteins and their phosphorylation in NF-κB signaling pathway. As shown in Fig. 3A and Fig. 3B, compared with control, LPS increased the protein expression of p65 and p-p65. Pretreatment with 10 ug/mL of PSE-NB reversed the increase in IPEC-J2 cells induced by LPS. When inflammatory signaling pathway were blocked by inhibitor, mRNA expression of IL-1, IL-6 and TNF-α decreased compared with LPS treatment ($P < 0.05$) in IPEC-J2 cells (Fig. 4). These results suggested that PSE-NB could inhibit LPS-induced inflammation by suppressing NF-κB signaling pathway in IPEC-J2 cells.

UPLC-QTOF-MS analysis of metabolic profiling

To investigate metabolic effect of LPS and LPS + PSE-NB on metabolic heterogeneity, a metabolic profiling analysis was performed on LPS vs control and LPS + PSE-NB vs LPS. UPLC-QTOF-MS system can obtain metabolic profiling of both groups with the total ion chromatogram (TIC). A total of 2088 after removing isotope peaks were detected in the positive ion mode respectively. Each feature is composed of exact mass, retention time, and average intensity. From the TICs, there are many different features in endo-metabolome between the two groups (Fig. 5). As shown in Fig. 5, the difference is mainly concentrated in the 4–10 minute between LPS vs control and LPS + PSE-NB vs LPS, indicating that more variables may come from this time range.

LPS distinctly perturbed metabolic patterns and intracellular metabolites in IPEC-J2 cells

For ESI-MS data, 2088 peaks of positive ions from 1 to 33 min of retention time were obtained after data pre-processing. To investigate the global metabolic alterations between LPS treatment and control, all

observations were integrated and analyzed using PCA. The score plot demonstrated that the two cell lines clustered closely within each group and separately from each other (Fig. 6A). To further explore the differences between two treatments, supervised PLS-DA was applied for chemometrics analysis (Fig. 6B). The LPS group can be separated from the control clearly, which reflected the remarkably distinct metabolic status of LPS and control cells. Many metabolites were severely perturbed when the cells were exposed to LPS. Finally 61 different metabolites and metabolic pathways were identified between LPS treatment and control (Table 3 and Fig. 7). A metabolic pathway analysis demonstrated that LPS affected glycine and serine metabolism (betaine, alanine, threonine, serine, sarcosine, phosphoserine, arginine and S-adenosylhomocysteine), methionine metabolism (betaine, serine, sarcosine, homoserine and S-adenosylhomocysteine), tryptophan metabolism (alanine, NADPH, quinolinic acid, tryptamine, kynurenine and S-adenosylhomocysteine), carnitine synthesis (carnitine, lysine and S-adenosylhomocysteine), nicotinate and nicotinamide metabolism (NADPH, quinolinic acid, 1-methylnicotinamidea and S-adenosylhomocysteine).

Table 3

Differentiating metabolites between LPS group and control from the data set

Compound	P-value [a]	FC [b]
S-(Hydroxymethyl)glutathione	1.03×10^{-7}	45.13
Phosphoserine	1.28×10^{-3}	3.49
Folic acid	7.86×10^{-7}	2.77
Salicylic acid	3.21×10^{-2}	2.58
Nicotine	4.28×10^{-2}	2.32
Selenomethionine	1.41×10^{-2}	2.05
3,5-Diiodo-L-thyronine	3.19×10^{-2}	1.99
L-Serine	1.48×10^{-3}	1.88
Guanosine	7.50×10^{-4}	1.66
L-Arginine	1.10×10^{-3}	1.57
L-Citrulline	4.67×10^{-2}	1.55
L-Isoleucine	1.10×10^{-4}	1.55
L-Norleucine	1.10×10^{-4}	1.55
Tryptamine	3.62×10^{-2}	1.54
Leucine	9.72×10^{-5}	1.53
4-Methylcatechol	3.79×10^{-2}	1.43
Guanine	1.02×10^{-2}	1.28
Phenylpyruvate	1.29×10^{-3}	1.28
1-Methylnicotinamide	4.15×10^{-2}	1.26
Thiamine pyrophosphate	3.37×10^{-2}	1.26

[a] P-values were calculated from a Student's t-test.

[b] Fold change was calculated by the average value of the LPS group compared to that of the control. FC with a value larger than 1 indicates a higher level of the metabolite in the LPS treatment; FC value lower than 1 indicates a lower level, compared to controls.

Compound	P-value [a]	FC [b]
Pyridoxine	1.57×10^{-3}	1.25
L-Threonine	3.38×10^{-2}	1.21
L-Homoserine	3.38×10^{-2}	1.21
L-allo-Threonine	3.38×10^{-2}	1.21
N-Methyl-D-aspartic acid	7.83×10^{-3}	1.21
1-Aminocyclopropanecarboxylic acid	3.88×10^{-2}	1.20
Creatinine	5.62×10^{-3}	0.86
L-Valine	1.41×10^{-2}	0.85
Betaine	1.41×10^{-2}	0.85
5-Aminovaleric acid	1.41×10^{-2}	0.85
4-Methylaminobutyrate	1.41×10^{-2}	0.85
NADPH	8.49×10^{-3}	0.83
Hypotaurine	4.70×10^{-3}	0.81
S-(5'-Adenosyl)-L-homocysteine	1.64×10^{-2}	0.80
CDP	3.76×10^{-2}	0.79
gefitinib	4.53×10^{-2}	0.78
1-Methyl-L-histidine	1.38×10^{-3}	0.77
Phosphocreatine	3.71×10^{-2}	0.76
L-Carnitine	2.52×10^{-3}	0.76
5-Hydroxylysine	2.73×10^{-3}	0.75
UMP	1.75×10^{-2}	0.74

[a] P-values were calculated from a Student's t-test.

[b] Fold change was calculated by the average value of the LPS group compared to that of the control. FC with a value larger than 1 indicates a higher level of the metabolite in the LPS treatment; FC value lower than 1 indicates a lower level, compared to controls.

Compound	P-value [a]	FC [b]
N-Acetyl-D-glucosamine	2.37×10^{-2}	0.73
N-Acetyl-D-galactosamine	2.37×10^{-2}	0.73
Cadaverine	4.04×10^{-2}	0.72
XMP	4.16×10^{-3}	0.71
Adenosine 3',5'-cyclic monophosphate	6.38×10^{-3}	0.71
Cyclic AMP	6.80×10^{-3}	0.71
Lumichrome	1.50×10^{-2}	0.68
Quinic acid	1.42×10^{-2}	0.66
Sarcosine	1.02×10^{-2}	0.65
Alanine	1.02×10^{-2}	0.65
Acetylcholine	1.51×10^{-6}	0.64
O-Succinyl-L-homoserine	1.22×10^{-3}	0.64
L-Tryptophanamide	3.05×10^{-3}	0.62
L-Lysine	0.89×10^{-3}	0.59
3-Hydroxyhexadecanoylcarnitine	2.31×10^{-2}	0.57
O-Acetyl-L-carnitine	7.52×10^{-5}	0.54
L-Kynurenine	0.06×10^{-2}	0.50
N-Acetyl-L-methionine	0.53×10^{-3}	0.50
N-Acetylputrescine	6.38×10^{-5}	0.46
2'-Deoxycytidine	2.65×10^{-2}	0.25

[a] P-values were calculated from a Student's t-test.

[b] Fold change was calculated by the average value of the LPS group compared to that of the control. FC with a value larger than 1 indicates a higher level of the metabolite in the LPS treatment; FC value lower than 1 indicates a lower level, compared to controls.

PSE-NB distinctly perturbed metabolic patterns and intracellular metabolites in IPEC-J2 cells

For ESI-MS data, 1854 peaks from 1 to 33 min of retention time were obtained after data pre-processing. To investigate the global metabolic alterations between LPS + PSE-NB treatment and LPS treatment, all observations were integrated and analyzed using PCA. The score plot demonstrated that the two cell lines clustered closely within each treatment and separately from each other (Fig. 8A). Further supervised PLS-DA suggested that PSE-NB have obvious effects on the metabolic pattern of cells exposed to LPS stimulus (Fig. 8B). Finally 174 different metabolites and metabolic pathways were identified between LPS + PSE-NB treatment and LPS treatment (Table 4, Fig. 9). A metabolic pathway analysis demonstrated that PSE-NB affected tryptophan metabolism (glutamic acid, alanine, NADP, NADPH, quinolinic acid, serotonin, tryptamine, 5-hydroxytryptophan, ATP, kynurenine, kynurenic acid, 5-hydroxyindoleacetic acid, xanthurenic acid, 2-aminobenzoic acid, N-acetylserotonin, melatonin, coenzyme A and 3-hydroxyanthranilic acid), purine metabolism (adenine, AMP, cyclic AMP, guanine, glutamic acid, hypoxanthine, aspartic acid, NADP, NADPH, uric acid, xanthine, xanthosine, ATP, dAMP, dGDP, GMP, AICAR, dATP and tetrahydrofolic acid), glutathione metabolism (glutathione, glutamic acid, alanine, ADP, NADPH, ATP, cysteine), thiamine metabolism (AMP, thiamine, ATP, thiamine pyrophosphate), phenylacetate metabolism (AMP, ATP, phenylacetic acid, coenzyme A).

Table 4

Differentiating metabolites between LPS group and LPS + PSE-NB group from the data set

Compound	P-value [a]	FC [b]
L-Methionine	2.20×10^{-7}	120.42
Lipoamide	7.08×10^{-2}	31.11
2-Aminoisobutyric acid	0.41	8.53
UTP	0.56×10^{-3}	6.64
Imidazoleacrylic acid	3.66×10^{-2}	4.12
Suberic acid	0.13	4.06
S-(Hydroxymethyl)glutathione	0.14×10^{-3}	3.30
nicotine	2.02×10^{-2}	2.98
2,5-Dimethylpyrazine	0.44	2.96
ATP	0.38	2.85
Salicylic acid	2.52×10^{-2}	2.80
Indole-3-acetamide	0.57	2.70
Arabinose	0.26	2.42
Lyxose	0.26	2.42
S-Carboxymethyl-L-cysteine	5.71×10^{-2}	2.14
3-Nitrotyrosine	0.11	2.07
1,4-Diaminobutane	2.43×10^{-3}	1.92
NADPH	0.63×10^{-3}	1.90
N-Acetylputrescine	4.64×10^{-2}	1.88
L-Asparagine	0.14	1.86
Homocysteine	4.96×10^{-2}	1.80

[a] P-values were calculated from a Student's t-test.

[b] Fold change was calculated by the average value of the LPS group compared to that of the LPS + PSE-NB group. FC with a value larger than 1 indicates a higher level of the metabolite in the LPS group; FC value lower than 1 indicates a lower level, compared to LPS + PSE-NB group.

Compound	P-value [a]	FC [b]
Selenomethionine	4.60	1.78
Glycerol	8.99×10^{-2}	1.74
Phosphoserine	3.23×10^{-2}	1.73
Uric acid	0.12	1.73
N-Acetyl-methionine	1.99×10^{-2}	1.71
Sarcosine	0.15	1.68
Alanine	0.15	1.68
D-Glucosaminic acid	0.16	1.62
dUMP	0.04	1.60
3-Methoxytyramine	0.31	1.60
O-Succinyl-L-homoserine	0.18	1.58
L-Homocystine	0.14	1.58
Thiamine	9.78×10^{-2}	1.54
dGDP	0.19	1.53
Phosphocreatine	0.279	1.51
Arabitol	0.109	1.47
Xylitol	0.109	1.47
Uridine	0.489	1.45
L-Phenylalanine	8.58×10^{-2}	1.42
D-Glucuronic acid	0.23	1.41
L-Cystine	0.28	1.40
L-Serine	0.19	1.39
6-Hydroxypyridine-3-carboxylic acid	0.12	1.38

[a] P-values were calculated from a Student's t-test.

[b] Fold change was calculated by the average value of the LPS group compared to that of the LPS + PSE-NB group. FC with a value larger than 1 indicates a higher level of the metabolite in the LPS group; FC value lower than 1 indicates a lower level, compared to LPS + PSE-NB group.

Compound	P-value [a]	FC [b]
CDP-ethanolamine	0.42	1.36
Kynurenine	0.29	1.36
Thiamine pyrophosphate	0.44	1.36
Nicotinic acid	0.46	1.35
Bilirubin	8.80×10^{-2}	1.35
1-Methylnicotinamide	0.29	1.35
Tryptamine	0.29	1.34
2,6-Diaminopimelic acid	0.35	1.34
Dulcitol	0.26	1.33
Trimethyllysine	0.24	1.33
2',4'-Dihydroxyacetophenone	0.46	1.32
N-Acetyl-L-alanine	0.27	1.30
Phenylpyruvate	0.27	1.29
3-Methylhistamine	0.23	1.28
GSH	0.31	1.28
N-Acetyl-glutamic acid	0.29	1.28
Pipecolinic acid	2.60×10^{-2}	1.27
L-Pipecolic acid	2.60×10^{-2}	1.27
L-allo-Threonine	0.32	1.27
4-Aminobenzoic acid	0.32	1.27
Anthranilic acid	0.32	1.27
L-Methionine sulfoximine	0.61	1.26
5-Hydroxylysine	0.35	1.26
5-Hydroxyindole-3-acetic acid	2.23×10^{-2}	1.25

[a] P-values were calculated from a Student's t-test.

[b] Fold change was calculated by the average value of the LPS group compared to that of the LPS + PSE-NB group. FC with a value larger than 1 indicates a higher level of the metabolite in the LPS group; FC value lower than 1 indicates a lower level, compared to LPS + PSE-NB group.

Compound	P-value [a]	FC [b]
Biotin	0.53	1.24
L-Arginine	1.96×10^{-2}	1.23
L-Cysteine	6.07×10^{-3}	1.23
Thiourea	0.56	1.23
AMP	0.45	1.22
Diethanolamine	0.45	1.21
Quinic acid	0.33	1.21
Pyridoxine	3.08×10^{-3}	1.20
Creatinine	0.60×10^{-2}	1.20
Glucosamine 6-phosphate	0.33	1.20
L-Kynurenine	0.39	1.19
L-Isoleucine	0.37×10^{-2}	1.19
Leucine	0.37×10^{-2}	1.19
Glutaryl-L-carnitine	0.33	1.18
GMP	0.56	1.18
N-Acetyl-L-leucine	0.74	1.17
4-Methylcatechol	0.56	1.17
N-Acetyl-D-glucosamine	0.40	1.16
N-Acetyl-D-galactosamine	0.40	1.16
Taurine	0.70	1.15
Tetrahydrofolic acid	0.47	0.84
Pyridoxal	9.68×10^{-2}	0.84
Trigonelline	0.71	0.84

[a] P-values were calculated from a Student's t-test.

[b] Fold change was calculated by the average value of the LPS group compared to that of the LPS + PSE-NB group. FC with a value larger than 1 indicates a higher level of the metabolite in the LPS group; FC value lower than 1 indicates a lower level, compared to LPS + PSE-NB group.

Compound	P-value [a]	FC [b]
Uridine 5'-diphospho-N-acetylgalactosamine	7.83×10^{-2}	0.84
Uridine 5'-diphospho-N-acetylglucosamine	7.83×10^{-2}	0.84
Xanthurenic acid	0.49	0.82
Aspartic acid	4.81×10^{-2}	0.82
Coenzyme A	0.31	0.82
L-Cystathionine	5.84×10^{-2}	0.81
Maleamic acid	0.28	0.81
Adenosine 3',5'-cyclic monophosphate	9.37×10^{-2}	0.80
cyclic AMP	9.37×10^{-2}	0.80
L-Citrulline	0.61	0.80
N-Acetylneuraminic acid	8.62×10^{-3}	0.80
Citric acid	0.48	0.79
Methyl beta-D-glucopyranoside	0.39	0.79
Kynurenic acid	0.45	0.79
Hydrocortisone acetate	0.49	0.79
L-Tyrosine	0.25	0.78
3-Hydroxyhexadecanoylcarnitine	0.32	0.78
Guanidinosuccinic acid	0.12	0.78
3-Hydroxy-kynurenine	0.20	0.77
NADP	3.02×10^{-2}	0.77
1-Methyl-L-histidine	0.35	0.77
Sorbitol	0.72	0.77
Mannitol	0.72	0.77

[a] P-values were calculated from a Student's t-test.

[b] Fold change was calculated by the average value of the LPS group compared to that of the LPS + PSE-NB group. FC with a value larger than 1 indicates a higher level of the metabolite in the LPS group; FC value lower than 1 indicates a lower level, compared to LPS + PSE-NB group.

Compound	P-value [a]	FC [b]
Histamine	0.29	0.76
Lauroyl-L-carnitine	0.64	0.75
Cytidine	0.28	0.75
Xanthine	5.66×10^{-3}	0.75
Glucose-6-phosphate	6.42×10^{-2}	0.74
Cysteic acid	0.28	0.74
3-Aminosalicylic acid	0.13	0.71
3-Hydroxyanthranilic acid	0.13	0.71
5-Hydroxy-L-tryptophan	0.24	0.71
3-Methyladenine	0.36	0.70
L-Lysine	1.61×10^{-2}	0.69
2,3-Pyridinedicarboxylic acid	6.61×10^{-2}	0.69
dAMP	0.21	0.68
trans-Aconitic acid	6.33×10^{-2}	0.66
Raffinose	0.11	0.64
Pyridoxamine	1.02×10^{-2}	0.63
4-Guanidinobutyric acid	0.31	0.62
Xanthosine	1.72×10^{-3}	0.62
1,3-Diaminopropane	0.12	0.61
Dihydrofolic acid	0.47	0.60
Theophylline	0.40	0.60
Theobromine	0.40	0.60
1,7-Dimethylxanthine	0.40	0.60

[a] P-values were calculated from a Student's t-test.

[b] Fold change was calculated by the average value of the LPS group compared to that of the LPS + PSE-NB group. FC with a value larger than 1 indicates a higher level of the metabolite in the LPS group; FC value lower than 1 indicates a lower level, compared to LPS + PSE-NB group.

Compound	P-value [a]	FC [b]
Methyl indole-3-acetate	0.23	0.60
5-Methylcytosine	1.50×10^{-2}	0.59
3-(2-Hydroxyethyl)indole	0.56	0.58
Glucosamine 6-sulfate	2.99×10^{-5}	0.57
Quinaldic acid	0.16	0.57
5'-Deoxyadenosine	0.12	0.56
Serotonin	0.30	0.56
7,8-Dihydro-L-biopterin	0.23	0.55
Riboflavin	0.19	0.55
Guanine	0.48	0.54
Reichstein's substance S	0.43	0.53
N-Methyl-D-aspartic acid	0.43	0.53
Nicotinic acid mononucleotide	1.90×10^{-4}	0.52
2'-Deoxycytidine	0.19	0.52
Dihydrouracil	1.20×10^{-2}	0.51
N-Acetyl-5-hydroxytryptamine	0.13	0.50
AICAR	0.31	0.50
L-Homocysteine thiolactone	0.10	0.49
Lumichrome	7.60×10^{-2}	0.48
dUTP	2.26×10^{-2}	0.47
Pimelic acid	0.17	0.47
2-Hydroxypyridine	0.13	0.45
4-Aetamidobutyric acid	0.42	0.45

[a] P-values were calculated from a Student’s t-test.

[b] Fold change was calculated by the average value of the LPS group compared to that of the LPS + PSE-NB group. FC with a value larger than 1 indicates a higher level of the metabolite in the LPS group; FC value lower than 1 indicates a lower level, compared to LPS + PSE-NB group.

Compound	P-value [a]	FC [b]
Hypoxanthine	4.36×10^{-3}	0.45
Adenine	2.49×10^{-3}	0.43
Uracil	4.55×10^{-3}	0.40
Cytosine	1.76×10^{-2}	0.37
Glutamic acid	0.21	0.30
O-Acetyl-L-serine	0.21	0.30
Phenylacetic acid	3.22×10^{-3}	0.30
Choline	3.10×10^{-2}	0.24
Nicotinamide	7.88×10^{-3}	0.17
Pregnenolone sulfate	0.15	0.08
DL-Normetanephrine	0.34	0.06
Epinephrine	0.34	0.06
dATP	0.34	0.04
Melatonin	8.44×10^{-2}	0.03

[a] P-values were calculated from a Student's t-test.

[b] Fold change was calculated by the average value of the LPS group compared to that of the LPS + PSE-NB group. FC with a value larger than 1 indicates a higher level of the metabolite in the LPS group; FC value lower than 1 indicates a lower level, compared to LPS + PSE-NB group.

Discussion

We studied the application of PSE in weaned pig diets, and found that PSE supplementation can improve the intestinal health by reducing the gut inflammation in piglet [9]. However, the potential anti-inflammatory effects of PSE on porcine intestinal cells remain to be elucidated. Therefore, the current study employed the IPEC-J2 cell line to investigate the protective effect and mechanism of PSE on LPS-induced inflammatory response in epithelial cells.

To clarify the anti-inflammatory active ingredients in the PSE, we investigated the anti-inflammatory activity of three PSEs by measuring the concentration of pro-inflammatory cytokines in IPEC-J2 cells induced by LPS. Among three PSEs, PSE-NB could significantly decrease the levels of IL-1, IL-6 and TNF-α in culture supernatants of IPEC-J2 cells induced by LPS (Table 2). Therefore, anti-inflammatory active

ingredients of PSE were mainly presented in PSE-NB. Our previous study found that PSE-NB contained higher contents of alkaloids and total phenolic than PSE-PE and PSE-EA [14], respectively. A number of studies suggested that alkaloids and phenolic phytochemicals have significant anti-inflammatory activity [15, 16]. So, the better anti-inflammatory properties of PSE-NB may be due to a higher content of alkaloids and total phenolic among the three PSEs. Similarly, Zakaria et al. found that the methanolic extracts of Piper sarmentosum leaves have exhibited anti-inflammatory activity [8].

We further evaluated the protective effect of PSE-NB on inflammatory response in IPEC-J2 cells induced by LPS. Cytokines, like IL-1, IL-6 and TNF-α, play key role in activating immune cells and amplifying inflammatory responses [17]. Previous studies have indicated that intestinal epithelial inflammation could damage tight junction by down-regulating the expression of tight junction proteins through inflammatory signaling transduction [18, 19]. The overproduction of pro-inflammatory cytokines, such as TNF-α, IL-1, and IL-6, has a negative impact on gut integrity and epithelial function [20]. Our results showed that LPS significantly increased mRNA expression of IL-1, IL-6 and TNF-α (Fig. 1), and reduced mRNA abundance of ZO-1, Claudin-1, Occludin and NHE3 (Fig. 2) in cells when compared with control, which are consistent with previous studies [21]. The PSE-NB treatments was able to attenuate the inflammatory responses and preserve barrier function of epithelial cells induced by LPS evidenced by modulating the mRNA expression of pro-inflammatory cytokines and tight junctions in cells (Fig. 1 and Fig. 2). The current results are consistent with previous animal experiment, which indicated that PSE supplementation down-regulated the mRNA expression of TNF-α, IL-1β, and IL-6 in the ileal mucosal layer of piglets [9]. Therefore, supplementation with PSE-NB might be a potential approach to reduce inflammatory responses in weaned piglets.

Generally, LPS triggers inflammatory and immune responses mainly through the TLR4 receptor, and the activation of TLR4 by LPS induces the activation of NF-κB pathway and ultimately results in the release of pro-inflammatory cytokines [22]. The NF-κB signaling pathway plays a critical role in regulating inflammation and immune which are involved in diverse biological responses [23]. Main family members of signaling pathway including p65 and I-κBα participate in the activation and production of pro-inflammatory mediators and cytokines [24, 25]. In LPS-stimulated inflammation, the activation and phosphorylation of NF-κB are crucial for up-regulated mRNA transcription and production of inflammatory mediators. In this study, our results are consistent with previous findings that LPS induced the activation of NF-κB and increased the phosphorylation level of p65 significantly (Fig. 3A and Fig. 3B). The pre-treatment by PSE-NB reduced protein expressions of p65, and p-p65 significantly. The specific kinase inhibitor, PDTC was used in our study to confirm the role of NF-κB signaling pathway in the inhibition of LPS-induced inflammatory response by PSB-NB in IPEC-J2 cells. When inflammatory signaling pathway was blocked, mRNA expressions of pro-inflammatory cytokines were down-regulated (Fig. 4). Previous studies suggested that the anti-inflammatory mechanism of alkaloids and polyphenolics are related to the mediation of NF-κB pathways in LPS-induced cells [26]. PSE-NB contained higher contents of alkaloids and total phenolic than PSE-PE and PSE-EA [14], and these results together suggested that PSE-NB exerted anti-inflammatory effect by the inhibition of NF-κB pathway in IPEC-J2 cells induced by LPS.

The metabolomics profiles of IPEC-J2 cells were significantly changed by LPS and PSE-NB. PCA and PLS-DA revealed that metabolic perturbations were different among control, LPS treatment and LPS + PSE-NB treatment (Fig. 6 and Fig. 8). Changes in metabolites were related to several metabolic pathways. Based on the data obtained from intracellular metabolites on LPS group vs control group, the enhanced production of pro-inflammatory cytokines and mediators markedly perturbed the levels of secretion of some metabolites and the metabolic pattern of the IPEC-J2 cells. These results suggested that LPS-induced inflammatory response promoted the biosynthesis of phosphoserine and serine through the involvement of gluconeogenesis and glycolysis to activate the innate immune system by controlling T cell proliferation capacity [27, 28], and reduced the levels of betaine and sarcosine to affect the biosynthesis of S-(5'-Adenosyl)-L-homocysteine. Soon afterwards, the lower levels of S-(5'-Adenosyl)-L-homocysteine disturbed the homeostasis of methionine metabolic cycle and intercellular transmethylation reactions [29]. Furthermore, the inhibition of intercellular transmethylation reactions reduced carnitine synthesis and affected fatty acid oxidation process [30]. Additionally, LPS-induced inflammatory response and disturbed nicotinate and nicotinamide anabolism and motivated oxidative stress state of cells due to decreased quinolinic acid production, which is an factor affected tryptophan metabolism [31].

Notably, LPS and PSE-NB both affected tryptophan metabolic pathway. As known that LPS perturbed tryptophan catabolism as well as reduced the production of kynurenine, quinolinic acid, alanine and NADPH and increased the production of tryptamine. We assured that tryptophan can be mostly converted into tryptamine and generate CO_2 simultaneously via the action of aromatic amino acid decarboxylase. Decreases in the levels of S-(5'-Adenosyl)-L-homocysteine and NADPH implied tryptamine catabolic disorders, which resulted in tryptamine accumulate. Then tryptamine probably released histamine, a kind of inflammatory mediators, which induced the production of inflammatory mediators and cytokines in different immune cells and affect inflammation of the immune system [32]. However, PSE-NB relatively upregulated the levels of kynurenine, quinolinic acid, alanine and NADPH, resulting in the higher levels of quinolinic acid, 3-hydroxyanthranilic acid, NADP, kynurenic acid, xanthurenic acid and glutamic acid compared to LPS-induced IPEC-J2 cells and showed the recovery of tryptophan catabolism. Meanwhile, PSE-NB relatively decreased the levels of tryptamine and enhanced the levels of 5-hydroxytryptophan, melatonin, serotonin, coenzyme A, 5-hydroxyindoleacetic acid and N-acetylserotonin (Fig. 10, http://smpdb.ca).

Some studies have reported that hydroxylated analogues of kynurenine tryptophan metabolites, such as 3-hydroxyanthranilic acid, xanthurenic acid and 5-hydroxytryptophan, possess antioxidant potential revealing a capability of these metabolites to suppress inflammatory condition [33]. Additionally, kynurenic acid, which is detoxified kynurenine, reduced the release of IL-4 through the specific activation of G protein-coupled receptor 35 (GPR35) [34], and quinolinic acid have a role in protect the body from inflammatory injury via a direct action on neutrophils or vascular permeability [35]. Melatonin, serotonin and N-acetylserotonin also have ability to inhibit production of inflammatory cytokines and superoxide [36, 37, 38]. Meanwhile, increase in the production of glutamic acid (glutamate) and NADP have a favor

role involved in the purine metabolism and glutathione metabolism while coenzyme A affect phenylacetate metabolism. Furthermore, we found that PSE-NB affected purine metabolism and heighted the levels of purine nucleotides and nucleobases to modulate energy metabolism which can satisfy the nutritional needs and decrease oxidative stress of LPS-induced IPEC-J2 cells [39, 40, 41].

In the glutathione metabolism, increased NADP production significantly implied that the reaction between oxidized glutathione and NADPH, resulting from the synthesis of NADP and glutathione. The higher levels of NADP proved the fact. Therefore, glutathione probably participates in anti-inflammatory and antioxidative response, resulting in the lower levels of it [42]. Moreover, the higher levels of coenzyme A and phenylacetic acid revealed that the process reduce energy consumption to satisfy the needs of anti-inflammatory of cells. In the same way, depletion of thiamine because of it probably was used for helping convert carbohydrates into energy.

Crosstalk between metabolic and inflammatory pathways is critical for cellular homeostasis. In the present study, we found that the connectivity between these processes in PSE-NB-mediating IPEC-J2 cells induced by LPS. Melatonin, which is the product of the tryptophan catabolic pathway affected by PSE-NB, has anti-inflammatory properties through inhibiting NF-κB activation consistent with previous results regarding to anti-inflammatory activity of PSE-NB on inflammatory signaling pathway [38]. As a result, PSE-NB' anti-inflammatory mechanisms between protein pathway and metabolic pathway are highly integrated and closely linked.

Conclusions

Collectively, our study for the first time demonstrates that PSE-NB effectively alleviates inflammatory responses and improves barrier function, and PSE-NB exerted anti-inflammatory effect by the inhibition of NF-κB pathway in IPEC-J2 cells induced by LPS. Meanwhile, metabolomics analysis further shows metabolic perturbations in IPEC-J2 cells caused by LPS, especially in amino acid metabolism. And PSE-NB could also regulate related metabolism toward the direction of anti-inflammatory and antioxidant, such as tryptophan metabolism and glutathione metabolism in IPEC-J2 cells induced by LPS.

Declarations

Acknowledgements

The authors would like to thank members of their laboratory colleagues for helpful and constructive advice.

Authors' contributions

DW and HZ designed the experiment. LZ and GH performed the experiment and collected samples. LZ analyzed samples. DW analyzed the data and wrote the paper. All authors reviewed and approved the final manuscript.

Funding

This work was financially supported by the Natural Science Fund Project of Hainan province, China (317266), and the Central Public-interest Scientific Institution Basal Research Fund for Chinese Academy of Tropical Agricultural Sciences (Projects No. 1630032017036).

Availability of data and materials

The datasets general or analyzed during this study are presented in this manuscript and available to readers.

Ethics approval

This study does not involve ethics approval.

Consent for publication

Not applicable.

Competing interests

The authors declare that that have no competing interests.

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Figures

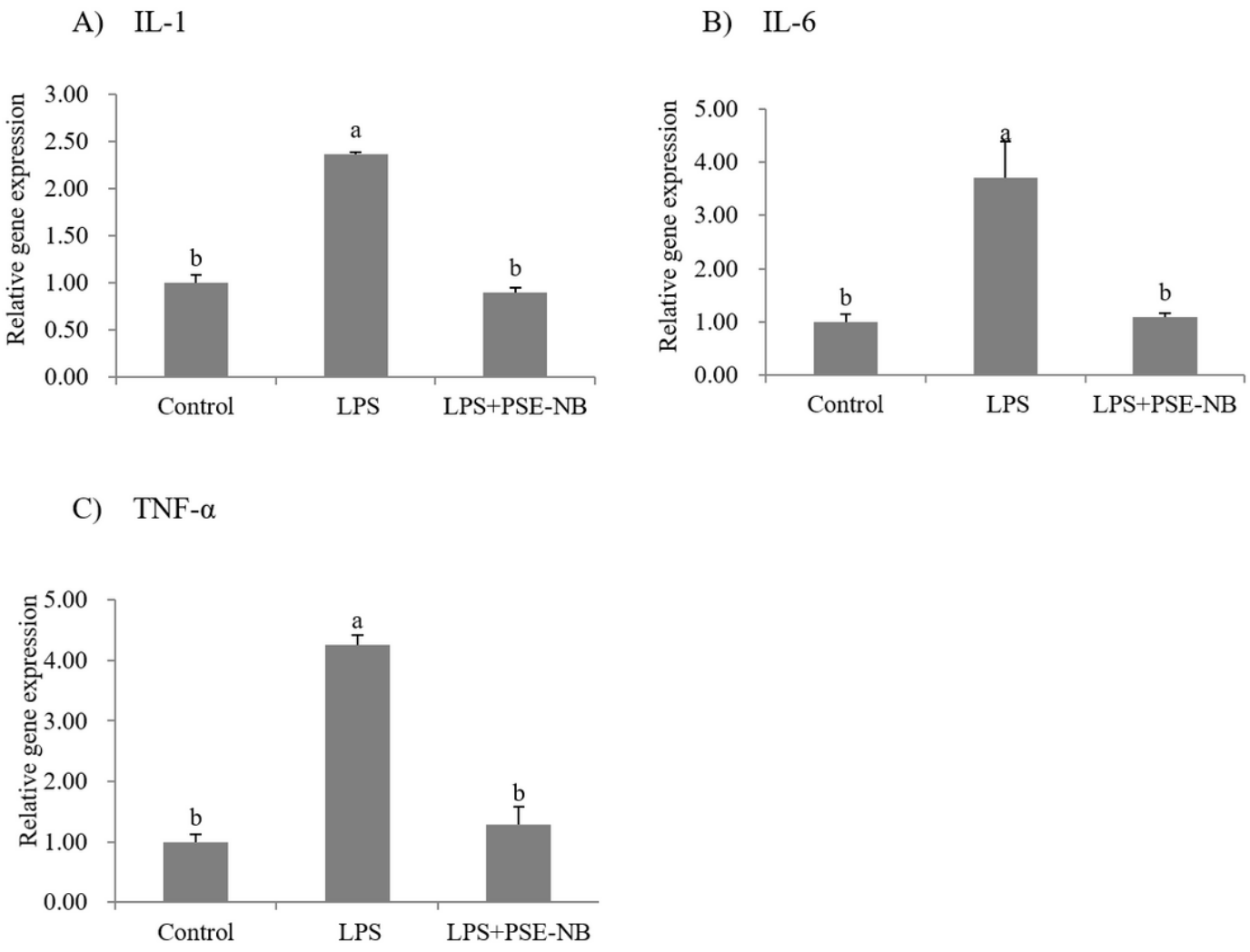


Figure 1

Effect of PSE-NB on LPS-induced cytokine gene expression. IPEC-J2 cells were cultured into a 6-well plate for 24 h. Cells were treated with 10 ug/mL PSE-NB (control and LPS treatment were treated by complete medium) for 24 h. Then, cells were washed with plain medium and then treated with 1 μg/mL of LPS (control was treated by complete medium) for 24 h. Total RNA was extracted from cells and mRNA abundance of IL-1 (A), IL-6 (B) and TNF-α (C) were detected by RT-PCR. The data were presented as mean ± SD, n=5. Different superscript letters represent a significant difference ($P < 0.05$).

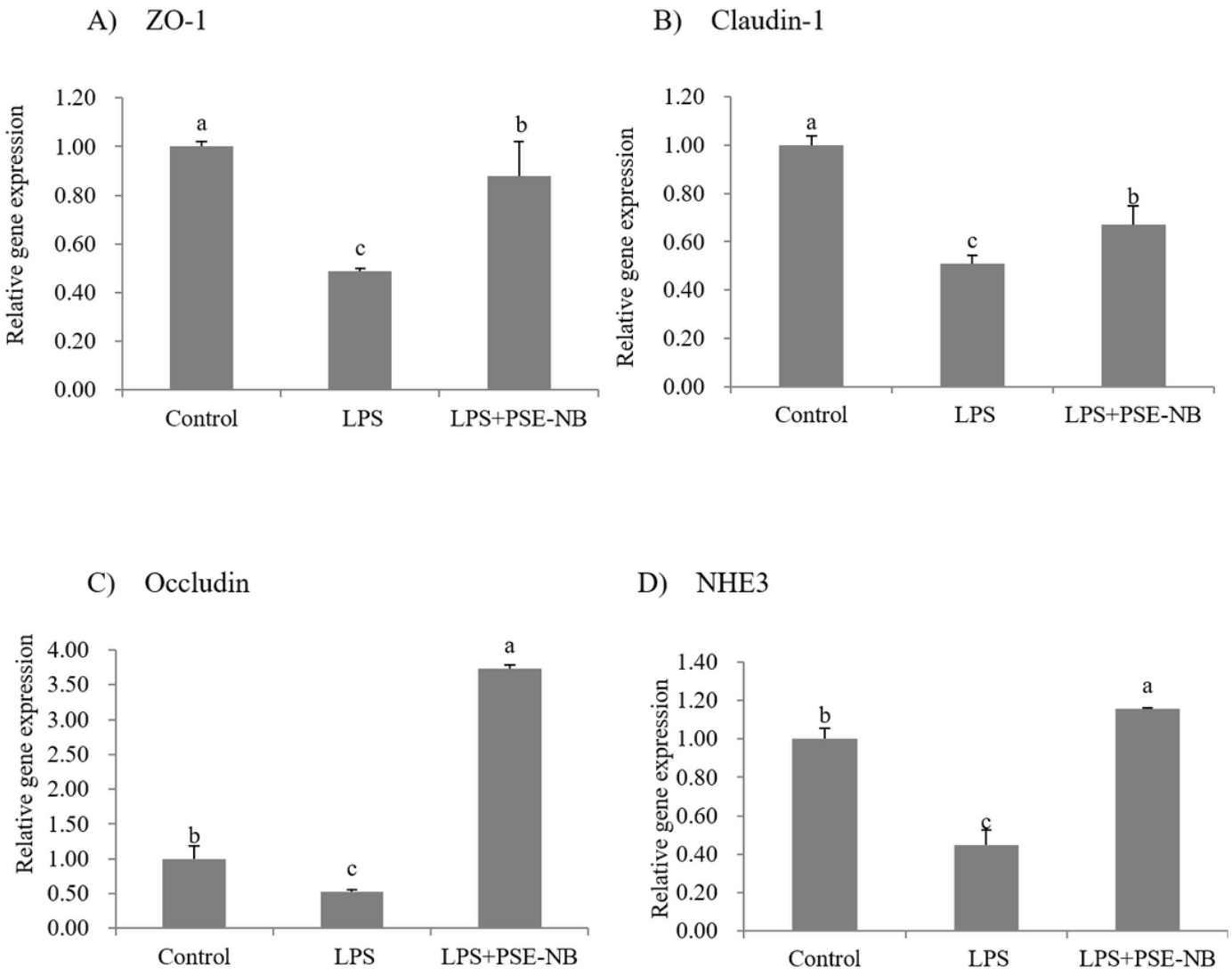


Figure 2

Effect of PSE-NB on LPS-induced tight junction protein gene expression. IPEC-J2 cells were cultured and treated using the same condition as Figure 1. Total RNA was extracted from cells and mRNA abundance of ZO-1(A), Claudin-1 (B), Occludin (C) and NHE3 (D) were detected by RT-PCR. The data were presented as mean ± SD, n=5. Different superscript letters represent a significant difference ($P < 0.05$).

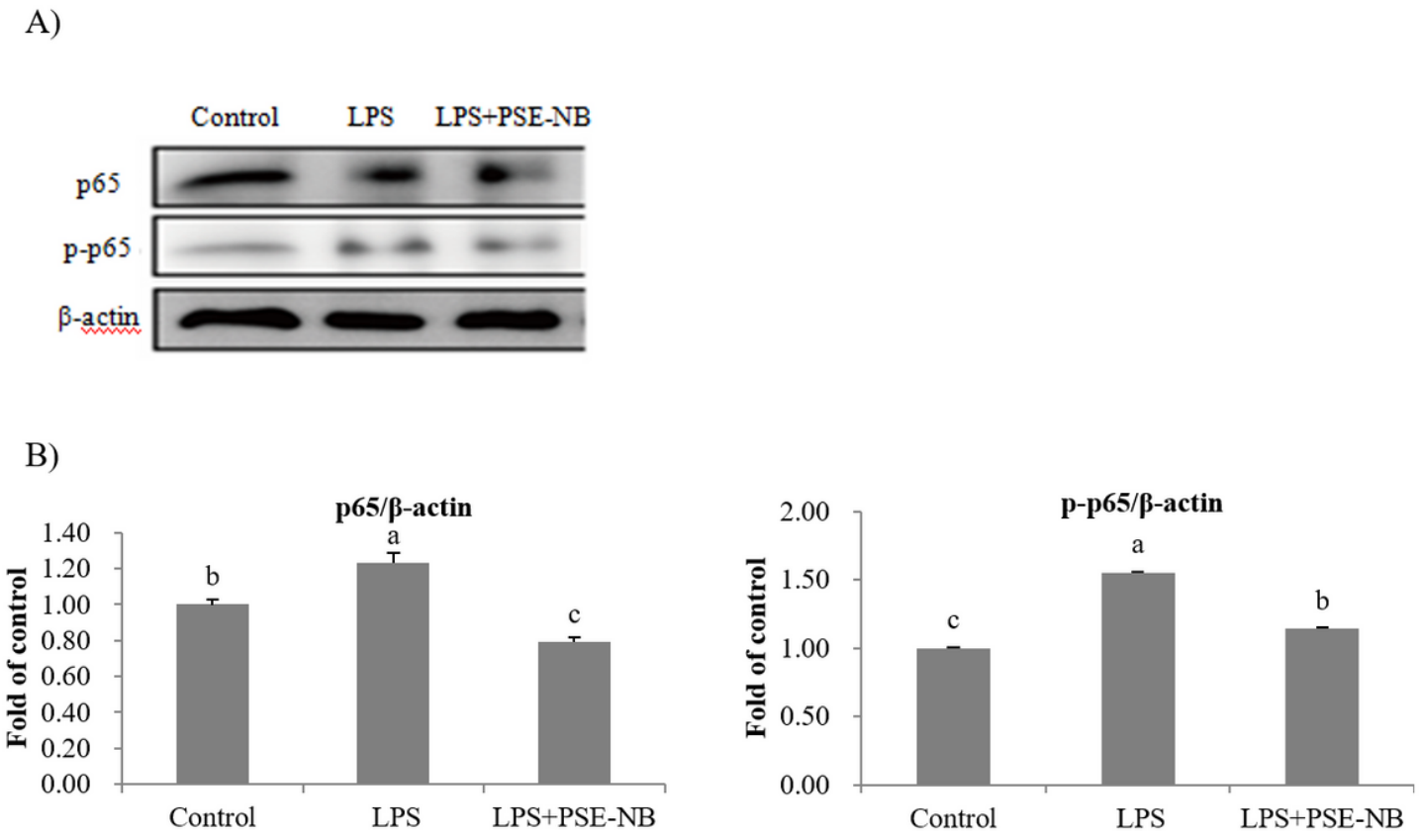


Figure 3

Effect of PSE-NB on protein expression in inflammatory signaling pathway induced by LPS. IPEC-J2 cells were cultured and treated using the same condition as Figure 1. Total protein was extracted from cells and protein expression of p65 and p-p65 were determined by Western blotting (A). The results were normalized to the β-actin standard (B). The data were presented as mean ± SD, n=5. Different superscript letters represent a significant difference ($P < 0.05$).

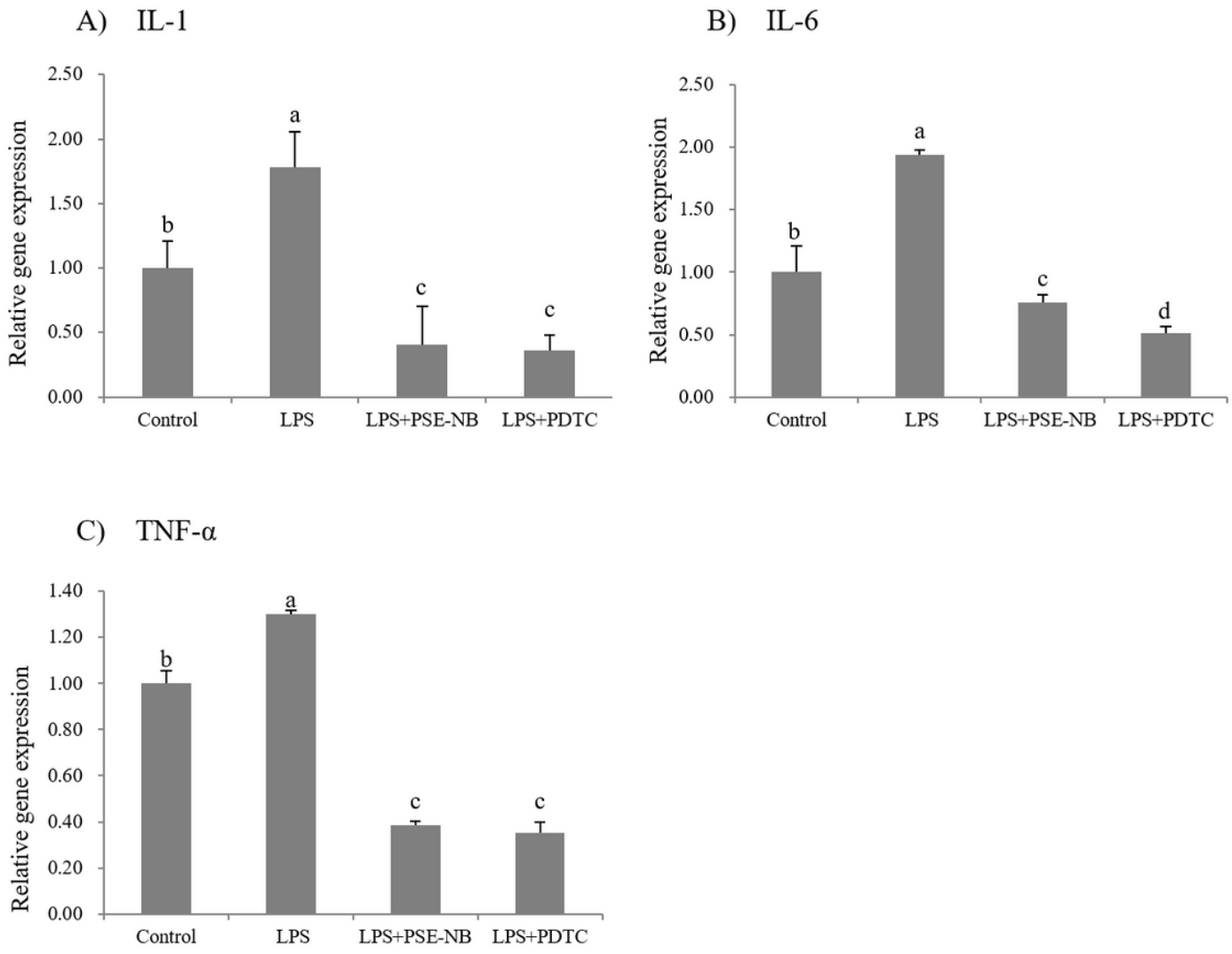
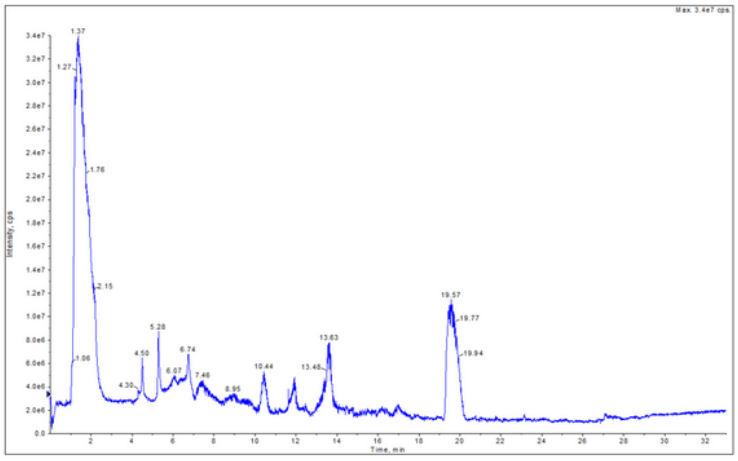


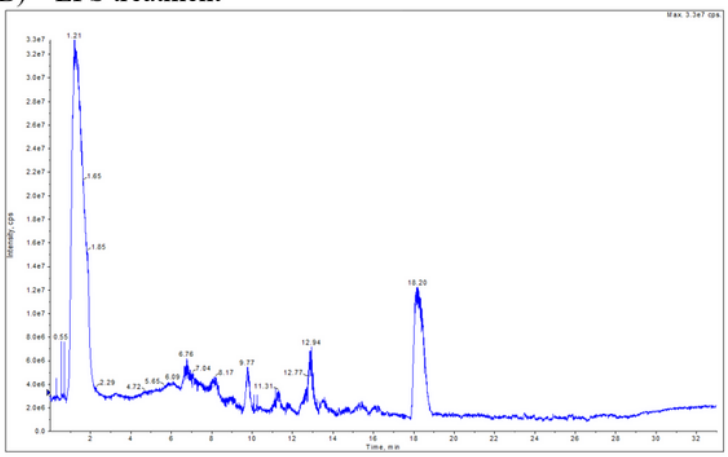
Figure 4

Effect of PSE-NB on LPS-induced cytokine gene expression after inflammatory signaling pathway were blocked. IPEC-J2 cells were cultured into a 6-well plate and were treated with inhibitors (control, LPS and LPS + PSE-NB treatments were treated by complete medium) for 30 min. Then, cells in LPS + PSE-NB treatment were treated with 10 μg/mL PSE-NB (other treatments were treated by complete medium) for 24 h. At last, cells were treated with 1 μg/mL of LPS (control was treated by complete medium) for 24 h. After treatment, total RNA was extracted from cells and mRNA abundance of IL-1 (A), IL-6 (B) and TNF-α (C) were detected by RT-PCR. The data were presented as mean ± SD, n=5. Different superscript letters represent a significant difference ($P < 0.05$).

A）control



B) LPS treatment



C) LPS+PSE-NB treatment

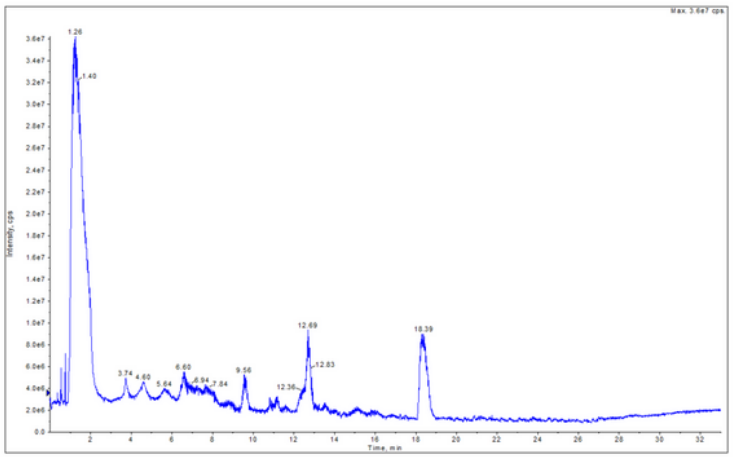
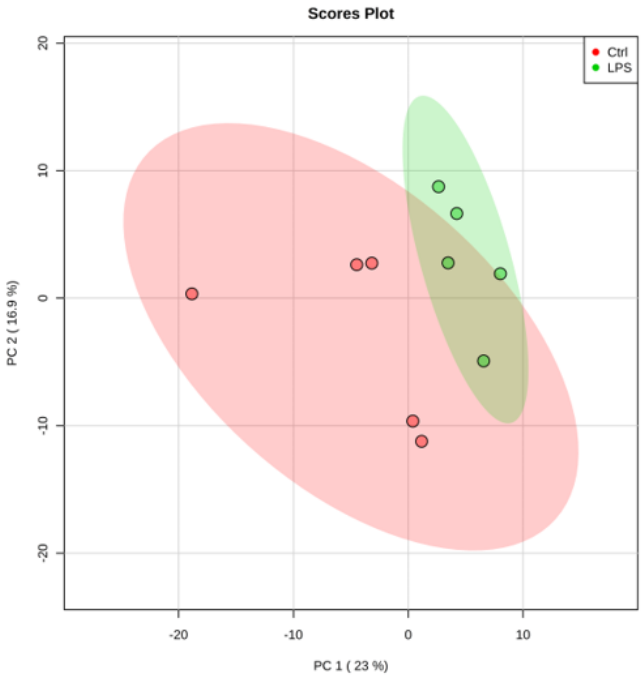


Figure 5

The total ion chromatogram of three treatments.

A) Score plot of the PCA model.



B) Score plot of the PLS model.

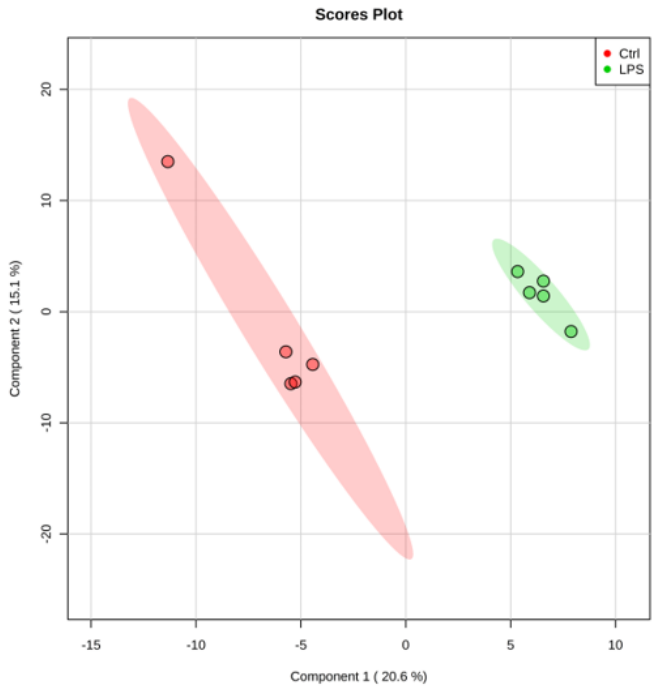


Figure 6

Score plot of the LPS treatment and control.

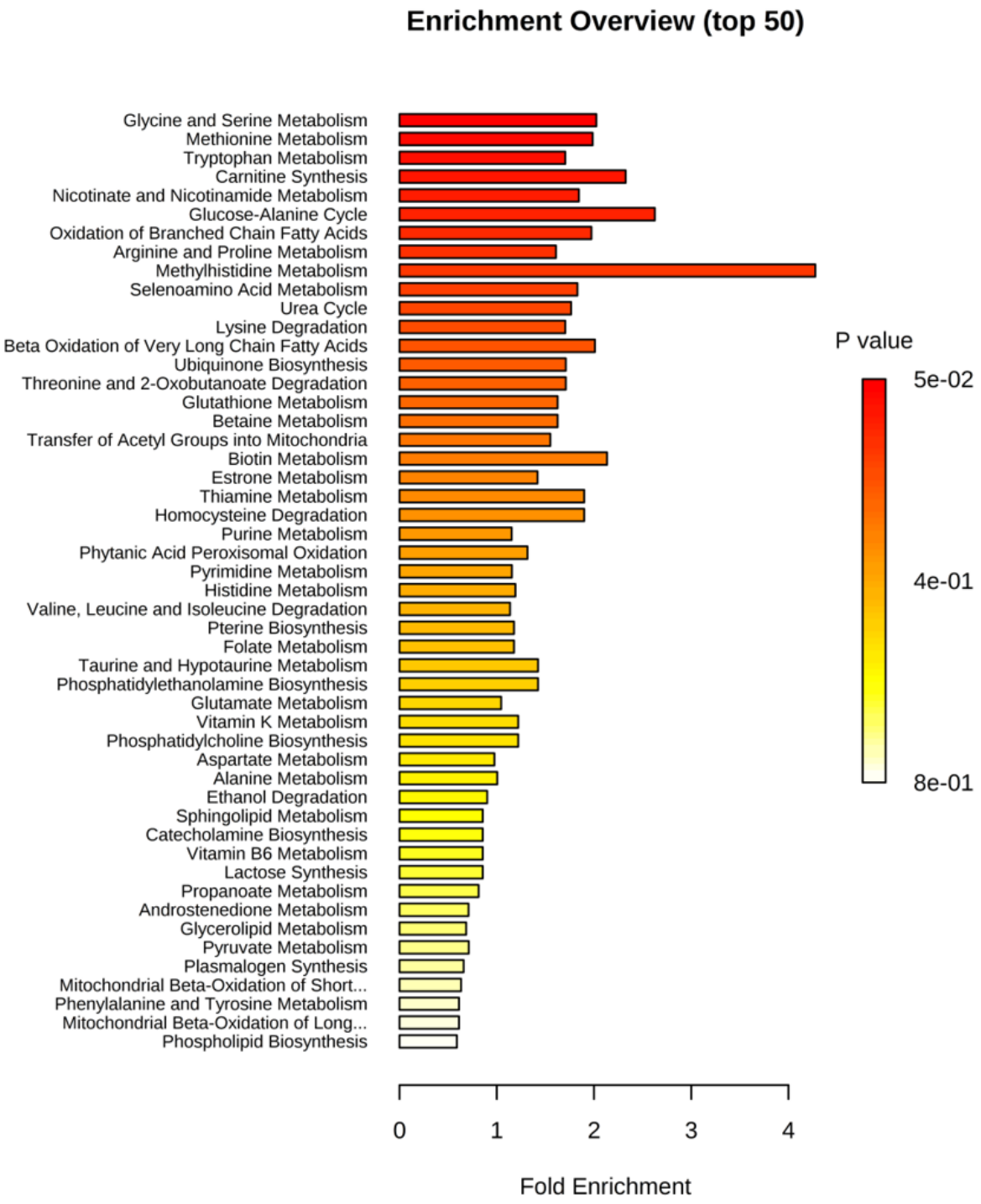
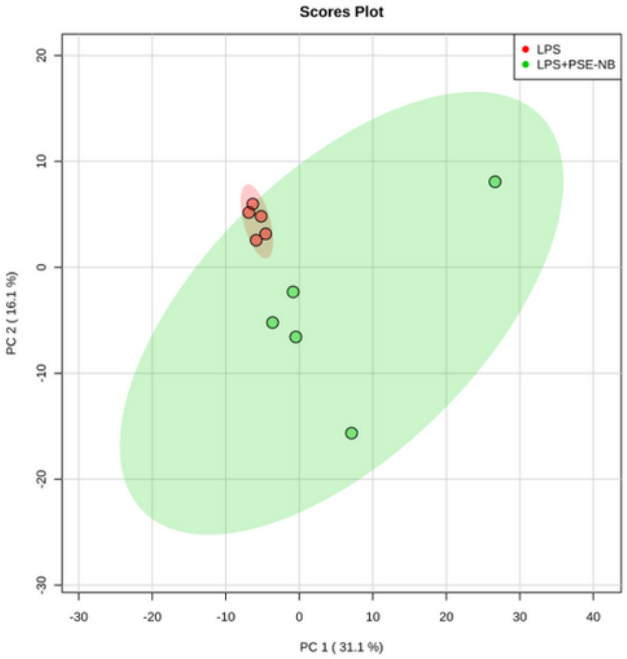


Figure 7

Overview of metabolites that were enriched in IPEC-J2 cells induced by LPS based on IPEC-J2 cell intracellular metabolites.

A) Score plot of the PCA model.



B) Score plot of the PLS model.

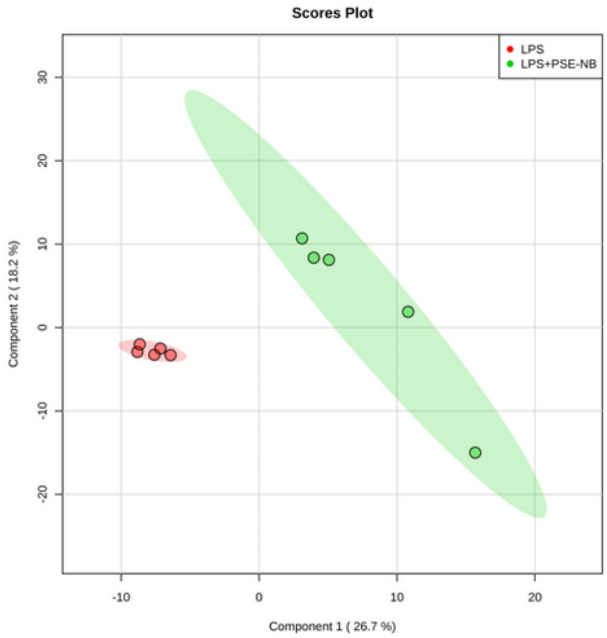


Figure 8

Score plot of the LPS and LPS+PSE-NB treatments.

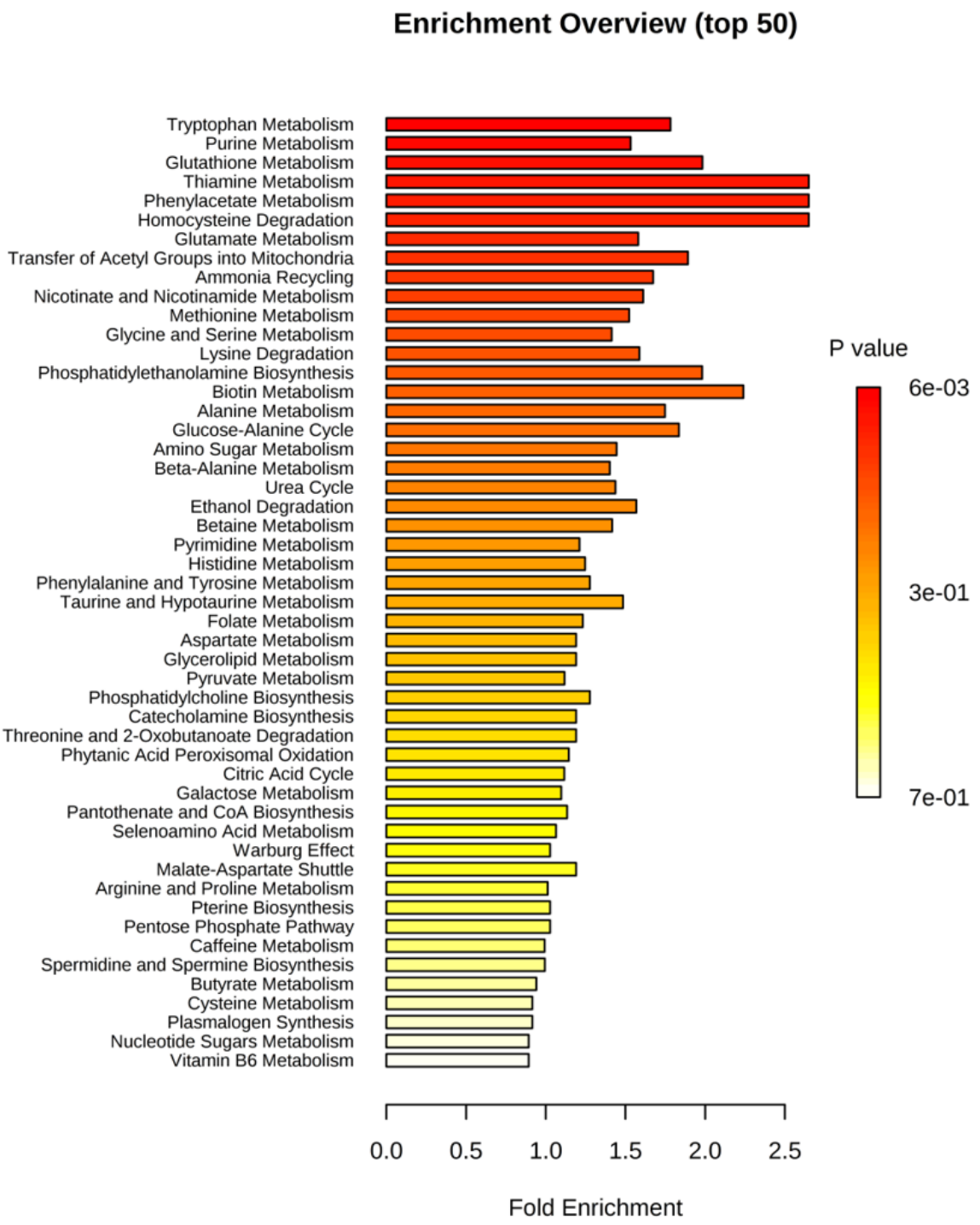


Figure 9

Overview of metabolites that were enriched in IPEC-J2 cells treated by LPS+PSE-NB based on IPEC-J2 cell intracellular metabolites.

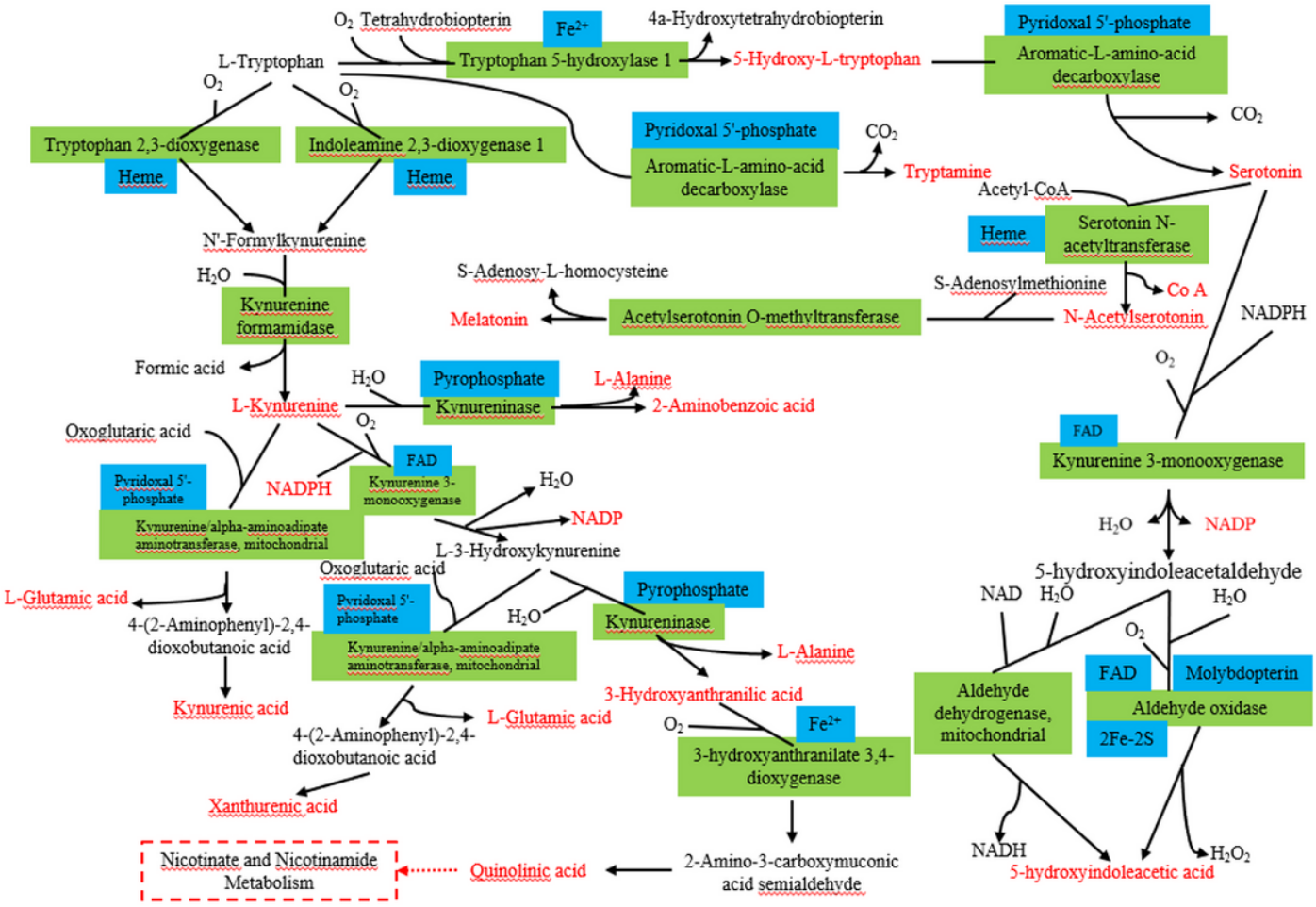


Figure 10

Tryptophan metabolism and its metabolites influenced by LPS and PSE-NB. Degradation of L-tryptophan is initiated by indoleamine 2, 3-dioxygenase, tryptophan 2, 3-dioxygenase, and tryptophan hydroxylase. Its metabolites influenced by LPS and PSE-NB include glutamic acid, alanine, NADP, NADPH, quinolinic acid, serotonin, tryptamine, 5-hydroxytryptophan, ATP, kynurenine, kynurenic acid, 5-hydroxyindoleacetic acid, xanthurenic acid, 2-aminobenzoic acid, N-acetylserotonin, melatonin, coenzyme A and 3-hydroxyanthranilic acid.