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# Effects of monolaurin on intestinal barrier, blood biochemical profile, immunity and antioxidant function in porcine epidemic diarrhoea virus-infected piglets

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## Abstract

The effects of monolaurin (ML) on the health of piglets infected with porcine epidemic diarrhoea virus (PEDV) have not been fully understood. This study aimed to investigate its role in blood biochemical profile, intestinal barrier function, antioxidant function and the expression of antiviral genes in piglets infected with PEDV. Thirty-two piglets were randomly divided into four groups: control group, ML group, PEDV group and ML + PEDV group. Piglets were orally administrated with ML at a dose of 100 mg/kg·BW for 7 d before PEDV infection. Results showed that PEDV infection significantly decreased D-xylose content and increased intestinal fatty acid-binding protein content, indicating that PEDV infection destroyed intestinal barrier and absorption function. While it could be repaired by ML administration. Moreover, ML administration significantly decreased plasma blood urea nitrogen and total protein content upon PEDV infection. These results suggested ML may increase protein utilisation efficiency. ML administration significantly decreased the number of large unstained cells and Hb and increased the number of leucocytes and eosinophils in the blood of PEDV-infected piglets, indicating ML could improve the immune defense function of the body. In the presence of PEDV infection, ML administration significantly increased superoxide dismutase and catalase activities in blood and colon, respectively, indicating ML could improve antioxidant capacity. Besides, ML administration reversed the expression of ISG15, IFIT3 and IL-29 throughout the small intestine and Mx1 in jejunum and ileum, indicating the body was in recovery from PEDV infection. This study suggests that ML could be used as a kind of feed additive to promote swine health upon PEDV infection.

**Keywords:** **Monolaurin: Piglets: PEDV: Intestine: Immunity: Antioxidant**

Pigs of all ages can be infected with porcine epidemic diarrhoea virus (PEDV), with newborn piglets being more susceptible, and the mortality rate could reach up to 90 %[1]. The main symptoms include severe vomiting, diarrhoea and dehydration. This virus colonises in intestinal villous epithelial cells and is excreted with the feces. PEDV is highly variable and can be co-infected with multiple pathogens (bacteria or viruses). The disease was first reported in the UK[2], followed by successive reports in multiple countries in Europe and Asia, including Canada, Germany and Japan[3,4]. In China, a similar case of acute diarrhoeal disease in pigs was found in Shanghai in 1973. However, this intestinal disease was not recognised as PEDV until 1984[5,6]. In 2010, a large outbreak of PED caused the death of millions of pigs in China[6]. Currently, vaccines are commonly used to prevent PEDV infection. However, due to PEDV's susceptibility to mutations, it is necessary to search for new potential preventive or therapeutic agents.

Monolaurin (ML), also known as 1-monolauroylglycerol, is a natural compound present in breast milk, palm oil or coconut oil. As a medium-chain fatty acid, ML can cross cell membranes and provide energy[7,8]. ML is generally used as safe and is commonly used as a food safety emulsifier. Studies have shown that ML is a broad-spectrum bacteriostatic agent, and its administration can improve the growth performance of animals, promote nutrient absorption and enhance immune function while reducing inflammatory cytokine production[9]. ML has also been shown to have a positive effect on parasitic and viral infection[10]. It can significantly alleviate the damage of herpes virus to the body[11]. In addition, ML administration can effectively prevent the spread of this virus and slow down the development of the disease[12]. It has been shown that adding ML to the diet of laying hens can promote the egg production rate and egg quality and has a positive effect on the intestinal morphological structure of laying hens[13]. Our previous study demonstrated that ML could

**Abbreviations:** CAT, catalase; DAO, diamine oxidase; EOS, eosinophils; GSH-Px, glutathione peroxidase; iFABP, intestinal fatty acid-binding protein; LUC, large unstained cells; ML, monolaurin; PEDV, porcine epidemic diarrhea virus; T-SOD, total superoxide dismutase.

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suppress PEDV replication and alleviate the diarrhoea caused by PEDV infection[14]. However, its effect on the health of PEDV-infected piglets has not been fully understood. Therefore, this study aimed to investigate whether ML supplementation could improve the overall health of PEDV-infected piglet, which would provide a theoretical basis for the application of ML in piglet production.

## Materials and methods

### Animal care and diets

The 7-day-old piglets used in this study were purchased from a PEDV-negative farm. All piglets were housed separately to prevent cross-infection, and each cage was equipped with a feeding tank and drinkers to allow piglets to eat and strictly disinfected every day. The temperature was maintained at 28–30°C. The piglets were exclusively fed artificial formula milk, and its nutrient components are shown in Table 1. The living environment followed animal welfare guidelines in the whole experimental period.

### Porcine epidemic diarrhoea virus and monolaurin

PEDV (Yunnan strain, GenBank accession number KT021228) was provided by State Key Laboratory of Microbiology, Huazhong Agricultural University. ML was purchased from Sinopharm Chemical Reagent Co., Ltd. Milk powder was purchased from Newruizi Food Co., Ltd.

### Experimental design

The animal protocol followed in this study was approved by the Animal Care and Committee of Hubei Province (WPU201904002). Thirty-two (Duroc × Landrace × Yorkshire) healthy piglets with an average body weight of (2·50 ± 0·15 kg) were randomly divided into four treatment groups: control group, ML group, PEDV group and ML + PEDV group, and each group contained eight piglets with half female and half male. The whole experimental period lasted 11 d, including a 3-day adaptation period and an 8-day experimental stage. On the 4th to 10th day of the experiment, piglets in ML group and ML + PEDV group were orally administered with 100 mg/kg BW ML (purity ≥ 98 %, dissolved in artificial milk) every day, and piglets in control group and PEDV group were orally administered with artificial milk; on the 8th day of the experiment, piglets in PEDV group and ML + PEDV group were orally administered with PEDV at the dose of $10^{4·5}$ $TCID_{50}$. The dose of ML and PEDV was chosen according to our previous study[14]. All the piglets were fasted at 22.00 on the 10th day of the experiment, and weighed at 07.00 on the next day. Then all the piglets were orally administered with 10 % D-xylose at a dose of 1 ml/kg BW. One hour later, blood was collected for biochemical analysis immediately, and piglets were anaesthetised intramuscularly with pentobarbital Na (50 mg/kg BW) and slaughtered for sampling.

**Table 1.** Nutrient reference value of experimental diets*

| Items | Per 100 g | NRV% |
|---|---|---|
| Energy | 2131 KJ | 25 % |
| Protein | 24·2 g | 40 % |
| Fat | 28·6 g | 48 % |
| Carbohydrate | 38·9 g | 13 % |
| Sodium | 264 mg | 13 % |
| Calcium | 875 mg | 109 % |

* Suckling piglets were fed a predominantly artificial milk-based diet formulated to meet all nutritional requirements recommended by the National Research Council (1998).

### Blood sample collection

Blood was collected from the anterior vena cava of piglets into vacuum blood collection tubes containing sodium heparin as anticoagulant (Becton-Dickinson V acutainer System). Then blood was allowed to sit at room temperature for 15 min before being centrifuged at 3000 rpm for 10 min at 4°C to obtain plasma. The plasma was collected and stored at −80°C[15].

### Intestinal sample collection

After the piglets were anaesthetised and slaughtered, they were dissected on ice and intestinal samples from each segment were removed[16,17]. The 10-cm intestinal segment was cut longitudinally along the mesentery and washed with normal saline. The washed intestinal segment was minced and packaged in sterilised foil paper on ice, snap-frozen in liquid nitrogen and stored at –80°C for subsequent analysis[18].

### Plasma biochemical parameters

Plasma biochemical parameters, including total bilirubin, total protein, albumin, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, total cholesterol, TAG, glucose, Ca, phosphorus (P), creatinine, LDL, LDL, glutamyl transpeptidase, blood urea nitrogen and creatine kinase were measured by Hitachi 7100 automatic biochemical analyser. These indicators were tested on the day of blood collection and repeated three times.

### Blood cell counts

The whole blood cell count analysis was performed using a Siemens ADVIA®2120iautomated blood cell analyser. The indicators detected were as follows: leucocyte, neutrophils, lymphocytes, eosinophils, basophils, large unstained cells (LUC), platelets, mean platelet volume, erythrocytes, Hb, mean corpuscular Hb concentration, mean corpuscular Hb. This experiment was conducted on the day of blood collection, and each sample was tested three times.

### Chemical analysis

Tissue samples were ground into powder in liquid nitrogen, then resuspended with cold saline at a mass: volume ratio of 1:9, homogenised using a frozen grinder, centrifuged at 3000 rpm for 10 min at 4°C, and the supernatant was collected and stored at −80°C.

The contents of diamine oxidase (DAO), D-xylose and intestinal fatty acid-binding protein (iFABP) in plasma were detected according to the manufacturer's instructions. These kits were purchased from Nanjing Jiancheng Bioengineering Institute.

**Table 2.** Primer sequences used in this study

| Genes* | | Primer sequences‡ |
|---|---|---|
| RPL4† | Forward | 5′-GAGAAACCGTCGCCGAAT-3′ |
| | Reverse | 5′-GCCCACCAGGAGCAAGTT-3′ |
| ISG15 | Forward | 5′-AGCATGGTCCTGTTGATGGTG-3′ |
| | Reverse | 5′-CAGAAATGGTCAGCTTGCACG-3′ |
| Mx1 | Forward | 5′-AGTGCGGCTGTTTACCAAG-3′ |
| | Reverse | 5′-TTCACAAACCCTGGCAACTC-3′ |
| IFIT3 | Forward | 5′-GCATTTTCCAGCCAGCATC-3′ |
| | Reverse | 5′-TCTGTTCCTTTCCTTTCCTTCCT-3′ |
| IL-29 | Forward | 5′-ACATCCACGTCGAACTTCAG-3′ |
| | Reverse | 5′-CAGCCTTGGGACTCTTTCTT-3′ |

* RPL4, ribosomal protein L4. ISG15, interferon-stimulating factor 15. MX1, antimyxovirus protein 1. IFIT3, interferon induced transmembrane protein 3. IL-29, interleukin-29.
† RPL4 used as a reference, the primer sequence starts at 5′ ends and ends at 3′ ends.
‡ To avoid the potentially contamination of genomic DNA, the primers were designed to span intron/exon boundaries.

Antioxidant enzyme activities in plasma and intestinal mucosa were analysed as described previously[19]. Catalase (CAT), total superoxide dismutase (T-SOD) and glutathione peroxidase (GSH-Px) detection kids were purchased from Nanjing Jiancheng Bioengineering Institute.

### *Real-time PCR*

Primer sequences used in this study are shown in Table 2. Total RNA was extracted using the RNAiso Plus. Reverse transcription was performed using the PrimeScript®RT reagent Kit with gDNA Eraser. Real-time PCR was performed using the SYBR® Premix Ex TaqTM (Tli RNaseHPlus) kit, which were purchased from Takara. The RPL4 gene was used as a reference in the experiment, the relative gene expression was calculated using $2^{-\Delta\Delta Ct}$ method as described by Hou et al[20]. The experiment was independently repeated at least three times with three technical replicates.

### *Statistical analysis*

All data were analysed by multivariate analysis of variance using SPSS 20.0 statistical software. When there was a significant interaction between groups, Duncan 's method was used for multiple comparisons. The test results were expressed as 'mean', and $P$ value was used as the judgement criteria for significant differences. $P < 0{\cdot}05$ indicated significant differences, and $P < 0{\cdot}01$ indicated extremely significant differences.

## Results

### *Effect of monolaurin on plasma diamine oxidase activity, D-xylose and intestinal fatty acid-binding protein concentrations in porcine epidemic diarrhoea virus-infected piglets*

As shown in Fig. 1, PEDV infection significantly decreased the concentration of D-xylose, increased the concentration of iFABP and slightly increased DAO activity in blood; ML administration significantly increased the concentration of D-xylose and tended to decrease the concentration of iFABP.

### *Effect of monolaurin on plasma biochemical parameters in porcine epidemic diarrhea virus-infected piglets*

As shown in Table 3, PEDV infection significantly decreased the contents of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, total cholesterol, P, HDL and LDL in blood ($P < 0{\cdot}05$) and significantly increased the contents of total bilirubin, total protein, ALB, creatinine, glutamyl transpeptidase and blood urea nitrogen ($P < 0{\cdot}05$) compared with the control group. While the contents of total bilirubin, total protein, CA, creatine kinase and blood urea nitrogen were significantly decreased ($P < 0{\cdot}05$), $P$ was increased significantly ($P < 0{\cdot}05$) with ML administration.

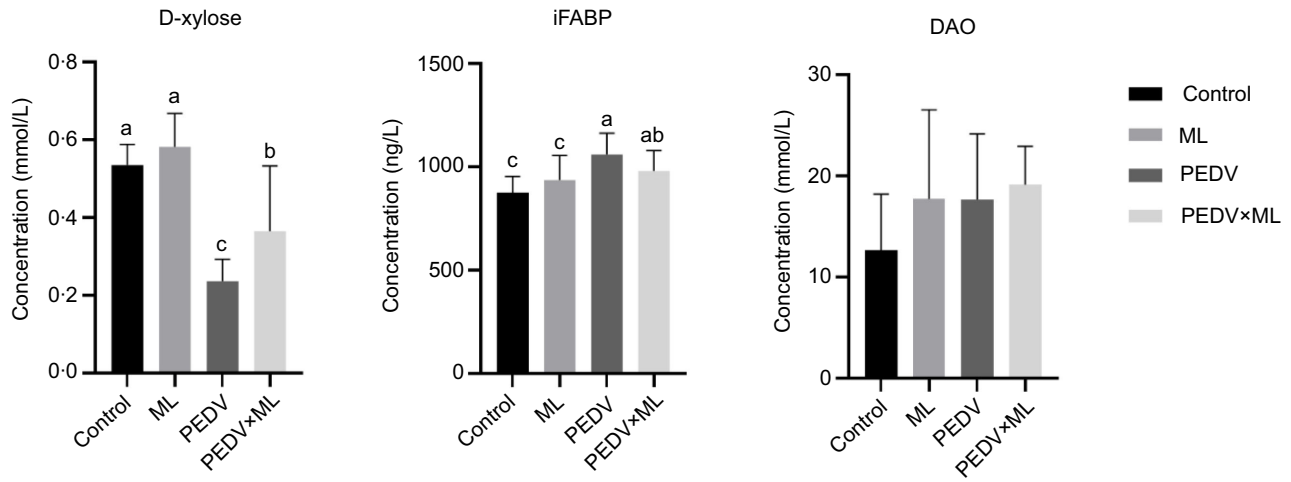


**Fig. 1.** Effect of ML administration on intestinal barrier function. ML, monolaurin; DAO, diamine oxidase; iFABP, intestinal fatty acid binding protein; a-c Means within rows with different superscripts differ ($P < 0{\cdot}05$).

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**Table 3.** Effects of ML on plasma biochemical parameters in PEDV-infected piglets§

| | –PEDV† | | +PEDV | | | *P* value | | |
|---|---|---|---|---|---|---|---|---|
| Items* | –ML | +ML | –ML | +ML | SEM‡ | PEDV | ML | PEDV × ML |
| Plasma | | | | | | | | |
| TB (mg/dl) | 0·100$^{bc}$ | 0·213$^{a}$ | 0·127$^{b}$ | 0·063$^{c}$ | 0·01 | 0·005 | 0·248 | < 0·001 |
| TP (g/dl) | 52·750$^{c}$ | 49·663$^{c}$ | 62·775$^{a}$ | 57·702$^{b}$ | 1·12 | <0·001 | 0·013 | 0·527 |
| ALB (g/dl) | 21·350$^{b}$ | 23·000$^{b}$ | 26·450$^{a}$ | 25·439$^{a}$ | 0·47 | <0·001 | 0·639 | 0·057 |
| AST (U/l) | 39·250$^{a}$ | 37·250$^{a}$ | 28·750$^{b}$ | 27·50$^{b}$ | 1·43 | <0·001 | 0·501 | 0·876 |
| ALT (U/l) | 29·444$^{a}$ | 27·625$^{ab}$ | 24·734$^{b}$ | 26·660$^{ab}$ | 0·60 | 0·014 | 0·962 | 0·097 |
| ALP (U/l) | 638·875$^{a}$ | 645·576$^{a}$ | 481·000$^{b}$ | 514·188$^{b}$ | 20·39 | <0·001 | 0·56 | 0·698 |
| TC (mg/dl) | 119·357$^{a}$ | 115·589$^{a}$ | 100·874$^{b}$ | 93·156$^{b}$ | 2·59 | <0·001 | 0·15 | 0·616 |
| TG (mg/dl) | 62·094 | 50·168 | 49·134 | 52·037 | 2·28 | 0·217 | 0·314 | 0·102 |
| GLU (mg/dl) | 108·713 | 104·755 | 113·000 | 106·478 | 2·22 | 0·51 | 0·25 | 0·78 |
| CA (mg/dl) | 10·939$^{ab}$ | 10·840$^{ab}$ | 11·051$^{a}$ | 10·641$^{b}$ | 0·06 | 0·715 | 0·037 | 0·193 |
| P (mg/dl) | 8·108$^{a}$ | 7·946$^{ab}$ | 6·777$^{c}$ | 7·613$^{b}$ | 0·12 | <0·001 | 0·047 | 0·004 |
| CREA (mg/dl) | 55·969$^{b}$ | 54·297$^{b}$ | 65·918$^{a}$ | 63·203$^{a}$ | 1·05 | <0·001 | 0·124 | 0·71 |
| HDL (mg/dl) | 85·031$^{a}$ | 86·913$^{a}$ | 61·511$^{b}$ | 58·838$^{b}$ | 2·94 | <0·001 | 0·924 | 0·583 |
| LDL (mg/dl) | 61·904 | 62·664 | 59·188 | 55·231 | 1·20 | 0·034 | 0·49 | 0·31 |
| GGT (U/l) | 34·469$^{ab}$ | 27·219$^{b}$ | 40·156$^{a}$ | 37·250$^{a}$ | 1·48 | 0·005 | 0·058 | 0·408 |
| BUN (mg/dl) | 1·089$^{c}$ | 1·283$^{c}$ | 6·029$^{a}$ | 5·023$^{b}$ | 0·40 | <0·001 | 0·177 | 0·050 |
| CK (U/l) | 306·000$^{ab}$ | 286·333$^{ab}$ | 374·556$^{a}$ | 251·889$^{b}$ | 20·10 | 0·665 | 0·077 | 0·196 |

* ML, monolaurin; TB, total bilirubin; TP, total protein; ALB, albumin; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; TC, total cholesterol; GLU, glucose;. CREA, creatinine; GGT, glutamyl transpeptidase; BUN, blood urea nitrogen; CK, creatine kinase.
† –PEDV, without PEDV infection. +PEDV, with PEDV infection. –ML, without ML addition. +ML, with ML addition.
‡ Values are mean and SD, SEM stands for standard error. *n* 8. $^{a-d}$ Means within rows with different superscripts differ ($P < 0·05$).
§ The experiment used a 2 × 2 factorial arrangement consisting of four treatment groups: Non-challenged Group: Piglets were fed artificial milk only and no other substances were added. ML-challenged Group: Piglets were fed artificial milk and supplemented with ML nutrients. PEDV-challenged Group: Piglets fed artificial milk and were fed with porcine epidemic diarrhoea virus. PEDV × ML challenged Group: Piglets were fed artificial milk, porcine epidemic diarrhoea virus and ML nutrients.

**Table 4.** The whole blood cell counts in piglets§

| | –PEDV† | | +PEDV | | | *P* value | | |
|---|---|---|---|---|---|---|---|---|
| Items* | –ML | +ML | –ML | +ML | SEM‡ | PEDV | ML | PEDV × ML |
| Plasma | | | | | | | | |
| WBC (10^3/μl) | 10·971$^{a}$ | 8·576$^{b}$ | 8·100$^{b}$ | 9·713$^{ab}$ | 0·32 | 0·117 | 0·472 | 0·001 |
| NEUT (10^3/μl) | 4·388$^{ab}$ | 3·540$^{ab}$ | 3·106$^{b}$ | 4·960$^{a}$ | 0·27 | 0·893 | 0·327 | 0·012 |
| LYMPH (10^3/μl) | 5·880 | 4·143 | 4·398 | 4·437 | 0·21 | 0·105 | 0·023 | 0·018 |
| EOS (10^3/μl) | 0·114$^{a}$ | 0·069$^{bc}$ | 0·048$^{c}$ | 0·086$^{ab}$ | 0·01 | 0·025 | 0·717 | <0·001 |
| BASO (10^3/μl) | 0·049 | 0·029 | 0·036 | 0·036 | 0·00 | 0·872 | 0·422 | 0·042 |
| LUC (10^3/μl) | 0·242$^{b}$ | 0·217$^{b}$ | 0·317$^{a}$ | 0·259$^{b}$ | 0·01 | 0·002 | 0·024 | 0·368 |
| LYMPH (%) | 56·962$^{a}$ | 51·026$^{b}$ | 53·903$^{ab}$ | 43·232$^{c}$ | 1·24 | 0·007 | <0·001 | 0·214 |
| EOS (%) | 1·000$^{a}$ | 0·812$^{a}$ | 0·581$^{b}$ | 0·817$^{a}$ | 0·04 | 0·011 | 0·757 | 0·009 |
| LUC (%) | 2·573$^{b}$ | 2·838$^{b}$ | 3·683$^{a}$ | 3·113$^{b}$ | 0·11 | 0·001 | 0·406 | 0·028 |
| PLT (10^3/μl) | 458·25 | 415·000 | 493·589 | 474·141 | 20·39 | 0·262 | 0·455 | 0·776 |
| MPV (fL) | 7·100$^{b}$ | 8·062$^{a}$ | 8·126$^{a}$ | 8·489$^{a}$ | 0·14 | 0·004 | 0·009 | 0·215 |
| RBC (10^6/μl) | 6·830$^{a}$ | 5·737$^{c}$ | 6·277$^{b}$ | 6·616$^{a}$ | 0·09 | 0·145 | 0·002 | <0·001 |
| HGB (g/dl) | 116·503$^{b}$ | 111·283$^{c}$ | 122·781$^{a}$ | 119·791$^{ab}$ | 1·11 | <0·001 | 0·026 | 0·532 |
| MCHC (g/dl) | 305·909$^{a}$ | 301·013$^{b}$ | 303·069$^{ab}$ | 296·579$^{c}$ | 0·80 | 0·004 | <0·001 | 0·502 |
| CHCM (g/dl) | 322·750$^{b}$ | 323·120$^{b}$ | 333·048$^{a}$ | 326·378$^{ab}$ | 1·47 | 0·017 | 0·252 | 0·202 |
| MCH (pg) | 18·467$^{ab}$ | 18·938$^{a}$ | 18·712$^{a}$ | 17·733$^{b}$ | 0·15 | 0·096 | 0·371 | 0·014 |

* ML, monolaurin; NEUT, neutrophils; LYMPH, lymphocytes; EOS, eosinophils; BASO, basophils; LUC, large unstained cells; PLT, platelets; MPV, mean platelet volume; RBC, erythrocytes; MCHC, mean corpuscular Hb concentration; MCH, mean corpuscular Hb.
† –PEDV, without PEDV infection. +PEDV, with PEDV infection. –ML, without ML addition. +ML, with ML addition.
‡ Values are mean and SD, SEM stands for standard error. *n* 8. $^{a-d}$ Means within rows with different superscripts differ ($P < 0·05$).
§ The experiment used a 2 × 2 factorial arrangement consisting of four treatment groups: Non-challenged Group: Piglets were fed artificial milk only and no other substances were added. ML-challenged Group: Piglets were fed artificial milk and supplemented with ML nutrients. PEDV-challenged Group: Piglets fed artificial milk and were fed with porcine epidemic diarrhea virus. PEDV × ML challenged Group: Piglets were fed artificial milk, porcine epidemic diarrhea virus and ML nutrients.

### *Effect of monolaurin on the whole blood cell count in porcine epidemic diarrhoea virus-infected piglets*

As shown in Table 4, PEDV infection significantly decreased the number of leucocytes, eosinophils and the percentage of eosinophils in blood ($P < 0·05$) and significantly increased the number of LUC, mean platelet volume, Hb, CHCM and the percentage of LUC ($P < 0·05$). The number of Hb, mean corpuscular Hb, LUC and the percentage of LUC and lymphocytes were significantly decreased ($P < 0·05$), and the number of erythrocytes, neutrophils, eosinophils and the percentage of eosinophils were significantly increased ($P < 0·05$) by ML administration. The number of leucocytes was increased by ML administration with no statistically significant difference.

**Table 5.** Levels of anti-oxidative enzymes in plasma and intestinal mucosa§

| | –PEDV† | | +PEDV | | | *P* value | | |
|---|---|---|---|---|---|---|---|---|
| Items* | –ML | +ML | –ML | +ML | SEM‡ | PEDV | ML | PEDV × ML |
| Plasma | | | | | | | | |
| CAT (U/ml) | 23·12$^{a}$ | 14·40$^{b}$ | 8·39$^{c}$ | 9·32$^{c}$ | 8·08 | <0·001 | 0·033 | 0·010 |
| T-SOD (U/ml) | 85·63$^{a}$ | 85·74$^{a}$ | 77·23$^{c}$ | 80·97$^{b}$ | 0·75 | <0·001 | 0·030 | 0·040 |
| GSH-Px (U/ml) | 269·68$^{a}$ | 237·10$^{b}$ | 242·83$^{b}$ | 229·64$^{b}$ | 4·05 | 0·011 | <0·001 | 0·136 |
| Duodenum | | | | | | | | |
| CAT (U/mg) | 16·19$^{a}$ | 12·64$^{b}$ | 8·00$^{c}$ | 9·16$^{c}$ | 0·73 | <0·001 | 0·224 | 0·021 |
| T-SOD (U/mg) | 381·52$^{a}$ | 331·55$^{bc}$ | 337·29$^{b}$ | 305·65$^{c}$ | 6·64 | <0·001 | <0·001 | 0·340 |
| GSH-Px (U/mg) | 67·50$^{ab}$ | 67·22$^{ab}$ | 82·66$^{a}$ | 49·06$^{b}$ | 4·65 | 0·863 | 0·061 | 0·065 |
| Jejunum | | | | | | | | |
| CAT (U/mg) | 8·58$^{a}$ | 6·91$^{b}$ | 3·78$^{c}$ | 4·70$^{c}$ | 0·39 | <0·001 | 0·387 | 0·005 |
| T-SOD (U/mg) | 329·7$^{ab}$ | 341·33$^{a}$ | 341·88$^{a}$ | 304·70$^{b}$ | 5·28 | 0·211 | 0·191 | 0·016 |
| GSH-Px (U/mg) | 60·13 | 42·95 | 56·23 | 51·88 | 4·01 | 0·758 | 0·194 | 0·435 |
| Ileum | | | | | | | | |
| CAT (U/mg) | 7·71 | 7·40 | 7·59 | 8·22 | 0·18 | 0·345 | 0·665 | 0·211 |
| T-SOD (U/mg) | 328·91 | 335·26 | 320·62 | 329·59 | 5·60 | 0·553 | 0·515 | 0·911 |
| GSH-Px (U/mg) | 79·74 | 73·81 | 80·55 | 73·57 | 2·66 | 0·958 | 0·247 | 0·924 |
| Colon | | | | | | | | |
| CAT (U/mg) | 12·35$^{a}$ | 10·30$^{b}$ | 3·91$^{d}$ | 7·68$^{c}$ | 0·63 | <0·001 | 0·138 | <0·001 |
| T-SOD (U/mg) | 341·63 | 322·73 | 320·23 | 303·57 | 7·17 | 0·164 | 0·220 | 0·938 |
| GSH-Px (U/mg) | 197·67$^{a}$ | 145·16$^{b}$ | 128·87$^{b}$ | 81·03$^{c}$ | 8·91 | <0·001 | <0·001 | 0·820 |

* ML, monolaurin; T-SOD, total superoxide dismutase; GSH-Px, glutathione peroxidase; CAT, catalase.
† –PEDV, without PEDV infection. +PEDV, with PEDV infection. –ML, without ML addition. +ML, with ML addition.
‡ Values are mean and SD, SEM stands for standard error. *n* 8. $^{a-d}$ Means within rows with different superscripts differ ($P < 0·05$).
§ The experiment used a 2 × 2 factorial arrangement consisting of four treatment groups: Non-challenged Group: Piglets were fed artificial milk only and no other substances were added. ML-challenged Group: Piglets were fed artificial milk and supplemented with ML nutrients. PEDV-challenged Group: Piglets fed artificial milk and were fed with porcine epidemic diarrhea virus. PEDV × ML challenged Group: Piglets were fed artificial milk, porcine epidemic diarrhea virus and ML nutrients.

**Table 6.** Results of real-time PCR for the detection of antiviral genes‡

| | –PEDV† | | +PEDV | | | *P* value | | |
|---|---|---|---|---|---|---|---|---|
| Items | –ML | +ML | –ML | +ML | SEM* | PEDV | ML | PEDV × ML |
| Duodenum | | | | | | | | |
| ISG15 | 1·000$^{ab}$ | 0·437$^{c}$ | 1·154$^{a}$ | 0·920$^{b}$ | 0·069 | <0·001 | <0·001 | 0·024 |
| Mx1 | 1·000 | 0·740 | 0·950 | 0·836 | 0·069 | 0·743 | 0·012 | 0·298 |
| IFIT3 | 1·000$^{c}$ | 0·888$^{c}$ | 2·156$^{a}$ | 1·474$^{b}$ | 0·076 | <0·001 | <0·001 | 0·001 |
| IL-29 | 1·000$^{b}$ | 0·975$^{b}$ | 1·378$^{a}$ | 1·000$^{b}$ | 0·087 | 0·028 | 0·028 | 0·053 |
| Jejunum | | | | | | | | |
| ISG15 | 1·000$^{c}$ | 0·649$^{c}$ | 3·488$^{a}$ | 2·162$^{b}$ | 0·168 | <0·001 | <0·001 | 0·007 |
| Mx1 | 1·000 | 0·742 | 2·010 | 1·596 | 0·104 | <0·001 | 0·003 | 0·457 |
| IFIT3 | 1·000$^{c}$ | 1·016$^{c}$ | 3·924$^{a}$ | 2·037$^{b}$ | 0·176 | <0·001 | <0·001 | <0·001 |
| IL-29 | 1·000$^{c}$ | 0·975$^{c}$ | 2·692$^{a}$ | 1·760$^{b}$ | 0·139 | <0·001 | 0·002 | 0·003 |
| Ileum | | | | | | | | |
| ISG15 | 1·000 | 0·651 | 2·064 | 1·668 | 0·105 | <0·001 | 0·001 | 0·821 |
| Mx1 | 1·000$^{b}$ | 1·116$^{b}$ | 1·516$^{a}$ | 1·003$^{b}$ | 0·085 | 0·025 | 0·027 | 0·001 |
| IFIT3 | 1·000$^{c}$ | 1·102$^{c}$ | 2·254$^{a}$ | 1·712$^{b}$ | 0·107 | <0·001 | 0·049 | 0·005 |
| IL-29 | 1·000$^{b}$ | 0·974$^{b}$ | 1·701$^{a}$ | 1·125$^{b}$ | 0·084 | <0·001 | 0·001 | 0·003 |

PEDV, porcine epidemic diarrhea virus; ML, monolaurin.
* Values are mean and SD, SEM stands for standard error. *n* 8. $^{a-d}$ Means within rows with different superscripts differ ($P < 0·05$).
† –PEDV, without PEDV infection. +PEDV, with PEDV infection. –ML, without ML addition. +ML, with ML addition.
‡ The experiment used a 2 × 2 factorial arrangement consisting of four treatment groups: Non-challenged Group: Piglets were fed artificial milk only and no other substances were added. ML-challenged Group: Piglets were fed artificial milk and supplemented with ML nutrients. PEDV-challenged Group: Piglets fed artificial milk and were fed with porcine epidemic diarrhea virus. PEDV × ML challenged Group: Piglets were fed artificial milk, porcine epidemic diarrhoea virus and ML nutrients.

### *Levels of anti-oxidative enzymes in plasma and intestinal mucosa*

As shown in Table 5, PEDV infection significantly decreased the activities of CAT, SOD and GSH-Px in plasma, CAT in jejunum, CAT and SOD in duodenum and CAT and GSH-Px in colon ($P < 0·05$). Upon PEDV infection, the activities of SOD in plasma and CAT in colon were increased significantly with ML administration ($P < 0·05$).

### *Effect of monolaurin on the expression of antiviral genes in porcine epidemic diarrhea virus-infected piglets*

As shown in Table 6, PEDV infection significantly increased the expression of ISG15, IFIT3, IL-29 in the small intestine ($P < 0·05$) and increased the expression of Mx1 in the jejunum and ileum. While their expression were reversed with ML administration.

## Discussion

Due to its high genetic diversity, PEDV has become a serious threat to the pig farms and poses a considerable challenge for prevention and control[21]. In this present study, we have demonstrated that ML can alleviate intestinal injury, enhance nutrient absorption, improve immune defense function and antioxidant capacity and reverse the expression of antiviral genes. Additionally, ML may be favourable for protein utilisation in the body. In summary, ML supplementation could promote piglet recovery from PEDV infection. Due to the anatomical, physiological and genomic similarities between pigs and humans, this study provides guidance on the application of ML to prevent virus, especially coronavirus infection in humans[22].

In healthy pigs, D-xylose is only absorbed into the blood by the small intestinal mucosa. When the intestine is damaged, the intestinal absorption function would be diminished, leading to the decrease of D-xylose content in the blood. Therefore, the content of D-xylose in the blood reflects the degree of intestinal injury and absorption ability[23]. DAO is an enzyme in intestinal epithelial cells, and its activity in the blood is typically very low. Upon stimulation, DAO is released from intestinal mucosal epithelial cells and enters the bloodstream. So, the level of DAO activity in the blood reflects whether the intestinal morphological structure of animals is intact[24]. iFABP is only present in intestinal cells, with strong tissue specificity. It could regulate the metabolism of fatty acids. Normally, the content of iFABP in the blood is very low. When the intestinal tract got injured, iFABP enters the blood, then increases the content of iFABP in the blood, so it is commonly used to reflect the degree of intestinal injury[25]. In this experiment, PEDV infection resulted in a significant decrease in D-xylose content, a significant increase in i-FABP content and a slightly increase in DAO activity in the plasma of piglets, indicating that PEDV infection destroyed the intestinal barrier and seriously affected the absorption function of the small intestine of piglets. These results are consistent with previous findings[26]. In the presence of PEDV infection, D-xylose content was significantly increased, and i-FABP content tended to be decreased after oral administration of ML, indicating that ML could protect the intestinal mucosa from damage and enhance the absorption of nutrients in intestine. Similar finding was observed in another study which found dietary ML supplementation could effectively ameliorate intestinal mucosal injury induced by LPS stimulation in broilers[27].

Blood biochemical indicators are important indicators reflecting cell permeability and metabolic function of the body, as well as liver function of the body[28]. Plasma total bilirubin is a product of Hb catabolism. Studies showed that total bilirubin is an endogenous antioxidant that protects against the development of oxidative stress damage[29]. In this study, it was found that plasma total bilirubin content of piglets was increased when ML was administered in the absence of PEDV infection, indicating that ML could enhance the antioxidant capacity of the body. Plasma total protein levels can reflect the capability of protein metabolism in the body[30]. In this study, the content of total protein was significantly increased in the plasma of PEDV-infected piglets, which may be due to the acceleration of protein synthesis or the dehydration result from diarrhoea[30]. While the content of total protein was decreased after ML administration. Plasma blood urea nitrogen content can reflect the protein metabolism and amino acid balance in the body, which is closely related to renal function in clinical practice. In the normal state, the blood urea nitrogen content in plasma is at a low level. While the plasma blood urea nitrogen is greatly increased when renal injury and metabolic dysfunction occurred[31]. In this study, the plasma blood urea nitrogen content of piglets was significantly increased after PEDV infection and significantly downregulated by ML administration, indicating that ML may increase the efficiency of protein utilisation in the body, keep amino acids in a balanced state and alleviate renal damage. Unfortunately, kidney sample was not collected in this experiment. This result suggests that further studies are warranted to verify the PEDV infection in the kidney and its effect on the renal function.

Leucocytes are important immune cells and are very important for maintaining the immune function of the body. They can cross the capillary wall through deformation, concentrate at the site of pathogen invasion and surround the pathogen for phagocytosis[32]. In this study, the number of white blood cells and eosinophils in the blood of piglets significantly decreased after PEDV infection, while the numbers of lymphocytes and Hb significantly increased. This may be attributed to immune stress on the body following viral infection, resulting in severe acute inflammation in the intestine, or excessive loss of body fluids due to severe diarrhoea, leading to haemoconcentration. The significant decrease in lymphocyte and Hb numbers and increase in white blood cell and eosinophil numbers following ML administration suggest that ML can improve immune defense function and reduce the degree of injury.

Reactive oxygen species free radicals can damage cell macromolecules through oxidative stress and cause a series of harmful biochemical reactions, resulting in protein damage, lipid peroxidation, DNA mutation, enzyme inactivation, etc. In order to prevent the destruction of oxygen-free radicals to cells, cells themselves have a complete set of protective system to scavenge various types of free radicals produced by metabolism[33]. Superoxide dismutase SOD, glutathione peroxidase GSH-Px and catalase CAT are considered the most critical antioxidant enzymes during resistance to oxidative stress[34]. In this experiment, PEDV infection weakened the antioxidant capacity by significantly decreasing the activities of CAT, SOD and GSH-Px in blood and intestinal tissues. Upon PEDV infection, ML administration significantly increased SOD and CAT activities in blood and colon, respectively, indicating that ML could improve the redox status of piglets.

PEDV enters the small intestine through the mouth and nose and triggers the shedding and atrophy of intestinal villi[35,36]. The Mx gene can reflect the biological activity of interferon and inhibit virus replication[37]. IFIT3 is normally expressed at low levels, but it is highly induced by stimulation, such as viral infections, interferon stimulation and lipopolysaccharide stimulation[38]. ISG15 is a secreted factor that can exist in a free form and act both intracellular and extracellular, and studies have shown that ISG15 can activate IFN and NF-κB, JNK and other signalling pathways involved in immune regulation

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and upregulation of the expression of IFN type I and ISG[39]. ISG15 is also a protein highly expressed when the body is strongly stimulated by viral infections. IL-29 is mainly produced by mature dendritic cells and macrophages, and studies have shown that IL-29 is involved in the pathogenesis of cancer and has antiviral, anti-proliferative and immunoregulatory abilities[40]. In this experiment, PEDV infection upregulated the expression levels of ISG15, Mx1, IFIT3 and IL-29 in intestine. However, ML administration reversed the expression of these antiviral genes. This may due to the virus load in the intestine were significantly declined by ML administration, which has been demonstrated in our previous study. Moreover, the change in the mRNA level for these genes fit well with their change in protein levels[14]. These results add another piece of evidence that ML administration could promote the restoration of homoeostasis by regulating the interferon pathway.

## Conclusion

In summary, ML supplementation could promote piglet's recovery from PEDV infection by repairing intestinal barrier integrity, increasing the efficiency of protein utilisation, improving antioxidant capacity and the immune defense function of the body. Therefore, ML could be used as a kind of feed additive to protect against PEDV infection.

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Y. H. designed the study. C. W., Q. Z. and C. Z. conducted the experiment. C. W., Q. Z., C. Z. and Y. H. carried out the sampling. Y. H., D.Y. and T. W. responsible for funding acquisition. C. W., Q. Z. and C. Z. wrote the original draft. L. W. and D. Z. analysed the data. Y. H. and Q. Z. reviewed the draft.

The authors declare that there is no conflict of interest regarding the publication of this paper.

All data used to support the findings of this study are available from the corresponding author upon request.

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