Maternal Dietary Linoleic Acid Altered Intestinal Barrier Function in Domestic Pigeons (Columba Livia)

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Research

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Abstract

Background

Linoleic acid (LA) is predominantly essential for poultry. Deficiency of LA in poultry were manifested in various aspects such as retarded growth and reduced resistance to disease. The effects of LA on intestinal health in vitro and in mammals has been studied, whereas research related to the effects of LA on intestine health in poultry was scanty. Intestinal health and immune function play an important role in pigeon squab growth. Considering squabs are fed by their parents, the purpose of this study was to explore the effects of maternal dietary LA on intestinal barrier function in squabs by determining intestinal morphology, gene expression of tight junction protein, immune cytokines, and microbial flora.

Results

A completely randomized design with a control group, 1% dietary LA supplementation group, 2% dietary LA supplementation group, and 4% dietary LA supplementation group was used. Six squabs from each treatment were randomly sampled at 21 posthatch. Results indicated that LA supplementation improved intestinal morphology as reflected by increased villus height, villus area and the ratio of villus to crypt, and the promotion at dosage of 1% was most significant. Besides, 1% LA supplementation elevated distribution density of goblet cell in intestine, and strengthened tight junction between enterocytes by up-regulating claudin3 and occludin gene expression, but down-regulating claudin2 gene expression. Moreover, 1% LA supplementation reduced secretion of pro-inflammation cytokines and increased anti-inflammation cytokines partly. The diversity index Chao1 of intestinal microbiota in 1% LA supplementation group was higher than other groups. And Butyrivibrio as beneficial bacteria was the biomarker of LA1%. However, excessive (4%) LA supplementation led to adverse impact on intestinal immunity and microbiota.

Conclusions

Maternal dietary LA in three levels all could improve intestinal morphology in squabs. Therein, appropriate dosage (1%) supplementation might enhance mucosal protection and epithelium barrier in squabs, and furthermore consolidated intestine immunity and luminal microbial environment. However, excessive (4%) LA supplementation might lead to adverse impact on immunity and microbiota. Maternal dietary LA might alter intestinal barrier function in pigeon squabs in a dose-dependent manner.

Background

Meat of pigeon squabs can well be compared with that of broilers in terms of nutritive value and acceptability. Pigeon squabs have been considered profitable sources of poultry meat from Columbia species as they grow more rapidly with minimum input.[1] Squabs have a high growth rate which is attributed to the special crop milk feeding. Several fatty acids available in the crop milk are necessary for
normal growth. Therein, linoleic acid (LA) is predominantly essential for poultry and its higher derivative arachidonate is the most important. [3–5]

The symptoms of LA deficiency in poultry were manifested in various aspects such as retarded growth, increased water consumption, enlarged liver with increased lipid content and reduced resistance to disease. In addition, study stated that the LA-enriched diet enhanced growth in hens immunized with M. butyricum after immunization. And maternal LA isomer regulated hepatic lipid metabolism via the AMPK signaling pathway in chick embryos. Also, our group previously found parental dietary LA supplementation altered lipid metabolism and antioxidant status in pigeon squabs in dose-dependent manner.

Researches in vivo and in vitro have focused on the effects of LA on intestinal health and immune function over the years. Li et al. indicated that in Caco2 cells, LA up-regulated both mRNA and protein level of ApoA-IV which was produced dominantly in intestine and functioned anti-inflammation. And in Ht-29 cells, LA isomer inhibits cell proliferation and stimulates apoptosis. Moreover, in human, duodenal ulcer was associated with deficiency of dietary LA intake. And in Interleukin-10-deficient mice, a LA metabolite ameliorated colonic inflammation. Study by Rodrigues et al. also indicated that LA might affect the course of inflammation. However, research related to the effects of LA on intestine health in poultry was scanty.

Consider that the crop milk produced by parent pigeons and regurgitated to squabs is gradually mixed with increasing grain particles, intestinal health and immune function play an important role in squab growth. Therefore, the purpose of this study was to explore the effects of maternal dietary LA on intestinal barrier function in squabs by determining intestinal morphology, gene expression of tight junction protein, immune cytokines, and microbial flora.

Materials And Methods

Birds and conditions

A total of 240-pair parent White King pigeons, 60 wk of age, were obtained from a commercial pigeon farm (Wenzhou, China). An artificial aviary with a perch and a nest was provided for each pair. Parent pigeons were randomly allocated to four treatments which consisted of 6 replications of 10-pair pigeons each. The four treatments are: control group, 1% LA supplementation group (LA1%), 2% LA supplementation group (LA2%), 4% LA supplementation group (LA4%). The LA (≥99.0% purity) was obtained from a commercial supplier (Hebei bawei biotechnology co., LTD, Handan, China). All experimental diets were isonitrogenous and isocaloric. The LA source was substituted for colseeseed oil by the same weight to equalize the total fat level among diets. The ingredients, nutrient levels and analyzed LA content of experimental diets for parent pigeons were shown in Table 1. The fatty acid composition of each diet analyzed according to Xie et al. were shown in Additional file 1.
pigeons were given to water *ad libitum* and were fed twice daily (7:00 a.m. and 3:00 p.m.) throughout the experiment.
Table 1
Ingredient compositions and nutrient levels of experimental diets for maternal pigeons (on as-fed basis)

| Items                                      | Control | LA1%  | LA2%  | LA4%  |
|--------------------------------------------|---------|-------|-------|-------|
| Ingredients of a whole-grain form feed (%) |         |       |       |       |
| Corn                                       | 56.27   | 56.27 | 56.27 | 56.27 |
| Pea                                        | 28.12   | 28.12 | 28.12 | 28.12 |
| Wheat                                      | 5.63    | 5.63  | 5.63  | 5.63  |
| Sorghum                                    | 5.63    | 5.63  | 5.63  | 5.63  |
| Colleseed oil                              | 4.35    | 3.26  | 2.17  | 0     |
| Linoleic acid                              | 0       | 1.09  | 2.18  | 4.35  |
| Total                                      | 100.00  | 100.00| 100.00| 100.00|
| Calculated nutrients (%)                   |         |       |       |       |
| Metabolizable energy (MJ/kg)               | 13.84   | 13.84 | 13.84 | 13.84 |
| Crude protein                              | 12.63   | 12.63 | 12.63 | 12.63 |
| Analyzed linoleic acid                     | 1.82    | 2.75  | 3.68  | 5.53  |
| Ingredients of grit meal (%)               |         |       |       |       |
| Limestone                                  | 52.93   | 52.93 | 52.93 | 52.93 |
| Shell meal                                 | 28.10   | 28.10 | 28.10 | 28.10 |
| Yellow mud                                 | 14.05   | 14.05 | 14.05 | 14.05 |
| Salt                                       | 1.41    | 1.41  | 1.41  | 1.41  |
| Ferrous sulfate (monohydrate)              | 0.23    | 0.23  | 0.23  | 0.23  |
| Premix                                      | 3.28    | 3.28  | 3.28  | 3.28  |
| Total                                      | 100.00  | 100.00| 100.00| 100.00|
| Calculated nutrients (%)                   |         |       |       |       |
| Calcium                                    | 27.79   | 27.79 | 27.79 | 27.79 |
| Phosphorus                                 | 0.01    | 0.01  | 0.01  | 0.01  |
| Sodium                                     | 0.03    | 0.03  | 0.03  | 0.03  |
| Chlorine                                   | 0.59    | 0.59  | 0.59  | 0.59  |
| Items          | Control | LA1% | LA2% | LA4% |
|---------------|---------|------|------|------|
| Ferrum        | 0.30    | 0.30 | 0.30 | 0.30 |

1. Control = control group; LA1% = 1% linoleic acid addition group; LA2% = 2% linoleic acid addition group; LA4% = 4% linoleic acid addition group.

2. All feeds were fed in a whole-grain form at 07:00 and 15:00 a day, and grit meal was offered to the birds on a continuous basis.

3. Nutrient values were calculated from tables of feed composition and nutritive values in China (twenty-eighth edition, 2017).

4. Metabolizable energy values determined in pigeons were calculated from those reported for chickens in accordance with a previous study (Hullar et al., 1999), which observed that the metabolizable energy values of feed in pigeons did not differ significantly from those in chickens.

5. The premix provided the following per kg of diet: Vitamin A 5000 IU, Vitamin E 50 IU, Vitamin D₃ 2000 IU, copper sulfate 15 mg, manganese sulfate 45 mg, zinc sulfate 90 mg.

Each pair of parent pigeons laid two eggs in a nest. Eggs were picked out by hand and meanwhile fake eggs were put into nests to meet parents' broodiness. The picked eggs were transferred to an incubator for an 18-day artificially incubation. On the day of hatching, 480 artificially hatched pigeon squabs with similar BW were selected from this pigeon farm. These squabs were randomly pair-matched and assigned into the nests of the selected parent pigeons to replace the fake eggs. Each parent pair adopted a pair of squabs. Parent pigeons were fed with experimental diets continually in the next 21 days. Squabs were fed with crop milk which was secreted by parent pigeons. The ambient temperature was 18 to 26 °C. The relative humidity was 60 to 70%. And the photoperiod was 12 L:12 D throughout the total experiment period.

**Sample collection**

On the day 21 posthatch, six squabs per treatment (one for each replication) were selected for sampling. The squabs were fasted for 12 h before weighing and slaughter. And they were sedated before cervical dislocation. Ileal contents were sampled into sterile plastic tubes with a sterile spatula, immediately frozen in liquid nitrogen, then stored at -80 °C for subsequent analyses. Three segments of the small intestine (duodenum, jejunum and ileum) were sampled, flushed with 0.9% saline to remove all the content, and processed for morphological examination. The collected segments were the loop of the duodenum (duodenum), the tract before Meckel's diverticulum (jejunum), the final segment of small intestine (ileum). The mucosa of each intestinal segment was scraped off with a glass slide carefully, frozen in liquid nitrogen rapidly, and then stored at -80 °C for subsequent analyses.

**Secretory IgA and cytokine analysis**
The homogenates of intestinal mucosa were prepared with phosphate buffered saline for secretory IgA (sIgA) and cytokine analyses. The sIgA concentration and the concentrations of tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), IL-6, IL-10, and IL-4 were determined by absorbance changes at a wavelength of 450 nm with commercial ELISA kits (Shanghai Mlbio Institute, Shanghai, China) according to the manufacture's protocol. The final sIgA and cytokine concentrations were expressed as units per milligram of protein.

**RNA extraction and quantitative PCR analysis**

Total RNA of intestinal mucosa was extracted using TRIzol procedure (Invitrogen, Carlsbad, CA) according to the instructions of the manufacturer. The extracted RNA was quantified by NanoDrop ND-2000 spectrophotometer (NanoDrop Technologies Inc., Wilmington, USA). The RNA integrity was verified by native RNA electrophoresis on 1.0% agarose gel. The complementary DNA was synthesized from 2-µg total RNA by M-MLV reverse transcriptase (Takara, Dalian, China) with oligo dT-adaptor primer at 42 °C for 60 min following the protocol of the manufacturer. The abundance of mRNA was assayed on a StepOne Plus Real-Time PCR system (ABI 7500, Applied Biosystems, Foster City, CA). The specific primers used were indicated in Table 2. The SYBR Green Realtime PCR Master Mix (Toyobo Co., Ltd., Osaka, Japan) was used for PCR reaction, consisting of an initial DNA denaturation of 95 °C for 60 s, followed by 40 cycles of 95 °C for 15 s, and 60 °C for 60 s. The GAPDH was considered as an appropriate endogenous reference. The average gene expression relative to the endogenous reference for each sample was calculated according to the $2^{-\Delta\Delta Ct}$ method [19]. The calibrator for each gene in study was the average ΔCt value of control group.

**Table 2**

| Gene | Primer | Sequence (5’→3’) | Amplicon size (bp) | Accession number |
|------|--------|------------------|--------------------|------------------|
| CLDN2 | CLDN2-F | GTGCAGATGGGAACAAGGT | 119 | XM_021283269.1 |
| | CLDN2-R | GAGCCAAGGAAGCTACGG | | |
| CLDN3 | CLDN3-F | ACCTCATCCCCGTCTCCT | 109 | XM_005515008.2 |
| | CLDN3-R | CAGCCACGTAGAGCGA | | |
| OCLN | OCLN-F | CAGGACGTGGCAGAGGA | 105 | XM_005509325.2 |
| | OCLN-R | GTGGAAGAGCTTGTTGCGT | | |
| GAPDH | GAPDH-F | CCCAATGTCTCTGTGGTGGG | 116 | NM_001282835.1 |
| | GAPDH-R | TATGCCAGGATGCCCTT | | |

1 CLDN2 = Claudin2; CLDN3 = Claudin 3; OCLN = Occludin; GAPDH = glyceraldehyde-3-phosphate dehydrogenase.
Intestinal morphological examination

Approximately 1-cm intestinal samples of each segment were collected and fixed in 10% neutral-buffered formalin solution. Each sample was dehydrated, cleared, and embedded in paraffin. Serial sections (5 µm) were placed on glass slides and submitted to two different staining. The hematoxylin and eosin staining was for the identification of villus height, villus area, crypt depth, and enterocyte. The periodic-acid Schiff staining was for the identification of goblet cell (GC). The examination was performed with light microscopy (Nikon Corp., Tokyo, Japan) using the Image-pro plus 6.0 (Media Cybernetics, Inc., Rockville, MD, USA). The density of enterocyte was expressed as number per millimeter of epithelial layer length. The density of GC was expressed as number per 100 enterocytes according to Tamura et al. [20].

16S rDNA high-throughput sequencing

The total microbial genomic DNA was extracted from the ileal contents of squabs from each treatment using the E.Z.N.A.® Stool DNA Kit (Omega Bio-Tek, USA). The concentration and quality of the extracted DNA were assessed with 2% agarose gel electrophoresis and a NanoDrop ND-2000 spectrophotometer (NanoDrop Technologies Inc., Wilmington, USA). The V3-V4 hyper-variable region of the 16S rRNA genes was amplified by specific degenerate primers (338F: ACTCCTACGGGAGGCAGCAG, 806R: GGACTACHVGGGTWTCTAAT). The PCR program was conducted following the conditions: initial denaturation step, 98 °C, 30 s; denaturation, 35 cycles, 98 °C, 10 s; annealing, 54 °C, 30 s; elongation, 72 °C, 45 s; and final extension, 72 °C, 10 min. The PCR amplification products were detected by 2% agarose gel electrophoresis. The target fragments were recovered using AMPure XT Beads (Beckman Coulter Genomics, Danvers, MA, USA). Qubit was used to quantify the purified PCR products, and the qualified library concentration should be more than 2 nM. Sequencing was performed with V3 chemistry and generated 2 × 300 bp double-end reads using MiSeq Reagent Kit V3 with the MiSeq Illumina platform (Illumina, Santa Clara, CA, USA) at Hangzhou Personal Biotechnology Co., Ltd. (Hangzhou, China).

Statistical analyses

The data obtained from slgA and cytokine analysis, quantitative PCR analyses, and intestinal morphological examination were subjected to one-way analysis of variance in SPSS 24.0 (SPSS Inc., Chicago, USA) for Windows. The significance level was set at $P < 0.05$. When significant differences were found, Tukey post-hoc tests were performed. Plotting was placed with GraphPad Prism 7.0 (GraphPad Software Inc., San Diego, CA, USA).

The raw sequence data obtained from the Illumina MiSeq platform were quality-filtered and demultiplexed using Quantitative Insights into Microbial Ecology (QIIME), version 1.8.0-dev (http://qiime.org/index.html). After trimming and assembling, the final clean data were obtained and assigned into bacterial operational taxonomic units (OTUs) using the UCLUST function in QIIME (http://qiime.org/scripts/pick_otus) according to a 97% similarity threshold. The data were categorized
with the Ribosomal Database Project (RDP), version 2.2 (http://sourceforge.net/projects/rdp-classifier/). The alpha diversity measures, including the observed species, Chao 1, and Shannon indices were calculated using MicrobiomeAnalyst (https://www.microbiomeanalyst.ca/MicrobiomeAnalyst/faces/home.xhtml). Venn diagram was performed in Venny 2.1 (https://bioinfogp.cnb.csic.es/tools/venny/index.html). Principal coordinate analysis (PCoA) was conducted using Galaxy (http://huttenhower.sph.harvard.edu/galaxy/) according to Unweighted Unifra representing Beta diversity values. Kolmogorov-Smirnov program is used to verify the normality of data. Data in line with normal distribution were counted by one-way ANOVA, and multiple comparisons were conducted by Tukey post-hoc tests. Nonnormal distribution data were analyzed by Kruskal-Wallis test and Duncan's multiple range tests. \( P < 0.05 \) means significant difference.

Results

Intestinal morphometric trait

Hematoxylin and eosin staining of intestine (duodenum, jejunum, and ileum) in the four treatment groups has shown the effects of maternal dietary LA on the intestinal morphometric trait in squabs (Fig. 1). In duodenum, the villus height, villus area and ratio of villus height to crypt depth (VCR) responded to increasing supplementation of LA in linear (\( P < 0.01, P = 0.01 \) and \( P < 0.01 \), respectively) and quadratic (all \( P < 0.01 \)) manner; their maximal response were observed in LA1%. In jejunum, the villus height and villus area emerged linear (\( P < 0.01 \) and \( P = 0.01 \), respectively) and quadratic (both \( P < 0.01 \)) trends with their maximum observed in LA1% as LA supplementation was increased. While the crypt depth showed a linear (\( P < 0.01 \)) increase with the level of LA supplementation increasing. In ileum, the villus height and VCR responded to increasing supplementation of LA in linear (both \( P < 0.01 \)) and quadratic (both \( P < 0.01 \)) manner, and their maximum were observed in LA1%. The villus area displayed a quadratic trend with the maximum in LA1% as LA supplementation was increased. In addition, the VCR in jejunum, crypt depth in duodenum and ileum had no significance among four groups. There was no significance (\( P > 0.05 \)) in enterocyte density among four experimental groups.

Tight junction protein gene expression

Effects of maternal dietary LA on the mRNA expression of three tight junction proteins have shown in Fig. 2. In duodenum, the mRNA expression of Claudin 3 (CLDN3) emerged a quadratic (\( P = 0.03 \)) trend with the maximum observed in LA1% as the LA supplementation was increased. Claudin 2 (CLDN2) mRNA expression responded to increasing supplementation of LA in linear (\( P = 0.02 \)) and quadratic (both \( P < 0.01 \)) manner with the minimum in LA1%. Although maternal dietary LA had no significant effect (\( P > 0.05 \)) on mRNA expression of Occludin (OCLN), a quadratic (\( P = 0.01 \)) trend was observed with the maximum response in LA2%. In jejunum, the mRNA expression of CLDN2 emerged a quadratic (\( P < 0.01 \)) trend with the minimum in LA1% as the supplementation of LA was increased. However, CLDN3 and OCLN in jejunum had no significance (\( P > 0.05 \)) among four treatments. In ileum, CLDN2 mRNA
expression displayed linear \( (P<0.01) \) and quadratic \( (P<0.01) \) trends with the maximum observed in LA4\% as the LA supplementation was increased. Moreover, CLDN3 and OCLN mRNA expression responded to increasing supplementation of LA in linear (both \( P<0.01 \)) and quadratic (both \( P<0.01 \)) manner; their maximal response were observed in LA1\%.

**Goblet cell density**

Periodic-acid Schiff staining of intestine (duodenum, jejunum, and ileum) in the four treatment groups has shown effects of maternal dietary LA on the density of GC (Fig. 3). The GC density in duodenum and jejunum responded to increasing supplementation of LA linearly (both \( P<0.01 \)), with the maximum observed in LA1\% and Control, respectively. The GC density in ileum displayed linear \( (P=0.02) \) and quadratic \( (P<0.01) \) trends with the maximum density in LA1\%.

**SIgA and cytokine analysis**

Effects of maternal dietary LA on the concentrations of slgA and cytokines have shown in Fig. 4. In duodenum, slgA and IL-10 concentrations responded to increasing LA supplementation quadratically (both \( P<0.01 \)) with their maximum observed in LA1\%. IL-6 concentration emerged linear \( (P<0.01) \) and quadratic \( (P<0.05) \) trends with the minimum in LA1\% as the LA supplementation was increased. TNF-\( \alpha \) and IL-1\( \beta \) concentrations increased linearly (both \( P<0.01 \)) with LA supplementation increasing, whereas IL-4 concentration decreased linearly \( (P<0.01) \). In jejunum, TNF-\( \alpha \) concentration emerged linear \( (P<0.01) \) and quadratic \( (P=0.01) \) trend with the minimum in LA1\% as the LA supplementation was increased. While IL-10 concentration emerged linear \( (P=0.01) \) and quadratic \( (P<0.01) \) trend with the maximum in LA1\% as the LA supplementation was increased. IL-6 concentrations responded to increasing LA supplementation quadratically \( (P<0.01) \) with the minimum observed in LA1\%. While IL-4 concentration responded to increasing LA supplementation quadratically \( (P<0.05) \) with their maximum observed in LA1\%. There was no significance \( (P>0.05) \) in slgA concentrations in jejunum among four experimental groups. In ileum, TNF-\( \alpha \) and IL-1\( \beta \) concentrations increased linearly \( (P<0.01 \) and \( P=0.04 \), respectively) with LA supplementation increasing, whereas IL-10 concentration decreased linearly \( (P=0.03) \). IL-4 concentration responded to increasing LA supplementation quadratically \( (P<0.01) \) with their maximum observed in LA1\%. While IL-6 concentration responded to increasing LA supplementation quadratically \( (P<0.01) \) with their minimum observed in LA1\%. There was no significance \( (P>0.05) \) in slgA concentration in ileum among four experimental groups.

**Ileal microbiota analysis**

The ileal microbiota analyses of 24 samples were performed. An average of 33218 raw sequences per sample was obtained. After trimming and assembling, an average of 29573 valid sequences per sample
was remained. Further, an average of 421 OTUs based on a 97% sequence similarity was identified from these sequences.

Effects of maternal dietary LA on the alpha diversity of ileal microbiota in domestic pigeon squabs are shown in Fig. 5. Rarefaction curves for the observed species (Fig. 5a) approached a plateau, indicating the availability of sufficient reads to reflect each microbiome community. The total OTU number of ileal contents was maximum in LA1%, and so was the unique OTU number (Fig. 5b). No significant difference was observed in alpha diversity index Chao1 among four groups (Fig. 5c). The alpha diversity index Shannon was significant increased in LA1% and LA2% compared with control group, whereas there was no significance between control group and LA4% (Fig. 5d). Effects of maternal dietary LA on the beta diversity of ileal microbiota in domestic pigeon squabs are shown in Fig. 6. As shown in Fig. 6a, the microbial communities from each treatment were mainly divided into 4 groups. Clustering analysis in phylum level was shown in Fig. 6b. Firmicutes and Proteobacteria were predominant phyla in ileal microbiota, followed by Actinobacteria, Cyanobacteria and Bacteroides. All samples in phylum level were not clustered into four groups. However, clustering analysis in genes level (Fig. 6c) showed that samples in genes level were clearly clustered into four groups, though one sample in LA4% was clustered with LA2%. Lactobacillus was predominant genes in most samples. A cladogram representative of the dominant microflora called biomarkers identified from phyla to genera was shown in Fig. 7. It was observed that Lactobacillus was the biomarker of control group and Butyrivibrio was the biomarker of LA1% in genes level. Besides, the biomarkers of LA2% were Aeriscardovia, Deinococcus, Streptococcus, Rhodopseudomonas, Methyllobacterium, Sphingomonas, Ralstonia and Pseudomonas. The biomarkers of LA4% were Chlorophyta, Enterococcus and Escherichia.

Discussion

The intestine played crucial role in nutrient digestion and absorption [21]. And intestinal functions were usually reflected by intestinal morphology such as villus height, villus area and crypt depth [22]. In the present study, maternal dietary LA supplementation resulted in higher villus height and villus area compared with those of control group whatever intestinal site. And the highest was observed in LA1%. Previous study indicated that the increased villus area allowed animals to absorb more nutrients into the body [23]. And the increase in villi height might be linked with improved health status of animals [24]. Therefore, our results suggested that maternal dietary LA especially at 1% level could improve the intestinal morphology of squabs, indicating that their intestinal development was strengthened. Study by Thomson et al. indicated that LA-deficiency rats had significantly lower villus height in ileum than control, proving the importance of dietary LA [25]. The higher the VCR, the higher the maturity and functional capacity of enterocyte [26]. In the current study, the VCR both in duodenum and ileum were significantly increased in squabs by maternal dietary LA supplementation. While VCR in jejunum had no significant change by LA supplementation because the crypt depth in jejunum was significant increased compared with control. Taken together, LA enhanced the development of duodenum and ileum more obviously and the effects of LA on jejunum in pigeon squabs might be complicated. In view of information about the
relationship between LA and intestinal morphology in poultry is not available, further studies are needed to explore the mechanisms.

The intestinal epithelium served as the front protective barrier between the host and the external environment against enteric pathogens, food antigens, and physiochemical stresses [27, 28]. Enterocytes which were closely connected with one another were the main components of intestinal epithelium [29]. In our study, the density of enterocytes in epithelial layer had no significant effect in three intestinal tracts of squabs by maternal LA supplementation, meaning the density of enterocytes was not affected by increased villus height. This result further confirmed that LA promoted villi growth. GC in intestine played an indispensable part in mucosal protection mainly by secreting mucus that together with water, irons and peptides formed a viscous gel reticular mucus layer overlying epithelial cell surface [30]. And the density of GC indicated the capability of mucus secretion [31]. In the present study, the maximum density of GC in duodenum and ileum were observed in LA1%. While the maximum in jejunum was in control, and the GC density of jejunum in LA4% was significantly lower than that in control. These results suggested that moderate maternal LA (1%) supplementation might be beneficial to GC formation and distribution in duodenum and ileum thus enhancing mucosal protection, whereas excessive LA (4%) supplementation might have the opposite effect in jejunum. We conjectured that LA might alter mucus layer of intestine in pigeon squabs in a site-dependent and dose-dependent manner. As a whole, GC density increased with distal progression along the intestine in squabs, which was similar with chicken [32].

Between the individual enterocyte presented tight junctions [33]. The tight junctions acted as a regulatory barrier in the intercellular space and maintain a palisade between the apical and basolateral domains of the plasma membrane [34]. The protein composition of tight junctions included claudins and occludins [33]. Claudins performed different functions and could roughly be divided into two types, those involved in barrier formation, and those important in channel formation [35]. Therein, CLDN2 expression was associated with increased tight junction permeability, and decreased the tightness of the epithelial barrier [36]. In inflammatory bowel disease, CLDN2 expression correlated positively with inflammatory activity [37]. In the current study, lowest mRNA expression of CLDN2 were observed in LA1% whatever intestinal site, whereas CLDN2 mRNA expression of ileum in LA4% was significantly higher than control group. The results indicated that moderate maternal dietary level (1%) of LA could strengthen tight junction by decreasing its permeability. While excessive LA supplementation might weaken tight junction. CLDN3 was known to be a “tightening” claudin [38]. And CLDN3 expression decreased in association with increased permeability in hens [39]. In the present study, highest mRNA expression of CLDN3 in duodenum and ileum were observed in LA1%, whereas CLDN3 mRNA expression of ileum in LA4% was significantly lower than control group, suggesting similar effects with the results of CLDN2 mRNA expression. OCLN played an important role in the assembly and maintenance of tight junctions [40]. In our study, OCLN mRNA expression was up-regulated in ileum by supplementation of three LA levels. The result was inconsistent with that of CLDN2 and CLDN3, suggesting that different tight junction proteins had different expression pattern. And LA might regulate tight junction of intestine in pigeon squabs in a dose-dependent manner and in an intestinal site-specific pattern.
Intestine is not only the nutrients digestion and absorption centers, but also an important place to play immune function [41]. sIgA is a vital immune effector molecule on the intestinal mucosal surface, which is the first line of defense against the adhesion of pathogenic bacteria in the intestinal mucosa [42]. In present study, the sIgA in duodenum was maximum in LA1%, which indicated that maternal dietary LA supplementation at 1% might enhance the ‘immune exclusion’ ability by stimulating the secretion of sIgA to some extent. Cytokines are important mediators and regulators of host against foreign antigens, and their main function is to coordinate the functional activities of immune system cells [43]. IL-1β, TNF-α, and IL-6 generated by macrophages were pro-inflammatory cytokines that exert multiple roles in both physiological and pathological conditions [44, 45]. IL-4 and IL-10 are classified as anti-inflammatory cytokines because they prevent the production of proinflammatory cytokines such as IL-2 and IL-12 by stimulated monocytes/macrophage [46]. In our study, the IL-6 in intestine was minimum by supplementation with 1% maternal dietary LA, and the IL-4 and IL-10 were maximum in this group, suggesting that 1% maternal dietary LA supplementation could reduce inflammation partly. And the decreased IL-6 was consistent with the result of CLDN2 expression since previous study has shown that IL-6 markedly induced CLDN2 expression which is associated with increased tight junction permeability, undermining the integrity of the intestinal barrier [47]. Overall speaking, appropriate LA supplementation consolidated intestinal function. However, excessive (4%) LA supplementation might lead to adverse impact. In the present study, IL-1β and IL-6 in duodenum as well as TNF-α in intestine of squabs in LA4% were significantly higher than control group, whereas IL-4 in duodenum was lower. A previous study displayed that higher than normal ratio of pro-inflammatory and anti-inflammatory factors promoted pathological changes in tissues [48]. Moreover, IL-1β, TNF-α, and IL-6 were expanded in the inflammatory bowel disease [45]. These results suggested that 4% maternal dietary LA supplementation might enhance the intestinal pathology.

Intestine contains a enormous amount of microorganisms primarily consisting of bacteria, which plays a crucial part in animal health [49]. Dietary composition not only affects the intestinal epithelial barrier, but also significantly influences the microbial community [50]. In our study, maternal dietary LA at 1% and 2% increased the diversity of intestinal microbiota in squabs. It is widely believed that the higher the diversity, the better the intestine health, because the diversity is one of the key determinants of colonization resistance against invading pathogens [51]. The result indicated that squabs had greater resistance in LA1% and LA2%. In addition, Butyrivibrio was the biomarker of LA1%, which means the relative abundance of Butyrivibrio was significantly higher compared with other groups. The bacteria genes can produce butyrate which as a short-chain fatty acid can lower the pH of intestinal lumen and regulate the microbial composition especially stimulate the growth of beneficial bacteria [52, 53]. The result further revealed the maintaining of intestine health was strengthened in squabs by supplementing maternal dietary LA at 1%. However, the changes of microbial community in LA2% was complicated. On the one hand, the biomarkers of LA2% included beneficial bacteria. Therein, Aeriscardovia belongs to Bifidobacteria which can protect from enteropathogenic infection through acetate production [54]. And Streptococcus was observed to be negatively related with inflammation in previous research [55]. While on the other hand, the biomarkers of LA2% also included opportunistic pathogens such as
Methylobacterium, Ralstonia and Pseudomonas [56–58]. Thus, it was vital to maintain the intestinal lumen environment and the balance between these bacteria in LA2%. Unfortunately, pathogenic Escherichia was observed in the biomarkers of LA4%. Previous studies showed that inflammation caused by Crohn's disease and colitis was characterized by the increased abundance of Escherichia in intestinal microbiota [59–61]. And as mentioned above, 4% maternal dietary LA supplementation promote inflammation. Thus, excessive (4%) LA supplementation might result in breakdown of homeostasis between mucosal immune system and the intestinal microbiota.

**Conclusion**

In conclusion, maternal dietary LA in three levels all could improve intestinal morphology in squabs. Therein, appropriate dosage (1%) supplementation might enhance mucosal protection and epithelium barrier in squabs by promoting GC formation and distribution in intestine, and strengthening tight junction between enterocytes. Moreover, moderate supplementation consolidated intestine immunity and luminal microbial environment by reducing inflammation partly, and promoting the growth of beneficial bacteria. However, unfortunately, excessive (4%) LA supplementation might lead to adverse impact on immunity and microbiota. We conjectured that LA might alter intestinal barrier function in pigeon squabs in a dose-dependent manner. The reason might partly rely on that moderate LA satisfied squabs, whereas excessive addition impacted the balance between ω-6 and ω-3 polyunsaturated fatty acids, which need further study to verify.

**Abbreviations**

LA: linoleic acid  
LA1%: 1% dietary LA supplementation group  
LA2%: 2% dietary LA supplementation group  
LA4%: 4% dietary LA supplementation group  
slgA: secretory IgA  
TNF-α: tumor necrosis factor-α  
IL: interleukin  
GC: goblet cell  
QIIME: Quantitative Insights into Microbial Ecology  
OTU: operational taxonomic unit  
RDP: Ribosomal Database Project
PCoA: Principal coordinate analysis

VCR: ratio of villus height to crypt depth

CLDN3: Claudin 3

CLDN2: Claudin 2

OCLN: Occludin

Declarations

Ethics approval and consent to participate

All experimental protocols involving animals were approved by the Animal Care and Welfare Committee of Animal Science College and the Scientific Ethical Committee of the Zhejiang University (No. ZJU2013105002) (Hangzhou, China).

Consent for publication

Not applicable.

Availability of data and materials

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

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

The article was mainly conceived and designed by XD and XZ. QX performed the experiments and analyzed the data. The manuscript was mainly written by QX. XD and XZ revised it critically for important content. All authors have read and approved the final manuscript.

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References

1. Bhuyan P, Nath DR, Hazarika M. Influence of age and sex on nutritive value (proximate composition) of squab and pigeon meat. Indian Vet J. 1999;76:530-2.

2. Desmeth M. Lipid composition of pigeon cropmilk—II. Fatty acids. Comp Biochem Physiol. 1980;66:135-8.

3. Reiser R. The Essential Role of Fatty Acids in Rations for Growing Chicks. J Nutr. 1950;42:319-23.

4. Mohrhauser H, Holman RT. The Effect of Dose Level of Essential Fatty Acids Upon Fatty Acid Composition of the Rat Liver. J Lipid Res. 1963;4:151-9.

5. Watkins BA. Importance of Essential Fatty Acids and Their Derivatives in Poultry. J Nutr. 1991;121:1475-85.

6. Sijben, JWC, De-Groot H, Nieuwland MGB, Schrama JW, Parmentier HK. Dietary Linoleic Acid Divergently Affects Immune Responsiveness of Growing Layer Hens. Poult Sci. 2000;79:1106-15.

7. Fu C, Zhang Y, Yao Q, Wei X, Shi T, Yan P, Liu X. Maternal conjugated linoleic acid alters hepatic lipid metabolism via the AMPK signaling pathway in chick embryos. Poult Sci. 2020;99:224-34.

8. Xu QQ, Ma XW, Dong XY, Tao ZR, Lu LZ, Zou XT. Effects of parental dietary linoleic acid on growth performance, antioxidant capacity, and lipid metabolism in domestic pigeons (Columba livia). Poult Sci. 2020;99:1471-82.

9. Vowinkel T, Mori M, Krieglstein CF, Russell J, Saijo F, Bharwani S, Turnage RH, Davidson WS, Tso P, Granger DN, Kalogeris TJ. Apolipoprotein A-IV inhibits experimental colitis. J Clin Invest. 2004;114:260-9.

10. Li X, Xu M, Liu M, Ji Y, Li Z. TNF-alpha and IL-6 inhibit apolipoprotein A-IV production induced by linoleic acid in human intestinal Caco2 cells. J Inflammation. 2015;12:1-8.

11. Park JH, Cho HJ, Kim WK, Kim EJ, Tyner AL. Conjugated linoleic acid (CLA) induces apoptosis and inhibits coupling of heregulin (HRG) signaling to phosphatidylinositol 3-kinase (PI3K) and Akt via ErbB3 phosphorylation in human colon cancer Ht-29 cells. Gastroenterol. 2003;124:A281.

12. Grant HW, Palmer KR, Riemensma RR, Oliver MF. Duodenal ulcer is associated with low dietary linoleic acid intake. Gut. 1990;31:997-8.

13. Okabe M, Matsuura M, Kitamoto H, Yamada S, Honzawa Y, Yamamoto S, Honzawa Y, Yamamoto S, Seno H, Kishino S, Ogawa J. Su1947-Hya, a Metabolite of Linoleic Acid by the Commensal Bacteria, Ameliorates Colonic Inflammation in Il-10-Deficient Mice Through the Alteration of Macrophage Function. Gastroenterol. 2019;156:S-669.

14. Rodrigues HG, Vinolo MAR, Magdalon J, Fujiwara H, Cavalcanti DMH, Farsky SHP, Calder PC, Hatanaka E, Curi R. Dietary Free Oleic and Linoleic Acid Enhances Neutrophil Function and Modulates the Inflammatory Response in Rats. Lipids. 2010;45:809-19.

15. Sales J, Janssens GPJ. Nutrition of the domestic pigeon (Columba livia domestica). Worlds Poult Sci J. 2003;59:221-32.
16. Gillespie MJ, Crowley TM, Haring VR, Wilson SL, Harper JA, Payne JS, Green D, Monaghan P, Stanley D, Donald JA, Nicholas KR, Moore RJ. Transcriptome analysis of pigeon milk production – role of cornification and triglyceride synthesis genes. BMC Genomics 2013;14:169.

17. Qi X, Wu S, Zhang H, Yue H, Xu S, Ji F, Qi G. Effects of dietary conjugated linoleic acids on lipid metabolism and antioxidant capacity in laying hens. Arch Anim Nutr. 2011;65:354-65.

18. Xie P, Wang Y, Wang C, Yuan C, Zou X. Effect of different fat sources in maternal diets on growth performance, villus morphology, digestive enzymes and colorectal microbiota in pigeon squabs. Arch Anim Nutr. 2013;67:147-60.

19. Schmittgen TD. Real-time quantitative PCR. Methods. 2001;25:383-5.

20. Tamura A, Soga H, Yaguchi K, Yamagishi M, Toyota T, Sato J, Oka Y, Itoh T. Distribution of two types of lymphocytes (intraepithelial and lamina-propria-associated) in the murine small intestine. Cell Tissue Res. 2003;313:47-53.

21. Kim YS, Ho SB. Intestinal Goblet Cells and Mucins in Health and Disease: Recent Insights and Progress. Curr Gastroenterol Rep. 2010;12:319-30.

22. Li J, Yin L, Wang L, Li J, Huang P, Yang H, Yin Y. Effects of vitamin B6 on growth, diarrhea rate, intestinal morphology, function, and inflammatory factors expression in a high-protein diet fed to weaned piglets. J Anim Sci. 2019;97:4865-74.

23. Wang Z, Li J, Wang Y, Wang L, Yin Y, Yin L, Yang H, Yin Y. Dietary vitamin A affects growth performance, intestinal development and functions in weaned piglets by affecting intestinal stem cells. J Anim Sci. 2020;98:skaa020.

24. Upadhaya SD, Jiao Y, Kim YM, Lee KY, Kim IH. Coated sodium butyrate supplementation to a reduced nutrient diet enhanced the performance and positively impacted villus height and faecal and digesta bacterial composition in weaner pigs. Anim Feed Sci Technol. 2020;265:114534.

25. Thomson AB, Keelan M, Clandinin MT, Walker K. Dietary fat selectively alters transport properties of rat jejunum. J Clin Invest. 1986;77:279-88.

26. Chee SH, Iji PA, Choct M, Mikkelsen LL, Kocher A. Functional interactions of manno-oligosaccharides with dietary threonine in chicken gastrointestinal tract. II. Mucosal development, mucin dynamics and nutrient utilisation. Br Poult Sci. 2010;51:667-676.

27. Pinton P, Tsybulskyy D, Lucioli J, Laffitte J, Callu P, Lyazhri F, Grosjean F, Bracarense P, Kolf-Clauw M, Oswald IP. Toxicity of Deoxynivalenol and Its Acetylated Derivatives on the Intestine: Differential Effects on Morphology, Barrier Function, Tight Junction Proteins, and Mitogen-Activated Protein Kinases. Toxicol. Sci. 2012;130:180-90.

28. Buzza MS, Netzel-Arnett S, Shea-Donohue T, Zhao A, Lin CY, List K, Szabo R, Fasano A, Bugge TH, Antalis TM. Membrane-anchored serine protease matriptase regulates epithelial barrier formation and permeability in the intestine. Proc. Natl. Acad. Sci. 2010;107:4200-5.

29. Hui K, Ren Q, Cao J. Insights into the intestine immune of marsupenaeus japonicus under the white spot syndrome virus challenge using ma sequencing. Vet Immunol Immunopathol. 2019;208:25-33.
30. Calik A, Ergün A. Effect of lactulose supplementation on growth performance, intestinal histomorphology, cecal microbial population, and short-chain fatty acid composition of broiler chickens. Poult Sci. 2015;94:2173-82.

31. Kridtayopas C, Rakangtong C, Bunchasak C, Loongyai W. Effect of prebiotic and synbiotic supplementation in diet on growth performance, small intestinal morphology, stress, and bacterial population under high stocking density condition of broiler chickens. Poult Sci. 2019;98:4595-605.

32. Reynolds KL, Cloft SE, Wong EA. Changes with age in density of goblet cells in the small intestine of broiler chicks. Poult Sci. 2020;99:2342-8.

33. Karcher DM, Applegate T. Survey of Enterocyte Morphology and Tight Junction Formation in the Small Intestine of Avian Embryos. Poult Sci. 2008;87:339-50.

34. Anderson, J. M. Molecular Structure of Tight Junctions and Their Role in Epithelial Transport. Physiology. 2001;16:126-30.

35. Colegio OR, Van Itallie CM, Mccrea HJ, Rahner C, Anderson JM. Claudins create charge-selective channels in the paracellular pathway between epithelial cells. Am J Physiol Cell Physiol. 2002;283:C142-7.

36. Suzuki T, Yoshinaga N, Tanabe S. Interleukin-6 (il-6) regulates claudin-2 expression and tight junction permeability in intestinal epithelium. J Biol Chem. 2011;286:31263.

37. Weber CR, Nalle SC, Tretiakova M, Rubin DT, Turner JR. Claudin-1 and claudin-2 expression is elevated in inflammatory bowel disease and may contribute to early neoplastic transformation. Lab Invest. 2008;88:1110-20.

38. Milatz S, Krug SM, Rosenthal R, Günzel D, Müller D, Schulzke JD, Amasheh S, Fromm M. Claudin-3 acts as a sealing component of the tight junction for ions of either charge and uncharged solutes. Biochim. Biophys. Acta, Biomembr. 2010;1798:2048-57.

39. Ariyadi B, Isobe N, Yoshimura Y. Expression of tight junction molecule “claudins” in the lower oviductal segments and their changes with egg-laying phase and gonadal steroid stimulation in hens. Theriogenology. 2013;79:211-8.

40. Gadde U, Oh ST, Lee YS, Davis E, Zimmerman N, Rehberger T, Lillehoj HS. The Effects of Direct-fed Microbial Supplementation, as an Alternative to Antibiotics, on Growth Performance, Intestinal Immune Status, and Epithelial Barrier Gene Expression in Broiler Chickens. Probiotics Antimicrob Proteins. 2017;9:397-405.

41. Wittig BM, Zeitz M. The gut as an organ of immunology. Int J Colorectal Dis 2003;18:181-7.

42. Pabst O. New concepts in the generation and functions of IgA. Nat Rev Immunol. 2012;12:821-32.

43. Liu H, Zhang M, Han H, Yuan J, Li Z. Comparison of the expression of cytokine genes in the bursal tissues of the chickens following challenge with infectious bursal disease viruses of varying virulence. Virol J. 2010;7:364.

44. Arranz L, Arriero M del M, Villatoro A. Interleukin-1β as emerging therapeutic target in hematological malignancies and potentially in their complications. Blood Rev. 2017;31:306-17.
45. Maloy KJ, Powrie F. Intestinal homeostasis and its breakdown in inflammatory bowel disease. Nature. 2011;474:298-306.

46. Nathan N, Denizot Y. Anti-inflammatory cytokines (IL-4, IL-10, IL-13) in plasma during mesenteric infarction. Mediators Inflammation. 1998;7:119.

47. Suzuki T, Yoshinaga N, Tanabe S. Interleukin-6 (IL-6) Regulates Claudin-2 Expression and Tight Junction Permeability in Intestinal Epithelium. J. Biol. Chem. 2011;286:31263-71.

48. Xu, ZY, Yu Y, Liu Y, Ou CB, Zhang YH, Liu TY, Wang QX, Ma JY. Differential expression of pro-inflammatory and anti-inflammatory genes of layer chicken bursa after experimental infection with infectious bursal disease virus. Poult Sci. 2019;98:5307-14.

49. Li Y, Fu X, Ma X, Geng S, Jiang X, Huang Q, Hu C, Han X. Intestinal Microbiome-Metabolome Responses to Essential Oils in Piglets. Frontiers in Microbiology 2018;9:1988.

50. Guzman JR, Conlin VS, Jobin C. Diet, Microbiome, and the Intestinal Epithelium: An Essential Triumvirate? BioMed Res Int. 2013;7405:425146.

51. Dong XY, Azzam MMM, Zou XT. Effects of dietary threonine supplementation on intestinal barrier function and gut microbiota of laying hens. Poult Sci. 2017;96:3654-63.

52. Le Leu RK, Brown IL, Hu Y, Bird AR, Jackson M, Esterman A, Young GPA. Synbiotic Combination of Resistant Starch and Bifidobacterium lactis Facilitates Apoptotic Deletion of Carcinogen-Damaged Cells in Rat Colon. J Nutr. 2005;135:996-1001.

53. Louis P, Flint HJ. Diversity, metabolism and microbial ecology of butyrate-producing bacteria from the human large intestine. FEMS Microbiol Lett. 2009;294:1-8.

54. Fukuda S, Toh H, Hase K, Oshima K, Nakanishi Y, Yoshimura K, Tobe T, Clarke JM, Topping DL, Suzuki T, Taylor TD, Itoh K, Kikuchi J, Morita H, Hattori M, Ohno H. Bifidobacteria can protect from enteropathogenic infection through production of acetate. Nature. 2011;469:543-7.

55. Fernández J, Redondo-Blanco S, Gutiérrez-del-Río I, Miguélez EM, Villar CJ, Lombó F. Colon microbiota fermentation of dietary prebiotics towards short-chain fatty acids and their roles as anti-inflammatory and antitumour agents: A review. J Funct Foods. 2016;25:511-22.

56. Truant AL, Gulati R, Giger O, Satishchandran V, Caya JG. Methylobacterium Species: An Increasingly Important Opportunistic Pathogen. Lab Med. 1998;29:704-10.

57. Denny T. Plant pathogenic Ralstonia species. Plant-Associated Bacteria. Springer Netherlands; 2007.

58. Hayward AC. Biology and Epidemiology of Bacterial Wilt Caused by Pseudomonas Solanacearum. Annu Rev Phytopathol. 1991;29:65-87.

59. Darfeuille-Michaud A. Adherent-invasive Escherichia coli: a putative new E. coli pathotype associated with Crohn's disease. Int J Med Microbiol. 2002;292:185-93.

60. Carvalho FA, Koren O, Goodrich JK, Johansson MEV, Nalbantoglu I, Aitken JD, Su Y, Chassaing B, Walters WA, González A, Clemente JC, Cullender TC, Barnich N, Darfeuille-Michaud A, Vijay-Kumar M, Knight R, Ley RE, Gewirtz AT. Transient Inability to Manage Proteobacteria Promotes Chronic Gut Inflammation in TLR5-Deficient Mice. Cell Host Microbe. 2012;12:139-52.
61. Zhang H, Xu B, Wang H, Xu B, Wang G, Jiang M, Lei C, Ding M, Yu P, Nie Y, Wu K, Sha S, Li M. IL-17 is a protection effector against the adherent-invasive Escherichia coli in murine colitis. Mol Immunol. 2018;93:166-72.

Figures

Figure 1
Effects of maternal dietary LA on intestinal morphometric trait in domestic pigeon squabs. a Villus height, b Crypt depth, c Villus area, d Ratio of villus height to crypt depth (VCR), e Density of enterocyte, f Hematoxylin and eosin staining of intestine (duodenum, jejunum, and ileum) in the four treatment groups. The arrow points to enterocyte. Bar = 100 μm. Control = control group; LA1% = 1% linoleic acid supplementation group; LA2% = 2% linoleic acid supplementation group; LA4% = 4% linoleic acid supplementation group. Values are means ± SEM of 6 squabs, n=6. a,b,c Means sharing no common superscripts differ significantly (Tukey test, P < 0.05).

Figure 2

Effects of maternal dietary linoleic acid on mRNA expression of tight junction proteins in domestic pigeon squabs. a mRNA expression of Claudin2 (CLDN2), b mRNA expression of Claudin3 (CLDN3), c mRNA expression of Occludin (OCLN). Control = control group; LA1% = 1% linoleic acid supplementation group; LA2% = 2% linoleic acid supplementation group; LA4% = 4% linoleic acid supplementation group. Values are means ± SEM of 6 squabs, n=6. a,b,c Means sharing no common superscripts differ significantly (Tukey test, P < 0.05).
Figure 3

Effects of maternal dietary linoleic acid on the density of intestinal goblet cell (GC) in domestic pigeon squabs. a Density of GC, b Periodic-acid Schiff staining of intestine (duodenum, jejunum, and ileum) in the four treatment groups. The arrow points to GC. Bar = 50 μm. Control = control group; LA1% = 1% linoleic acid supplementation group; LA2% = 2% linoleic acid supplementation group; LA4% = 4% linoleic acid supplementation group. Values are means ± SEM of 6 squabs, n=6. a,b Means sharing no common superscripts differ significantly (Tukey test, P < 0.05).
Figure 4

Effects of maternal dietary linoleic acid on intestinal slgA and cytokine concentrations in domestic pigeon squabs. a slgA, b TNF-α, c IL-4, d IL-6, e IL-10, f IL-1β. Control = control group; LA1% = 1% linoleic acid supplementation group; LA2% = 2% linoleic acid supplementation group; LA4% = 4% linoleic acid supplementation group. Values are means ± SEM of 6 squabs, n=6. a,b Means sharing no common superscripts differ significantly (Tukey test, P < 0.05).
Figure 5

Effects of maternal dietary linoleic acid on the alpha diversity of ileal microbiota in domestic pigeon squabs among groups. a Rarefaction curves, b OTU distribution, c alpha diversity index: Chao1, d alpha diversity index: Shannon. Control = control group; LA1% = 1% linoleic acid supplementation group; LA2% = 2% linoleic acid supplementation group; LA4% = 4% linoleic acid supplementation group. Values are means ± SEM of 6 squabs, n=6. a,b Means sharing no common superscripts differ significantly (Kruskal-Wallis test and Duncan’s test, P < 0.05).
Figure 6

Effects of maternal dietary linoleic acid on the beta diversity of ileal microbiota in domestic pigeon squabs among groups (n=6). a Principal coordinates analysis, b Clustering analysis in phylum level, c Clustering analysis in genes level. Control = control group; LA1% = 1% linoleic acid supplementation group; LA2% = 2% linoleic acid supplementation group; LA4% = 4% linoleic acid supplementation group.
Figure 7

Effects of maternal dietary linoleic acid on the microbial biomarkers of ileum in domestic pigeon squabs (n=6). Control = control group; LA1% = 1% linoleic acid supplementation group; LA2% = 2% linoleic acid supplementation group; LA4% = 4% linoleic acid supplementation group. Linear discriminant analysis effect size analysis shows differentially abundant genera as biomarkers determined using Kruskal-Wallis test (P < 0.05) with the logarithmic linear discriminant analysis score > 2.0.

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