Effects of coated sodium butyrate on production performance, egg quality, serum biochemistry, digestive enzyme activity, and intestinal health of laying hens

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ABSTRACT
The present study was conducted to investigate the effects of coated sodium butyrate (CSB) on production performance, egg quality, serum biochemistry, digestive enzyme activity, intestinal health of laying hens. 720 hens at 52 weeks were assigned in five groups including basal diet (control) and a basal diet supplemented with 250, 500, 750, and 1000 mg/kg CSB for 8 weeks. The results showed that the 500 mg/kg CSB group increased laying rate but 500 and 750 mg/kg groups decreased feed conversion ratio \( (p < .05) \). At the fourth week, dietary CSB at more than or equal to 500 mg/kg level increased the Haugh unit \( (p < .01) \); At the eighth week, 500 and 1000 mg/kg CSB groups improved the eggshell strength, Haught unit and albumen height \( (p < .05) \). Dietary CSB administration except for the 250 mg/kg group increased the serum albumin and calcium and decreased the triglyceride content \( (p < .05) \). 500 and 750 mg/kg CSB groups increased the activities of trypsin and amylase in the pancreas and duodenum \( (p < .05) \). The groups of 500 and 750 mg/kg increased the villus height and villus height to crypt depth ratio \( (V/C) \) in the jejunum and ileum \( (p < .05) \). In the jejunum, at more than or equal to 500 mg/kg CSB groups increased the expressions of ZO-1 and Occludin \( (p < .05) \); In the ileum, 500 and 750 mg/kg CSB groups increased the expressions of claudin-1 and occludin and ZO-2 \( (p < .05) \). Conclusively, dietary supplementation of CSB can improve egg production, egg quality, digestive enzyme activity and present a positive effect on improving villi and intestinal mechanical barrier function.

HIGHLIGHTS
- A significant improvement in production performance and egg quality was observed in birds fed diet with coated sodium butyrate.
- Administration of coated sodium butyrate enhanced protein and mineral availability and lipid metabolism of serum.
- Supplementation of coated sodium butyrate presented a positive impact on regulating intestinal health.

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Introduction
The growth and production of poultry rely on the intestinal digestion and absorption of the diet (Chocht 2009). Intestinal health is necessary to maintain efficient and sustainable gastrointestinal tract (GIT) physiology (Salois et al. 2016). The GIT has powerful multifunction, including digestion, absorption, metabolism, immunity, and endocrine (Svihus 2014). If the intestine health and its functions are damaged, the whole organism will be affected (Oviedo-Rondón 2019). Therefore, in poultry nutrition, we need to use supplements that improve the bird’s performance through promoting their intestine health. Sodium butyrate gets more and more focus due to its biological effects on the intestinal tissues and gut health (Elnesr et al. 2020).

The main component of sodium butyrate is butyric acid, which can be produced by the fermentation of dietary fibre by gastrointestinal microflora, in addition,
to obtain from butyrate (Pryde et al. 2002). Butyric acid is unstable and volatile, which is made into relatively stable sodium butyrate and applied in animal husbandry in feed production. It was reported that sodium butyrate had a variety of biological functions, including anti-oxidative (Ahmed 2018), anti-inflammatory (Hu et al. 2014), and antineoplastic (Wang et al. 2013). In addition, butyric acid has received more and more attention because of its regulation of intestinal health (Bedford and Gong 2018). Butyrate is the most important energy substance in colonic epithelial cells (Roediger 1982), which can regulate the differentiation and proliferation of gastrointestinal epithelial cells (Galfi and Neogrady 2001) and promote the apoptosis of genetically disordered cells (Leu et al. 2009). Sodium butyrate was found to protect the intestinal barrier by regulating the expression of tight junction protein and intestinal permeability (Song et al. 2017). Moreover, numerous studies reported that sodium butyrate had a variety of biological functions, including anti-oxidative (Ahmed 2018), anti-inflammatory (Hu et al. 2014), and antineoplastic (Wang et al. 2013). In addition, butyric acid has received more and more attention because of its regulation of intestinal health (Bedford and Gong 2018). Butyrate is the most important energy substance in colonic epithelial cells (Roediger 1982), which can regulate the differentiation and proliferation of gastrointestinal epithelial cells (Galfi and Neogrady 2001) and promote the apoptosis of genetically disordered cells (Leu et al. 2009). Sodium butyrate was found to protect the intestinal barrier by regulating the expression of tight junction protein and intestinal permeability (Song et al. 2017). Moreover, numerous studies reported that sodium butyrate is conducive to improve the development of the intestinal mucosa and morphological structures as well as regulate intestinal flora balance (Hu and Guo 2007; Smulikowska et al. 2009; Wu et al. 2018). However, uncoated sodium butyrate with a special lipid odour has a limiting effect on feed take (Lacorn et al. 2010) and is hard to reach the distal portion of the GIT (Claus et al. 2007; Piva et al. 2007). Dietary supplementation with coated sodium butyrate could delay the release of substance along with the GIT, stimulating hind-gut absorption and exerting its antimicrobial effect (Warnecke and Gill 2005; Bortoluzzi 2017). Moreover, the efficacy of butyrate was enhanced when it is fed in a coated form (Smith 2012). Considering the multifunctional and high efficiency, CSB is a candidate strategy to improve poultry production and health.

To our knowledge, the application of sodium butyrate in laying hens has been conducted actually, but recent advances on this issue are lacking. Therefore, dietary coated sodium butyrate was hypothesised to improve the performance of laying hens by ameliorating intestinal morphology, enhancing gut barrier function, and promoting nutrient absorption. The objective of the present trial is to evaluate the influence of coated sodium butyrate on egg production, egg quality, serum biochemistry, digestive enzyme activity, intestinal morphology, and barrier function of laying hens.

### Material and methods

**Experimental design, animals, and diets**

In this study, a total of 720 commercial hens of Huafeng at the age of 52 weeks were randomly assigned to five treatment groups and each group with six replicates. Four hens were housed in individual stainless steel cages (50 × 50 × 50 cm), which were equipped with two nipple drinkers and one feeder. All laying hens were kept in three-layer stepped cages, and six cages in the same layer were regarded as a replicate. The laying hens were housed in an enclosed, ventilated, and conventional room. Feed and water were provided ad libitum. The experiment lasted 9 weeks, including 1 week for acclimation and 8 weeks for the experiment. The five groups were divided into the control group and four treatment groups. The control group was fed basal diet, the hens in the other four experimental groups were fed basal diets supplemented with 250, 500, 750, and 1000 mg, respectively, and in the control without additional sodium butyrate.

### Sample collections

During the trial, the eggs from each replicate were counted and weighted daily to calculate the daily egg production, egg weight. Feed consumption was recorded weekly on a replication basis and the average daily feed intake (ADFI) and feed conversion ratio (FCR) were calculated. At the end of the experiment.

### Table 1. Ingredient compositions and nutrient levels of basal diet for hens.

| Items                          | Composition |
|-------------------------------|-------------|
| Ingredients                   | Content (%) |
| Corn                          | 62          |
| Soybean meal                  | 24.50       |
| Soybean oil                   | 0.50        |
| Limestone                     | 8           |
| Premix <sup>a</sup>           | 5           |
| Total                         | 100         |
| Nutrient <sup>b</sup>         |             |
| Metabolism energy, MJ/kg      | 10.99       |
| Crude protein, %              | 15.67       |
| Lysine, %                     | 0.80        |
| Methionine, %                 | 0.34        |
| Calcium, %                    | 3.69        |
| Total phosphorus, %           | 0.54        |

<sup>a</sup>The premix provided following per kilogram of diet: vitamin A, 7500 IU; vitamin D₃, 2500 IU; vitamin E, 49.5 mg; vitamin K₃, 2.5 mg; vitamin B₁, 1.5 mg; vitamin B₂, 4 mg; vitamin B₆, 2 mg; vitamin B₁₂, 0.02 mg; niacin, 30 mg; folic acid, 1.1 mg; pantothenic acid, 10 mg; biotin, 0.16 mg; chloride choline, 400 mg; Sodium chloride, 2500 mg; Fe, 80 mg; Cu, 20 mg; Mn, 60 mg; Zn, 80 mg; I, 0.8 mg; Se, 0.3 mg.

<sup>b</sup>The premix in five treatments provided per kilogram of diet: sodium butyrate, 250, 500, 750, and 1000 mg, respectively, and in the control without additional sodium butyrate.

<sup>c</sup>Values were calculated from Chinese feed database provided with tables of feed composition and nutritive values in China (21th edition).
two birds per replicate (12 birds each treatment) were chosen. After 12 h of fasting (water was offered ad libitum), the blood sample was obtained from the wing vein and centrifuged at 3000 x g for 15 min at 4 °C to separate serum for biochemical analysis. Then birds were euthanised by cervical dislocation. The small intestine and pancreas were collected immediately. Part of the small intestine was fixed in 4% paraformaldehyde and kept at 4 °C for histological evaluation. The pancreas and other parts of intestine were stored at −80 °C for further analysis.

**Egg quality**

In the 4th and the 8th weeks of the experiment, egg quality was measured immediately on three eggs collected randomly from each replicate. Eggshell thickness (without shell membrane) was measured by Egg Shell Thickness Gauge (ESTG-1, Orka Food Technology Ltd., Ramat Hasharon, Israel). Albumen height, Haugh unit, yolk colour, and eggshell strength were determined by a multi-functional egg quality analyser (DET-6000, Nabel Co., Ltd., Kyoto, Japan).

**Serum biochemical indicators**

The concentrations of total protein (TP), albumin (ALB), calcium (Ca), phosphorus (P), triglyceride (TG), total cholesterol (T-CHO), along with the activities of aspartate aminotransferase (GOT) and alanine aminotransferase (GPT) in the serum were assayed and calculated followed by the instructions of commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

**Determination of digestive enzyme activities**

The weight of the pancreas and duodenum chyme and the volume of Phosphate Buffered Saline was used to prepare the tissue homogenate at 1:9. The tissue homogenate was centrifuged at 3500rpm for 15 min, and the supernatant was collected and stored at −80 °C. The activities of lipase, amylase, and trypsin were determined by spectrophotometer according to the kit instructions (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). The protein concentrations were determined using the Coomassie Brilliant Blue G-250 reagent with BSA as a standard.

**Histopathological analysis**

About 1-cm segment of the duodenum, jejunum, and ileum fixed with 4% paraformaldehyde was trimmed and embedded in paraffin wax. The paraffin sections were cut into 6μm thick using a microtome (Leica Microsystems, RM2016), then stained with haematoxylin and eosin (H&E) for histopathological observation. Villus height and crypt depth of 12 villi in each intestinal sample were calculated by optical microscopy (Nikon Eclipse 80i, Nikon, Tokyo, Japan).

**Real-time PCR**

The detailed processes of total RNA extraction and complementary DNA (cDNA) synthesis are as described previously (Miao et al. 2020 (Miao et al., 2020)). The gene expressions of ZO-1, ZO-2, claudin-1, claudin-4, and occludin in jejunum and ileum were determined on a real-time polymerase chain reaction (PCR) system (ABI 7500; Applied Biosystems, Foster City, CA, USA) following the protocol of SYBR qPCR Master Mix kit (Vazyme Biotech Co., Ltd, Nanjing, Jiangsu, China). The expression of target genes was normalised to that of β-Actin. The Gene-specific primers are presented in Table 2. There were six samples in each group, and each sample was made in duplicate, excluding the template control. The 2−ΔΔCT method was used to

| Gene symbol | Gene name       | Primer sequence (5'-3')          | Accession No. |
|-------------|-----------------|----------------------------------|---------------|
| β-Actin     | β-Actin         | F: TCCCTGGAGAAGAGCTATGAA         | NM_205518.1   |
|             |                 | R: CAGGACTCTCCATACCCAAAGAAG      |               |
| ZO-1        | Zonula Occludens 1 | F: TTAGGCACAGCGAAGGGG          | XM_015278975  |
|             |                 | R: CTTGAATGCTCTTTTTTTG          |               |
| Claudin-4   | Claudin 4       | F: GAAGGCTGAACCGGATACCAA       | AY435420      |
|             |                 | R: TCTCTCTCTCTCTCTCTCT           |               |
| Occludin    | Occludin        | F: TACGCTGCTGCTTGCTCTG          | NM_205128     |
|             |                 | R: TCTCTCTCTCTCTCTCTCTCT         |               |
| Claudin-1   | Claudin 1       | F: TGGAGATGACCACTGAGAAG         | NM_001013611  |
|             |                 | R: CGAGCACTGCTGGCTGCTG          |               |
| ZO-2        | Zonula Occludens 2 | F: ACGGCTGCTGCCCAAAATGAGATG    | NM_204918     |
|             |                 | R: CCCAGTCTGCCACTCACAAGA        |               |

F: forward; R: reverse.

*p*β-Actin served as endogenous reference gene.

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Table 2. Primer sequences used for RT-qPCR analysis.
calculate the average mRNA expression level relative to the β-Actin (Livak and Schmittgen, 2001).

**Statistical analysis**

The data were statistically analysed by one-way ANOVA using SPSS 20.0 (SPSS Inc., Chicago, IL, USA) and expressed as means and SEM. Linear and quadratic effects were tested and considered significant at \( p < .05 \). Tukey post-hoc test was used to compare the significant differences \( (p < .05) \) between means. Before analysis, the laying rate was subjected to an arcsine transformation.

**Results**

**Growth performance**

The effects of CSB on production performance are summarised in Table 3. The laying rate was increased quadratically \( (p < .01) \) with the increasing of the dietary CSB levels, and did best in the group of 500 mg/kg. However, the feed conversion ratio (FCR) showed a linear and quadratic decrease \( (p < .01) \) after CSB treatments. In addition, no significant differences were found in egg weight and ADFI \( (p > .05) \). 

**Egg quality**

The effects of CSB on egg quality are shown in Table 4. The Haugh unit (HU) presented a linear and quadratic increase after CSB treatments \( (p < .01) \) at the end of the fourth week. After 8 weeks of the feeding trial, the eggshell strength, Haugh unit, and Albumen height were significantly increased in a linear or quadratic manner due to the addition of CSB in the diet \( (p < .01) \).

**Serum biochemistry**

Serum biochemical indices are presented in Table 5. The level of ALB and Ca were significantly enhanced \( (p < .05) \) in a linear and quadratic manner with dietary supplementation of CSB. On the contrary, the level of TG showed a quadratic decrease as the dietary CSB level increased \( (p < .01) \). There are no significant differences in levels of serum TP, P, T-CHO, GOT, and GPT among all groups.

**Digestive enzyme activity in the pancreas and duodenum chyme**

The digestive enzyme activity in the pancreas and duodenum is shown in Table 6. In the pancreas, the activity of amylase was linearly and quadratically

### Table 3. Effect of coated sodium butyrate (CSB) on production performance of laying hens.

| Items              | Control | 250 | 500 | 750 | 1000 | SEM  | \( p \)-Value | Linear Quadratic |
|--------------------|---------|-----|-----|-----|------|------|---------------|------------------|
| Laying rate, %     | 73.60b  | 74.62ab| 76.93a| 76.29ab| 75.11ab| 1.07 | 0.030         | 0.076            |
| Egg weight, g      | 49.56   | 49.47| 49.68| 49.21| 49.47| 0.47 | 0.894         | 0.098            |
| ADFI, g            | 89.25   | 88.04| 89.65| 89.28| 90.21| 2.05 | 0.874         | 0.480            |
| FCR                | 2.50b   | 2.50a| 2.33b| 2.34b| 2.39ab| 0.05 | 0.003         | 0.007            |

SEM: standard error of the means; ADFI: average daily feed intake; FCR: feed conversion ratio. Values are represented as the mean & SEM \( (n = 6) \). \( a,b \)Means within a column with different superscripts are significantly different \( (p < .05) \).

### Table 4. Effects of coated sodium butyrate (CSB) on egg quality of laying hen.

| Items              | Control | 250   | 500   | 750   | 1000  | SEM  | \( p \)-Value | Linear Quadratic |
|--------------------|---------|-------|-------|-------|-------|------|---------------|------------------|
| 4th week           |         |       |       |       |       |      |               |                  |
| Eggshell strength, kgf/m² | 3.97   | 4.01  | 4.10  | 4.07  | 4.03  | 0.10 | 0.691         | 0.373            |
| Eggshell thickness, mm | 0.34   | 0.34  | 0.34  | 0.34  | 0.35  | 0.05 | 0.586         | 0.132            |
| Haugh unit         | 73.15b  | 74.72ab| 76.88a| 76.65a| 76.93a| 1.10 | 0.006         | 0.001            |
| Albumen height, mm | 5.17    | 5.11  | 5.24  | 5.20  | 5.37  | 0.16 | 0.575         | 0.160            |
| Yolk colour        | 6.56    | 6.61  | 6.39  | 6.44  | 6.33  | 0.17 | 0.441         | 0.103            |
| 8th week           |         |       |       |       |       |      |               |                  |
| Eggshell strength, kgf/m² | 3.73   | 3.90ab| 4.00a | 4.03a | 3.92a | 0.06 | 0.001         | <0.001           |
| Eggshell thickness, mm | 0.38   | 0.39  | 0.39  | 0.38  | 0.38  | 0.01 | 0.411         | 0.736            |
| Haugh unit         | 67.14b  | 70.76ab| 73.27a| 72.76ab| 73.63a| 2.07 | 0.022         | 0.003            |
| Albumen height, mm | 4.85b   | 5.12ab| 5.31a | 5.27ab| 5.47a | 0.14 | 0.003         | <0.001           |
| Yolk colour        | 6.17    | 6.5   | 6.33  | 6.17  | 6.17  | 0.27 | 0.659         | 0.575            |

SEM: standard error of the means. Values are represented as the mean & SEM \( (n = 18) \). \( a,b \)Means within a column with different superscripts are significantly different \( (p < .05) \).
increased ($p < .01$) by increasing the CSB levels in the diet, whereas the activity of trypsin responded quadratically ($p < .01$). In the duodenum, the activity of trypsin and amylase showed a quadratic increase as dietary CSB levels increased ($p < .01$). No significant difference was observed among all groups in the activity of lipase ($p > .05$).

The morphology of intestinal tract

Histological changes in the small intestine were assessed by a light microscope. As shown in Table 7, in the jejunum, increasing dietary CSB concentration significantly increased the villus height and V/C in a linear or quadratic manner ($p < .01$). In the ileum, the villus height and V/C presented a quadratic increase ($p < .01$). No difference was exhibited in duodenum morphology ($p > .05$).

Gene expression of tight junctions (TJs) in jejunum and ileum

The expression of TJs in the jejunum and ileum is shown in Figure 1. In the jejunum, CSB treatment significantly upregulated the mRNA levels of ZO-1 and Occludin in a linear or quadratic manner ($p < .01$), but did not affect the mRNA expression levels of ZO-2, Claudin-1, and Claudin-4. In the ileum, the gene expressions of ZO-2, Claudin-1, and occludin showed a quadratic increase as the dietary CSB levels increased ($p < .01$), whereas this had no effects on the mRNA levels of ZO-1 and Claudin-4 ($p > .05$).

Discussion

Previous studies have found that dietary supplementation of sodium butyrate benefitted the performance and health condition of animals. Pardo et al. (2009) reported that dietary supplementation of coated sodium butyrate could significantly increase the daily gain, feed intake, and feed conversion rate of broiler in the middle and late period. Sikandar et al. (2017) observed that feeding sodium butyrate at 500 mg/kg diet could significantly improve the growth performance of broilers. Similarly, in the present study, different levels of CSB improved egg production and feed efficiency to varying degrees. These observed improvements in bird growth egg production and feed efficiency resulted from CSB supplementation can be, at least partially, explained by the following two distinct aspects. On the one hand, butyrate sodium has a beneficial effect on gut tissue and intestinal function (Kotunia, 2004; Hu and Guo, 2007). On the other hand, butyrate sodium indirectly enhanced the

| Table 5. Effect of coated sodium butyrate (CSB) on serum biochemistry of laying hens. |
|----------------|----------------|----------------|----------------|----------------|
| **Items**     | **Control**   | **Coated sodium butyrate (mg/kg)** | **SEM** | **p-Value** | **Linear** | **Quadratic** |
| **TP, g/L**   | 22.29         | 23.46           | 27.18          | 23.98          | 26.98       | 2.25         | 0.147         | 0.083         | 0.182         |
| **ALB, g/L**  | 19.96         | 23.42           | 24.97          | 24.61          | 24.40       | 1.38         | 0.008         | 0.036         | 0.004         |
| **Ca, mmol/L**| 3.29          | 3.44            | 3.56           | 3.61           | 3.52        | 0.08         | 0.031         | 0.011         | 0.034         |
| **P, mmol/L** | 1.25          | 1.27            | 1.30           | 1.31           | 1.29        | 0.05         | 0.799         | 0.283         | 0.426         |
| **TG, mmol/L**| 15.33         | 13.22           | 11.76          | 11.61          | 14.63       | 1.41         | 0.047         | 0.402         | 0.009         |
| **T-CHO, mmol/L** | 3.40   | 2.89            | 2.95           | 3.02           | 3.16        | 0.38         | 0.686         | 0.686         | 0.379         |
| **GOT, IU/L** | 23.07         | 22.87           | 25.27          | 24.13          | 22.93       | 1.76         | 0.613         | 0.802         | 0.480         |
| **GPT, IU/L** | 0.54          | 0.80            | 0.63           | 0.71           | 0.54        | 0.21         | 0.656         | 0.846         | 0.509         |

SEM: standard error of the means; TP: total protein; ALB: albumin; Ca: calcium; P: phosphorus; TG: triglyceride; T-CHO: total cholesterol; GOT: aspartate aminotransferase; GPT: alanine aminotransferase.

Values are represented as the mean & SEM (n = 12).

# Means within a column with different superscripts are significantly different ($p < .05$).

| Table 6. Effects of coated sodium butyrate (CSB) on the digestive enzyme activity of laying hens. |
|----------------|----------------|----------------|----------------|----------------|
| **Items**     | **Control**   | **Coated sodium butyrate (mg/kg)** | **SEM** | **p-Value** | **Linear** | **Quadratic** |
| **Pancreas**  |               |                             |           |             |           |               |
| Trypsin, U/mgprot | 334.15<sup>a</sup> | 394.46<sup>a</sup> | 450.80<sup>a</sup> | 463.27<sup>a</sup> | 386.66<sup>a</sup> | 37.13         | 0.022         | 0.102         | 0.004         |
| Lipase, U/gprot | 235.42        | 284.80          | 270.17          | 246.25          | 245.74      | 33.19         | 0.580         | 0.813         | 0.455         |
| Amylase, U/mgprot | 50.29<sup>b</sup> | 55.25<sup>a</sup> | 77.87<sup>a</sup> | 77.36<sup>a</sup> | 76.11<sup>a</sup> | 7.46          | 0.008         | 0.002         | 0.002         |
| **Duodenum**  |               |                             |           |             |           |               |
| Trypsin, U/mgprot | 238.39<sup>a</sup> | 335.81<sup>a</sup> | 357.23<sup>a</sup> | 352.97<sup>a</sup> | 309.12<sup>b</sup> | 31.20         | 0.010         | 0.093         | 0.001         |
| Lipase, U/gprot | 84.76         | 88.13           | 87.16           | 84.58           | 84.06       | 2.62          | 0.447         | 0.402         | 0.271         |
| Amylase, U/mgprot | 1.87<sup>bc</sup> | 3.11<sup>ab</sup> | 4.57<sup>a</sup> | 4.07<sup>a</sup> | 2.90<sup>ab</sup> | 0.64          | 0.013         | 0.170         | 0.002         |

SEM: standard error of the means.

Values are represented as the mean & SEM (n = 12).

<sup>a</sup>–<sup>c</sup>Means within a column with different superscripts are significantly different ($p < .05$).
digestion and absorption of nutrients of laying hens via increasing the activity of digestive enzymes, which can be proved that feeding the diet supplemented with CSB improved the trypsin and amylase activity. Moreover, we found a positive effect of CSB on the performance of hens is at relatively lower dietary sodium butyrate contents (500–750 mg/kg). This suggests that a dose–effect response exists between sodium butyrate and hens performance, which was supported by the non-significant effect of 1000 mg/kg coated sodium butyrate in this study and the similar result reported in broilers by Lan et al. (2020).

Eggs exist the high risk of eggshell damage in the process of collection, packaging, and transportation which cause huge economic losses to lay producer (Li et al. 2019). One of the main concerns is the value of eggshell strength is not enough big. In our study, CSB treatments led to a significant increase in eggshell strength as shown in Table 7.

**Table 7. Effects of coated sodium butyrate (CSB) on villi morphology of small intestine of laying hens.**

| Items          | Control | Coated sodium butyrate(mg/kg) | SEM | p-Value | Linear | Quadratic |
|----------------|---------|-------------------------------|-----|---------|--------|-----------|
| Duodenum       |         |                               |     |         |        |           |
| Villus height, μm | 1286.41 | 1281.04 | 1305.83 | 1296.82 | 1289.63 | 43.99 | 0.983 | 0.815 | 0.922 |
| Crypt depth, μm | 170.77  | 166.31 | 163.33 | 165.45 | 163.97 | 0.85 | 0.38 | 0.757 | 0.334 | 0.451 |
| V/C            | 7.54    | 7.74 | 8.03 | 7.85 | 7.89 | 0.38 | 0.38 | 0.757 | 0.334 | 0.451 |
| Jejunum        |         |                               |     |         |        |           |
| Villus height, μm | 996.23 | 1047.86 | 1190.44 | 1153.17 | 1137.26 | 48.37 | 0.003 | 0.003 | 0.001 |
| Crypt depth, μm | 139.65 | 133.18 | 136.70 | 135.24 | 138.02 | 0.34 | 0.34 | 0.001 | 0.003 | <0.001 |
| V/C            | 7.14    | 7.88 | 8.71 | 8.52 | 8.23 | 0.34 | 0.34 | 0.001 | 0.003 | <0.001 |
| Ileum          |         |                               |     |         |        |           |
| Villus height, μm | 723.05 | 775.73 | 807.69 | 778.18 | 778.94 | 21.29 | 0.008 | 0.028 | 0.001 |
| Crypt depth, μm | 87.12  | 81.65 | 82.63 | 85.48 | 86.61 | 3.04 | 0.30 | 0.700 | 0.170 |
| V/C            | 8.34    | 9.54 | 9.78 | 9.26 | 9.02 | 0.44 | 0.44 | 0.028 | 0.341 | 0.009 |

SEM: standard error of the means; V/C: Villus height/crypt depth. Values are represented as the mean & SEM (n = 12).

Means within a column with different superscripts are significantly different (p < .05).

**Figure 1.** Effects of coated sodium butyrate (CSB) on mRNA expression of ZO-1, ZO-2, claudin-1, claudin-2, and occludin of laying hens. Method of 2^-ΔΔCt_ was applied for calculation of relative gene expression with β-actin as the endogenous control and the average ΔCt value of control group as the calibrator to normalise the signal. Values were expressed as mean with range (n = 6). Columns with different superscript letters were significantly different (p < .05). PL and PQ represented linear and quadratic analysis, respectively.

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| Crypt depth, μm | 170.77  | 166.31 | 163.33 | 165.45 | 163.97 | 0.85 | 0.38 | 0.757 | 0.334 | 0.451 |
| V/C            | 7.54    | 7.74 | 8.03 | 7.85 | 7.89 | 0.38 | 0.38 | 0.757 | 0.334 | 0.451 |
| Jejunum        |         |                               |     |         |        |           |
| Villus height, μm | 996.23 | 1047.86 | 1190.44 | 1153.17 | 1137.26 | 48.37 | 0.003 | 0.003 | 0.001 |
| Crypt depth, μm | 139.65 | 133.18 | 136.70 | 135.24 | 138.02 | 0.34 | 0.34 | 0.001 | 0.003 | <0.001 |
| V/C            | 7.14    | 7.88 | 8.71 | 8.52 | 8.23 | 0.34 | 0.34 | 0.001 | 0.003 | <0.001 |
| Ileum          |         |                               |     |         |        |           |
| Villus height, μm | 723.05 | 775.73 | 807.69 | 778.18 | 778.94 | 21.29 | 0.008 | 0.028 | 0.001 |
| Crypt depth, μm | 87.12  | 81.65 | 82.63 | 85.48 | 86.61 | 3.04 | 0.30 | 0.700 | 0.170 |
| V/C            | 8.34    | 9.54 | 9.78 | 9.26 | 9.02 | 0.44 | 0.44 | 0.028 | 0.341 | 0.009 |

SEM: standard error of the means; V/C: Villus height/crypt depth. Values are represented as the mean & SEM (n = 12).

Means within a column with different superscripts are significantly different (p < .05).
Weaver (2017). Therefore, we speculated that the enhanced bioavailability of calcium (Whisner and and lowered the pH value of the intestine, and butyrate) increased the eggshell breaking strength, demonstrated that dietary supplementation of SCFA (mainly butyrate). Sengor et al. (2007) also demonstrated that dietary inclusion of CSB (mainly butyrate) significantly reduced the TG content. Results from our study showed that dietary CSB significantly increased Haught unit and album height, suggesting that CSB is effective in persevering the freshness of eggs. There is no difference in other egg quality traits, indicating that CSB had no effect on egg yellow pigmentation. Based on the above results, dietary supplementation with CSB is meaningful to the transportation and preservation of eggs.

Serum biochemical indexes of animals can be used as diagnostic tools to reflect the physiological health status of the body (Nyblom et al. 2004; Prvulovic et al. 2012). The TP and ALB contents can reflect the state of protein absorption and metabolism of the body (Liu et al. 2013). Results from our experiment revealed that dietary inclusion of CSB significantly promoted serum ALB levels. The reason may be that butyric acid can increase body bioavailability and preserve proteins by increasing the absorption of certain essential amino acids in the intestine (Roberts 2004; Ng and Koh 2017). In poultry, calcium is the key element of eggshell formation, which takes place along the gastrointestinal tract (Sugiyama et al. 2007). Sodium butyrate, as an SCFA, could irritate epithelial cell proliferation and intestinal morphology (García et al. 2007), and then effectively make use of the calcium (Boling-Frankenbach et al. 2001; Soltan 2008). There was also evidence that sodium butyrate can increase the villus height and total mucosa of ileum (Zou et al. 2019). In our study, the serum calcium was enhanced by supplementing with CSB, which is consistent with the change in eggshell. The concentrations of T-CHO and TG can reflect the status of body fat metabolism. Results from our study found that the supplementation of CSB significantly reduced the TG content. Triglycerides are synthesised in the liver, whose level can be used as an indicator of liver function (Osman et al. 2010; Deng et al. 2011). We surmised that CSB may reduce TG levels by inhibiting hepatic lipogenesis. To sum up, CSB is beneficial to enhance protein and mineral availability and lipid metabolism, but more in-depth studies need to be explored.

The digestion and absorption of food require the coordination and cooperation of the various organs of the digestive system. The activities of digestive enzymes are valuable indicators to evaluate feed utilisation efficiency and performance of domestic animals (Yi et al. 2013). Interestingly, the current study indicated that dietary supplementation CSB significantly increased the trypsin and amylase activities in the pancreas and duodenum. These outcomes were in line with previous studies, which showed that organic acid can enhance digestive enzymes and or inhibit pathogenic bacteria due to its slight acidifying properties (Castillo et al. 2014; Hoseinifar et al. 2017). These results revealed that CSB addition improved the digestibility of the protein and starch components of the feed. Furthermore, these data explained the improved feed conversion efficiency observed in hens supplemented with CSB. These outcomes were consistent with previous studies, which provided evidence that dietary CSB supplementation can improve nutrient digestibility in piglet and broiler (Zhong et al. 2009; Yang et al. 2011).

The morphology of the villi and crypts of the intestinal mucosa are essential indicators of intestinal health (Chen et al. 2021). The longer the villus height and the shallower the crypt depth in the small intestine, the greater the ability to absorb nutrients. The ratio of villus height to crypt depth can comprehensively reflect the functional status of the gut. Increased ratio indicated favourable intestinal mucosa and enhanced digestion and absorption capacity (Chee et al. 2010). A report showed that sodium butyrate could significantly improve the histomorphological indexes of jejunum and duodenum of broilers (Sikandar et al. 2017). Czerwinski et al. (2012) demonstrated that the villus height of jejunum in broilers was significantly increased by adding coated sodium butyrate. Same as previous studies, our results revealed that dietary CSB intake increased the villus height and ratio of villi height to the crypt depth of the jejunum and ileum, promoting the intestinal health. The possible reason is that the addition of butyric acid increased the content of SCFA, which facilitated the proliferation of intestinal epithelial cells leading to longer villi (Tomaszewksa et al. 2018). Meanwhile, our result found that CSB presented better on the hindgut morphology because CSB is a coated
product to achieve protection and sustained release of biological activity.

A major component of the intestinal barrier is the formation of tight junctions between epithelial cells (Liu et al. 2019). Tight junctions are complex composed of a variety of proteins, mainly including transmembrane proteins (claudin family and occludin) and cytoplasmic proteins (ZOs), which play an important role in maintaining the polarity of intestinal mucosal epithelial cells and regulating the permeability of the intestinal barrier (Camilleri et al. 2012). Occludin and claudin protein has strong adhesion to close cell voids and protect intestinal barriers. Simultaneously, ZOs can enhance the formation of tight junctions between cells, thus preventing macromolecules, such as bacteria and toxins from entering the body and maintaining the physical barrier function of the intestinal tract (Xu et al. 2016). Our results showed that the supplementation of CSB could significantly affect intestinal integrity and increase the gene expressions of ZO-1, ZO-2, Claudin-1, and Occludin, indicating that CSB could enhance the intestinal barrier. This was consistent with a previous study that the addition of sodium butyrate increased the intestinal gene expression of claudin and ZO-1 in fish (Liu et al. 2019). Ma et al. (2012) also reported that sodium butyrate increased the gene expression of Zos, which could promote wound healing. A recent report (Peng et al. 2009) has shown that butyrate accelerated the assembly of tight junctions by activating AMP-activated protein kinase (AMPK) to enhance intestinal barrier function. AMPK is a serine/threonine kinase, which relates to the synthesis of glucose and fatty acid metabolism and protein (Hardie et al. 2006). Tight junction assembly is impaired when AMPK activity is down-regulated by gene manipulation strategies (Zhang et al. 2006; Zheng and Cantley 2007). We hypothesised that the increased expression of genes involved in the intestinal barrier was associated with the up-regulation of AMPK activity. However, the concrete mechanisms remain to be expounded.

Conclusions

In conclusion, the current study indicated that dietary coated sodium butyrate supplementation could improve egg production and egg quality, enhance protein and mineral availability, and lipid metabolism. Furthermore, coated sodium butyrate could improve digestive enzyme activity, intestinal morphology, and intestinal barrier function. In this experiment, 500 mg/kg is the suitable added concentration of coated sodium butyrate on hens’ diet according to quadratic regression analysis.

Ethical approval

The current study was 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).

Disclosure statement

The authors declare no conflict of interest.

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