Supplemental effect of bamboo charcoal and bamboo vinegar, alone or in combination, on laying hen performance, egg quality, intestinal bacterial populations and alteration of intestinal villi

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Abstract

This experiment aimed to determine the effects of bamboo charcoal (BC), bamboo vinegar (BV) and their combinations in laying hen diet on performance, egg quality, intestinal bacterial populations and alteration of intestinal villi. A total of 450 Hy-Line Brown hens aged 40 weeks were divided into 9 treatment groups, with 5 replicates per treatment and 10 hens per replicate. They were fed diets with 0% BC and BV (control), 0.5% BC, 1% BC, 0.3% BV, 0.6% BV, 0.5% BC + 0.3% BV (BCV53), 0.5% BC + 0.6% BV (BCV56), 1% BC + 0.3% BV (BCV13) or 1% BC + 0.6% BV (BCV16) from 40 to 56 weeks of age. The overall feed conversion ratio was better in all supplemented groups than in the control (p < .05). Eggshell strength and shell thickness at week 56 were higher in the 0.3% BV, 0.6% BV, BCV56 and BCV16 groups when compared to the control group (p < .05). In the ileal digesta, population of Salmonella spp. decreased in the 0.3% BV, 0.6% BV, BCV56 and BCV16 groups (p < .05) and the population of Lactobacillus spp. increased in the 0.3% BV, 0.6% BV and BCV56 groups (p < .05). Villus height and areas of the duodenum were higher in the groups receiving BV (p < .05), and villus height and areas of jejunum were higher in all groups receiving BV at 0.6% level (p < .05). The present results indicate that feeding of BV alone at 0.3% level was sufficient to improve feed efficiency, eggshell quality, growth of Lactobacillus spp. and function of duodenal villi in laying hens. Supplementation with BV at 0.6% level, either alone or in combination with BC in the diet, had an additional positive effect in stimulating jejunal villi.

Highlights

- Supplementation of BV alone in the diet at 0.3% level was sufficient to improve feed efficiency, eggshell quality and intestinal microbial balance.
- Supplementation of BV at 0.6% level, either alone or in combination with BC in the diet, had a stimulant effect on the functioning of intestinal villi.

Introduction

In the poultry industry, antibiotics have been widely used to maintain health and improve productivity. However, with bans on antibiotics in animal feed and increasing concerns over food safety, there is a growing interest in finding alternatives to antibiotics. Some natural substances are possible alternatives to antibiotics for health promotion and performance improvement in poultry. Charcoal has been known as a good adsorbent material because it easily links to various types of molecules. Activated charcoal has the characteristics of large specific surface area, great porosity, and high adsorption capacity. Activated bamboo charcoal made by the dry distillation of bamboo stem is a universal adsorbent that can bind with various molecules. Bamboo charcoal (BC) is four times more porous than general wood charcoal, which significantly increases its effectiveness (Zhao et al. 2008). It has been used as an adsorbent for the management of patients with drug overdoses and poisonings. Charcoal has been used in animal feed as a feed additive due to its properties for absorbing toxins and...
gases and eliminating the poisons from the gastrointestinal tract of animals. The use of BC in animal feeding seems to be a possible way to improve the performance of animals. Studies have reported that supplementing BC in the diet improved the body weight gain and feed efficiency of broiler chickens (Kim et al. 2011) and the fattening pigs (Chu et al. 2013). Goats fed a diet containing 0.5 g of BC per kg of body weight grew faster than the controls (Van et al. 2006). Bamboo vinegar (BV) is a condensate obtained by cooling the smoke while making bamboo charcoal and contains more than 200 chemical components, such as organic acids, phenolic compounds, alcohols, furans, aldehydes and ketones, among which the organic acids are the main component (Rattanawut et al. 2018). Pyroligneous acid has diverse applications as an antimicrobial, antioxidant, anti-inflammatory, pesticide, herbicide and plant growth stimulator. Bamboo vinegar is used in animal nutrition as a feed acidifier. It is added to diets to reduce feed buffering capacity and maintain the optimum pH of feed and gastrointestinal tract. The primary purpose of dietary vinegar in animal feed is to reduce the pathogens or increase beneficial bacteria, both in the feed and in the gastrointestinal tract to support enteric health and performance of animals (Partanen and Mroz 1999; Griggs and Jacob 2005). Studies have reported that supplementation of BV in the diet could increase nutrient digestibility, body weight gain and reduce the faecal Escherichia coli population of growing pigs (Ahmed et al. 1970; Wang et al. 2012). Recently, we demonstrated that feeding BV at 0.4% improved production performance in egg-laying ducks (Rattanawut et al. 2019) and reduced damaged egg rate in aged laying hens (Rattanawut et al. 2018). However, there has been little information on the potential effects of BC or BC mixed with BV on layer performance and egg quality. Mixtures may be more beneficial than single components as they act on different sites and provide different modes of action that could create synergistic effects. The objectives of this study were, therefore, to evaluate the potential effects of both substances (alone or in combination), on egg production performance, egg quality, intestinal bacterial populations and alteration of intestinal villi in laying hens, and to demonstrate which alternative is the most effective.

Materials and methods

Experimental design and animals

A total of 450 Hy-Line Brown hens (aged 40 weeks) with similar mean body weight and egg production levels were randomly assigned to nine experimental diets, with 5 replicates per treatment and 10 hens per replicate. The rearing cage dimensions were 40 × 45 × 35 cm (900 cm² for each bird), equipped with nipple drinkers and tube feeders. During the experiment, the hens had free access to mash feed and clean water. The lighting program was set at 16 h of light and 8 h of darkness throughout the study. The basal diet was an antibiotic-free diet based on corn and soybean meal (Table 1) and balanced to meet the nutrient requirements for laying hens (Hy-Line Brown Management Guide). They were fed on (1) a control diet, (2) the control diet + 0.5% BC (0.5% BC), (3) the control diet + 1% BC (1% BC), (4) the control diet + 0.3% BV (0.3% BV), (5) the control diet + 0.6% BV (0.6% BV), (6) the control diet + 0.5% BC + 0.3% BV (BCV53), (7) the control diet + 0.5% BC + 0.6% BV (BCV56), (8) the control diet + 1% BC + 0.3% BV (BCV13) or (9) the control diet + 1% BC + 0.6% BV (BCV16). Commercial BC (pH = 9.6) and BV (pH = 3.1) used in this study were purchased from a community enterprise located in Surat Thani province, Thailand. The chemical properties of BV and the composition of BC were analysed in the university laboratory, and the results are presented in Tables 2 and 3, respectively. Laying hens were fed an experimental diet from 40 to 56 weeks of age.

Laying performance

Bodyweight change was calculated from the initial body weight (40 weeks of age) and the final body

| Ingredient | Amount, % |
|------------|-----------|
| Corn       | 56.94     |
| Soybean meal | 22.48   |
| Rice bran  | 4.00      |
| Fish meal  | 4.00      |
| Oyster shell | 3.00   |
| Dicalcium phosphate (18% P) | 2.00 |
| Plant oil  | 2.55      |
| DL-Methionine | 0.13 |
| Salt       | 0.30      |
| Premix a   | 0.30      |

Calculated analysis

| Item                  | Amount, % |
|-----------------------|-----------|
| Crude protein (CP)    | 16.5      |
| Metabolizable energy, kcal/kg | 2800    |
| Crude fibre           | 3.43      |
| Crude fat             | 5.69      |
| Calcium               | 4.08      |
| Available phosphorus  | 0.45      |
| Lysine                | 0.88      |

Fixed and measured ingredients of the basal diet (as-fed basis).

*Premix: 2.0 MIU vitamin A, 0.32 MIU vitamin D₃, 2,000 µg vitamin E, 330 µg vitamin K₃, 220 µg vit B₁, 450 µg vitamin B₂, 4.5 mg vitamin B₁₂, 600 µg niacin, 100 µg copper, 150 µg iodine, 130 µg cobalt, 10 g iron, 8.8 g manganese, 8.8 g zinc, 25 g preservative, up to 1 kg filter.
weight (56 weeks of age). Laying performance was determined every 8 weeks by recording egg production, egg weight, egg mass, feed intake and feed conversion ratio. Eggs were collected daily and the weight was recorded to calculate mean egg weight. Egg production was expressed as average hen-day production. Egg mass was calculated by multiplying egg weight by egg production. Feed intake was determined by subtracting the remaining feed from the original amount of feed at the end of each week. The feed conversion ratio was calculated by dividing feed intake by the egg mass.

**Measurement of egg quality**

Egg quality was estimated every 8 weeks (weeks 48 and 56) by measuring the whole egg weight, eggshell weight, eggshell strength, eggshell thickness, yolk weight, albumen weight, Haugh unit and yolk colour of each egg, obtained on the last day of each period of the experiment. The weight of the collected eggs from each group was recorded. The eggshell breaking strength was measured using an eggshell strength tester. After the eggs were broken on a plate, the shell and egg yolk weights were measured using a digital balance. Albumen’s weight was calculated by subtracting the weight of the yolk and shells of the whole egg weight. Eggshell thickness was estimated as the mean from three measured locations (air cell, equator, and sharp end), and was measured by a digital micrometer (Mitutoyo Corporation, Kanagawa, Japan). Yolk colour and Haugh units were determined using the digital egg tester (DET6000, NABEL Co., Ltd, Kyoto, Japan). Haugh units were automatically calculated using the formula: \[ HU = 100 \log[H - 1.7 W^{0.37} + 7.6] \]
in which \( HU \) = Haugh unit, \( H \) = albumen height (mm) and \( W \) = egg weight (g).

**Intestinal microflora analyses**

At 56 weeks of age, samples of ileal digesta contents (5 samples/diet) were collected aseptically in vinyl
One gram of ileal content was suspended in a sterile test tube containing 9 ml sterile buffered peptone water and homogenised by an electric touch mixer. Samples were then serially diluted from $10^{-2}$ to $10^{-5}$. One-tenth millilitre from each dilution was withdrawn and spread on the appropriate agar media. 

Escherichia coli and Salmonella spp. were assayed using Eosin Methylene Blue agar and Salmonella-Shigella agar, respectively, and incubated at 37°C for 24 h. Lactobacillus spp. was assessed using lactobacillus MRS agar, and incubated at 37°C for 48 h. The colonies on each plate were counted, and the results are expressed in log10 CFU per gram of fresh sample.

### Intestinal sampling and measurement of intestinal villi

At the end of the experimental period (56 weeks of age), 5 birds per group were used for intestinal morphological observations of the villi in each intestinal segment. The birds were immediately eviscerated for the collection of intestinal samples. The midpoint of the duodenum, jejunum and ileum were fixed in 10% neutral-buffered formalin. After dehydration in graded alcohol, intestinal segments were embedded in Paraplast. Transverse 5 μm sections were cut, and stained with haematoxylin and eosin. Villus height and villus area were evaluated using an automatic image analyser (Olympus DP73 camera, Olympus Corporation, Tokyo, Japan) connected to a light microscope. The height of each villus was measured from the top of the villus to the crypt transition. The heights of 16 villi in each segment were measured per bird. The mean villus height from five birds is given as the mean villus height for a treatment group. Villus area was calculated from the villus height, basal width, and apical width. A total of 16 calculations of the villus area were made for each bird, and the mean of five birds is given as the mean villus area for one treatment group.

### Statistical analysis

The experimental data were statistically analysed using one-way ANOVA, and significant differences between treatments were determined with Duncan’s multiple range test using SPSS 16.0 statistical software (SPSS Inc., Chicago, IL, USA). The results are expressed as the mean and the pooled standard error of the mean (SEM). $p < .05$ was considered significant, and $p < .10$ was considered a tendency.

### Results

#### Laying performance

Performances of laying hens are presented in Table 4. Although egg production and egg weight were not significantly different among the groups ($p > .05$), egg mass was higher in all supplemented groups during 49–56 weeks of age ($p < .05$). Feed intake decreased

| Parameters          | Control | 0.5% BC | 1.0% BC | 0.3% BV | 0.6% BV |
|---------------------|---------|---------|---------|---------|---------|
| Body weight change, g | 141.50  | 142.20  | 120.70  | 124.90  | 128.70  | 136.60  | 126.20  | 138.20  | 124.90  | 2.56  | .363 |
| Egg production, %   |         |         |         |         |         |         |         |         |         |      |     |
| 41–48 weeks         | 88.06   | 88.79   | 90.22   | 91.07   | 89.64   | 88.82   | 90.79   | 88.44   | 89.24   | 0.38  | .602 |
| 49–56 weeks         | 87.50   | 90.71   | 91.42   | 91.43   | 90.71   | 91.07   | 91.07   | 91.78   | 90.71   | 0.34  | .150 |
| Egg weight, g       |         |         |         |         |         |         |         |         |         |      |     |
| 41–48 weeks         | 87.78   | 89.75   | 90.83   | 91.24   | 90.17   | 89.94   | 90.93   | 90.11   | 89.98   | 0.29  | .236 |
| 49–56 weeks         | 61.24   | 61.01   | 61.37   | 61.15   | 61.26   | 61.42   | 61.20   | 60.94   | 61.43   | 0.06  | .550 |
| 41–56 weeks         | 61.94   | 61.91   | 62.07   | 62.14   | 62.39   | 62.38   | 62.59   | 62.48   | 62.49   | 0.04  | .492 |
| Egg mass, g         |         |         |         |         |         |         |         |         |         |      |     |
| 41–48 weeks         | 53.94   | 54.17   | 55.37   | 55.69   | 54.91   | 54.54   | 55.56   | 53.90   | 54.83   | 0.23  | .517 |
| 49–56 weeks         | 54.20b  | 56.16b  | 56.74b  | 56.81b  | 56.59b  | 56.80b  | 56.99b  | 57.34b  | 56.68b  | 0.21  | .031 |
| 41–56 weeks         | 54.07   | 55.16   | 56.06   | 56.25   | 55.75   | 55.67   | 56.28   | 56.61   | 55.75   | 0.18  | .116 |
| Feed intake, g/day  |         |         |         |         |         |         |         |         |         |      |     |
| 41–48 weeks         | 110.28  | 109.75  | 107.66  | 108.55  | 109.23  | 108.70  | 107.67  | 108.21  | 108.47  | 0.34  | .701 |
| 49–56 weeks         | 111.60a | 111.76a | 108.15a | 111.32a | 111.31a | 111.22ab| 109.43ab| 109.96ab| 110.19ab| 0.26  | .010 |
| 41–56 weeks         | 110.94  | 110.75  | 107.91  | 109.93  | 110.27  | 109.46  | 108.55  | 109.09  | 109.33  | 0.25  | .059 |
| Feed conversion ratio|         |         |         |         |         |         |         |         |         |      |     |
| 41–48 weeks         | 2.04    | 2.02    | 1.94    | 1.95    | 1.99    | 1.99    | 1.93    | 2.01    | 1.98    | 0.02  | .298 |
| 49–56 weeks         | 2.05b   | 1.99ab  | 1.90b   | 1.96b   | 1.96b   | 1.94b   | 1.92b   | 1.91b   | 1.94b   | 0.01  | .009 |
| 41–56 weeks         | 2.05a   | 2.00ab  | 1.92c   | 1.95bc  | 1.97bc  | 1.97bc  | 1.93c   | 1.96bc  | 1.96bc  | 0.01  | .010 |

Values with different superscripts in the same row are significantly different ($p < .05$). Values are means of 5 replicates.
in the 1% BV fed group during 49–56 weeks of age (p < .05) and tended to be lower in supplemented groups for the overall period (p = .059). The feed conversion ratio was better in all supplemented groups during 49–56 weeks of age and overall (p < .05) except for the 0.5% BC group that was similar to the control group. Bodyweight changes were not significantly different among the groups (p > .05).

Egg quality traits

The effects of dietary BC, BV and their combinations on egg quality are presented in Table 5. Eggshell strength was higher in the 0.3% BV, 0.6% BV, BCV56 and BCV16 groups at week 56 (p < .05). Compared to the control group, the eggshell thickness was higher for hens fed the 0.3% BV, 0.6% BV and BCV56 diets at week 56 (p < .05). There were no statistically significant differences between groups in whole egg weight, albumen weight, yolk weight, eggshell weight, yolk colour, or Haugh units (p > .05).

Ileal microflora population

Table 6 shows ileal microflora counts. The population of ileal Salmonella spp. decreased in the 0.3% BV, 0.6% BV, BCV56 and BCV16 groups (p < .05). The number of Lactobacillus spp. significantly increased in the hens fed 0.3% BV, 0.6% BV and BCV56 diets (p < .05). The ileal E. coli colony counts did not differ significantly among the treatments (p > .05).

Intestinal villi morphology

Villus height and villus area are shown in Table 7. Compared to the control group, villus height and area in duodenum were higher in the 0.3% BV, 0.6% BV and all the mixture groups (p < .05). Villus height and

Table 5. Effects of bamboo charcoal (BC), bamboo vinegar (BV) and their combination on egg quality traits of laying hens at 48 and 56 weeks of age.

| Parameters            | Control | 0.5% BC | 1.0% BC | 0.3% BV | 0.6% BV | 0.5% BC + 0.3% BV | 0.5% BC + 0.6% BV | 1.0% BC + 0.3% BV | 1.0% BC + 0.6% BV | SEM | p-Value |
|-----------------------|---------|---------|---------|---------|---------|-------------------|-------------------|-------------------|-------------------|-----|---------|
| Egg weight, g         |         |         |         |         |         |                   |                   |                   |                   |     |         |
| 48 weeks              | 61.36   | 61.44   | 61.28   | 61.20   | 61.18   | 61.24             | 61.24             | 61.18             | 61.24             | 0.05| .556    |
| 56 weeks              | 62.62   | 62.40   | 63.16   | 62.38   | 62.72   | 63.18             | 62.36             | 62.64             | 62.46             | 0.20| .985    |
| Eggshell weight, g    |         |         |         |         |         |                   |                   |                   |                   |     |         |
| 48 weeks              | 6.94    | 6.63    | 6.77    | 6.91    | 6.68    | 7.06              | 6.88              | 6.59              | 7.04              | 0.05| .346    |
| 56 weeks              | 7.06    | 7.26    | 7.30    | 7.06    | 7.13    | 7.05              | 7.10              | 7.23              | 7.08              | 0.05| .966    |
| Yolk weight, g        |         |         |         |         |         |                   |                   |                   |                   |     |         |
| 48 weeks              | 14.10   | 14.01   | 14.02   | 14.06   | 14.01   | 14.13             | 14.06             | 14.14             | 14.38             | 0.07| .975    |
| 56 weeks              | 15.02   | 14.44   | 15.54   | 15.02   | 15.33   | 15.29             | 15.21             | 15.19             | 15.10             | 0.11| .504    |
| Albumen weight, g     |         |         |         |         |         |                   |                   |                   |                   |     |         |
| 48 weeks              | 40.30   | 40.79   | 40.48   | 40.21   | 40.47   | 40.23             | 40.29             | 40.44             | 40.24             | 0.08| .880    |
| 56 weeks              | 40.54   | 40.68   | 40.30   | 40.29   | 40.04   | 40.83             | 40.04             | 40.31             | 40.27             | 0.18| .990    |
| Eggshell strength, kg/cm² |       |         |         |         |         |                   |                   |                   |                   |     |         |
| 48 weeks              | 4.24    | 4.52    | 4.55    | 4.56    | 4.63    | 4.69              | 4.87              | 4.40              | 4.47              | 0.08| .875    |
| 56 weeks              | 3.84abcd| 3.82abcd| 3.86abcd| 4.40abcd| 4.65abcd| 4.29abcd          | 4.39abcd          | 4.26abcd          | 4.44abcd          | 0.06| .007    |
| Eggshell thickness, mm |         |         |         |         |         |                   |                   |                   |                   |     |         |
| 48 weeks              | 0.364   | 0.360   | 0.368   | 0.378   | 0.382   | 0.382             | 0.364             | 0.366             | 0.370             | 0.002| .305    |
| 56 weeks              | 0.362abcd| 0.360abcd| 0.364abcd| 0.380abc| 0.386abcd| 0.366bcd          | 0.382abcd         | 0.368bcd          | 0.384abcd         | 0.002| .001    |
| Yolk colour score     |         |         |         |         |         |                   |                   |                   |                   |     |         |
| 48 weeks              | 8.02    | 7.88    | 8.26    | 8.30    | 8.24    | 8.20              | 8.18              | 7.84              | 7.86              | 0.06| .554    |
| 56 weeks              | 7.58    | 7.88    | 7.76    | 7.72    | 8.00    | 8.10              | 7.66              | 7.52              | 7.82              | 0.08| .843    |
| Haugh unit            |         |         |         |         |         |                   |                   |                   |                   |     |         |
| 48 weeks              | 93.74   | 94.22   | 94.87   | 95.80   | 94.44   | 95.28             | 93.28             | 93.01             | 94.03             | 0.41| .921    |
| 56 weeks              | 94.70   | 92.12   | 94.58   | 95.08   | 94.66   | 94.16             | 93.80             | 93.40             | 93.46             | 0.43| .886    |

Table 6. Ileal microflora counts of hens fed dietary bamboo charcoal (BC), bamboo vinegar (BV) and their combination (log10 cfu/g of wet digesta).

| Parameters               | Control | 0.5% BC | 1.0% BC | 0.3% BV | 0.6% BV | 0.5% BC + 0.3% BV | 0.5% BC + 0.6% BV | 1.0% BC + 0.3% BV | 1.0% BC + 0.6% BV | SEM | p-Value |
|-------------------------|---------|---------|---------|---------|---------|-------------------|-------------------|-------------------|-------------------|-----|---------|
| Escherichia coli        | 5.16    | 5.02    | 5.04    | 4.94    | 4.92    | 4.98              | 4.96              | 5.04              | 4.96              | 0.02| .187    |
| Salmonella spp.         | 4.70abcd| 4.68abcd| 4.56abcd| 4.46abcd| 4.36abcd| 4.34abcd          | 4.46abcd          | 4.56abcd          | 4.38abcd          | 0.02| .001    |
| Lactobacillus spp.      | 6.88abcd| 6.86abcd| 6.86abcd| 7.10abcd| 7.14abcd| 7.04abcd          | 7.06abcd          | 7.00abcd          | 7.04abcd          | 0.02| .041    |

a,bValues with different superscripts in the same row are significantly different (p < .05).
Values are means of 5 replicates.
Table 7. Villus height and villus area of the duodenum, jejunum and ileum in hens fed dietary bamboo charcoal (BC), bamboo vinegar (BV) and their combination.

| Parameters | Control | 0.5% BC | 1.0% BC | 0.3% BV | 0.6% BV | 0.5% BC + 0.3% BV | 0.5% BC + 0.6% BV | 0.5% BC + 0.3% BV | 0.5% BC + 0.6% BV | SEM | p-Value |
|------------|---------|---------|---------|---------|---------|-------------------|-------------------|-------------------|-------------------|-----|---------|
| Villus height, mm | | | | | | | | | | | |
| Duodenum | 1.31<sup>c</sup> | 1.30<sup>c</sup> | 1.35<sup>d</sup> | 1.37<sup>a</sup> | 1.39<sup>a</sup> | 1.38<sup>a</sup> | 1.39<sup>a</sup> | 1.39<sup>a</sup> | 1.40<sup>a</sup> | 0.01 | .001 |
| Jejunum | 0.85<sup>b</sup> | 0.85<sup>b</sup> | 0.87<sup>ab</sup> | 0.88<sup>ab</sup> | 0.89<sup>b</sup> | 0.87<sup>ab</sup> | 0.89<sup>b</sup> | 0.87<sup>ab</sup> | 0.90<sup>b</sup> | 0.01 | .043 |
| Ileum | 0.47 | 0.46 | 0.48 | 0.49 | 0.50 | 0.48 | 0.49 | 0.48 | 0.49 | 0.01 | .285 |
| Villus area, mm<sup>2</sup> | | | | | | | | | | | |
| Duodenum | 0.113<sup>c</sup> | 0.109<sup>c</sup> | 0.115<sup>c</sup> | 0.117<sup>bc</sup> | 0.124<sup>ab</sup> | 0.125<sup>bc</sup> | 0.124<sup>ab</sup> | 0.126<sup>a</sup> | 0.126<sup>a</sup> | 0.001 | .001 |
| Jejunum | 0.073<sup>b</sup> | 0.072<sup>b</sup> | 0.074<sup>ab</sup> | 0.075<sup>ab</sup> | 0.079<sup>a</sup> | 0.075<sup>ab</sup> | 0.078<sup>a</sup> | 0.076<sup>ab</sup> | 0.078<sup>a</sup> | 0.001 | .001 |
| Ileum | 0.033 | 0.032 | 0.033 | 0.035 | 0.035 | 0.034 | 0.034 | 0.034 | 0.035 | 0.001 | .738 |

<sup>a,b,c</sup>Values with different superscripts in the same row are significantly different (p < .05). Values are means of 5 replicates.

Discussion

In the present study, although egg production and egg weight did not improve in all treated groups, egg mass significantly increased in all supplemented groups during 49–56 weeks of age. These results reveal that prolonged feeding of BC or BV alone or in combination with the diet can improve the egg yield of laying hens. Increased egg mass in supplemented groups was directly related to higher egg production and egg weight in the treated groups, although those values were not significantly different. The overall feed conversion ratio was also better in all treated groups. The positive effects of BV and BC in feed on egg yield and feed efficiency could be attributed to the beneficial effects of BC and BV in improving gut health, as they are known to improve digestion and absorption of nutrients. BC is known to have a higher adsorption ability than conventional charcoal because of the special micro-pore structure of bamboo stems. BC may capture, bind, and remove gut irritants and toxins in the gastrointestinal tract, consequently improving digestibility and availability of nutrients. BC may capture, bind, and remove gut irritants and toxins in the gastrointestinal tract, consequently improving digestibility and availability of nutrients. Van et al. (2006) reported that dry matter and protein digestibility significantly increased by dietary supplementation of BC (0.5 g charcoal/kg body weight) in growing goats. Watarai and Tana (2005) also found that activated charcoal could reduce pathogenic bacteria (Salmonella spp.) and minimise the removal of normal bacterial flora (E. faecium) in the intestine of White Leghorn chickens. These changes had a positive effect on improving the gut health of the hens. BV contains many ingredients, including organic acids, phenols, furans, aldehydes, ketones, alcohols and heterocyclic aromatic compounds (Table 2). Organic acids are the predominant ingredient in BV, which is characterised by its antimicrobial (Ryssel et al. 2009) and antifungal (Guimaraes et al. 2018) activities. The proposed mode of action of BV is related to reducing intestinal pH, and controlling the balance of the intestinal ecosystem. In addition, the organic acids reportedly increase the activities of digestive enzymes in the intestinal tract, improve the digestibility of proteins (Ahiwe et al. 2020), and reduce the growth of pathogenic bacteria in the gut (Jacela et al. 2009; Roofchaei et al. 2019). These changes are expected to improve the digestibility of nutrients and to improve egg yield. In our study, although overall feed intake tended to be lower in treated groups when compared to the control group, egg production and egg weight were not affected. The reduction in feed intake might be explained by more efficient digestion and absorption of nutrients in the supplemented birds. Supplementation of BC at 1% level induced a small reduction in feed intake compared to the other groups. A possible explanation for the reduced feed intake due to BC supplementation could be that high levels of charcoal might have a negative effect on the palatability of the diet and appetite of birds. In the present study, feeding BV alone at 0.3 and 0.6% levels increased eggshell thickness and strength at week 56, while the mixture groups showed a dominant effect in the 0.6% BV supplemented groups. Eggshell thickness and eggshell strength are important factors for the preservation and transportation of eggs. Improved eggshell quality with BV supplementation could decrease damaged egg rate and increase marketable table eggs. The improvement of eggshell quality in the current study can probably be explained by more efficient digestion and absorption of minerals, particularly calcium, brought about by a decrease in pH in the intestinal tract. Organic acids reportedly increase digestive enzyme activities and increase the utilisation of calcium and phosphorus in the intestine of laying hen (Świątkiewicz et al. 2010). Dietary inclusion of organic...
Organic acids can also improve the digestibility of minerals and increase phytate phosphorus utilisation (Khan and Iqbal 2016). Studies have reported that organic acids could increase eggshell thickness through increased calcium solubility and absorption in the intestinal tract (Park et al. 2002; Fouladi et al. 2018). Youssef et al. (2013) also reported that supplementing organic acids to the laying hen diet at the level of 0.06% increases eggshell thickness. In this study, therefore, BV may have improved eggshell quality through decreasing gut pH and enhancing digestion and increasing utilisation of minerals. *Salmonella*, *Escherichia coli*, and *Clostridium* are the most common bacteria that affect the intestinal health of poultry. These bacteria compete with the host for the nutrients and produce different metabolites like ammonia and amines, leading to reduced performance of the poultry. In the present study, feeding BV alone at 0.3 or 0.6% levels could decrease the ileal *Salmonella* spp., while mixture groups showed a dominant effect in the cases supplemented with 0.6% BV. This result reveals that the use of BV alone at 0.3% level was sufficient to reduce pathogenic bacteria, while the combination with BC requires a higher level of BV. This may be due to an increase in pH level in the gut from feeding BC. At the same time, the number of *Lactobacillus* spp. increased in the hens fed 0.3% BV, 0.6% BV and BCV56 diets. This may be due to the organic acids in BV stimulating the growth of *Lactobacillus* spp. to dominate in the gastrointestinal tract since *Lactobacillus* species are considered intrinsically resistant to acidic environments. In addition, decreased *Salmonella* spp. counts in the current study may be due to an increase in the population of *Lactobacillus* spp. that produced lactic acid in the gut after feeding BV. Hinton et al. (2000) reported that low pH and a higher number of lactobacilli lower the incidence of *Salmonella* in the crop of broilers. Wang et al. (2010) also described that supplementation of organic acids in poultry diets reduced gram-negative bacterial counts but increased *Lactobacillus* spp. in the digestive tract. Lactic acid bacteria are known probiotics with many health benefits, including improving normal microflora, inhibiting infectious diseases, and alleviating intestinal bowel disease symptoms (Galdeano et al. 2007). The increase in beneficial bacteria in the gut positively affects the gut and overall host health (Janczyk et al. 2009). The small intestine is the most important absorbing organ in the gastrointestinal tract. The majority of nutrient absorption takes place in the small intestine. Longer intestinal villi are associated with an increase in the absorptive surface of the intestine while lowering the height of intestinal villi results in a reduction in the absorption of nutrients. Increased height of villus corresponds to improved nutrient digestion and absorption (Montagne et al. 2003). Concerning the effects of BC and BV on intestinal villi, we found that feeding BV alone or in combination with BC in laying hen diet improved gut development by increasing the villus height and area in the duodenum, while supplementation with BV at 0.6% level, either alone or in combination with BC in the diet had an additional beneficial effect in stimulating the jejunal villi. It has been reported that lactic acid bacteria can increase the villus height via the digestion of carbohydrates, production of volatile fatty acids, and consequent nourishment of the intestinal villi (Shah et al. 2019), while pathogenic bacteria damage the villi of the intestinal mucosa and inhibit the secretion of digestive enzymes (Chot 2009). *Salmonella* is a pathogenic organism that causes disease manifested by irritation of the intestinal wall and decreased villi number and length, which impair nutrient absorption (Pelicano et al. 2005). Organic acids are known to inhibit the growth of pathogenic bacteria such as *Campylobacter*, *E. coli* and *Salmonella*, in poultry’s feed and in their gastrointestinal tracts, which is of great importance for poultry health, thus enhancing the performance of poultry. An increase in villus height and area of the intestine in the present study may be due to the antimicrobial properties of the BV, which lowered the number of pathogenic bacteria while stimulating the growth of beneficial bacteria in the intestine, leading to reduced inflammatory processes at the intestinal mucosa. These improvements in gut structure and function allow better utilisation of the available dietary nutrients, including protein and minerals, which subsequently drive the improvements in feed efficiency, egg yield, and eggshell quality in this study. In the present study, supplementation of BC or BV in the diet did not affect ileal morphology. This may be explained by the fact that the major absorption of nutrients occurs in the upper segment of the small intestine. Most digestive enzymes enter the small intestine in the duodenum, while most digestion and nutrient absorption takes place in the jejunum (Yamauchi 2007).

Conclusions

According to the results of the current study, feeding of BV alone at 0.3% level was sufficient to improve feed efficiency, eggshell quality, growth of beneficial gut bacteria and function of duodenal villi, while feeding of BV at 0.6% level, either alone or in combination
with BC had a stimulant effect on the functioning of jejunal villi in laying hens.

**Ethical approval**

The experimental protocols were approved by the Institutional Animal Care and Use Committee of Prince of Songkla University, Thailand (approval number: MHESI 6800.11/1418).

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**Disclosure statement**

No potential conflict of interest has been reported by the author(s).

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