

## Production Performance, Egg Quality, and Fecal Bacterial Population of Laying Ducks Fed Ration Supplemented by Bamboo Vinegar

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### ABSTRACT

With the ban on the use of antibiotics in poultry feed, it is required to produce alternative substances to maintain poultry health and performance. The present study was carried out to investigate the effects of dietary supplementation at various levels of bamboo vinegar (BV) on performance, egg quality, and fecal bacterial population of laying ducks. One hundred and five 50-week-old crossbred laying ducks (Khaki Campbell × Native) were randomly allocated into five treatment groups, each with seven replicates of three ducks. The experimental ducks were fed a basal diet (antibiotic-free diet) supplemented with BV at the levels of 0%, 0.2%, 0.4%, 0.6%, or 0.8% of diet until 62 weeks of age. For the overall period (from 50 to 62 weeks of age), the dietary supplementation of BV at the levels of 0.4% and 0.6% of diet increased egg production and egg mass, and improved feed efficiency of laying ducks ( $p < 0.01$ ). Supplementation of BV at the level of 0.8% in the diet decreased feed intake ( $p < 0.01$ ). Egg quality parameters were not significantly affected by supplementation of BV ( $p > 0.05$ ). The population of fecal *Escherichia coli* was significantly decreased in the experimental ducks fed diet supplemented with BV at the levels of 0.4%, 0.6%, and 0.8% compared to the control group ( $p < 0.01$ ), while the population of *Lactobacillus* spp. remained unchanged. The present results indicate that a level of 0.4% BV supplementation in laying duck diet is sufficient for reducing fecal *E. coli* and improves productive performance of egg laying ducks.

**Keywords:** bamboo vinegar; egg quality; fecal bacteria; laying ducks

### INTRODUCTION

Antibiotics have been commonly included at the sub-therapeutic doses in poultry diets for improving performance and maintaining the optimal health of the birds. However, there is a growing concern that the use of antibiotics in poultry feed selects for the evolution of antibiotic-resistant pathogens, as well as causes antibiotic residue problems in poultry products. As a result, the poultry industry has been compelled to find alternatives for antibiotics. Dietary acidifiers appear to be potential alternatives to the antibiotics for improving poultry performance and preventing diseases. The beneficial effects of acidifiers include the enhancing growth rate through the improved gut health through the reduced pH, promoting the growth of beneficial bacteria, and increasing the digestibility of nutrients through the improved pancreatic secretion (Papatsiros & Christodoulopoulos, 2011). Besides, acidifiers have antimicrobial activity in controlling the bacterial populations in the gut and inhibiting the growth of pathogenic bacteria (Kil *et al.*, 2011). It was shown that acidifiers in broiler diets increased weight gain and improved

feed efficiency (Hedayati *et al.*, 2013). In laying hens, acidifiers in the diets also improve egg production and eggshell quality (Swiatkiewicz *et al.*, 2010). Bamboo vinegar (BV) is an acidic by-product of bamboo charcoal production. It is composed of water, and many other organic constituents, and has a pH of 2.5 to 3.0 (Akakabe *et al.*, 2006; Velmurugan *et al.*, 2009; Huo *et al.*, 2016). Akakabe *et al.* (2006) reported that there were many similarities between the components of bamboo vinegar and those of wood vinegar. Wood vinegar exhibits high antimicrobial activities against various microorganisms, along with a significant antioxidant activity (Yang *et al.*, 2016). Bamboo vinegar also acts as an insecticide and bactericide and is used as a component in folk medicine (Akakabe *et al.*, 2006). Positive effects of feeding BV to pigs have been reported from several studies (Yan *et al.*, 2012; Chu *et al.*, 2013; Huo *et al.*, 2016). However, the applications of BV in poultry have not been vigorously investigated, and there was no prior study examining the effects of BV supplementation in laying ducks. The objective of this study, therefore, was to examine the effects of dietary supplementation of BV at various levels on egg production performance, internal and external

egg qualities, and fecal bacterial populations in laying ducks.

## MATERIALS AND METHODS

### Birds and Management

The experiment in its totality was conducted following the guidelines and rules for animal experiments of Prince of Songkla University, Thailand. A total of 105 crossbred laying ducks (Khaki Campbell × Native) at 50 weeks of age were randomly assigned into five treatments, and each treatment group consisted of 7 replicates with 3 birds in each replicate. The ducks were housed in 40 cm × 45 cm × 35 cm cages (three ducks per cage), in an environmentally controlled room under a photoperiod of 18 h of light. The basal diet (Table 1) was formulated to meet the nutrient recommendations of the NRC (1994). The ducks were fed a basal diet supplemented with BV at the levels of 0%, 0.2%, 0.4%, 0.6%, or 0.8% of diet (v/w) until 62 weeks of age. Commercial BV (pH= 3.0) was purchased from a company (Charcoal Home Co., Ltd., Bangkok, Thailand) and select physicochemical characteristics of this bamboo vinegar are shown in Table 2. Feed and water were provided *ad libitum* throughout the 12 weeks of treatment.

### Performance and Egg Quality Parameters

Production performance was examined every 4 weeks by measuring feed intake, egg production, egg

Table 1. Feed formulation and chemical composition of experimental diet (as-fed basis)

Item	Amount (%)
<b>Ingredient</b>	
Corn	59.25
Soybean meal (44% CP)	24.40
Raw rice bran	4.00
Fish meal (55% CP)	2.00
Calcium carbonate (38% Ca)	7.95
Dicalcium phosphate (18% P)	1.40
Rice bran oil	0.40
Salt	0.30
Premix <sup>1</sup>	0.30
<b>Calculated analysis</b>	
Crude protein	17.00
Metabolizable energy (kcal/kg)	2,700
Crude fiber	3.61
Crude fat	3.57
Calcium	3.60
Available phosphorus	0.35
Lysine	0.90
Methionine	0.40
Methionine+Cysteine	0.70

<sup>1</sup>Composition per 1 kg premix: 20.0 MIU vitamin A, 4.0 MIU vitamin D3, 22,000 mg vitamin E, 4 g vitamin K3, 5 g vitamin B1, 10 g vitamin B2, 6 g vitamin B6, 0.06 g vitamin B12, 50 g nicotinic acid, 20 g pantothenic acid, 0.24 g biotin, 3 g folic acid, 15 g vitamin C, 23.4 g feed additives, up to 1 kg filter.

Table 2. Chemical properties of bamboo vinegar liquid

Compound	Relative concentration (%)
<b>Organic acids</b>	
Acetic acid	47.59
Formic acid	0.33
Propionic acid	3.31
4-oxo-pentanoic acid	0.40
Benzoic acid	0.35
Total organic acids	51.98
<b>Alcohols</b>	
Benzene methanol	1.90
Total alcohols	1.90
<b>Furans</b>	
2-methyl-furan	
Tetrahydro-2-furanmethanol	0.29
Dihydroxy-2(3H)-furanone	3.35
2-furanmethanol	0.36
3-hydroxy-2methyl-2H-pyran-4-one	1.69
2-furancarboxaldehyde	0.97
5-methyl-2-furancarboxaldehyde	0.32
2-(2,3-epoxypropyl)-tetrahydrofuran	5.60
1,4:3,6-dianhydro-alpha-d-glucopyranose	0.36
Total furans	12.94
<b>Phenol derivatives</b>	
Phenol	5.14
3-methylphenol	0.44
Total phenol derivatives	5.58
<b>Methoxy phenol derivatives</b>	
2-methoxyphenol	2.26
4-methyl-2-methoxyphenol	0.70
4-ethyl-2-methoxyphenol	0.38
4-methylsyringol	4.08
Total methoxy phenol derivatives	7.42
<b>Aldehyde and ketones</b>	
1-hydroxy-2-butanone	0.36
1-hydroxy-2-propanone	0.91
3-methyl-2-cyclopenten-1-one	0.47
2,3-dimethyl-2-cyclopenten-1-one	0.51
2-hydroxy-3methyl-2 cyclopenten-1-one	1.32
3-ethyl-2-hydroxy-2-cyclopenten-1-one	0.31
1-(2-furanyl)-ethanone	0.26
2-nitro-propionate(ester)ethanol	0.47
Total aldehyde and ketones	4.61
<b>Heterocyclic aromatic compound</b>	
Pyridine	1.37
2-methylpyridine	0.25
3-methylpyridine	0.25
3-methoxypyridine	1.06
11,2,3-trimethoxy-5-methylbenzene	0.27
Total heterocyclic aromatic compounds	3.20
<b>Other compounds</b>	
3,5-dimethoxy-4-hydroxytoluene	0.39
Phospholane	0.33
1-ethoxy-10-methylundec-1-yne	0.29
Total other compounds	1.01

weight, egg mass, and feed efficiency. Body weight was recorded at the beginning and the end of experiments (at 50 and 62 weeks of age, respectively). Egg quality was estimated every 4 weeks (weeks 54, 58, and 62) by measuring the egg weight, eggshell weight, eggshell percent, eggshell strength, eggshell thickness, yolk percent, albumen percent, yolk color score, and Haugh unit. The weights of the eggs from each group were recorded, and the breaking strength of the eggshell (kg/cm<sup>2</sup>) was measured using a breaking strength measuring device (DET6000, NABEL Co., Ltd, Kyoto, Japan). The weights of the shell, albumen, and egg yolk were measured using an electronic digital balance and are reported as percentages. Eggshell thickness was measured by a digital micrometer (Mitutoyo Corporation, Japan). The pigmentation of the egg yolk and Haugh unit scores were measured using the digital egg tester (DET6000, NABEL Co., Ltd).

**Fecal Bacterial Analyses**

At 62 weeks of age, samples of fresh feces (7 samples/diet) were collected aseptically in vinyl bags, and a 1 g subsample was transferred to a test tube. The subsample was mixed with 9 mL of phosphate-buffered

saline and homogenized at 2,500 rpm for 30 sec. The homogenized sample was serially diluted between 10<sup>-2</sup> to 10<sup>-5</sup> fold. *Escherichia coli* was grown on EMB agar (BBL, Sparks, MD, USA) and incubated at 37°C for 24 h. *Lactobacillus* spp. was grown on lactobacillus MRS agar (HiMedia LAB, Mumbai, India) and incubated at 37°C for 48 h. The colonies on each plate were counted, and the results were expressed as log<sub>10</sub> CFU per gram of fresh sample.

**Statistical Analysis**

Collected data were subjected to one-way analysis of variance, followed by Duncan’s multiple range test to find out the level of significance in differences between the mean values. Differences with p<0.05 were considered significant.

**RESULTS**

**Performance**

Table 3 shows the production performances of laying ducks. Compared to the control group, egg production significantly increased in the ducks fed diet

Table 3. Performance of laying ducks during 50-62 weeks of age supplemented by different levels of dietary bamboo vinegar (BV)

Variables	Dietary bamboo vinegar (%)					SEM	p-value
	0	0.2	0.4	0.6	0.8		
Initial body weight (g)	1,319.30	1,325.00	1,330.00	1,328.60	1,314.30	17.08	0.990
Final body weight (g)	1,425.00	1,402.10	1,422.10	1,424.30	1,400.00	16.31	0.980
Body weight change (g)	105.70	77.10	92.10	95.70	85.70	6.63	0.743
Hen-day egg production (%)							
51-54 weeks	72.45	73.31	79.59	76.53	71.43	1.06	0.073
55-58 weeks	75.00 <sup>bc</sup>	75.51 <sup>bc</sup>	82.65 <sup>a</sup>	81.63 <sup>ab</sup>	72.45 <sup>c</sup>	1.18	0.014
59-62 weeks	73.47 <sup>abc</sup>	72.45 <sup>bc</sup>	80.61 <sup>a</sup>	78.57 <sup>ab</sup>	70.41 <sup>c</sup>	1.25	0.040
51-62 weeks	73.64 <sup>b</sup>	73.75 <sup>b</sup>	80.95 <sup>a</sup>	78.91 <sup>a</sup>	71.43 <sup>b</sup>	0.93	0.001
Egg weight (g)							
51-54 weeks	61.52	61.38	61.29	62.63	63.73	0.47	0.434
55-58 weeks	62.28	62.76	61.39	62.37	63.56	0.30	0.249
59-62 weeks	62.71	63.25	63.49	64.01	63.84	0.16	0.100
51-62 weeks	62.17	62.46	62.05	63.00	63.71	0.27	0.290
Egg mass (g/hen/day)							
51-54 weeks	44.57	44.99	48.78	47.93	45.52	0.76	0.177
55-58 weeks	46.71 <sup>ab</sup>	47.39 <sup>ab</sup>	50.73 <sup>a</sup>	50.91 <sup>a</sup>	46.04 <sup>b</sup>	0.68	0.048
59-62 weeks	46.07 <sup>bc</sup>	45.82 <sup>bc</sup>	51.17 <sup>a</sup>	50.29 <sup>ab</sup>	44.94 <sup>c</sup>	0.79	0.023
51-62 weeks	45.78 <sup>b</sup>	46.06 <sup>b</sup>	50.22 <sup>a</sup>	49.71 <sup>a</sup>	45.50 <sup>b</sup>	0.58	0.004
Feed intake (g/d)							
51-54 weeks	140.36	138.16	139.85	138.64	137.40	0.38	0.071
55-58 weeks	139.03 <sup>a</sup>	139.90 <sup>a</sup>	139.80 <sup>a</sup>	137.83 <sup>a</sup>	132.86 <sup>b</sup>	0.53	0.001
59-62 weeks	137.65 <sup>a</sup>	137.04 <sup>a</sup>	139.08 <sup>a</sup>	138.11 <sup>a</sup>	134.08 <sup>b</sup>	0.40	0.001
51-62 weeks	139.01 <sup>a</sup>	138.36 <sup>a</sup>	139.57 <sup>a</sup>	138.19 <sup>a</sup>	134.78 <sup>b</sup>	0.33	0.001
Feed efficiency (g of egg mass/g of feed consumed)							
51-54 weeks	0.317	0.325	0.348	0.345	0.331	0.005	0.166
55-58 weeks	0.335	0.338	0.362	0.367	0.335	0.004	0.081
59-62 weeks	0.334	0.334	0.367	0.364	0.335	0.005	0.056
51-62 weeks	0.328 <sup>b</sup>	0.332 <sup>b</sup>	0.359 <sup>a</sup>	0.358 <sup>a</sup>	0.333 <sup>b</sup>	0.004	0.004

Note: Means in the same row with different superscripts differ significantly (p<0.05). Values are means of 7 replicates.

supplemented with BV at the level of 0.4%, during the 55-58 and 59-62 weeks-of-age periods ( $p < 0.05$ ), and the overall egg production was elevated in the laying ducks fed diet supplemented with BV at the levels of 0.4% and 0.6% ( $p < 0.01$ ). The highest egg mass was produced by the ducks fed diet supplemented with 0.4% BV, during 59-62 weeks-of-age period ( $p < 0.05$ ), and the highest overall egg mass was produced by ducks fed diets supplemented with 0.4 and 0.6% BV ( $p < 0.01$ ). Feed intake decreased in the ducks fed diet supplemented with BV at the level of 0.8% during 55-62 weeks-of-age, and across the overall period ( $p < 0.01$ ). Overall feed efficiency was better in the ducks fed diet supplemented with BV at the levels of 0.4% and 0.6% ( $p < 0.01$ ). Body weight and egg weight did not differ significantly between the different levels of BV supplementations in the diet ( $p > 0.05$ ).

### Egg Quality Parameters

The BV supplementation in the diet did not affect egg weight, shell weight, shell strength, shell thickness, albumen percent, yolk percent, yolk color score, or Haugh unit ( $p > 0.05$ ) (Table 4).

### Fecal Bacterial Population

As shown in Table 5, dietary supplementation of BV at the levels of 0.4%, 0.6%, and 0.8% significantly reduced the population of *E. coli* in the feces compared to the control ( $p < 0.01$ ). However, the fecal *Lactobacillus* counts did not differ significantly between the treatment groups ( $p > 0.05$ ).

Table 4. Egg quality traits of laying ducks at 54, 58, and 62 weeks of age supplemented by different levels of dietary bamboo vinegar (BV)

Variables	Dietary bamboo vinegar (%)					SEM	p-value
	0	0.2	0.4	0.6	0.8		
Egg weight (g)							
54 weeks	61.38	61.60	61.75	62.48	62.77	0.25	0.361
58 weeks	61.88	62.05	61.60	62.38	63.08	0.30	0.601
62 weeks	63.05	63.35	63.57	64.20	63.97	0.30	0.779
Eggshell weight (g)							
54 weeks	7.12	7.07	7.05	7.10	7.18	0.03	0.831
58 weeks	6.91	7.05	6.97	6.91	6.98	0.03	0.711
62 weeks	7.14	7.17	7.24	7.22	7.21	0.03	0.825
Eggshell percent (%)							
54 weeks	11.59	11.47	11.41	11.36	11.43	0.06	0.756
58 weeks	11.16	11.36	11.31	11.07	11.06	0.18	0.685
62 weeks	11.32	11.31	11.38	11.24	11.27	0.03	0.815
Eggshell thickness (mm)							
54 weeks	0.396	0.397	0.398	0.393	0.394	0.001	0.452
58 weeks	0.384	0.385	0.395	0.385	0.384	0.002	0.309
62 weeks	0.378	0.383	0.385	0.383	0.380	0.001	0.591
Eggshell strength (kg/cm <sup>2</sup> )							
54 weeks	4.64	4.77	4.86	4.68	4.61	0.10	0.949
58 weeks	4.34	4.58	4.59	4.24	4.22	0.11	0.732
62 weeks	4.16	4.36	4.30	4.38	4.29	0.07	0.895
Albumen percent (%)							
54 weeks	55.46	56.39	56.02	55.50	55.59	0.25	0.746
58 weeks	57.44	56.85	56.46	56.23	56.67	0.25	0.644
62 weeks	57.36	57.16	56.68	56.62	56.71	0.21	0.759
Yolk percent (%)							
54 weeks	32.95	32.14	32.57	33.14	32.98	0.25	0.755
58 weeks	31.40	31.79	32.23	32.70	32.27	0.24	0.522
62 weeks	31.32	31.53	31.94	32.14	32.02	0.21	0.742
Yolk color score							
54 weeks	7.58	7.27	7.60	7.25	7.47	0.06	0.278
58 weeks	7.37	7.07	7.81	7.25	7.15	0.10	0.141
62 weeks	7.28	7.64	7.38	7.44	7.75	0.08	0.382
Haugh unit							
54 weeks	88.87	89.58	88.67	89.75	90.10	0.58	0.941
58 weeks	83.68	85.08	84.84	85.60	85.20	0.51	0.837
62 weeks	84.52	85.32	86.14	86.35	85.44	0.44	0.734

Note: Values are means of 7 replicates.

Table 5. Fecal bacteria count (log<sub>10</sub> cfu/g of fresh feces) of ducks fed diet supplemented by bamboo vinegar (BV) at the levels of 0%, 0.2%, 0.4%, 0.6%, and 0.8%

Variables	Dietary bamboo vinegar (%)					SEM	p-value
	0	0.2	0.4	0.6	0.8		
<i>Escherichia coli</i>	8.86 <sup>a</sup>	8.87 <sup>a</sup>	8.83 <sup>b</sup>	8.82 <sup>b</sup>	8.81 <sup>b</sup>	0.005	0.001
<i>Lactobacillus</i> spp.	9.01	9.02	9.02	9.03	9.02	0.004	0.480

Note: Means in the same row with different superscripts differ significantly ( $p < 0.01$ ). Values are means of 7 replicates.

## DISCUSSION

In a recent study with aged laying hens, we found that feeding BV at the level of 0.4% or 0.8% of the diet beneficially affected damaged egg rate, but did not influence egg production (Rattanawut *et al.*, 2018). In the present study, adding 0.4% or 0.6% BV in the feed increased egg production performance and improved feed efficiency of ducks during the whole experiment (51-62 weeks of age). The improved laying performance as a result of dietary BV supplementation might be due to the beneficial effects of BV through the improved gastrointestinal tract environment. Bamboo vinegar contains more than 200 accessory ingredients, including organic acids, phenols, ketones, and aldehydes (Akakabe *et al.*, 2006). Organic acids are the main component in bamboo vinegar (Table 2). The main actions of organic acids in the gut are modifying the pH and controlling the balance of intestinal microflora and pathogens. A low gizzard pH improves pepsin activity and increases the solubility of the mineral fraction of the diet (Mabelebele *et al.*, 2014). These changes, in turn, are expected to improve the digestibility of nutrients and to enhance product performance. The concentration of phenolic compounds in bamboo vinegar is 3-4 folds that in wood vinegar (Velmurugan *et al.*, 2009). Some phenolic compounds have been reported to inhibit pathogenic bacteria (Pacheco-Ordaz *et al.*, 2017). Chan *et al.* (2012) reported that wood vinegar displayed potent antibacterial activity against the strains of Gram-negative *Escherichia coli*, *Salmonella typhi*, and *Pseudomonas aeruginosa*. In this study, bamboo vinegar might also have a direct effect on the digestive tract bacterial populations, reducing the number of some pathogenic bacteria and controlling the populations of certain types of bacteria that compete with the host bird for nutrients. Therefore, the positive effects of 0.4% and 0.6% BV supplementation on productive performance observed in this study might be related to the bioactive components in BV, such as organic acids, phenolic compounds, and heterocyclic compounds. In the present study, supplementing the diet with 0.8% BV decreased the feed intake. The reduction in the feed intake might be due to the strong taste associated with BV decreasing palatability of the feed. In this study, supplementation of BV at 0.4%, 0.6%, and 0.8% levels decreased the *E. coli* population in the laying ducks, while the presence of *Lactobacillus* spp. remained unchanged. This result may be due to the active ingredients in bamboo vinegar that reduces the growth of harmful bacteria but maintains the existence of beneficial bacteria. Yan *et al.* (2012) found that addition of BV in the diet of fattening pigs

could decrease the number of *E. coli* in feces caused by the rich phenols, ketones, and organic acids contents in the BV products. Watarai & Tana (2005) also found that wood vinegar added to the diet of chickens inhibited the growth of pathogenic bacteria, but promoted the growth of beneficial bacteria. In the study of Chu *et al.* (2013) the populations of coliform bacteria and *Salmonella* in the feces were lower in pigs fed diet supplemented with BV. However, a higher level of BV in the diet may affect the balance of gut microflora. Wang *et al.* (2012) also stated that the quantity and diversity index of fecal bacteria were reduced in pigs fed diet with high levels of BV, indicating that the buffering capacity of intestinal microflora might be affected by the high doses of BV. This might be another reason why the ducks fed diet supplemented with BV at the level of 0.8% had similar performance to the control ducks, in addition to the reduced feed intake.

## CONCLUSION

The results of this study demonstrate that a level of 0.4% BV supplementation in laying duck diet is sufficient for reducing pathogenic gut bacteria and improves the productive performance of egg laying ducks.

## CONFLICT OF INTEREST

We declare there is no conflict of interest with any financial, personal, or other relationships with other people or organization related to the material discussed in the manuscript.

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