Eggshell quality, eggshell structure and small intestinal histology in laying hens fed dietary Pantoea-6® and plant extracts

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Abstract

The present study aims to investigate the effects of dietary Pantoea-6® (extract of fermented wheat flour with Pantoea agglomerans) and plant extracts (red clover and garlic) on eggshell quality and structure and intestinal histology. Sixty-six Boris Brown laying hens (30 weeks old) were allotted to 3 groups, each with eleven replicates of two chickens. The control group was fed a basal diet (18% crude protein, 2850 kcal/kg ME) and the other groups were fed the basal diet supplemented with 0.1% Pantoea-6® (including 0.06 g/kg lipopolysaccharide) and 0.1% plant extracts, respectively. There were no significant differences in laying performance and egg quality. However, these adverse effects occurred in the egg and albumen weight and eggshell breaking strength of the Pantoea-6® and plant extracts groups (P<0.05). Shell weight of the Pantoea-6® group was significantly higher than the other groups (P<0.05). Compared with the control, eggshell structure tended to have greater thickness in both dietary Pantoea-6® and plant extracts groups. The duodenum and jejunum of both Pantoea-6® and plant extracts groups showed higher values for cell area than those of the control (P<0.05). Moreover, cells on the villus tip surface were protuberated in both dietary Pantoea-6® and plant extracts groups, resulting in a rough surface. This study shows that Pantoea-6® and plant extracts at a 0.1% level might have a beneficial effect on egg and albumen weight, eggshell quality and structure parameters, as well as on small intestine histological parameters.

Introduction

In the egg industry, it is estimated that 8 to 11% of the eggs produced are lost due to damaged eggshell quality problems (Dunn et al., 2005). Thus, eggshell provides protection for the contents, influences the economic profitability of egg production, impacts on the containers used for marketing the eggs and provides a unique package for a valuable food (Hunton, 2005). Hen eggshell consists of ceramic materials constituted by a three-layered structure, namely, cuticle on the outer surface, a spongy (calcareaous) layer and an inner lamellar (or mammillary) layer (Stadelman, 2000). The outer surface of the eggshell is covered with a mucin protein that acts as a soluble plug for the pores in the shell. The cuticle is also permeable to gas transmission (Tsai et al., 2006). The spongy and the inner lamellar layers form a matrix composed of protein fibres bonded to calcite (calcium carbonate) crystals. The two layers are also constructed in such a manner that there are numerous circular openings (pores). This structure permits gaseous exchange throughout the shell. The chemical composition of eggshell has been reported as: calcium carbonate (94%), magnesium carbonate (1%), calcium phosphate (1%) and organic matter (4%) (Stadelman, 2000; Hunton, 2005). These chemical elements from the hen’s diet must be broken down in the digestive system and then re-synthesised in the shell gland to form the eggshell. Dietary supplementation with natural substances, e.g. garlic, has been demonstrated to improve eggshell and egg quality (Qatramiz, 2006). Supplementation with black cumin (Nigella sativa L.) can increase the shell strength of eggs (Aydin et al., 2008). Radwan et al. (2008) indicated that feeding dietary Cucumis Longa to laying hens could increase shell weight and thickness because it could improve the micro environment in the uterus which was the site of calcium deposition. Furthermore, the active substance was lipopolysaccharide (LPS), which is derived from the cell walls of Pantoea agglomerans, gram-negative bacteria that grow symbiotically with wheat (Kohchi et al., 2006). Suzuki et al. (1992) reported that feeding dietary LPS enhanced eggshell strength. Gastrointestinal tract development and health are the key to productivity in all farm animals and poultry (Abdullah et al., 2010). The digestive functions could be considered the most limiting factors in production. Meimandipour et al. (2010) and Yamauchi et al. (2010) reported that small intestinal histology is markedly affected by dietary components and the histological changes in the small intestine correlate with small intestinal function. Thus, it is also possible that the presence of extraction of fermented wheat flour with Pantoea agglomerans and herb mixture could alter the intestinal histology and quality of eggshell. The purpose of this study was to evaluate the effect of adding Pantoea-6® (extract of fermented wheat flour with Pantoea agglomerans) and plant extracts (red clover and garlic) supplements on the laying performance, egg quality, eggshell structure and small intestinal histology of laying hens.

Materials and methods

Birds and management

The experiment was conducted according to the guidelines for the care and use of laboratory animals established by Kagawa University, Japan. In total, 66 Boris Brown laying hens (30 weeks old) were randomly divided into 3 experimental groups of 22 birds each as follows: the control group was fed a basal diet (Table 1) and the other groups were fed the basal diet supplemented with 0.1% Pantoea-6® and 0.1% plant extracts (red clover and garlic). During the experiment, all birds were maintained in individual laying cages in an environmentally controlled room (at a temperature of around 27°C with a photoperiod of 16L:8D). Each group was fed ad libitum with its own diet for a period of 8 weeks. Water was continuously available from nipple drinkers. Feed intake and refusal were recorded daily and body weight was measured weekly.
Egg quality examination

Eleven eggs from each group were collected weekly to measure egg quality. At first, the egg weight was recorded using an electronic digital balance. The shell-breaking strength was measured using an eggshell strength meter (accuracy: 0.1 kg/cm²; Fujihira Industry Co. Ltd., Tokyo, Japan). The eggs were broken onto a metal plate and the height of the albumen was measured as the distance between the metal plate and the electrode placed on top of the thick part of the egg. Then, the weights of the albumen, egg yolk and eggshell were measured using an electronic digital balance. Eggshell thickness was recorded as the mean value of measurements at three locations on the egg (air cell, equator and sharp end), measured by a dial gauge (Peacock Ozaki, Tokyo, Japan).

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\[ \text{Haugh unit} = 100 \log (H - 1.7 W^{-0.37} + 7.6) \]

where H is the observed height of the albumen (mm) and W is the weight of eggs (g).

Examination of phosphorus, calcium and magnesium in eggshell

The phosphorus, calcium and magnesium contents of eggshell were measured at the end of the experiment by the Japan Food Research Laboratories (December 6, 2011, No. 111127760001-01-111127760006-01; Tokyo, Japan).

Blood component examination

At 38th week of age, 15 birds (5 birds/treatment) were randomly selected. Blood was drawn from the wing vein for determining total protein, albumin, albumin to globulin ratio (A/G), total amount of bilirubin (T-BIL), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LD), glyc eride, total cholesterol (T-cho), blood urea nitrogen (BUN), creatinin, calcium, inorganic phosphorus and glucose. Chemical elements were measured at a commercial laboratory (Wakamatsu Medical Research Laboratory, Kitakyushu, Japan).

Eggshell examination

At 38th week of age, three eggs were randomly chosen from each treatment for eggshell sampling. The samples of the air cell, equator and sharp end of the eggshell were cut transversely approximately 0.5x0.5 cm in size. The pieces were mounted on aluminium stubs with electrically conductive carbon paste, coated (E-1030 Ion Sputter; Hitachi Ltd., Tokyo, Japan) and viewed under a scanning electron microscope (SEM) (Hitachi S-4300SE/N; Hitachi Ltd.) at 8 kV. Images were used to observe the microstructure of the eggshells.

Tissue sampling

At 38th week of age, four chickens with similar mean body weight and egg production level were chosen from each treatment. They were euthanised under anesthesia with diethyl ether to obtain tissues for microscopic assessment. The whole small intestine was removed immediately and placed into a mixture of 3% glutaraldehyde and 4% paraformaldehyde fixative solution in 0.1 M cacodylate buffer (pH 7.4). The intestinal segments were cut from the duodenum, jejunum and ileum as follows: i) the duodenum segment was cut from the gizzard to the pancreatic and bile ducts; ii) the jejunum segment was cut from the duct to the Meckel’s diverticulum; and iii) the ileum segment was cut from the diverticulum to the ileocecal-colonic junction. Tissue samples were taken from each part.

Light microscopy examination

A 2 cm long section of each intestine was fixed in Bouin’s solution for 1 d and dehydrated in a graded series of alcohol solutions. Finally, each segment was embedded in paraffin wax using standard techniques. Eight transverse sections from each intestinal segment cut at a thickness of 4 μm were fixed in each slide and stained with haematoxylin-eosin and the subsequent values were measured using an image analyser (Nikon Cosmopage 1 S; Nikon Co., Tokyo, Japan). Images were used to measure villus height, villus area, cell area and cell mitosis.

Measurement of villus height

Two villi having a lamina propria were randomly selected per transverse section. The villi were measured and placed into the T-BIL. The average villi heights from the three birds (16 villi from eight different sections in each segment, per bird) were expressed as a mean villus height for one treatment group.

Measurement of villus area

Two villi with lamina propria were chosen from each section and the width of the villus was measured at the basal and apical parts. The widths of 16 villi at the basal and apical parts were measured from different sections in each bird. The villus area was calculated from the villus height, basal width and apical width as follows:

\[
\text{villus area} = \frac{\text{basal width} + \text{apical width}}{2} \times \text{villus height}
\]

The mean villus areas from the three birds (16 calculations of the villus area from eight different sections in each section, per bird) were expressed as a mean villus area for one group.

Measurement of epithelial cell area

The area of the epithelial cell layer was randomly measured at the middle part of the villus and the cell nuclei within the cell layer were counted. Then, the area of the layer was divided by the number of cell nuclei. A total calculation of cell areas was measured from four different sections per bird and these four values were expressed as a mean cell area in one bird. These four mean cell areas from the four birds were expressed as a mean cell area for one treatment group.

Measurement of cell mitosis in the crypt

Mitotic cells having homogenous, intensely stained, basophilic nuclei with hematoxylin (Tarachai and Yamauchi, 2000) were counted. Total mitosis numbers were counted from four different sections per bird and an average of

Table 1. Composition and analysis of basal diet.

| Ingredients, % | Value |
|----------------|-------|
| Corn           | 65.00 |
| Soybean meal   | 12.00 |
| Fish meal      | 9.00  |
| Wheat bran     | 5.35  |
| Alfalfa meal   | 3.00  |
| Calcium carbonate | 4.00 |
| Dicalcium phosphate | 0.40 |
| Salt (NaCl)    | 0.30  |
| Vitamin-mineral mixture° | 0.30 |
| Vegetable cooking oil (soybean oil) | 0.50 |
| Paprika extracts | 0.15 |
| Total          | 100   |
| Calculated composition |
| Crude protein, % | 18.00 |
| Crude fibre, %  | 6.00  |
| Crude fat, %    | 2.00  |
| Crude ash, %    | 13.00 |
| Calcium, %      | 3.50  |
| Available phosphorus, % | 0.55 |
| ME, kcal/kg     | 2850  |

ME, metabolisable energy; °Vitamin-mineral mixture provided the following per kg of diet: vitamin A, 4000 U; vitamin D₃, 1200 U; vitamin E, 6 mg; vitamin K₃, 1.5 mg; vitamin B₁₂, 3 mg; vitamin B₆, 4.5 mg; potassium B₆, 4 mg; vitamin B₉, 6 µg; nicotinic acid, 9 mg; pantothenic acid, 6 mg; choline, 240 mg; folic acid, 0.06 mg; manganese, 54 mg; iron, 18 mg; cobalt, 0.6 mg; copper, 3.6 mg; zinc, 20.1 mg.

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these values was expressed as a mean cell mitosis number for each bird. Finally, these mean cell mitosis numbers from the three birds were expressed as a mean cell mitosis number for one group.

**Scanning electron microscopy examination**

A 2-cm long section of the intestine was slit longitudinally, opened and washed with 0.1 M phosphate buffered saline (pH 7.4). The tissue sample was pinned flat and fixed in this flattened position in a mixture of 3% glutaraldehyde and 4% paraformaldehyde fixative solution in 0.1% cacodylate buffer (pH 7.4) for 1 h at room temperature, cut into 4×4 mm squares. It continued this fixing for 1 h. The pieces were rinsed with 0.1 M sodium cacodylate buffer and were post-fixed with 1% osmium tetroxide in a 0.1 M ice-cold sodium cacodylate buffer for 2 h. The specimens were dried in a critical point drying apparatus. The dried specimens were coated with platinum and observed at 8 kV with SEM (Hitachi S-4300SE/N; Hitachi Ltd.). Morphological alterations of the epithelial cells on the villus apical surface were compared between each treatment group.

**Statistical analysis**

**Statistical analysis of the laying performance, egg quality, phosphorus, calcium and magnesium of the eggshell, blood component and light microscopy examination (villus and villus area, absorptive epithelial cell area and cell mitosis) were statistically analysed by one-way analysis of variance (ANOVA) with the Statistical Package for the Social Sciences (SPSS) software (version 10.0 for Windows; SPSS Inc., Chicago, IL, USA). Differences among the treatment groups were tested by Tukey’s studentised range test and differences were considered significant at P<0.05.

**Results and discussion**

**Laying performance and egg quality**

The effect of dietary Pantoea-6® and plant extracts on laying performance and egg quality are presented in Table 2. Compared with the control, the egg weight, albumen weight and eggshell breaking strength were significantly higher in the Pantoea-6® and plant extracts groups (P<0.05). Eggshell weight was significantly higher in the Pantoea-6® group (P<0.05).

**Phosphorus, calcium and magnesium in eggshell**

The effect of dietary Pantoea-6® and plant extracts on the phosphorus, calcium and magnesium contents in eggshell is shown in Table 3. There were no significant (P>0.05) differences in these blood components among the dietary treatments.

**Blood component**

The effect of dietary Pantoea-6® and plant extracts on total protein, albumin, A/G, T-BIL, AST, ALT, ALP, LD, glyceride, T-cho, BUN, creatinin, calcium, inorganic phosphorus and glucose is given in Table 4. In this case too, there were no significant (P>0.05) differences in these blood components among the dietary treatments.

**Eggshell examination**

The effect of dietary Pantoea-6® and plant extracts on eggshell structure is shown in Figures 1-3. Eggshell structure (air cell, equator, conical region) was determined by measuring the length, width, and height of the eggshell surface.

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### Table 2. Laying performance and egg quality in control, 0.1% Pantoea-6®, and 0.1% plant extracts (mean±SE, n=11) (8 weeks after feeding).

|                      | Control               | 0.1% Pantoea-6®       | 0.1% plant extracts | P   |
|----------------------|-----------------------|-----------------------|---------------------|-----|
| Initial body weight, g | 1785.23±26.93         | 1787.50±26.17         | 1783.64±23.47       | 1.000 |
| Egg production, %     | 93.83±1.08            | 92.05±1.17            | 91.23±1.24          | 0.280 |
| Feed intake, g/hen/d  | 99.38±14.22           | 90.19±15.65           | 90.94±12.42         | 0.063 |
| Final body weight, g  | 2012.95±36.63         | 2084.55±31.59         | 2088.64±28.08       | 0.184 |
| Feed efficiency       | 0.44±0.01             | 0.52±0.04             | 0.52±0.05           | 0.172 |
| Egg weight, g         | 60.31±0.92⁴           | 66.74±1.17⁴           | 66.04±1.81⁴         | 0.004 |
| Eggshell breaking strength, kg/cm² | 3.15±0.25⁵ | 3.84±0.19⁵ | 4.09±0.20⁵ | 0.012 |
| Eggshell thickness, mm | 0.35±0.01            | 0.37±0.01             | 0.38±0.01           | 0.208 |
| Shell weight, g       | 6.44±0.18⁴            | 7.18±0.16⁴            | 6.97±0.26⁴          | 0.046 |
| Yolk weight, g        | 16.72±0.29            | 17.85±0.41            | 17.00±0.47          | 0.139 |
| Albumen weight, g     | 37.08±0.86⁴           | 41.68±0.83⁴           | 42.07±1.37⁴         | 0.003 |
| Roche yolk colour fan | 10.36±0.15            | 10.55±0.16            | 10.36±0.15          | 0.633 |
| Haugh units           | 93.12±1.42            | 92.74±0.96            | 92.43±3.15          | 0.289 |

*⁴Means in a row with different superscripts are different at P<0.05.

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![Figure 1. Eggshell surface (air cell) in laying hens fed commercial mash diet (A), commercial mash diet supplemented with 0.1% Pantoea-6® (B), and 0.1% plant extracts (C). Scale bar=50 µm (x200).](image)
Table 3. Phosphorus, calcium and magnesium in eggshell in control, 0.1% Pantoea-6®, and 0.1% plant extracts (mean±SE, n=2).

|                     | Control       | 0.1% Pantoea-6® | 0.1% plant extracts | P   |
|---------------------|---------------|-----------------|---------------------|-----|
| Phosphorus, mg/100 g| 119.50±18.50  | 120.00±4.00     | 125.50±5.50         | 0.920 |
| Calcium, g/100 g    | 36.00±1.00    | 37.50±0.50      | 36.00±0.01          | 0.306 |
| Magnesium, mg/100 g | 344.00±14.00  | 351.00±10.00    | 378.00±1.00         | 0.177 |

Table 4. Blood component in hens fed control, 0.1% Pantoea-6®, and 0.1% plant extracts (mean±SE, n=5).

|                      | Control       | 0.1% Pantoea-6® | 0.1% plant extracts | P   |
|----------------------|---------------|-----------------|---------------------|-----|
| Total protein, g/dL  | 5.24±0.20     | 5.06±0.21       | 5.12±0.26           | 0.851 |
| Albumin, g/dL        | 2.16±0.51     | 2.06±0.09       | 2.14±0.07           | 0.581 |
| A/G                  | 0.70±0.02     | 0.69±0.03       | 0.74±0.04           | 0.484 |
| T-BIL, mg/dL         | 0.45±0.05     | 0.37±0.04       | 0.40±0.08           | 0.665 |
| AST (GOT), U/L       | 141.80±6.92   | 146.80±8.94     | 358.00±200.66       | 0.355 |
| ALT (GPT), U/L       | 0.40±0.40     | 1.80±0.37       | 3.20±5.50           | 0.422 |
| ALP, U/L             | 3051.80±686.88| 3197.00±961.05  | 3331.20±1178.81     | 0.445 |
| LD, U/L              | 138.80±13.08  | 119.00±13.32    | 107.60±17.35        | 0.349 |
| Glyceride, mg/dL     | 247.20±269.21 | 1852.20±252.26  | 1598.60±356.31      | 0.143 |
| T-cho, mg/dL         | 138.00±13.08  | 119.00±13.32    | 107.60±17.35        | 0.349 |
| BUN, mg/dL           | 1.94±0.35     | 1.98±0.35       | 1.38±0.22           | 0.358 |
| Creatinin, mg/dL     | 0.22±0.02     | 0.27±0.02       | 0.25±0.03           | 0.443 |
| Calcium, mg/dL       | 28.18±1.04    | 27.58±0.52      | 26.90±1.15          | 0.643 |
| Inorganic phosphorus, mg/dL | 4.98±0.31 | 4.94±0.29 | 5.12±0.25 | 0.895 |
| Glucose, mg/dL       | 235.40±6.33   | 232.00±6.97     | 238.20±4.22         | 0.856 |

A/G, albumin to globulin ratio; T-BIL, total amount of bilirubin; AST, aspartate aminotransferase; GOT, glutamic oxaloacetic transaminase; ALT, alanine aminotransferase; GPT, glutamic-pyruvic transaminase; ALP, alkaline phosphatase; LD, lactate dehydrogenase; T-cho, total cholesterol; BUN, blood urea nitrogen.

Figure 2. Eggshell surface (equator) in laying hens fed commercial mash diet (A), commercial mash diet supplemented with 0.1% Pantoea-6® (B), and 0.1% plant extracts (C). Scale bar=50 µm (×200).

Figure 3. Eggshell surface (sharp end) in laying hens fed commercial mash diet (A), commercial mash diet supplemented with 0.1% Pantoea-6® (B), and 0.1% plant extracts (C). Scale bar=50 µm (×200).
and sharp end) can be clearly seen in all groups: it tended to have greater thickness in both dietary Pantoea-6® and plant extracts groups than in the control.

**Light microscopic examination**

Villus height and area, cell area and mitosis in all intestinal segments tended to be higher in all laying hens in the dietary Pantoea-6® and plant extracts groups than in the control (Figure 4). The duodenum and jejunum of both treatment groups showed higher cell area values than those of the control (P<0.05).

**Scanning electron microscopy examination**

In the duodenum, in the villus apex of the control (Figure 5A), flat cell areas (small arrows) were found. In treatment groups (Figure 5 B,C), such flat cells developed into clearly protuberated cells (large arrows). The same can be seen on the jejunal villus apical surface of the control group (Figure 6 A-C). On the ileal villus apex surface of the control (Figure 7A) flat cell areas (small arrows) were observed too. However, the cell protuberances cells (large arrows) continued to be present in the Pantoea-6® and plant extracts groups (Figure 7 B,C).

**General remarks**

The results of the present study showed that supplementation of the diet with Pantoea-6® and plant extracts did not negatively influence egg production, feed intake, feed efficiency, final body weight, eggshell thickness, yolk weight, yolk colour and Haugh units of the laying hens. These data correspond with no changes to final body weight (Qureshi et al., 1983; Abdullah et al., 2010) and feed conversion ratio in broiler chicks fed dietary garlic (Konjufca et al., 1997; Abdullah et al., 2010). Interestingly, the egg weight, albumen weight and eggshell breaking strength of the treatment groups were significantly higher than in the control. Eggshell weight of the Pantoea-6® group was significantly higher than in the other groups. As the nutritional composition of diets in all groups was almost the same, the tendency to better egg quality seems to have been induced by Pantoea-6® and plant extracts. In this study, Pantoea-6® was made from extraction of fermented wheat flour with Pantoea agglomerans, which is gram-negative bacteria (Hebishima et al., 2010). Rajput et al. (2013) reported that LPS is a structural component of the outer membrane of gram-negative bacteria. Xie et al. (2000) found that LPS increased the total plasma protein concentration in chickens. Kohchi et al. (2006) reported

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Figure 4. (A) Villus height, (B) Villus area, (C) cell area and (D) cell mitosis of each intestinal segment (duodenum, jejunum and ileum) in laying hens fed commercial mash diet (control group), commercial mash diet supplemented with 0.1% Pantoea-6®, and 0.1% plant extracts (mean±SE, n=4). a and b, means with varying lowercase letters differ significantly at P<0.05.
that feeding LPS serves to strengthen the prophylactic ability. Moreover, LPS activates macrophages and the innate immune system. Thus, the improvement in egg and albumen weight might be attributed to the effect of LPS on nutrient digestibility. However, studies on the effects of LPS on poultry are limited compared with studies using mammals. Therefore, further study is being carried out to address this issue.

In previous studies, garlic supplementation improved egg (Yalcin et al., 2006; Mahmoud et al., 2010) and albumen weight (Qatramiz, 2006; Mahmoud et al., 2010). The mechanisms of garlic have been accredited to its effective antioxidant action (Yang et al., 1993), and its ability to stimulate immunological responsiveness (Reeve et al., 1993). Ramakrishna et al. (2003) reported that garlic supplementation probably enhanced the activities of the pancreatic enzymes and provided a micro-environment for better nutrient utilisation in rats. Supplementation with black cumin (*Nigella sativa* L.) can increase the shell strength of
eggs (Aydin et al., 2008). Radwan et al. (2008) indicated that feeding dietary Curcuma Longa to laying hens could increase the eggshell weight and thickness because it could improve the micro environment in uterus (site of calcium deposition). Hernandez et al. (2004) and Jang et al. (2004) reported that dietary feeding of essential oil extracted from herbs improved the secretion of digestive enzymes thus improving the digestibility of the feeds. In the current study, the eggshell structure (air cell, equator and sharp end) can be clearly seen in both Pantoea-6® and plant extracts groups. Density of mamillary knobs tended to be lower in both treatment groups. Van Toledo et al. (1982) reported that weak eggshells generally have a higher mamillary knobs density. Moreover, the eggshell structure tended to have greater thickness in both the dietary Pantoea-6® and plant extracts groups than in the control. Increased thickness of the eggshell by the addition of Pantoea-6® and plant extracts may be attributed to the components of these supplements which have antioxidant activities (Abdullah et al., 2010).

The morphological differences among the intestinal parts would be induced by the nutrients in the diets (Mekbungwan et al., 2003; Yamauchi et al., 2006) and the intestinal absorptive function of each segment (Rattanawut and Yamauchi, 2012). The present results show that most light microscopy parameters (villus height and area, cell area and mitosis) in all intestinal segments tended to have higher values in both treatment groups than in the control. In particular, the cell area in the duodenum and jejunum of the Pantoea-6® and plant extracts groups had higher values than the control. Under normal circumstances, the major absorption of nutrients occurs in the duodenum and jejunum (Noy and Sklan, 1995). Based on the results, we conclude that the absorptive function of cells in the duodenum and jejunum was sufficient in both treatment groups, whereas the ileum did not play a significant role in absorption. The results reported in this study are in complete agreement with what has been reported in the literature. Santin et al. (2001) reported that when birds were treated with 0.2% of yeast cell walls, the villus height increased. Adibmoradi et al. (2006) reported that garlic administration enhanced the villus height and crypt depth and decreased the epithelial thickness and goblet cells number in the duodenum, jejunum and ileum of birds; similar results were reported by Nusairate (2007). Garlic administration stimulated the selective population of intestinal cells (Abdullah et al., 2010). Gupta and Sandhu (1998) found that feeding garlic agglutinin to rats caused lengthening intestinal villi due to cellular hypertrophy and hyperplasia. Increased villus size indicates an increased villus length (Laurenore et al., 2000) and provides a greater surface area for the absorption of available nutrients (Onderci et al., 2006). The ingested nutrients are absorbed into the blood or lymphatic system after being transported from the intestinal lumen through the mucosal epithelial cell barrier (Caspary, 1992).

The epithelial cells react morphologically to ingested diets more quickly than the intestinal villi (Tarachai and Yamauchi, 2000; Maneewat and Yamauchi, 2004), because the histological reaction to ingested diets differs between the micro (epithelium) and macro (villi) levels. In the present study, protuberated cells were observed on the villus apical surface in both the dietary Pantoea-6® and plant extracts groups. It is possible that both dietary Pantoea-6® and plant extracts are able to stimulate gut cellular proliferation by direct interaction with a selective population of intestinal cells or indirectly via gut endocrine cells. The present dietary amounts of Pantoea-6® and plant extracts would also increase the light microscopy parameters and protuberated cells, resulting in increased digestion and absorption.

**Conclusions**

This study shows that supplementing Pantoea-6® and plant extracts at a 0.1% level might have a beneficial effect on egg and albumen weight, eggshell quality and eggshell structure parameters, as well as on small intestine histological parameters.

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