ISSN 1682-8356
ansinet.org/ijps

INTERNATIONAL JOURNAL OF

POULTRY SCIENCE

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International Journal of Poultry Science 8 (11): 1078-1085, 2009
ISSN 1682-8356

Production Performance, Egg Quality and Intestinal Histology in Laying Hens Fed Dietary Dried Fermented Ginger

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Abstract: To evaluate the effect of dietary Dried Fermented Ginger (DFG) on production performance, egg quality and intestinal histology, 27 White Leghorn laying hens (24 weeks of age) were assigned into three groups of 9 birds each as follows: the control group was fed a basal diet (CP, 17%; ME, 2,850 kcal/kg) and the other groups were fed the basal diet supplemented with 1% or 5% DFG. Compared with the control, feed consumption tended to increase in DFG groups and increased significantly in the 1% DFG group ($p<0.05$). Hen-day egg production and egg mass are better in both DFG groups. Feed efficiency tended to be a little higher value in the DFG diets. There were no significant ($p>0.05$) differences in shell-breaking strength, shell thickness, shell ratio, albumen ratio, yolk ratio, yolk color and Haugh unit among the dietary treatments. Villus height, villus area, cell area and cell mitosis in all intestinal segments tended to be higher in DFG groups than in the control group and jejunal cell area of the 1% DFG group as well as jejunal cell mitosis of the both DFG groups showed significant increases. Moreover, the cells on the villus tip surface were protuberated in all DFG groups, resulting in a rough surface. In addition, segmented filamentous bacteria were observed in the ileum of the 5% DFG group. These results suggest that dietary DFG increase the production performance of layer chickens due to hypertrophy of intestinal villi and epithelial cells at 1% DFG.

Key words: Dried fermented ginger, egg quality, histological intestinal changes, production performance, white leghorn hens

INTRODUCTION

Silage juice has high concentrations of organic acid, such as lactic acid and acetic acid (Hang *et al*., 2003). These organic acids were reported to reduce digestive pH and increase pancreatic secretion and to have trophic effects on gastrointestinal mucosa in piglets and poultry (Dibner and Buttin, 2002). In particular, lactic acid bacteria were affected by silage products; these bacteria increased during the stages of ensiling (McDonald *et al*., 2002). Among herbal plants, Japanese mugwort (*Artemisia princeps* Pamp.) is one of the most popular wild herbal plants. Young leaves have a characteristic greenish aroma and the green juice of leaves is also used in traditional Japanese folk medicine to treat skin injuries (Umano *et al*., 2000). Moreover, it is known as a common weed and a very vigorous plant, which grows in desolate area such as the mountains, fields, roadsides and banks. Craft Co., Ltd (Kagawa prefecture, Japan) has made a Japanese Mugwort Silage Juice (JMS) and identified lactic acid bacteria, yeast fungus, photosynthetic bacteria, ray-fungus, hyperthermal bacteria, *Aspergillus* and *Bacillus subtilis*. Recently, live microorganisms are used to confer a health benefit on the host when microorganisms administered in adequate amounts (Fuller, 1989). As dietary *Lactobacillus sporogenes* (Panda *et al*., 2006) and fungus (Huang *et al*., 2004) enhanced growth performance, the JMS seems to induce the production performance of chickens.

Ginger (*Z. officinale* Rosc.) has been traditionally used from time immemorial for varied human ailments in different parts of the globe, to aid digestion and treat stomach upset, diarrhoea and nausea (Shukla and Singh, 2007). Its rhizome also has the stimulating effect on peptic juices, such as bile and salivary, gastric, pancreatic and intestinal juices (Stoilova *et al*., 2007). The oleoresin from the rhizome contained 6-gingerol and its homologs which have been shown to possess anti-inflammatory, antipyretic, antihepatotoxic, analgesic and cardiotonic properties (Surh, 1999). Ginger acetone extract and its constituents have been shown to enhance the transport of a charcoal meal in mice (Yamahara *et al*., 1990). Moorthy *et al*. (2009) have been reported that dried ginger powder increased body weight of broilers when included in the diet at 2% level. In Kochi prefecture, ginger has been widely cultivated and processed into various products. About 30~50 tones of ginger by-product are thrown away from one company as waste without using effectively. Many researchers have worked on the biological activities and medicine of ginger. However, there are few works on use of ginger by-product as supplements in animal diets.

In 2008, we formulated Dried Fermented Ginger (DFG), a fermented product comprised of ginger and JMS and obtained positive results in broiler growth performance. Therefore, the present study was designed to determine the effects of DFG diets on production performance, egg

quality and intestinal villi histology in laying hens, from 24-43 weeks of age.

MATERIALS AND METHODS

DFG Preparation: JMS was provided by Craft Co., Ltd. (Kagawa prefecture, Japan). Japanese mugwort was collected and ensiled at room temperature to get silage juice. The present JMS included natural microorganisms, such as lactic acid bacteria, yeast fungus, photosynthetic bacteria, ray-fungus, hyperthermal bacteria, *Aspergillus* and *Bacillus subtilis*. DFG was produced from ginger by-product fermented with JMS as follows: Ginger was ground with an electric grinder and placed into plastic bags. JMS was added to the bags containing ground ginger. These bags were kept under anaerobic conditions at room temperature for 4-5 d. Fresh fermented mixture was then dried in a hot-air oven at 50°C for 1-2 d. Finally, it was again ground using an electric grinder and kept at room temperature until mixed with the basal diet. Gross energy was measured with a bomb calorimeter (IKA C5000, IKA® Werke GmbH and Co. KG, Staufen, Germany). Dry matter, crude protein, crude fat, crude fiber, crude ash, calcium and phosphorus were determined by methods according to the Association of Official Analytical Chemists (AOAC, 1984) and shown in Table 1. We obtained informal consent regarding the safety of DFG from the Ministry of Agriculture, Forestry and Fisheries of the Japanese Government.

Table 1: Proximate composition of Dried Fermented Ginger (DFG)

Items	(%)
Dry matter	87.44
Crude protein	7.29
Crude fat	6.51
Crude fiber	22.10
Crude ash	13.40
Calcium	0.46
Phosphorus	0.84
Gross energy (kcal/kg)	3,908

All parameters of DFG samples were determined in triplicate

Hens, diets and housing: Twenty-seven White Leghorn laying hens (24 weeks of age) were selected and assigned into three experimental groups of 9 birds each as follows: the control group was fed a basal diet (Table 2; Nippon Formula Feed Mfg. Co., Ltd., Kanagawa-ken, Japan) and the other groups were fed the basal diet supplemented with 1% or 5% DFG. Feed and water were available *ad libitum* in each dietary group. All birds were maintained in individual laying cages in an environmentally controlled room. The photoperiod was set at 17L: 7D during the experiment.

Egg quality: Eggs were collected and counted twice per week to obtain data on egg production and feed consumption for each replicate was recorded weekly for the entire study. Eggs from each group were collected weekly to measure egg quality, for criteria such as shell-breaking strength, shell thickness, shell ratio, albumen ratio, yolk ratio, yolk color and Haugh unit. At first, egg weight was recorded by using an electronic digital balance. The shell-breaking strength was measured using an eggshell strength meter (accuracy: 0.1 kg/cm^2; Fujihira Industry Co., Ltd.). The eggs were broken onto a metal plate and the height of the albumin was measured by the distance between the metal plate and the electrode placed on top of the thick egg white of the broken egg. Then, the weights of the albumin, egg yolk and eggshell were measured using an electronic digital balance. Shell thickness was a mean value of measurements at three locations on the egg (air cell, equator and sharp end), measured by a dial thickness gauge (Peacock, Tokyo, Japan). Yolk color was measured visually by using the Roche Yolk Color Fan (Roche Lte., Basel, Switzerland). The values of the shell ratio, albumen ratio, yolk ratio and Haugh unit were calculated for each individual egg using the following formula:

$$\text{Shell ratio} = \frac{\text{Shell weight}}{\text{Egg weight}} \times 100$$

Table 2: Ingredients and nutrient compositions of the basal diet

Items	
Ingredients (%)	
Maize	61.00
Milo	1.00
Soybean meal	16.00
Rapeseed meal	4.00
Gluten meal	3.00
Chicken meal	2.50
Fish meal	0.50
Rice bran	1.00
Animal fat	1.30
Calcium carbonate	8.57
Dicalcium phosphate	0.40
Salt	0.20
Paprika	0.03
Concentrate mixture[1]	0.50
Calculated nutrient composition	
Crude protein (%)	17.00
Crude fat (%)	3.00
Crude fiber (%)	6.00
Crude ash (%)	14.00
Calcium (%)	3.10
Available phosphorus (%)	0.45
Metabolizable energy, kcal/kg	2,850

[1]Concentrate mixture including (per kg of diet): vitamin A, 10,000 IU; vitamin D_3, 2,000 IU; vitamin E, 15.63 mg; vitamin K, 2.6 mg; vitamin B_1, 2.88 mg; vitamin B_2, 3.7 mg; vitamin B_6, 7.48 mg; vitamin B_{12}, 0.01 mg; biotin, 0.14 mg; pantothenic acid, 16.12 mg; folic acid, 1.0 mg; niacin, 53.68 mg; choline, 1,400 mg; zinc, 75.00 mg; copper, 15.79 mg; manganese, 81.75 mg; iron, 170.76 mg; iodine, 0.35 mg; selenium, 0.15 mg

$$\text{Albumen ratio} = \frac{\text{Albumen weight}}{\text{Egg weight}} \text{x} 100$$

$$\text{Yolk ratio} = \frac{\text{Yolk weight}}{\text{Egg weight}} \text{x} 100$$

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

Where
H = Observed height of the albumen in mm
W = Weight of egg (g)

Tissue sampling procedure: At 43 weeks of age, three chickens with similar body weights were chosen from each treatment and killed for tissue sampling. The whole small intestine was removed immediately and placed into a mixture of 3% glutaraldehyde and 4% paraformaldehyde fixative solution in 0.1M cacodylate buffer (pH 7.4). The intestinal segments were cut from the duodenum, jejunum and ileum as follows: 1) the duodenum segment was from the gizzard to the pancreatic and bile ducts; 2) the jejunum segment was from the duct to Meckel's diverticulum and 3) the ileum segment was from the diverticulum to the ileo-caecal-colonic junction. The tissue samples were taken from the midpoint of each part.

Sample preparation for light microscopy: A 2-cm length of each intestinal segment was fixed in Bouin's solution for 1 day 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 hematoxylin-eosin and the subsequent values were measured using an image analyzer (Nikon Cosmozone IzS, Nikon Co., Tokyo, Japan).

Measurement of villus height: Two villi having a lamina propria were randomly selected per transverse section. The villus was measured from the tip to the base excluding the crypt. The average villus 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 per 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:

$$\text{Villus area} = \frac{\text{Basal width} + \text{Apical width}}{2} \text{x Villus height}$$

The mean villus areas from the three birds (16 calculations of the villus area from eight different sections in each segment, 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 then the cell nuclei within this cell layer were counted. Next, the area of the layer was divided by the number of cell nuclei. A total calculation of cell areas were measured from four different sections per bird and these four values were expressed as a mean cell area in one bird. These three mean cell areas from the three 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 these values was expressed as a mean cell mitosis number for each bird. Finally, these three mean cell mitosis numbers from the three birds were expressed as a mean cell mitosis number for one group.

Scanning electron microscopy: A 2-cm length of the intestinal segment was slit longitudinally, opened and washed with 0.1 M phosphate buffered saline (pH 7.4). The samples were pinned flat to prevent curling in a mixture of 3% glutaraldehyde and 4% paraformaldehyde in 0.1 mol/L cacodylate buffer (pH 7.4) at room temperature for 1 h. Afterward, the tissue block was cut into 5 x 6 mm square and fixed for a further 1 h. The samples were rinsed with 0.1 mol/L sodium cacodylate buffer (pH 7.4) and post-fixed with 1% osmium tetroxide in an ice-cold buffer for 2 h. Then, these specimens were washed in deionized distilled water, stored in 45% ethanol for 24 h and kept in 70% ethanol. Just before the specimens were freeze-dried, they were transferred to 80, 90 and 100% ethanol solution (120, 60 and 15 min, respectively) and stored in *t*-butyl alcohol (15 min; three times). The dried samples 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 (Hitachi S-4300SE/N, Hitachi Ltd., Tokyo, Japan) at 8 kV. Morphological alterations of the epithelial cells on the villus apical surface were compared between each treatment group.

Statistical analysis: The results were reported as means±SE and data on production performance, egg quality and light microscopic examination (villus height, villus area, absorptive epithelial cell area and cell mitosis) were statistically analyzed by one-way analysis

Table 3: Effect of dietary Dried Fermented Ginger (DFG) on production performance of laying hens from 24-43 weeks of age

Items	Control	1% DFG	5% DFG
Feed consumption, g/(hen*day)	102.93[b]±1.05	110.63[a]±1.73	104.40[b]±2.14
Initial weight, g	1437±34.5	1431±10.5	1444±27.2
Final weight, g	1484±17.3	1526±10.8	1517±49.7
Hen-day egg production (%)	87.98±3.72	96.03±0.89	94.80±1.03
Egg mass, g egg/(hen*day)	53.15±3.13	59.49±1.19	56.48±4.79
Feed efficiency	0.52±0.03	0.55±0.15	0.56±0.04

[a,b]Mean values within a row having different superscripts are significantly different by least significant difference test ($p<0.05$)

Table 4: Effect of dietary Dried Fermented Ginger (DFG) on egg quality of laying hens from 24-43 weeks of age

Items	Control	1% DFG	5% DFG
Shell-breaking strength, kg/cm^2	4.79±0.16	4.49±0.16	4.64±0.05
Shell thickness, mm	0.39±0.005	0.38±0.003	0.38±0.005
Shell ratio (%)	12.95±0.42	11.96±0.39	11.78±0.27
Albumen ratio (%)	60.57±0.36	62.23±0.74	61.24±0.29
Yolk ratio (%)	26.48±0.27	25.81±0.45	26.98±0.44
Yolk color	11.25±0.18	11.22±0.31	11.07±0.19
Haugh unit	91.21±0.82	91.55±1.90	91.29±0.80

of variance (ANOVA) of SPSS statistical software package (version 10.0 for windows, SPSS, Inc., Chicago, IL). Differences among the treatment groups were tested by Duncan's multiple range test and differences were considered significant at $p<0.05$.

RESULTS

Production performance: The effect of dietary DFG on production performance of laying hens from 24-43 weeks of age is presented in Table 3. Compared with the control, feed consumption tended to increase in DFG groups and increased significantly in the 1% DFG group ($p<0.05$). Hen-day egg production, egg mass and feed efficiency tended to be better in both dietary DFG groups.

Egg quality: The effect of dietary DFG on the egg quality of laying hens from 24-43 weeks of age is shown in Table 4. There were no significant ($p>0.05$) differences in shell-breaking strength, shell thickness, shell ratio, albumen ratio, yolk ratio, yolk color, or Haugh unit, among the dietary treatments.

Light microscopic examination: Villus height, villus area, cell area and cell mitosis in all intestinal segments tended to be higher in all laying hens in the dietary DFG groups than in the control group (Fig. 1). For cell area, the jejunum of the 1% DFG groups showed higher values than that of the control ($p<0.05$). Also, for cell mitosis numbers, the jejunum of the 1 and 5% DFG groups was increased ($p<0.05$). Most of these light microscopic parameters were higher in the DFG groups than in the control group.

Epithelial cells: In the duodenum, in the villus apex of the control (Fig. 2A), flat cell areas (arrowheads) and an unclear central sulcus (small arrow) were found. In the 1 and 5% DFG groups (Fig. 2B and C), such flat cells developed into clearly protuberated cells (large arrows) and a clear central sulcus (small arrow) appeared. On the jejunal villus apical surface of the control group (Fig. 3A), most cells were flat (arrowheads). In the dietary DFG groups (Fig. 3B and C), the flat cells on the villus tip developed to protuberated cells (large arrows), resulting in a rough surface. On the ileal villus apex surface of the control (Fig. 4A), flat cell areas (arrowheads) were observed. However, the cell protuberances cells (large arrows) continued to be present in the DFG groups (Fig. 4B and C). In addition, segmented filamentous bacteria (arrows with F) were found in the 5% DFG group (Fig. 4C).

DISCUSSION

The hen-day egg production and egg mass tended to be better in both dietary DFG groups, although no statistically significant differences. The hen-day egg production is 9.15% higher in 1% DFG group and 7.75% higher in 5% DFG group than the control. The egg mass is also 11.93% higher in 1% DFG group and 6.26% higher in 5% DFG group than the control. As the nutritional composition of diets in all groups was almost the same, the tendency of the present better egg performance seems to be induced by DFG. Such higher egg performance than that of the control is thought to need more basal metabolism. Consequently, the birds showing higher egg performance resulted in more energy requirement. This corresponds with the result feed consumption tended to increase in dietary DFG groups and increased significantly in the 1% DFG group. This result suggests that the higher percentage of DFG than 1% might be not good for chicken production. As the body weight of DFG groups is almost similar to the control, the ingested nutrient might be used for egg performance. On the other hand, the dietary DFG had no significant effect ($p>0.05$) on egg qualities such as shell-breaking strength, shell thickness, shell ratio, albumen ratio, yolk ratio, yolk color and Haugh unit. Consideration

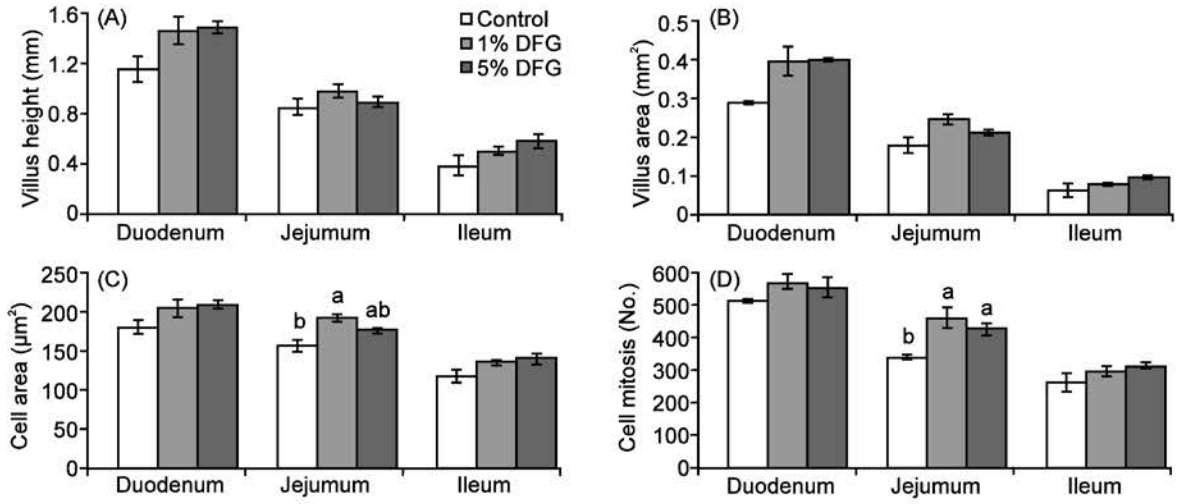

Fig. 1: (A) Villus height, (B) Villus area, (C) Cell area and (D) Cell mitosis of each intestinal segment in laying hens fed a commercial mash diet (control group), the commercial mash diet supplemented with 1% and 5% DFG (mean±SE). [a,b]Means with varying superscripts differ significantly at $p<0.05$

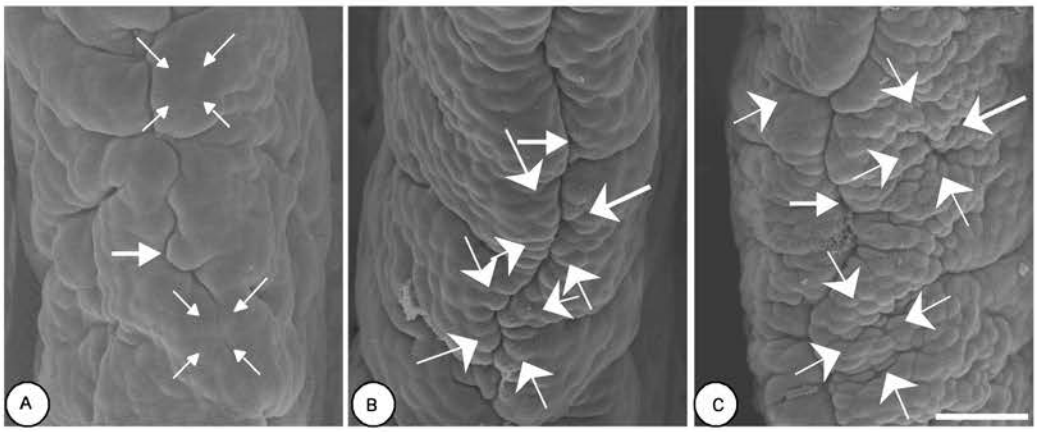

Fig. 2: Duodenal villus tip surface in laying hens fed a commercial mash diet (A; small arrow, central sulcus; arrowheads, flat cell area), the commercial mash diet supplemented with 1% (B; small arrow, central sulcus; large arrows, protuberating cell area) and 5% DFG (C; small arrow, central sulcus; large arrows, protuberating cell area). Scale bar = 50 µm (x600)

of the current egg quality parameters suggest that DFG would have no detrimental effect on egg quality, with up to 5% dietary DFG.

The tissues of gastrointestinal tract in the chickens respond very quickly to the alterations in the status of the nutrients and feed intake and intestinal mucosal structure is changed (Dou *et al*., 2002). Histology of the intestinal villi and epithelial cells on the villous apical surface in chickens (Yamauchi *et al*., 2006) and in piglets (Mekbungwan *et al*., 2003) is well known to be affected by dietary feed components. It has been suggested that long villi result in an increased surface area that is capable of greater absorption of available nutrients (Caspary, 1992), while greater villus height and more numerous cell mitosis in the intestine are indicators that the function of intestinal villi is activated (Langhout *et al*., 1999; Yasar and Forbes, 1999). The present results show that villus height, villus area, cell area and cell mitosis in all intestinal segments had higher values in the DFG groups than in the control group. Considerations for the present increased light microscopic parameters in the DFG groups might have consequences relative to the hypertrophy of the villi.

As the stem cells located in the intestinal crypt produce enterocytes and they ascend along the villus surface upward to replace old cells which are exfoliated at the tip, intestinal epithelial cells are composed of a continuously renewable cell population (Wice and Gordon, 1992). Then these cells are extruded into the intestinal lumen within a few days (Imondi and Bird,

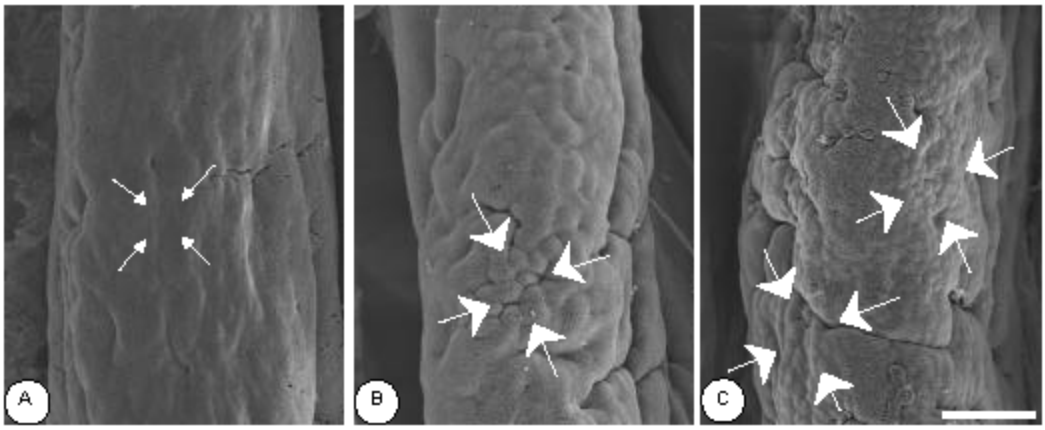

Fig. 3: Jejunal villus tip surface in laying hens fed a commercial mash diet (A; arrowheads, flat cell area), the commercial mash diet supplemented with 1% (B; large arrows, protuberating cell area) and 5% DFG (C; large arrows, protuberating cell area). Scale bar = 50 μm (x600)

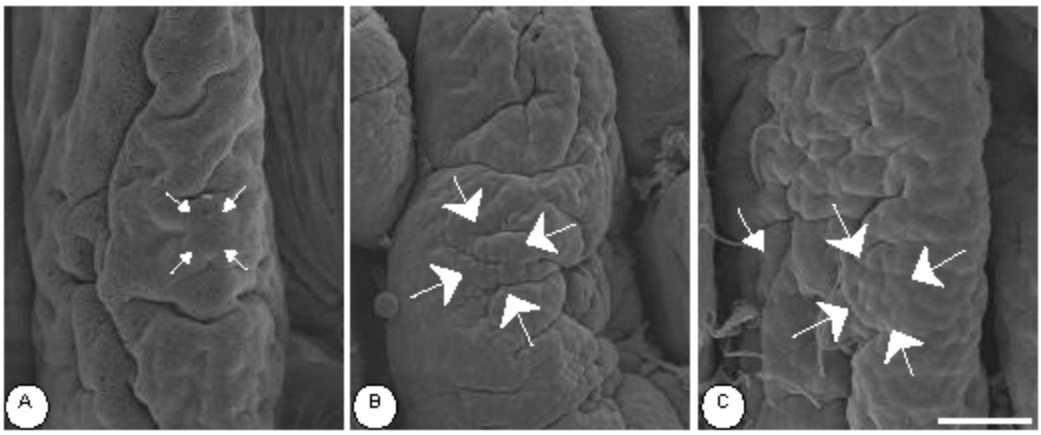

Fig. 4: Ileal villus tip surface in laying hens fed a commercial mash diet (A; arrowheads, flat cell area), the commercial mash diet supplemented with 1% (B; large arrows, protuberating cell area) and 5% DFG (C; large arrows, protuberating cell area; arrow with F, segmented filamentous bacteria). Scale bar = 50 μm (x600)

1966) or 48 h after formation (Potten, 1998). The present results show that the control epithelial cells were flat and the cells of the DFG groups were protuberated into the lumen in each intestinal segment suggest that the cells of the DFG groups might be more hypertrophied than those of the control.

In addition, segmented filamentous bacteria were found in the 5% DFG group. Segmented filamentous bacteria are autochthonous bacteria colonizing the ileum of many young animals by attaching to intestinal epithelial cells (Yamauchi *et al*., 1990). These non-pathogenic bacteria strongly stimulate the host immune response by elevating IgA in the intestine (Klaasen *et al*., 1993) and have a possible protective role against infection with *Salmonella enteritidis* in rats (Garland *et al*., 1982) and enteropathogenic *Escherichia coli* O103 disease in rabbits (Heczko *et al*., 2000). It is not clear at present why segmented filamentous bacteria appeared in the 5% DFG groups, but it is possibly related to the components of DFG.

Ginger has been used as a household remedy for dyspepsia, flatulence, colic and diarrhea (Borrelli *et al*., 2004) and as an appetite stimulant (White, 2007). Its rhizome also has the stimulating effect on peptic juices, such as bile and salivary, gastric, pancreatic and intestinal juices (Stoilova *et al*., 2007). The oleoresin from the rhizome contained 6-gingerol and its homologs which have been shown to possess anti-inflammatory, antipyretic, antihepatotoxic, analgesic and cardiotonic properties (Surh, 1999). Ginger acetone extract and its constituents have been shown to enhance the transport of a charcoal meal in mice (Yamahara *et al*., 1990). Moorthy *et al*. (2009) have been reported that dried ginger powder increased body weight of broilers when included in the diet at 2% level. On the other hand, silage juice has high concentrations of organic acid, such as

lactic acid and acetic acid (Hang *et al*., 2003), which were reported to have several beneficial effects on piglet and poultry performance, including reduction in digestion pH, increased pancreatic secretion and trophic effects on the gastrointestinal mucosa (Dibner and Buttin, 2002). The present DFG would also enhance the stimulating effect on digestive enzymes and thereby increase digestion and absorption, resulting in the increased light microscopic parameters and protuberated epithelial cells.

Conclusion: The current histological hypertrophied villi and epithelial cells in the DFG groups suggests that intestinal absorptive function might be activated after feeding a DFG diet, resulting in the improved production performance, especially in the 1% DFG group, without a detrimental effect on egg quality. Dietary DFG can be used as a natural feed additive to stimulate intestinal function at 1% DFG.

REFERENCES

AOAC, 1984. Official Methods of Analysis, 14th Edn., Association of Official Analytical Chemists. Washington, D.C., USA.

Borrelli, F., R. Capasso, A. Pinto and A.A. Izzo, 2004. Inhibitory effect of ginger (*Zingiber officinale*) on rat ileal motility *in vitro*. Life Sci., 74: 2889-2896.

Caspary, W.F., 1992. Physiology and pathophysiology of intestinal absorption. Am. J. Clin. Nutr., 55: 299s-308s.

Dibner, J.J. and P. Buttin, 2002. Use of organic acids as a model to study the impact of gut microflora on nutrition and metabolism. J. Applied Poult. Res., 11: 453-463.

Dou, Y., S. Gregersen, J. Zhao, F. Zhuang and H. Gregerseen, 2002. Morphometric and biochemical intestinal remodeling induced by fasting in rats. Dig. Dis. Sci., 47: 1158-1168.

Fuller, R., 1989. A Review. Probiotics in man and animals. J. Applied Bacteriol., 66: 365-378.

Garland, C.D., A. Lee and M.R. Dickson, 1982. Segmented filamentous bacteria in the rodent small intestine: their colonization of growing animals and possible role in host resistance to *Salmonella*. Microb. Ecol., 8: 181-190.

Hang, Y.D., E.E. Woodams and L.F. Hang, 2003. Utilization of corn silage juice by Klyuveromyces marxianus. Bioresource Technol., 86: 305-307.

Heczko, U., A. Abe and B.B. Finlay, 2000. *In vivo* interactions of rabbit enteropathogenic Escherichia coli O103 with its host: an electron microscopic and histopathologic study. Microbes. Infect., 2: 5-16.

Huang, M.K., Y.J. Choi, R. Houde, J.W. Lee, B. Lee and X. Zhao, 2004. Effect of lactobacilli and an acidophilic fungus on the production performance and immune responses in broiler chickens. Poult. Sci., 83: 788-795.

Imondi, A.R. and F.H. Bird, 1966. The turnover of intestinal epithelium in the chick. Poult. Sci., 45: 142-147.

Klaasen, H.L.B.M., P.J. Van der Heijden, W. Stok, F.J.G. Poelma, J.P. Koopman, M.E. Van der Brink, M.H. Bakker, W.M.C. Eling and A.C. Beynen, 1993. A pathogenic, intestinal, segmented, filamentous bacteria stimulate the mucosal immune system of mice. Infect. Immun., 61: 303-306.

Langhout, D.J., J.B. Schutte, P. Van Leeuwen, J. Wiebenga and S. Tamminga, 1999. Effect of dietary high and low methyllated citrus pectin on the activity of the ileal microflora and morphology of the small intestinal wall of broiler chickens. Br. Poult. Sci., 40: 340-347.

McDonald, P., R.A. Edwards, J.F.D. Greenhalgh and C.A. Morgan, 2002. Animal Nutrition. 6th Edn. Longman Group, Oxford.

Mekbungwan, A., K. Yamauchi and N. Thongwittaya, 2003. Histological alterations of intestinal villi in growing pigs fed soybean and pigeon pea seed meals. Can. J. Anim. Sci., 83: 755-760.

Moorthy, M., S. Ravi, M. Ravikumar, K. Viswanathan and S.C. Edwin, 2009. Ginger, pepper and curry leaf powder as feed additives in broiler diet. Int. J. Poult. Sci., 8: 779-782.

Panda, A.K., S.V.R. Rama, M.V.L.N. Raju and S.R. Sharma, 2006. Dietary supplementation of Lactobacillus sporogenes on performance and serum biochemico-lipid profile of broiler chickens. J. Poult. Sci., 43: 235-240.

Potten, C.S., 1998. Stem cells in the gastrointestinal epithelium: numbers, characteristics and death. Philos. Trans. R. Soc. Lond. B, Biol. Sci., 353: 821-830.

Shukla, Y. and M. Singh, 2007. Cancer preventive properties of ginger: A brief review. Food Chem. Toxicol., 45: 683-690.

Stoilova, I., A. Krastanov, A. Stoyanova, P. Denev and S. Gargova, 2007. Antioxidant activity of a ginger extract (*Zingiber officinale*). Food Chem., 102: 764-770.

Surh, Y.J., 1999. Molecular mechanisms of chemopreventive effects of selected dietary and medicinal phenolic substances. Mutat. Res., 428: 305-327.

Tarachai, P. and K. Yamauchi, 2000. Effects of luminal nutrient absorption, intraluminal physical stimulation and intravenous perenteral alimentation on the recovery responses of duodenal villus morphology following feed withdrawal in chickens. Poult. Sci., 79: 1578-1585.

Umano, K., Y. Hagi, K. Nakahara, A. Shoji and T. Shibamoto, 2000. Volatile chemicals identified in extracts from leaves of Japanese mugwort (*Artemisia princes* pamp.). J. Agric. Food Chem., 48: 3463-3469.

Wice, B.M. and J.I. Gordon, 1992. A strategy for isolation of cDNAs encoding proteins affecting human intestinal epithelial cell growth and differentiation: characterization of a novel gut-specific N-myristoylated annexin. J. Cell Biol., 116: 405-422.

White, B., 2007. Ginger: an overview. Am. Fam. Physician, 75: 1689-1691.

Yamauchi, K., Y. Isshiki, Z.-X. Zhou and Y. Nagahiro, 1990. Scanning and transmission electron microscopic observations of bacteria adhering to ileal epithelial cells in growing broiler and White Leghorn chickens. Br. Poult. Sci., 31: 129-137.

Yamauchi, K., T. Buwjoom, K. Koge and T. Ebashi, 2006. Histological intestinal recovery in chickens refed dietary sugar cane extract. Poult. Sci., 85: 645-651.

Yamahara, J., Q.R. Huang, Y. Li, L. Xu and H. Fujimura, 1990. Gastrointestinal motility enhancing effect of ginger and its active constituents. Chem. Pharm. Bull. (Tokyo), 38: 430-431.

Yasar, S. and J.M. Forbes, 1999. Performance and gastro-intestinal response of broiler chicks fed on cereal gain-based foods soaked in water. Br. Poult. Sci., 40: 65-76.