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Growth performance and intestinal histomorphology in egg-type growing roosters fed recycled food waste containing effective microorganisms

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Abstract: To evaluate the effects of a recycled food waste (eco-feed) containing effective microorganisms derived from Japanese mugwort silage juice (EJP) on growth performance and intestinal histomorphology, 48 male chickens of 14 days old were divided into 0%, 20%, 40%, and 60% EJP diet groups. At 20 weeks of age, body weight gain was similar in the 20% EJP group but lower in the 40% and 60% EJP groups ($P < 0.05$) compared to that in the control group. The relative total intestinal length and weight tended to be long and heavy, respectively, in the 20% and 40% EJP groups, but short and light, respectively, in the 60% EJP group. Although numerous villi were found in the jejunum and ileum of all EJP groups, the villus height and size observed in these groups were smaller. Cell area and cell mitosis number in the duodenum and jejunum of all EJP groups were lower than those of the control group ($P < 0.01$). Epithelial cells on the villus apical surface of the 20% EJP group showed morphology similar to that of cells on the villus apical surface of the control group in all of the intestinal segments. The growth performance data suggested that EJP could be incorporated at up to a level of 20% in chicken diets. The intestinal histological results suggested that long-term feeding of an EJP diet at up to the 40% level may result in a long and heavy intestine by increasing the numbers of miniature intestinal villi.

Key words: Eco-feed, effective microorganisms, chicken, intestinal villi, epithelial cell

Introduction

Feed represents the greatest cost in poultry production, accounting for up to 70% of the total broiler production cost (1). Due to price hikes in feed, several strategies should be developed to reduce these feeding costs. At the same time, there has been a considerable increase in environmental pollution from food waste, food remnants, or expired food from restaurants, supermarkets, and convenience stores. These wastes have been incinerated and dumped into landfills. This process results in the emission of global warming gases and toxic substances such as dioxins and heavy metals. Eco-feed (a portmanteau of "eco" from ecology and economy and "feed"), feed produced from recycled food waste, has been produced in many countries to solve both problems. Feeding eco-feed to livestock is not a recent innovation, as it has been practiced throughout the world and is often concentrated around metropolitan centers. The use of conventional eco-feed as animal feed has been reported in pigs (2,3) and chickens (4). However, no studies have reported the effects of eco-feed containing effective microorganisms on animal growth performance and intestinal histomorphology.

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The beneficial effects of dietary probiotics in poultry production are well known. Recently, the useful effects of probiotics on growth performance (5), nutrient digestibility (6), modulation of intestinal microflora (7), and pathogen inhibition (8) have been reported in chickens. In a previous study, we reported increased body weight in broilers after being fed dried fermented ginger made from ginger and Japanese mugwort silage juice (JMSJ) (9). The latter, JMSJ, included effective microorganisms such as lactic acid bacteria, yeast, phototrophic bacteria, hyperthermal bacteria, *Actinomycetes*, *Aspergillus*, and *Bacillus subtilis* at 10^{6-7} cfu/mL or more. A new eco-feed infused with effective microorganisms by immersing eco-feed into JMSJ (EJP) was developed in Japan as an animal feed supplement to promote the quality of conventional eco-feed used as a chicken feed supplement.

Because intestinal histomorphology is markedly affected by dietary feed components and the histomorphological changes in the intestine correlate with intestinal function (10,11), feeding EJP may influence certain intestinal morphological features. Long-term feeding of dietary EJP is a suitable model to determine the intestinal histological alterations related to the diets. The objective of this experiment was to obtain fundamental data on the effects of dietary EJP on growth performance and intestinal histomorphology in chickens.

Materials and methods

EJP preparation

The EJP used in this study (Table 1) was produced by Public Co., Ltd. (Kagawa, Japan) as follows. Food remnants such as bread (13.1%), noodles (13.8%), fruits (17.3%), vegetables (7.2%), rice (5.6%), meat (7.5%), fish (6.5%), and other mixed foods (29%) were collected from restaurants, convenience stores, and supermarkets in the Kagawa prefecture. Impurities such as plastic, metal, paper, and animal bones were removed manually from the collected food remnants. The eco-feed sources (300 kg/day) were separately placed into 3 miniature containers, which were filled with JMSJ (1000 L water, 20 kg molasses, 10 kg salt, 1 L milk; pH 4 or less; Craft Co., Ltd, Kagawa, Japan) until all of the eco-feed sources were submerged. After 2 days, the JMSJ was drained from the containers, and the eco-feed sources, now saturated with JMSJ, were placed into a fermentation drying machine (Jetter type C, Wing Farm Co., Ltd, Hiroshima, Japan), in which the contents revolved at 70 °C for 24 h. Subsequently, they were ground through a sieve, removed, and cooled.

Table 1. Chemical composition of the eco-feed containing effective microorganisms derived from Japanese mugwort silage juice*.

Items	Percentage
Dry matter	82.06
Crude protein	15.78
Gross energy (kcal/kg)	4009
Crude fiber	2.01
Crude fat	7.96
Crude ash	3.94
Calcium	0.12
Phosphorus	0.23

*Values are expressed on an as-fed basis. Each value is an average of analyses done on 2 samples.

Birds, housing, and feeding

All of the experiments were conducted according to the guidelines for the care and use of laboratory animals established by Kagawa University. Egg-type male Sanuki Cochin chickens were obtained from the animal science farm of Kagawa prefecture at 1 day of age. The chicks were housed in electrically heated brooder cages under continuous light for 2 weeks and provided with ad libitum access to water and a conventional starter mash diet. At 14 days of age, the birds were weighed individually; the heaviest and lightest were discarded, and the remaining birds were randomly divided into 4 groups with 4 replicates of 3 birds, based on similar body weight. The birds were transferred to individual cages in an environmentally controlled room under a photoperiod of 14 h of light. The commercial basal diets (Table 2) were replaced with 0%, 20%, 40%, or 60% EJP for a total of 4 treatments. The birds were fed a starter diet until 28 days of age, followed by a grower diet from 29 to 70 days of age and a finisher diet from 71 to 140 days of

Table 2. Composition of the basal diets (%).

Ingredients	Starter, 1 to 28 days	Grower, 29 to 70 days	Finisher, 71 to 140 days
Maize	59.0	53.0	63.0
Milo	2.0	5.0	2.0
Soybean meal	27.0	20.0	15.0
Rapeseed meal	-	3.0	4.0
Gluten meal	2.0	13.0	6.0
Fish meal	7.0	3.0	1.0
Rice bran	-	-	6.0
Animal fat	1.1	0.8	0.5
Calcium carbonate	1.0	1.2	1.5
Dicalcium phosphate	0.3	0.4	0.4
Salt	0.2	0.2	0.2
Vitamin and trace mineral mixture*	0.4	0.4	0.4
Calculated chemical component			
Crude protein	21.0	18.0	15.0
Metabolizable energy (kcal/kg)	3000	2850	2800
Crude fat	3.0	3.0	2.5
Crude fiber	6.0	6.0	8.0
Crude ash	8.0	9.0	9.0
Calcium	0.7	0.7	0.55
Available phosphorus	0.5	0.5	0.45

*Vitamin and trace mineral mixture including (per kg of diet): 7020 IU of vitamin A, 1400 IU of vitamin D_3, 12.5 mg of vitamin E, 1.5 mg of vitamin K_3, 2.6 mg of vitamin B_1, 2.7 mg of vitamin B_2, 6 mg of vitamin B_6, 9 μg of vitamin B_{12}, 0.2 mg of biotin, 0.5 mg of folic acid, 15 mg of pantothenic acid, 22 mg of nicotinic acid, 1000 mg of choline, 1.05 mg of iodine, 50 mg of manganese, 160 mg of iron, 70 mg of zinc, and 8 mg of copper.

age. The birds were provided with feed and water ad libitum. Feed intake and body weight were measured weekly.

Protocol for gross anatomical evaluation of the carcass and visceral organs

At the end of the experiment, the chickens were killed by decapitation under light anesthesia with diethyl ether. Subsequently, the carcass, heart, liver, proventriculus, gizzard, pancreas, and each of the intestinal regions were collected from 4 birds per group to observe gross anatomical changes. Organ weights and intestinal lengths are presented relative to body weight (g/100 g body weight and cm/100 g body weight, respectively).

Tissue sampling

Another 4 birds per group were used for morphometrical and histological observations of the villi in each intestinal segment. From the viewpoint of animal welfare, 3 animals are known to be enough for histological observations. In this study, we used 4 birds for the histological study. After decapitation, the entire small intestine was excised and fixed in a mixture of 3% glutaraldehyde and 4% paraformaldehyde fixative solution (pH 7.4). The same fixative solution was injected into the intestinal lumen of the middle part of each intestinal segment. The intestinal segment from the gizzard to pancreatic and bile duct was regarded as duodenum, the jejunum

was that from the duct to Meckel's diverticulum, and the ileum was that from the diverticulum to the ileo-cecal-colonic junction. The tissue samples were taken from the middle part of each intestinal segment. A 2-cm length of each intestinal segment was excised for scanning electron microscopic observations and placed in the same fixative solution described above. Another 2-cm length of each intestinal segment was cut for light microscopic observations and kept in Bouin's solution. Light microscopic samples were taken immediately proximal to the block collected for scanning electron microscopy.

Light microscopy for villus height, villus area, cell area, and cell mitosis number

After dehydration in graded alcohol, each intestinal segment was embedded in Paraplast. Transverse sections were cut into 4-μm sections, and every 10th section was collected. After staining with hematoxylin and eosin, the following values were measured using an image analyzer (Nikon Labophot-2, Tokyo, Japan).

Measurement of the villus height: The highest 2 villi having the lamina propria were randomly selected per transverse section. The villus height was measured from the villus tip to the bottom. The mean villus heights from 4 birds (16 villi from 8 different sections in each segment per bird) were expressed as the mean villus height for 1 group.

Measurement of the villus area: The width of the villus was measured at the basal and apical parts and 2 villi were selected from each section. The 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. The average of these was expressed as the mean for each bird. Finally, the 4 bird means were expressed as the mean villus area for 1 group.

Measurement of the 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 measured epithelial cell layer were counted. Finally, the area of the layer was divided by the number of cell nuclei to obtain an epithelial cell area. A total of 16 samples per bird were counted in each group.

Measurement of the cell mitosis number: Mitotic cells having homogeneous, intensely stained basophilic nuclei with hematoxylin in one transverse section were counted. The total mitosis numbers were counted from 4 different sections for each bird and these 4 values were used to calculate the mean for 1 bird. Finally, these 4 means from 4 birds were expressed as the mean cell mitosis in 1 group.

Scanning electron microscopy for epithelial cells

A 2-cm tissue sample of each intestinal segment was transversely cut, slit longitudinally, opened, and washed with 0.1 M phosphate buffered saline (pH 7.4). To prevent curling, the edges of tissue sample were pinned flat to the paraffin-covered bottom of a Petri dish containing a mixture of 3% glutaraldehyde and 4% paraformaldehyde fixative solution (pH 7.4) at room temperature for 2 h. The samples were then cut into 4 × 7-mm^2 squares, washed with a 0.1 M cacodylate buffer, and postfixed with 1% osmium tetroxide for 2 h. The specimens were washed in distilled deionized water and dehydrated in ethanol of increasing concentrations (45%, 70%, 80%, 90%, and 100%). These specimens were then freeze-dried in a critical point drying apparatus (Hitachi Freeze Dryer, Hitachi Ltd., Tokyo, Japan), sputter coated with platinum (Hitachi E-1030 Ion Sputter, Hitachi Ltd.), and observed with a scanning electron microscope (Hitachi S-4300SE/N, Hitachi Ltd.). The morphological alterations of the epithelial cells on the villus tip surface were compared among the groups. To obtain objective results for the villi numbers and villi widths, they were counted on a scanning electron microscope micrograph of 1360 × 1800 μm.

Statistical analysis

All of the experimental data were analyzed with one-way ANOVA using the general linear model of SAS software (12), and the results were expressed as the mean and the pooled standard error of the mean (SEM). Separate analysis was performed, employing orthogonal polynomial contrast, to determine the effects of the graded levels of dietary EJP. $P < 0.05$ was considered significant and $P < 0.1$ was considered trend.

Results

Bird performance

The growth performance of the chickens as affected by the different levels of EJP during the starter, grower, and finisher periods are presented in Table 3. Compared with the control birds, chickens fed the 40% and 60% EJP diets during the 2-week starter period consumed less feed ($P < 0.01$). However, during the grower and finisher periods, feed intakes were not significantly different among the groups.

At the end of the starter period, the body weight gain of the birds fed the EJP diets was similar to that of the control birds. During the grower period, the chickens fed the 40% and 60% EJP diets gained less body weight than the control birds ($P < 0.01$), but the chickens fed the 20% EJP diet showed no difference. The overall body weight gain slightly increased in the birds fed the 20% EJP diet, whereas it decreased linearly in the birds fed the 40% and 60% EJP diets ($P < 0.05$).

All of the birds fed the 4 diets had similar feed conversion ratios during the starter period. However, during the grower period, the feed conversion ratios of the birds fed the control and 20% EJP diets were better ($P < 0.01$) than those of the birds fed the 40% and 60% EJP diets. Over the entire experimental period, the feed conversion ratios of the birds fed the EJP diets were similar to those of the control birds.

Gross anatomical observations of the carcass and visceral organs

The relative intestinal lengths, as well as the relative weight of the carcass and visceral organs, were normal in all of the groups (Table 4). However, the chickens fed the 20% and 40% EJP diets tended to have greater total intestinal length and weight, whereas the length and weight were lower in the chickens fed the 60% EJP diet compared with those of the control birds ($P < 0.1$, quadratic effects).

Villus height, villus area, cell area, and cell mitosis number

Villus height and villus area in all of the intestinal segments were not significantly different among the groups (Table 5). Compared with the control group, cell area and cell mitosis of the duodenum and jejunum decreased linearly in all of the EJP groups ($P < 0.01$).

Table 3. Influence of 0%, 20%, 40%, and 60% dietary eco-feed containing effective microorganisms derived from Japanese mugwort silage juice (EJP) on chicken performance during the starter, grower, and finisher periods.

Items	Dietary EJP (%)				SEM	P-value	
	0	20	40	60		Linear	Quadratic
Feed intake (kg/head)							
2-4 weeks	0.478	0.483	0.472	0.467	0.002	<0.01	0.06
5-10 weeks	3.561	3.608	3.528	3.510	0.021	0.24	0.45
11-20 weeks	13.470	13.545	13.252	13.004	0.095	0.06	0.37
Body weight gain (kg/head)							
2-4 weeks	0.201	0.204	0.198	0.195	0.002	0.22	0.18
5-10 weeks	1.289	1.303	1.204	1.166	0.017	<0.01	0.20
11-20 weeks	2.928	2.949	2.816	2.764	0.031	0.02	0.49
Feed conversion ratio							
2-4 weeks	2.378	2.367	2.383	2.441	0.033	0.65	0.38
5-10 weeks	2.762	2.768	2.930	3.010	0.034	<0.01	0.43
11-20 weeks	4.600	4.593	4.705	4.704	0.051	0.39	0.95

Data are the means of 4 replicates.

Table 4. Influence of 0%, 20%, 40%, and 60% dietary eco-feed containing effective microorganisms derived from Japanese mugwort silage juice (EJP) on carcass and visceral organs in chickens.

Items	Dietary EJP (%)				SEM	P-value	
	0	20	40	60		Linear	Quadratic
Length (cm/100 g BW)							
Duodenum	1.07	1.04	1.07	0.96	0.02	0.23	0.47
Jejunum	2.05	2.15	2.00	1.94	0.04	0.28	0.43
Ileum	1.92	2.08	2.11	1.88	0.03	0.75	0.06
Total intestinal length	5.04	5.27	5.18	4.78	0.08	0.26	0.08
Weight (g/100 g BW)							
Carcass	75.24	74.83	73.81	74.66	0.33	0.92	0.09
Duodenum	0.31	0.34	0.39	0.30	0.01	0.81	0.03
Jejunum	0.50	0.56	0.58	0.49	0.02	0.07	0.93
Ileum	0.55	0.56	0.55	0.44	0.02	0.14	0.25
Total intestinal weight	1.36	1.46	1.52	1.23	0.04	0.35	0.06
Heart	0.71	0.81	0.74	0.83	0.02	0.28	0.94
Liver	1.21	1.27	1.25	1.31	0.02	0.31	0.95
Proventriculus	0.30	0.28	0.30	0.25	0.01	0.20	0.33
Gizzard	1.62	1.68	1.54	1.53	0.06	0.49	0.76
Pancreas	0.15	0.16	0.16	0.13	0.01	0.37	0.23

Data are the means of 4 replicates.

Table 5. Villus height, villus area, cell area, and cell mitosis number of the duodenum, jejunum, and ileum in chickens fed 0%, 20%, 40%, and 60% dietary eco-feed containing effective microorganisms derived from Japanese mugwort silage juice (EJP) diets.

Items	Dietary EJP (%)				SEM	P-value	
	0	20	40	60		Linear	Quadratic
Villus height (mm)							
Duodenum	1.60	1.63	1.57	1.57	0.02	0.60	0.83
Jejunum	1.31	1.26	1.21	1.22	0.02	0.18	0.22
Ileum	0.80	0.81	0.80	0.79	0.01	0.71	0.45
Villus area (mm^2)							
Duodenum	0.259	0.263	0.254	0.248	0.005	0.45	0.66
Jejunum	0.189	0.178	0.175	0.171	0.012	0.06	0.54
Ileum	0.110	0.109	0.108	0.107	0.004	0.57	0.92
Cell area (μm^2)							
Duodenum	271.65	267.45	259.69	254.99	1.83	<0.01	0.22
Jejunum	238.75	236.64	235.00	226.60	1.70	<0.01	0.27
Ileum	195.35	195.87	193.19	191.70	0.78	0.07	0.50
Cell mitosis (number)							
Duodenum	796.43	785.25	750.56	735.50	9.33	<0.01	0.90
Jejunum	691.37	671.25	630.12	602.75	9.25	<0.01	0.75
Ileum	494.50	487.56	465.38	467.06	4.80	0.06	0.59

Data are the means of 4 replicates.

Villi observations using scanning electron microscopy

The number and size of villi in the duodenum of the chickens fed the 20%, 40%, and 60% EJP diets were similar to those of the control birds. However, the villi numbers were numerically greater ($P > 0.05$), whereas the villi widths in the jejunum and ileum of the EJP groups were smaller ($P < 0.1$) than those of the control group (Table 6).

Alterations in the epithelial cells on the villus apical surface

Protuberant cells (stars) were clearly observed on the duodenal villus apical surface in the control group (Figure 1A). These cells were also found in the 20% EJP group (Figure 1B). However, protuberant cells (arrows) were only faintly present in the 40% and 60% EJP groups (Figures 1C and 1D).

The jejunal villus apical surfaces in the control and 20% EJP groups (Figures 2A and 2B) were covered with protuberating cells (stars), thus showing a rough surface, whereas those of the 40% and 60% EJP groups (Figures 2C and 2D) were covered with flat cells (arrows), thus showing a smooth surface.

All of the groups showed a similar morphology of the villus tips in the ileum (pictures not shown).

Discussion

A wide fluctuation in ingredient costs has led to a greater emphasis on poultry production economics in recent years. If food wastes could be used in animal diets, this would solve the cost problem by helping farmers reduce feeding costs while reducing environmental problems arising from food waste disposal. Westendorf et al. (2) reported that most of the nutrients in recycled food waste have a digestibility similar to or greater than that of a basal diet composed of corn and soybean meal. Therefore, the digestibility of recycled food waste should not be a limiting factor for use in animal feed. We found decreased feed intake during the starter period in the 40% and 60% EJP groups, which may have been related to the bulk density of the diets. The bulk of the 40% and 60% EJP diets may have been too large for small chicks, as the feed intake was not significantly different among the groups as the chicks aged. The decreased body weight gain in the 40% and 60% EJP groups during the grower and finisher periods may have resulted from the low crude protein (CP) content in the diets fed during the starter and grower periods. These observations suggest that EJP can be used as a feed ingredient in chicken diets up to a level of approximately 20%.

The beneficial effects of probiotics on improved growth performance have previously been reported

Table 6. Villi numbers and villi widths on a scanning electron microscopic micrograph of 1360 × 1800 μm in the duodenum, jejunum, and ileum in chickens receiving 0%, 20%, 40%, and 60% dietary eco-feed containing effective microorganisms derived from Japanese mugwort silage juice (EJP).

Items	Dietary EJP (%)				SEM	P-value	
	0	20	40	60		Linear	Quadratic
Villi numbers							
Duodenum	24.50	24.50	26.25	23.00	0.73	0.68	0.29
Jejunum	34.50	36.50	39.75	37.50	1.39	0.33	0.50
Ileum	50.25	65.25	61.25	58.50	2.78	0.40	0.12
Villi widths (mm)							
Duodenum	0.71	0.75	0.68	0.70	0.02	0.63	0.76
Jejunum	0.77	0.64	0.63	0.61	0.02	0.06	0.28
Ileum	0.52	0.39	0.42	0.41	0.02	0.05	0.06

Data are the means of 4 replicates.

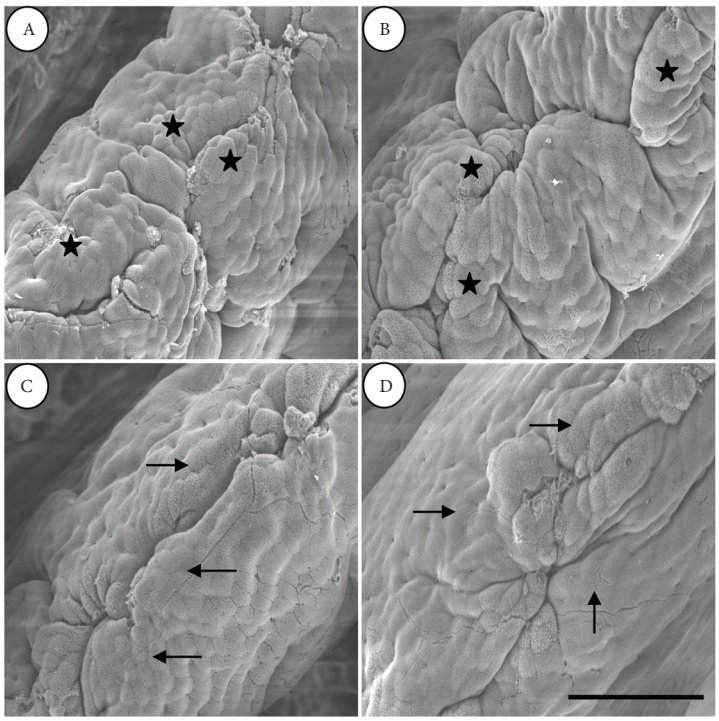

Figure 1. Epithelial cells on the duodenal villus apical surface of the chickens fed 0% (A: stars, protuberant cells), 20% (B: stars, protuberant cells), 40% (C: arrows, faint protuberant cells), and 60% (D: arrows, faint protuberant cells) dietary eco-feed containing effective microorganisms derived from Japanese mugwort silage juice diets. Scale bar = 50 μm.

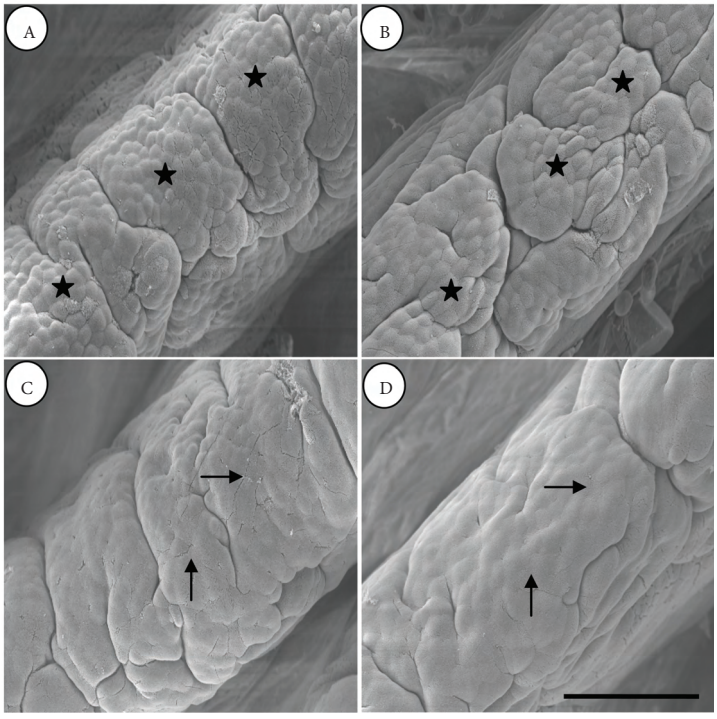

Figure 2. Epithelial cells on the jejunal villus apical surface of the chickens fed 0% (A: stars, protuberating cells), 20% (B: stars, protuberating cells), 40% (C: arrows, flat cell area), and 60% (D: arrows, flat cell area) dietary eco-feed containing effective microorganisms derived from Japanese mugwort silage juice diets. Scale bar = 50 μm.

(8,10,13,14). Those studies reported that probiotics constitute a functional nutritional approach by helping to maintain a healthy gastrointestinal environment and improving intestinal function. Thus, it is likely that the improved intestinal function in the chickens fed the 20% EJP diet in the current study was mainly due to the effects of effective microorganisms on improving the gastrointestinal tract environment. The ingested nutrients are absorbed into the blood or lymphatic system after being transported from the intestinal lumen through the mucosal epithelial cell barrier (15). The epithelial cells react morphologically to ingested diets more quickly than the intestinal villi (16,17), because the histological reaction to ingested diets differs between the micro (epithelium) and macro (villi) levels. At the micro level, the present epithelial cells of the duodenum and jejunum were protuberant in the control and 20% EJP groups but were flat in the 40% and 60% EJP groups. These protuberant cells have been observed among the hypertrophied cells in chickens with increased body weight (9), suggesting that the cells in the 20% EJP group may have an absorptive function similar to that of the cells in the control group. However, in the ileum, the epithelial cells were not morphologically different among the groups. These morphological differences among the intestinal parts would be induced by the nutrients in the diets fed and the intestinal absorptive function of each segment. The CP contents of the 0%, 20%, 40%, and 60% EJP diets during the finisher period were 15.0%, 15.2%, 15.3%, and 15.5%, respectively. Their metabolizable energy levels were 2.800, 2.812, 2.944, and 3.126 kcal/kg, respectively (18). Although these values increased with increasing dietary EJP levels, the total nutrients such as minerals and vitamins in the EJP groups were assumed to be lower than those in the control group. Moreover, it might be related to the protein quality (amino acid content) of dietary EJP. It has been reported that the duodenum and initial segment of the jejunum absorb lipids, whereas the ileum does not play a significant role in lipid absorption (19), indicating that nutrients are absorbed in the upper segment of the intestine (20) and that nitrogen is absorbed beyond the ileum (21). Thus, the ileum appears to have relatively less intestinal absorptive function (22), and under normal circumstances, the major absorption of nutrients occurs in the duodenum and jejunum (23). Based on the present results and those of related studies, we conclude that the absorptive function of cells in the duodenum and jejunum was sufficient in the 20% EJP group, and that the ileum did not play a significant role in absorption. At the macro level, it is well known that intestinal villi respond very quickly to changes in nutrient status and feed intake. In this study, the villus width in the duodenum was not different, even in the 60% EJP group. However, it tended to be narrower in the jejunum and ileum of all of the EJP groups, corresponding with the light microscopic parameters of the villus area and villus height (except in the ileum). In contrast, the villi numbers increased numerically in the jejunum and ileum of all of the EJP groups. Such increased numbers of villi would induce longer and heavier intestines in the 20% and 40% EJP groups than in the control group. Thus, the intestine may optimally function but obtain insufficient nutrients because of the chickens being raised on a long-term hyponutrient diet during the starter and grower periods, resulting in compensatory intestinal enlargement. Previous transmission electron microscopic observations of the epithelial cells in fasted chickens indicate that intracellular digestion through liposomal autophagy appeared to obtain the deficient nutrients (16). These results suggest that the intestine adapts to a homeostatic mechanism corresponding to the environmental change in the intestinal lumen. However, the total intestinal length and weight decreased in the 60% EJP group. Intestinal function may diminish when chickens are raised on a long-term hyponutrient diet during the starter and grower periods of 60% EJP. Reduced weight of the gastrointestinal organs has also been reported in chickens fed with restricted nutrients early in life (24).

In conclusion, the result that feed intake and body weight gain in the 20% EJP group were not different compared to those in the control group suggests that EJP could be incorporated as a feed ingredient at a level of up to 20% in chicken diets. Histological results indicated that there were greater villi numbers but shorter villus heights and sizes in the longer jejunum and ileum of the 20% and 40% EJP groups, suggesting that feeding of the EJP diet at up to a level of 40% may result in a long and heavy intestine due to increasing numbers of miniature intestinal villi.

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