A comparison of intestinal integrity, digestive function, and egg quality in laying hens with different ages

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ABSTRACT Intestinal integrity, digestive enzyme activity, nutrient utilization, and egg quality of laying hens at different ages were evaluated and compared in this study. A total of 192 Hy-line Brown laying hens at 195-d-old (D195 group), 340-d-old (D340 group), and 525-d-old (D525 group) were allocated into one of 3 groups in accordance with their ages. Each group had 8 replicates of 8 birds each, and all birds were fed a maize-soybean meal basal diet for a 2-wk experiment. Compared with the D195 group, intestinal villus height and ratio of villus height to crypt depth, as well as serum D-lactate content increased in the D525 group ($P < 0.05$). The sucrase and maltase activities in the jejunal mucosa, amylase activity in the pancreas, and trypsin activity in the jejunal chyme of 525-d-old hens were lower than their 195-d-old counterparts ($P < 0.05$). In addition, there was a decline of trypsin and lipase activities in the ileal chyme of hens from D525 group in comparison with D195 or D340 group ($P < 0.05$). Apparent retention of dry matter and crude protein of birds in D340 and D525 group decreased when compared with the D195 group ($P < 0.05$). Moreover, birds in the D525 group exhibited a lower level of ether extract retention, and higher contents of several excreted amino acids than those in the D195 group ($P < 0.05$). Compared with the D195 group, eggs harvested from D525 group exhibited lower albumen height, eggshell strength and thickness, and a higher egg weight ($P < 0.05$). In conclusion, increased intestinal permeability (higher serum D-lactate content), compromised digestive function (lower digestive enzyme activities and apparent nutrient retention, and higher concentrations of excreted amino acids), and poor egg quality (lower albumen height, eggshell strength, and thickness) were observed with increasing age in the laying hens.

Key words: age, digestive function, egg quality, intestinal integrity, laying hen

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INTRODUCTION

It is generally acknowledged that aging has adverse consequences on egg production of laying hens after the peak laying stage (Tumova and Gous, 2012). In addition to being regulated by the reproductive and endocrine system (Johnson et al., 1986; Braw-Tal et al., 2004), animal productive performance is also closely correlated with their gastrointestinal functionality and health, such as structure and function of intestinal barrier, digestive function, and commensal microbiota (Biagi et al., 2013; Celi et al., 2017). In an experiment performed on the mice at 3 different ages (3-mo-old, 12-mo-old, and 24-mo-old), it has been identified that thinner mucosal muscularis, shortened and scattered villus, and reduced villus density can be observed from the ileal histological structure in the aging mice, which may be related to the mucosal atrophy, increased bacteria counts, and decreased expression of epithelial tight junction proteins (Ren et al., 2014). Similarly, another report has also demonstrated that 24-mo-old rats exhibit the distorted and destructed state of jejunum, as well as the disrupted mucosal antioxidant defense system (Hassan et al., 2017), suggesting the negative effects of aging on gut function. In humans, a literature has summarized that aging is accompanied by the continuous damage to morphology and function of gastrointestinal tract, which would impair the nutrient absorption and protection against ingested pathogens (Soenen et al., 2016). Besides, digestive organs in animals such as the stomach, pancreas, liver, and intestine tend to show functional decline imperceptibly as the age increases, and thus cause the compromised ingestion and absorption of nutrients (Drozdowski and Thomson, 2006). The

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digestive enzymes, mainly scattered in the pancreas and intestinal lumen, are of great importance to catalyze the splitting of proteins, carbohydrates, lipids, and other nutrients for more digestible molecules. Researchers have found that aging can decrease the ability to degrade unfold proteins in the pancreas, and subsequently lead to the reduction of lipase activity in the male mice (Yamamoto et al., 2014). In accordance with a published literature, the activities of dipeptidyl peptidase, lactase, sucrase, maltase, and alkaline phosphatase in the intestinal mucosa decline significantly with increasing age of mice, implying that aging may reduce the absorption of peptides and disaccharides, and impair intestinal integrity in rodent animals (Detel et al., 2008). Moreover, a previous study in humans has indicated that aging is characterized by the moderate slowdown in gastric emptying, which negatively affects the digestive physiology and health of individuals (Soenen et al., 2015). In an investigation of canines, older individuals tend to exhibit lower apparent total tract digestibility of protein and fat when compared with the adult animals, probably due to their degraded intestinal digestive function (Maria et al., 2017).

As for laying hens, the egg quality parameters (e.g., egg weight, Haugh unit, eggshell strength, and shell color) are observed to be associated with increasing age (Hy-Line International; http://www.hyline.com). Liu et al. (2018) has demonstrated that aging can downregulate the mRNA expression of genes responsible for fat acid synthesis, which would then decrease the formation of hepatic yolk precursors, and thus lead to the quality reduction of yolk and albumen. In addition, eggshell quality is also evidenced to be related with advanced age in laying hens (Jiang et al., 2014). Besides, older hens tend to be more vulnerable to immune challenge (Jing et al., 2014), and exhibit degenerated liver antioxidant capacity when compared with their young counterparts (Liu et al., 2018), probably indicating the compromised ability against stress with increasing age. However, little is known about the effects of age on intestinal barrier and digestive physiology of laying hens with different ages. In accordance with the findings aforementioned, we then hypothesized that the deterioration of intestinal barrier and digestive function of laying hens may become more obvious with increasing age after peak laying stage, which may account for the poor productive performance and egg quality of laying hens during the late laying period. This study was therefore conducted to compare intestinal integrity, digestive enzyme activity, nutrient utilization, and egg quality of laying hens at 195-d, 340-d and 525-d old, and the findings of which would provide a reference for improving nutritional regulation and performance in laying hen production.

MATERIALS AND METHODS

Animals, Diet, Experimental Design, and Housing

The experimental design and procedures were approved by the Animal Care and Use Committee of Nanjing Agricultural University. A total of 192 Hy-line Brown laying hens with 3 different ages from the same parental generation were obtained from Tiancheng Group (Jiangsu, P.R. China), where these hens were fed a same basal diet and reared in 3 different houses based on their ages. The birds were 195-d-old (D195 group), 340-d-old (D340 group), and 525-d-old (D525 group), and their egg production rate was 95.8, 90.3, and 81.5%, respectively. The hens were, thereafter, allocated into one of 3 groups as per their ages, with each group being composed of 8 replicates of 8 birds with the same age. All birds were fed a maize-soybean meal basal diet, and the ingredient composition and nutrient level of basal diet are presented in Table 1. The laying hens from each replicate (8 birds/replicate) were reared in 2 adjacent stainless-steel cages (60 × 50 × 40 cm, 4 birds per cage) with plastic floors, received 16 h of light and 8 h of darkness, and subjected to the conventional feeding program in a chicken house with free access to the mash diets and water for a 2-wk experiment. The house temperature and humidity were automatically controlled at 18°C–25°C and 40–60%, respectively.

Sample Collection

The collection trays were installed under the cages for excreta collection. In the 2-wk feeding trial, excreta without feathers and feed was collected every day, weighed to approximate 1.0 g using an analytical scale, stored at −20°C, and finally pooled for the analysis of apparent nutrient retention and excreted amino acid concentration. At the end of feeding period, one healthy laying hen from each replicate was randomly selected

Table 1. Ingredients and nutrient composition of the basal diet (g/kg, as fed basis).

Items	Contents
Ingredients	
Maize	618
Soybean meal	249
Soybean oil	8
Limestone	85
Premix[1]	40
Calculated nutrient levels	
Apparent metabolizable energy (MJ/kg)	11.15
Crude protein	164
Ether extract	35
Calcium	38
Total phosphorus	6.2
Available phosphorus	3.7
Lysine	8.2
Methionine	3.6
Total sulfur amino acids	6.4
Analyzed nutrient levels	
Crude protein	159
Ether extract	31

[1]Premix provided per kilogram of diet: transretinyl acetate, 10,000 IU; cholecalciferol, 3,000 IU; all-rac-α-tocopherol, 30 IU; menadione, 1 mg; thiamin, 1 mg; riboflavin, 6 mg; nicotinamide, 40 mg; choline chloride, 350 mg; calcium pantothenate, 10 mg; pyridoxine HCl, 3 mg; biotin, 0.1 mg; folic acid, 0.3 mg; cobalamine, 0.01 mg; Cu (copper sulfate), 8 mg; Fe (ferrous sulfate), 80 mg; Mn (manganese sulfate), 100 mg; I (calcium iodate), 1 mg; Se (sodium selenite), 0.3 mg; calcium, 6.25 g; phosphorus, 3 g; methionine, 1 g.

(one bird per replicate, and 24 birds in total), and blood samples were obtained via the wing vein puncture. The serum was separated from blood sample after a centrifugation at 4,000 g for 15 min at 4°C and stored at −20°C before the subsequent determination. Then birds were euthanized by exsanguination and necropsied, and then the pancreas, duodenum, jejunum, and ileum were separated immediately. Approximately 1-cm proximal sections of the duodenum, jejunum, and ileum were excised and flushed with ice-cold phosphate-buffered saline (pH = 7.4), and placed in the 10% formalin solution for intestinal tissue fixation and histological measurement. Chyme in the jejunal and ileal lumen and the pancreas tissues were collected and stored at −80°C for further analysis. The duodenal, jejunal, and ileal mucosae were scratched carefully by a sterile glass microscope slide, frozen in liquid nitrogen, and stored at −80°C until subsequent determination. Twenty-four eggs were randomly collected from each group (3 eggs per replicate) at the end of the feeding experiment for the measurement of egg quality.

Histological Measurement

Fixed intestinal segments in the formalin solution were dehydrated, hyalinized, and embedded in paraffin. Thereafter, the intestinal sections were cut at 5 μm for slices, deparaffinized, rehydrated, and stained with hematoxylin and eosin. Villus height and crypt depth of 15 intact villi-crypt in each sample were measured using a Nikon ECLIPSE 80i light microscope equipped with an ocular micrometer (Nikon Corporation, Tokyo, Japan).

Serum Diamine Oxidase Activity and D-lactate (D-LA) Content

The diamine oxidase (DAO) activity in the serum was assayed in accordance with the method described by Hosoda et al. (1989). In brief, 0.5 mL of serum sample was mixed with 3.0 mL of phosphate buffer (0.2 mol/L, pH = 7.4), 0.1 mL of horseradish peroxidase solution (0.04 g/L), 0.1 mL of o-dianisidine methanol solution (5.0 g/L odianisidine in methanol), and 0.1 mL of substrate solution (1.75 g/L cadaverine dihydrochloride). This mixture was then incubated for 30 min at 37°C, and DAO activity was measured as the absorbance at 436 nm. The reagents used for DAO activity assay were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, P. R. China). Based on the enzyme-linked immunosorbent assay technique (Engvall and Perlmann, 1972), the D-LA concentration in the serum was determined using commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, P. R. China) as per the manufacturer's protocols.

Intestinal Mucosal Disaccharidase Activity

The intestinal mucosa samples were homogenized (1:9, wt/vol) with ice-cold sodium chloride solution (154 mmol/L) using an Ultra-Turrax homogenizer (Tekmar Co., Cincinnati, OH). The tissue homogenates were then centrifuged at 4,000 g for 15 min at 4°C for the supernatant, which was used for the subsequent disaccharidase activity assay. Intestinal mucosal disaccharidase activity was determined using a Tris-glucose oxidase reagent in accordance with the method described by Dahlqvist (1964). One unit of disaccharidase was defined as the activity splitting 1 μmol disaccharide (sucrose or maltose) per minute. All disaccharidase activities were normalized against total protein for inner comparison, and the concentration of which was measured by the method described by Bradford (1976).

Digestive Enzymes Activities in the Pancreas and Intestinal Chyme

The pancreas and intestinal chyme samples were preliminarily processed for the supernatant as described above. Briefly, amylase activity was measured in accordance with the iodometric method of Somogyi (1960), and one unit of amylase activity was defined the amount of enzyme required for the hydrolyzation of 10 mg starch in 30 min. Lipase activity was determined using the turbidimetric method of Verduin et al. (1973), and the amount of enzyme hydrolyzing 1.0 μmol olive oil per minute was exactly the one unit of lipase. Before the evaluation of pancreatic trypsin activity, the enterokinase was added into the homogenate for the activation of zymogens in accordance with the procedure recommended by Glazer and Steer (1977). Trypsin activity was determined by using N-benzoyl-l-arginine ethyl ester as a substrate for reacting with trypsin (Schwert and Takenaka, 1955). One trypsin unit was expressed as the amount of enzyme increasing the absorbance of the product by 0.003 per minute at 253 nm. All enzyme activities were calibrated against the total protein concentration as described above.

Apparent Nutrient Retention

The collected feed and excreta samples were dried for 48 h at 65°C and then placed in the atmospheric environment for 24 h to reach the equilibrium. After that, feed and excreta were ground and particles of 0.45 mm were screened for the standard chemical analysis according to AOAC (2000). The dry matter was analyzed by drying the sample to a constant weight at 105°C for 4 h (method 934.01). The ash content was calculated after the sample was combusted at 550°C for 8 h (method 942.05), and the organic matter was expressed as the weight loss after ashing. The crude protein was calculated to be 6.25 times the nitrogen content, which was measured after the catalytic reaction with sulfuric acid (method 988.05). The ether extract was determined using a Soxhlet extractor (method 920.39). The apparent nutrient retention was calculated using acid-insoluble ash as the indicator (Vogtmann et al., 1975).

Excreted Amino Acid Analysis

Amino acid analysis referred to the method of Yin et al. (2009). Approximately 0.1 g dried sample was reacted with 6 mol/L hydrochloric acid at 110°C for 24 h. Then the liquid sample was distilled by rotary evaporation at 65°C for removing water and hydrochloric acid, and the concentrated samples were diluted to the appropriate concentration with 0.02 mol/L hydrochloric acid. After being filtered through the semipermeable membrane, the sample was determined by automatic amino acid analyzer (Hitachi L-8080; Hitachi, Tokyo, Japan).

Egg Quality

Measurement of egg quality indices referred to the method described by Su et al. (2018). Eggshell strength, defined as breaking strength from the blunt end to the sharp end, was measured by the compression tester (Model-II; Robotmation, Japan). The egg weight, albumen height, Haugh unit, and yolk color were analyzed by the egg multitester (EMT-5200; Robotmation, Japan). Then the detached eggshell was used for determination of eggshell thickness, which was calculated as the mean value of measurements at 3 locations on the egg (air cell, equator, and sharp end) using a spiral micrometer.

Statistical Analysis

Statistical data were analyzed by one-way analysis of variance using SPSS statistical software (ver.19.0 for Windows, SPSS Inc., Chicago, IL), with Tukey's multiple range test for post hoc comparison. The means and their pooled standard errors were presented in the tables and difference was considered to be significant when P value was less than 0.05, otherwise it is not significant (P value was equal or greater than 0.05).

RESULTS

Intestinal Mucosal Morphology

Compared with the D195 group, villus height and ratio between villus height and crypt depth in the duodenum, jejunum, and ileum were increased in the D525 group (Table 2, $P < 0.05$), and all statistical data of intestinal mucosal morphology of 340-d-old birds were intermediate among 3 groups ($P > 0.05$).

Intestinal Permeability Biomarkers

The effects of age on the serum biomarkers of intestinal permeability were presented in Table 2. The birds in the D525 group exhibited a higher D-LA concentration when compared with the D195 group ($P < 0.05$), and the value of this parameter in the 340 group was intermediate among 3 groups ($P > 0.05$). However, the serum DAO activity was similar among treatments ($P > 0.05$).

Digestive Enzyme Activities

Compared with birds in the D195 group, 525-d-old laying hens exhibited lower sucrase and maltase activities in the jejunal mucosa (Table 3, $P < 0.05$). The disaccharidase activities in the duodenal and ileal mucosa also reduced numerically when the age of birds increased, although their difference did not reach a significant level ($P > 0.05$).

Pancreatic amylase activity of birds in the D525 group decreased in comparison with the D195 group ($P < 0.05$). The 525-d-old birds exhibited a lower trypsin activity in the jejunal chyme than their 195-d-old counterparts ($P < 0.05$). Similarly, there was a decrease of trypsin and lipase activity in the ileal chyme of 525-d-old laying hens when compared with those in D195 or D340 group ($P < 0.05$).

Table 2. Age-related changes in intestinal mucosal morphology and serum diamine oxidase activity and D-lactate concentration of laying hens.

	Treatments[1]				
Items	D195	D340	D525	SEM	*P*-value
Villus height (μm)					
Duodenum	1,150.66^{b}	1,357.06^{a,b}	1,484.25^{a}	45.155	0.004
Jejunum	1,051.15^{b}	1,183.82^{a,b}	1,267.52^{a}	28.079	0.002
Ileum	853.43^{b}	886.38^{a,b}	988.04^{a}	21.668	0.022
Crypt depth (μm)					
Duodenum	267.67	266.28	253.65	7.070	0.691
Jejunum	265.43	247.06	226.67	9.144	0.231
Ileum	215.96	198.36	190.58	7.336	0.367
Villus height: crypt depth					
Duodenum	4.33^{b}	5.19^{a,b}	5.99^{a}	0.245	0.014
Jejunum	4.16^{b}	4.95^{a,b}	5.65^{a}	0.235	0.027
Ileum	4.02^{b}	4.52^{a,b}	5.40^{a}	0.203	0.012
Serum					
Diamine oxidase (U/L)	10.17	10.04	11.26	0.541	0.620
D-lactate (mmol/L)	0.70^{a}	0.82^{a,b}	1.00^{b}	0.051	0.044

^{a,b}Means within a row with different superscripts are different at $P < 0.05$.
[1]D195, 195-d-old laying hens; D340, 340-d-old laying hens; D525, 525-d-old laying hens.

Table 3. Age-related changes in disaccharidase activity in the intestinal mucosa and digestive enzyme activity in the pancreas and intestinal chyme of laying hens.

Items	Treatments[1]			SEM	*P*-value
	D195	D340	D525		
Sucrase (U/mg protein)					
Duodenum	27.53	24.27	21.91	2.133	0.579
Jejunum	66.97^{a}	61.94^{a,b}	52.42^{b}	2.264	0.021
Ileum	41.17	38.25	33.07	2.273	0.353
Maltase (U/mg protein)					
Duodenum	385.66	353.99	324.93	18.134	0.410
Jejunum	731.49^{a}	610.12^{a,b}	487.56^{b}	35.926	0.014
Ileum	509.67	460.31	446.22	24.086	0.549
Amylase (U/mg protein)					
Pancreas	250.29^{a}	205.50^{a,b}	197.37^{b}	8.657	0.019
Jejunal chyme	34.34	33.30	30.77	1.928	0.756
Ileal chyme	25.61	23.42	19.39	1.243	0.114
Trypsin (U/mg protein)					
Pancreas	397.18	388.11	376.54	13.782	0.841
Jejunal chyme	406.07^{a}	357.48^{a,b}	319.56^{b}	12.166	0.008
Ileal chyme	396.15^{a}	375.98^{a}	240.77^{b}	24.520	0.012
Lipase (U/g protein)					
Pancreas	510.87	499.08	492.17	14.030	0.870
Jejunal chyme	109.23	101.61	98.93	4.821	0.683
Ileal chyme	91.70^{a}	82.91^{a}	51.18^{b}	5.243	0.001

^{a,b}Means within a row with different superscripts are different at $P < 0.05$.
[1]D195, 195-d-old laying hens; D340, 340-d-old laying hens; D525, 525-d-old laying hens.

Apparent Nutrient Retention

Nutrient retention of dry matter and crude protein of birds in D340 and D525 group decreased when compared with the D195 group (Table 4, $P < 0.05$), but no differences were observed between D340 and D525 groups ($P > 0.05$). In addition, 195-d-old birds exhibited a higher retention of ether extract than that in the D525 group ($P < 0.05$), with the level of 340-d-old birds being intermediate among 3 groups ($P > 0.05$).

Excreted Amino Acids Contents

As indicated in Table 4, excreted threonine, methionine, and leucine concentrations of 525-d-old birds were higher than those in the 195 group ($P < 0.05$). There is a growing numerical trend in the contents of other measured amino acids as the age increased, although their differences did not reach significant levels ($P > 0.05$).

Egg Quality

Albumen height, eggshell strength, and thickness of eggs from laying hens in the D525 group all decreased when compared with those in the D195 group (Table 5, $P < 0.05$). In addition, the 525-d-old birds produced the heaviest eggs in the 3 groups ($P < 0.05$). However, there was no difference in Haugh unit and yolk color among 3 groups ($P > 0.05$).

DISCUSSION

The small intestine is an organ of vital importance to maintain digestive, endocrine, metabolic, and immune function for domestic animals. The intestinal physical barrier formed by intestinal epithelial cells and junctional complexes plays a crucial role in the absorption of nutrients and protection of gut from harmful substances such as endotoxins and pathogens (Suzuki, 2013). Changes of intestinal morphology (e.g., villus height and crypt depth), the important indicators and

Table 4. Age-related changes in apparent nutrient retention and excreted amino acid concentrations of laying hens.

Items	Treatments[1]			SEM	*P*-value
	D195	D340	D525		
Apparent nutrient retention (%)					
Crude protein	58.19^{a}	45.66^{b}	43.13^{b}	2.221	0.006
Dry matter	62.94^{a}	56.65^{b}	56.97^{b}	0.810	<0.001
Ether extract	59.84^{a}	56.33^{a,b}	42.87^{b}	2.987	0.042
Organic matter	72.25	66.22	68.20	1.069	0.056
Essential amino acids (mg/g, dry matter basis)					
Arginine	2.45	2.27	2.64	0.093	0.295
Histidine	1.02	1.10	1.10	0.030	0.482
Isoleucine	2.68	2.81	3.19	0.124	0.228
Leucine	7.30^{b}	7.93^{a,b}	8.54^{a}	0.195	0.023
Lysine	3.77	3.81	4.30	0.146	0.266
Methionine	0.73^{b}	0.89^{a,b}	0.99^{a}	0.041	0.022
Phenylalanine	2.13	2.44	2.70	0.110	0.103
Threonine	4.04^{b}	4.17^{a,b}	4.55^{a}	0.085	0.032
Tyrosine	3.63	3.81	3.99	0.105	0.391
Valine	4.28	4.05	4.99	0.221	0.203
Nonessential amino acids (mg/g, dry matter basis)					
Alanine	6.04	5.94	7.08	0.248	0.101
Aspartic acid	7.86	8.02	8.76	0.248	0.300
Glutamic acid	10.35	10.67	11.88	0.310	0.096
Glycine	5.82	6.43	6.79	0.217	0.190
Serine	4.18	5.16	4.88	0.180	0.067

^{a,b}Means within a row with different superscripts are different at $P < 0.05$.
[1]D195, 195-d-old laying hens; D340, 340-d-old laying hens; D525, 525-d-old laying hens.

Table 5. Age-related changes in egg quality of laying hens.

Items	Treatments[1]			SEM	*P*-value
	D195	D340	D525		
Egg weight (g)	60.65[b]	62.16[b]	66.73[a]	0.830	0.003
Albumen height (mm)	6.85[a]	6.09[a,b]	5.66[b]	0.193	0.031
Yolk color	5.83	5.63	5.38	0.229	0.741
Haugh unit	79.36	77.64	73.58	1.484	0.273
Eggshell strength (kg/cm^2)	4.14[a]	3.85[a,b]	3.62[b]	0.084	0.033
Eggshell thickness (μm)	348.38[a]	317.46[a,b]	308.46[b]	6.268	0.017

[a,b]Means within a row with different superscripts are different at $P < 0.05$.
[1]D195, 195-d-old laying hens; D340, 340-d-old laying hens; D525, 525-d-old laying hens.

reflections of the digestive and absorptive capacity, can be used to partially assess intestinal functionality (Van der Hulst et al., 1998). In the present study, the effect of aging on the intestinal morphology was revealed by the simultaneous increase of villus height and the ratio of villus height to crypt depth. This hyperproliferative state of crypts and villi, possibly caused by the high expression of intestinal proliferating cell nuclear antigen, is a natural phenomenon happening in the aging intestine with no specific morphological function, but it may reduce the absorptive capacity of the elderly individuals (Corazza et al., 1998). A similar result was also observed by Moorefield et al. (2017), who have found that old mice (18- to 22-mo-old) would exhibit a marked increase in intestinal villus height, and they consider it to be a compensatory reaction of aging intestine against the reduced efficiency of nutrient absorption. Circulating DAO activity and D-LA content have been proved to be convincing biomarkers of intestinal damage and permeability (Ma et al., 2018), and a higher value of serum D-LA in 525-d-old laying hens was observed in the present study, indicating the compromised state of the aging intestine of laying hens. In addition, disaccharidases are defined as membrane glycoproteins located on or within the microvilli, which are essential for the hydrolyzation and absorption of disaccharides. Several researches have confirmed that reduced duodenal disaccharidase activity is related with morphological abnormality (Langman and Rowland, 1990), and villous atrophy and malnutrition (Nichols et al., 2000). The present study showed that 525-day-old laying hens exhibited lower sucrase and maltase activities in the jejunal mucosa when compared with the D195 group, implying that aging may negatively affect intestinal mucosal barrier function. Consistently, Jang et al. (2000) has examined the effects of aging on the specific enzyme activities in the small intestinal brush border membrane of rats with different ages (2.5-wk-old, 5-wk-old, 5-mo-old, and 23-mo-old), and they demonstrate that 23-mo-old rats would exhibit lower mucosal lactase and sucrase activities than their 5-wk-old counterparts. Combining these results of intestinal morphology and permeability and mucosal disaccharidase activities, we could infer that age-related decline of intestinal barrier function might occur in the aging birds. This alteration may negatively regulate the transport of ions and some hydrophilic and lipophilic compounds, and impact the normal function of intestinal transporters that help nutrients such as sugars and amino acids cross the epithelial cells (Vancamelbeke and Vermeire, 2017). As a consequence, declined nutrient digestibility and intestinal functionality would occur in the aging individuals (Van der Hulst et al., 1998).

As for poultry, digestive enzymes, such as amylase, protease, and lipase, are mainly secreted from the pancreas, and distributed and activated in the duodenal and jejunal sections of small intestine for nutrient digestion and absorption (Rideau et al., 1983). In this trial, as the age increased, the trypsin and lipase activities in the intestinal chyme and amylase in the pancreas all decreased, accompanied by the corresponding decline in the apparent retention of dry matter, crude protein, and ether extract. Because most digestive enzymes are sensitive to the acidity-alkalinity degree of the substrate, the less acidic environment in the intestinal lumen of senescent animals may be responsible for the decreased digestive enzyme activities in this study (Ikuma et al., 1996). Subsequently, these changes of digestive enzymes can lead to the lower efficiency of catalysis and reduced decomposition of ingested feed, resulting in the decreased digestibility of nutrients in the laying hens. In addition, in regard to lipid digestion, the declined secretion of bile acid in the enteric physiology environment of aging hens can also contribute to the decreased lipid solubilization and absorption, thereby adversely affecting the lipid absorption (Holt and Balint, 1993). With respect to the digestion of proteins, Gilani and Sepehr (2003) have demonstrated that protein digestibility of different protein products in old rats (20-mo-old) is considerably lower than that in young rats (5-wk-old), indicating the negative impact of aging on protein ingestion and absorption, and a similar result has been observed in the turkeys (Palander et al., 2005). Meanwhile, large amounts of amino acids, which are split from proteins through the catalysis of digestive enzymes, are of great importance to the maintenance, growth, and production of birds, while most of them are inefficiently used and excreted through the cloaca. As the lower trypsin activity and crude protein retention have been observed in the aging laying hens in the current study, the excreted amino acids profile might be affected. Correspondingly, the contents of excreted threonine, methionine, and leucine of 525-d-old birds were higher than

those of 195-d-old birds, suggesting the possible reduced apparent retention of amino acids in the older birds. In an in vitro study investigating the nutrient uptake efficiency of the small intestine isolated from young (7.6-mo-old) and aged (24.8-mo-old) mice, Ferraris and Vinnakota (1993) have found that transport and absorptive capacity of amino acids declines with increasing age, which may result in the decreased deposition of these nutrients in the organisms and elevated accumulation of unutilized nutrients in the excrement. One possible explanation for this phenomenon is probably imputable to the age-induced altered expression of amino acid transporters in the intestinal mucosa of old laying hens (Dato et al., 2019).

Based on the observations of declined intestinal barrier function and compromised digestive physiology of aging laying hens, absorbed nutrients that involved in the formation of eggs might change, and it can be one of the major reasons for the poor egg quality of old layers (Zhang et al., 2020). In agreement with a previous study (Silversides and Scott, 2001), egg weight was observed to increase with age in the present experiment, and the higher weight ratio of yolk to white might contribute to this effect of aging on egg weight (Ahn et al., 1997). In addition, probably due to the reduced albumen weight with increasing age (Izat et al., 1986), a lower value of albumen height was observed in the aging birds from this study, which might in part account for the lower freshness of egg laid by the old birds. As for eggshell quality, the present study showed that eggs harvested from 525-d-old hens exhibited lower eggshell strength and thickness, implying the frangibility of eggshells from older laying hens. As a consequence of age-related decline of intestinal function, calcium uptake may be reduced in the intestine of laying hens (Albatshan et al., 1994), and it would possibly inhibit the ion transmission and endometrial formation, resulting in the degradation of structural properties of eggshells, such as lower breaking strength and greater variability (Rodriguez-Navarro et al., 2002).

In conclusion, age could decline the intestinal barrier function and lead to the lower digestive enzyme activities and nutrient retention, and negatively affect the egg quality of laying hens after the peak laying stage. These findings indicate that nutritional intervention needs to be more concerned in the late-phase laying hens.

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DISCLOSURES

The authors declare no conflict of interest with respect to the authorship and publication of this article.

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