Effect of different concentrations of neohesperidin on performance, egg quality, serum biochemistry and intestinal morphology in laying hens

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ABSTRACT In recent years, neohesperidin (**NH**), as a class of natural flavonoids, has received more and more attention in nutrition research. However, the research on the application of neohesperidin in the laying hens is rarely reported. This study was conducted to determine the effects that different concentrations of dietary NH have on the production performance, egg quality, serum biochemistry and intestinal morphology of laying hens. A total of 240 Lohmann commercial laying hens (66 wk old) were divided into 4 groups, with each group's diet containing a different concentration of NH (0, 100 mg/kg, 200 mg/kg, and 400 mg/kg). Significant associations were found between NH consumption and both higher egg production ($P = 0.050$) and lower FCR ($P = 0.028$) after 12 wk NH feed. At 12 wk, eggs produced by hens consuming a 200 mg/kg NH diet had significantly thicker eggshells ($P = 0.059$) than those produced by hens consuming a 400 mg/kg diet. Dietary NH addition improved albumen height and Haugh unit after 15 d of storage ($P < 0.01$). However, no significant associations between NH consumption and these factors were identified after 12 wk. Dietary NH addition had no significant effects apparent of gel properties at 12 wk. In addition, NH can effectively reduce the content of total cholesterol (**TC**) ($P = 0.042$) and Groups treated with 100 mg/kg NH supplementation showed significantly increased T-AOC concentrations compared to control ($P = 0.013$) in serum. Hens fed an NH-supplemented diet exhibited a longer villus height and a higher villus/crypt ratio in the ileum ($P < 0.01$) as compared to controls, as well as lower crypt depth in the duodenum, jejunum and ileum. These results indicate that, as compared to a control diet, an NH-supplemented diet results in higher egg production and quality, as well as improvement in egg gel properties, serum biochemistry and intestinal morphology.

Key words: neohesperidin, egg quality, gel properties, serum biochemistry, intestinal morphology

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INTRODUCTION

Neohesperidin (**NH**) is a hydrogenated flavonoid derivative which can be extracted from natural citrus, orange and other plants, and has wide applications as a low-calorie artificial sweetener in various foods and beverages. In recent years, NH has received increased attention in medical, food and nutritional research. NH has antioxidant, antimicrobial and anti-inflammatory properties in animals (Yeomans et al., 2007; Marti et al., 2008). Some research indicates that NH can lead to a significant reduction in the plasma cholesterol and neutral lipid content of rats. Additionally, NH can significantly increase plasma high-density lipoprotein (**HDL**) content, thereby promoting cholesterol metabolism and effectively treating or preventing diseases associated with elevated lipid levels (Bok et al., 2004). Takii et al. (1997) indicated that NH can reduce blood sugar levels of mice. Kristian et al. (2014) found that adding NH to the swine diet, led to a significant increase in levels of Lactobacillus at the entrance of the cecum, which can, in turn, affect the commensal intestinal flora, regulate immunity, reduce the risk of intestinal diseases, and improve production performance. The above studies show that NH has an effect on lipid level and may have an effect on improving fat accumulation and production performance in animals. There are a series of problems in the body health of laying hens in the late period of laying, for example, the absorption and utilization of nutrients in the intestine is significantly reduced, and the ability of the fallopian tube to secrete mucus is decreased. The accumulation of reactive oxygen species in cells leads to organ dysfunction, reduces production performance, large amount of liver fat deposition, prone to fatty liver syndrome, and the egg quality gradually

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decreases, the eggshell becomes thinner, the Haugh unit of the protein decreases, and the shelf-life is shortened. However, whether NH will affect the performance, serum and intestinal morphology of laying hens, and what is the optimal amount of NH added to the diet remains a question worthy of study. Therefore, the objective of this study was to investigate the effect of different concentrations of NH supplementation on production performance, egg quality, gel properties, serum biochemistry and intestinal morphology in laying hens.

MATERIALS AND METHODS

Birds, Experimental Design, and Management

The experimental protocol used in the study was approved by the Animal Care and Use Committee of Sichuan Agricultural University. A total of 240 Lohmann commercial laying hens from 66 to 77 wk of age were randomly allocated to 1 of 4 treatment groups. Each treatment had 6 replicates with 10 birds per replicate. Birds were housed in pairs in stainless steel cages. The room environment was controlled at 20 ± 4°C and the daily lighting schedule was standardized (16 h light, 8 h dark). The dietary treatments were basal diets (Table 1) supplemented with 0 (control), 100 mg/kg, 200 mg/kg, and 400 mg/kg NH, respectively. Neohesperidin was provided by Shandong Baixing Biotechnology Co., Ltd.

Table 1. Composition and nutrient level of basal diet (as-fed basis).

Ingredients, %	Amount
Corn	58.11
Soybean meal,43% CP	24.50
Wheat bran	3.50
Soybean oil	2.00
DL-Methionine	0.11
Calcium carbonate	9.60
Calcium hydrophosphate	1.15
Sodium chloride	0.40
Choline chloride, 50%	0.10
Mineral premix[1]	0.50
Vitamin premix[2]	0.03
Total	100.00
Calculated nutrient levels	
Metabolisable energy, kcal/kg	2660.00
Crude protein,%	15.70
Calcium,%	4.00
Total phosphorus,%	0.54
Available phosphorus,%	0.32
Lysine,%	0.72
Methionine,%	0.33
Tryptophan,%	0.16
Threonine,%	0.53

[1]Provided per kg of diet: 60 mg Mn (as $MnSO_4$); 80 mg Zn (as $ZnSO_4$); 8 mg Cu (as $CuSO_4 \cdot 5H_2O$); 60 mg Fe (as $FeSO_4 \cdot 7H_2O$); 0. 35 mg I(as KI), and 0. 30 mg Se (as $Na2SeO_3 \cdot 5H_2O$).

[2]Provided per kilogram of diet: vitamin A, 8, 000 IU; vitamin D_3, 1, 600 IU; vitamin K, 0. 5 mg; vitamin B1, 0. 8 mg; vitamin B2, 2. 5 mg; vitamin B6, 3. 0 mg; vitamin B_{12}, 0. 004 mg; folic acid, 0. 25 mg; niacin, 20 mg; Capantothenate acid, 2. 2 mg and biotin, 0. 10mg; vitamin E, according to the amount of each treatment (0, 20 or 200 mg).

Sample Collection and Measurements

Egg number and total egg weight of each replicate were recorded every day. Feed conversion ratio (**FCR**) was calculated as grams of total feed intake per broiler/ grams of total egg mass per hen. A total of 24 eggs were randomly collected from each treatment group in the 12 wk, respectively, and used to determine egg quality and gel properties. At the end of 12 wk, 24 eggs were randomly collected from each treatment and stored for 15 d to determine the storage egg quality. At the end of 12 wk, 24 broilers (6 replicates of each treatment) were weighted individually, and blood samples were collected from the wing vein via sterile syringe. Samples were then centrifuged at 3,000 × g for 15 min, and serum was stored at −20°C until analysis. After blood collection, hens were anesthetized and sacrificed by cervical dislocation, after which their abdomens were dissected. The duodenum, jejunum and ileum were fixed in 4% neutral formaldehyde.

Egg Quality

Eggshell strength was evaluated using an eggshell force gauge (model II, Robotmation Co., Ltd., Tokyo, Japan). Egg yolk color and Haugh unit were evaluated using an egg multi tester (EMT-7300, Robotmation Co., Ltd., Tokyom Japan). Egg shell color was measured in triplicate by the CIE L∗a∗b∗ system using a Minolta Chroma Meter CR-400 (Konica Minolta Sensing, Inc., Tokyo, Japan). Egg quality was measured according to Sauveur (1988).

Gel properties were measured according to Houska et al. (2004) with minor modifications. Twenty mL egg white was measured into a 25 mL beaker, sealed with plastic wrap, and heated in an 80°C water bath for 45 min. Then, the solution was quickly taken out, incubated at 4°C for 24 h, and allowed to equilibrate to room temperature before the measurement. After the preparation was completed, the texture analyzer was used in gel analysis mode under the following conditions: premeasuring speed 2.0 mm/s, test speed 2.0 mm/s, postmeasuring speed 5.0 mm/s, distance 10 mm, interval time 5S, data acquisition rate 200 pps, and probe P/0.5 cylinder type in order to measure the hardness, adhesiveness, springiness, cohesiveness, gumminess, chewiness, resilience and peak force of the gel.

Intestinal Morphology

Intestinal segments were flushed clean and fixed in 10% NoToX (a nonformalin tissue fixative). Histological slides were prepared from 3 cross-sections (5 μm thick) of each intestinal sample, which were processed in low-melt paraffin and stained with hematoxylin-eosin (AR; GL01-GMPC; Tokyo, Japan). Villus height (**V**) and crypt depth (**C**) were measured using the ImagePro Plus as described in detail by Touchette et al. (2002), and the V:C ratio was calculated.

Table 2. Effect of dietary feed different concentration of NH on production performance of laying hens.[1]

Item		NH level (mg/kg)				SEM	*P*-Value
		Control	100	200	400		
1−8 wk	Egg production, %	77.17	79.70	82.20	82.14	2.12	0.310
	Egg weight, g	65.16	65.62	64.73	64.81	0.06	0.703
	FCR	2.30	2.22	2.16	2.19	0.54	0.348
	ADFI,g	114.64	114.71	114.25	115.97	1.33	0.815
1−12 wk	Egg production, %	75.52[b]	78.73[ab]	82.39[a]	82.99[a]	2.00	0.050
	Egg weight, g	65.14	65.61	64.77	64.90	0.54	0.705
	FCR	2.33[a]	2.21[ab]	2.11[b]	2.13[b]	0.05	0.028
	ADFI,g	111.81	111.87	110.61	112.99	1.17	0.570

[a,b]Means with different superscripts within a column differ significantly ($P < 0.05$).
[1]Each mean represents 6 replicates, with 4 eggs/replicate.
Abbreviations: 100, 100 mg/kg neohesperidin; 200, 200 mg/kg neohesperidin; 400, 400 mg/kg neohesperidin; ADFI, average daily feed intake; FCR, feed conversion ratio; NH, neohesperidin.

Serum Biochemistry and Antioxidant Capacity

The serum concentrations of total protein, globulin, albumin, aspartate aminotransferase (**AST**), alanine aminotransferase (**ALT**), total cholesterol (**TC**), triglyceride, high-density lipoprotein (**HDL**), and low-density lipoprotein (**LDL**) were measured using an automatic biochemical analyzer (Cobas 8000, Roche-Diagnostics, Switzerland). The very-low-density-lipoprotein cholesterol (**VLDL**) concentration was determined using an ELISA kit (Ground work Biotechnology Diagnosticate Ltd.) with a Multiskan Spectrum spectrophotometer (Thermo Scientific, Franklin, MA, USA). The antioxidant status superoxide dismutase (**SOD**), total antioxidant capacity (**T-AOC**) and malondialdehyde (**MDA**) content in serum was measured by means of commercial kits (SOD, A001-1; T-AOC, A015; MDA, A003-1; Nanjing Jiancheng Biotechnology, Nanjing, China).

Statistical Analysis

Statistical analysis was performed using SAS 9.2 (version 9.2, SAS Institute Inc., Cary, NC). One-way analysis of variance (ANOVA) was performed for significance analysis among groups. For significant effects, means were compared by Duncan's multiple comparison test to determine specific differences between means. Statistical significance was assigned at $P < 0.05$.

RESULTS

Production Performance

Dietary supplementation with NH increased egg production and decreased FCR between 1 and 8 wk as compared with the control group ($P > 0.05$, Table 2), and egg production significantly increased and FCR significantly decreased ($P < 0.05$) at 12 wk. Supplementation with NH for 1 to 12 wk did not significantly affect either egg weight or ADFI.

Egg Quality

Overall feeding chicken with an NH-supplemented diet tended to significantly influenced ($P < 0.1$, Table 3) on egg albumen height and Haugh unit in the stored for 15 d, but had no impact in the 12-wk samples. NH consumption increased yolk color after 15 d of storage. Meanwhile, NH has a tendency to increase eggshell strength, but had no statistical significance. Supplementation with NH had no effect on yolk weight, lightness, redness or yellowness.

In the current experiment, the results of our measurements shown in Table 4, the dietary supplementation of 400 mg/kg NH had a higher cohesiveness compared with the control group, though no effect to other factors was detected at 12 wk.

Intestinal Morphology

In the duodenum of animals fed with 100 mg/kg NH supplementation, a higher villus height was observed as compared to control group, with this height also significantly increased ($P = 0.0308$, Table 5) when compared to that found in the 200 and 400 mg/kg NH treatment groups. Animals fed 400 mg/kg NH tended to have a lower crypt depth than the control group. As for the jejunum, the supplementation of NH in diet decreased crypt depth, but not to a significant degree ($P = 0.0705$). The 100 mg/kg NH dosage was found to result in increased villus height, higher villus/crypt (**V/C**) ratio and decreased ileum crypt depth ($P < 0.01$).

Serum Biochemistry and Antioxidant Capacity

The NH-supplemented treatment groups tended to have lower ALT ($P = 0.06$, Table 6) and VLDL ($P = 0.1$) concentrations when compared to the control group. Groups treated with 100 mg/kg or 200 mg/kg NH supplementation showed significantly decreased TC concentrations compared to control ($P = 0.042$). No differences in the concentration of total protein, globulin, albumin, aspartate aminotransferase, HDL, triglyceride

Table 3. Effect of dietary feed different concentration of NH on egg quality of laying hens.[1]

Item		NH level (mg/kg) Control	100	200	400	SEM	*P*-Value
12 wk	Eggshell strength, kg/cm^3	4.15	3.86	4.03	4.47	0.15	0.06
	Albumen height, mm	7.05	7.33	7.58	7.78	0.33	0.447
	York color	6.39	6.66	6.7	6.4	0.14	0.293
	Haugh unit	80.11	84.33	85.34	86.54	1.98	0.147
	Yolk weight, g	18.56	18.96	19.01	18.98	0.48	0.9
	L	79.89	81.55	81.69	81.72	0.66	0.175
	A	4.46^a	4.33^a	3.28^b	3.782^b	0.22	0.004
	B	18.47	16.93	17.26	17.99	0.53	0.192
stored 15 d	Eggshell strength, kg/cm^3	3.86	4.3	4.16	4.14	0.17	0.375
	Albumen height, mm	3.84^b	4.05^b	5.21^a	4.47^{ab}	0.27	0.012
	York color	6.94^b	7.31^a	7.36^a	7.29^a	0.09	0.014
	Haugh unit	49.42^c	55.91^{bc}	66.65^a	60.58^{ab}	2.96	0.004
	Yolk weight, g	20.84	20.47	21.61	21.16	0.61	0.603
	L	83.38	83.08	83.79	82.63	0.85	0.804
	a	3.82	3.61	3.33	3.86	0.31	0.618
	b	18.23	17.85	17.31	18.31	0.69	0.737

[a,b,c]Means with different superscripts within a column differ significantly ($P < 0.05$).
[1]Each mean represents 6 replicates, with 4 eggs/replicate.
Abbreviations: 100, 100 mg/kg neohesperidin; 200, 200 mg/kg neohesperidin; 400, 400 mg/kg neohesperidin; a, redness; b, yellowness; L, lightness; NH, neohesperidin.

Table 4. Effect of dietary feed different concentration of NH on the egg white gels properties of laying hens.[1]

Item		NH level (mg/kg) Control	100	200	400	SEM	*P*-Value
12 wk	Hardness(g)	496.37	466.32	428.61	458.27	18.91	0.123
	Adhesiveness(mJ)	−142.71	−130.92	−121.55	−124.6	12.58	0.652
	Springiness(mm)	0.95	0.95	0.93	0.92	0.02	0.403
	Cohesiveness(mJ)	0.46	0.47	0.45	0.47	0.01	0.058
	Gumminess(g)	209.65	220.29	184.99	217.59	11.9	0.175
	Chewiness(g)	200.32	210.07	171.18	200.65	12.6	0.181
	Resilience(mJ)	0.12	0.13	0.13	0.13	0.01	0.345
	Peak Force(g)	481.88	439.26	401.5	425.12	24.85	0.171

[a,b]Means with different superscripts within a column differ significantly ($P < 0.05$).
[1]Each mean represents 6 replicates, with 4 eggs/replicate.
Abbreviations: 100, 100 mg/kg neohesperidin; 200, 200 mg/kg neohesperidin; 400, 400 mg/kg neohesperidin; NH, neohesperidin.

Table 5. Effect of dietary feed different concentration of NH on Intestinal Morphology of laying hens.[1]

Intestine	Item	NH level (mg/kg) Control	100	200	400	SEM	*P*-Value
Duodenum	Villus height (μm)	1010.89^{ab}	1138.82^a	950.12^b	904.09^b	53.46	0.031
	crypt depth (μm)	162.41	161.58	153.4	136.64	7.35	0.077
	VH:CD	6.32	7.12	6.83	6.06	0.42	0.288
Jejunum	Villus height (μm)	839.04	848.08	842.91	840.71	41.93	0.999
	Crypt depth (μm)	147.3	129.06	118.15	135.07	7.35	0.071
	VH:CD	5.84	6.31	6.84	6.31	0.34	0.273
Ileum	Villus height (μm)	375.49^b	662.80^a	457.06^b	416.96^b	45.06	0.001
	Crypt depth (μm)	101.77	117.98	116.56	89.76	8.19	0.076
	VH:CD	3.84^b	5.62^a	4.10^b	4.79^{ab}	0.34	0.007

[a,b]Means with different superscripts within a column differ significantly ($P < 0.05$).
[1]Each mean represents 6 replicates, with 4 eggs/replicate.
Abbreviations: 100, 100 mg/kg neohesperidin; 200, 200 mg/kg neohesperidin; 400, 400 mg/kg neohesperidin; NH, neohesperidin; VH:CD, villus height/crypt depth.

and LDL were detected between control and treatment groups. Groups treated with 100 mg/kg NH supplementation showed significantly increased T-AOC concentrations compared to control ($P = 0.013$, Table 7). Supplementation with NH f did not significantly affect either SOD or MDA.

DISCUSSION

There are only a few studies on NH in animals, For example, Cromwell et al. (2008) found that adding NH in weaned pigs diets can improve the performance of pigs. Meanwhile, NH is a flavonoid compound. There

Table 6. Effect of dietary feed different concentration of NH on serum biochemistry of laying hens.[1]

Item	NH level (mg/kg)				SEM	*P*-Value
	Control	100	200	400		
Total protein(g/L)	70.24	61.62	69.77	61.95	6.39	0.653
Globulin(g/L)	46.72	40.87	49.55	40.87	6.01	0.645
Albumin(g/L)	23.52	20.75	20.22	21.65	0.91	0.088
AST(U/L)	151.10	145.12	144.77	146.92	8.67	0.952
ALT(U/L)	2.14	1.35	1.02	1.33	0.28	0.062
TC (mmol/L)	4.22[a]	2.79[b]	3.00[b]	3.64[ab]	0.36	0.042
HDL (mmol/L)	0.52	0.46	0.33	0.51	0.09	0.423
Triglyceride (mmol/L)	12.89	10.93	12.84	13.36	1.15	0.470
LDL (mmol/L)	0.37	0.25	0.29	0.33	0.05	0.464
VLDL (mmol/L)	3.09	2.19	2.38	2.81	0.26	0.100

[a,b]Means with different superscripts within a column differ significantly ($P < 0.05$).
[1]Each mean represents 6 replicates, with 4 eggs/replicate.
Abbreviations: AST, aspartate aminotransfera; ALT, alanine aminotransferase; 100, 100 mg/kg neohesperidin; 200, 200 mg/kg neohesperidin; 400, 400 mg/kg neohesperidin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NH, neohesperidin; TC, total cholesterol; VLDL, very-low-density lipoprotein.

are relatively studies on similar flavonoid compounds. Recent studies showed that adding flavonoids to the diet can improve the performance of laying hens. Brisibe et al. (2008) reported that dried Artemisia annua leaves, which are known to contain high levels of flavonoids, increase egg production of laying hens. Propolis, also rich in flavonoids, has been shown to increase egg production of laying hens (Galal et al., 2008; Seven, 2008). Liu et al. (2014) have indicated that the addition of quercetin to the diet can significantly increase the egg production and the FCR of laying hens. Our study is in consistent with the results of this observation. Egg weight had no significant difference in this study, which is in agreement with the report by Yildiz et al. (2006) that hens supplied with dietary Jerusalem artichoke, which contains flavonoids did not affect egg weight. The results of this experiment show that the NHDC can significantly increase the egg production and reduce the FCR. We speculate that it may be related to the antioxidant properties of neohesperidin dihydrochalcone. We use the late period of laying. As the age increases, the ovarian reproductive function of the laying hens deteriorates and is prone to oxidative stress, but NHDC improves the body's antioxidant capacity. Thereby reducing the occurrence of oxidative stress, at the same time NHDC can promote the absorption of nutrients in the intestine of laying hens, thereby increasing the egg production rate. In previous studies, NHDC can significantly increase the feed intake of piglets and sows, thereby improving production performance. This is related to the physical properties of NHDC. The high sweetness of NHDC can stimulate pigs to eat, but in our experiment, although the feed intake of the NHDC diet group increased, it was not significant. We speculated that this was related to the lower taste sensitivity of poultry. According to reports by A Boushy (1987), the number of taste buds in chicken is 0.16% of that in pigs, NHDC cannot effectively stimulate layer intake through its sweet taste, so there is no significant effect on feed intake.

Previous studies have indicated that different flavonoids have specific effects on egg quality. Iskender et al. (2017) reported that hesperidin, naringin, and quercetin can all cause decreased albumen index and Haugh unit.In contrast, Liu et al. (2014) found that quercetin had no effect on albumen index. Furthermore, Goliomytis et al. (2019) reported that Haugh unit was not influenced by either naringin or hesperidin supplementation. In our study, NH-supplemented treatment groups had a significantly higher albumen height and Haugh unit as compared to controls. This result may be related to the antioxidant capacity of NH, which can effectively scavenge free radicals and achieve the purpose of slowing down the rate of oxidation (Suarez et al., 1998). This is in agreement with the work of El−Tarabany (2018), who reported that albumen height

Table 7. Effect of dietary feed different concentration of NH on serum antioxidant capacity of laying hens.[1]

Item	NH level (mg/kg)				SEM	*P*-Value
	Control	100	200	400		
T-AOC	2.88[b]	5.78[a]	4.43[ab]	3.64[b]	0.57	0.013
SOD	8.66	7.95	8.57	8.14	0.47	0.675
MDA	3.96	3.86	4.02	5.39	0.94	0.623

[a,b]Means with different superscripts within a column differ significantly ($P < 0.05$).
[1]Each mean represents 6 replicates, with 4 eggs/replicate.
Abbreviations: 100, 100 mg/kg neohesperidin; 200, 200 mg/kg neohesperidin; 400, 400 mg/kg neohesperidin; MDA, malondialdehyde; NH, neohesperidin; SOD, superoxide dismutase; T-AOC, total antioxidant capacity.

and Haugh unit in both Royal-Jelly treated groups were significantly greater than those in the control group. At the same time, NH-supplemented treatment groups also significantly higher yolk color after 15 d of storage. We think it may be because NH increases the activity of oxidase in the body and prevents lutein from being oxidized, which increases pigment deposition. Yuan et al. (2016) showed that adding antioxidants to layer feed can significantly improve the color of egg yolk. The improvement of egg yolk color by NHDC may be related to its antioxidant function, which reduces the effects of lipid peroxides and free radicals on organisms. The destruction of the membrane makes the egg yolk color increased. The different flavonoids have different effects on egg quality is perhaps unsurprising, because different classes of flavonoids have different antioxidant capacities and different breed, type of housing, age of breeders and differences in gut microbiota and environmental conditions Is also a possible factor leading to this deviation.

Gel properties are one of the important characteristics of egg albumen. Adding different media will change the gel temperature of an egg white. In industrial egg processing, metal ions and sucrose are often added. Additionally, some functional groups change the gel temperature of the egg white (Yoo et al., 1990;Hatta et al., 1986; Wang et al., 2010). At present, there is no article on the effect of adding flavonoids on the gel properties of egg white. During storage, there are a series of changes in the internal quality of eggs under different storage conditions. The most obvious phenomenon is that egg whites will lose their viscosity which called egg white dilution, egg white dilution will have a huge impact on the functional properties of eggs, our study found that the addition of neohesperidin in the diet can improve the hardness, gumminess and chewiness of the gel. This may be related to the neohesperidin into the body, which can improve the body's antioxidant capacity, and thus the egg white protein thinning has a certain alleviation effect, but the specific reasons need further research.

In this study, positive effects on intestinal morphology were observed in groups fed NH-supplemented diets. Kristian et al. (2014) have indicated that adding NH to the diet of swine can significantly increase cecal Lactobacilli, affecting the intestinal flora and promoting intestinal health. Basir et al. (2017) have reported that flavonoids can lead to both decreased jejunal crypt depth and greater V/C, with no effects detected on the ileal morphometric features of broiler chicks. This study is partially consistent with the experimental results reported here. In our study, only lower crypt depth was observed in the jejunum, while we found significant increases in villus height and V/C and decreases in ileum crypt depth. Katherine et al. (2016) have summarized the ability of flavonoids to alleviate intestinal inflammation and improve intestinal flora. In our study, the effect of NH on intestinal villus height and crypt depth was demonstrated, indicating that NH consumption can improve intestinal morphology and promote intestinal health.

In general, the idea that flavonoids can improve serum biochemistry has been reported in previous studies. ROZA (2007) indicated that people take flavonoids daily for 4 wk could significantly reduced total cholesterol, LDL and triglycerides, while HDL levels remained unchanged. However, Samani and Farrokhi (2014) reported that flavonoids had no effect on total cholesterol, triglycerides, HDL or LDL of people. Hu et al. (2014) found that NH can significantly alleviate the increase of ALT of mice caused by CCL4 injection; this is consistent with our work, in which we demonstrate that dietary NH supplementation can reduce ALT content. NH can also reduce the content of total cholesterol in the serum. We speculate that NH can increase the expression level of lipid metabolism-related genes in the liver, thereby reducing the total cholesterol content in the serum, but whether it is affected by this pathway needs further research. At the same time, although the impact on LDL and VLDL has not reached a significant level, from a trend point of view, NH also has a reducing effect on it. As for antioxidant capacity, this investigation demonstrated that serum T-AOC level was increased by NHDC supplementation, which was in accordance with the results of Seo et al. (2003) and Kim et al. (2004). NHDC is flavonoids and have structures that are rich in hydroxyl (OH) groups capable of supplying hydrogen atoms for free radicals to block the oxidation chain reaction (Van Acker et al. 2000; Miyake et al. 2003) Another antioxidant mechanism of flavonoids may result from the ability to produce chelates with metal ions such as iron and copper. Some authors proved the antioxidant ability of NHDC through the coordination test of hesperidin and iron ion, Fernandez et al (2002) indicated that by changing the oxidation state of the metal to observe the redox reaction, the flavonoids are oxidized, thereby enhancing the antioxidant capacity.

As a whole, our findings indicate that dietary supplementation with NH improves egg production rate, feed conversion ratio, albumen height and Haugh unit. It also increases villus height and V/C in the ileum, while simultaneously decreasing crypt depth in the small intestine. NH is suggested to increase hardness, gumminess, and chewiness of the gel. In addition, a decrease in ALT and TC content is linked to NH. Further research is needed to explain the mode for the observed changes caused by NH.

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DISCLOSURES

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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