Dietary resistant potato starch improves growth performance and feather development in Pekin ducks fed a low phosphorus diet

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ABSTRACT This study investigated whether dietary resistant potato starch (**RPS**) inclusion could ameliorate the negative impact of a low nonphytate phosphorus (**nPP**) diet on growth performance, feather growth, feather follicles (**FF**) development, and carcass traits by improving nutrient utilization and cecal microbiome fermentation capacity in Pekin ducks. The experiment was performed with a 2 × 2 randomized block design with 2 levels of RPS (0 or 12%) and 2 levels of nPP (low or normal, low: 0.22% at 1–14 d and 0.18% at 15–35 d of age; normal: 0.40% at 1–14 d and 0.35% at 15–35 d of age) for a total of 4 treatments, each with 8 replicate pens per treatment of 12 birds per pen. As regards growth performance and carcass traits, RPS inclusion markedly increased ($P < 0.05$) BW of 14 and 35 d, BWG and FI of 1–14 d, 15–35 d, and 1–35 d as well as abdominal fat and breast meat percentage of 35 d in ducks fed low nPP diets; moreover, RSP inclusion significantly reduced ($P < 0.05$) mortality in ducks fed low nPP diets. As regards feather growth and follicles development of 35 d, RPS inclusion significantly increased ($P < 0.05$) the fourth primary feather length, absolute feather weight, and the density of primary FF in the back skin in ducks fed low nPP diets. In regard to nutrition utilization, RPS supplementation significantly increased ($P < 0.05$) the availability of DM, CP, and energy, as well as dietary AME at 35 d of age in ducks fed low nPP diets. However, RPS supplementation had no effect ($P > 0.05$) on the concentration of cecal short-chain fatty acids and the activities of cecal phytase and cellulase in ducks fed low nPP diets. These results indicate that RPS can improve nutrient availability to ameliorate the negative effects on performance and feather development caused by a low nPP diet in Pekin ducks.

Key words: duck, feather growth, feather follicles development, non-phytate phosphorus, nutrient utilization, resistant potato starch

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INTRODUCTION

Feather is an important economic trait for duck production (Zhao et al., 2018). The application of genetic manipulation strategies to reduce broiler rearing period to market weight has been observed to cause problems with feather growth, including poor down quality, feather coverage, and feather-picking (Lopen-Coello, 2003). Similar to mammal (Feng and Gun, 2020), the feather follicles (**FF**) population shows a strong correlation with feather density, with greater FF numbers corresponding to a thicker density of feathers. Many factors such as nutritional deficiencies, toxicity, nutrient concentration, and feed restriction may affect feathering (Barbi et al., 2003). Nazem et al. (2015) reported that high dose (50 mg) of DL-Methionine significantly increased the density and diameter of chicken embryo FF compared with those obtained with 20 mg of DL-Methionine. Dietary crude protein (**CP**) (Urdaneta-Rincon and Lesson, 2004), amino acids (**AA**) (Ansari-Renani et al., 2011; Zeng et al., 2015a; Bajpai et al., 2016), mineral elements zinc (**Zn**) (Drew et al., 2007), and natrium (**Na**) (Yang and Cotsarelis, 2010) were also reported to affect the feather growth of birds to varying degrees, and the lack of CP, AA, minerals causes feather wear and breakage. Therefore, how to improve the digestion and absorption of these nutrients is very key to feather growth or FF development in ducks.

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Many studies have indicated that dietary resistant starch (**RS**) inclusion could increase the absorption of minerals such as Zn (Yonekura and Suzuki, 2005), Ca, and Fe (Morais et al., 1996) in animals. Resistant starch is defined as the portion of starch that escapes degradation and absorption in the small intestine and reaches the hindgut to be used as a fermentation substrate and to produce short-chain fatty acids (**SCFA**) that can benefit intestinal health (Englyst et al., 1992; Topping and Clifton, 2001; Birt et al., 2013). In Pekin ducks, previous studies by Qin et al. (2019, 2020) found ducks can ferment dietary resistant potato starch (**RPS**) at 12% dosage to produce SCFA by modulating cecal microbiota, which subsequently enhanced the intestinal barrier function, as well as improved the growth performance. Yin et al. (2019) also found that RS supplementation can spare AA from catabolism in the gut mucosa of broiler chickens. However, inconsistent results showed that ileal nitrogen (**N**) digestibility tended to decrease and fecal N excretion tended to increase in pigs fed 6% dietary raw or retrograded high-amylose corn starch; RPS consumption tended to reduce the apparent fecal fat digestibility in rats as well as apparent ileal and fecal fat digestibility in pigs (Schrijver et al., 1999). However, to the best of our knowledge, there is limited literature addressing the impact of dietary RS inclusion on feather growth and nutrient utilization in poultry. Therefore, we hypothesized that dietary RS inclusion can improve feather growth and FF development by increasing nutrient availability and absorption, especially N and P utilization.

Phosphorus is one of the most important minerals in poultry diets to obtain optimal productivity. In our previous study, Xu et al. (2019) found that a low nonphytate phosphorus (**nPP**) diet damaged the intestinal digestion and absorption function in Pekin duck, and low dietary nPP contents (0.22% nPP) affected the diversity of cecal microbiota and had a significant effect in modifying the bacterial community in the ceca of ducks (Dai et al., 2018), which indicated that dietary P deficiency impaired the structure and function of cecal microbiome as well as intestinal health of ducks. Therefore, in the present study, we established a model showing the damaged intestinal health induced by a P-deficient diet to investigate whether dietary RPS inclusion could improve growth performance, carcass traits, feather growth, and FF development by improving nutrient utilization and indicators of cecal microbiome fermentation capacity, such as cecal SCFA content and cecal phytase and cellulase activities, in Pekin ducks.

MATERIALS AND METHODS

The Institutional Animal Care and Use Committee of Sichuan Agricultural University approved all procedures used in the study (SAU-PN-2019-10).

Birds, Experimental Design, Diets, and Management

A total of 384 1-day-old male Cherry Valley ducklings were bought from a local commercial hatchery (Sichuan Mianying Breeding Duck Co. Ltd. Mianyang, China) and were weighed and assigned randomly to the experimental treatments on arrival. The experimental treatments consisted of 2 × 2 factorial arrangements with 2 levels of nPP (low or normal, low: 0.22% at 1–14 d and 0.18% at 15–35 d of age; normal: 0.40% at 1–14 d and 0.35% at 15–35 d of age) and 2 levels of RPS (0 or 12%, AVEBE Ltd, Holland) for a total of 4 treatments, each with 8 replicate pens of 12 ducks. The trial lasted for 35 d.

Four isocaloric and isonitrogenous diets were formulated on the basis of digestible AAs to meet the nutrient requirements of ducks suggested in NRC (1994) and Qin et al. (2019) (Table 1). All diets were offered in pelleted form; the diameter of the feed particles was 2.0 mm for ducks aged 1–14 d, and 3.5 mm for birds aged from 15–35 d. All ducks were reared in pens (1.00 m × 1.00 m × 0.55 m) in a temperature- and humidity-controlled room with free access to water and feed throughout the experimental period. The room temperature was controlled by infrared lamps, and the temperature was maintained at 30°C∼32°C for 1∼3 d and 28°C∼30°C for 4∼7 d, after which the temperature was reduced by 2°C per week until it reached room temperature. Humidity was controlled within a range of 60∼70%.

Data Collection

On day 14 and 35, and after 12 h of feed withdrawal, ducks were weighed, and feed consumption was obtained for each pen. Body weight (**BW**) and body weight gain (**BWG**), feed intake (**FI**), and feed to gain ratio (**F: G**) were calculated accordingly at the following intervals: 1–14 d, 15–35 d, and 1–35 d. Feed waste was recorded daily, and the data were used for calculations of feed consumption. Birds that died during the experiment were weighed, and the data were used for calculations of F: G.

Feather Condition Measurements

At the end of the experiment (day 35), a total of 8 birds (one duck per pen) were randomly selected for the analysis of feather condition, which included measurements of the length of the left fourth (fourth) primary feather, score of back feathers, absolute and relative feather weight. The length of the fourth primary feather counted from the left-wing tip was measured in millimeters with a ruler. The back-feather score was measured using the method described by Gustafson et al. (2007). All selected birds were then weighed and euthanized by exsanguination after stunning, after which all feathers were removed and weighed to record the absolute feather weight (g) and to calculate the

Table 1. Composition and nutrient levels of the diets (as fed-basis).

	1–14 d				15–35 d			
Ingredients	LP[1]	LP + RPS	NP	NP + RPS	LP	LP + RPS	NP	NP + RPS
Ingredients, %								
Corn	54.13	37.61	54.13	37.61	50.79	34.28	50.79	34.28
Soybean oil	1.62	3.17	1.62	3.17	2.44	3.98	2.44	3.98
Raw potato starch[2]	-	12.00	-	12.00	-	12.00	-	12.00
Rice bran	8.40	8.40	8.40	8.40	20.60	20.60	20.60	20.60
Rapeseed meal	3.20	3.20	3.20	3.20	6.00	6.00	6.00	6.00
Soybean meal	28.86	31.87	28.86	31.87	16.84	19.85	16.84	19.85
L-Lysine-HCL	0.13	0.07	0.13	0.07	0.07	0.02	0.07	0.02
DL-Methionine	0.16	0.17	0.16	0.17	0.14	0.14	0.14	0.14
Calcium carbonate	1.62	1.59	1.34	1.30	1.47	1.44	1.20	1.16
Mono calcium phosphate	0.33	0.38	1.13	1.18	0.13	0.18	0.89	0.94
Sodium chloride	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
Choline chloride	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Vitamin premix[3]	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Mineral premix[4]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Bentonite	0.52	0.51	0.00	0.00	0.49	0.48	0.00	0.00
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Calculated nutrients levels								
AME, Kcal/kg	2,850	2,850	2,850	2,850	2,900	2,900	2,900	2,900
CP, %	19.50	19.50	19.50	19.50	16.50	16.50	16.50	16.50
Ca, %	0.80	0.80	0.80	0.80	0.70	0.70	0.70	0.70
Total P, %	0.55	0.55	0.73	0.73	0.61	0.61	0.72	0.72
Nonphytate P, %	0.22	0.40	0.22	0.40	0.18	0.35	0.18	0.35
Lys, %	1.10	1.10	1.10	1.10	0.85	0.85	0.85	0.85
Met, %	0.75	0.75	0.75	0.75	0.40	0.75	0.75	0.75
Thr, %	0.45	0.45	0.45	0.45	0.61	0.61	0.61	0.61
Trp, %	0.22	0.22	0.22	0.22	0.18	0.18	0.18	0.18
Analyzed levels								
CP, %	19.15	19.12	19.08	19.03	16.58	16.55	16.51	16.53
Ca, %	0.84	0.83	0.84	0.82	0.72	0.73	0.74	0.76
Total P, %	0.55	0.54	0.70	0.68	0.62	0.62	0.73	0.73

[1]LP, low nonphytate phosphorus diet; LP + RPS, low nonphytate phosphorus diet with 12% raw potato starch; NP, normal nonphytate phosphorus diet; NP + RPS, normal nonphytate phosphorus diet with 12% raw potato starch.
[2]The apparent metabolizable energy (AME) of raw potato starch is 2.76 Mcal/kg.
[3]Provided per kilogram of diet: vitamin A, 6,875 IU; vitamin D_3, 1,640 IU; vitamin E, 30.01 mg; thiamine, 1 mg; riboflavin, 3.9 mg; pyridoxine, 3.375 mg; vitamin B_{12}, 0.01 mg; calcium pantothenate, 8.85 mg; folate, 0.5 mg; biotin, 0.1 mg; niacin, 49.25 mg.
[4]Provided per kilogram of diet: Cu ($CuSO_4 \cdot 5H_2O$), 8 mg; Fe ($FeSO_4 \cdot 7H_2O$), 80 mg; Zn ($ZnSO_4 \cdot 7H_2O$), 90 mg; Mn ($MnSO_4 \cdot H_2O$), 70 mg; Se ($NaSeO_3$), 0.3 mg; I (KI), 0.4 mg.

relative feather weight (feather yield) = the weight of all feathers/live BW × 100%.

Primary and Secondary Feather Follicles Measurements

Eight ducks were sampled at random from each treatment group on day 35. An approximately 1.5 cm^2 sample from the center of the thorax and back skin was cut for morphological examination and fixed by immersion in a 10% buffered formalin solution and subsequently routinely processed and embedded in paraffin. Sections of tissue, 6–8 μm thick, were made transversally along the feather tracks, placed on glass slides, and stained with hematoxylin and eosin. Images were obtained at 100 × magnification using an Olympus BX41 microscope with an Olympus DP71 camera (Tokyo, Japan). The density and diameter of the primary and secondary FF were measured.

Carcass Traits Evaluation

Carcass traits (n = 8) were evaluated at 35 d of age in accordance with the protocol described in previous study (Zeng et al., 2015b). Briefly, the carcass weight, weight of the eviscerated yield with giblets, weight of eviscerated carcass, breast meat weight, leg meat weight, and abdominal fat weight were obtained with an electronic scale (measuring range: 0∼3,000 g, accuracy: 0.1 g; Laihui Electronics Co. Ltd., Shenzhen, China). Carcass yield was determined as the carcass weight in relation to slaughter weight and expressed as a percentage of slaughter weight. Eviscerated yield with giblets and eviscerated carcass rate as well as breast meat, leg meat, and abdominal fat percentages were expressed as percentages of the carcass weight.

Serum Uric Acid, Cecal Short-Chain Fatty Acid Content, and Cecal Enzyme Activity Measurements

The blood samples were centrifuged at 3,000 g for 15 min at 4°C, and serum was collected and stored at −80°C until it was analyzed for uric acid. Eight replicate serum samples were analyzed for uric acid using an auto-analyzer (Yellow Springs Instrument Co., Inc., Yellow Springs, OH). The enzyme activities of phytase and cellulase in cecal digesta were determined

Table 2. The effect of low nonphytate phosphorus levels diet supplemented with resistant starch on growth performance of ducks.

		BW, g			BWG, g			F: G			FI, g			Mortality, %	
nPP level, %	RPS level, %	1 d	14 d	35 d	1–14 d	15–35 d	1–35 d	1–14 d	15–35 d	1–35 d	1–14 d	15–35 d	1–35 d	1–14 d	15–35 d
0.22/0.18	0	55.89[1]	384.4^{d}	1,200^{c}	328.5^{d}	812^{c}	1,144^{c}	1.62	2.03	1.82	529.5^{c}	1,648^{c}	2,078^{c}	29.0^{a}	29.0^{a}
0.22/0.18	12	55.73	604.7^{c}	1,997^{b}	549.0^{c}	1,383^{b}	1,941^{b}	1.45	2.01	1.77	798.0^{b}	2,785^{b}	3,433^{b}	3.0^{b}	2.0^{b}
0.40/0.35	0	55.87	688.6^{b}	2,620^{a}	632.7^{b}	1,928^{a}	2,564^{a}	1.31	1.92	1.67	827.3^{b}	3,684^{a}	4,287^{a}	0.0^{b}	0.0^{b}
0.40/0.35	12	55.92	804.0^{a}	2,714^{a}	748.0^{a}	1,910^{a}	2,658^{a}	1.22	1.82	1.55	912.0^{a}	3,466^{a}	4,127^{a}	0.0^{b}	2.0^{b}
SEM		0.08	20.49	56.10	56.10	52.38	56.10	0.02	0.07	0.03	28.93	106.13	105.95	3.00	4.00
Main effect means															
nPP level, %															
0.22/0.18		55.81	494.6^{b}	1,598^{b}	438.7^{b}	1,098^{b}	1,543^{b}	1.53^{a}	2.02^{a}	1.79^{a}	663.7^{b}	2,216^{b}	2,756^{b}	16.0^{a}	16.5^{a}
0.40/0.35		55.89	746.3^{a}	2,667^{a}	690.4^{a}	1,919^{a}	2,611^{a}	1.26^{b}	1.87^{b}	1.61^{b}	869.7^{a}	3,575^{a}	4,270^{a}	0.0^{b}	1.0^{b}
RPS level, %															
0		55.88	536.5^{b}	1,910^{b}	480.6^{b}	1,370^{b}	1,854^{b}	1.46^{a}	1.98	1.75^{a}	678.4^{b}	2,666^{b}	3,183^{b}	14.5^{a}	14.5^{a}
12		55.82	704.3^{a}	2,356^{a}	648.5^{a}	1,647^{a}	2,300^{a}	1.34^{b}	1.91	1.66^{b}	855.0^{a}	3,125^{a}	3,780^{a}	1.5^{b}	1.5^{b}
P-values															
nPP level		0.33	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
RPS level		0.51	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	0.41	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
nPP*RPS		0.24	<0.05	<0.05	0.02	<0.05	<0.05	0.08	0.59	0.33	<0.05	<0.05	<0.05	<0.05	<0.05

[a–d]Values within a column with no common superscripts differ significantly ($P < 0.05$).
[1]Values are the means of 8 replicates of 12 ducks each (n = 8).

Table 3. The effect of low nonphytate phosphorus levels diet supplemented with resistant starch on feather and follicle growth at 35 d of ducks.

nPP level, %	RPS level, %	Score of back feathers	The 4th primary feather length, cm	Absolute feather weight, g	Feather yield, %	Density of primary feather follicle, number/cm^2		Diameter of primary feather follicle, μm		Density of secondary feather follicle, number/mm^2		Diameter of secondary feather follicle, μm	
						Thorax	Back	Thorax	Back	Thorax	Back	Thorax	Back
0.22/0.18	0	7.46[1]	0.00^{c}	44.48^{c}	3.39	18	3^{b}	36.83	56.34^{a}	14	31	12.52^{b}	9.58
0.22/0.18	12	7.81	5.63^{b}	68.33^{b}	3.34	17	11^{a}	41.28	52.84^{a}	9	26	13.32^{b}	8.95
0.40/0.35	0	9.33	7.38^{a,b}	78.85^{a}	3.08	11	16^{a}	44.03	41.80^{b}	16	16	18.18^{a}	11.97
0.40/0.35	12	8.60	8.75^{a}	82.60^{a}	3.16	19	13^{a}	41.66	52.63^{a}	16	19	10.94^{b}	14.26
SEM		0.40	0.66	6.36	0.12	2	2	1.79	1.88	3	5	0.79	0.97
Main effect means													
nPP level, %													
0.22/0.18		7.64^{b}	2.81^{b}	60.44^{b}	3.36^{a}	18	7^{b}	39.06^{b}	54.59^{a}	12	28	12.92^{b}	9.27^{b}
0.40/0.35		8.97^{a}	8.06^{a}	81.03^{a}	3.12^{b}	15	14^{a}	42.84^{a}	47.21^{b}	16	18	14.56^{a}	13.11^{a}
RPS level, %													
0		8.40	3.69^{b}	62.86^{b}	3.23	15	10	41.47	49.07	15	24	15.35^{a}	10.77
12		8.21	7.19^{a}	75.73^{a}	3.25	18	12	40.43	52.73	13	22	12.13^{b}	11.61
P-values													
nPP level		<0.05	<0.05	<0.05	0.05	0.41	<0.05	<0.05	<0.05	0.19	0.05	<0.05	<0.05
RPS level		0.64	<0.05	<0.05	0.87	0.27	0.31	0.57	0.07	0.53	0.81	<0.05	0.40
nPP*RPS		0.18	<0.05	<0.05	0.56	0.10	<0.05	0.07	<0.05	0.41	0.44	<0.05	0.15

[a–c]Values within a column with no common superscripts differ significantly ($P < 0.05$).
[1]Values are the means of 8 replicates of one duck each (n = 8).

spectrophotometrically with commercial kits purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, Jiangsu, People's Republic of China) as per the instructions of the manufacturer. The cecal SCFA content was determined using the method described in our previous study (Qin et al., 2020). Briefly, approximately 0.5 g of cecal digesta was diluted with 2 mL ultrapure water, mixed and centrifuged (3,000 × *g*, 15 min). The supernatant (1 mL) was mixed with 0.2 mL ice-cold 25% (w/v) metaphosphoric acid solution and mixed at 4°C for 30 min, then centrifuged. Concentrations of acetate, propionate, and butyrate were measured by gas chromatographic system (Varian CP-3800, America).

Metabolizable Trial

On day 12 and 33, excreta samples from each cage were collected for three consecutive days (day 12∼14 and day 33∼35, 100 g per day). Acid insoluble ash was used as an endogenous indicator, and its content was determined according to the methods described by Van Keulen and Young (1977). Excreta samples were weighed and then stored at −20°C immediately. These fecal samples were dried at 65°C ± 5°C for 24 h, and then weighed and crushed to pass through a 40-mesh sieve for assessments of dry matter (**DM**), ester extract, crude protein (**CP**), calcium (**Ca**), total phosphorus (**TP**), and energy apparent digestibility as per the protocols described by Adeola et al. (1997) and Zeng et al. (2015c). In addition, the apparent metabolizable energy (**AME**) was calculated on the basis of the energy efficiency.

Statistical Analysis

All data were analyzed using SAS statistical software (version 9.2, SAS Institute Inc., 2004). All data were subjected to a two-way analysis of variance followed by Duncan's multiple range tests to determine significant differences among the treatments at $P < 0.05$ level. Data are presented as the mean and standard error of mean.

RESULTS

Growth Performance

As shown in Table 2, dietary nPP and RPS levels showed significant interaction ($P < 0.05$) in relation to the mortality rate, BW of 14 and 35 d, BWG and FI of all intervals. Resistant potato starch inclusion markedly ameliorated ($P < 0.05$) growth performance and reduced ($P < 0.05$) mortality in ducks fed low nPP diets. Moreover, RPS supplementation also significantly improved ($P < 0.05$) BW of 14 d, BWG and FI of 1–14 d in ducks fed normal nPP diets.

Feather Growth and Follicles Development

As shown in Table 3, we did not measure the fourth primary feather length of ducks fed a low nPP diet without RPS addition since ducks in this group did not show primary feathers. Dietary nPP levels and RPS inclusion showed significant interactions ($P < 0.05$) in relation to the fourth primary feather length, absolute feather weight, the density and diameter of primary FF in the back skin as well as the diameter of the secondary FF in the thorax skin of ducks. Resistant potato starch inclusion significantly increased ($P < 0.05$) the fourth primary feather length, absolute feather weight, and the density of primary FF in the back skin in ducks fed low nPP diets, but not in ducks fed normal nPP diets (Table 3 and Figure 1). Resistant potato starch inclusion significantly also increased ($P < 0.05$) the diameter of primary FF in the back skin and diameter of secondary FF in the thorax skin.

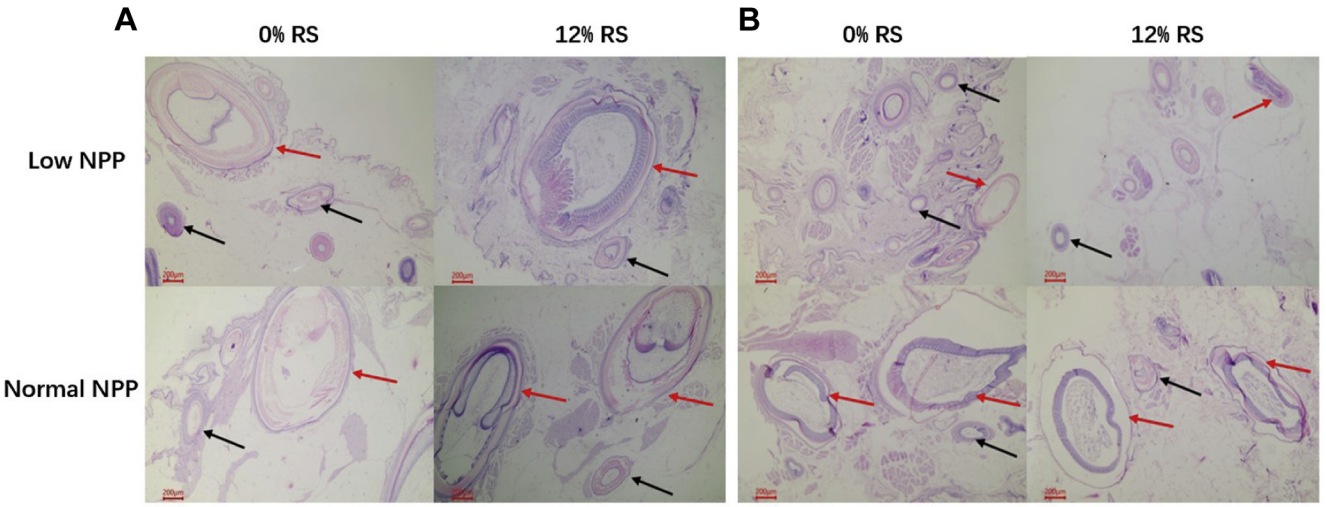

Figure 1. The effect of low nonphytate phosphorus levels diet supplemented with resistant starch on feather follicle growth of thorax (A) and back (B) skin at 35 d of ducks. The red arrow indicates the primary follicle and the black arrow indicates the secondary follicle (n = 8).

Table 4. The effect of low nonphytate phosphorus levels diet supplemented with resistant starch on carcass trait at 35 d of ducks.

nPP level, %	RPS level, %	Carcass rate, %	Eviscerated yield with giblet rate, %	Eviscerated carcass rate, %	Abdominal fat percentage, %	Breast meat percentage, %	Leg meat percentage, %
0.22/0.18	0	89.10[1]	81.61	72.90	0.53[b]	4.15[c]	14.33
0.22/0.18	12	88.50	81.36	73.76	1.10[a]	7.11[b]	11.80
0.40/0.35	0	89.48	79.43	72.11	1.18[a]	8.81[a]	11.98
0.40/0.35	12	89.43	82.78	75.65	1.27[a]	10.38[a]	11.69
SEM		0.54	1.79	0.09	0.57	0.60	0.27
Main effect means							
nPP level, %							
0.22/0.18		88.80	81.49	73.33	0.81[b]	5.63[b]	13.07[a]
0.40/0.35		89.45	81.10	73.88	1.22[a]	9.60[a]	11.83[b]
RPS level, %							
0		89.29	80.52	72.51	0.85[b]	7.26	13.15[a]
12		88.96	82.07	74.71	1.18[a]	7.96	11.75[b]
P-Value							
nPP level		0.24	0.83	0.76	<0.05	<0.05	<0.05
RPS level		0.55	0.39	0.23	<0.05	0.23	<0.05
nPP*RPS		0.62	0.32	0.46	<0.05	<0.05	0.07

[a–c]Values within a column with no common superscripts differ significantly ($P < 0.05$).
[1]Values are the means of 8 replicates of one duck each (n = 8).

Carcass Traits

Table 4 indicates that nPP levels and RPS inclusion showed a significant interaction ($P < 0.05$) in relation to the abdominal fat and breast meat percentage. Resistant potato starch inclusion significantly increased ($P < 0.05$) abdominal fat and breast meat percentage in ducks fed low nPP diets, but not in ducks fed normal nPP diets.

Serum Uric Acid Content

As shown in Table 5, reducing dietary nPP levels significantly increased ($P < 0.05$) serum uric acid content at 14 or 35 d of age. However, RPS inclusion significantly decreased ($P < 0.05$) serum uric acid content only at 14 d of age in Pekin ducks.

Table 5. The effect of low nonphytate phosphorus levels diet supplemented with resistant starch on serum uric acid content, cecal short chain fatty acid content, and cecal enzyme activities of ducks.

		Serum uric acid, nmol/l		Cecal acetate , mmol/L		Cecal propionate, mmol/L		Cecal butyrate, mmol/L		Cecal phytase, U/L	Cecal cellulase, U/L
nPP level (%)	RPS level (%)	14 d	35 d	14 d	35 d	14 d	35 d	14 d	35 d	35 d	35 d
0.22/0.18	0	409.8[1]	352.1	41.86	63.14	14.82	20.44	16.38	10.66	100.79	284.52
0.22/0.18	12	244.3	501.9	67.46	70.32	22.06	33.60	23.96	13.60	108.83	312.27
0.40/0.35	0	200.8	147.5	42.07	63.52	13.98	24.94	15.49	14.13	118.23	339.57
0.40/0.35	12	169.4	181.3	70.98	75.91	19.45	37.30	19.61	19.37	126.02	368.20
SEM		35.20	45.73	4.69	5.96	1.76	3.31	1.66	1.64	5.84	16.78
Main effect											
nPP level, %											
0.22/0.18		327.0[a]	427.0[a]	54.66	66.73	18.44	27.02	20.17	12.13[b]	104.81[b]	298.40[b]
0.40/0.35		185.1[b]	164.4[b]	56.52	69.71	16.71	31.12	17.55	16.75[a]	122.13[a]	353.89[a]
RPS level, %											
0		305.3[a]	249.8	41.97[b]	63.33	14.40[b]	22.69[b]	15.94[b]	12.40[b]	109.51	312.05
12		206.8[b]	341.6	69.22[a]	73.12	20.76[a]	35.45[a]	21.79[a]	16.49[a]	117.43	340.24
P-Value											
nPP		<0.01	<0.01	0.69	0.62	0.33	0.23	0.12	<0.01	<0.01	<0.01
RPS		<0.01	0.06	<0.01	0.11	<0.01	<0.01	<0.01	0.02	0.19	0.10
nPP*RS		0.07	0.22	0.73	0.67	0.62	0.91	0.31	0.49	0.98	0.98

[a–b]Values within a column with no common superscripts differ significantly ($P < 0.05$).
[1]Values are the means of 8 replicates of one duck each (n = 8).

Table 6. The effect of low nonphytate phosphorus levels diet supplemented with resistant starch on nutrient utilization of ducks.

		Dry matter, %		Crude fat, %		Crude protein, %		Calcium, %		Phosphorus, %		Energy, %		AME, kcal/g	
nPP level, %	RPS level, %	14 d	35 d	14 d	35 d	14 d	35 d	14 d	35 d	14 d	35 d	14 d	35 d	14 d	35 d
0.22/0.18	0	74.05[1,b]	75.67[c]	84.04	87.23	70.41	70.61[c]	29.40	28.60	40.68[c]	27.31	79.52[b]	81.03[c]	3121[b]	3,239[c]
0.22/0.18	12	74.13[b]	79.44[b]	90.56	91.17	73.95	76.43[b]	41.66	34.71	42.26[b,c]	39.44	79.26[b]	84.04[b]	3132[b]	3,419[b]
0.40/0.35	0	74.23[b]	81.50[a]	84.34	93.16	74.57	81.08[a]	42.97	31.37	45.43[b]	37.99	77.69[b]	85.73[a]	3078[b]	3,466[a,b]
0.40/0.35	12	78.58[a]	82.54[a]	92.92	94.21	77.07	77.50[a,b]	48.02	32.59	53.89[a]	45.54	82.21[a]	86.10[a]	3293[a]	3,492[a]
SEM		0.85	0.59	1.20	0.82	1.61	1.28	2.12	1.51	1.42	2.35	0.75	0.44	29.96	17.59
Main effect means															
nPP level, %															
0.22/0.18		74.09[b]	77.55[b]	87.30[b]	89.20[b]	72.18[b]	73.52[b]	35.53[b]	31.65	41.47[b]	33.38[b]	79.39	82.54[b]	3,127	3,329[b]
0.40/0.35		76.40[a]	82.02[a]	91.13[a]	93.69[a]	75.82[a]	79.29[a]	45.50[a]	31.98	49.66[a]	41.77[a]	79.95	85.92[a]	3,186	3,479[a]
RPS level, %															
0		74.14[b]	78.58[b]	86.69[b]	90.19[b]	72.49	75.84	36.18[b]	29.98[b]	43.06[b]	32.65[b]	78.60[b]	83.38[b]	3099[b]	3,352[b]
12		76.35[a]	80.99[a]	91.74[a]	92.69[a]	75.51	76.97	44.84[a]	33.65[a]	48.08[a]	42.49[a]	80.74[a]	85.07[a]	3213[a]	3,455[a]
P-values															
nPP level		<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	0.83	<0.05	<0.05	0.46	<0.05	0.06	<0.05
RPS level		<0.05	<0.05	<0.05	<0.05	0.07	0.39	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
nPP*RPS		<0.05	<0.05	0.23	0.09	0.75	<0.05	0.10	0.11	<0.05	0.34	<0.05	<0.05	<0.05	<0.05

[a–c]Values within a column with no common superscripts differ significantly ($P < 0.05$).
[1]Values are the means of 8 replicates of 12 ducks each (n = 8).

Cecal Short-Chain Fatty Acid Content and Phytase and Cellulase Activities

Based on main effect, RPS inclusion significantly increased ($P < 0.05$) cecal acetate (14 d), propionate (day 14 and 35 d), and butyrate (14 and 35 d) concentrations in Pekin duck, and had no effect ($P > 0.05$) on cecal phytase and cellulase activities (Table 5); Low nPP diets significantly decreased ($P < 0.05$) cecal butyrate concentration, cecal phytase and cellulase activities at 35 d of age (Table 5).

Nutrition Utilization

As shown in Table 6, dietary nPP levels and RPS inclusion showed significant interaction ($P < 0.05$) in relation to the availability of DM (14 and 35 d), CP (35 d), TP (14 d), and energy (14 and 35 d) as well as AME (14 and 35 d). Resistant potato starch supplementation significantly increased ($P < 0.05$) the availability of DM, CP, and energy, as well as dietary AME at 35 d of age in ducks fed low nPP diets, but not in ducks fed normal nPP diets. However, RPS supplementation to normal nPP diets significantly increased ($P < 0.05$) the availability of DM, TP, and energy, as well as dietary AME at 14 d of age.

DISCUSSION

Numerous studies have demonstrated that reduced dietary nPP levels can decrease growth performance and nutrient availability in poultry (Adeola, 2018; Xu et al., 2019), consistent with the findings of the present study. One reason for the decrease in the growth performance of ducks fed a P-deficient diet was the higher Ca: nPP ratio in such diets. Higher dietary Ca: P ratios decrease the FI of birds (Rao et al., 2003, 2006; Powell et al., 2011) and can also affect P absorption and utilization. The other important reason for the decrease in the growth performance was that ducks' gut microbial communities obtained P from phytate degradation when the dietary P was deficient (Dai et al., 2018), necessitating greater energy expenditure on the maintenance of the basic metabolism and leaving less energy to spend on nutrient utilization and growth. Indeed, we found that low nPP diets reduced P utilization and cecal butyrate concentration as well as cecal phytase and cellulase activities in the present study. Dai et al. (2018) also showed that a low nPP diet (0.22% nPP) decreased *Ruminococcaceae* UCG-014 abundance, which has been associated with the maintenance of gut health and has the enzymatic capability to degrade cellulose and hemicellulose (Biddle et al., 2013), as well as *Lachnospiraceae* abundance, which is known to degrade complex polysaccharides to SCFA. P is an essential nutrient for a variety of metabolic processes in bacterial cell. Therefore, P deficiency can inhibit the metabolic and fermented capacity of bacterial cell (Durand and Komisarczuk, 1988).

Dietary RPS supplementation improved growth performance and breast meat deposition of ducks fed low nPP diets in this study. Martinez-puig et al. (2003) and Fang et al. (2014) reported that in comparison with corn starch, 25% RPS significantly increased the FI and reduced fat synthesis levels in the adipose tissue of growing pigs. This may be attributed to the fact that RS, as a functional dietary fiber, can stimulate intestinal movement and increase the FI and absorption and utilization of nutrients in the intestines, thus improving growth performance. We also observed that dietary RPS significantly increased the utilization of DM, CP, energy, and numerical increased the utilization of crude fat, Ca, and P in ducks fed low nPP diets in the present study. Knudsen et al. (2012) reported that increasing the fermentable carbohydrate content in the diet could reduce the concentration of protein metabolites (such as nitrogen, etc.) in pig large intestinal digesta. Yin et al. (2019) also found that amylose supplementation can spare AA from catabolism in the gut mucosa of broiler chickens. These findings suggest that RPS inclusion in a low nPP diet improved the nitrogen metabolism and deposition in Pekin ducks. Moreover, RPS addition numerical improved cecal microbiome fermentation capacity to increase cecal SCFA content in ducks fed a low nPP diet. Short-chain fatty acid from hindgut microbial fermentation are important for energy metabolism and the normal development of intestinal epithelial cells and plays a beneficial role in gut health (Wächtershäuser and Stein, 2000; Arpaia and Rudensky, 2014).

Interestingly, this is the first study to show that supplementation of 12% RPS significantly increased the fourth primary feather length, absolute feather weight, and the density of the primary FF in ducks fed a low nPP diet. Chen et al. (2020) showed that ovo injection of methionine improved feather follicle development by activating Wnt/β-catenin signaling, and thereby promoting feather growth in broiler chickens. Zhu et al. (2019) also found that the addition of dietary methionine could significantly increase hair follicles density on the dorsal skin in rabbits. Rex rabbit fur is well known for its hair fineness, which has a strong correlation with the hair follicles density and diameter. Lower the hair follicles diameter is higher the value of Rex rabbit fur. Similarly, higher the hair follicles density is, higher the value of Rex rabbit fur. Feng and Gun (2020) showed that melatonin improved fur quality in offspring rabbits by reducing the diameter of primary and secondary hair follicles, and increasing the hair follicles population. These findings suggest that dietary RPS supplementation can improve FF development and feather quality and quantity in Pekin ducks. These effects may be attributed to the increased dietary CP, Ca, P, and trace mineral availability and absorption as a result of RPS addition. Yonekura and Suzuki (2005) found that RS supplementation reduced the pH value in the gut and increased Zn bioavailability in mice. Dietary P deficiency or RPS addition

affecting feather growth and FF development may be related to the intestinal microbiome and intestinal health, which requires further study.

CONCLUSIONS

In summary, this is the first study to demonstrate that dietary P deficiency depressed feather growth and follicles development, which was related to the decrease of cecal microbiome fermentation capacity, such as decreasing cecal butyrate content and phytase or cellulase activities, and nutrient utilization. The supplementation of 12% RPS to a P-deficient diet could improve growth performance, carcass traits, feather growth, and feather follicles development by increasing nutrient utilization. This study provides new insights into nutritional regulations for feather development in waterfowl.

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DISCLOSURES

None of the authors has any conflicts of interest to declare.

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