JPSA
Japan Poultry Science Association

http://www.jstage.jst.go.jp/browse/jpsa
doi:10.2141/jpsa.0200082

Effects of Persimmon Peel on Laying Performance, Nitrogen Availability, and Egg Quality in Laying Hens Provided with Shrimp Meal Diets

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To determine whether persimmon peel (PP) showing high chitinase activity could alleviate the detrimental dietary effects of chitin-rich shrimp meal (SM), we assessed the laying performance, nitrogen (N) balance, and egg quality of laying hens provided with SM diets containing PP. We also examined the color and antioxidant properties of egg yolk, as we anticipated these would be improved by providing SM and PP. Seventy-two laying hens (45 weeks of age) were allotted to one of the nine dietary treatments (eight hens each), namely three levels of SM (0%, 10%, and 15%)×three levels of PP (0%, 6%, and 8%), and fed with the experimental diets over a period of 6 weeks. Hen-day egg production, feed intake, egg mass, feed conversion ratio, and N balance reduced with increasing levels of SM, whereas the reductions were recovered in a dose-dependent manner in response to increasing levels of PP; however, the SM0% treatment showed that PP exerted little effects. Notably, reductions in the Haugh unit and albumen height of eggs with increasing SM levels, and recovery by provision of increasing levels of dietary PP, were observed. Yolk color was improved by SM, although PP exerted little effect, whereas the antioxidant properties of yolk were enhanced by the inclusion of both SM and PP in diets. Furthermore, eggshell strength, weight, and thickness were enhanced with increasing levels of SM, whereas dietary PP had little effect on these parameters. Thus, we suggest that PP can alleviate the negative effects of dietary SM and improve egg quality, without causing a reduction in laying performance, provided that the level of supplementary PP in diets is less than 8%. These findings accordingly indicate that PP is a promising feed constituent for laying hens fed with SM diets.

Key words: egg quality, laying performance, nitrogen balance, persimmon peel, shrimp meal

J. Poult. Sci., 58: 238−244, 2021

Introduction

As a consequence of rapid world population growth in recent years, particularly in developing countries, the issue of food supply security has gained prominence, and research on the development of novel feed resources has accordingly received increasing attention. However, the further expansion of agricultural land for feed production is deemed undesirable, given that this would lead to a further destruction of areas of natural vegetation, such as rainforests. In this regard, the use of feed prepared from generally inedible organic residues can reduce the disposal and accumulation of waste products, thereby diminishing the associated environmental burden. To date, several types of inedible organic residue, including dried papaya skins (Fouzder *et al*., 1999), dried kitchen waste (Kojima, 2005), fermented banana peel meal (Sugiharto *et al*., 2020), grape pomace (Brenes *et al*., 2008), and shrimp meal (SM) (Khempaka *et al*., 2006a, 2006b; Rahman and Koh, 2016), have been examined for their suitably as chicken feed ingredients. Among these, SM appears to be a promising waste product, given that it could potentially be used as an alternative to expensive protein sources, such as soybean meal.

To date, however, SM has not been widely used commercially, as chickens provided with feed supplemented with this product tend to show deteriorations in laying and growth performance (Khempaka *et al*., 2006a, 2006b; Rahman and Koh, 2018), which is suggested to be attributable to high levels of chitin, a low-digestible amino-polysaccharide. In-

Received: August 23, 2020, Accepted: November 16, 2020
Released Online Advance Publication: January 25, 2021
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deed, our previous studies have revealed that formic acid treatment, which reduces chitin levels, alleviated the negative effects of SM on chicken performance (Rahman and Koh, 2016). However, although promising, this method requires the use of a chemical acid, and thus from an environmental perspective is deemed undesirable. As a more environmentally friendly alternative, we examined the efficacy of persimmon peel (PP), a by-product of dried persimmon production known to have chitinolytic activity (Takii *et al*., 2010; Sangkaew and Koh, 2021), as a chitin digestion promotor, and revealed that the *in vitro* crude protein (CP) digestibility of SM diets reached a maximum when supplanted with 6% PP (Sangkaew and Koh, 2021). These observations accordingly indicated that PP could serve as a valuable constituent in high-chitin feed. To the best of our knowledge, however, there have been no reports on use of PP for improvement of dietary chitin digestion in chickens.

In the present study, we thus sought to determine the laying performance, nitrogen (N) balance, and egg quality in laying hens fed with SM diets containing PP, and to assess whether dietary PP alleviates the negative effects of SM. Additionally, we examined the effects of dietary PP on the color and antioxidant properties of egg yolk, as these may be affected by the high carotenoid (Takahashi *et al*., 2006) and tannin (Fitri *et al*., 2020) contents in PP. We also determined the levels of ammonia excretion as an indicator of the activity of unfavorable intestinal bacteria, as tannins can reduce the populations of these bacteria (Smith and Mackie, 2004; Jamroz *et al*., 2009).

Materials and Methods

The present study was conducted in accordance with the guidelines for regulation of animal experimentation of Shinshu University, Japan (Approval number 290085).

Test Products

Sun-dried PP was purchased commercially from a local market in Nagano Prefecture, Japan, and sun-dried SM, prepared from the heads and hulls of the whiteleg shrimp (*Litopenaeus vannamei*), was purchased commercially from Thailand. Samples of PP and SM were ground to pass through a 1.0-mm aperture and maintained at room temperature until used for analysis (Table 1).

Hens, Diets, and Sampling Procedures

Seventy-two Lohmann LSL-Lite laying hens (45 weeks of age), were divided into nine groups (each containing eight hens) with an average initial laying rate of 98%, and reared in individual cages under a 16L:8D photoperiod. Hens were allocated to one of the nine dietary treatments according to a 3×3 factorial arrangement i.e., three levels of SM (0%, 10%, and 15%)×three levels of PP (0%, 6%, and 8%) (Table 2). SM and PP were added at the expenses of soybean meal and maize, respectively. Diets were formulated to meet or slightly exceed the nutrient requirements of laying hens recommended by the NRC (1994). However, chitin N was not included in the calculation of CP, as there is no evidence to indicate that birds utilize this source of N. The hens were fed with the experimental diets for 6 weeks, with the first week being used for adaptation and the subsequent five weeks for data collection. Both feed and water were provided *ad libitum*. The excreta of each hen were collected over the last 3 days of the second, fourth, and sixth weeks of the experimental period and stored at −20℃ until used for analysis.

Laying Performance and Egg Quality

Egg production and feed intake (FI) were recorded daily. Hen-day egg production was calculated on a hen per day basis, and egg mass was calculated from egg production and egg weight, using the following equation: egg mass (g/hen) =(egg production×egg weight)/period (day). The feed conversion ratio (FCR) was calculated as the ratio of feed consumed to egg mass, and changes in body weight (BW) were calculated as the difference between the initial and final BWs. Egg weight, eggshell strength, Haugh unit, albumen height, and yolk color were measured daily using a digital egg tester (Nabel Co., Ltd., Kyoto, Japan). Eggshells were weighed after drying at 100℃ for 2 h, and their thickness was measured using a micrometer (PK-1012CPX; Mitutoyo Corporation, Kanagawa, Japan).

Chemical Analysis

Samples of SM, PP, diets, and excreta were analyzed for proximate composition following standard methodology (AOAC, 1990). Chitin levels were measured using the method described by Ghanem *et al*. (2003) and chitinase activity was measured according to our previously described methods (Sangkaew and Koh, 2021). Yolk lipid oxidation

Table 1. **Chemical composition of shrimp meal, persimmon peel, and maize**

Parameters (% of dry matter)	Shrimp meal[1]	Persimmon peel[1]	Maize[2]
Crude protein	49.7	3.7	8.8
Ether extract	3.4	1.7	4.4
Crude fiber	16.9	15.2	2.0
Crude ash	29.4	3.1	1.4
Nitrogen free extract	0.6	76.3	83.4
Chitin	14.5	—	—
Chitinase activity (U/g protein)	—	1,291	—

[1] The values of each parameter represent the mean values of triplicate analyses (in dry matter).
[2] Standard Table of Feed Composition in Japan (NARO, 2009).

Table 2. **Composition and nutrient levels of the experimental diets[1,2,3] (as-fed basis)**

Dietary groups		Ingredients, g/kg							Analyzed value	
		Maize	PP	Soybean meal	Corn oil	SM	Tricalcium phosphate	Calcium carbonate	Chitin (g/kg)	Chitinase activity (U/g protein)
0% SM	0% PP	266	0	162	34	0	27	42	0	0
	6% PP	190	60	171	41					77.5
	8% PP	164	80	175	43					103.9
10% SM	0% PP	253	0	76	47	100	22	33	15	0
	6% PP	175	60	87	54					77.5
	8% PP	151	80	89	56					103.9
15% SM	0% PP	248	0	32	53	150	19	29	22	0
	6% PP	171	60	42	60					77.4
	8% PP	147	80	44	62					103.7

[1] SM=shrimp meal; PP=persimmon peel.
[2] All diets contained 450 g/kg of commercial diet [crude protein (CP)>17%, ME>2,850 kcal/kg, Nippon Formula Feed Mfg., Kanagawa, Japan] and 19 g/kg of vit-min premix providing the following nutrients per kg of diet: vitamin A, 10,000 IU; vitamin D_3, 2,100 IU; vitamin E, 15 IU; thiamine, 10 mg; riboflavin, 7 mg; pantothenic acid, 15 mg; pyridoxine, 3 mg; niacin, 32 mg; choline chloride, 500 mg; folic acid, 0.6 mg; biotin, 0.1 mg; manganese, 75 mg; iron, 50 mg; zinc, 60 mg; copper, 5 mg; and iodine, 2 mg.
[3] CP in all diets ranged from 17.0% to 17.2% (analyzed value), ME in all diets ranged from 2,901 to 2,903 kcal/kg, and Ca was 3.6% (calculated values).

was determined weekly using the thiobarbituric acid reactive substance assay (TBARS), following the method described by Cherian *et al*. (1996). Briefly, fresh egg yolk (2 g) was homogenized with 18 mL of 3.86% perchloric acid and 50 μL of butylated hydroxytoluene. After filtration, the filtrate (2 mL) was mixed with 2 mL of 2 mM thiobarbituric acid and heated in a boiling water bath for 30 min, after which the absorbance was measured at 531 nm. Data were expressed in terms of nanograms of malondialdehyde (MDA) per gram of yolk. The amount of ammonia in fresh excreta was determined using the indophenol method (Scheiner, 1976).

Statistical Analysis

The data were subjected to two-way ANOVA using the GLM procedure (SAS Institute, 2015). When an interaction was significant, the Tukey–Kramer test was performed, and when not significant, the data were combined over main effects and then subjected to the Tukey–Kramer test. In all cases, a *P*-value <0.05 was considered to be indicative of a statistically significant difference.

Results

Laying Performance (Table 3)

Hen-day egg production and FI were found to be significantly affected by both SM and PP, and their interaction. In the PP0% groups, hen-day egg production was 97.7% in the absence of SM but decreased significantly with increasing levels of SM ($P<0.05$). However, with the exception of the SM0% group in which PP had no appreciable effect, the reduction in these values was dose-dependently reversed by increasing the level of dietary PP. A similar pattern was observed with respect to FI and egg mass. Although the interactive effect in the case of egg mass was not significant, the *P*-value was close to 0.05, and therefore the data were not combined. Given that there was no significant interaction, the FCR data were combined over main effects, as follows: the effect of SM levels (SM0% 1.95 ± 0.02^{b}, SM10% 1.96 ± 0.02^{b}, and SM15% 2.03 ± 0.02^{a}) and the effect of PP levels (PP0% 2.04 ± 0.03^{a}, PP6% 1.96 ± 0.01^{b}, and PP8% 1.95 ± 0.02^{b}) ($^{a-b}$ mean values followed by different superscript letter differ significantly at the $P<0.05$ level). These data indicated that FCR increased with increasing levels of SM and decreased with increasing levels of PP. In contrast, neither SM nor PP had a significant effect on the changes in BW.

N Balance (Table 4)

All parameters relating to N balance were affected significantly by both SM and PP, as well as their interaction. The pattern of change in N intake was found to be similar to that of FI, which reflects that fact that all diets were isonitrogenous. N excretion in the PP0% groups increased significantly with increasing levels of SM ($P<0.05$), although this increasing tendency became less evident with an increase in the level of PP; the exception being the SM0% group, in which N excretion was unaffected by PP. Furthermore, whereas there were significant reductions in N retention in the PP0% groups with increasing levels of SM ($P<0.05$), the reduced values were recovered dose-dependently by increasing the level of PP, with the SM0% group, in which PP had little effect, being the exception.

Egg and Eggshell Quality (Table 5)

The results of two-way ANOVA reveled that there was no significant interactions in any of the parameters of egg and eggshell quality (data not shown), and therefore each parameter was combined over the main effects. Whereas neither SM nor PP had any significant effects on egg weight, we observed significant reductions and increases in the Haugh unit with increasing levels of SM and PP, respectively ($P<0.05$). Similarly, albumen height tended to decrease

Table 3. **Laying performance of hens fed with diets containing shrimp meal and persimmon peel[1,2]**

Dietary groups		Hen-day egg production (%)	Feed intake (g/hen/day)	Egg mass (g/hen/day)	FCR (g feed/g egg)	Body weight change (g/hen/6 weeks)
SM (%)	PP (%)					
0	0	97.7±0.6[a]	114.5±1.2[ab]	58.2±1.1[a]	1.96±0.03	40.6±29.7
	6	97.3±0.8[a]	115.4±1.0[ab]	59.6±0.9[a]	1.94±0.02	14.4±29.0
	8	96.4±1.0[ab]	116.2±1.2[a]	59.3±1.6[a]	1.97±0.04	−26.9±26.1
10	0	87.2±1.4[cd]	107.4±1.1[cd]	52.9±1.2[bc]	2.04±0.06	21.3±16.2
	6	95.9±0.7[ab]	112.9±1.4[ab]	58.0±0.6[a]	1.95±0.02	15.0±22.9
	8	98.2±0.8[a]	116.1±0.8[a]	59.8±1.2[a]	1.90±0.02	18.1±31.7
15	0	82.8±1.1[d]	102.7±1.3[d]	50.1±0.7[c]	2.12±0.02	−17.5±28.4
	6	89.0±0.9[c]	110.2±1.1[bc]	55.4±0.6[ab]	2.00±0.02	3.1±23.7
	8	91.8±1.3[bc]	113.2±0.8[ab]	57.5±1.1[ab]	1.97±0.03	4.4±24.4
Source of variation		---------- *P*-value ----------				
SM		0.0001	0.0001	0.0001	0.0081	0.7697
PP		0.0001	0.0001	0.0001	0.0020	0.8507
SM×PP		0.0001	0.0036	0.0624	0.1166	0.7971

[a–d] Mean values within the same column followed by different superscript letters are significantly different at the $P<0.05$ level.
[1] SM=shrimp meal; PP=persimmon peel; FCR=feed conversion ratio.
[2] The values for each parameter represent mean±standard error of eight observations.

Table 4. **Nitrogen balance in laying hens fed with diets containing shrimp meal and persimmon peel[1,2]**

Dietary groups		N intake (g/bird/day)	N excretion (g/bird/day)	N retention (g/bird/day)
SM (%)	PP (%)			
0	0	3.16±0.03[abc]	0.97±0.04[c]	2.19±0.04[ab]
	6	3.20±0.03[ab]	0.99±0.03[bc]	2.21±0.03[ab]
	8	3.25±0.04[a]	1.01±0.04[bc]	2.24±0.05[ab]
10	0	2.99±0.03[d]	1.11±0.03[ab]	1.88±0.04[d]
	6	3.17±0.04[abc]	0.92±0.03[cd]	2.25±0.05[ab]
	8	3.19±0.02[ab]	0.83±0.02[d]	2.36±0.02[a]
15	0	2.81±0.04[e]	1.19±0.02[a]	1.63±0.04[e]
	6	3.04±0.03[cd]	1.04±0.02[bc]	2.00±0.04[cd]
	8	3.10±0.02[bcd]	0.99±0.03[bc]	2.11±0.03[bc]
Source of variation		---------- *P*-value ----------		
SM		0.0001	0.0001	0.0001
PP		0.0001	0.0001	0.0001
SM×PP		0.0171	0.0001	0.0001

[a–e] Mean values within the same column followed by different superscript letters are significantly different at the $P<0.05$ level.
[1] SM=shrimp meal; PP=persimmon peel; N=nitrogen.
[2] The values for each parameter represent mean±standard error of eight observations.

with increasing levels of SM and increased with increasing levels of PP ($P=0.0507$). In contrast, we observed that there was a significant increase in yolk color with increasing levels of dietary SM ($P<0.05$), although not PP. Moreover, there were reductions in the levels of MDA in response to inclusion of SM and PP in the diets ($P<0.05$). Additionally, we detected significant increases in eggshell parameters with increasing levels of SM ($P<0.05$), whereas PP appeared to have little effect on eggshells.

Ammonia Excretion (Fig. 1)

We observed significant reductions in ammonia excretion with increasing levels of PP ($P<0.05$), whereas the effects of SM and the interaction between SM and PP were non-significant.

Discussion

To the best of our knowledge, this study is the first to report on an improvement in the nutritional quality of SM diets of laying hens as a consequence of PP supplementation.

In the SM0% group, however, we observed that PP had relatively little effect on hen-day egg production, FI, egg mass, and N balance. These findings lead us to propose the following two hypotheses: (1) there is no significant difference in the nutritional values of PP and maize, the latter

Table 5. **The results of the main effects on egg and eggshell quality in laying hens[1]**

Main effects	Egg weight[2] (g)	Haugh units[2]	Albumen height[2] (mm)	Yolk		Eggshell		
				Color[2]	MDA[3] (ng/g yolk)	Strength[2] (kgf/cm^2)	Weight[3] (g)	Thickness[3] (mm)
SM levels (%)								
0	60.9±0.53	93.6±0.44^a	8.8±0.12	8.5±0.07^c	37.2±1.31^a	5.5±0.06^b	6.2±0.05^b	0.55±0.003^b
10	60.7±0.36	93.0±0.60^{ab}	8.6±0.17	9.8±0.11^b	24.1±1.24^b	6.0±0.09^a	6.9±0.08^a	0.58±0.009^{ab}
15	60.3±0.39	91.3±0.61^b	8.3±0.11	11.2±0.10^a	21.7±1.24^b	6.3±0.11^a	7.0±0.10^a	0.60±0.013^a
PP levels (%)								
0	60.4±0.40	91.3±0.67^b	8.4±0.14	9.7±0.22	34.9±1.73^a	6.0±0.11	6.8±0.11	0.58±0.012
6	60.5±0.34	92.7±0.43^{ab}	8.5±0.11	9.9±0.25	24.6±1.65^b	5.9±0.11	6.7±0.12	0.57±0.009
8	60.9±0.53	93.9±0.51^a	8.9±0.16	10.0±0.26	23.5±1.63^b	5.9±0.12	6.7±0.13	0.57±0.011

$^{a-c}$ Mean values within the SM and PP groups followed by different superscript letters are significantly different at the $P<0.05$ level.
[1] SM=shrimp meal; PP=persimmon peel; MDA=malondialdehyde.
[2] The values for each parameter represent mean±standard error of 24 observations.
[3] The values for each parameter represent mean±standard error of 18 observations.

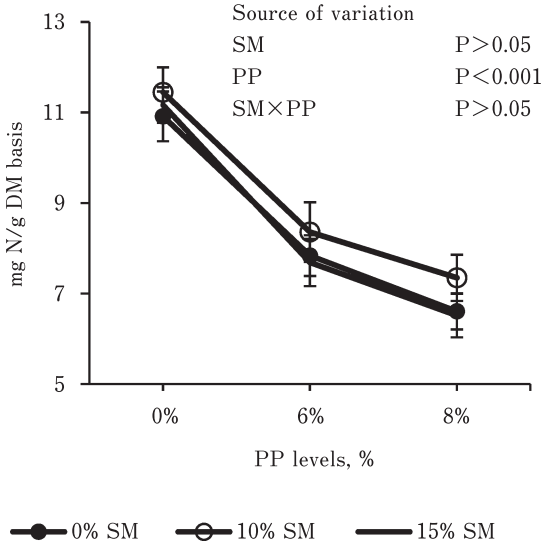

Fig. 1. **Ammonia excretion in laying hens fed with diets containing shrimp meal and persimmon peel.** SM=shrimp meal; PP=persimmon peel. Data represent mean±standard error (n=8).

which was replaced with former in the experimental diets (Table 1), and (2) PP has little effect on the digestibility and utilization of CP in laying hens, provided that the level of supplementation is less than 8%. In this regard, Sangkaew and Koh (2021) have reported that there was a reduction in the *in vitro* CP digestibility of an SM-free diet when supplemented with 8% PP, which we suggest could be attributable to the high levels of tannins, the binding of which to digestive enzymes results in an increase in pancreatic enzyme secretion (Griffiths and Moseley, 1980).

With respect to the PP0% group, we observed reductions in hen-day egg production, FI, N intake, egg mass, and N retention, and increases in FCR and N excretion, in response to increasing levels of dietary SM, which is consistent with the findings reported by Rahman and Koh (2016, 2018). These results thus tend to indicate that the negative effects of SM are attributable not only to a reduction in FI but also to an increase in FCR, namely, a reduction in nutritional utilization. In line with expectations, we found that the negative effects of SM were alleviated by the addition of PP, with supplementation at the 8% level almost completely negating the detrimental effects of the SM10% diet, although this level of PP was not as effective in the case of the SM15% diet. We suggest that this ameliorative effect of PP can be explained in terms of chitin digestion by PP chitinase, although this was not empirically determined. Dietary PP can potentially serve as an alternative to formic acid treatment for reducing chitin levels, although as previously indicated, it would appear that PP contents in excess of 8% may be necessary in the case of diets comprising more than 10% SM. The development of such high SM diets is desirable, given that this can not only enhance the utilization of SM, a by-product of shrimp-producing industries, but also enables a reduction in the use of soybean meal, an expensive imported CP source. However, although it may be feasible to increase the level of dietary SM by also increasing the content of PP, there may be an upper limit to beneficial PP supplementation, owing to the inhibitory effects of tannins on digestion.

We also observed reductions in the Haugh unit and albumen height of the eggs of hens fed with diets containing increasing levels of SM, with these effects being reversed by the supplementation of increasing levels of PP. In this regard, Oh *et al*. (2013) consistently found that dietary PP improved the Haugh unit of eggs after 7 days of storage. Taking these observation into account, it would appear that although SM has a negative effect on albumen quality, the inclusion of PP in diets has a beneficial effect. We suggest that the observed improvement in albumen quality can be attributed to the high ascorbic acid content of PP (Lee *et al*., 2006; Sorifa Akter *et al*., 2010), which has previously been reported to improve albumen quality (Keshavarz, 1996).

Consistent with the findings of Rahman and Koh (2018), we found that yolk color was enhanced by dietary supplementation with SM, whereas PP appeared to have relatively little effect. The coloring effect of SM may be attributable to the astaxanthin content of this product (Sowmya and Sachindra, 2012), which has previously been reported to enhance yolk color (Akiba *et al*., 2000; Anderson *et al*., 2008). Although PP is known to contain carotenoids that would be assumed to affect yolk coloring (Karunajeewa *et al*., 1984), we obtained no evidence for this under the conditions of the present study. Oh *et al*. (2013) have similarly reported that yolk color is little affected by dietary PP or an ethanol extract of PP. We also detected a significant reduction in the MDA content of the eggs laid by hens fed with diets supplemented with SM and PP, which is consistent with the findings of previous studies indicating an increase in antioxidant activity attributable to carotenoid extracts obtained from shrimp processing waste (Sowmya and Sachindra, 2012), as well as from fresh and dried persimmon (Jung *et al*., 2005). Consequently, by feeding hens diets supplemented with SM and PP, it may possibly be to produce value-added eggs with high antioxidant activity.

Similarly consistent with the finding reported by Rahman and Koh (2018), we found that supplementing hen diets with SM had the effects of enhancing eggshell strength, weight, and thickness, whereas comparatively, PP had little effect, as also reported by Oh *et al*. (2013). These observations thus tend to indicate that PP may not adversely affect eggshell quality, provided that the level of supplementation remains below 8%. It has also been reported that dietary polyphenols and tannins have the effects of reducing intestinal putrefactive products, including ammonia, via a reduction in the populations of unfavorable bacteria in the intestine (Terada *et al*., 1993; Hara *et al*., 1995). Therefore, we assume that the reduction in ammonia excretion observed in hens receiving PP-supplemented diets in the present study can be attributed to the inhibitory effects of PP tannins on such bacteria. However, given that ammonia in the excreta is not exclusively derived from the activities of intestinal bacteria, further studies will be necessary to clarify the effects of PP on the intestinal bacteria of laying hens.

In conclusion, in this study, we established that persimmon peel could alleviate the detrimental effects of dietary shrimp meal on the quality and performance of laying hens and could enhance the quality of eggs, including Haugh unit values and antioxidant properties, without causing a reduction in laying performance, provided that the levels of persimmon peel and shrimp meal supplementation did not exceed 8% and 10%, respectively. Additionally, it plausible that dietary PP has the effect of reducing unfavorable intestinal bacteria. Collectively, our findings indicate that persimmon peel can be effectively utilized as a quality improving constituent of SM-supplemented diets.

Acknowledgment

This study was supported by JSPS KAKENHI Grant Number JP20K06341.

Conflicts of Interest

The authors declare no conflict of interest.

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