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[Research Note]

Powdered red wine residue addition to hen feed promotes egg-laying rate

Yoshinori NAKAO[1*], Tomoya HATA[1], Tohru OKUDA[2], and Yoshiaki HAYASHI[1]

[1]Experimental Farm, Faculty of Agriculture, Meijo University, Takaki Kasugai, Aichi 486-0804, Japan.

[2]The Institute of Enology and Viticulture, University of Yamanashi, 1-13-1 Kitashin, Kofu, Yamanashi 400-0005, Japan.

Abstract: Grape skin and seeds are produced in large quantities as the byproducts of red wine making. Although they are known to contain useful substances, such as anthocyanins, there are few studies of their effective use. In this study, we examined their utility as feed additive for egg-laying hens. The byproducts in the form of powdered red wine residue were mixed with commercial feed at the concentration of 1% or 5% and fed to egg-laying hens for 30 day. Anthocyanin content in red wine residue after freeze-drying treatment was higher than that after air-drying treatment. Daily feed intake was decreased by the addition of powdered red wine residue at the beginning of feeding, but became almost unchanged regardless of treatment after 30 day. Egg weight, egg-laying rate, and eggshell strength were increased significantly, whereas yolk color became slightly bright yellow with the addition of 5% powdered red wine residue. The addition of 1% powdered red wine residue decreased blood glucose levels, but the decrease was within the normal physiological range. Body weights of egg-laying hens were almost the same regardless of treatment. The results show that the addition of powdered red wine residue to feed increased egg-laying rate.

Key words: Anthocyanin, Egg-laying rate, Egg quality, Feed additive

Introduction

Anthocyanins are flavonoids present in grape skin and play important roles in the color and taste of red wine. Proanthocyanidins are polyphenols present at high levels in grape seed, and are responsible for the bitter taste. Anthocyanins and proanthocyanidins are antioxidants that exhibit radical scavenging activity and inhibitory effects on lipid peroxidation induced by ultraviolet irradiation (Tsuda et al. 1996). They also improve visual function (Ohguro et al. 2007), regenerate rhodopsin (Matsumoto et al. 2003), and inhibit tumor cell growth in human (Meiers et al. 2001). In

*Corresponding auther (email: nakaoy@meijo-u.ac.jp)

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addition, they lower the incidence of coronary heart disease and arteriosclerosis (Renaud and De Logeril 1992), and increase resistance to bad cholesterol (LDL-Co) oxidation in blood (Yu 2009). Egg-laying hens are susceptible to infection and environmental stress, and the addition of anthocyanins to feed may increase resistance to infection and enhance egg-laying rate. Grape skin and seeds, which are the byproducts of wine fermentation, contain anthocyanins. Most of the grape skin and seed residues are discarded as industrial waste, whereas some are used as compost. However, the residues may contain many useful substances. It has been shown that white wine byproduct did not affect egg-laying rate (Ishiguro and Mikami 2009) when silage was used as one of the main feeds and not as an additive. Egg weight and egg-laying rate did not change significantly, whereas hen body weight and yolk color difference (a value and b value) were clearly decreased. Generally, these hen feeds are easy to handle and preserved in the dried form. There are few studies of wine residue as feed additive for egg-laying hens. We hypothesized that feeding powdered red wine residue containing such useful substances as anthocyanins would change the physical constitution of egg-laying hens and improve egg quality. In the present study, we determined anthocyanin content in powered red wine residue, and the suitability of powdered red wine residue as feed additive for egg-laying hens.

Materials and Methods

'Muscat Bailey A' is a cultivar certified by the Office International de la Vigne et du Vin, and its residue was used in the present study. Grape skin and seed residue after fermentation was stored at −28°C at the Institute of Enology and Viticulture, University of Yamanashi.

Wine residue treatment and quantitative analysis of anthocyanins

Anthocyanin content was measured in the wine residue after air-drying, freeze-drying, or no treatment. The air-dried sample was prepared by spreading the wine residue on a stainless steel mesh tray and drying at 60°C for three day in an air drier. The freeze-dried sample was prepared in a lyophilizer (FD-5N, Eyela Co.). Each sample included skin and seeds and was ground in a mill. Anthocyanins were extracted from 0.5 g of each sample by using 50% acetic acid, 5% acetic acid in 50% methanol, or 5% formic acid in methanol at 4°C. After a 24 hr extraction, the sample was centrifuged (10,000 *g*, 5 min), the supernatant was removed, and the precipitate was re-extracted twice with 3 mL of extraction solvent at 4°C for 10 min. The supernatants were combined and adjusted to 10 mL by adding the extraction solvent.

Anthocyanin content was analyzed by HPLC with photodiode array detection (SPD-M20A, Shimadzu Co.) at 525 nm after passing through a 0.45 μm pore size filter. Anthocyanins were identified from the retention times and the absorption spectra of commercial

specimens and previously reported results (Tsubosaka and Nishimura 1991, Sato et al. 2000, Downey and Simone 2008). The injection volume was 20 μL. HPLC separation of anthocyanins from the extract was performed using 25% acetonitrile, 1.5% phosphoric acid, and 20% acetic acid in water (Solvent A) and 1.5% phosphoric acid in water (Solvent B) at the flow rate of 1.0 mL/min. Column (Inertsil ODS-2, 5 μm, 4.6 × 250 mm, Shodex, Japan) temperature was maintained at 39°C. The gradient conditions were as follows: 0–30 min, 15% Solvent B; 30–45 min, a linear gradient to 60% Solvent B; and 45–55 min, 60% Solvent B. Compounds detected by HPLC were quantified as cyanidin-3-glucoside by integration of peak height.

Egg-laying rate, blood analysis, and body weight of egg-laying hens

Two feed treatments, namely, addition of 1% and 5% powdered red wine residue, were examined, and no addition was used as control. Egg-laying white leghorn hens (479 day old) were individually kept in cages. After 10 day, 27 hens were selected as high egg layers. The selected hens were divided into three groups of nine hens each. Each group of nine hens was further divided into three groups of three hens each. The egg-laying rate of each group was approximately 80%. Cages of each treatment placed two cages apart. After a two-day familiarization period, feeding was began on September 13, 2010 and continued for 30 day. The hens were fed 55.0 g twice a day at 9 a.m. and 3 p.m. Pulverized oyster shell was mixed with the feed every morning at the rate of 5.0 g/hen. In all other respects, hen management followed routine methods.

All remaining feed was collected every three day, dried in the air drier at 100°C, and weighed to determine daily feed intake. Eggs were collected at 10:30 a.m. every day. Egg-laying rate was calculated as the total number of eggs produced per treatment during the study period. Body weight was measured at the beginning and the end of the study period. At the end of the study period, blood (6 mL) was sampled from wing vein, immediately centrifuged (5 min at 10,000 *g*) at 10°C, and stored at −18°C until analysis. Analyses of total protein, total cholesterol, and glucose in the blood samples were performed by Oriental Yeast Co., Ltd., Japan.

Egg quality

All eggs were weighed immediately after collection. Eggshell strength was measured with an eggshell strength meter (Fujihira Co., Japan). Thickness of the eggshell without the membrane was measured at three points using a dial gauge. Yolk color was measured with a yolk color fan (Roche Co., Switzerland), and yolk diameter was measured with digital calipers. Albumen diameter was measured at the shortest and longest points, and the mean value was calculated. Yolk and albumen heights were measured with an egg quality meter (NFN381, Fujihira Co., Japan.) on a horizontal flat glass board. Albumen height was measured at a point 1.0 cm away from yolk edge.

Results and Discussion

Total anthocyanins in powdered red wine residue were calculated from the peaks in the HPLC chart (Fig. 1). Each peak was identified on the basis of the retention time and/or absorption spectrum (Tsubosaka and Nishimura 1991, Sato et al. 2000, Downey and Simone 2008). The HPLC peak appearing at the retention time of 48.1 min had λmax at 527 nm and 347 nm in the absorption spectrum, and was identified as malvidin-3-glucoside. The peak appearing at the retention time of 45.4 min was identified as peonidin-3-glucoside on the basis of λmax at 518 nm and 278 nm. Delphinidin-3-glucoside like substance, cyanidin-3-glucoside like substance, and petunidin-3-glucoside like substance were identified on the basis of the peaks appearing at the retention times of 42.0, 44.5, and 45.4 min, respectively. Each anthocyanin content of a major peak was converted into cyanidin-3-glucoside. Total anthocyanin content was determined by adding up the anthocyanin contents. As regards the extraction solvent, 50% acetic acid had the highest extraction efficiency (Table 1). The high extraction efficiency of 50% acetic acid, which does not contain alcohol, indicates that this solvent is appropriate for extracting anthocyanins. Anthocyanin contents in the freeze-dried residues were higher than those in the air-dried residues regardless of extraction solvent.

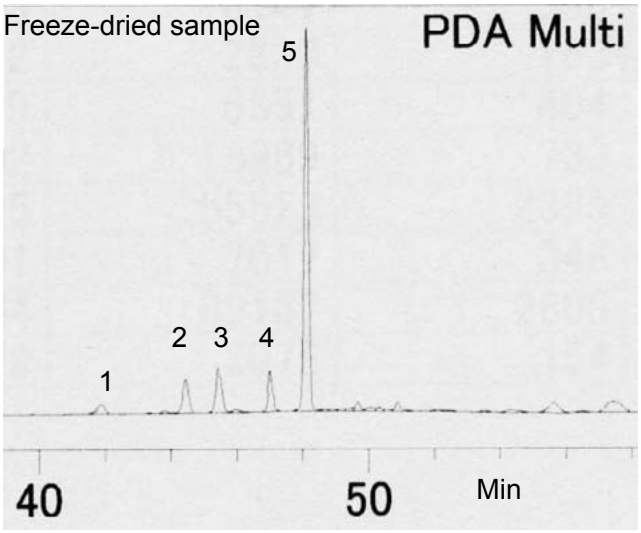

Fig. 1. HPLC chart of extract from Muscat Bailey A wine residue after red wine making. HPLC analysis was carried out with monitoring at 525 nm to selectively detect the following anthocyanins: 1, delphinidin-3-glucoside like substance; 2, cyanidin-3-glucoside like substance; 3, petunidin-3-glucoside like substance; 4, peonidin-3-glucoside; 5, malvidin-3-glucoside.

Anthocyanins have unstable chemical structures that are easily changed by ultraviolet irradiation, temperature, and pH. Studies have been conducted on an-

Table 1. Anthocyanin content of 'Muscat Bailey A' wine residue extracted with 50% acetic acid, 50% methanol containing 5% acetic acid, and methanol containing 5% formic acid, respectively.

	Control[z]	Freeze-dried	Air-dried
50% acetic acid	6.2[y]±0.8[x]	20.0±1.3	5.3±0.9
50% methanol containing 5% acetic acid	4.7±1.4	10.7±0.7	3.3±1.0
Methanol containing 5% formic acid	6.2±0.2	10.5±0.4	3.7±0.1

z: Raw red wine residue.
y: mg/100 g (grape skin in the red wine residue) fresh weight.
x: Mean±SE.

thocyanin stability in plants and food (Tsukui et al. 1999, Gradinaru et al. 2003, Ankit et al. 2010). Tsukui (1998) reported that the amount of anthocyanins extracted from strawberries was decreased by approximately 75% when the extraction was carried out at 90°C, pH 3.16 for 5 hr. Tsubosaka and Nishimura (1991) showed that the amounts of anthocyanins extracted from strawberries and grapes were decreased by approximately 25% and 45%, respectively, when the extraction was carried out at 50°C, pH 3.0 for two day. In the present study, the air-drying treatment required three day at 60°C. Therefore, anthocyanin content in the wine residue was decreased by air-drying, and freeze-drying was preferred for the efficient extraction of anthocyanins.

The control group (no addition of powdered red wine residue) showed high initial daily feed intake (Fig. 2). Daily feed intake for the 5% residue group was lower than those for the 1% residue group and the control on September 15; however, the daily feed intake for all the three groups was almost the same at the end of the study period. The smell and/or bitter taste of the powdered red wine residue might have initially influenced the appetite of egg-laying hens, indicating that the addition of more than 5% powdered red wine residue would decrease the appetite of egg-laying hens. Total protein and total cholesterol in blood and body weight were not significantly different among the three groups (Table 2). No glucose, fructose, sucrose, or sorbitol was present in the residue (data not shown) as those sugars were metabolized by yeast during fermentation. Liver triglyceride levels increase when carbohydrate levels in feed are high (Tanaka et al.

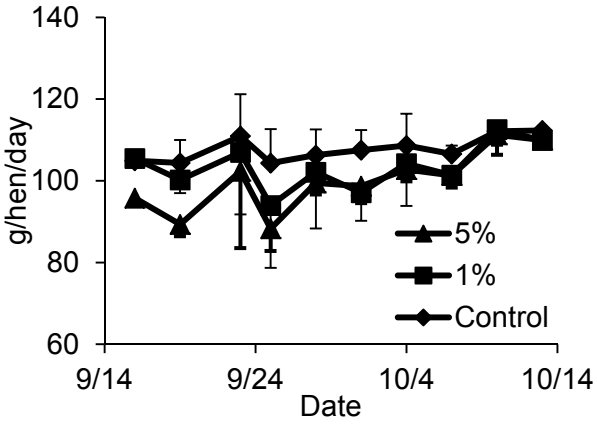

Fig. 2. Daily feed intake of egg-laying hens. Control, 1%, and 5% indicate 0, 1%, and 5% powdered red wine residue mixed with commercial feed, respectively. Vertical bars indicate SD.

Table 2. Blood total protein, total cholesterol, and glucose levels, body weights, and egg-laying rates of hens fed or not fed powdered red wine residue for 30 day.

	Total protein (g/dL)	Total cholesterol (mg/dL)	Glucose (mg/dL)	Body weight (kg)	Egg-laying rate (%)
5%[z]	5.8±0.3[y]	119.7±10.5	232.0±2.0	1.71±0.14	84.4±5.2*
1%	6.1±0.1	131.0±34.2	222.3±5.1*	1.68±0.16	81.9±4.3
Control	6.0±0.0	100.6±7.5	239.7±9.1	1.60±0.20	74.9±11.8

z: The percentages of powdered red wine residue.
y: Mean±SE.
* indicates significant difference from control at $p<0.05$ by the t-test.

1982); however, this grape residue from wine fermentation did not contain soluble sugars. Urquiage et al. (2015) reported that the intake of wine residue decreased blood glucose levels in humans as polyphenols and dietary fibers in the residue exerted a beneficial effect on health. In the present study, blood glucose level was decreased significantly in the 1% residue group. Nakatani and Goto (1961) reported changes of blood glucose levels between 148 and 188 mg/dL due to fasting, and Morita et al. (1973) indicated that the blood glucose levels of egg-laying hens were between 216 and 268 mg/L. The blood glucose levels observed in the present study were within the normal physiological range.

Egg weight for the 5% residue group was significantly increased mainly due to the eggshell becoming harder ($p<0.05$, Table 3). Furthermore, the egg-laying rate for this group was significantly higher ($p<0.05$, Table 2) than those for the 1% residue group and the control group. Ishiguro and Mikami (2009) fed hens white grape wine residue silage for four weeks. Their feed contained 30% grape wine residue (dry weight). The silage was used as the main feed and not as an additive. Feed intake did not change but body weight was decreased. Silage has high water content, whereas hens typically prefer dry feed. In regards to egg quality, yolk color became slightly bright yellow. In the present study, the reason why body weight was not decreased and egg-laying rate was increased might be the use of the red wine residue as an additive and not as the main feed. Feed containing carotenoids changes yolk color to a dark hue (Rowghani et al. 2006). There were hardly any carotenoids in the red wine residue. The reason why yolk color became slightly bright yellow was the presence of polyphenols in the red wine residue.

The present study showed that egg-laying rate and egg weight were higher in the 5% residue group than in the 1% residue group and the control group, whereas body weight as well as total protein and total cholesterol levels were not significantly different among the three groups. However, whether anthocyanins are the major contributor to these effects requires further study. The addition of powdered red wine residue to

Table 3. Egg quality after giving feed containing 1% or 5% powdered red wine residue for 30 day.

	Weight (g)	Egg shell		Yolk			Egg albumen	
		Strength (kg/cm)	Thickness (mm)	Color[z]	Diameter (mm)	Height (mm)	Diameter (mm)	Height (mm)
5%[y]	65.2±2.2[x]*	3.2±0.4*	0.46±0.1	9.8±0.4**	42.0±2.8	17.2±1.2	70.0±5.3**	7.1±1.3
1%	63.5±2.8	3.0±0.1	0.43±0.1	10.1±0.6*	42.3±1.0	17.4±0.9	74.7±3.4*	7.6±0.6**
Control	61.0±2.5	2.7±0.3	0.43±0.1	10.9±0.4	42.3±1.4	17.8±0.9	79.1±3.4	6.6±0.5

z: Yolk color fan value (Roche Co.).
y: The percentages of powdered red wine residue.
x: Mean±SE.
* and ** indicate significant difference from control at $p<0.05$, 0.01 by the t-test, respectively.

feed had no influence on the health of egg-laying hens even though it improved egg-laying rate. To conclude, we found that powdered red wine residue has potential for use as natural feed additive.

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採卵鶏用飼料への赤ワイン残渣の乾燥粉末の添加による鶏卵生産性への影響

中尾義則[1]*・畑 知哉[1]・奥田 徹[2]・林 義明[1]

[1]名城大学農学部附属農場

[2]山梨大学ワイン科学研究センター

要約

ワイン醸造残渣に含まれるブドウ果皮と種子は廃棄物として処理されることが多いが、その果皮と種子にはアントシアニンなどの有効成分が含まれている。そこで、ワイン残渣が採卵鶏用の飼料添加剤としての利用可能性かどうかを検討した。凍結乾燥したワイン残渣は風乾したワイン残渣より機能性成分の一つであるアントシアニン含量が高く維持された。この凍結乾燥試料を粉末にし、市販の採卵鶏用飼料に 1%または 5%添加した。試料の摂取量は、5%区では給餌開始直後は減少傾向にあるが、約 2 週間後から増加し、30 日後には無添加区と差はなかった。卵重、産卵率、卵殻強度は 5%添加区で有意に高かったが、卵黄色がやや薄く明るくなった。1 か月後の血中のグルコース値に有意差はあったものの採卵鶏の標準値内となった。凍結乾燥処理した赤ワイン残渣は採卵鶏の産卵率向上に有効であることが示された。