

Effect Of Replacing Different Levels Of Corn With Raisin By-Product On Production Properties And Digestibility In Old Laying Hens (Hy-Line Strain W-36)

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Abstract

An experiment was performed to investigate the possibility of replacing different levels of corn with raisin by-product (Shekarak) and its effect on production properties and digestibility in laying hens (Hy-Line W-36) after molting. A completely randomized design with a factorial arrangement of 2*5 with 5 levels of by-product (0, 5, 10, 15 and 20%) and 2 enzyme levels (0 and 0.03%), 8 replications and 4 hens per replication and a total of 320 laying hens (Hy-line W-36) at 78-week-old in three 21-day experimental periods (A total of 63 days). The results of this study showed that feed consumption in the experimental group, 10% by-product with 0.03% enzyme significantly increased ($P<0.05$) and in the first and second periods, the average feed consumption and egg production percentage compared to the third period has increased significantly. The percentage of egg production in the control group and 0.03% phytase enzyme was increased ($P<0.05$) significantly, also the control group with 5% by-product, 10% by-product and the experimental group 10% by-product with the phytase enzyme increased the ability of protein digestion. Also, the effect of period on egg quality properties was significant and the shell weight in the first and third periods and yolk color index in the first period increased significantly ($P<0.05$). In this study, the use of by-product at the level of 10%, if it replaces corn at the level mentioned, in addition to improve the quality of egg, it also effects in reducing the feed costs in laying hens.

Keywords: Egg, Performance, Raisin By-Product, Laying Hens.

Introduction

The poultry industry has faced several challenges in recent years, including rising input prices and limited supply of corn and soybeans (Mazaheri et al. 2014). Since the most important costs of poultry farming units are related to feed, so any nutritional measures that lead to a reduction in feed costs can also be effective in the profitability of poultry farming. One way to reduce feed costs in poultry industry is to replace the main feed items with low-priced items (Nobakht. 2013). In Iran, about 90% of soybean meal and 55% of corn consumed in diet of laying hens are imported, and in studies, about 78% of the cost eggs is related to feed prices; on the other hand, the food and health quality of imported raw materials or domestic production has many fluctuations that have strongly affected production efficiency and health of the bird (Mazheri et al. 2014). One of the nutritional strategies in laying hens is use of agricultural waste (Nobakht. 2013). By-product is one of these wastes that is waste of raisin in raisin factories. Raisin, ripe and dried fruits of different grape belong to species of *Vitis vinifera* L. related to Vitaceae family (Chamorro et al. 2013). Raisin by-product consists of small pieces of raisin that is obtained after separation of factory packaging (Alipour et al. 2010). Iran ranks third for raisin production in the world and about a quarter of all Iranian raisins are produced in Malayer (Yari et al. 2015). In fact, the production of raisin by-product is about 135 tons per year, which is disposed in the environment without any processing (Besharati and Taghizadeh. 2009). Using raisin as a feed for livestock and poultry, while preventing environmental pollution due to its nutrient content, can also provide part of their nutritional needs (Alipour et al. 2010). Use of raisin waste (by-product) most studies have been done on ruminants and no specific research has been done on poultry and specifically laying hens. Therefore, the purpose of this study is use waste of raisin factories (by-product) as substitute for corn and the effect of phytase enzyme in its better utilization on performance characteristics in laying hens.

Materials and Methods

Housing and experimental diets

This experimental was conducted in a completely randomized design with 2 × 5 factorial arrangement with 5 levels of by-product and 2 levels of enzyme, in 10 experimental groups, 8 replications and 4 hens per replication (per cage) and a total of 320 laying hens at 78-week-old with an average weight of 1450 g, was designed in the poultry farm in the Department of Animal Sciences of Malayer University (the dimensions of each cage in this study had 35 cm high and 46 cm wide). A ten days was done to adaptation of chickens to proposed diets. After this period, the main stage of this experimental with designing experimental diets, respectively, includes 1: basic diet (diet based on corn and soybeans) without by-product and phytase enzyme, 2: diet with 0.03% of phytase enzyme, 3: diet with 5% by-product, 4: diet with 5% by-product and 0.03% phytase enzyme, 5: diet with 10% by-product, 6: diet with 10% by-product and 0.03% phytase

enzyme, 7: diet with 15% by-product , 8: diet with 15% by-product and 0.03% phytase enzyme, 9: diet with 20% by-product , and 10: diet with 20% by-product and 0.03% phytase enzyme. The phytase enzyme in this study had trade name of PHYTASE 5000 (SMIZYME PT PHYTASE, CHINA). During the rearing period, the birds had free access to water and food, and the experimental diets were adjusted according to recommendations of Commercial Laying Hens Manual (HY-LINE Strain W-36, 2013) at 78week-old using WUFFDA software (table 1).

The lightening program was also based on the guide of breeding white leghorn laying hens (HY-LINE strain W-36, 2013), in the form of 16 hours of light (10 lux was adjusted using a luxmeter), and 8 hours of darkness were set. Raisin by-product was first collected from raisin preparation factories in Malayer city and the amount of its chemical compounds was analyzed in the laboratory of Malayer University Department of Animal Sciences in order to prepare diet, (AOAC 2000), (table 2).

Study of performance properties

In order to evaluate performance properties, after the end of acclimatization period, every day after feeding, operations related to collecting, counting and weighing eggs were performed and the end of each week, the remaining feed from previous week was collected and weighed in each repetition and then was replaced with fresh feed and information was recorded weekly. The eggs of each experimental unit were collected, counted and recorded daily at 4 pm. After collecting and counting the eggs, they were weighed and recorded with a scale with an accuracy of one gram. The number of possible casualties per replicate was recorded daily.

Study of quantitative and qualitative properties of eggs

For this purpose, at the end of each experimental period, among the eggs produced on last day of each period, 2 eggs from each replication were randomly selected, numbered and transferred to laboratory of Jihad Agricultural Research Center of Karaj. In the first stage, the factors of egg weight, length and width, egg white and yolk height, shell thickness and strength and egg shell weight were determined. Then, to determine the egg white quality, an egg was collected on last day of each experimental period and two eggs from each replicate were used to determine the egg white quality. The Egg Multi Tester (EMT-5200) was used to determine quality of whites. Units are actually indices in which egg white height is corrected for egg weight (Kara and Kocaoglu-Guclu. 2012). To determine the shell thickness of Thickness Gauge Ultrasonic Echometer device (ECHOMETER 1062-MAKS-USA) was used and finally the shell thickness in millimeters was considered for each egg (Carlm. 1984). Egg Shell Force Gauge (MODEL-II-MAKS-USA) was used to measure egg shell resistance. To do this, the egg is placed in the desired location of the machine with its wide end facing upwards, then it is cracked by making a blow to its end, and thus the amount of force applied in kilograms per square centimeter is recorded in the device (Kara and Kocaoglu-Guclu. 2012). At the end of each experimental period, two eggs were randomly selected from each replication and after weighing, their specific weight were determined using the immersion method (Floating Method) in a solution of brine with concentrations of 1.064, 1.068, 1,

1.072, 1.076, 1.08, 1.084, 1.08, 1.092, 1.096 and 1.1 mg / ml. In this method, different concentrations of Salt water solutions were prepared using a densitometer in plastic buckets and the concentration the contents of each bucket was written on it, and finally the buckets were arranged next to each other and the eggs were arranged in order. Chickens sampled from each experimental unit were first placed inside the thinnest of them. If any of the eggs is floated on the water, the concentration was recorded as the specific weight of the eggs, and if it was not float, it was taken out of the bucket and placed in next thicker solution. At the end, the egg weight of each replicate was calculated (Kara and Kocaoglu-Guclu. 2012). Yolk color was also measured by the ROCHE unit; color Fan Yolk was used in this study (Carlm. 1984).

Measurement of ileal protein digestibility

To evaluate the ileal digestibility of protein, one chicken was randomly slaughtered from each replicate and contents of the ileum area (2 cm after the macula up to 4 cm above the ileoscal valve) and was gently emptied into a special container and placed for -20 °C for further experiments. Samples of ileum digest and feed were dried at 55 °C by mill, powder and sieved through a 0.2 mm whole for greater homogeneity of the material. The feed samples and ileum contents were measured based on AOAC, 2000 to determine the ileal digestibility of crude protein.

Data analysis and statistical model

The obtained data were performed in a completely randomized design with 2 × 5 factorial arrangement with 5 levels of by-product and 2 levels of enzyme, 8 replications and 4 pieces of chicken in each replication. The statistical model used for the design using SAS (2001) software version 9.1 and mixed procedure was as follows and for properties that were measured periodically (once every 21 days) during the experimental, data were analyzed repeatedly. The means were compared with Tukey test.

$$(1) \quad Y_{ijk} = \mu + A_i + B_j + (AB)_{ij} + e_{ijk} + Se_{ijk}$$

In the above formula, Y_{ijk} is the observe k related to the level j of factor B and level i of factor A , μ : mean effect, A_i is the effect of level i of factor A , B_j is the effect of level j of factor B , $(AB)_{ij}$ is the interaction of factor A and Factor B , e_{ijk} is a test error and Se_{ijk} is a sampling error.

Results and Discussion

Performance parameters

The effect of different experimental groups on the performance of laying hens is reported in Table 3. The results showed that experimental group receiving 10% by-product significantly ($P < 0.05$) increased feed intake compared to group containing 20% by-product, 5% by-product and control group. In this study, the effect of period on performance of laying hens was studied and it was found that in the first and second periods, average feed intake and egg production percentage increased significantly ($P < 0.05$) compared to the third period Table 3.

The effect of adding experimental groups, different levels of by-product and different periods on the performance of laying hens. In each column, the means with dissimilar letters have a significant difference ($P < 0.05$). In line with the results of present study and in accordance with the previous research that was reported, the use of 4% grape pomace along with multivalent in laying hens significantly increased egg weight, egg mass and egg quality properties (Carlm. 1984). In contrast, it has been stated that adding grape pomace at the level of 1 g/kg of feed does not have a significant effect on performance and growth (Cross et al. 2008). Another study reported that the addition of a multivalent enzyme to the diet of laying hens did not have a positive effect on performance (Kara and Kochoaglu-Goklu. 2012). Since the production of egg masses depends on the weight of egg and its production percentage, these parameters have been improved by using grape pomace. In the present study, despite the lack of significant difference in feed intake and feed conversion ratio, the highest feed intake was observed 5% by-product and 0.03% of phytase enzyme in the experimental group that according to previous findings, the presence of proanthocyanins in grape pomace has antioxidant properties and is more than 50 times higher than vitamin E, which improves metabolism in chickens (Sayago-Ayerdi et al. 2009).

Antioxidants in grape pomace improved the physical condition of the gastrointestinal tract by preventing the oxidation of oxidation-sensitive nutrients and improved digestion and absorption of nutrients, thus improving poultry performance (Carlm. 1984). The results of several studies show that phenolic compounds in grapes improved intestinal microbial population and affect the production of microbial metabolites, increase nitrogen digestibility, increase digestion and absorption of nutrients, and improve performance properties (Shen. 2009). Antimicrobial properties in grape extract, which has the greatest effect against *E.coli* and improves performance of gastrointestinal tract of poultry and improves the absorption of nutrients by eliminating harmful intestinal bacteria (Perumalla et al. 2011). In another study, grape pomace increased antioxidant activity in birds, which increased growth hormone concentrations, reduced stress, and increased immune function, all of which improve performance (Liu et al. 2014). The use of grape pomace in diet increases the height of intestinal villi and increases the level of absorption and absorbs more nutrients, which ultimately improves production performance (Viveros et al. 2011). On the other hand, the lack of significant effect of adding by-product in the present study can be attributed to some feed conversion ratio, average egg weight and egg mass in laying hens during the breeding period. The effect of different experimental groups on the quality properties of eggs is presented in table 4.

The results of this study showed that the effect of period on egg quality properties was significant ($P < 0.05$) and shell weight in the first and third periods of yolk index in the first period was significantly higher ($P < 0.05$). In this study, the interactions of by-product and period were investigated and it was observed that the units in the experimental group of by-products increased by 5% by 20% in the second period and in the third period, it increased significantly ($P < 0.05$), compared to the other experimental groups. In this study, it was found that different levels of by-product, different levels of enzyme, interactions of by-product and enzyme and interactions of enzyme and period did not show any significant effect on the quality of eggs ($P < 0.05$).

In each column, the means with dissimilar letters have a significant difference ($P < 0.05$). Line with the results of the other researchers, the use of grape pomace has improved the quality properties of eggs (Kocaoglu-Guclu and Kara. 2012). Another study reported the use of herbal supplements improves production performance in poultry (Garcia et al. 2006). In another study, it was reported that the use of commercial multi enzymes increases egg weight in laying hens (Scheideler et al. 2005). The results of another study showed that the addition of commercial enzymes to the diet of laying hens caused a significant increase in egg specific weight (Gordon and Roland. 1997). No side effects of similar nutrients (tomato pomace, rice, bran) have been reported on egg quality properties in laying hens (Nobakht et al. 2008). Research shows that herbal compounds increase the thickness of the eggshell (Radwan et al. 2008). In contrast, a study reported that the use of grape supplements at the level of 0.5, 1.5 and 1.5% in quail diet did not have a significant effect on egg units and quail egg weight, shell thickness and yolk color of quail eggs (Silici et al. 2011). The results of another study showed that diets containing herbal supplements did not have a significant effect on the quality properties of eggs (Liu et al. 2014). Consumption of phytase enzyme in laying hen diets did not have a significant effect on eggshell quality and egg weight (Safamehr et al. 2010). The main use of commercial multi-enzymes in poultry diets is to supplement the effects of internal enzymes in reducing the effects of food inhibitors and increasing the digestion and absorption of nutrients in diets, which in turn improves the quality properties of eggs (Safamehr et al. 2010). The use of multi-enzyme increases digestion, absorption and transfer of more pigments of food items into the egg and improves the yolk color index. Also, grape pomace along with multi-enzyme and improving digestibility and absorption causes more transfer of calcium to the ovarian duct, more of it is deposited in the shell and increases the weight of the egg (Safamehr et al. 2010). Polyphenols are part of plant anti-nutrients and are highly active chemical compounds that react with dietary protein (Antunes et al. 1995). In one study, researchers found that grape pomace reduced the activity of protein-degrading enzymes, thereby reduced protein digestibility in diets with high grape pomace (Moreno et al. 2003). Researchers have also attributed the increase of digestibility in diets containing grape pomace to the antioxidant activity of grape pomace, which is directly related to its consumption level in poultry diets (Brenes et al. 2010). In general, it can be said that herbal products rich in phenolic compounds have antimicrobial effects and have digestive stimulating properties and improve the use of nutrients in poultry feed (Chamorro et al. 2013). Antioxidants in the pomace prevent oxidation of oxidation-sensitive nutrients and potassium content with providing the required potassium and proper electrolyte balance and their pectin content with improving the physical condition of the gastrointestinal tract causes more digestion and absorption of nutrients and therefore improves production properties. (Chamorro et al. 2013). In fact, herbal compounds increase the secretion of various digestive enzymes and improve the condition of the intestine to absorb various nutrients, including calcium, and increases the weight of the units, which indicates the quality of egg whites; the higher consistency of the white, the higher its height and the greater the number of eggs. A protein called oocyte causes the gel structure in egg whites; so that with the increase of oocyte, egg whites increase. Herbal compounds such as by-product and grape

pomace, which have antioxidant properties, can increase the thickness of the shell. Herbal products contain xanthophyll pigment. Their use in poultry diets causes the transfer of xanthophyll pigments to eggs and their deposition in the yolk, causes the yolk to change color. The color of the egg yolk depends on the composition of the poultry diet. Because plants contain xanthophyll pigments and carotenoids, they cause the egg yolk to become more pigmented. On the other hand, fructo-oligosaccharides in herbal compounds have stimulating effects on digestive enzymes of the stomach, pancreas and intestinal mucus, which lead to improved digestibility and ileal absorption and increase the absorption of these pigments (Radwan et al. 2008). In another study, herbal products were reported to have stimulant effects on the digestive system of poultry. Compounds in plants increase the production of digestive enzymes and increase the efficiency of utilization of dietary nutrients by improving liver function and affect egg weight (Williams et al. 2001). In this study, different levels of by-product and enzyme did not have a significant effect on the quality properties of eggs, which may be due to low levels of enzyme used or production capacity of chickens. It may also be due to the breeding conditions, the composition of the basal diet and the level of by-product used in their diet (Kara and Kocaoglu-Guclu. 2012).

Protein digestibility

The effect of different experimental groups on the percentage of protein digestibility is presented in table 5.

In this study, the effect of different levels of by-product in comparison with the control group on protein digestibility was investigated and the results showed that the control group, 5% by-product and 10% by-product significantly increased protein digestibility compared to the experiment group containing 15% by-product and 20% by-product. In the study of the effects of different levels of by-product with enzyme, it was found that the experiment group containing 10% by-product with enzyme significantly increased protein digestibility ($P<0.05$). The effect of different experimental groups on the percentage of protein digestibility showed that the control group, with 5% by-product and 10% by-product significantly increased protein digestibility ($P<0.05$) compared to the experimental groups containing 15% by-product and 20% by-product. In the study of the effects of different levels of by-product with enzyme, it was found that the experiment group containing 10% by-product with 0.03% enzyme significantly increased protein digestibility in comparison with other experimental groups ($P<0.05$). In a study with the same results of the present study, the researchers found that the addition of grape pomace increased protein digestibility in broilers (Brenes et al. 2010). In another study, the positive effects of polyphenols have been reported on the digestibility of poultry (Shi et al. 2004). In another study, researchers found that the use of 6% grape pomace in poultry feed did not have a negative effect on the digestibility of proteins and amino acids (Chamorro et al. 2013). The use of 5 g / kg grape pomace in poultry diets significantly reduced the digestibility of proteins and amino acids (Brenes et al. 2010). Another study examined the effect of vitamin E and grape pomace on protein digestibility in broilers and reported that the ileal digestibility of proteins and amino acids in diets containing grape pomace and vitamin E was not affected (González-Centeno. 2010). The results of

this study showed that with increasing the level of by-product in the diet of laying hens, the digestibility of protein is reduced due to the pectin and tannins in grape pomace. 37% to 54% of the cell wall of grape pomace is made of polysaccharides, which reduce the digestibility of proteins (González-Centeno. 2010). In each column, the means with dissimilar letters have a significant difference ($P<0.05$). Structure and weight of polyphenols in grape pomace play an important role in protein digestion. Tanins in grapes pomac are able to bind to proteins before being broken down by pancreatic enzymes (Yari et al. 2015).

Conclusion

In general, according to the results of the present study, it can be stated that in laying hens, the use of by-product at the level of 10% if it replaces corn at the mentioned level, in addition to improve the quality of eggs, has been effective in reducing feed costs of laying hens.

Conflict of Interest Declaration

Authors have no conflict of interests to declare.

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Table 1: ingredients and chemical compositions of basal and experiment diets

Ingredients (%)	Raisin by-Products
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	0%	5%	10%	15%	20%
Corn	57.9	52.2	46.1	40.3	34.4
Soy bean	26	26.2	27.1	27.7	28.3
Bran	3	3	3	3	3
Vegetable oil	1.6	1.8	2	2.2	2.4
Oyster shell	3	3	3	3	3
Lime stone	6	6	6	6	6
DCP	1.4	1.4	1.4	1.4	1.4
Salt	0.3	0.3	0.3	0.3	0.3
Sodium Bicarbonate	0.3	0.3	0.3	0.3	0.3
Vitamin and Mineral premix	0.5	0.5	0.5	0.5	0.5
Methionine	0.11	0.11	0.11	0.11	0.11
Lysine	0.04	0.04	0.04	0.04	0.04

¹Vitamin and mineral mix supplied the following per kilogram of diet: vitamin A, 11,000 IU; vitamin D3, 1,800 IU; vitamin E, 11 mg; vitamin K3, 2 mg; vitamin B2, 5.7 mg; vitamin B6, 2 mg; vitamin B12, 0.024 mg; nicotinic acid, 28 mg; folic acid 0.5 mg; pantothenic acid, 12 mg; choline chloride, 250 mg; Mn, 100 mg; Zn, 65 mg; Cu, 5 mg; Se, 0.22 mg; I, 0.5 mg; and Co, 0.5 mg, Anti-oxidant 4g/kg.

Table 2 - Chemical composition of by-product (in terms of dry matter percentage)

Composition	Phosphorus (%)	Calcium (%)	Ash (%)	Ether Extract (%)	Gross Energy (Kcal/Kg)	Crude Protein (%)	Dry Matter (%)	Tannins
Raisin by-products	0.10	1.91	0.20	1.60	3876	8.20	91.90	6.50

Table 3. The effect of adding experimental groups, different levels of by-product and different periods on the performance of laying hens

Raisin Byproduct (%)	Egg mass (g/hen/day)	Average egg weight (g)	Hen day egg production (%)	Feed conversion ratio	Average feed intake (g/hen/day)
0	32.94	65.15	55.89	2.97	105.7 ^d
5	31.92	64.76	50.89	3.38	108.68 ^c
10	37.22	65.15	52.59	3.19	111.64 ^a
15	33.28	66.37	52.03	3.55	110.10 ^{ab}
20	30.48	65.61	53.57	3.22	102.9 ^{bc}

P-value	0.31	0.40	0.56	0.34	0.0001
SEM	2.28	0.61	2.19	0.19	0.33
Enzyme level (%)					
0	34.06	65.62	53.08	3.28	108.6 ^b
0.03	32.28	65.21	52.88	3.22	110.1 ^a
P-value	0.38	0.44	0.91	0.12	0.01
SEM	1.44	0.38	1.38	0.69	0.21
Periods					
1	33.91	65.98	54.58 ^a	3.22	109.45 ^a
2	34.66	65.60	54.82 ^a	3.20	109.91 ^a
3	30.93	64.67	49.55 ^b	3.33	107.2 ^b
P-value	0.29	0.13	0.04	0.77	0.0002
SEM	1.77	0.47	1.69	0.14	0.26

In each column, the means with different superscript have a significant difference ($p < 0.05$).

Table 4. The effect of experimental groups, different levels of by-product and different periods on the quality properties of eggs in laying hens

Raisin by products (%)	Yolk color index (Rosche)	Shell weight	Haugh unit score	Egg weight (g)	Shell strength (kg/cm ²)	Shell thickness (mm)
0	4.45	5.99	88.16	64.61	3.24	0.28
5	4.58	5.86	89.12	65.79	3.26	0.28
10	4.54	5.65	91.07	64.80	3.26	0.28
15	4.70	5.65	88.43	65.49	3.30	0.28
20	4.70	5.80	90.31	66.07	3.25	0.27
P-value	0.36	0.64	0.83	0.76	0.38	0.44
SEM	0.10	0.15	2.08	0.91	0.02	0.004
Enzyme levels (%)						
0	4.63	5.80	89.10	64.27	3.27	0.28
0.03	4.56	5.71	89.74	65.98	3.26	0.28
P-value	0.47	0.40	0.73	0.12	0.63	0.59
SEM	0.06	0.07	1.13	0.58	0.01	0.003
Periods						
1	4.90 ^a	5.81 ^a	90.56	66.40	3.25	0.28
2	4.27 ^c	5.44 ^b	89.90	64.14	3.25	0.28
3	4.62 ^b	6.10 ^a	87.80	65.22	3.26	0.27
P-value	0.001	0.001	0.64	0.17	0.87	0.43

SEM	0.08	0.11	1.60	0.83	0.01	0.003
In each column, the means with different superscript have a significant difference ($p < 0.05$)						
Table 5. The effect of different experimental groups on the percentage of protein digestibility in laying hens						
Raisin byproducts (%)			Protein digestibility (%)			
	0				87.25 ^a	
	5				87.75 ^a	
	10				90.50 ^a	
	15				77.37 ^b	
	20				72.75 ^b	
	P-value				0.004	
	SEM				2.03	
Enzyme level (%)						
	0				84.05	
	0.03				81.20	
	P-value				0.06	
	SEM				1.28	

In each column, the means with different superscript have a significant difference ($P < 0.05$).