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Technical Note

Grape (Vitis Vinifera) Pomace Flour as Pigment Agent of Quail Eggs

■Author(s)

Fróes HG[I]
Jácome IMTD[I]
Tavares RA[I]
Garcia RG[II]
Domingues CHF[III]
Bevilaqua TMS[II]
Martinelli M[I]
Naas IA[II]
Borille R[II]

[I] Animal Science Department, Federal University of Santa Maria, UFSM/CESNORS, Palmeira das Missões, RS, Brasil
[II] College of Agrarian Sciences, FCA/UFGD, Dourados, MS, Brasil
[III] College of Administration and Economic Science, FACE/UFGD, Dourados, MS, Brasil

■Mail Address

Corresponding author e-mail address
Carla Heloisa de Faria Domingues
Rua Cláudio Goelzer n° 1500, Residencial Itamaracá, Bairro Parque Alvorada, CEP: 79823-352, Dourados, MS, Brasil.
Tel: (67) 3410-2063
Email: carlafariadomingues@hotmail.com

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ABSTRACT

The aim of the study was to evaluate the effect of dietary grape pomace flour (GPF) inclusion on the performance and egg quality of European quails (*Coturnix coturnix coturnix*) in lay. One hundred ninety-two (192), 42-day-old quails were distributed in a completely randomized design with four treatments (0, 2, 4 and 6% grape pomace flour inclusion) with six replicates of eight birds each. The performance parameters egg weight (g), average feed intake (g/bird), total feed intake (g/bird), egg production (%) and feed conversion ratio (kg/dozen) were evaluated, as well as egg quality traits Haugh unit, albumen weight (g), yolk weight (g), eggshell thickness (mm), egg specific gravity (g/cm^3), and yolk pigmentation. There was no effect (p>0.05) of dietary GPF levels on egg production, feed intake, or feed conversion ratio. Egg weight, albumen weight, and egg specific gravity linearly decreased (p<0.05) as GPM levels increased in the diet. Haugh unit and eggshell thickness were not affected (p>0.05) by the treatments. A quadratic effect (p<0.05) was observed for yolk weight, with an estimated maximal inclusion level of grape pomace flour of 4.18% to obtain the highest yolk weight. A quadratic effect (p<0.05) was also observed on yolk pigmentation, with an estimated minimal level of 3.2% for this parameter. The minimal inclusion of 3.2% of grape pomace flour in quail diets influenced the egg yolk pigmentation, demonstrating its efficiency as a pigmentation additive.

INTRODUCTION

New management technologies adopted by the industry have allowed the growth of Brazilian quail production. There have also been advancements in the commercialization of quail products, such as eggs and meat, supported by improved genetic strains. Increased activity has been observed in academic studies in the areas of breeding, nutrition, and husbandry (Keener *et al*., 2000; Ipek *et al*., 2003). Egg production remains the primary purpose of quail rearing. In 2015, Brazilian quail egg production reached nearly 340 million dozen, showing an increase of 20.2% over 2014 (IBGE, 2015), evidencing the economic importance of this product.

Consumers associate yolk color with egg quality and adequate nutrition, and prefer yellow-orange yolks (Hernández *et al.,* 2001). Pigments and colorants are sometimes added to poultry feeds to enhance yolk color. These additives may be of natural or synthetic origin. In poultry, canthaxanthin, a synthetic red carotenoid pigment, is predominantly used. However, there is a worldwide consumer trend of preferring natural pigment sources and rejecting synthetic ones.

Natural pigments have already been applied in poultry production, and demonstrated satisfactory results. Zahrrojian *et al*. (2013) studied the effects of the dietary addition of marine algae (*Spirulina platensis*) at 0, 1.5, 2.0 and 2.5% on egg quality and production performance of laying chickens and observed a significant increase in egg yolk color when hens were fed with *Spirulina*. The authors suggest the addition of 2.0-2.5% Spirulina to the diet to produce an aesthetically pleasing yolk color. Mirzah & Djulardi (2017) conducted an experiment to determine the effect of marigold (*Tagetes erecta*) flower extract (MFE) as a feed additive on performance and egg quality of laying Japanese quails (*Coturnix coturnix japonica*). The authors concluded that 15 ppm MFE in the diet improved the performance, improved egg quality, reduced egg cholesterol levels and increased egg yolk color of quail eggs.

No studies using grape pomace flour as a feed additive for quails were found in literature. The grape is a natural pigment and may be possibly be used an alternative to synthetic additives. Grape pomace is the residue left from fruit processing and contains the skins, seeds, and pulp that remains after crushing grapes for wine and juice production (Bagchi *et al*., 2000; Shrikhande, 2000; Llobera & Canellas, 2007). This by-product contains large quantities of phenolic compounds – around 20-30% in the skin and 60-70% in the seeds – which include anthocyanins, which are pigments that give the purple color to grapes and their products (Monrad *et al*., 2010).

The aim of the present study was to evaluate the effects of using grape pomace flour on performance and egg quality of quails.

MATERIAL AND METHODS

The experiment was conducted in the Poultry Section of the Department of Animal Science and Biological Sciences, Universidade Federal de Santa Maria, Palmeira das Missões, RS, Brazil, located at 634m altitude, 27° 53′ 58″S latitude, and 53° 26′ 45″ W longitude. The experimental protocol was approved by the Animal Ethics Committee (CEUA) of the Universidade Federal de Santa Maria (UFSM), Palmeira das Missões, Brazil, under protocol number 015/2015.

A total of 192 European quails (*Coturnix coturnix coturnix*), with ±180g initial weight and 42 days initial age, were used in the experiment. The quails were housed in cages (eight birds per cage), measuring 0.50m x 0.50m x 0.15m and equipped with automatic drinkers and feeders, in an environmentally-controlled room. Water was available *ad libitum*, and the feed was provided twice daily at an allowance 30g/bird/day during the experimental period. A continuous lighting program of 16 hours of light daily was applied using artificial lighting. The quails were distributed in a completely randomized design with four treatments with six replicates (cages) of eight birds each, totaling 24 cages. The experiment lasted 84 days and was divided into three periods of 28 days each.

The experimental diets (Table 1) were based on corn and soybean meal and formulated according to the recommendations of Rostagno *et al*. (2011). The variable portion consisted grape pomace flour added at the expense of kaolin, according to treatment. The experimental treatments consisted of a basal diet and basal diets with the addition of 2%, 4%, or 6% grape pomace flour.

The pomace from a *Vitis vinifera* grape variety was obtained from wine industry of Santa Maria, state of Rio Grande do Sul. The pomace was weighed and spread in a thin layer on perforated trays. The pomace was then placed in a greenhouse with air circulation at 70 °C for 2h for drying. After drying, the pomace was ground in an analytical mill (Table 2). The grape pomace flour chemically analyzed for moisture, ash, crude protein, ether extract, and crude fiber contents according to the methods of the AOAC (2007). Moisture content was determined by drying the samples in an oven at 105 °C ± 5 °C until constant weight. Ash content was obtained by burning the samples in a muffle at 550 °C until a constant weight. Crude protein content was determined by the Kjeldahl method, and the obtained nitrogen content was multiplied by a factor 6.25 to convert the result into crude protein. Ether extract content were obtained by the Soxhlet extraction method. Crude fiber content was determined by the enzymatic-gravimetric method.

Performance and egg quality were evaluated for each 28-d period. Performance parameters included egg weight (g), average daily feed intake (g/bird), total feed intake (g/bird), egg production (%), and feed conversion ratio (kg feed/dozen eggs). On the last day of each 28-day period, four eggs replicate were collected to determine the following egg quality traits: albumen weight (g), yolk weight (g), eggshell thickness (mm), egg specific gravity (g/cm^3), yolk pigmentation, and Haugh units, as 100 log (h + 7.57 – 1.7 W 0.37), where: h = albumen height in mm and W = egg weight in g.

Table 1 – Ingredients and calculated nutritional composition of the experimental diets.

Ingredients	Grape pomace flour levels (%)			
	0	2	4	6
Corn	48.60	48.60	48.60	48.60
*Commercial concentrate for laying quails (41%CP)	39.89	39.89	39.89	39.89
Dicalcium phosphate	1.72	1.72	1.72	1.72
Limestone	3.44	3.62	3.79	3.79
Salt	0.35	0.18	-	-
Kaolin	6.00	4.00	2.00	-
Grape pomace flour	-	2.00	4.00	6.00
TOTAL	100	100	100	100
Nutritional composition				
Metabolizable energy (kcal/kg)	2800	2800	2800	2800
Crude protein (%)	20.19	20.28	20.37	20.46
Digestible lysine (%)	0.97	0.97	0.97	0.97
Digestible methionine (%)	0.47	0.47	0.47	0.47
Calcium (%)	3.17	3.23	3.30	3.30
Total phosphorus (P) (%)	1.06	1.06	1.07	1.07
Available P (%)	0.97	0.97	0.97	0.97
Digestible P (%)	0.27	0.27	0.27	0.27
Potassium (%)	0.14	0.14	0.14	0.14
Sodium (%)	0.15	0.15	0.15	0.22
Chloride (%)	0.24	0.13	0.03	0.03
Crude Fiber (%)	4.83	5.21	5.59	5.97
Ether extract (%)	2.57	2.61	2.65	2.69
Ash (%)	14.85	14.88	14.91	14.94

*Commercial Concentrate for laying quails (CP 41%), with mineral and vitamin premix. Guaranteed levels = Mg: 0.01 %; Mn: 108 mg/kg; Zn: 57.6 mg/kg; Fe: 72 mg/kg; Cu: 14.4 mg/kg; I: 1.0 mg/kg; Se: 0.4 mg/kg; A vit.: 14400 UI/kg; D3 vit.: 2880 UI/kg; E vit.: 36 UI/kg; K3 vit.: 2.32 mg/kg; B1 vit.: 2.32 mg/kg; B2 vit.: 7.2 mg/kg; Pantothenic acid: 14.4 mg/kg; B6 vit.: 4.32; B12 vit.: 23.04 mcg/kg; Folic acid: 0.72 mg/kg; Choline: 160 mg/kg.

Table 2 – Chemical composition of the grape pomace flour included in the experimental diets.

	%/kg
Humidity	8
Ash	4.7
Crude Protein	4.5
Ether extract	2
Crude fiber	19

Feed residues were weighed during the last three days of each cycle, and total feed intake was calculated as the difference between total feed offer and feed residues. Feed conversion ratio in kg/dozen eggs was calculated as total feed intake (kg) divided by the dozens of eggs produced in each experimental unit. Egg production, eggs were collected daily for 17 hours per 28-d period, and at the end of each period the total production was counted, and the percentage relative to the total period production of total eggs produced was calculated.

Egg specific gravity was determined by immersing eggs in graded NaCl solutions with densities ranging between 1.065 and 1.100 g/cm^{3}at0.005 gradients, according to the methodology of Moreng and Avens (1990). Yolk pigmentation was evaluated using the colorimetric scale of the DSM yolk color fan (DSM® Nutritional Products,) of 1 to 15 points corresponding increasing pigmentation of egg yolks.

Data were analyzed by analysis of variance at 95% confidence level, and submitted to polynomial regressions against GPF levels, using the statistical software Minitab 16.1 (2010).

RESULTS AND DISCUSSION

There was no effect (p>0.05) of grape pomace flour levels on egg production, feed intake (both average and total), or feed conversion ratio. According to Leeson & Summers (2001), voluntary feed intake is directly related to the palatability of the feed. In this study, feed intake was not affected (p>0.05), indicating that the addition of grape pomace flour to the diet did not change the palatability of the experimental diets. These results are in agreement with those of Rossi *et al*. (2015),who reported that the addition of increasing sweet pepper levels (0, 75, 125, and 225ppm) to laying chicken diets did not affect egg production, feed intake, egg mass, or feed conversion ratio.

Several studies report the lack of influence of natural sources of antioxidants on the feed intake (Radwan *et al*., 2008; Zhao *et al.,* 2011; Özeku *et al.,* 2011; Bozkurt *et al*., 2012) and laying performance (Özeku *et al.,* 2011; Bozkurt *et al*., 2012). However, Radwan *et al.* (2008) found that although feed intake was not affected, the dietary inclusion of 1% of oregano, rosemary or thyme, or 0.5% turmeric promoted better productive performance in laying hens.

Egg weight linearly decreased with increasing dietary GPF levels (Figure 1). A possible explanation for this result is that grape pomace is particularly rich in a wide range of polyphenols. One these polyphenols formerly known as tannins are considered anti-nutritional and has adverse effects on animal nutrition, as discussed below. According to Brenes (2016), the main limitations of the inclusion of grape pomace in monogastric feeds are its high levels of lignified cell wall fraction and tannins. On the other hand, Salici *et al*. (2011), in a study evaluating the inclusion of grape seed flour (0.5, 1.0, and 1.5%) in Japanese quail diets, did not observe any effect on egg weight, possibly due to the low levels of grape seed flour evaluated.

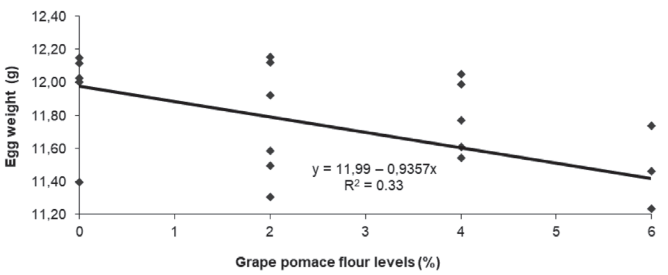

Figure 1 – Egg weight of quails fed diets containing different levels of grape pomace flour during lay.

As to egg quality traits, there was no effect (p>0.05) of treatments on Haugh units or eggshell thickness. However, there was a decreasing linear effect on albumen weight and specific gravity (Figure 2) as GPF inclusion levels increased. Grape pomace flour has high fiber content, and non-ruminant animals (with the exception of horses and rabbits) have limited utilization of dietary fiber as their large intestine do not present an active cellulolytic microbiota, and, therefore, high dietary fiber content may reduce the digestibility of ingredients (Betercchini, 2012). It has been shown that the inclusion of polyphenolic grape extracts in poultry diets may reduce the digestibility of fat (Brenes *et al*., 2008), protein, and some amino acids, such as proline and cystine (Chamorro *et al*., 2013). Tannins may potentially precipitate proteins, thereby reducing protein and amino acid digestibility. The binding of tannins to both dietary and endogenous proteins (such as digestive enzymes and proteins located at the luminal side of the intestinal tract) has been used to explain the reduced protein and amino acid digestibility in tannin-containing diets (Gilani *et al*., 2012).

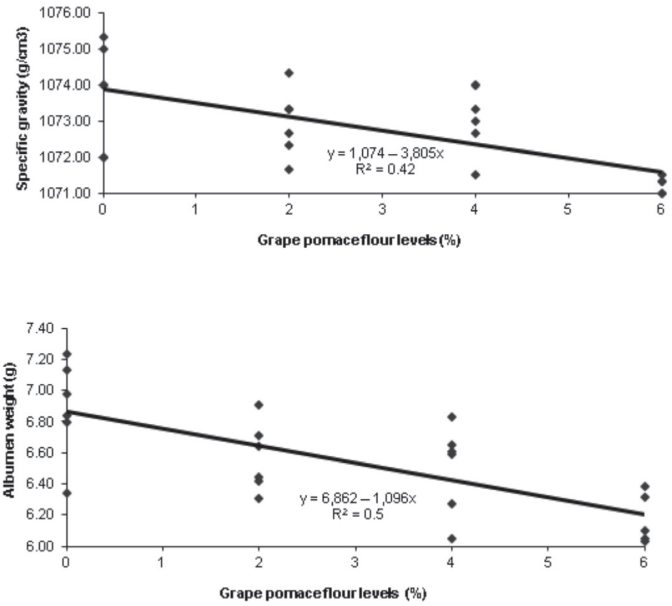

Figure 2 – Albumen weight (g) and egg specific gravity (g/cm3) of quails fed diets containing different levels of grape pomace flour during lay.

Kara *et al*. (2016) included 4% and 6% grape pomace in laying hen diets, but did not observe any significant effects on egg quality. On the other hand, Ozgan (2008), reported an increase in albumen height and albumen index with the addition of 2% grape pomace to laying hen diets.

A quadratic effect of GPF inclusion on yolk weight was observed, with the highest yolk weight obtained at an estimated maximal GPR inclusion level of 4.18%. Yolk pigmentation also presented a quadratic response, with minimal pigmentation achieved at an estimated GPM level of 3.2%.

The increasing density of yolk pigmentation observed in this study may be related to the presence of anthocyanins in the grape pomace flour. Anthocyanins are pigments that provide the purple color to grapes and their products (Abe, 2007). In addition of their coloring potential, these compounds present antioxidants and antibacterial properties (Ho *et al*., 2010). Antioxidant supplements improve the quality of foods of animal origin in terms of color, oxidative stability, and storage properties (Kara *et al.,* 2016).

In the commercial hen egg market, richer-colored yolks are desirable, and this characteristic depends exclusively on the feed, because even though hens are not able to synthesize pigments, they are able to

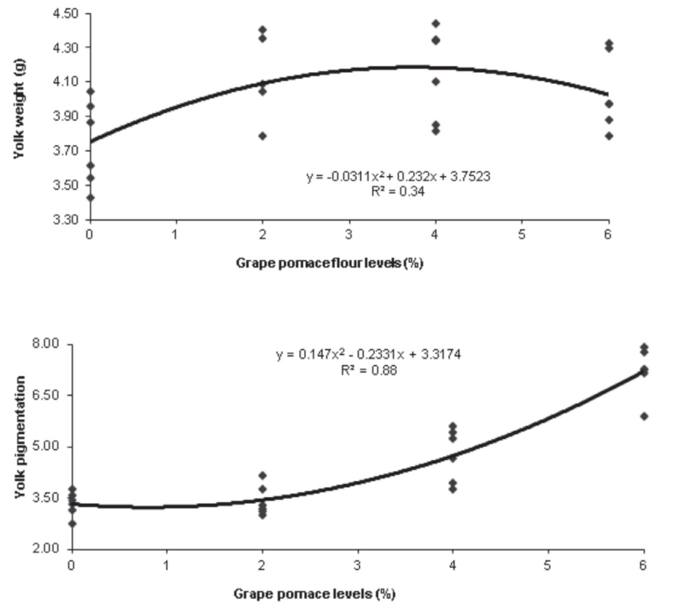

Figure 3 – Yolk weight (g) and yolk pigmentation of quails fed with diets containing different levels of grape pomace flour during production.

absorb between 20% and 60% of the feed pigments (Moura *et al*., 2011). According to Klassing (1998), pigment deposition in specific tissues depends on its levels in the diet, tissue growth rate, and the bird's ability to digest, absorb, and metabolize it.

The impact of the inclusion of grape pomace on performance and egg quality of poultry can be influenced by several factors. Such factors include its total polyphenol content, which depends on the grape variety and the soil where they were grown, as well as the processing methods from which the pomace is derived, *e.g*., for the production of wine, vinegar, or grape juice (Kara *et al*., 2016).

CONCLUSIONS

Egg yolk pigmentation improved at a minimal inclusion level of 3.2% of grape pomace flour in the diet of quails in lay, demonstrating its efficiency of as pigmentation additive. The inclusion of grape pomace flour up to 4.18% of the diet increased yolk weight. However, the dietary inclusion of grape pomace flour compromised egg weight, albumen weight, and egg specific gravity. Further studies are required aiming at minimizing the possible adverse effects of the inclusion of grape pomace flour in quail diets.

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