Effect of Grape Pomace Concentrate and Vitamin E on Digestibility of Polyphenols and Antioxidant Activity in Chickens

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ABSTRACT Grape pomace provides a rich source of polyphenols that have the capacity to act as powerful antioxidants. An experiment was conducted to study the effect of inclusion of grape pomace concentrate (GPC) at levels of 15, 30, and 60 g/kg and α-tocopheryl acetate (200 mg/kg) in broiler chicks (21 to 42 d of age) on performance; digestive organ sizes; protein; fat; hydrolyzable polyphenol and condensed tannin digestibilities; the antioxidant activity of diet, serum, ileal content, and excreta; and the susceptibility to oxidation of breast meat during refrigerated storage. The inclusion of GPC did not affect the performance; the apparent ileal digestibility of CP; the relative abdominal fat, liver, pancreas, and spleen weight; and the relative intestinal length. Fat digestibility was reduced in birds fed control and GPC diets compared with birds fed vitamin E. Ileal and fecal digestibility of hydrolyzable polyphenols and condensed tannins reached values in a range of 56 to 73% and 14 to 47%, respectively. The GPC diets reduced ileal and fecal digestibility of hydrolyzable polyphenols. Antioxidant activity in GPC diet, ileal content, and excreta [2, 2-azinobis (3-ethilenzotiazolin)-6-sulfonate method] and GPC diet (ferric antioxidant power method) exhibited higher scavenging free radical capacity than control and vitamin E diets. The lipid oxidation in breast meat was lower in the birds fed the supplemented vitamin E diet than the control diet after 1, 4, and 7 d of refrigerated storage. Oxidative stability in breast meat at 1, 4, and 7 d of storage was equivalent in GPC diets compared with the vitamin E diet. In conclusion, the inclusion of GPC (up to 60 g/kg) did not impair chicken growth performance, digestive organ sizes, and protein digestibility. Hydrolyzable polyphenols were more bioavailable than condensed tannins. Antioxidant activity in diet, excreta, ileal content, and breast muscle were increased in GPC diets. The GPC supplementation was equally as effective in antioxidant potential as vitamin E. On the basis of these observations, we concluded that GPC could be a new source of antioxidant in animal nutrition.

Key words: grape pomace concentrate, polyphenol, digestibility, antioxidant activity, chick

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INTRODUCTION

Grape (*Vitis vinifera*) is one of the largest fruit crops in the world, with an approximate annual production of 61 million metric tons (FAO STAT Database, www.fao.org; Schieber et al., 2002). The main by-products are collected during destemming (stems), grape crushing, and pressing (skins, seeds, and lees). Grape pomace consists mainly of peels, stems, and seeds and accounts for about 20% of the weight of the grape processed into wine (Llobera and Cañellas, 2007). Recent investigations have stressed the importance of by-products from wine processing as plant materials particularly rich in a wide range of polyphenols (Bonilla et al., 1999; Alonso et al., 2002; Torres et al., 2002). Grape skins and seeds are rich sources of flavonoids including monomeric phenolic compounds, such as (+)-catechins, (−)-epicatechin, and (−)-epicatechin-3-*O*-gallate and dimeric, trimeric, and tetrameric procyanidins. Studies have shown flavonoids have the capacity to act as powerful antioxidants by scavenging free radicals and terminating oxidative reactions (Gonzalez-Paramás et al., 2004; Yilmaz and Toledo, 2004; Ruberto et al., 2007). In the zones of wine production, great quantities of residues are generated, causing problems in both economical and ecological terms. Thus, any useful production from these by-products could represent an interesting advance in the maintenance of the environmental equilibrium and also an economic revaluation of the raw material. However, studies on grape seeds are more limited, in spite of their richness in polyphenolic substances, mainly monomeric and oligomeric flavanols.

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Animal nutrition is currently evolving toward n-3 polyunsaturated fatty acid (**PUFA**)-enriched diets to improve animal fat healthfulness (Bourre, 2005), but this nutritional strategy has been associated with an increase of lipoperoxidation in s.c. and i.m. lipids, as well as in plasma (Scislowski et al., 2005; Fellenberg and Speisky, 2006). Vitamin E is the antioxidant most commonly used in animal nutrition, but it presents some drawbacks, including its synthetic origin, its limited bioefficiency when n-3 PUFA intake is too high (Allard et al., 1997), its potential antioxidant prooxidant action (Mukai et al., 1993), and its nonhomogeneous distribution between tissues. Research for new bioefficient antioxidants has particularly focused on natural antioxidants to respect the consumer concerns over safety and toxicity. Plant extracts rich in polyphenols are good candidates, because they are easily obtained from natural sources and they efficiently prevent lipid oxidation in food products. Grape seed extract has been evaluated for its antioxidative effect on a few meat types and has been reported to improve the oxidative stability of cooked beef (Ahn et al., 2002), turkey patties, and cooled stored turkey meat (Lau and King, 2003; Mielnik et al., 2006). Use of such natural antioxidants in animal nutrition could be limited due to the low bioavailability of polyphenols and that many types of polyphenols can lose part of their antioxidant capacity in vivo (Manach et al., 2004). Feeding studies recently conducted with poultry (Giannenas et al., 2005) showed that plant extract obtained from oregano prevented lipoperoxidation in muscle tissue and may be complementary to vitamin E. A recent report by Gladine et al. (2007) confirmed in rats the ability of plant-rich polyphenols, including grape extract, to exhibit a significant antioxidative protective effect in plasma and liver.

Previous experiments in our laboratory (Goñi et al., 2007) have shown an increase in the antioxidative activity of broiler diet, excreta, and meat as a result of the dietary administration of grape pomace concentrate (**GPC**) and vitamin E in broilers at 3 wk of age. The aim of this study was to assess the effects of higher dietary concentrations of polyphenols by the addition of GPC in broiler chickens from 21 to 42 d on performance and on ileal and fecal digestibility of nutrients, hydrolyzable polyphenols (**HP**), and condensed tannins. The antioxidant activity of diet, serum, ileal content, excreta, and breast tissue were also determined to investigate the digestibility of polyphenols and oxidative stability of animal products, which is a major interest for the meat industry.

MATERIALS AND METHODS

Test Product

Red GPC (*Vitis vinifera* var. Cencibel) was obtained by a patented procedure (F. Saura-Calixto and J. A. Larrauri. Consejo Superior de Investigaciones Científicas. Dietary fiber concentrate from grape. ES 2430092 A1). Proximate composition of GPC is shown in Table 1. The GPC was used as a source of dietary fiber and polyphenols in the chicken diets. The α-tocopheryl acetate used in the diets was donated by DSM Nutritional Products Iberia S.A., Madrid, Spain.

Table 1. Proximate composition of grape pomace concentrate[1]

Item	DM (g/kg)
Protein	138.50 ± 1.20
Soluble sugars	20.70 ± 0.30
Fat	9.87 ± 0.17
Fiber	151.80 ± 0.72
Ash	24.10 ± 0.30
Extractable polyphenols	48.70 ± 0.07
Hydrolyzable polyphenols	26.60 ± 0.05
Condensed tannins	150.90 ± 0.05

[1]Data are the mean of 4 determinations ± SD.

Birds, Feeding, and Management

A total of 180 one-day-old male broiler chicks (Cobb strain) were obtained from a commercial hatchery. The birds were housed in electrically heated starter battery brooders in an environmentally controlled room with 23 h of constant overhead fluorescent lighting and were fed on a commercial broiler starter diet from 1 to 21 d. At the end of wk 3, chickens were weighed and moved to grower-finisher batteries from 21 to 42 d. Chicks were allocated to 30 cages, each cage containing 6 chicks, to receive 5 dietary treatments with 6 replicates of each treatment. Diets in mash form and water were provided for ad libitum consumption. Celite (Celite Corp., Lompoc, CA), a source of acid insoluble ash (**AIA**), was added at 10 g/kg to all diets as an indigestible marker. All diets were formulated to meet or exceed the minimum NRC (1994) requirements for broiler chickens. At the end of the experimental period, birds were weighed, and feed consumption was recorded for feed efficiency computation. All housing and handling were approved by the University Complutense of Madrid Animal Care and Ethics Committee in compliance with the Ministry of Agriculture, Fishery and Food for the Care and Use of Animals for Scientific Purposes. Ingredients and nutrient composition of diets are shown in Table 2. Experimental diets were as follows: 1) control corn-soybean diet (CS), 2) CS + vitamin E (200 mg/kg of α-tocopheryl acetate), 3) CS + 15 g/kg of GPC, 4) CS + 30 g/kg of GPC, and 5) CS + 60 g/kg of GPC. The cellulose was substituted by GPC in the experimental diets. To eliminate in the analysis the possible interference of nutrients (protein and carbohydrate), the determination of condensed tannins in the experimental diets was calculated based on the total concentration of this polyphenol in GPC.

Collection of Samples and Measurements

At 42 d of age, 8 birds were randomly selected from each treatment, and blood samples were obtained by cardiac puncture for subsequent determination of antioxidant activity. The blood samples were allowed to clot in polypropylene tubes for 2 h at room temperature. The

Table 2. Ingredients and nutrient composition of experimental diets (g/kg as fed)

Ingredients	Control	Control + vitamin E	Control + 15 GPC[1]	Control + 30 GPC	Control + 60 GPC
Corn (8.1% CP)	485.9	485.9	493.1	499.0	509.5
Soybean (48% CP)	336.0	336.0	330.8	325.7	315.8
Sunflower oil	82.4	82.4	80.6	80.0	80.0
Cellulose	60.0	60.0	45.0	30.0	—
GPC (13.8% CP)	—	—	15.0	30.0	60.0
Dicalcium phosphate	15.5	15.5	15.5	15.5	15.5
Calcium carbonate	10.0	10.0	9.8	9.6	9.1
Salt	3.0	3.0	3.0	3.0	3.0
Vitamin-mineral premix[2]	5.0	5.0	5.0	5.0	5.0
DL-Met	1.2	1.2	1.2	1.2	1.1
Celite[3]	1.0	1.0	1.0	1.0	1.0
Analyzed composition					
CP	201.5	201.3	199.1	198.5	200.3
Crude fat	107.7	108.9	103.8	105.8	106.2
Extractable polyphenols	1.8	1.9	2.7	3.9	5.2
Hydrolyzable polyphenols	14.2	13.5	13.8	15.3	16.9
Condensed tannins	—	—	2.25	4.51	9.0
Calculated composition					
AME[4] (Kcal/kg)	3,000	3,000	3,000	3,000	3,000
Met + cystine	7.3	7.3	7.3	7.3	7.3
Ca	8.7	8.7	8.7	8.7	8.7
Available P	3.7	3.7	3.7	3.7	3.7

[1]GPC = grape pomace concentrate.

[2]Vitamin-mineral mix supplied the following per kilogram of diet: vitamin A, 8,250 IU; cholecalciferol, 1,000 IU; vitamin E, 11 IU; vitamin K, 1.1 mg; vitamin B_{12}, 12.5 μg; riboflavin, 5.5 mg; Ca panthothenate, 11 mg; niacin, 53.3 mg; choline chloride, 1,020 mg; folic acid, 0.75 mg; biotin, 0,25 mg; delquin, 125 mg; DL-Met, 500 mg; amprol, 1 g; Mn, 55 mg; Zn, 50 mg; Fe, 80 mg; Cu, 5 mg; Se, 0.1 mg; I, 0.18 mg; and NaCl, 2,500 mg.

[3]Celite Corp, Lompoc, CA.

[4]Calculated value (FEDNA Tables, 2003).

tubes were centrifuged at 1,500 × g for 10 min, and the supernatant was removed and stored at –20°C until assayed. After sacrificing the chicks by cervical dislocation (12 randomly selected chicks, 2 per replicate, per treatment), liver, pancreas, spleen, and abdominal fat were weighed, and the length of duodenum, jejunum, ileum, and ceca were measured. The ileum was quickly dissected out and the content expressed by gentle manipulation into a plastic container and stored at –20°C. Digesta were pooled from 2 birds of each replicate within the same treatment. Ileal contents were freeze-dried and ground (1-mm screen) and subsequently analyzed for N-Kjeldahl, celite, HP, condensed tannins, and antioxidant activity. Clean stainless steel collection trays were also placed under each cage, and excreta from the birds were collected for 48 h. A subsample of excreta was collected in polyethylene bags and freeze-dried for subsequent determination of celite, fat, HP, condensed tannins, and antioxidant activity. Eight birds per treatment were slaughtered, and carcasses were immediately trimmed for breast meat. This tissue was individually sliced and sampled for lipid oxidation studies. Tissue samples, breast excluding skin, were wrapped in transparent oxygen-permeable polyvinyl chloride film (13,500 cm^3/m^2 per d), frozen, and stored at –20°C until required. After thawed, the progress of lipid oxidation in the breast meat samples during storage was determined after 1, 4, and 7 d in a nonilluminated refrigerated cabinet at 4°C.

Chemical Analysis

Dry matter (930.15), CP (976.05), crude fiber (978.10), and ash (942.05) were analyzed according to the methods of the AOAC (1995). Crude fat (**CF**) was determined by extraction in petroleum ether after acidification with 4 *N* HCl solution (Wiseman et al., 1992). The AIA contents of diet, ileal content, and excreta were measured after ashing the samples and treating the ash with boiling 4 *M* HCl (Siriwan et al., 1993). Samples of diets, ileal content, and excreta were extracted by shaking at room temperature with methanol-water (50:50 vol/vol, 50 mL/g of sample during 60 min) and acetone-water (70:30 vol/vol, 50 mL/g of sample during 60 min). After centrifugation (15 min, 3,000 × *g*), supernatants were combined and used to measure the extractable polyphenols and the antioxidant capacity by the 2, 2-azinobis (3-ethilenzotiazolin)-6-sulfonate (**ABTS**) method. Extractable polyphenols were determined in GPC and diets by Folin-Ciocalteau procedure (Montreau, 1972) using gallic acid as standard. In the residues from the extract, condensed tannins-proanthocyanidins and HP were determined separately. Residues from the methanol-acetone-water extraction were treated with 5 mL/L of HCl-butanol during 3 h at 100°C (Reed et al., 1982) for condensed tannin (**CT**) determination. Condensed tannins were calculated from the absorbance at 550 nm of the anthocyanidin solutions. Condensed tannins from Mediterranean carob pod (*Ceratonia siliqua* L.) supplied by Nestlé S.A. (Vevey, Switzerland) were

Table 3. Performance of broiler chicks (21 to 42 d) fed diets containing grape pomace concentrate (GPC)[1] and vitamin E

Treatments	Weight gain (g)	Feed consumption (g)	Feed:gain ratio
Control	1,551	2,694	1.74[a]
Control + vitamin E	1,560	2,630	1.69[b]
Control + 15 GPC	1,574	2,744	1.74[ab]
Control + 30 GPC	1,566	2,699	1.72[ab]
Control + 60 GPC	1,483	2,652	1.79[a]
Pooled SEM	79	106	0.05
Statistical significance[2] (*P*-value of contrast)			
Control vs. vitamin E	NS	NS	NS
GP[3] vs. no GPC	NS	NS	NS
Vitamin E vs. GPC	NS	NS	0.05
Type of response due to percentage of GPC in diet			
Linear	NS	NS	NS
Quadratic	0.05	NS	NS

[1]Data are means of 6 pens of 6 chicks.

[2]NS = $P > 0.05$.

[3]GP = grape pomace.

treated under the same conditions to obtain standard curves. Hydrolyzable polyphenols were hydrolyzed by a methanol-H_2SO_4 90:10 (vol/vol) treatment from the residues of the methanol-acetone-water extraction at 85°C for 20 h (Hartzfeld et al., 2002). Phenolic content was determined in the hydrolysates by Folin-Ciocalteau procedure (Montreau, 1972).

The ABTS assay was determined in extracted samples (GPC, diet, ileal content, and excreta) and serum. The antioxidant activity was estimated after the procedure described by Re et al. (1999) with some modifications. The ABTS radical cation (**ABTS$^+$**) was produced by reacting 7 m*M* ABTS stock solution with 2.45 m*M* potassium persulfate and allowing the mixture to stand in the dark at room temperature for 12 to 16 h before use. The $ABTS^{+\cdot}$ solution was diluted with methanol to an absorbance of 0.70 ± 0.02 at 658 nm. After addition of 100 μL of extracted samples or Trolox standard to 3.9 mL of diluted $ABTS^{+\cdot}$ solution, absorbance readings were taken every 20 s using a Beckman DU-640 (Beckman Instruments Inc, Fullerton, CA). The reaction was monitored for 6 min. The percentage inhibition of absorbance vs. time was plotted, and the area below the curve (0 to 6 min) was calculated. Methanolic solutions of known Trolox concentrations were used for calibration the measurement of extractable polyphenol antioxidant activity. The ABTS determination on serum was similar to the method previously indicated but adding 10 μL of serum to 3 mL of $ABTS^+$ solution and using aqueous solution of Trolox for calibration of the measurement of antioxidant activity.

The ferric antioxidant power (**FRAP**; FRAP assay) of the samples (diets, ileal content, and excreta) was estimated according to the procedure previously described (Benzie and Strain, 1996; Pulido et al., 2000). Briefly, FRAP reagent was mixed with distilled water and either the sample or appropriate reagent blank. Readings at 30 min were selected for calculation of FRAP values. Reduction power activities were as micromolars of Trolox equivalents per gram of DM.

The extent of lipid oxidation was determined by measuring the thiobarbituric acid-reacting substances at 1, 4, and 7 d of storage and was expressed as micrograms of malondialdehyde (**MDA**) per gram of muscle using the procedure described by Salih et al. (1987). Ten grams of ground meat was homogenized with 35 mL of 3.86% perchloric acid in an Ultraturrax at 21,280 × g for 1 min. Butylated hydroxyanisole was added before homogenization at a level of 125 μg/mg of fat. The blended sample was filtered through a Whatman No. 2V filter (Whatman International Ltd, Maidstone, UK) into 50-mL Erlenmeyer flasks. Five microliters of the filtrate was mixed with 5 mL of 0.02 *M* TBA in distilled water in capped test tubes. Tubes were incubated at room temperature in the dark for 15 to 17 h or heated in boiling water for 30 min. The absorbance was determined at 531 nm against a blank containing 5 mL of distilled water and 5 mL of 0.02 *M* TBA solution.

Calculations and Statistical Analysis

Apparent ileal CP, CF, and HP and CT digestibility were calculated using the following formula: 100% – [100% × (AIA concentration in feed/AIA concentration in ileal digesta or excreta) × (CP, CF, HP and CT concentration in ileal content and in excreta/CP, CF, HP and CT concentration in feed)]. Data were subjected to ANOVA using the GLM procedures of SAS (SAS Institute, 2003), and single degree of freedom linear contrast was used to separate treatments. Linear and quadratic effects were also analyzed. Significant differences among treatment means were determined at $P < 0.05$ by Duncan's multiple-range test.

RESULTS

Growth Performance

The addition of increasing concentration of GPC in the chicken diets did not change the growth performance

Table 4. Apparent ileal digestibility (%) of protein and fat and relative weights and lengths of digestive organs of broiler chicks (42 d) fed grape pomace concentrate (GPC) and vitamin E

	Digestibility (%)		Relative weight[1] (%)				Relative length[1] (%)			
Treatments	Protein[2]	Fat[3]	Abdominal fat	Liver	Pancreas	Spleen	Duodenum	Jejunum	Ileum	Ceca
Control	85.12	84.53^{bc}	0.76^{b}	2.27	0.17	0.11^{ab}	1.40^{ab}	2.71	2.70	0.80^{ab}
Control + vitamin E	85.93	86.20^{a}	0.80^{ab}	2.14	0.16	0.10^{b}	1.35^{b}	2.66	2.64	0.77^{b}
Control + 15 GPC	83.50	85.03^{ab}	0.90^{a}	2.15	0.17	0.12^{a}	1.45^{a}	2.88	2.85	0.85^{a}
Control + 30 GPC	84.88	83.32^{cd}	0.80^{ab}	2.27	0.16	0.11^{ab}	1.38^{ab}	2.81	2.83	0.78^{ab}
Control + 60 GPC	83.67	82.35^{d}	0.84^{ab}	2.21	0.17	0.11^{ab}	1.39^{ab}	2.64	2.84	0.84^{ab}
Pooled SEM	3.14	1.19	0.13	0.20	0.02	0.02	0.11	0.27	0.29	0.09
Statistical significance[4] (*P*-value of contrast)										
Control vs. vitamin E	NS	0.001	NS	NS	NS	NS	NS	NS	NS	NS
GP[5] vs. no GPC	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Vitamin E vs. GPC	NS	0.001	NS	NS	NS	0.05	NS	NS	0.05	NS
Type of response due to percentage of GPC in diet										
Linear	NS	0.01	NS	NS	NS	NS	NS	NS	0.05	NS
Quadratic	NS	0.05	NS	NS	NS	NS	NS	NS	NS	NS

$^{a–d}$Means in columns with no common superscript differ significantly ($P < 0.05$).

[1]Data are means of 12 chicks for each treatment.

[2]Data are means of 6 samples corresponding to 12 birds.

[3]Data are means of 6 pens of 6 chicks each.

[4]NS = $P > 0.05$.

[5]GP = grape pomace.

(Table 3). Feed:gain ratio was reduced (up to 6%; $P < 0.05$) in the vitamin E diet compared with those birds fed GPC diets. A quadratic effect ($P < 0.05$) was observed in weight gain with increasing dietary GPC.

Apparent Digestibility of Protein and Fat, Relative Weight, and Length of Digestive Organs

The inclusion of graded concentrations of GPC did not affect the AID of CP; the relative abdominal fat, liver, pancreas, and spleen weight; and the relative duodenum, jejunum, ileum, and ceca length (Table 4). Fat digestibility was reduced 2% and up to 5% ($P < 0.001$) in birds fed control and GPC diets, respectively, compared with birds fed vitamin E. Relative spleen weight and relative ileum length were increased (up to 20 and 8%, respectively; $P < 0.05$) in the birds fed GPC compared with those fed vitamin E diet. No differences were observed between birds fed GPC and no-GPC diets. A linear effect was observed in fat digestibility and relative ileum length, and a quadratic effect was observed in fat digestibility with increasing dietary GPC.

Digestibility of HP and Condensed Tannins

Ileal and fecal digestibility of HP and condensed tannins reached values in a range of 56 to 78% and 13 to 47%, respectively. The inclusion of GPC in the chicken diets increased total intake (up to 18%; $P < 0.05$) and reduced ($P < 0.001$) ileal (up to 26%) and fecal (up to 29%) digestibility of HP compared with those fed the control diet (Table 5). Total intake was increased (up to 23%; $P < 0.01$), and ileal (up to 26%; $P < 0.001$) and fecal (up to 28%; $P < 0.01$) digestibility of HP were reduced in birds fed GPC diets compared with those fed the vitamin E diet. A linear effect was observed in total intake ($P < 0.001$) and ileal ($P < 0.01$) and fecal ($P < 0.001$) digestibility of HP with increasing dietary GPC. A quadratic effect ($P < 0.001$) was observed in total intake and ileal digestibility of HP with increasing dietary GPC. The ileal digestibility of condensed tannins was not modified among the different concentrations of GPC reaching values in a range of 41 to 47%. A linear and quadratic effect was observed in total intake and a quadratic effect of fecal digestibility of condensed tannins with increasing dietary GPC.

Antioxidant Activity in Diets, Ileal Content, Excreta, and Serum

Diet (99%; $P < 0.001$) and excreta (23%; $P < 0.05$) antioxidant activity using the ABTS method were increased in chicks fed the vitamin E diet compared with those fed the control diet (Table 6). Antioxidant activity in diet (up to 6.5 times; $P < 0.001$), ileal content (up to 2.8 times; $P < 0.001$), and excreta (up to 1.4 times; $P < 0.01$) were increased in birds fed GPC diets compared with those fed control diets. Antioxidant activity in diet (up to 3.3 times; $P < 0.01$) and ileal content (up to 2.0 times; $P < 0.05$) were increased in birds fed the GPC diet compared with those fed the vitamin E diet. The dietary treatment did not affect the antioxidant activity measured on serum. A linear ($P < 0.001$) and quadratic ($P < 0.01$) effect in diet and a linear effect ($P < 0.01$) in ileal content using the ABTS method was observed increasing dietary GPC. Diet antioxidant activity, using the FRAP method, was increased ($P < 0.01$) in GPC diets compared with control (up to 5.5 times) and vitamin E (up to 4.5 times) diets. A linear and quadratic effect ($P < 0.01$) in ileal content diet and a linear effect ($P < 0.01$) in diet using FRAP method were observed increasing dietary GPC.

Table 5. Total intake and ileal and fecal digestibility of hydrolyzable polyphenols and condensed tannins of broilers chicks (42 d) fed grape pomace concentrate (GPC) and vitamin E

	Hydrolyzable polyphenols			Condensed tannins		
Treatments	Total intake (g)	Ileal digestibility[1] (%)	Fecal digestibility[2] (%)	Total intake (g)	Ileal digestibility[1] (%)	Fecal digestibility[2] (%)
Control	37.99[c]	76.16[a]	77.78[a]	—	—	—
Control + vitamin E	36.37[c]	76.81[a]	77.00[a]	—	—	—
Control + 15 GPC	37.87[c]	67.92[b]	72.80[b]	6.13[c]	40.92	13.81[b]
Control + 30 GPC	41.30[b]	67.45[b]	64.80[c]	12.18[b]	46.50	33.63[a]
Control + 60 GPC	44.82[a]	56.73[c]	55.62[d]	23.87[a]	47.37	25.42[a]
Pooled SEM	1.41	1.97	2.82	0.40	5.07	7.84
Statistical significance[3] (*P*-value of contrast)						
Control vs. vitamin E	NS	NS	NS	—	—	—
GP[4] vs. no GPC	0.05	0.001	0.001	—	—	—
Vitamin E vs. GPC	0.01	0.001	0.01	—	—	—
Type of response due to percentage of GPC in diet						
Linear	0.001	0.01	0.001	0.001	NS	NS
Quadratic	0.001	0.001	NS	0.001	NS	0.01

[a–d]Means in columns with no common superscript differ significantly ($P < 0.05$).

[1]Data are means of 6 samples corresponding to 12 birds.

[2]Data are means of 6 pens of 6 chicks each.

[3]NS = $P > 0.05$.

[4]GP = grape pomace.

MDA Concentration

The extent of lipid oxidation, as measured by MDA formation, in breast meat, was significantly lower in the supplemented vitamin E diet, in a range of 25 to 44%, than the control group after 1 ($P < 0.001$), 4 ($P < 0.05$), and 7 ($P < 0.001$) days of refrigerated storage (Table 7). The inclusion of GPC in the diets significantly reduced MDA values in breast samples after 1 (up to 40.9%; $P < 0.001$,), 4 (up to 28%; $P < 0.05$,) and 7 d (up to 36%; $P < 0.01$) of refrigerated storage compared with samples obtained from birds fed the control diet. There were not significant differences in MDA concentration between the chicks fed GPC diet compared with those fed vitamin E diet. A linear effect was observed in MDA concentrations at 1 ($P < 0.01$), 4 ($P < 0.05$), and 7 d ($P < 0.01$) increasing dietary GPC.

DISCUSSION

The present study demonstrated that the inclusion of concentrations of GPC in chicken diets up to 60 g/kg did

Table 6. Antioxidant activity of extractable polyphenols in diets, ileal content, excreta, and serum of broiler chicks (42 d) fed diets containing grape pomace concentrate (GPC) and vitamin E

	Antioxidant activity (μmol of Trolox equivalent/g)						
	ABTS[1] method				FRAP[2] method		
Treatments	Diet	Ileum[3]	Excreta[4]	Serum[5]	Diet	Ileum[3]	Excreta[4]
Control	4.0[d]	15.8[d]	74.7[c]	336.9[c]	3.3[c]	19.9[b]	58.3
Control + vitamin E	7.9[c]	21.3[cd]	91.9[abc]	344.9[bc]	4.0[c]	19.7[b]	63.3
Control + 15 GPC	13.0[b]	24.6[bc]	86.4[bc]	326.1[c]	8.1[b]	18.4[b]	60.0
Control + 30 GPC	14.4[b]	28.2[b]	104.3[ab]	371.9[ab]	10.2[b]	20.6[b]	60.5
Control + 60 GPC	26.1[a]	43.5[a]	107.2[a]	384.5[a]	18.0[a]	32.7[a]	63.5
Pooled SEM	1.87	5.11	14.26	29.40	2.78	2.49	5.01
Statistical significance[7] (*P*-value of contrast)							
Control vs. vitamin E	0.001	NS	0.05	NS	NS	NS	NS
GP[5] vs. no GPC	0.001	0.001	0.01	NS	0.01	NS	NS
Vitamin E vs. GPC	0.01	0.05	NS	NS	0.01	NS	NS
Type of response due to percentage of GPC in diet							
Linear	0.001	0.01	NS	NS	0.01	0.01	NS
Quadratic	0.01	NS	NS	NS	NS	0.01	NS

[a–d]Means in columns with no common superscript differ significantly ($P < 0.05$).

[1]ABTS = 2,2-azinobis (3-ethilenzotiazolin)-6-sulfonate.

[2]FRAP = ferric antioxidant power.

[3]Data are means of 6 samples corresponding to 12 birds.

[4]Data are means of 6 pens of 6 chicks each.

[5]Data are means of 8 chicks for each treatment.

[6]NS = $P > 0.05$.

[7]GP = grape pomace.

Table 7. Effect of refrigerated storage on lipid oxidation of breast meat of broiler chicks (42 d) fed diets containing grape pomace concentrate (GPC)[1] and vitamin E

	Malondialdehyde concentration (mg/kg of meat)		
Treatments	d 1	d 4	d 7
Control	0.22[a]	0.32[a]	0.72[a]
Control + vitamin E	0.15[c]	0.24[b]	0.41[b]
Control + 15 GPC	0.19[b]	0.30[a]	0.66[b]
Control + 30 GPC	0.15[c]	0.23[b]	0.52[b]
Control + 60 GPC	0.13[c]	0.26[ab]	0.46[b]
Pooled SEM	0.02	0.06	0.09
Statistical significance[2] (*P*-value of contrast)			
Control vs. vitamin E	0.001	0.05	0.001
GP[3] vs. no GPC	0.001	0.05	0.01
Vitamin E vs. GPC	NS	NS	NS
Type of response due to percentage of GPC in diet			
Linear	0.001	0.05	0.001
Quadratic	NS	NS	NS

[a–c]Means in columns with no common superscript differ significantly ($P < 0.05$).

[1]Data are means of 8 chicks for each treatment.

[2]NS = $P > 0.05$.

[3]GP = grape pomace.

not change the growth performance (3 to 6 wk of age) and the organ size. Similar results have been obtained in our laboratory with the addition of GPC up to 30 g/kg (3 wk of age; Goñi et al., 2007). There are few references in the literature in relation to the use of grape by-products in chicken feed. The growth depression obtained with the use a grape seed extract reported by Hughes et al. (2005) and Lau and King (2003) was justified, because grape seed extract was a pure form containing 90.2% of total phenolics, expressed as gallic acid equivalent by the Folin method, and incorporated in the diet at 30 g/kg. In the current experiment, GPC contained 4.87% of total polyphenols by the Folin method. Thus, the total extractable polyphenols in the diet containing the highest proportion of GPC were 0.52%. Similarly, the concentration of condensed tannins present in the higher concentration of GPC diet could be relatively low to produce a growth depression effect. The effect of polyphenols has also been studied in chickens using ingredients like sorghum and faba bean. In general, relatively high dietary concentrations of polyphenols by the addition of these ingredients reduced performance in chickens as well as other livestock (Jansman et al., 1989; Nyachotti et al., 1997).

Binding of polyphenolic compounds to both dietary and endogenous protein, such as digestive enzymes and proteins located at the luminal side of the intestinal tract, have been used to explain the reduced apparent digestibility of protein in polyphenol-containing diets. Polyphenols are known to form complexes with protein due to the interaction of their reactive hydroxyl groups with the carbonyl group of protein. As a consequence of this complexation, protein and amino acid digestibility were reduced by the inclusion in chicken and pig diets of sorghum and faba bean polyphenols (Jansman et al., 1989; Ortiz et al., 1993). In the current experiment, AID of protein was not affected. This lack of effect could be attributed to the low content of polyphenols in the experimental diets to cause detrimental effect.

Result in this study also showed a significant reduction in the fat digestibility in those birds fed GPC diets. The inclusion of polyphenolic compounds has been associated with an increase in the lipid excretion in rats (Bravo et al., 1994). The mechanism of action of polyphenols on lipid metabolism is not well defined. It has been observed that condensed tannins could bind biliary salts and cholesterol with a concomitant reduction in their absorption and an increase in the fecal excretion (Roy and Schneeman, 1981). Bile salts are known in chickens to be a limiting factor for efficient fat digestion (Krogdahl, 1985). Another mechanism whereby nutrients are rendered less digestible by polyphenols is through the inactivation of digestive enzymes. Only few works were devoted to the effects of polyphenols on digestive enzymes. Proanthocyanidin extracts from bean greatly inhibited all 3 digestive enzymes (trypsin, α-amylase, and lipase) in young chicks (Longstaff and McNab, 1991). Naturally occurring polyphenols, and in particular condensed tannins, can also inhibit in vitro a number of digestive enzymes including trypsin, lipase, and α-amylase. The inhibition of digestive enzymes may be explained with the ability of condensed tannins to form insoluble complexes with proteins in the gastrointestinal tract (Griffiths, 1986; Horigome et al., 1988). Moreno et al. (2003) also demonstrated in vitro the inhibitory effects of grape seed extract on fat-metabolizing enzymes and lipoprotein lipase. In addition, the increase of fat digestibility in birds fed vitamin E diet compared with control diets is in agreement with those recently reported by Chae et al. (2006) in broilers fed on a commercial diet supplemented with 100 or 200 mg of α-tocopheryl acetate/kg.

There are many references in the literature to the composition and antioxidant properties of grape polyphenols (Gonzalez-Paramás et al., 2004; Yilmaz and Toledo, 2004), but there have been very few studies on the digestibility and intestinal degradation of polyphenols and other major grape constituents. The effects of dietary polyphenols have been confined so far to effects observed on processes in

the lumen of the digestive tract. Whether dietary polyphenols also cause systemic effects in the animal is related to the question of whether these compounds are absorbed from the digestive tract. It is noteworthy that most reports on the beneficial effects of polyphenols have been obtained from in vitro studies, and more detailed investigations are required to extrapolate these results to in vivo situations. This is particularly relevant in view of the fact that polyphenols are known to undergo various biochemical transformations that affect their bioavailability as well as bioefficacy.

In the current experiment, the ileal and fecal digestibility of HP reached values in a range of 56 to 73% in those birds fed GPC diets. This suggests that polyphenols, or their metabolites, could be bioefficient in some tissues. In the literature revised, we have not found information relative to polyphenol digestibility in chickens, but similar or superior digestibilities have also been reported in rats by Goñi and Serrano (2005). The nutritional effects of polyphenols would be a consequence of the absorbed monomers and aromatic acid, the interaction of unabsorbed polyphenols with components of the intestinal tract, or both. As reducing agents, they may be active in the gastrointestinal tract and modify the intestinal environment (Scalbert and Williamson, 2000). Goñi et al. (2005) reported that intestinal bacteria showed a high capacity to degrade extractable polyphenols in rats. Deprez et al. (2000) and Ward et al. (2004) also showed that major polyphenolic constituents of grape seed (polymeric proanthocyanidins) were degraded by human colonic microflora into smaller compounds including phenolic acids that could be absorbed and metabolized. The low excreta digestibility of condensed tannins (in a range of 14 to 47%) found in our experiment could also be justified by the different effect of the avian ceca microbiota to metabolize these polyphenols compared with rat colon and ceca microbiota. Moreover, the antioxidant activity remaining in the excreta in birds fed GPC diets (Table 6) would appear to confirm their resistance to bacterial degradation. Similar studies in chickens indicated that sorghum polymeric fractions free of monomers were not absorbed (Jimenez-Ramsey et al., 1994). Available data on the absorption and metabolism of condensed tannins reported by Donovon et al. (2002) and Gonthier et al. (2003) suggested negligible bioavailability of polymeric proanthocyanidins in rats. These compounds are poorly absorbed in the intestine due to their high molecular weight (Santos-Buelga and Scalbert, 1998; Gonthier et al., 2003). However, García et al. (2006) and Gladine et al. (2007) demonstrated in rats, using synthetic oligomeric proanthocyanidin, which dimeric proanthocyanidins are rapidly absorbed and found in physiologically relevant amounts in plasma and liver as highly methylated forms.

On the basis of these observations, relatively significant quantities of polyphenols could be absorbed in the small intestine, and a fraction remain in the lumen, where they may exert biological activity. Moreover, extract plant procyanidins may have biological effects protecting biomolecules from possible oxidative damage during digestion, sparing other antioxidants (vitamin E) and enhancing the overall antioxidant status of tissue (Frank, 2005; Silbergberg et al., 2006; Goñi et al., 2007).

Nutritional interest in polyphenolic compounds has increased greatly in light of their antioxidant capacity (Scalbert and Williamson, 2000). The relative contribution of polyphenols to the total antioxidant activity in excreta and ileal content obtained by the FRAP and ABTS methods depended on the diet. In this study, because feedstuffs contain both oil-soluble and water-soluble compounds with antioxidant capacity, 2 different methods have been selected for the evaluation of the antioxidant capacity. One determines the total reduction power (FRAP), and the other measures the capacity of a compound to capture the radical cation ABTS. The antioxidant compounds present in grape have already been identified as phenolic acids (benzoic and hydroxycinnamic acids), stilbene derivatives, flavan-3-ols (catechin and epicatechin), flavonols (quercetin and myricetin), and anthocyanidins (Caillet et al., 2006). In the current experiment, the GPC diets exhibited the highest antioxidant activity in diet, ileal content, and excreta using mainly ABTS method. These results are similar to those reported in chickens and rats by Goñi et al. (2007) and Goñi and Serrano, (2005). Previous studies also demonstrated that the antioxidant properties of plant extracts can be achieved by the activation of the liver antioxidant enzymes (Alia et al., 2003). The mechanisms underlying the activation of antioxidant enzymes by polyphenols are not fully understood, but it was recently demonstrated that grape seed procyanidins are able to affect the gene expression of antioxidant enzymes by interacting with element promoter in DNA (Puiggross et al., 2005). Saura-Calixto and Díaz-Rubio (2007) reported that 35 to 61% of wine polyphenols are associated to dietary fiber, and it may not be bioavailable in the upper portion of the intestine and may contribute to an antioxidant environment.

The susceptibility of lipids to peroxidation in tissue depends on 3 main factors: the proportion of PUFA in lipid bilayers, the amount of reactive oxygen species produced, and the level of antioxidants that can be of endogenous or nutritional origin. Results in this study confirm that dietary GPC (30 and 60 g/kg) and vitamin E can delay lipid oxidation at 1, 4, and 7 d in breast meat. Similarly, Goñi et al. (2007) reported an increase in the oxidative stability of breast and thigh chicken meats. Giannenas et al. (2005), Tang et al. (2000, 2001), Maraschiello et al. (1999), and De Winne and Dirinck (1996) have also demonstrated that using plant extracts, tea catechins, and vitamin E in chicken diets prevented lipoperoxidation in muscle tissue. Our study also corroborates in vitro observations that the addition of wine polyphenols to various food systems (fish lipids, frozen fish, and turkey meat) inhibits lipid oxidation (Lau and King, 2003; Pazos et al., 2005; Mielnik et al., 2006) and are contrary to those that provide an enhancement of the antioxidant defense potential in plasma of rats by flavonol-rich red wine and plant extracts rich in proanthocyanidin (Fremont et al., 2000; Rodrigo et al., 2002, 2005; Gladine et al., 2007). Recently, Gladine et al. (2007) also reported that the lipoperoxidation intensity was not sig-

nificantly modified by plant extract-ich polyphenols in muscle tissue of rats, suggesting that the short time of the dietary treatment (3 wk) was not sufficient to significantly modify the intensity of lipoperoxidation in extrahepatic tissues. Using malonaldehyde concentration as an index of absorption of polyphenols and based on the digestibility values obtained in the current experiment, this study showed that polyphenolic antioxidant compounds in GPC were distributed, retained, and remained functional in muscle. Because there is no method with sensitivity available so far for the identification of the antioxidant constituents deposited in muscle, the presence of these compounds cannot yet be directly demonstrated.

Overall, it may be concluded that the inclusion of GPC up to 60 g/kg did not impair performance, digestive organ sizes, and protein digestibility. Our findings also suggest that polyphenols present in GPC were absorbed at sufficient levels to contribute to the protection of PUFA in membranes and to modulate the antioxidant activity in ileal content, excreta, and muscle tissue. Grape pomace concentrate supplementation was as equal in antioxidant potential as vitamin E. On the basis of these observations as well as the previous one (Goñi et al., 2007), we concluded that GPC rich in polyphenols could represent antioxidants of great interest for animal nutrition.

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