Food Research International 96 (2017) 226–234

Contents lists available at ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

# Addition of exogenous enzymes to diets containing grape pomace: Effects on intestinal utilization of catechins and antioxidant status of chickens

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ARTICLE INFO

*Article history:*
Received 5 January 2017
Received in revised form 14 February 2017
Accepted 17 February 2017
Available online 22 February 2017

*Keywords:*
Grape polyphenols
Catechins
Tannase
Carbohydrase
Antioxidant
Minerals
Digestibility
Chickens

ABSTRACT

Grape pomace (GP) is a rich source of polyphenols with antioxidant capacity. An experiment was conducted to study the effect of GP phenolic compounds included at 5 and 10%, and the addition (individually or combined) of hydrolyzing enzymes (carbohydrase enzyme complex and tannase at 500 ppm) on intestinal utilization of catechins and antioxidant status in broiler chickens. A diet supplemented with 200 ppm of α-tocopheryl acetate was also used. Our findings demonstrate the capacity of chickens to digest the monomeric (catechin, epicatechin, gallic acid, and epicatechin-*O*-gallate) and dimeric (procyanidin B1 and procyanidin B2) catechins present in grape pomace. The addition of enzymes (mainly tannase) hydrolyzed the polymeric structures into smaller catechins, but also promoted a lower digestibility of the monomeric and dimeric catechins suggesting that polymeric structures might favour the intestinal utilization of these catechins. The intestinal accumulation of phenolic compounds generated with tannase and with 10% GP reversed the antimicrobial effect against *Clostridium perfringens* observed with 5% of GP. Grape pomace improved the antioxidant status of the bird, increasing the α-tocopherol and reducing the iron content on plasma, not affecting the plasma gluthatione. Enzymes modified the intestinal utilization of catechins but not additional protective effect was detected on any of the parameters analyzed to evaluate the antioxidant status.



## 1. Introduction

Grapes are one of the world's largest fruit crops, and approximately 80% of their yield is used for winemaking. Grape pomace (GP) is a wine by-product consisting of peels (skins), seeds and stems particularly rich in a wide range of polyphenols. The compounds found in the greatest proportion are the flavanols, which include simple monomers of (*epi*) catechin, oligomeric (from 2 to 5 units) and polymeric (>5 units) procyanidins, commonly known as condensed tannins (Jara-Palacios, Hernanz, Escudero-Filete, & Heredia, 2014; Ky, Lorrain, Kolbas, Crozier, & Teissedre, 2014; Ferri et al., 2016). The phytochemicals present in wine pomace are known to have antioxidant, preservative and health-promoting effects in different biological and food systems (Fontana, Antoniolli, & Bottini, 2013; Teixeira et al., 2014 and Jara-Palacios et al., 2014; Brenes, Viveros, Chamorro, & Arija, 2016). Antioxidant activity is the most notable bioactivity of phenolic compounds from GP (Georgiev, Ananga, & Tsolova, 2014; Xia, Deng, Guo, & Li, 2010). The antioxidant potential has been demonstrated when GP was directly added to meat products (Sáyago-Ayerdi, Brenes, Viveros, & Goñi, 2009a; García-Lomillo, Gonzalez-Sanjose, Del Pino-Garcia, Ortega-Heras, & Muniz-Rodriguez, 2017). This beneficial effect on meat was also demonstrated when GP was incorporated to the diet in pigs (Yan & Kim, 2011) and chickens (Brenes et al., 2008; Goñi et al., 2007; Iqbal et al., 2014; Sáyago-Ayerdi, Brenes, & Goñi, 2009b). These studies indicated that the intake of grape by-products increased the antioxidant activity in diet, excreta and meat. In this sense, the inclusion of GP in chicken diets rich in polyunsaturated fatty acids (more susceptible to oxidative processes) delayed the meat lipid oxidation (Chamorro et al., 2015). This antioxidant effect was linked to modifications in the fatty acid profile, expressed by an increase in the polyunsaturated and a decrease in the monounsaturated fatty acid content of meat. Gladine, Morand, Rock, Bauchart, and Durand (2007a) and Gladine, Morand, Rock, Gruffat, Bauchart and Durand (2007b) also suggest that tissues might be differently affected by polyphenols supplements. Thus, the present experiment aims to support our previous studies by investigating the effect of grape polyphenols on plasma parameters (vitamins, essential minerals and enzymes) implicated in the maintenance of the antioxidant defense system.

Studies dealing with the bioavailability of polyphenols suggest that the intestinal utilization of polyphenolic compounds depends on the degree of polymerization and galloylation, being less digestible polymeric

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http://dx.doi.org/10.1016/j.foodres.2017.02.010
0963-9969/

than monomeric structures (Manach, Scalbert, Morand, & Jimenez, 2004; Tsang et al., 2005; Henning, Cho, & Heber, 2008). A relatively low digestibility of polymeric grape procyanidins (hydrolyzable and condensed tannins) has also been reported in chickens (Brenes et al., 2008). However, polyphenols which are not absorbed in the intestine can be metabolized by the intestinal microbiota into microbial derived phenolic acids with different physiological significance (Monagas et al., 2010; Selma, Espin, & Tomás-Barberán, 2009).

Addition of enzyme preparations to animal feeds is a common practice for improving the nutritional characteristics of by-products. Cell-wall degrading enzymes can improve the extraction of phenols. A previous in vitro study (Chamorro, Viveros, Alvárez, Vega, & Brenes, 2012) reported that the addition of carbohydrases (pectinases and cellulases) and tannase hydrolyzed and released polyphenols and polysaccharides entrapped in GP cell wall, enhancing the antioxidant capacity. Likewise, a subsequent in vivo study (Chamorro et al., 2015) also showed that the addition of enzymes in grape pomace containing diets increased the content of total phenolic compounds in the chicken intestine. However, this increment was not correlated with an improvement in the antioxidant activity in meat, and the beneficial antioxidant effects obtained with the intake of GP were reversed with the addition of enzymes. These results suggested that the polyphenols generated in the intestine with the inclusion of enzymes were less active than those naturally presented in GP.

Thus, the aim of this study was to investigate the intestinal utilization of the polyphenolic compounds present in grape pomace and the structural changes obtained with the inclusion of dietary enzymes. Finally, the effect of the inclusion of GP and the addition of enzymes on the chicken antioxidant status (alpha-tocopherol, antioxidant activity, glutathione and minerals content in plasma) will be also determined.

## 2. Materials and methods

### 2.1. Test product and enzymes

Red GP (*Vitis vinifera* var. Cencibel) was obtained from Grupo Matarromera (San Bernardo-Valbuena de Duero, Valladolid, Spain). Whole grape pomace was dried in a convection oven at 60 °C, grounded to pass a 1 mm-mesh screen and directly incorporated to the experimental diets. Proximate composition of GP is shown in Table 1. GP was used as a source of dietary fiber and polyphenols in the chicken diets. The α-tocopheryl acetate (αT) used in the diets was provided by DSM Nutritional Products Iberia S.A. (Alcalá de Henares, Madrid, Spain). Two different enzymes were selected on the basis of GP structural composition. A feed enzyme complex (EC) Avyzyme® 1505, donated by Danisco Animal Nutrition (Marlborough, UK) and containing endo-1,4-beta-xylanase (1500 U/g, EC 3.2.1.8), α-amylase (2000 U/g, EC 3.2.1.1) and subtilisin (20,000 U/g, EC 3.4.21.62), and another enzyme preparation with tannase activity (T), supplied by Kikkoman Foods Products Company (Edogawa Plant, Japan) and containing tannin acylhydrolase (500 U/g, EC 3.1.20) were added to diets containing GP.

### 2.2. Solvents and reagents

All solvents used for HPLC analysis were of liquid chromatography grade and the water ultrapure. Standards for catechin (C), epicatechin (EC) and epicatechin-*O*-gallate (ECG) procyanidin dimer B1 (PB1) and B2 (PB2) were purchased from Extrasynthèse (Genay, France). Gallic acid (GA), Folin–Ciocalteu reagent, α-tocopherol and trolox, were obtained from Sigma–Aldrich (St. Louis, MO). Acetone, butanol, isopropanol, hexane, acetonitrile and methanol were obtained from Panreac (Castellar del Vallés, Barcelona, Spain).

### 2.3. Birds and diets

A total of three hundred 1-day-old male broiler Cobb chicks were housed in electrically-heated starter battery cages in an environmentally-controlled room with 23 h of constant overhead fluorescent lighting for three weeks. The chicks were allocated to 50 cages containing six chicks to receive one of the 10 dietary treatments (five replicates per treatment) during the whole experimental period (21 days). Diets in mash form and water were provided for ad libitum consumption. All diets were formulated to meet or exceed the minimum National Research Council (1994) requirements for broiler chickens. Celite, a source of acid-insoluble ashes (AIA), was added at 10 g/kg to all diets as an indigestible marker. Experimental procedures were approved by the University Complutense of Madrid Animal Care and Ethics Committee in compliance with guidelines of the Ministry of Agriculture, Fishery and Food for the Care and Use of Animals for Scientific Purposes. Experimental diets were as follows: 1. Control maize soybean diet (C); 2. C + α-T (200 mg/kg of α-tocopheryl acetate); 3. C + 5% GP; 4. C + 5% GP + enzyme complex; 5. C + 5% GP + tannase; 6. C + 5% GP + enzyme complex + tannase; 7. C + 10% GP; 8. C + 10% GP + enzyme complex; 9. C + 10% GP + tannase, and 10. C + 10% GP + enzyme complex + tannase. Diets were isocaloric and isoproteic and due to the high doses of GP used in this study, all the diets were also formulated to contain the same fiber content by substituting straw for GP. Ingredients and nutrient composition of diets have been reported in Chamorro et al. (2015).

### 2.4. Collection of samples and measurements

At 19 days of age, clean stainless steel collection trays were 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 polyphenol content.

At 21 days of age, 14 chicks from each treatment group were randomly selected after an overnight fast. Blood, obtained from cardiac puncture, from two birds fed the same dietary treatment was pooled (7 replicates/treatment) and plasma was prepared for subsequent determination of α-tocopherol, antioxidant activity by photochemiluminescence, glutathione (GSH) and minerals. The blood was collected in EDTA Vacutainer tubes. Tubes were centrifuged at 2500 ×*g* for 15 min at 4 °C, and the supernatant was

**Table 1**
Concentration of total extractable polyphenols (g gallic acid equivalent/100 g DM), and phenolic profile (mg/100 g DM) of grape pomace (GP) and experimental diets containing GP at 5 and 10% (C + 5GP and C + 10GP, respectively).

| | Control (C) | C + 5GP | C + 10GP | GP |
|---|---|---|---|---|
| Total extractable polyphenols | 0.11 ± 0.007 | 0.14 ± 0.003 | 0.19 ± 0.007 | 2.34 ± 0.7 |
| Phenolic profile | | | | |
| Gallic acid | nd | 0.86 ± 0.06 | 1.54 ± 0.15 | 19.3 ± 0.12 |
| Catechin | nd | 0.63 ± 0.07 | 1.15 ± 0.11 | 12.2 ± 0.18 |
| Epicatechin | nd | 0.71 ± 0.07 | 1.27 ± 0.14 | 12.0 ± 0.48 |
| Procyanidin B1 | nd | 1.13 ± 0.07 | 1.88 ± 0.10 | 20.2 ± 2.1 |
| Procyanidin B2 | nd | 1.79 ± 0.15 | 3.59 ± 0.28 | 36.9 ± 1.3 |
| Epicatechin-*O*-gallate | nd | 0.08 ± 0.01 | 0.14 ± 0.02 | 1.32 ± 0.03 |

Data are the mean of 4 determinations ± standard deviation.