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Influence of dietary enzyme addition on polyphenol utilization and meat lipid oxidation of chicks fed grape pomace

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ABSTRACT

Grape pomace provides a rich source of polyphenols that have the capacity to act as powerful antioxidant. An experiment with ten dietary treatments (30 birds/treatment), was conducted to study the effect of inclusion of phenolic compounds from grape pomace (GP) added at different levels (0, 5, and 10%) and the addition (individually or combined) of hydrolyzing enzymes (carbohydrase enzyme complex and tannase at 500 ppm) in broiler chicks (1 to 21 days of age). A diet supplemented with 200 ppm of α-tocopheryl acetate (αT) was also used. Growth performance, ileal and fecal total polyphenol content, thigh meat lipid oxidation, and α-tocopherol and fatty acid content of thigh meat were determined. No differences were observed in body weight, feed consumption and feed efficiency among the different treatments. Birds fed GP diets showed a higher ileal and fecal polyphenol content. The inclusion of tannase in GP diets increased ileal polyphenol content and did not affect the fecal polyphenol content. Oxidative stability of thigh meat after 1 and 4 days of refrigerated storage increased with dietary addition of αT and GP, and was worsened when GP was supplemented with carbohydrases. Meat α-tocopherol content was increased by dietary addition of αT. Birds fed αT and GP diets showed higher meat polyunsaturated fatty acid content, while monounsaturated fatty acid was reduced. The addition of tannase to GP diets reversed the beneficial effect observed on fatty acid content obtained in GP diets. In conclusion, dietary GP reached the protective effect of α-tocopherol by reducing the susceptibility of meat to lipid oxidation and increasing the PUFA content. The inclusion of tannase in diets containing GP increased the amount of total polyphenol released in the intestine, but did not improve the stability to meat lipid oxidation.

1. Introduction

Supplementation of domestic animals with n-3 polyunsaturated fatty acids (n-3 PUFA) is becoming an accepted practice to improve the nutritional quality of lipids in animal products. However, this nutritional strategy also enhances the susceptibility to the lipoperoxidation of the meat. Poultry meat is susceptible to oxidative deterioration due to its high content of polyunsaturated fatty acids (Arshad et al., 2011; Huff-Lonergan & Lonergan, 2005; Tavárez et al., 2011). The oxidative stability of poultry meat may be improved by dietary addition of α-tocopherol, the active antioxidant form of Vitamin E (Avila-Ramos et al., 2012; Carreras et al., 2004). However, the bioefficiency of this vitamin is limited when PUFA intake is increased (Allard, Kurian, Aghdassi, Muggli, & Royall, 1997) and added at high doses, α-tocopherol would be catabolized or excreted in feces and urine (Aurousseau, 2002) and not retained in tissues. On the basis of these observations, there is an increasing interest to improve the endogenous protection against negative effects of reactive oxygen species, especially in young animals, by supplementing various phytogenic antioxidant preparations containing among others flavonoids (Frank et al., 2006; Gobert et al., 2009). Grape pomace (GP) is a wine by-product consisting of pressed seeds, skins, and stems, and is rich source in flavonoids including monomeric phenolic compounds, such as (+)-catechins, (−)-epicatechin, and (−)-epicatechin-3-gallate and dimeric and oligomeric proanthocyanidins. These compounds have the capacity to act as powerful antioxidants by scavenging free radicals and terminating oxidative reactions (Yilmaz & Toledo, 2004). Previous reports (Brenes et al., 2008; Goñi et al., 2007) indicated that the intake of grape pomace increases the antioxidant capacity in breast and thigh meat of broiler of chickens in the same way as added vitamin E in experimental diets. The antioxidant activity of polyphenols in biological tissues is currently associated with their capacity to scavenge free radicals, chelate active redox metals and protection of the endogenous antioxidant system (Lourenco, Gago, Barbosa, Freitas, & Laranjinha, 2008; Pazos, Gallardo, Torres, & Medina, 2005).

The growing interest in the use of natural antioxidant grape-products in diets with high PUFA content may contribute to an improved oxidative stability of meat and meat products and provide greater potential for developing quality poultry foods for human consumption. However, the use of such natural antioxidants in animal nutrition could be limited

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due to the low bioavailability of grape polyphenols, and might be improved by the use of exogenous enzymes. Enzymatic supplementation is a technique with increasing applicability for improving the nutritional characteristic of by-products and it is widely used in animal nutrition. Cell-wall degrading enzymes can improve the extraction of phenols. The GP cell wall is a complex network composed of 30% of neutral polysaccharides (cellulose, xyloglucan, arabinan, galactan, xylan and mannan), 20% of acidic pectin substances, 15% of insoluble proanthocyanidins, lignin and structural proteins and phenols, these two latter cross-linked to the lignin–carbohydrate framework (Pinelo, Arnous, & Meyer, 2006). A recent in vitro study (Chamorro, Viveros, Alvárez, Vega, & Brenes, 2012) reported that the addition of carbohydrases (pectinases and cellulases) and tannase released polyphenols and polysaccharides entrapped in grape pomace cell wall increasing its antioxidant activity. The hydrolysis of the complex polysaccharides and polyphenols into more digestible sugars and phenols might increase the amount of active substances that can be easily metabolized improving its nutritional value and render this by-product more suitable to be used as an animal ingredient. To our knowledge there is no information about the effect of the addition of enzymes to chicken diets containing grape pomace on the antioxidant status and lipid peroxidation of meat. The objective of this experiment was to study if dietary addition of enzymes could improve the polyphenol utilization, the antioxidant status and meat quality of chickens fed grape pomace.

2. Material 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). 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 pomace, consisting of stems, skins and seeds from red grapes, was dried in a convection oven at 60 °C. Proximate composition of grape pomace was: protein 138.9 g/kg; fat 9.87 g/kg; fiber 343.0 g/kg and ash 24.1 g/kg. 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 enzymatic preparations were selected on the basis of the structural composition of grape pomace. A feed enzyme complex (EC) Avyzyme®1505, donated by Danisco Animal Nutrition (Marlborough, UK), 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) was added to diets containing GP. Another enzyme preparation with tannase activity (T) supplied by Kikkoman Foods Products Company (Edogawa Plant, Japan) containing tannin acylhydrolase (500 U/g, EC 3.1.20) was used.

Table 1
Ingredients and nutrient composition of experimental diets (% as fed).

	Control (C)	C + 5GP	C + 10GP
Maize	49.1	50.0	50.8
Soybean	19.0	16.9	14.4
Sunflower oil	9.70	9.64	10.5
Soybean concentrate	9.10	9.40	9.09
Straw	7.78	3.77	0
Grape pomace	0	5.0	10.0
Monocalcium phosphate	1.95	1.96	1.98
Calcium carbonate	1.42	1.37	1.31
Salt	0.30	0.30	0.30
Vitamin–mineral premix[a]	0.50	0.50	0.50
DL-Methionine	0.14	0.13	0.12
Enzymes[b]	–	−/+	−/+
Chemical composition			
Crude protein	21.1	21.1	21.1
Ether extract	12.0	12.0	12.0
Crude fiber	5.16	5.10	5.16
Ca	1.0	1.0	1.0
Available P	0.495	0.493	0.495
AME[c](Kcal/kg)	3000	3000	3000
SFA [d]	1.36	1.50	1.49
MUFA[d]	1.79	1.67	1.54
PUFA[d]	0.42	0.41	0.34

[a] Vitamin and mineral mix supplied the following per kilogram of diet: vitamin A, 8250 IU; cholecalciferol, 1000 IU; vitamin E, 11 IU; vitamin K, 1.1 mg; vitamin B_{12}, 12.5 μg; riboflavin, 5.5 mg; Calcium pantothenate, 11 mg; niacin, 53.3 mg; choline chloride, 1020 mg; folic acid, 0.75 mg; biotin, 0,25 mg; ethoxyquin, 125 mg; DL-methionine, 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, 2500 mg.
[b] Enzyme added to the diets were 500 mg/kg of enzyme complex (EC), 500 mg/kg of tannase (T) or the combination of both.
[c] AME: apparent metabolizable energy; calculated values (FEDNA Tables, 2003).
[d] Analyzed composition of saturated fatty acid (SFA), monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA).

2.2. Solvents and reagents

All solvents used for HPLC analysis were of liquid chromatography grade and the water was ultrapure. Standards for catechin (C), epicatechin (EC) and epicatechin O-gallate (ECG) procyanidin dimer B1 (PB1) and B2 (PB2) and cyanidin-3-O-glucoside were purchased from Extrasynthèse (Genay, France). Gallic acid (GA), Folin–Ciocalteu reagent, α-tocopherol, trolox, butylated hydroxytoluene, and 1,1,3,3-tetraethoxy propane 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 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 for 3 weeks. The chicks were allocated to 50 pens, each pen containing six chicks, to receive 10 dietary treatments during 21 days with five replicates per treatment. Diets in mash form and water were provided ad libitum. The diets were stored in a dark and cool dry location during the experimental period. Ingredients and nutrient composition of diets are shown in Table 1. All diets were formulated to meet or exceed the minimum (National Research Council, 1994) requirements for broiler chickens. Experimental procedures 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. Experimental diets were as follows: 1. Control corn soybean diet (C); 2. C + αT (200 mg/kg of α-tocopheryl acetate); 3. C + 5% of 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. At the end of the experimental period, birds were weighed and feed consumption was recorded for feed efficiency computation.

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 (Telstar, Terrasa, Spain) for subsequent determination of polyphenol content.

At 21 days of age, fifteen birds per treatment were euthanized by carbon dioxide (100%), 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 three birds of each replicate within the same treatment. Ileal contents were freeze-dried and ground (1 mm screen) and used to determine the polyphenol content. Carcasses from ten birds per treatment were also immediately trimmed for thigh