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Novel feed including bioactive compounds from winery wastes improved broilers' redox status in blood and tissues of vital organs

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ABSTRACT

Currently, there is a great interest in the production of animal feed with antioxidant activity. The aim of this study was to examine the potential antioxidant effects of a feed supplemented with grape pomace (GP), a winery by-product with high environmental load, in chickens. Broilers of 15 days post birth were separated into two groups fed either with standard diet or with diet supplemented with GP for 35 days. Blood and tissues collections were performed after feeding for 15 and 35 days with the experimental diet (i.e. at 30 and 50 days post birth). Free radical toxicity markers, namely thiobarbituric acid reactive substances, protein carbonyls, total antioxidant capacity, reduced glutathione, catalase activity and rate of H_2O_2 decomposition were determined in blood and tissues of vital organs. The results indicated that feed supplemented with GP decreased oxidative stress-induced toxic effects and improved chickens' redox status, and so it may also improve their wellness and productivity. On the other hand, this exploitation of GP may solve problems of environmental pollution in areas with wineries.

1. Introduction

It is a common knowledge that diet is a key factor in animal production, since it does not only affect the health and productivity of farm animals, but also the cost of livestock products. In recent years, natural bioactive compounds are incorporated to animal feed in order to develop biofunctional products which would optimize the efficiency, production and welfare of livestock (Pinotti et al., 2014). Moreover, a considerable amount of evidence suggested that the administration of antioxidants to farm animals may improve their welfare and productivity (Lykkesfeldt and Svendsen, 2007). Thus, administration of antioxidants can be an alternative and cost-effective intervention for the treatment of oxidative stress-induced pathological conditions of livestock (Lykkesfeldt and Svendsen, 2007). Studies have also indicated that young animals have reduced antioxidant mechanisms compared with older animals, and thus it is more necessary for the former to obtain antioxidants for disease protection (Chan et al., 2013; Jain and Flora, 2012).

Oxidative stress has been recognized as a major health problem of broilers in modern intensive production. It is regarded as the cause of several pathologies (e.g. microbial infections, heat stress and stress caused by management) affecting poultry growth (Avanzo et al., 2001). Therefore, finding out methods to prevent free radicals' overproduction and treat oxidative stress is an important issue for chicken farming. Several antioxidants have been used to attenuate toxicity caused by free radicals in poultries (Zhang et al., 2009). For example, in a previous study we have shown that feed

Abbreviations: ARE, antioxidant response element; CARB, protein carbonyls; CAT, catalase; DNPH, 2,4-dinitrophenylhydrazine; DPPH, 2,2-diphenyl-1-picrylhydrazyl; DTNB, 5,5′-dithiobis-2 nitrobenzoate; EDTA, ethylenediaminetetraacetic acid; EpREs, electrophiles response elements; GCL, gamma-glutamylcysteine synthetase; GP, grape pomace; GSH, glutathione; GST, glutathione S-transferase; Hb, hemoglobin; HiCN, hemiglobincyanide; IC50, half-maximal inhibitory concentration; MDA, malondialdehyde; OMWW, olive mill waste waters; PBS, phosphate buffered saline; RBCL, red blood cell lysate; RSC, radical scavenging capacity; TAC, total antioxidant capacity; TBARS, thiobarbituric acid reactive species; TCA, trichloroacetic acid.

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enriched with polyphenolic compounds from olive mill waste waters improved the antioxidant capacity in broiler chickens (Gerasopoulos et al., 2015a).

Grapes are the world's largest fruit crop with more than 60 million tons produced annually. About 80% of the total grape production is used in winemaking (Mossa et al., 2015). Grape pomace (GP), a winemaking by-product, represents approximately 20% of the weight of grapes processed from wineries (Mossa et al., 2015; Souad El Gengaihi et al., 2013). GP is the solid remains of grapes after pressing for extracting juice or oil, containing skins, pulp, seeds, and stems. GP has traditionally been used to produce brandy and grape seed oil. Nowadays, GP is mostly used as fodder or fertilizer. GP is characterized by high content in polyphenols, bioactive compounds with strong antioxidant activity, due to poor extraction during winemaking (Amico et al., 2004; Ju and Howard, 2005; Kammerer et al., 2005; Spigno et al., 2007). Polyphenols contained in the GP have been shown to reduce toxicity caused by free radicals and prevent oxidative damage of biological macromolecules. Moreover, they contribute significantly to defense of animal organisms by increasing the levels of endogenous antioxidant molecules and enzymes such as glutathione (GSH) and catalase (CAT), and consequently enhancing their immune system. Thus, GP constitutes a cheap source for antioxidant polyphenols, which can be used as dietary supplements or the production of biofunctional foods having high added value (Alonso et al., 2002).

Thus, the present study aimed at exploiting GP for making biofunctional feed of high added value for broilers in order to enhance their antioxidant defense, and thus leading to the optimization of their welfare and productivity. Specifically, feed enriched with GP administrated to broiler chickens of 15 days old for 35 days. Blood and tissues were collected after 15 and 35 days of feed administration. Then, chickens' redox status was assessed using toxicity biomarkers, namely total antioxidant capacity (TAC), glutathione levels (GSH), catalase activity (CAT), thiobarbituric acid reactive species (TBARS), protein carbonyl levels (CARB) and rate of H_2O_2 decomposition. Moreover, since by-products of winemaking such as GP are pollutants for the environment, their exploitation for developing biofunctional feeds may reduce these environmental problems.

2. Materials and methods

2.1. Winery by-product

Red grape pomace (*Vitis vinifera* L. *var. Moschato*) was obtained from a winery in Tyrnavos (Larissa prefecture, Greece) in September 2014.

2.2. Silage and broilers' feed preparation

GP was added in broilers' feed as corn silage. Based on a previous study, the proportion of the ingredients was such that the final silage contained 60% solids and 40% water (Gerasopoulos et al., 2015a). The 60% of solids in the experimental feed (which has been patented) contained the ingredients from GP (Table 1). Standard commercial formulation (*11CFT Pioneer, Buxtehude, Germany*) of lactic acid bacteria was used for the lactic fermentation of corn and the preparation of corn silage. The lactic acid bacteria had been dissolved in water (10% w/v) by stirring and warmed to 40 °C in order to be activated prior to their mixing with corn. After activation, lactic acid bacteria were mixed with corn (1 g of bacteria with 100 kg of corn). The resulting silage was placed into vacuum bags and just before feed administration was mixed with other ingredients for making the final broiler's feed (Table 1).

Table 1
Ingredients and nutrient composition of the experimental diet.

	Composition (%w/w)
corn	55.2[a]
soybean 42/8	31.8
fat powder (Lecithin)	5.0
fishmeal 70/10	4.0
broiler balancer 2.5%	2.5
marble powder	1.5
Total	100.0

[a] Corn contained 60% solids and 40% water in the control feed; 51% solids, 9% GP and 40% water in the experimental feed.

2.3. Animals and feed administration

The experiment was reviewed and approved by the institutional review board of the University of Thessaly. Thirty female broilers (Hubbard) 2 days old were purchased from the 'Agrafiotis' aviary', (Tyrnavos, Greece). Chickens were housed under controlled environmental conditions (12-hour light/dark cycle, temperature 18–21 °C, humidity 50–70%) in standard single cages (area 1500 cm^2/cage). Standard ration and water were provided *ad libitum* for 15 days. Then, the chickens were randomly divided into two experimental groups (15 broilers per group) as follows: (a) broilers fed with standard ration (control group) and (b) broilers fed with ration containing silage with GP (GP group). At day 30 post birth (i.e. after 15 days of feeding), six broilers from each group (control and GP) were sacrificed in order to collect their blood and tissues. At day 50 post birth (i.e. after 35 days of feeding), the remaining 9 broilers from each group were also sacrificed for collecting blood and tissues. Thus, two time-points of sacrifice were chosen, at 30 d and 50 d post birth. At the first time-point, the chickens were at a relatively young age at which the antioxidant defense mechanisms are considered weak. Thus, it was examined at this young age how the experimental feed affected the antioxidant mechanisms and/or protected from oxidative damage. At the second time-point, the chickens were close to the age at which they are slaughtered for their meat. So, it was also checked at this 'mature' age the effects of the experimental feed on chickens' redox status.

2.4. Blood and tissues collection

Blood and tissues samples were drawn at 30 and 50 days post birth (i.e. 15 and 35 days after administration of the experimental feed). For blood collection, chickens were restrained manually and 4 ml of blood were collected from branchial vein and placed in ethylenediaminetetraacetic acid (EDTA) tubes (8.1 μL). Blood samples were centrifuged immediately at 1370 g for 10 min at 4 °C and the plasma was collected and used for measuring TAC, TBARS and CARB. The packed erythrocytes were lysed with distilled water (1:1 v/v), inverted vigorously, centrifuged at 4000 g for 15 min at 4 °C and the erythrocyte lysate was collected for the measurement of GSH and catalase activity. For measurement of GSH, 400 μL of erythrocyte lysate were added in 400 μL 5% trichloroacetic acid (TCA), inverted vigorously, centrifuged at 22,000 g for 5 min at 5 °C, and the supernatant was collected. Then, 90 μL of 5% TCA were added in each tube, inverted again vigorously, centrifuged at same conditions as above and finally, the clear supernatant was collected.

The results of the markers in the erythrocyte lysate were given as per mg of hemoglobin (Hb). Hemoglobin concentrations were determined by the hemiglobincyanide (HiCN) method using a commercial kit (Dutch Diagnostics, Zutphen, Holland). Briefly, 5 μL of the red blood cell lysate (RBCL) was added in 1 ml of working hemoglobin reagent (reagent R1). The reagent R1 (pH 7.3) was