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Feed supplemented with byproducts from olive oil mill wastewater processing increases antioxidant capacity in broiler chickens

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ABSTRACT

In the present study, a ceramic membrane microfiltration method was used for the separation of two liquid products, the downstream permeate and the upstream retentate, from olive mill wastewater (OMWW). These liquid products were examined for their antioxidant activity by incorporating them into broilers' feed. Twenty four broilers 13 d old were divided into two feeding groups receiving supplementation with OMWW retentate or permeate for 37 d. Blood was drawn at 17, 27 and 37 d, while tissues (muscle, heart, liver) were collected at 37 d. The antioxidant effects were assessed by measuring oxidative stress biomarkers in blood and tissues. The results showed that broilers given feed supplemented with OMWW retentate or permeate had significantly lower protein oxidation and lipid peroxidation levels and higher total antioxidant capacity in plasma and tissues compared to control group. In both OMWW groups, catalase activity in erythrocytes and tissues was significantly increased compared to control group. OMWW retentate administration increased significantly GSH in erythrocytes in broilers with low GSH, although both OMWW products significantly reduced GSH in broilers with high GSH. Thus, it has been demonstrated for the first time that supplementation with OMWW processing residues could be used for enhancing broilers' redox status.

1. Introduction

Olive oil obtained from olive tree fruit (*Olea europaea* L.) is a main part of the Mediterranean diet. Several studies have shown that olive oil consumption is associated with lower risk of certain diseases such as cardiovascular disease and cancer (Cárdeno et al., 2013; Scoditti et al., 2014). Many of these beneficial effects of olive oil on human health are attributed to its polyphenolic compounds (e.g. tyrosol, hydroxytyrosol, oleuropein and pinoresinol) having potent antioxidant properties (Cárdeno et al., 2013; Scoditti et al., 2014). Apart from olive oil itself, some of the byproducts of olive oil production such as olive oil mill wastewater (OMWW) present important bioactivities (Cardinali et al., 2010, 2012; Hamden et al., 2009).

OMWW is a liquid effluent derived mainly from the water used for the various stages of oil production and vegetable water from the fruit, and amounts to 0.5–3.25 m^3 per 1000 kg of olives (Kapellakis et al., 2012; Paraskeva and Diamadopoulos, 2006). Since the annual worldwide olive oil production is estimated at about 2×10^6 tons, the OMWW would amount to about 4×10^6 m^3 (Agalias et al., 2007). Moreover, OMWW has a dark brown colour, high organic content [chemical oxygen demand (COD) 45–170 g/L and biochemical oxygen demand (BOD) 35–110 g/L], suspended solids (SS) 1–9 g/L, strong specific olive oil smell and acidic pH (3–6) (Paraskeva and Diamadopoulos, 2006). OMWW also contains tannins, lignins, long-chain fatty acids, reduced sugars, proteins and phenolic compounds which are toxic to microorganisms and plants (Paixao and Anselmo, 2002; Paraskeva and Diamadopoulos, 2006). Furthermore, disposal of OMWW causes serious environmental problems such as soil contamination, water body pollution, underground seepage and odour (Rinaldi et al., 2003). Thus, the discharge of large quantities of OMWW in the sewage system is not possible without any treatment such as aerobic treatment, anaerobic digestion and composting (Aly et al., 2014; Rinaldi et al., 2003). However, an environmentally safe and cost-effective treatment of OMWW has not yet been found (Zagklis et al., 2013).

Abbreviations: ARE, antioxidant response element; BOD, biochemical oxygen demand; COD, chemical oxygen demand; DNPH, 2,4-dinitro-phenyl-hydrazine; DPPH, 2,2-diphenyl-1-picrylhydrazyl; DTNB, 5,5′-dithiobis-(2-nitrobenzoic acid); EDTA, ethylenediamine tetraacetic acid; GSH, reduced glutathione; OMWW, olive mill wastewater; RO, reverse osmosis; ROS, reactive oxygen species; TAC, total antioxidant capacity; TBARS, thiobarbituric acid reactive substances; TCA, trichloroacetic acid.

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As already mentioned, a large part of the toxic effects of OMWW are attributed to its polyphenolic content causing phytotoxicity, toxicity against aquatic organisms and suppression of soil microorganisms (Aliotta et al., 2002; Fiorentino et al., 2003; Kotsou et al., 2004). The phenolic compounds found in OMWW can be simple phenols or polyphenols resulting from polymerization of simple phenols and their concentration varies from 0.5 to 24 g/L (Frankel et al., 2013).

The polyphenols identified in OMWW include hydroxytyrosol and tyrosol as the major components, as well as p-coumaric acid, homovanillic acid, caffeic acid, protocatechuic acid, 3,4-dihydroxymandelic acid, vanillic acid and ferulic acid (Frankel et al., 2013). It should be noted that polyphenols in OMWW represent about 50% of the total polyphenols found in olive fruit (Rodis et al., 2002). Despite the toxic effects exhibited at high concentrations, these polyphenols also possess antioxidant activity (Frankel et al., 2013). Therefore, isolation of the polyphenolic content of OMWW could represent an antioxidant source in food, pharmaceutical and cosmetic industry, while reducing the environmental toxicity of OMWW.

Our research group has developed a patented methodology (patent application number: 20120100569 – Greek Industrial Property Organisation) for obtaining polyphenols from OMWW based on the use of ceramic membrane microfiltration. A major problem of this methodology was that a large quantity of byproducts were produced. These byproducts contain a part of the OMWW polyphenols and have to be discharged in an eco-friendly and sustainable way. For this purpose, in the present study, these byproducts were used for making broilers' feed supplemented with antioxidant compounds as a disposable method. In farm animals, oxidative stress may be involved in several pathological conditions (e.g. pneumonia, sepsis) affecting animal production and general welfare (Basu and Eriksson, 2000; Lauritzen et al., 2003; Lykkesfeldt and Svendsen, 2007). For example, the hot and humid environment in aviaries may cause heat-induced oxidative stress in chickens which in turn it reduces growth and meat quality (Rhoads et al., 2013). Moreover, low feed efficiency in broilers is associated with high oxidative stress in breast muscle, liver and intestine (Iqbal et al., 2004). Thus, administration of natural antioxidant compounds to chickens has been proposed as a means for reducing the oxidative stress-induced adverse effects (Chen et al., 2013; Oskoueian et al., 2014). Although olive oil-supplemented diet has been shown to protect chicken skeletal muscle from heat stress-induced oxidative stress (Mujahid et al., 2009), OMWW has not been used so far in chickens' feed. Thus, the administration of antioxidants to farm animals has been suggested for protecting them from such pathologies or mitigating their symptoms (Abuelo et al., 2014; Deaton et al., 2004; Lykkesfeldt and Svendsen, 2007). The antioxidant effects of supplementation with OMWW of the processing byproducts were assessed by measuring oxidative stress biomarkers in broilers' blood and different tissues (i.e. muscle, cardiac and liver).

2. Materials and methods

2.1. Preparation and isolation of byproducts containing polyphenolic compounds from OMWW processing

As a part of a patented OMWW polyphenol powder production scheme, two byproducts containing antioxidant polyphenolic compounds are produced which have to be discharged in an eco-friendly and sustainable way. For this purpose, in this work, the production of animal feed was selected as a disposable technology of these two byproducts. According to the patented production scheme (Fig. 1) the raw OMWW was first passed through a finisher in order to separate suspended particles in the form of a heavy sludge. The finisher that was used for this first clarification step of the OMWW was a standard one step butterfly type finisher, operating at 1200 rpm/min and equipped with a cylindrical stainless steel sieve with openings of 150 μm diameter. The suspended particles from the OMWW raw material were removed by the finisher, in order to avoid clogging of the ceramic microfiltration membranes in the subsequent steps.

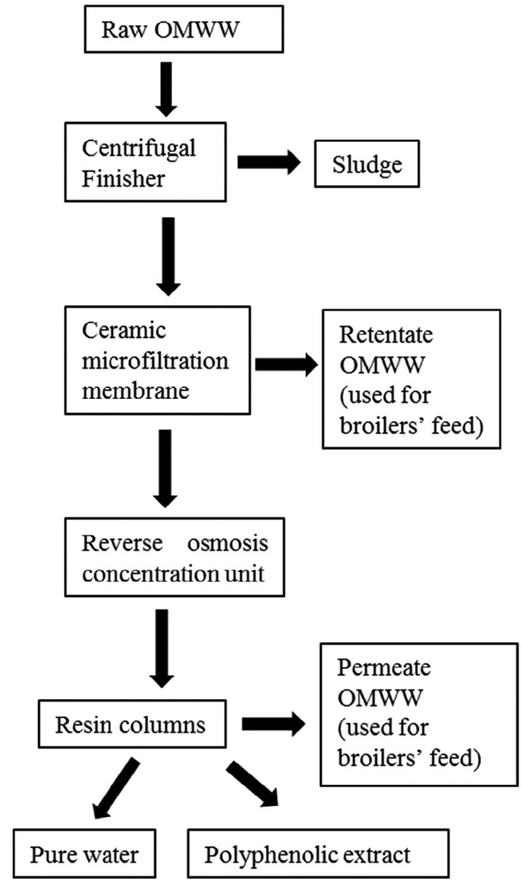

Fig. 1. Patented OMWW polyphenol powder production scheme.

Subsequently, the clarified OMWW was passed through a ceramic microfiltration unit where 30% of the input OMWW stream was separated as retentate (upstream) (this was used for silage production and designated hereafter as OMWW retentate) and 70% as permeate (downstream) in a tangential mode of operation. The microfiltration unit which was used as a part of the production of the OMWW liquid was supplied by Jiangu Jiuwu Hi-Tech Co. Ltd (Nanjing, China). This consisted of a membrane module with three pieces of ceramic microfiltration membranes with a total area of 0.69 m^2. The type of membrane was the CMF19040. The unit was fed with a positive displacement pump ensuring fluid velocity of 10 m/s in order to avoid membrane fouling.

The microfiltration permeate was then concentrated at low temperature by using a standard reverse osmosis (RO) membrane unit to a concentration factor 4:1 by using a pressure of 20 bar and a spiral type membrane module, thus producing a concentrate rich in polyphenols. Then, this was passed through a XAD4 resin column to isolate the main product which was the polyphenol extract that could be used as raw material for antioxidant powder production. The liquid material which was the leftover of the polyphenol absorption from the resins and which contained approximately 20 to 30% of the initial polyphenolic content of the input material was the second byproduct (designated hereafter as OMWW permeate) to be used for silage preparation. The above-mentioned two byproducts of the patented polyphenol powder production scheme were then utilized for silage production by mixing them with corn. This processing of the two byproducts ensured a total discharge of the OMWW and complete protection of the ecosystem as only pure water is disposed to the environment.

2.2. Silage and broilers' feed preparation

OMWW retentate and permeate were used for making silage corn. For this purpose, corn was mixed with OMWW retentate or permeate in a ratio of 24:1. Thus, corn was made that contained 56% solids, 4% OMWW retentate or permeate and 40% liquid. Then, standard commercial formulation of lactic bacteria was used for the lactic fermentation of corn. The lactic 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 bacteria were mixed with corn (1 g of bacteria with 100 kg of corn). The resulting silages were then mixed with other ingredients for making the final broilers' feed (Table 1).