Animal Feed Science and Technology 209 (2015) 90–97

Contents lists available at ScienceDirect

Animal Feed Science and Technology

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

Effects of dietary dried Greek Oregano (*Origanum vulgare* ssp. *hirtum*) supplementation on blood and milk enzymatic antioxidant indices, on milk total antioxidant capacity and on productivity in goats

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ARTICLE INFO

Article history:
Received 3 March 2015
Received in revised form 31 August 2015
Accepted 1 September 2015

Keywords:
Antioxidant enzymes
Antioxidant capacity
Essential oil
Goat milk
Oregano
Oxidative stress

ABSTRACT

The aim of the present study was to evaluate the effects of the dietary supplementation of dried Greek oregano (*Origanum vulgare* ssp. *hirtum*) as a whole plant on various blood and milk oxidative stress enzymatic indices, on milk total antioxidant capacity and on productivity in dairy goats. Twelve Alpine goats were used in a 4-week experiment and were allocated to 1 of 2 groups (CON, OR). The animals were fed 1.2 kg of alfalfa hay and 1.2 kg of concentrate mixtures (50% basal and 50% lactation ration) daily. The concentrate mixtures for the CON group were not supplemented with oregano, while oregano plants were incorporated into the lactation ration of the OR group, at a level of 30 g equivalent to a daily dosage of 1 ml of essential oil per animal. The goats were milked twice per day and the milk yield was recorded. At the end of each week of the experimental period, individual milk samples were obtained and analyzed for fat, milk and protein contents. Additional blood and milk samples were taken during the 3rd and the 4th week of the experimental period. The activities of the following antioxidant enzymatic indices were measured: superoxide dismutase, glutathione peroxidase, glutathione reductase, catalase in blood and milk, glutathione transferase in blood and lactoperoxidase in milk. The OR group showed a significant increase in glutathione peroxidase and glutathione reductase both in blood ($P < 0.01$ and $P < 0.001$, respectively) and milk ($P < 0.001$ and $P < 0.001$, respectively). In addition, the dietary oregano supplementation effectively enhanced FRAP values ($P < 0.001$) of the milk. It can be concluded that the dietary intake of dried oregano plants positively affected at least partially, some enzymatic and non enzymatic antioxidant defenses of blood and milk and thus, contributed to enhanced antioxidant capacity of milk.

1. Introduction

The production of reactive oxygen species (ROS) during aerobic metabolism is a natural and inevitable phenomenon (Jóźwik et al., 2012). Although ROS are involved in various physiological functions (Nordeberg and Arner, 2001), when they

Abbreviations: ADF, acid detergent fiber; BW, body weight; CLA, conjugated linoleic acid; CON, control; DM, dry matter; EO, essential oil; FRAP, ferric reducing antioxidant power; GC/MS, gas chromatography–mass spectrometry; NADPH, nicotinamide adenine dinucleotide phosphate reduced form; NDF, neutral detergent fiber; NE_L, net energy for lactation; N, nitrogen; OR, oregano; ROS, reactive oxygen species.

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http://dx.doi.org/10.1016/j.anifeedsci.2015.09.001
0377-8401/

are not scavenged efficiently, oxidative stress develops (Lykkesfeldt and Svedsen, 2007). Oxidative stress is influenced by physiological status (gestation, lactation), animal performance and nutrition as well as environmental factors (Festila et al., 2012; Jóźwik et al., 2012). This type of stress, while it does not demonstrate clinical symptoms, it has severe consequences on the immune system function as it contributes to an increased incidence of post partum diseases including mastitis and also to diminished milk quality (Castillo et al., 2013). The latter, can be affected through the oxidation of proteins, vitamins and lipids leading to a reduced nutritive value and organoleptic characteristics (Smet et al., 2008).

In order to counteract these negative effects, the organism is equipped with an antioxidant defense system which can scavenge ROS sufficiently under normal physiological conditions (Jóźwik et al., 2012). The antioxidant defense system consists of non-enzymatic scavengers such as vitamin A, vitamin C and vitamin E and proteins including enzymatic scavengers of ROS (Lindmark-Mansson et al., 2001). The latter include primarily superoxide dismutase and catalase which decompose O_2^- and H_2O_2 (Lindmark-Mansson and Akesson, 2000), respectively. Glutathione peroxidase removes various organic peroxides including H_2O_2 (Rezapour and Taghinejad-Roudbaneh, 2011). Other antioxidant enzymes include glutathione transferase which catalyzes the conjugation of the reduced form of glutathione to various oxidative substrates (Hayes et al., 2005), glutathione reductase which reduces the oxidized glutathione using NADPH (Azambuja et al., 2011) and lactoperoxidase which catalyzes the oxidation of various organic and inorganic substrates by hydrogen peroxide (Kohler and Jenzer, 1989). All the aforementioned factors can be used as antioxidative indices.

Several *in vivo* studies showed that aromatic plants can improve the antioxidant system performance by means of increased activity of antioxidant enzymes activity in broiler chicken (Akbarian et al., 2014), rats (Lv et al., 2012) and fish (Azambuja et al., 2011). Furthermore, some *in vivo* studies showed that the intake of aromatic plants made a positive impact on the performance of dairy cows (Kraszewski et al., 2002; Tekippe et al., 2011). However there has been little or no research carried out on small ruminants, and more specifically goats (Heidarian Miri et al., 2013) in spite of the fact that goat milk consumption and derived dairy products are increasing worldwide due to the recognition of the beneficial effects of these products on human health (García et al., 2014). In addition, the farming of dairy goats plays an important role in the economy of many Mediterranean countries (Park et al., 2007).

Greek oregano (*Origanum vulgare* ssp. *hirtum*) is an aromatic plant that belongs to the Labiatae family and is commonly found in Mediterranean-type ecosystems (Vokou et al., 1993). Greek oregano contains high amounts of carvacrol or/and thymol which are phenolic compounds that demonstrate potent antioxidant properties (Milos and Makota, 2012). Essential oils (EOs) as well as other natural antioxidants, after absorption in the digestive tract can be transferred into the blood stream and consequently a minor part is incorporated into milk (Pizzoferato et al., 2007). Thus, the main objective of this study was to assess the effects of the consumption of dietary dried Greek oregano on blood and milk antioxidant enzymatic indices (superoxide dismutase, glutathione peroxidase, glutathione reductase, catalase, lactoperoxidase, glutathione transferase) and on susceptibility of milk to oxidation assessed by FRAP assay in lactating goats. The second objective was to evaluate the yield and chemical composition of the milk.

2. Materials and methods

2.1. Plant material

The oregano plants were collected from the Greek island of Ikaria which is located in the Aegean Sea, in July of 2013 during the midflowering stage. The collected plants (including stems, leaves, and inflorescences but excluding the roots) were naturally dried in a partly closed space for 30 days with an average ambient temperature of 30 °C.

2.2. Animals and diets

Twelve healthy and lactating (mid lactation; 120 ± 7 days in milk) multiparous Alpine goats balanced for age (4–5 years) were used in one 4 week experimental trial and animals were allocated to two groups, with an average body weight of 50.25 ± 3.20 kg (control group; CON) and 48.83 ± 2.95 kg (treatment group; OR). The experiment was conducted in agreement with the guidelines of the Agricultural University of Athens concerning the care and welfare of agricultural animals in order to avoid any unnecessary discomfort to the animals.

The animals were kept in tie stalls equipped for individual feeding and were fed 1.056 kg of DM/d of alfalfa hay and 1.068 kg of DM/d of concentrate mixtures which consisted of 50% basal and 50% lactation ration (Table 1). The OR group received the same rations as the CON group the only difference being the addition of 30 g of ground oregano plants in the lactation ration, in order to provide a daily dosage of 1 ml of oregano EO per animal. This dosage was chosen because some studies showed it has a positive effect on milk quality. Simitzis et al. (2007) reported a positive effect on the milk protein of lactating ewes when a dosage of 1 ml of oregano EO/kg of concentrate was applied. Similar results have been demonstrated by Offer et al. (2005) in dairy cows.

The mixing of the feedstuff and the ground oregano herbs took place every 15 days based on the results of a previous study (Paraskevakis et al., 2015) in order to minimize essential oil losses from the concentrate mixture during storage. Goats were weighted every week. All animals had *ad libitum* access to fresh water throughout the experimental period.