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The effect of dietary oregano essential oil on microbial growth of rabbit carcasses during refrigerated storage

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ABSTRACT

The effect of dietary supplementation with oregano essential oil on microbial growth of rabbit carcasses during refrigerated storage was investigated. A total of 45 weaned rabbits were separated into three equal groups with three subgroups each. One group was given the basal diet and served as control and the other two groups were administered diets supplemented with oregano essential oil at levels of 100 and 200 mg/kg diet, respectively (OR100 and OR200 groups). Total viable counts, *Pseudomonas* spp., lactic acid bacteria, *Brochothrix thermosphacta*, *Enterobacteriaceae* and yeast and mould counts, as well as off-odours and appearance of slime were all assessed on rabbit carcasses stored at 3 ± 1 °C for 12 days. The results showed that performance parameters were not affected ($p > 0.05$) whereas the dietary supplementation with oregano essential oil resulted in lower ($p < 0.05$) average microbial counts on the carcasses, compared to controls, throughout storage. Dietary supplementation with oregano essential oil at 200 mg/kg was more effective in inhibiting microbial growth compared with 100 mg/kg. Sensory evaluation scores indicated that the carcasses obtained from OR100 and OR200 groups gave a noticeable putrid odour after days 8 and 10, respectively, whereas the control carcasses developed off-odours after the 6th day of storage. Slime formation in the controls was observed after day 6, while the OR100 and OR200 groups were just beginning to show slime after days 8 and 10, respectively.

1. Introduction

Rabbits, with high fertility rates, fast growth rates, and short production cycles, are a good source of meat and much needed protein of animal origin. In addition, rabbit meat is a highly digestible, tasty, low-calorie food since its protein content is higher and fat and cholesterol contents are lower compared to other red meats (Dalle Zotte, 2002). The development of rabbit meat production requires high-speed and more automated slaughter lines which can lead to higher microbial risks due to possible cross-contamination during preslaughter (crating, transportation, and holding conditions) and processing (skinning and evisceration) operations (Cavani & Petracci, 2004).

Many attempts have been made to control microbial growth at the surface of meat and meat products with antimicrobial chemicals. Nowadays, consumers have demanded more natural foods, with low levels of chemical additives and food legislation has restricted the use of some synthetic antimicrobials based on a possible toxicity for consumers (Brul & Coote, 1999). In this context, the notable antioxidative, antibacterial and antifungal activity of essential oils of aromatic plants and spices has received considerable attention as a potential food preservative of natural origin. However, their practical application is often limited because of flavour considerations, as well as because their effectiveness is moderated due to interaction with food ingredients and structure (Juven, Kanner, Schved, & Weisslowicz, 1994; Skandamis, Tsigarida, & Nychas, 2000).

Dietary supplementation has been proved to be a simple, convenient and often more effective strategy to inhibit development of lipid oxidation than direct addition of the additive to the muscle food (Higgins, Kerry, Buckley, & Morrissey, 1998; Mitsumoto, Arnold, Schaefer, & Cassens, 1993). The essential oil of oregano, in particular, which is obtained by a steam-distillation process of leaves and flowers of *Origanum vulgare* subsp. *hirtum* plants, is a very promising dietary supplement since it exhibits substantial antioxidant as well as antimicrobial activity *in vitro* (Sivropoulou et al., 1996; Skandamis & Nychas, 2001). Several recent studies have shown that incorporation of this essential oil in chicken, turkey and rabbit diets improved the oxidative stability of the raw and cooked muscle tissues during refrigerated and long-term frozen storage (Botsoglou, Christaki, Fletouris, Florou-Paneri, & Spais, 2002; Botsoglou, Fletouris, Florou-Paneri, Christaki, & Spais, 2003; Botsoglou, Florou-Paneri, Christaki, Fletouris, & Spais, 2002; Botsoglou, Florou-Paneri, Christaki, Giannenas, & Spais, 2004; Botsoglou, Govaris, Botsoglou, Grigoropoulou, & Papageorgiou, 2003; Govaris, Botsoglou,

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Papageorgiou, Botsoglou, & Ambrosiadis, 2004). Furthermore, it exerted a coccidiostatic activity against *Eimeria tenella* in experimentally infected chickens (Giannenas et al., 2003). Govaris, Botsoglou, Florou-Paneri, Moulas, and Papageorgiou (2005) have also demonstrated that incorporation of oregano essential oil in turkeys' diet at the level of 100 mg/kg exerted an inhibitory effect on microbial growth of the breast fillets during refrigerated storage.

Dietary control of meat spoilage by natural extracts, such as oregano oil, that are harmless to consumers would be of value to the meat industry which shows a major concern in extending the shelf life of muscle food. The main objective of the present study was to evaluate the effect of dietary oregano essential oil supplementation on microbial growth of rabbit carcasses during refrigerated storage.

2. Materials and methods

2.1. Animals and diets

Forty-five Californian–New Zealand white rabbits, 42-days old, were divided by restricted randomisation into three equal groups with three subgroups, ensuring the groups were balanced for body weight. All nine subgroups were housed in separate stainless steel cages under controlled temperature and light conditions (18 h/6 h, light/dark photoperiod cycle).

To meet the nutrient requirements of the rabbits during the experimental period, a complete basal diet was formulated, which was fed pelleted (Table 1). One of the groups (control) was given the basal commercial diet, whereas the remaining two groups had the basal diet supplemented with 100 mg (OR100) or 200 mg (OR200) of oregano essential oil/kg diet.

Supplementation with oregano essential oil was achieved by incorporating an appropriate amount of Oregano-Stim (Meriden Animal Health Ltd., Luton, UK), a powder containing 5% essential oil of *O. vulgare* subsp. *hirtum* (Ecopharm Hellas, SA, Kilkis, Greece), and 95% feed grade inert carrier. Feed and drinking water were allowed to all rabbits *ad libitum*. All rabbits were weighed individually on weaning and thereafter on a weekly basis. Feed intake was monitored weekly on a subgroup basis and average feed intake (g/day) and feed conversion ratio were calculated.

After feeding for 42 days, rabbits were stunned and slaughtered. Throughout the trial, rabbits were handled according to the principles for the care of animals in experimentation (National Research Council, 1985).

Table 1
Composition of the basal diet.

Components	(g/kg feed)
Lucerne meal	390
Barley grains	200
Wheat bran	176
Sunflower seed meal	120
Soybean meal	54.0
Soybean oil	10.0
Corn gluten	22.5
Limestone, pulverized	9.9
Monocalcium phosphate	3.6
Biolysine-BASF	2.6
DL-Methionine	1.0
Sodium chloride, iodized	4.6
Choline chloride	0.8
Binder	1.0
Vitamin premix[a]	2.0
Trace-mineral premix[b]	2.0
Total	1000
Chemical analysis[c]	911.5
Dry matter	169.2
Crude protein	33.2
Ether extract	144.4
Crude fibre	76.2
Ash	
Calculated values	
Calcium	12.0
Phosphorus (total)	6.9
Lysine	7.5
Methionine + cystine	6.5
ME [MJ/kg]	10.0

[a] Provided per kg of diet: vit. A, 12,000 IU; vit. D_3, 2500 IU; vit. E, 20 mg; vit. B_1, 1.5 mg; vit. B_2, 7.5 mg; vit. B_6, 4.5 mg; vit. B_{12}, 30 μg; vit. K_3, 3 mg; nicotinic acid, 45 mg; pantothenic acid, 15 mg; folic acid, 0.8 mg; biotin, 0.08 mg; vit. C, 10 mg; choline chloride, 450 mg.

[b] Provided per kg of diet: Zn, 125 mg; Mn, 100 mg; Fe, 62 mg; Cu, 7.5 mg; Co, 0.2 mg; I, 2 mg; Se, 0.2 mg.

[c] According to AOAC (1990).

2.2. Storage and sampling procedure

Post-chill rabbit carcasses were packed in isothermal boxes and transported within 1 h to the laboratory where they were separately put on sterilised aluminium trays 200 mm × 111 mm (Ac-Pac Dhler, Germany) and covered with an oxygen permeable polyvinyl chloride (PVC) film. According to the supplier (Ver-Pack, Greece), the oxygen transmission rate of the web (polyvinyl chloride) was 250–300 ml/m^2 · 24 h atm) at 23 °C. The samples were kept at 3 ± 1 °C and tested at day 0 and at 2-day intervals for up to 12 days of storage.

For the microbiological analyses five rabbit carcasses of each subgroup were stored and the surface tissue was examined with a maceration method. Five pieces of surface tissue (0.5 cm deep) from the leg, breast, foreback, rearback and the regions around the visceral cavity obtained using a sterile cork borer (3.6 cm in diameter, 10 cm^2) which was punched through the packaging film. To balance out the different bacterial loads of the individual carcasses, a surface tissue sample was taken from each of five carcasses of each subgroup in rotation and mixed. To prevent dehydration, the punched film piece was replaced on the carcass, fixed and tightly sealed with a waterproof adhesive tape.

2.3. Microbiological methods

Five pieces of surface tissue were cut up coarsely with sterilized scissors in a sterile container and homogenized for 2 min in a stomacher (Lab blender 400, Seward Medical, London, UK), with 45 ml of sterile 0.1% peptone water. From the resulting dilution, appropriate decimal dilutions were prepared, using the same diluent (Acuff, 1992) and plated in duplicate to enumerate the following microorganisms: (1) total viable counts (TVC) were enumerated by the pour-plate method using Plate Count Agar (Oxoid). Plates were incubated at 25 °C for 3 days (ISO, 2003; Maturin & Peeler, 1998). (2) Lactic acid bacteria (LAB) were enumerated by the pour-overlay method using deMan Rogosa Sharpe (MRS) agar (Oxoid). Plates were incubated anaerobically at 30 °C for 3 days. (3) *Pseudomonas* spp. were enumerated using the surface-plate method on Pseudomonas agar base (Oxoid), plus the selective agent Pseudomonas C-F-C supplement (Oxoid). Plates were incubated at 25 °C for 2 days. (4) *Brochothrix thermosphacta* was enumerated using the Gardner's STAA agar (Oxoid) (Gardner, 1996; Sneath & Jones, 1986). After 3-day incubation at 25 °C, the plates were flooded with 1% tetramethyl-*p*-phenylene diaminedihydrochloride solution and uncoloured oxidase negative colonies were counted as *Br. thermosphacta*. (5) *Enterobacteriaceae* were enumerated by the pour-overlay method using Violet Red Bile Glucose agar (Merck). Plates were incubated at 37 °C for 24 h. Purple colonies surrounded by the purple zone, were enumerated and recorded as *Enterobacteriaceae* (ISO, 2004). (6) Yeast and mould