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In vivo and *in vitro* effects of selected antioxidants on rabbit meat microbiota

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ABSTRACT

The purpose of this study was to investigate the effect of dietary vitamin E or EconomasE™ supplementation on the growth of several background/pathogenic bacteria on rabbit carcasses and hamburgers during refrigerated storage. For 51 days, 270 New Zealand rabbits received either a basal diet, or experimental diets enriched with 100 or 200 mg/kg of vitamin E or EconomasE™. The bacteria studied were *Salmonella*, *Listeria monocytogenes*, *Pseudomonas*, *Enterobacteriaceae*, *Escherichia coli*, coagulase-positive staphylococci, plus both mesophilic and psychrotrophic aerobes. The growth of *Listeria monocytogenes* on contaminated patties was evaluated through a challenge test. The potential protective or antimicrobial effect of vitamin E or EconomasE™ on *Listeria monocytogenes* or *Pseudomonas aeruginosa* was assessed *in vitro*. Diet did not influence the concentrations of bacteria found on rabbit carcasses and developing on hamburgers. Vitamin E (*in vivo* and *in vitro*) and EconomasE™ *in vivo* had a protective antioxidant role, while EconomasE™ *in vitro* had strong antibacterial activity against *Listeria monocytogenes*, but not against *Pseudomonas aeruginosa*.

1. Introduction

Rabbit meat presents excellent nutritional and dietetic properties and meets the current demand for low-fat meat (Dalle Zotte & Szendrö, 2011). However, rabbit meat is expensive, time-consuming to prepare and rather perishable because it is prone to oxidative damage due to its high level of polyunsaturated fatty acids (Abdel-Khalek, 2013). Consequently, in order to expand the market, in addition to retail fresh cuts, many rabbit meat industries have tried to approach the consumer through the production of meat preparations, such as hamburgers or patties, which may benefit from antioxidants. Given the above, a relevant question might be raised, as already suggested by Sofos, Cabedo, Zerby, Belk, and Smith (2000): are those antioxidants able to protect bacterial cellular membranes as well, when present on the very same meat or meat preparation? There is plenty of literature regarding the effects of antioxidants on rabbit meat and meat preparations, but special attention has been paid to vitamin E, i.e. DL-α-tocopherol (VE) (Castellini, Dal Bosco, & Bernardini, 2001; Castellini, Dal Bosco, Bernardini, & Cyril, 1998; Dal Bosco, Castellini, Bianchi, & Mugnai, 2004; Lo Fiego et al., 2004). Due to its high antioxidant activity, VE, especially as α-tocopherol acetate, is commonly used in animal feed to promote growth and to improve meat quality; VE is deposited in muscle cell membranes and lipid depots, thus reducing lipid oxidation, which is one of the most significant causes of meat deterioration during refrigeration (Hu et al., 2015). Moreover, among the antioxidants it is possible to include the EconomasE™ (EcoE), a patented commercial premixture of nutritional additives consisting mainly of L-ascorbic acid (50,000 mg/kg) and organic selenium produced by Saccharomyces cerevisiae CNCMI-3060 (750,000 mg/kg). Selenium in yeast is incorporated into organic compounds, mainly selenomethionine, and low molecular weight seleno-components. Selenium is an essential trace element involved in various physiological functions; as an integral part of

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selenoproteins, it plays an important role in the antioxidant defense system against reactive molecules and free radicals (Ahmad et al., 2012; Mehdi, Hornick, Istasse, & Dufrasne, 2013). Despite its importance, to our knowledge, information about the effect of dietary supplementation of antioxidant on microbial growth in rabbit carcasses and meat preparations is almost non-existent. In detail, carcasses and meat preparations from rabbits fed additional levels of VE or EcoE have not been studied so far in terms of their microbial status.

At each step of the food chain, meat and meat preparations might be contaminated and cold storage does not always inhibit the growth of bacteria. In particular, *Listeria monocytogenes* is a ubiquitous pathogen, and is especially dangerous because it is able to grow also at refrigeration temperatures, unlike most other foodborne pathogens (EFSA, 2014; Swaminathan, Cabanes, Zhang, & Cossart, 2007). A wide variety of meats and processed products have been associated with L. *monocytogenes* contamination at a prevalence which can be high because of various conditions of storage, distribution and handling in addition to inadequate bacterial inactivation (Swaminathan et al., 2007). Furthermore, *L. monocytogenes* survives in foods for a long time, even under adverse conditions (Ramaswamy et al., 2007; Rocourt, BenEmbarek, Toyofuku, & Schlundt, 2003) and it causes severe symptoms and diseases (meningitis, septicemia and abortion) (Ramaswamy et al., 2007). It must also be remembered that human listeriosis cases in Europe have been increasing in recent years (EFSA & ECDC, 2014). In contrast, the *Pseudomonas* genus represents the dominant contaminant on rabbit carcasses and other packed meat (Bobbitt, 2002; Rodríguez-Calleja, Santos, Otero, & García-López, 2004). In particular, *Pseudomonas aeruginosa* is a food spoilage agent included in the list of bacteria carrying a biological risk, unlike all other species of *Pseudomonas*. In fact, the public health interest in these two microorganisms stems from the fact that they are both human pathogens and, according to regulations in Europe and the United States, these two bacteria are classified in risk group 2 on the basis of biohazard (EC, 2000; HHS, 2013).

The purpose of this work was to investigate the effect of dietary VE or EcoE supplementation on the growth of eight types of background or pathogenic bacteria on rabbit carcasses and rabbit meat preparations (hamburgers) during refrigerated storage. The growth of *Listeria monocytogenes* on contaminated patties was also evaluated through a challenge test. The potential protective or antimicrobial effect of VE or EcoE on L. *monocytogenes* or *Pseudomonas aeruginosa* was also assessed *in vitro*.

2. Materials and methods

This work represents the microbiological part of a multidisciplinary research project designed to evaluate the shelf-life of rabbit meat, including the study of carcass quality and the technological, nutritional and sensory quality of rabbit meat.

2.1. Animals and diets

Two hundred and seventy commercial New Zealand white rabbits (*Oryctolagus cuniculis*) provided by the Rabbit Genetic Centre of the Martini Group were selected for this study. Thirty-five-day-old males from a single breeding were randomly divided into five experimental units (*e.u.*) of 54 animals each. Every *e.u.* was housed under controlled temperature and light conditions (12 h light/12 h dark photoperiod cycle), equally and randomly divided into three cages (= replicates) having provision of *ad libitum* feeding and watering. A starter complete basal diet for growing rabbits and a subsequent finisher diet for fattening rabbits were formulated to meet the nutrient requirements of the animals during the experimental period (Table 1).

Two antioxidants in two different concentrations were tested in this work. The basal diets of two *e.u.* were supplemented with 100 or 200 mg/kg of DL-α-tocopherol acetate (Sigma-Aldrich, St. Louis, MO, USA) (indicated as VE 100 and VE 200, respectively) while the diets of other two *e.u.* were supplemented with 100 or 200 mg/kg of EcoE (Alltech Ireland Ltd., Dunboyne, Ireland) (indicated as EcoE 100 and EcoE 200, respectively), as suggested by the producer. The remaining *e.u.* was fed a normal diet and used as a control (CTRL). After 51 days, 256 animals (mortality 5.2%) were slaughtered in the Ma.Ge.Ma abattoir (Savignano sul Rubicone, FC, Italy); rabbits underwent electrical stunning followed by cutting of the carotid arteries and jugular veins. Two carcasses were discarded due to abscesses. Slaughter weights (g) ± standard errors (SE) were: 2991 ± 35.09 (CTRL); 2934 ± 29.18 (VE 100); 2867 ± 38.85 (VE 200); 2905 ± 37.15 (EcoE 100); and 2981 ± 30.31 (EcoE 200). The abattoir structure, layout and hygiene procedures were in compliance with European Union requirements (EC, 2004). All handling procedures followed the recommendations of the European Council Directive 86/609/EEC for the protection of animals used for experimental and other scientific purposes (EEC, 1986).

Ten carcasses were randomly selected out of each *e.u.* (total number = 50). The selected carcasses were transported to the DIMEVET laboratory of Food Hygiene and Technology in accordance with traceability and cold chain. After 24 h at 4 °C, carcass hygiene was tested and then carcasses were used to produce hamburgers and patties.

Table 1
Ingredients and chemical composition of the basal starter and growth diets (as fed).

	Starter diet	Finisher diet
Ingredients (g/kg)		
Wheat bran	250	160
Sunflower seed meal	195	215
Sugarbeet pulp	150	150
Lucerne meal	100	90
Dehydrated lucerne meal	80	30
Sugarcane molasses	50	70
Wheat middlings	50	50
Oats	40	40
Barley	-	85
Grape seed meal	-	50
Pomace oil	35	-
Palm oil	8	11
Soybean oil	3.3	3.3
Soybean hulls	-	20
Calcium carbonate	12	8
Sodium chloride	4	4
Vitamin-mineral premix[a]	22.7	13.7
Total	1000	1000
Chemical composition[b]		
Moisture	115	109
Crude protein	152	152
Crude fiber	170	161
Crude oils and fat	34	36
N-free extracts	453	473
Ash	76	69
Calcium	9.1	8.0
Phosphorus	5.2	5.2
Sodium	3.1	2.2

[a] Provided per kg of diet: 16.000 IU vitamin A; 1.600 IU vitamin D3; 60 mg vitamin E acetate; 66 mg robenidine hydrochloride (only for the starter diet); 39 mg cupric sulphate; 0.22 mg sodium selenite; 0.23 mg basic cobaltus monosodium carbonate; 4.6 mg anhydrous calcium iodate; 414 mg ferrous carbonate; 99 mg zinc oxide; 66 mg manganous oxide.
[b] N-free extracts were obtained by difference.

2.2. Microbiological analyses

Microbiological assays on rabbit carcasses and meat preparations were performed using international standard methods. Samples were prepared according to the ISO standard 6887-1 (ISO, 1999) and 6887-2 (ISO, 2003a) and were diluted with a solution of 0.1% tryptone (Oxoid Ltd., Basingstoke, England) and 0.85% NaCl (Oxoid Ltd.) in distilled water. ISO standard 6579 (ISO, 2007) and ISO 11290-1 (ISO, 2004a) were used respectively to detect *Salmonella* spp. and *L.*