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EFFECTS OF PLANT EXTRACTS AND ESSENTIAL OILS AS FEED SUPPLEMENTS ON QUALITY AND MICROBIAL TRAITS OF RABBIT MEAT

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Abstract: The effects of dietary supplementation with onion, cranberry and strawberry extracts and essentials oils on meat quality were analysed. Five groups of 48 Grimaud female weaned rabbits received the supplemented or the control ration; the experimental unit was a cage of 6 rabbits. Each experimental diet contained 10 ppm of added active ingredients. Rabbits were fed with the experimental diets for 4 wk before determining slaughter and carcass traits and determining the pH at 1 and 24 h post mortem (pHu) of the *Longissimus dorsi* (LD) and the *Biceps femoris* muscle, left and right, respectively. Cooking loss, drip loss and L*, a* and b* colour parameters were obtained for the right LD and for ground meat and antioxidant status (TBARS, DNPH, Folin Ciocalteu) was measured. Only the pHu of the LD muscle for the strawberry supplemented group was significantly lower when compared to the control group (P=0.04). However, we note that for the pH of the LD, the average was less than 6 for the meat of animals that received a diet enriched in polyphenols, compared to the control group. Plant extract supplementation did not influence meat quality traits, growth performance or oxidative stability. However, under aerobic and anaerobic conditions, our results indicate that dietary supplementation with extracts rich in polyphenols, especially with essential oils, had a small but sporadic positive effect in reducing bacterial microflora compared to the control group (P<0.05). In conclusion, plant extracts and essential oils can be used in a rabbit diet without adverse effects on performance and meat quality traits. This effect could be optimised by investigating higher doses.

Key Words: essential oils, plant extract, polyphenol, rabbit meat, shelf life.

INTRODUCTION

According to 2002 data, rabbit meat production is particularly developed in the Mediterranean countries of the EU, such as in France, with a consumption of 1.5 kg per person per year (Magdelaine, 2003), whereas other countries, such as the USA, only consume 0.02 kg per person per year (Cliche, 2010). In Canada, rabbit meat consumption per capita in 2013 was 0.024 kg (AAFC, 2012).

Rabbit meat is low in calories, high in protein and very rich in polyunsaturated fatty acids compared to other meats (Combes, 2004). Although the high concentration of polyunsaturated fat in rabbit meat is a nutritional advantage (ratio omega-6/omega-3=5.9; Combes, 2004), it makes the meat particularly sensitive to lipid oxidation and affects meat appearance and flavour. Its ultimate pH (pHu) is particularly high, compared to other meats, such as beef and pork (5.5-5.7), which makes it more prone to bacterial growth and spoilage (Jolley, 1990; Faucitano *et al.,* 2010). According to Blasco and Piles (1990), the pHu greatly influences meat quality and microbial growth, increasing the risk of foodborne infection. As reported in a number of studies, susceptibility to microbial growth is higher in carcasses with higher pH values (pH>6), resulting in Dark, Firm and Dry (DFD) meat (review by Faucitano *et al.,* 2010).

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The occurrence of foodborne illness is increasing in industrialised countries (King *et al.,* 2000). In Canada, Thomas *et al.* (2008) estimated the number of foodborne illness cases to be 11 million with an estimated annual cost of $3.7 billion (CAD). Hence, microbiological food safety continues to be a major concern for consumers, industries and governments (Sofos and Geornaras, 2010), and meat safety remains an important consideration, as meat is the most commonly incriminated food, accounting for 32.1% of reported cases of foodborne illnesses in Quebec (DGSAIA, 2012).

For these reasons, it is important to find effective solutions to produce safer meat. Some strategies include the addition of synthetic products to the meat, such as nitrates, phosphate, lactate, acetate and sodium diacetate. Other strategies, including the use of natural preservatives, have been of great interest to the meat industry in response to the growing consumer demand for food products without artificial preservatives. Many small fruits like cranberry and strawberry contain large amounts of phenolic compounds. Cranberry is very rich in proanthocyanidins, which have inhibitory effects on *Staphylococcus aureus* and *Escherichia coli* growth in meat (Daglia, 2012). Exogenous addition of cranberry powder to fresh turkey meat (Raghavan and Richards, 2006) and minced pork meat (Lee *et al.,* 2006) also decreased lipid oxidation. Studies on beef confirmed the efficacy of an onion extract dietary supplement in reducing microbial contamination (Irkin and Arslan, 2010). Essential oils extracted from various spices and herbs were successfully used in animal feed to increase the oxidative stability of meat (Botsoglou *et al.,* 2002, 2003, 2004). Soultos *et al.* (2009) demonstrated that adding oregano oil to the diet of rabbits reduced total mesophilic aerobes, *Pseudomona*s spp. and *Enterobacteriaceae* after 12 d of storage under aerobic conditions, and Fortier *et al.* (2012) obtained similar results with pigs.

In order to improve rabbit meat quality and shelf life, we examined the effects of feed supplementation with essential oils or onion, strawberry or cranberry extracts on carcass microbial contamination as well as meat quality and oxidation stability.

MATERIAL AND METHODS

Animal management and alimentation

Experimental design and animal handling procedures were approved by the Animal Use and Care Committee of Université Laval, which strictly adheres to the Guidelines of the Canadian Council on Animal Care (CCAC, 2009). A total of 240, 35-day-old, weaned, female Grimaud breed rabbits were obtained from a commercial farm (Laprodéo, Saint-Tite, Quebec, Canada) and kept in conventional commercial cages. Rabbits were placed 6/cage and the cage constituted the experimental unit. Eight cages were analysed per experimental group. Upon arrival, the animals were acclimatised to their new environment and received a control diet, followed by 4 wk on the experimental feed (Table 1).

Table 1: Nutritional values and composition[a] of the commercial and experimental diets.

Crude protein (min) (%)	16.8
Crude fat matter (min)(%)	4.37
Acid detergent fibre (max) (%)	24.4
Neutral detergent fibre (max) (%)	33.0
Crude fiber (max) (%)	18.1
Calcium (real) (%)	0.99
Phosphorous (real) (%)	0.48
Sodium (real) (%)	0.30
Vitamin A (min) UI/kg	5542
Vitamin D (min) UI/kg	755
Vitamin E (min) UI/kg	35.0
Added Selenium mg/kg	0.10
Extracts of active ingredients (%)	0.001

[a]Composition: Alfalfa, beet pulp, wheat, soybean meal, canola meal, corn gluten feed, molasses, mineral and vitamin premix.

A group without supplement served as the control group (commercial feed) and 4 experimental groups received a supplement of essential oils (Xtract™ Instant, Pancosma SA, Geneva, Switzerland), or onion, strawberry or cranberry extract (Nutra Canada Inc., Champlain, Québec, Canada). The level of Xtract (10 ppm of active ingredients) was added according to supplier recommendations to promote a healthy rabbit digestive system. It contains thyme oil, trans-anethole, thymol, eugenol, cinnamaldehyde, capsicum oleoresin, gum arabic and maltodextrins. The other groups were supplemented at the same level (10 ppm of total polyphenol) based on the content of total polyphenols measured by the Folin-Ciocalteu method according to Waterhouse (2003). Supplement was mixed directly with the other feed ingredients prior to pelleting at 60°C and cooling at 16°C within 2.5 min. Feed was manufactured in a commercial facility in separate 600 kg batches (Belisle Solution Nutrition, St-Mathias-sur-Richelieu, Quebec, Canada).

The animals were fed *at libitum*. They were weighed and the feed intake was measured weekly during the experimental period to determine the body weight (BW), the average daily weight gain (ADG), the average daily feed intake (ADFI) and the feed conversion ratio (FCR). Rabbits were slaughtered at an average weight of 3067±258 g. They were fasted 20 h before slaughter, including transport and lairage time, following current commercial practices to reduce transport-related sickness. They had access to water at all times prior to transport. The length of transport to the abattoir was 5 h with a third of the distance consisting of rural roads. The animals were slaughtered in a federally inspected establishment according to the Canadian Food Inspection Agency rules and the Meat Hygiene Manual of Procedures.

Meat quality measurement

The muscular pH of the *Biceps femoris* (BF) and the *Longissimus dorsi* (LD) muscles were measured post mortem after 1 (pH1) and 24 h (pHu; Blasco and Ouhayoun, 1996) using a portable pH meter (ROSS, Orion Star A221, Thermo Scientific, Beverly, CA, USA) combined with an Orion Kniphe pH electrode (ThermoFisher, Nepean, ON, Canada) and a temperature compensation probe (928 007 MD, micro probes ATC, Maryland, USA). Meat colour was evaluated 24 h after slaughter on the LD and the exposed surface of the BF using a Chromameter (Chromameter CR 300 Minolta Ltd., Osaka, Japan) equipped with a D65 light source and a 0° viewing angle geometry according to the reflectance coordinates (L*, a*, b*; CIE, 2004), after exposing the muscle surface for 20 min blooming time (Faucitano *et al.,* 2010). The drip loss was measured on a piece of LD (about 2 cm thick×2.5 cm in diameter, from the front part of the carcass) by weight difference, according to the EZ-Driploss method (Rasmussen and Anderson, 1996), where samples are stored at 4°C for 48 h. The meat exudate lost (%) during cold storage was also measured by weight difference of the thighs upon microbial analysis. The cooking loss was also determined on a similar piece of LD muscle (Pla, 1999) and is expressed as a percentage of the initial weight loss. Each sample was placed into an 18-oz Whirl-Pak bag (Nasco Whirl- Pak®, USA) and immersed in a water bath at 70°C for 15 min after removing the air from the bag. The samples were then removed from the bag, patted dry with filter paper and weighed (Vergara *et al.,* 2005; Apata *et al.,* 2012).

Muscle Sampling

One leg per animal was packaged aerobically in a Styrofoam tray (14×24×4.5 cm, width×length×height) with an absorbent pad, sealed with an oxygen-permeable polyethylene film (132 L; oxygen transmission 825 cc/645 cm^2 per 24 h at 23°C; water vapour transmission rate 24 g/645 cm^2 per 24 h at 38°C and 90% relative humidity [RH]) obtained from a local food equipment distributor (Emballage L. Boucher, Quebec, QC, Canada) and stored at 4°C for 0, 5, 10 or 15 d. The other leg was vacuum packaged (Sipromac, St-Germain, QC, Canada) in bags (nylon [23%] and polyethylene [77%; seven multilayers] of 1136 L; oxygen transmission 3.3 cc/645 cm^2 per 24 h at 23°C; water vapour transmission rate 0.5 g/645 cm^2 per 24 h at 38°C and 90% RH; Sealed Air Co, Mississauga, ON, Canada) and stored also at 4°C for 0, 15, 30 or 45 d. The rest of the carcass was deboned and the meat was ground and stored at –30°C (Electric meat Grinder, No RE50255, IPNO IPXI, China).

Microbial Analysis

For microbial enumeration of the thighs, a sampling procedure similar to the one described by Brichta-Harhay *et al.* (2007) for whole poultry carcasses was used. One leg from the rabbits (6 per cage) coming from the same cage (our experimental unit; 8 cages for each experimental group) was randomly taken at each sampling time. Each cage was sampled at every sampling time and conditions (aerobic and anaerobic). Thigh was aseptically placed in a sterile Stomacher bag (Stomacher® 400C, Seward Laboratory Systems Inc., London, UK), weighed (see details on meat exudate lost during cold storage above) and sealed after addition of 300 mL of 0.1% (weight/volume) peptone water (Bacto peptone, Difco Laboratories, Inc., Detroit, MI, USA). The bag was placed on a rotary shaker (Boekel Scientific Orbitron Rotator II, model 260250, New York, USA) for 1 min on each side. The samples were then manually massaged for 30 s to remove microorganisms from the surface. Ten-fold dilutions were done in 0.1% peptone water for enumeration on appropriate agar plates (Saucier *et al.,* 2000). Total Aerobic Mesophilic (TAM) counts were performed on Plate Count Agar medium (PCA; Difco Laboratories Inc.) incubated at 35°C for 48 h (MFHPB-18, Health Canada, 2001). Presumptive Lactic Acid Bacteria (LAB) were enumerated on deMan, Rogosa and Sharp (MRS; Difco

Laboratories Inc.; Saucier *et al.,* 2000) and the plates were incubated anaerobically for 48 h at 25°C using anaerobic jars with an envelope generator of H_2 and CO_2 (AnaeroGen™ 2.5L, AN0025A, Oxoid Company, Nepean, ON, Canada). Presumptive *Pseudomonas* spp. were determined on Heart Infusion Broth agar (Difco Laboratories Inc.) after adding a Cetrimide-Fucidin-Cephalosporin supplement (Oxoid, No.SR0103E) and plates were incubated at 25°C for 48 h (Mead and Adams, 1977; Gill and Greer, 1993). Coliforms and *E. coli* counts were determined using 3M Petrifilm™ plates after incubating at 35°C for 18-24 h (MFHPB-34, Health Canada, 2013). Presumptive *S. aureus* were evaluated on 3M Petrifilm™ plates and incubated at 37°C for 26 h (MFLP-21, Health Canada, 2004). Enterobacteriaceae counts were performed on 3M Petrifilm™ (MFLP-09, Health Canada, 2007) and incubated at 37°C for 24 h. Measurements were performed in duplicate and all bacterial counts were transformed to a $\log_{10}$ value of colony forming units per gram of thigh weight ($\log_{10}$ CFU/g) prior to statistical analysis according to Gill (2000).

Proximate analysis

Ground meat samples (100 g) were lyophilised (Model 6203-3005-0L, Virtis Co., Gardiner, NY, USA) for 7 d. The fat content was measured using a Soxtec system (Soxtec system HT 1043 Tecator, Hoganas, Sweden) using procedure 991.36 of the Association of Official Analytical Chemists (AOAC, 1995). Total protein was quantified using procedure 992.15 of the AOAC (1995) with a LECO® protein analyser (model FP-2000, Leco Corp., St. Joseph, MO, USA). The results are expressed on the basis of wet weight and the analyses were performed in triplicate.

Determination of muscle antioxidant status

Lipid oxidation

Products of lipid oxidation were measured in minced meat, quantitated using the thiobarbituric acid reactive substances (TBARS) method and are expressed as malondialdehyde (MDA) equivalents according to the method of Ermis *et al.* (2005) with the following modifications. Briefly, 10 g of minced meat was homogenised with 10 mL of Phosphate Buffered Saline solution (PBS, Sigma-Aldrich, St-Louis, MO, USA). After centrifugation (3000×*g*, 4°C, 15 min), 12.5 µL of butylated hydroxytoluene (BHT) solution was added to 500 µL of supernatant and vortexed. Then, 250 µL of trichloroacetic acid (TCA) was added to the mixture and placed on ice for 30 min. After centrifugation, (3000×*g*, 4°C, 10 min), 500 µL of the supernatant was added to 37.5 µL of EDTA (ethylenediaminetetraacetic) and 125 µL of thiobarbituric acid in 0.05N NaOH followed by 15 min in boiling water (100°C) to allow the colour reaction to develop. After heating, the samples were cooled and centrifuged for 10 min at 3000×*g* and 4°C. Absorbance (100 µL) was measured at 530 nm using a spectrophotometer (Varioskan™, Microplate instrumentation Thermo Electron Corporation, Vantaa, Finland). The results were expressed as nanomoles of MDA per g of meat. Measures were performed in triplicate for each meat sample.

Total phenol content

Total phenol content was measured using the method of Jang *et al.* (2008). Each meat sample (5 g) was homogenised in distilled water (15 mL) and chloroform (9 mL), then centrifuged at 3000×*g* for 5 min at room temperature (21°C). Chloroform was added to remove the lipids. The total phenol content in the aqueous supernatant was estimated by the Folin-Ciocalteu method (Subramanian *et al.,* 1965). A 1 mL aliquot of diluted sample (1:4, v/v) was added to 500 mL of 2N Folin-Ciocalteu's phenol reagent (Sigma-Aldrich, St.Louis, MO, USA) followed by the addition of 1 mL of $NaCO_3$ (10%). Reaction mixture was vortexed and the absorbance was measured with a spectrophotometer (Varioskan™) at 700 nm after incubating for 1 h at room temperature (21°C). Quantification was based on a standard curve generated with gallic acid. All measurements were performed in triplicate.

Carbonyl content measurement

Protein carbonyl groups were evaluated on 5 g of ground meat using an assay kit from Cayman Chemical Company (Item No.10005020, Ann Arbor, MI, USA). Nucleic acids were removed according to the manufacturer's instructions. Absorbance was measured at 370 nm (Varioskan™) and the results are expressed as nanomoles of 2,4-dinitrophenylhydrazine (DNPH) fixed per mg of protein. All measurements were performed in triplicate.

Statistical analysis

To determine whether treatment, time, and their interactions had an effect on the microbiological analysis, data were analysed by an analysis of variance (ANOVA) using the MIXED procedure of SAS software. The 5 treatments were analysed independently to determine the overall effect of supplementation with plant extracts (onion, cranberry, and strawberry) and essentials oils *vs.* the control treatment. For these analyses, the storage time under aerobic (0, 5, 10 or 15 d) and anaerobic conditions (0, 15, 30 or 45 d) was taken into consideration (SAS Institute Inc., 2002). Logistic regression models, using the GLIMMIX procedure, were used to evaluate the effect of dietary treatments on the presence or absence of coliforms, *E. coli,* presumptive *S. aureus* and Enterobacteriacea, in both anaerobic and aerobic storage conditions. Frequency distributions for coliforms, *E. coli,* presumptive *S. aureus* and Enterobacteriacea were obtained with FREQ procedure. Significant difference was declared at $P<0.05$.

RESULTS

Growth performance

The effect of dietary supplementation with plant extracts and essential oils on the rabbits' growth performance is presented in Table 2. Overall, there was no significant difference between the control and the experimental groups

Table 2: Growth performance of weaned rabbits fed experimental diets.

							P-value			
	CTL	EO	C	O	S	SEM	CTL *vs.* EO	CTL *vs.* C	CTL *vs.* O	CTL *vs.* S
Initial BW (g)	1698	1736	1719	1745	1729	36	NS	NS	NS	NS
Week 1										
BW on d 7 (g)	2115	2177	2181	2141	2160	32	NS	NS	NS	NS
ADG (g/d)	59.6	63.1	66.1	56.6	61.4	13.1	NS	NS	NS	NS
ADFI (g/d)	178	172	176	178	187	12	NS	NS	NS	NS
FCR	0.332	0.368	0.376	0.320	0.345	0.059	NS	NS	NS	NS
Week 2										
BW on d 14 (g)	2437	2450	2505	2455	2534	41	NS	NS	NS	0.097
ADG (g/d)	45.9	44.9	46.3	44.9	47.5	3.5	NS	NS	NS	NS
ADFI (g/d)	175	170	175	183	186	9	NS	NS	NS	NS
FCR	0.264	0.265	0.263	0.247	0.258	0.015	NS	NS	NS	NS
Week 3										
BW on d 21 (g)	2729	2780	2792	2757	2827	46	NS	NS	NS	NS
ADG (g/d)	41.7	47.1	40.9	43.1	41.7	2.2	0.088	NS	NS	NS
ADFI (g/d)	176	180	178	182	181	8	NS	NS	NS	NS
FCR	0.238	0.264	0.230	0.241	0.232	0.015	NS	NS	NS	NS
Week 4										
BW on d 28 (g)	3046	3101	3062	3032	3098	48	NS	NS	NS	NS
ADG (g/d)	45.3	45.7	38.6	39.3	38.7	2.5	NS	0.071	NS	0.077
ADFI (g/d)	151	157	155	148	153	6	NS	NS	NS	NS
FCR	0.297	0.291	0.251	0.266	0.253	0.023	NS	0.050	NS	0.065
Global										
ADG (g/d)	48.1	50.1	47.9	45.9	47.4	4.3	NS	NS	NS	NS
ADFI (g/d)	170	170	171	173	175	7	NS	NS	NS	NS
FCR	0.282	0.296	0.281	0.268	0.272	0.019	NS	NS	NS	NS

CTL: control; EO: essential oils; C: cranberry; O: onion; S: strawberry; SEM: standard error of the mean; n=8 rabbit cages, a cage of 6 rabbits is the experimental unit. BW: body weight; ADG: average daily weight gain; ADFI: average daily feed intake; FCR: feed conversion ratio. CTL *vs.* EO: Contrast *a priori* to determine the effect of treatment control *vs.* Essential oils; CTL *vs.* C: control *vs.* cranberry; CTL *vs.* O: control *vs.* onion; CTL *vs.* S: control *vs.* strawberry.
NS: not significant.

except for the feed conversion ratio with cranberry supplementation on week 4 (P=0.050). Overall, during the whole feeding period, growth was similar for all experimental groups as slaughter weight was not significantly different.

Proximate composition and pH of the meat

The effect of the dietary treatments on physicochemical analyses and meat quality parameters of the rabbit meat are presented in Table 3. Protein content (17.9 to 19.50%), fat (11.2 to 14.3%), moisture (66.5 to 68.4%) and cooking loss (30.0 to 30.6%) were not influenced significantly by the dietary treatments (P>0.05). Furthermore, drip loss, measured 48 h after slaughter, was not affected by supplementation compared to the control group (P>0.05). Overall, water loss was very small (0.03-0.09%) and only a relevant but no significant difference was observed during anaerobic storage (45 d; P=0.09). Nevertheless, a negative linear effect of time was observed throughout the storage period in aerobic (P=0.0017) and anaerobic (P=0.0001) conditions.

No significant difference was observed for the pH in the LD and BF muscle 1 h after slaughter (6.39 to 6.75 and 6.40 to 6.76, respectively; Table 4). The pHu in LD muscle for the strawberry supplemented group was significantly lower than the control group (P=0.04), but only a no significant downward was measured in the BF muscle (P=0.08). It is important to note, however, that the average pHu in LD muscle for all groups supplemented with plant extract were below 6 compared to the control (Table 4). A pH of 6 is the threshold pH value for DFD meat (Faucitano *et al.,* 2010).

Colour of the meat

Colour parameters of the LD muscle: L* (lightness), a* (redness), b* (yellowness) were not significantly influenced by the dietary enrichment in polyphenols (P>0.05; Table 4). When evaluating BF muscle, meat from the rabbits fed rations supplemented with cranberry extract had a significantly higher red colour index (a*=3.23; P<0.03) compared to the control (a*=2.11). When the ration was supplemented with strawberry extract, the lightness index (L*=50.6; P=0.03) was significantly darker than in the control (L*=53.6). No effects caused by treatments were found in the b* values of the rabbit meat.

Table 3: Effect of the dietary treatment on physicochemical analyses and meat quality parameters in rabbit meat.

							P-value			
	CTL	EO	C	O	S	SEM	CTL *vs.* EO	CTL *vs.* C	CTL *vs.* O	CTL *vs.* S
% Protein	18.4	17.9	19.5	18.6	18.4	0.7	NS	NS	NS	NS
% Moisture	68.4	67.6	66.5	66.9	67.3	1.6	NS	NS	NS	NS
% Drip loss	0.33	0.03	0.04	0.03	0.09	0.15	NS	NS	NS	NS
% Cooking loss	30.6	30.6	30.3	30.6	30.0	1.5	NS	NS	NS	NS
Water loss aerobic 5-15 d[a]										
d5	2.38	1.56	2.08	2.24	2.18	0.25	NS	NS	NS	NS
d10	2.10	2.01	2.19	2.13	1.97	0.10	NS	NS	NS	NS
d15	1.96	1.71	1.07	1.64	1.74	0.29	NS	NS	NS	NS
Water loss anaerobic 15-45 d[b]										
d15	1.92	2.30	2.48	2.26	2.31	0.25	NS	NS	NS	NS
d30	1.11	1.53	1.13	1.21	0.98	0.41	NS	NS	NS	NS
d45	0.39	0.59	1.22	0.31	0.91	0.42	NS	0.09	NS	NS

CTL: control; EO: essential oils; C: cranberry; O: onion; S: strawberry; SEM: standard error of the mean; n=8 rabbit cages, a cage of 6 rabbits is the experimental unit; CTL *vs.* EO: Contrast *a priori* to determine the effect of treatment control *vs.* Essential oils; CTL *vs.* C: control *vs.* cranberry; CTL *vs.* O: control *vs.* onion; CTL *vs.* S: control *vs.* strawberry. NS: not significant.

[a]Linear effect of time on water loss under aerobic conditions, P<0.0017.

[b]Linear effect of time on water loss under anaerobic conditions, P<0.0001.

Table 4: Effect of dietary treatments on physical characteristics of the *Longissimus dorsi* and the *Biceps femoris* muscles, and on lipid oxidation, carbonyl and total phenol content in raw meat.

							P-value			
	CTL	EO	C	O	S	SEM	CTL *vs.* EO	CTL *vs.* C	CTL *vs.* O	CTL *vs.* S
pH (1 h)										
LD	6.63	6.69	6.75	6.39	6.54	0.18	NS	NS	NS	NS
BF	6.63	6.51	6.76	6.40	6.65	0.10	NS	NS	0.06	NS
pHu (24 h)										
LD	6.05	5.92	5.98	5.96	5.84	0.10	NS	NS	NS	0.04
BF	6.23	6.13	6.09	6.13	6.07	0.09	NS	NS	NS	0.08
LD color										
a*	2.65	2.34	2.58	2.95	2.88	0.44	NS	NS	NS	NS
b*	0.61	0.49	0.27	0.39	0.65	0.30	NS	NS	NS	NS
L*	52.7	52.5	51.9	52.5	52.4	2.3	NS	NS	NS	NS
BF color										
a*	2.11	2.39	3.23	2.45	2.90	0.45	NS	0.02	NS	NS
b*	0.70	0.16	0.59	0.42	0.36	0.33	NS	NS	NS	NS
L*	53.6	51.5	52.3	52.5	50.6	1.4	NS	NS	NS	0.03
TBARSA (nmol/g MDA)	2.16	1.79	2.12	2.50	2.28	0.32	NS	NS	NS	NS
Carbonyls (nmol/mg protein)	2.95	2.58	2.81	2.65	2.79	0.51	NS	NS	NS	NS
Total phenols (nmol/g)	1.04	1.03	1.05	1.09	1.05	0.03	NS	NS	NS	NS

CTL: control; EO: essential oils; C: cranberry; O: onion; S: strawberry; Each value represents the mean of 8 samples with SEM: standard error of the mean; n=8 rabbit cages, a cage of 6 rabbits is the experimental unit. CTL *vs.* EO: Contrast *a priori* to determine the effect of treatment control *vs.* essential oils; CTL *vs.* C: treatment control *vs.* cranberry; CTL *vs.* O: treatment control *vs.* onion. CTL *vs.* S : treatment control *vs.* strawberry. AAll data of TBARS are presented as mean Malondialdehyde (MDA) values from 3 analyses performed in triplicate. TBARS: thiobarbituric acid reactive substances.
NS: not significant.

Antioxidant status of meat.

The effects of dietary supplementation with plant extracts and essential oils on lipid oxidation, carbonyl and total phenol content in raw meat are shown in Table 4. No significant differences ($P>0.05$) were observed between the dietary treatments and the control.

Microbial analysis and shelf life

The evolution of the microflora on rabbit thighs from animals fed rations supplemented with plant extracts or essential oils when packaged under aerobic and anaerobic conditions is presented in Tables 5 and 6, respectively. Microbial analysis of the refrigerated rabbit meat indicated that for all tests, under both aerobic and anaerobic storage conditions, the cell counts increased significantly over time ($P<0.05$). Overall, the difference between the control and the experimental groups, supplemented with various sources of polyphenol, was sporadic and at the most of 1.5 log CFU/g for the meat stored under aerobic and anaerobic conditions (Tables 5 and 6, respectively). However, under aerobic conditions, the control value was always close to or greater than that of the experimental groups, except for presumptive *Pseudomonas* at day 5 for the group supplemented with onion extract (Table 5). At day 0, the presumptive LAB in the control group were significantly higher ($P>0.05$) than all 4 experimental groups. A non-parametric analysis was carried out on the presence or absence of coliforms, *E. coli,* presumptive *S. aureus* and Enterobacteriacea*,* under both aerobic and anaerobic storage conditions. No significant differences were detected between the control group and the groups receiving enriched rations (data not shown, $P>0.05$).

We observed significant linear and quadratic time effects during the aerobic and anaerobic storage periods ($P<0.0001$). Generally, after an increase, the bacterial count reached a plateau under aerobic (10 d) and anaerobic (30 d) conditions, as normally seen in a typical growth curve. A significant interaction between time and treatment for LAB was observed ($P<0.01$) under aerobic conditions. The interaction is explained by the lowest level of LAB in the

Table 5: Total aerobic mesophilic (TAM), presumptive *Pseudomonas* spp. and presumptive lactic acid bacteria (LAB) counts (log CFU/g) on rabbit thighs between 0 and 15 d of storage at 4°C under aerobic conditions.

							P-value			
	CTL	EO	C	O	S	SEM	CTL *vs.* EO	CTL *vs.* C	CTL *vs.* O	CTL *vs.* S
TAM[a]										
d0	2.15±0.63	1.45±0.23	1.83±0.44	1.58±0.30	1.62±0.42	0.18	0.004	0.08	0.02	0.005
d5	4.79±0.39	4.14±0.55	4.39±0.50	4.59±0.80	4.45±0.56	0.19	0.003	0.05	NS	NS
d10	9.31±0.22	8.78±0.90	8.92±0.69	9.19±0.50	9.22±0.30	0.20	0.08	NS	NS	NS
d15	10.49±0.49	10.23±0.42	9.90±0.35	9.50±0.73	9.76±0.21	0.19	NS	0.01	0.001	0.003
Presumptive Pseudomonas[a]										
d0	1.03±0.38	0.93±0.36	0.86±0.37	0.82±0.25	0.90±0.48	0.18	NS	NS	NS	NS
d5	4.65±0.81	4.23±0.58	4.56±0.42	4.80±0.62	4.50±0.34	0.19	0.03	NS	NS	NS
d10	10.33±0.19	9.91±0.35	9.93±0.24	9.96±0.26	10.12±0.16	0.12	0.001	0.002	0.004	NS
d15	10.92±0.10	10.65±0.46	10.79±0.15	10.73±0.09	10.78±0.06	0.08	0.01	NS	0.09	NS
Presumptive LAB[ab]										
d0	1.67±0.65	0.91±0.27	1.22±0.34	1.16±0.44	1.04±0.26	0.19	0.004	0.02	0.01	0.02
d5	4.83±0.73	3.33±0.80	4.33±0.50	4.20±1.10	4.61±0.89	0.24	0.0001	0.004	0.01	NS
d10	9.42±0.68	9.40±0.42	8.81±0.92	9.41±0.82	9.14±0.24	0.23	NS	0.08	NS	NS
d15	10.49±0.34	9.76±0.52	10.17±0.37	10.21±0.46	10.27±0.40	0.12	0.0001	0.01	0.02	0.08

CTL: control; EO: essential oils; C: cranberry; O: onion; S: strawberry. Each value represents the mean of 8 samples with standard deviation. SEM: standard error of the mean; n=8 rabbit cages, a cage of 6 rabbits is the experimental unit, NS: not significant, log cfu/g: colony forming units per gram. CTL *vs.* EO: Contrast *a priori* to determine the effect of treatment control *vs.* essential oils; CTL *vs.* C: treatment control *vs.* cranberry; CTL *vs.* O: treatment control *vs.* onion. CTL *vs.* S: treatment control *vs.* strawberry.

[a]Linear and quadratic time effect, $P<0.0001$.

[b]Time×treatment interaction, $P<0.01$.

supplemented group of plant extracts and essential oils compared to the control group at 0 d, 5 d and 15 d, except at 10 d, and for strawberry at 5 d, 10 d and 15 d. A significant interaction between time and treatment for TAM and presumptive LAB was also observed ($P<0.009$) under anaerobic conditions. For TAM, the effect of treatment was not significantly different for the cranberry group compared to the control, at all time, and for the other treatments, it was significant at day 0. Presumptive LAB interaction is explained by the significant differences of treatment supplemented with plant extracts and essential oils compared to the control group at 0 d. But over time, these significant differences only remained for the group supplemented with essential oils.

Despite limited microbial count differences obtained with a supplementation as low as 10 ppm of active ingredients compared to the control group, some significant differences were observed under both aerobic and anaerobic conditions over time. Compared to the control, onion-, strawberry- and essential oils-supplemented groups had lower TAM counts at day 0 ($P<0.02$, $P<0.005$, $P<0.004$, respectively), although differences were rather limited (<0.7 log CFU/g). Under aerobic conditions, reduced TAM counts (compared to the control group) were also observed at 5 d for the rations containing essential oils ($P=0.003$) and cranberry ($P=0.05$), and at 15 d for cranberry ($P=0.01$), onion- ($P=0.001$) and strawberry-supplemented groups ($P=0.003$; Table 5). Presumptive *Pseudomonas* counts were reduced on day 5, 10 and 15 by the essential oils supplement ($P<0.03$) and on day 10 by cranberry ($P=0.002$) and onion ($P<0.004$). Overall, a small but positive effect was observed for the dietary treatments compared to the control diet when the meat samples were placed under aerobic conditions (Table 5).

Under anaerobic conditions (Table 6), the rations supplemented with essential oils reduced significantly TAM counts only at 0 and 45 d ($P=0.03$). The other supplemented groups were not significantly different from the control at 15, 30 and 45 d ($P>0.09$). Reduction of LAB counts over time was only observed with essential oils under anaerobic conditions ($P>0.09$). Essential oils supplement reduced presumptive *Pseudomonas* counts at 45 d ($P=0.03$) and onion at 15 d ($P=0.02$). Overall, more significant differences were observed with the presumptive LAB compared to

Table 6: Total aerobic mesophilic (TAM), presumptive *Pseudomonas* spp. and presumptive lactic acid bacteria (LAB) counts (log CFU/g) on rabbit thighs between 0 and 45 d of storage at 4°C under vacuum.

							P-value			
	CTL	EO	C	O	S	SEM	CTL *vs.* EO	CTL *vs.* C	CTL *vs.* O	CTL *vs.* S
TAM[ab]										
d0	2.15±0.63	1.45±0.23	1.83±0.44	1.58±0.30	1.62±0.42	0.18	0.004	0.08	0.02	0.005
d15	7.81±0.49	8.10±0.56	7.68±0.70	7.53±0.58	7.61±0.42	0.15	0.07	NS	0.09	NS
d30	9.26±0.53	9.33±0.19	9.15±0.62	9.42±0.34	9.45±0.21	0.17	NS	NS	NS	NS
d45	9.71±0.23	9.55±0.27	9.70±0.32	9.64±0.30	9.76±0.22	0.06	0.03	NS	NS	NS
Presumptive Pseudomonas[a]										
d0	1.03±0.38	0.93±0.36	0.86±0.37	0.82±0.25	0.90±0.48	0.18	NS	NS	NS	NS
d15	8.17±0.38	8.12±0.25	8.02±0.27	7.90±0.27	8.14±0.13	0.11	NS	NS	0.02	NS
d30	8.92±0.39	8.76±0.17	8.72±0.54	8.65±0.11	8.89±0.19	0.16	NS	NS	NS	NS
d45	9.49±0.33	9.12±0.42	9.41±0.33	9.44±0.36	9.36±0.50	0.17	0.03	NS	NS	NS
Presumptive LAB[ab]										
d0	1.67±0.65	0.91±0.27	1.22±0.34	1.16±0.44	1.04±0.26	0.17	0.004	0.02	0.01	0.02
d15	8.26±0.51	8.55±0.18	8.36±0.17	8.39±0.18	8.33±0.20	0.12	0.03	NS	NS	NS
d30	9.83±0.54	9.41±0.54	9.88±0.58	10.00±0.60	9.86±0.65	0.15	0.01	NS	NS	NS
d45	10.16±0.43	9.80±0.43	9.99±0.59	10.13±0.42	10.20±0.38	0.10	0.009	0.09	NS	NS

CTL: control; EO: essential oils; C: cranberry; O: onion; S: strawberry. Each value represents the mean of 8 samples with standard deviation SEM: standard error of the mean, n=8 rabbit cages, a cage of 6 rabbits is the experimental unit, NS: not significant, log CFU/g: colony forming units per gram. CTL *vs.* EO: Contrast *a priori* to determine the effect of treatment control *vs.* essential oils; CTL *vs.* C: treatment control *vs.* cranberry; CTL *vs.* O: treatment control *vs.* onion. CTL *vs.* S: treatment control *vs.* strawberry.
[a]Linear and quadratic time effect, $P<0.0001$.
[b]Time×treatment interaction, $P<0.009$.

the other microbial groups analysed, and the essential oils supplemented group seems more effective (Table 5 and 6). Under anaerobic conditions, the effect of supplements is not as important as under aerobic conditions.

DISCUSSION

The effects of dietary supplementation with plant extracts and essential oils on the quality of rabbit meat were studied for their antimicrobial and antioxidant effects. Growth performance of the rabbits was consistent with the studies of Botsoglou *et al.* (2004), who did not observe significant differences when oregano essential oil was provided to rabbits at much higher doses of 100 and 200 mg per kg of feed. Similarly, rabbits consuming a diet supplemented with 3 levels (0, 10 or 15%) of chia seeds (*Salvia hispanica* L.) also showed no effect on growth performance (Peiretti and Meineri, 2008). However, Rotolo *et al.* (2013) did observe a positive effect with dried oregano and sage leaves. Hence, at the low level tested (10 ppm of active ingredients), the plant extracts did not perform as growth promoters like antibiotics administered at sub-therapeutic doses (Richard *et al.,* 2000; Dibner and Richards, 2005).

Colour is generally accepted as one of the major features that consumers evaluate when making a purchase decision. The colour parameters of meat are related to pHu, which influences the oxidation of the meat's haem pigments. According to Fraysse and Darre (1989), low pH causes discoloration of the meat, whereas high pH gives the meat a darker colour. At high pH levels, oxymyoglobin rapidly turns into a dark red, reduced myoglobin (Ouhayoun and Dalle Zotte, 1993) and the muscle structure is less reflective because of its less compact structure (Bízková and Tůmová, 2010). But in this study, the lower L* values of strawberry extract in BF (darkest meat, pHu=6.07) had a pHu lower than the control group (pHu =6.23); which is contrary to the literature. Dabbou *et al.* (2014) showed that different levels of artichoke bracts supplementation did not significantly affect the pHu, L* and other colour parameters of

rabbit meat. The water loss values we obtained decreased over time, which is also contrary to what is observed in pig *Longissimus* muscle, where values have been reported to increase with time (Simitzis *et al.,* 2010).

Oxidation is a process that affects various constituents including proteins, carbohydrates, lipids, pigments, vitamins and DNA in muscle and fatty tissue. Oxidation also increases with time and shortens the shelf life of meat and meat products (Smet *et al.,* 2008). Under our experimental conditions, we observed no treatment effects on TBARS, carbonyl and total phenol content, indicating that the supplementation, or the level of active ingredient used, had little influence on the level of raw meat oxidation at day 0. The TBARS values obtained are similar to those published by Botsoglou *et al.* (2004) at day 0 using relatively high levels of oregano essential oils (100 and 200 mg/kg feed rabbit). In this study, the control group's TBARS mean was 155.41 ng/g MDA value, the essential oil group was 130.99 ng/g, cranberry was 153.16 ng/g, onion was 180.38 ng/g and strawberry was 158.58 ng/g, compared to 115 ng/g and 137 ng/g for 100 and 200 mg of oregano essential oil/kg of feed, respectively, for Botsoglou *et al.* (2004). Rotolo *et al.* (2013), who used oregano and sage dried leaves, also came to the same conclusion. Alarcón-Rojo *et al.* (2013), however, reported that lipid oxidation in pork was inhibited by 1000 ppm of essential oils in the diet. The lack of difference between the experimental groups and the control could be due to the low dose used, despite the higher fat content of our rabbit meat. Indeed, our results obtained for fat (11.2 to 14.3 g/100 g) correspond to the maximum values obtained by Salvini *et al.* (1998; 0.6 to 14.4 g/100 g). The low dose used (10 ppm), could also explain the absence of increased levels of total phenols at day 0.

Rabbit meat protein values between 17.9 and 19.5 g/100 g observed in this study are comparable to published values between 18.1 and 23.7 g/100 g (Salvini *et al.,* 1998). In agreement with our results, Peiretti *et al.* (2013) showed that different levels of tomato pomace supplementation did not significantly affect the chemical composition of rabbit meat.

Jolley (1990) indicated that rabbit meat has characteristics similar to DFD meat and rapidly deteriorates. Rabbit meat obtained in this experiment also had characteristics similar to DFD meat. This resulted in a high water retention capacity and very low water loss (drip loss) and is corroborated by Hulot and Ouhayoun (1999). An inverse relationship exists between drip loss and pH, so when pH is low, drip loss is high (Melody *et al.,* 2004). This is in agreement with previous studies, which reported that no significant differences to drip loss occurred with dietary supplementation of spirulina (*Arthrospira platensis*) and thyme leaves in rabbit feed (Dalle Zotte *et al.,* 2014).

Meat contains sufficient nutrients to support microbial growth, even at refrigeration temperature. Several intrinsic and extrinsic factors affect growth rate (Greer, 1989; Holmer *et al.,* 2009). The faster spoilage of DFD pork is promoted by a high ultimate pH but also by its lower content of glucose and glycolytic intermediates, which forces organisms to use amino acids and leads to spoilage and off-odours, notably by the production of malodorous compounds such as biogenic amines putrescin and cadaverin, produced in low quantity, and volatile metabolites, including sulphur compounds, aldehydes and ketones (Newton and Gill, 1981). In rabbits, the pHu was higher in BF muscle than LD and was attributable to its lower glycolytic potential (Hulot and Ouhayoun, 1999). Compared to the control group, a significantly lower pH was only observed with the strawberry-supplemented ration in the BF muscle (pHu=5.84; Table 4). The fact that the means of the pHu for the LD muscle were below 6 for meat from rabbits fed supplemented rations suggests conducting other experiments at a higher level of supplementation. Other studies, however, did not observe significant effects for dietary supplementation of rabbit feed with rosemary (0.2%), oregano extract (0.2%) and rosemary (0.1%) plus oregano extract (0.1%) on pHu values (Cardinali *et al.,* 2012). Therefore, based on published studies, it is uncertain if higher incorporation levels would significantly affect this parameter. Improved pre-slaughter management to lower animal stress would be a more sound approach to reduce the pHu of the meat (Hulot and Ouhayoun, 1999).

The microbiological analyses do not reveal clear activity of dietary supplemented cranberry, strawberry, onion and essentials oils rations on rabbit meat, as significant differences were observed sporadically at the low supplementation level used. Overall, under aerobic conditions, presumptive *Pseudomonas* spp., presumptive LAB and TAM were close or reduced in all experimental groups, compared to the control group. Based on a maximum of 7-log CFU/g for TAM, we obtained a shelf life between 5-10 d. Soultos *et al.* (2009) also came to the same conclusion with a dietary supplementation of 100 or 200 mg/kg oregano oil and indicated that, according to both appearance and odour, shelf life was estimated at between 8-12 days under aerobic conditions.

As observed by Soultos *et al.* (2009) with oregano oil, supplementation of the diet with polyphenol-rich extracts influenced the microbial status of the meat under aerobic more favourably compared to anaerobic conditions. Despite a limited effect of the experimental diets compared to the control group (<1.5 log CFU/g, essential oils compared to the control group at day 5 under aerobic conditions), our results suggest that more research is needed, using higher concentrations of active substances. Because of the low toxicity of plant extracts (Shoji *et al.,* 2004), a higher degree of supplementation must be tested to optimise the positive effects observed.

CONCLUSION

The results of this study show that meat from rabbits receiving feed without supplements had the characteristics of DFD meat, which is more susceptible to microbial spoilage due to its high ultimate pH. The addition (10 ppm of active ingredients) of various dietary supplements containing polyphenols appears to influence the microbial quality of the rabbit thighs during storage favourably, especially under aerobic conditions and with essential oils. However, higher doses of extracts should be tested before concluding to an effective antimicrobial activity of practical and commercial value.

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