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Dietary Supplementation of Oregano and Sage Dried Leaves on Performances and Meat Quality of Rabbits

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Abstract

The aim of this research was to evaluate the dietary supplementation 1% (w/w) of oregano and sage dried leaves on performances and meat quality of broiler rabbits. A feeding trial, which lasted 48 d, was carried out on 105 male Bianca Italiana rabbits randomly divided in seven groups and fed *ad libitum*. At the end of the trial ten animals per group were slaughtered and samples of dorsal muscle were taken in order to perform laboratory analysis. Mortality rate did not statistically differ between groups. Growth performances of animals fed diets supplemented with aromatic plants were higher (P<0.05) than those of animals of control group, whereas carcass parameters were not affected by treatments excepting for the slaughter weight that showed the same trend as growth performances. Meat quality traits, oxidative lipid stability and fatty acid profile were not influenced by aromatic plant supplementation. In conclusion, oregano and sage in form of dried leaves can be used in rabbit without adverse effects on performance, carcass characteristics and meat quality traits.

Key words: oregano, sage, lipid oxidation, fatty acids, meat quality, rabbits

INTRODUCTION

In Mediterranean area, rabbit meat consumption belongs to eating habits from several generations. Amongst the European countries, Italy represents the most important producer with more than 240 000 t of meat per year (Food and Agriculture Organization of the United Nations 2012). In commercial rabbit breeding, as well as in other livestock animals, productivity is measured as a combination of reproduction efficiency, growth rate and consequently meat production rate. Moreover, feed efficiency, pathological conditions associated with high mortality, and acceptable meat quality standard have become limiting factors for the economic output of a farm unit. In 2006, the European Union (EU) banned the use of antibiotics growth promoters (AGP) in animal feeding and rabbits gastroenteric diseases significantly increased, mainly on 35-50 d old rabbits (Zoccarato *et al*. 2008). Use of phytoadditives or active plant components, such as tannins, in rabbit husbandry can be an acceptable way to improve welfare, health and meat quality of animals (Fichi *et al*. 2007; Liu *et al.* 2009). Among Mediterranean plants of potential use as phytoadditives, oregano and sage are important candidates with well-known biological (Friedman *et al*. 2002; Hassawi and Kharma 2006) and antioxidant effects (Botsoglou *et al*. 2002a, b; Papageorgiou *et al*. 2003; Nadia *et al*. 2008). Oregano (*Origanum vulgare* L.) is an ar-

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omatic plant and its essential oil contains more than 30 constituents, which are mainly phenolic antioxidants with antimicrobial and cytotoxic activity (Sivropoulou *et al*. 1996). The genus *Salvia* (sage) encompasses about 900 species of plants belonging to the Lamiaceae family, typically used for food flavouring, as well as in cosmetics, perfumery and the pharmaceutical industries (Tepe *et al*. 2006). Generally, for these purposes, plants are commonly dried at 40-60°C for preserving their quality (Tambunan *et al*. 2001). Nevertheless, some references indicated that drying technique modifies biological active components of herbs leaves (Figiel *et al*. 2010; Tibaldi *et al*. 2010) and consequently affects their potential use as phytoadditives. Some studies were carried out to investigate the effects of dietary Lamiaceae essential oil inclusion both on the productive traits and the oxidative stability of meat (Botsoglou *et al*. 2002a, b, 2003, 2004; Papageorgiou *et al*. 2003) in different monogastric animals.

Rabbit meat, characterized by a high proportion of unsaturated fatty acids, is susceptible to oxidative damage causing rancidity, warmed-over flavour and colour deterioration (Dalle Zotte 2002; Combes 2004). In the light of these considerations, in order to improve rabbit husbandry welfare and control meat-derived product stability, it is important to find effective, safe and low cost solutions. In this regard, dietary oregano and sage usage in form of dried leaves as phytoadditives could be an economical alternative to the use of essential oil. Considering also that until now a few number of studies on broiler rabbits are available, the evaluation of the dietary inclusion of different-thermally treated aromatic plants, on live performance, meat quality traits, oxidative lipid stability, and fatty acid profile of heavy broiler rabbits was performed.

RESULTS AND DISCUSSION

Essential oils composition of herbs

From oregano samples 29 compounds were detected in the essential oil (EO) extracted from dehumidifying process (DH) herbs and 27 were detected in the EO extracted from oven-drying process (OD) herbs, with carvacrol being the most abundant with values ranging between 23.5% (DH) and 24.7% (OD) of the EO, followed by *cis*-sabinene hydrate (Table 1). From sage *Salvia officinalis* 'Extrakta' (SE) samples, 18 compounds were detected in both the EO extracted from DH herbs and OD herbs, with camphor, manool and α-thujone being the most abundant components with values ranging between 19.8% (DH) and 20.3% (OD) of the EO for camphor, between 16% (DH) and 19.2% (OD) of the EO for manool, between 19.8% (DH) and 20.3% (OD) of the EO for α-thujone (Table 2). From sage *S. officinalis* subsp. *lavandulifolia* (SL) samples, 17 compounds were detected in both the EO extracted from DH herbs and OD herbs, with camphor and α-thujone being the most abundant components with values ranging between 19.7% (DH) and 21.3% (OD) of the EO for camphor, between 13.4% (DH) and 15.2% (OD) of the EO for α-thujone (Table 2).

Table 1 Essential oil (EO) composition of oregano leaves subjected to different drying systems

Item RT (min)	EO component	OR-DH (% EO)	OR-OD (% EO)
3.83	α-Thujene	0.39	0.36
3.96	α-Pinene	0.23	0.24
4.28	Camphene	0.02	0.03
4.92	Sabinene	2.58	1.81
5.22	1-Octen-3-ol	0.35	-
5.44	Myrcene	0.98	0.58
6.05	α-Terpinene	0.89	2.68
6.29	Cymene	2.76	1.33
6.38	α-Phellandrene	1.02	0.83
6.45	1,8-Cineole	1.31	0.45
7.28	γ-Terpinene	4.90	7.08
7.53	*Trans*-Sabinene hydrate	2.18	1.94
8.07	α-Terpinolene	0.38	1.19
8.60	*Cis*-Sabinene hydrate	20.5	8.72
8.66	α-Thujone	2.45	2.62
8.94	β-Thujone	2.21	1.24
9.55	Camphor	0.79	0.76
10.30	Borneol	2.78	0.98
10.66	Terpinen-4-ol	3.12	7.38
10.98	α-Terpineol	1.48	0.05
12.89	Lynalil Acetate	1.52	2.43
14.38	Carvacrol	23.5	24.7
14.45	Teresantol	1.67	-
16.80	*Trans*-Caryophyllene	1.50	2.44
17.73	α-Humulene	0.50	1.01
18.40	Germacrene	0.91	1.50
18.80	Bicyclogermacrene	1.47	2.44
19.10	β-Bisabolene	2.61	3.86
29.40	Phytol	1.75	4.18
	Total	86.75	82.83

RT, retention time; OR, *Origanum vulgare* subsp. *hirtum*; DH, dehumidifying process; OD, oven-drying process. The same as below.

Table 2 Essential oil (EO) composition of sage leaves subjected to different drying systems[1)]

Item RT	EO component	SE-DH (% EO)	SE-OD (% EO)	SL-DH (% EO)	SL-OD (% EO)
3.96	α-Pinene	3.68	3.83	5.15	5.25
4.28	Camphene	3.86	3.04	4.22	4.31
4.88	β-Pinene	1.61	0.93	1.64	1.42
5.44	Myrcene	0.59	0.33	0.53	0.23
6.05	α-Terpinene	0.11	0.18	0.13	0.10
6.29	Cymene	0.14	0.19	-	-
6.48	1,8-Cineole	7.09	5.77	9.68	10.2
7.28	γ-Terpinene	0.36	0.28	-	-
8.07	α-Terpinolene	-	-	0.38	0.41
8.66	α-Thujone	15.5	17.3	13.4	15.2
8.94	β-Thujone	4.70	5.64	2.26	2.56
9.55	Camphor	19.8	20.3	19.7	21.3
10.30	Borneol	2.02	1.91	3.38	3.11
10.66	Terpinen-4-ol	0.52	0.40	0.35	0.22
13.50	Bornyl acetate	1.68	1.69	1.92	1.85
16.80	*Trans*-Caryophyllene	3.92	3.16	4.73	4.16
17.73	α-Humulene	7.81	6.02	7.58	8.12
21.04	Verdiflorol	8.08	7.95	9.16	9.45
28.97	Manool	16.0	19.2	9.50	11.2
	Total	97.47	98.12	93.71	99.09

[1)] SE, *Salvia officinalis* 'Extrakta'; SL, *S. officinalis* subsp. *lavandulifolia*. The same as below.

Live performance, mortality and carcass traits

Live performance of animals are reported in Table 2. Mortality rate did not statistically differ among groups. Conversely, live performance parameters were affected by treatments. As reported, the growth and consumption parameters were lower than those typically reported in intensive rearing conditions of northern Italy (Lazzaroni *et al.* 2009). This could be due to the suboptimal environmental temperatures that have characterized the period of the trial. Indeed for all the period, maximum temperatures in the experimental facility exceeded 30°C and it is known that high temperature is related to a substantial decrease in both intake and growth in rabbits (Cervera *et al*. 1997). In the rearing conditions used in the experiment, the aromatic plants supplementation improved the final body weights for all treated groups, with the highest final body weight achieved by OR-OD group (2 273 g). Average daily feed intake (ADFI) of the control group (C) resulted lower than that of all the other groups, while the highest values were found for OR-DH and SE-OD groups (85.02 and 84.77 g d^{-1}, respectively), although not statistically higher than SE-DH, SL-DH and SL-OD. As reported for the final body weights, the slaughter weights of rabbits showed differences between groups (Table 3). This result is in agreement with the study by Giannenas *et al.* (2005), who showed that dehydrated oregano plants given as single supplement at the level of 0.5% exerted a growth-promoting effect in broiler chickens. To our knowledge, most of the works reported in literature are related to the use of phytogenics as dietary supplementation in essential oil form, while *in-vivo* studies comparing the dietary use of dried oregano and sage leaves for rabbit species are quite limited. Nonetheless, some evidences in pigs and poultry highlighted negative effects of phytogenics on feed intake, and indicated as main affecting causes the choice of proper herb, its extraction technique and dietary dose (Windisch 2009; Symeon *et al*. 2010). Szaboova *et al*. (2008) showed that provision of sage extract in drinking water improved feed intake and weight gain on 35-d old male rabbits. Conversely, Botsoglou *et al*. (2004) observed a lack of beneficial effect in rabbit fed 100 and 200 mg oregano essential oil kg^{-1} diet. In a previous work, Botsoglou *et al*. (2002a) did not find differences in body weight and feed conversion ratio (FCR) on Cobb broilers fed 50 and 100 mg oregano essential oil kg^{-1} wheat-soybean meal. Similarly, Papageorgiu *et al.* (2003) showed that in turkeys fed 100 and 200 mg oregano essential oil kg^{-1} diet live performance parameters were unaffected. This is in accordance with Florou-Paneri *et al*. (2005) who indicated a lack of growth promoting effect of oregano in turkeys fed 5 and 10 g kg^{-1} of oregano herb.

Physical traits, chemical composition and oxidative stability of LD muscle

Meat composition, pH_{24}, colour, cooking losses, and shear force of the *Longissimus dorsi* (LD) muscle are reported in Table 4. The dietary treatment did not affect any of the parameters investigated. These results are in accordance with Simonova *et al*. (2010) that did not observe differences in colour parameters on rabbits supplemented with oregano extract in drinking water. Similarly, in pigs, Janz *et al*. (2007) and Simitzis *et al*. (2010) observed no significant influence of dietary oregano oil supplementation on meat colour with data ranged within normal limits.

Dietary use of phytobiotics has been demonstrated as a good strategy to prevent oxidation in animal derived products. Moreover, interesting results were reported when comparing this strategy to direct spread-

Table 3 Effect of aromatic plants on live performance, mortality (n=15) and carcass traits (n=10) of rabbits

Item	C[1)]	OR-DH	OR-OD	SE-DH	SE-OD	SL-DH	SL-OD
Initial body weight (g)	871.52±43.08	855.35±32.66	974.25±43.11	923.87±32.80	866.09±32.68	900.27±31.46	873.75±29.59
Final body weight (g)	2000.19±70.93 c	2 177.72±41.52 ab	2 273.70±39.66 a	2170.55±33.20 ab	2094.09±44.06 bc	2252.97± 0.96 a	2212.24±30.30 ab
Average daily gain (g)	23.19±1.23 c	27.09±0.88 ab	27.07±0.99 ab	26.23±0.52 ab	25.30±0.65 bc	28.15±0.59 a	28.05±0.60 a
Average daily feed intake (g d^{-1})	80.07±2.02 c	85.02±0.34 a	80.76±0.62 bc	82.42±0.67 abc	84.77±1.63 a	82.69±1.47 abc	83.87±0.63a bc
Feed conversion ratio (g g^{-1})	3.54±0.15 a	3.18±0.10b c	3.04±0.12 c	3.15±0.05 bc	3.38±0.10 ab	2.98±0.05 c	3.00±0.06 c
Mortality (animals)	0	3	0	2	1	1	1
Slaughter weight (SW, g)	2 125.50±61.64 d	2255.88±36.90 bc	2 382.75±26.64 a	2248.50±20.47 bc	2206.38±24.72 cd	2343.00±43.58 ab	2279.00±31.10 abc
Dressing out (% SW)	48.76±0.64	46.51±0.98	45.67±0.71	46.99±0.75	48.18±0.77	46.89±1.33	44.02±2.48
Liver (% SW)	2.32±0.08	2.28±0.07	2.22±0.05	2.23±0.05	2.46±0.05	2.36±0.13	2.33±0.07
Kidneys (% SW)	0.58±0.03	0.56±0.02	0.63±0.03	0.58±0.01	0.59±0.02	0.57±0.02	0.57±0.02
Heart and lungs (% SW)	1.00±0.04	1.02±0.06	0.95±0.04	0.99±0.05	0.99±0.05	0.93±0.05	0.94±0.02
Skin, tail, feet and paws (% SW)	21.31±0.37	21.38±0.44	21.66±0.53	22.75±0.47	20.89±0.41	22.21±0.70	21.83±0.35
Full gastrointestinal tract (% SW)	23.54±0.61 a	21.28±0.54 ab	20.21±0.55 ab	20.43±0.57 ab	23.25±0.57 a	20.83±0.72 ab	18.35±2.38 b

[1)]C, control.

Data are mean±standard error. Different letters in the same row differs significantly. Significance was accepted for $P<0.05$.

The same as below.

ing of oregano-based solution on meat right after slaughter (Govaris *et al.* 2004). Nevertheless, in our experimental conditions, no treatment effects on TBARS results were found, indicating that herb type supplementation did not delay the lipid oxidation in LD muscle frozen at -20°C. This is in accordance with Simitzis *et al*. (2010) who did not observe a decrease in meat lipid oxidation when pigs were supplemented with oregano essential oil. Similar results were reported by Botsoglou *et al*. (2004) and Florou-Paneri *et al*. (2005) who did not observe effects in rabbit LD and turkey muscles when oregano essential oil was supplemented in the diet. On the contrary, in broiler a supplementation of 500 mg kg^{-1} of rosemary and sage oleoresin improved the stability of the meat (Lopez-Bote *et al*. 1998).

Fatty acid composition of diet and rabbit meat

Fatty acid (FA) composition of diet is reported in Table 5. The pattern of FAs in the diet was characterised by three dominant FAs: palmitic acid (PA, C16:0), oleic acid (OA, C18:1n-9) and linoleic acid (LA, C18:2n-6). PA ranged from 12.8 to 17.6, OA ranged from 20.9 to 23.9 while LA ranged from 49.3 to 52.8 of total FA, respectively. From a nutritional point of view, rabbit meat is recognised as high digestible, lean and rich in high biological value proteins. With regard to human dietary guidance, its high-unsaturated lipid profile (60% of total FAs) makes this meat interesting for following healthy lifestyle (Combes 2004; Dalle Zotte *et al*. 2011). FA composition of LD muscle is reported in Table 6. In the LD muscle, the most representative saturated fatty acids was palmitic acid (C16:0) which showed similar results across the experimental groups irrespectively of the treatment. Percentages of this FA oscillated around 33-34%. Monounsaturated fatty acid (MUFA) fractions were not affected by treatments and this can be attributed to the content of the oleic acid (C18:1n-9) that resulted homogenous in all groups. Typically, rabbit meat is rich in oleic (C18:1n-9) and palmitoleic (C16:1) FA and their sum is higher than 20% of all FA (Combes 2004). On the whole, the treatments did not affect the most representative FAs (C16:0, C18:1, C18:2n-6) and were consequently ineffective on modifying the FA pattern.

CONCLUSION

In conclusion, we can confirm that oregano and sage in form of dried leaves, regardless of the type of drying systems, can

Table 4 Effect of aromatic plants on physical traits, chemical composition and oxidative stability of LD muscle (n=10)

Item	C	OR-DH	OR-OD	SE-DH	SE-OD	SL-DH	SL-OD
pH_{24}	6.27±0.07	6.31±0.11	6.26±0.16	6.20±0.11	6.31±0.18	6.28±0.13	6.36±0.15
L* (lightness)	61.75±0.35	62.72±0.63	61.16±1.03	61.35±0.60	61.51±0.90	62.20±0.73	61.34±0.54
a* (redness)	9.38±0.53	9.47±0.71	9.89±1.06	9.90±0.83	9.45±0.52	9.36±0.82	9.88±0.55
b* (yellowness)	7.81±0.28	7.93±0.24	7.18±0.32	7.80±0.25	8.25±0.28	7.78±0.31	7.95±0.25
Chroma	12.24±0.49	12.43±0.52	12.38±0.82	12.69±0.68	12.59±0.43	12.28±0.62	12.72±0.45
Hue	40.04±1.62	40.56±2.36	37.18±3.15	38.97±2.37	41.38±1.92	40.59±2.99	39.15±1.82
Cooking losses (%)	28.18±0.70	29.11±0.59	28.89±1.09	27.62±0.83	29.22±0.76	27.68±0.73	27.98±0.40
Shear force (N)	13.63±0.84	13.85±1.60	15.53±1.94	11.42±0.92	14.90±0.85	13.85±1.15	16.07±2.65
Dry matter (% as basis)	23.99±0.27	23.26±0.40	24.03±0.40	24.30±0.40	24.18±0.43	24.67±0.49	23.98±0.34
Ether extract (% as basis)	0.27±0.03	0.28±0.03	0.32±0.07	0.41±0.10	0.42±0.07	0.43±0.06	0.40±0.06
Crude protein (% as basis)	22.54±0.41	22.40±0.63	22.78±0.38	22.45±0.61	22.56±0.49	22.96±0.47	22.49±0.38
Ash (% as basis)	1.45±0.02	1.41±0.03	1.41±0.03	1.41±0.03	1.47±0.03	1.39±0.04	1.40±0.05
TBARS (mg MDA kg^{-1} muscle tissue)	0.304±0.049	0.509±0.098	0.398±0.066	0.450±0.084	0.414±0.114	0.470±0.098	0.403±0.086

Table 5 Fatty acids composition of experimental diets (% on total FAs)

Item[1)]	C	OR-DH	OR-OD	SE-DH	SE-OD	SL-DH	SL-OD
C14:0	1.88	1.52	0.98	1.35	1.38	0.26	0.22
C15:0	0.21	0.12	0.12	0.12	0.14	0.16	0.14
C16:0	17.55	12.75	12.81	12.92	14.38	15.09	13.54
C16:1	0.67	0.23	0.20	0.26	0.42	0.44	0.24
C18:0	3.68	2.88	2.88	2.95	3.18	3.36	2.87
C18:1n-9	21.28	21.50	21.60	20.87	22.95	23.93	21.85
C18:2n-6	52.28	52.46	52.40	52.68	49.34	49.26	52.83
C18:3n-3	4.64	6.44	6.97	6.51	6.04	5.62	6.56
SFA	23.58	17.49	16.98	17.54	19.27	19.06	16.95
MUFA	23.42	22.84	22.88	22.41	24.50	25.22	22.87
PUFA	58.01	59.67	60.12	60.07	56.23	55.72	60.18
n3 PUFA	4.64	6.44	6.97	6.51	6.04	5.62	6.56
n6 PUFA	53.37	53.23	53.15	53.56	50.19	50.10	53.62

[1)] C12:0, C17:0, C14:1, C15:1, C17:1, C20:1n-9, C18:3n-6, C20:3n-6 were also detected but not reported in the Table because negligible. SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; n3 PUFA, polyunsaturated fatty acids series n-3; n6 PUFA, polyunsaturated fatty acids series n-6. The same as below.

Table 6 Effect of aromatic plants on fatty acids composition of LD muscle (as % on total FAs; n=10)

Fatty acid[1)]	C	OR-DH	OR-OD	SE-DH	SE-OD	SL-DH	SL-OD
C14:0	2.86±0.16	2.53±0.17	2.61±0.14	2.79±0.11	2.94±0.18	2.81±0.10	2.65±0.16
C15:0	1.01±0.10	1.15±0.12	1.11±0.15	0.86±0.04	1.02±0.09	0.90±0.06	0.96±0.08
C16:0	33.67±0.45	34.58±0.30	33.54±0.70	34.31±0.50	34.07±0.77	34.33±0.52	34.57±0.46
C16:1	1.60±0.20	1.52±0.10	1.93±0.16	1.91±0.18	1.64±0.11	1.98±0.08	1.69±0.15
C18:0	7.20±0.23	7.09±0.21	6.74±0.24	7.08±0.28	6.81±0.25	7.12±0.25	6.97±0.13
C18:1n-9	21.76±0.35	20.68±0.28	21.54±0.23	21.50±0.25	21.38±0.31	20.99±0.45	21.11±0.21
C18:2n-6	25.28±0.84	24.16±0.44	25.79±0.70	25.18±0.73	24.91±1.03	25.48±0.35	25.70±0.45
C18:3n-3	1.62±0.16	1.47±0.12	1.82±0.16	1.72±0.11	1.63±0.15	1.81±0.08	1.76±0.12
C20:4n-6	2.09±0.34	2.87±0.31	2.08±0.29	1.82±0.23	2.29±0.31	1.74±0.19	1.79±0.22
SFA	46.23±0.49	47.42±0.34	45.49±0.70	46.43±0.79	46.54±0.95	46.55±0.50	46.66±0.54
MUFA	23.79±0.54	22.74±0.28	23.80±0.34	23.86±0.38	23.52±0.39	23.42±0.45	23.18±0.26
PUFA	30.00±0.66	29.84±0.28	30.71±0.64	29.71±0.71	29.96±0.90	30.05±0.46	30.17±0.44
n3 PUFA	2.12±0.10	2.33±0.09	2.36±0.12	2.25±0.09	2.28±0.10	2.39±0.08	2.18±0.12
n6 PUFA	27.88±0.64	27.51±0.20	28.34±0.55	27.46±0.66	27.68±0.83	27.66±0.42	27.99±0.42

[1)] C10:0, C12:0, C17:0, C20:0, C14:1, C15:1, C17:1, C20:1n-9, C18:3n-6, C20:2n-6, C20:3n-6, C20:5n-3, C22:5n-3, C22:6n-3 were also detected but not reported in the Table because negligible.

be used in rabbit rearing without reporting adverse effects on performance, carcass characteristics and meat quality traits. Probably, the lack of differences amongst the postharvest managements of the herbs on meat quality could be related to the low herb inclusion level in feeds. Further research will be necessary to clarify the absorption and metabolism of sage and oregano bioactive components, and to test the combination of different dietary supplementation levels on this species.

MATERIALS AND METHODS

Plant materials and essential oil extraction

The study was carried out in the experimental centre at the Faculty of Agriculture of the Turin University, located in Carmagnola (44°53´11.67´´N, 7°41´7.00´´E, NW Italy). Greek oregano (OR, *Origanum vulgare* L. subsp. *hirtum*) and two types of sage herbages *Salvia officinalis* 'Extrakta' (SE, Pharmaplant, Germany), and *S. officinalis* subsp. *lavandulifolia* (SL) were used in this experimentation. Sage green shoots and oregano branches were hand harvested and collected. For each plant, phytomasses were split into two postharvest managements. The processed herbs were obtained either through a heat pump-assisted dehumidified air drying (dehumidifying, DH) process, with herbs in a single layer at 25°C or a conventional hot air dryer (oven-drying, OD) process, with herbs in bulk at 60°C, both of which were conducted in commercial drying structures at the experimental centre at the Faculty of Agriculture, as described by Tibaldi *et al*. (2013). The two drying systems are based on different principles and give rise to different herb drying duration and dry matter, depending on herbal structure, but in general the DH is faster than the OD (Tibaldi *et al*. 2010, 2011, 2013). For oregano, DH process lasted 2 d and phytomass reached a dry matter of 20%, while OD lasted 4 d and phytomass reached a dry matter of 17%. For sage, DH process lasted 4 d and phytomass reached a dry matter of 35%, while OD lasted 4 d and phytomass reached a dry matter of 15%. Afterwards, samples were crushed for subsequent inclusion in the experimental diets and prepared for the essential oil (EO) profile determination. EO extraction was obtained *via* the hydrodistillation technique according to Tibaldi *et al*. (2010, 2011), as described by Orio *et al*. (2012). All values were expressed as % of extracted EO. EO compositions of oregano and sage leaves were analyzed with an Agilent 5973 N GC-MS System (Agilent Technologies Inc., Palo Alto, CA, USA) as described by Tibaldi *et al*. (2010, 2011, 2013).

Animals and diets

105 male Bianca Italiana rabbits were randomly divided into seven groups at the weaning stage (30 d of age), single housed in three-floor cages, and fed *ad libitum* until the end of the trial (78 d of age). In order to meet the nutrient requirements of the rabbits a basal diet (dry matter 88.5% DM, crude protein 19.7% CP, ether extract 3.3% EE, neutral detergent fibre 36.9% NDF, acid detergent fibre 20.6% ADF, digestible energy 11.71 MJ kg^{-1} DE on DM) was formulated, and fed pelleted. A control group (C) was assigned to the basal diet. The other six groups were fed with the same diet supplemented with 1% (w/w) of dried herbs, processed either DH or OD and crushed, as follows: Greek oregano (OR-DH and OR-OD, respectively), *S. officinalis* 'Extrakta' (SE) (SE-DH and SE-OD, respectively) and *S. officinalis* subsp. *lavandulifolia* (SL) (SL-DH and SL-OD, respectively). The chemical composition of the experimental diets was analysed according to AOAC (2000).

Growth performance

Rabbits weight and feed intake were recorded fortnightly during the whole experimental period. Data on average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR) were calculated.

Slaughter procedures

At the end of the feeding period, which lasted 48 d, ten rabbits per group were slaughtered at a mean weight of (2 209±186) g in an experimental slaughterhouse without fasting. The carcasses were prepared by removing the skin, feet, paws, genital organs, urinary bladder, and digestive tract, as recommended by Blasco *et al*. (1993). Carcass, skin, tail, feet, paws, liver, kidneys, heart, lungs, and full gastrointestinal tract were weighed and weights were recorded and expressed as a percentage of slaughter weight (SW). After 24 h of chilling, the carcasses were halved into sides and the *Longissimus dorsi* (LD) muscle was excised. LD muscle taken from left side was divided into two parts. The fore part was used to measure cooking losses and colour and the hind part was frozen and used to measure shear force. LD muscle taken from right side was vacuum-packed and frozen at -20°C until the chemical composition, thiobarbituric-acid reactive substances (TBARS), and fatty acid composition were determined.

pH and colour measurements

pH of the meat was measured at 24 h post-mortem (pH_{24}) at the level of 13th thoracic rib at 20°C and recorded with a Crison MicropH 2001 (Crison Instruments, Barcelona, Spain) using a combined electrode penetrating 3 mm. Meat colour was measured on the surface of the *Longissimus dorsi* muscle using a portable Minolta CR-331C Colorimeter (ϕ25 mm measuring area, 45° circumferential illumination/0° viewing angle geometry; Minolta Camera, Osaka, Japan) with the D_{65} illuminant and 2° standard observer. The results were expressed in terms of lightness (L*), redness (a*) and yellowness (b*) in the CIELAB colour space model (CIE 1976). Chroma ($C^*=(a^{*2}+b^{*2})^{0.5}$) and Hue ($H_0=\tan^{-1}(b^*/a^*)$) were calculated according to Boccard *et al*. (1981). The values obtained were the mean of three different measurements per meat sample.

Cooking losses

For each rabbit, a sample of left LD muscle fore part (about 22 g) was weighed (F), vacuum packed in plastic bag and cooked at 80°C for 1 h by immersion in a water bath (Ramirez *et al*. 2004). Cooked samples were cooled under running water for 30 min. The samples were then removed from the bags, blotted and weighed (C). Cooking losses were calculated as (F-C)×100/F.

Shear force

The frozen samples were thawed over night (approximately 16 h) in a refrigerated chamber at 4°C and then cooked as described by Combes *et al.* (2004). The loins were cut into rectangular cross-section strips (1 cm thick×1 cm wide×2 cm along the fibre axis) and were shared perpendicular to the muscle fibre direction using Warner-Bratzler shear device attached to the Instron 5543 equipped with a 1 kN load cell and a crosshead speed of 100 mm min^{-1} (Honikel 1998). The maximum force measured to shear the strips was expressed as Newtons (N).

Chemical composition

The proximate composition in terms of dry matter (950.46), crude protein (981.10), ether extract (960.39) and ash (900.02) content of LD muscle was determined according to the methods of the AOAC (2000) and values were expressed on wet weight basis.

Lipid oxidation

Lipid oxidation was determined using thiobarbituric acid (TBA) analysis according to the iron-induced TBA reactive substances (TBARS) procedure described by Huang and Miller (1993). The assay was performed after 180 min of incubation and absorbance was read at 532 nm. Liquid 1,1,3,3-tetramethoxy propane (Aldrich Chemical Co. Ltd., Dorset, UK) was used as the standard to determine the linear standard response and recovery. TBARS values were expressed as mg of malonaldehyde (MDA) per kilogram of muscle tissue.

Fatty acid composition of diets and *Longissimus dorsi* muscle

Diet fat content (963.15) was determined in accordance with AOAC (2000), while lipids were extracted according to Folch *et al*. (1957) for determining fatty acid composition. For lipid diets and LD samples, fatty acid methyl esters (969.33) were prepared following AOAC (2000) and analysed by gas chromatography. A SHIMADZU-GC 17A chromatograph equipped with a HP88 capillary column (100 m×0.25 mm i.d., 0.2 μm film thickness; Agilent Technologies, Santa Clara, USA) was used. The column temperature was held at 45°C for 5 min and then rose at a rate of 20°C min^{-1} to a final temperature of 195°C, where it remained for 50 min. Temperatures of the injector and flame-ionization detector were maintained at 250°C and 280°C, respectively. The injected volume was 0.1 μL with a 1:50 split ratio. An Hamilton Microliter™ Syringes (HAMILTON Bonaduz AG, Switzerland) was used. Nitrogen column flow was 1.4 mL min^{-1} with a nitrogen average speed of 22 cm s^{-1}. The individual fatty acids were identified by comparing their retention times with those of standard fatty acids (FAME Mix 30 mg mL^{-1} methylene chloride, Restek Corporation-110 Benner Circle, Bellefonte, PA 16823, USA).

Statistical analysis

On all parameters, a one-way ANOVA was performed in order to test the diet effect. Differences amongst groups were evaluated with Duncan's test. Fisher's exact test was used to compare mortality rate of groups. Significance was accepted for $P<0.05$.

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