

PAPER

Effects of Chia (Salvia hispanica L.) seed supplementation on rabbit meat quality, oxidative stability and sensory traits

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Abstract

Chia (Salvia hispanica L.) seed (SHS) dietary supplementation is effective in improving the nutritional quality of rabbit meat for consumers and could contribute to the novel concept of "functional food" in human nutrition. A trial has been conducted in order to verify the effects of three levels (0, 10, or 15%) of SHS inclusion in a rabbit diet on the meat quality, oxidative stability and sensory traits. The dietary treatment did not induce any differences in the ultimate pH, chemical composition, drip losses of the longissimus dorsi muscle or the initial and ultimate pH of the biceps femoris muscle, but the SHS supplementation increased cooking losses of the rabbit meat. The inclusion of SHS also reduced oxidative stability during meat storage. No adverse effects were observed on the meat quality or customer acceptability. The inclusion of SHS in rabbit diets, which is effective in improving the n-3 polyunsaturated fatty acids content of meat, increased the lipid oxidation in the hind leg meat. An improvement in tissue oxidative stability could be obtained by feeding rabbits with higher levels of antioxidants.

Introduction

Rabbit meat is considered richer in polyunsaturated fatty acids (PUFA; 23% of total fatty acids) than other meats, poultry included (Dalle Zotte, 2002). Different studies have pointed out that rabbit meat has a high nutri-

tional value (Dalle Zotte et al., 2001; Hernández and Gondret, 2006). This means rabbit meat can play an active role in the prevention and management of several pathologies (Simopoulos, 2000). Nevertheless, with the current interest in increasing n-3 PUFA levels in animal products and decreasing the n-6/n-3 ratio to 5/1 (Lunn and Theobald, 2006), oil enriched diets are very common. However, the n-6/n-3 ratio in the rabbit meat usually is higher than 5/1 and reaches 7/1 in the loin (Dal Bosco et al., 2004) and 11/1 in the meat of hind leg (Ramírez et al., 2005), therefore the objective of Chia seed supplementation is to increase n-3 fatty acids and to decrease the n-6/n-3 ratio.

The dietary oil inclusion level and source play different roles in the carcass composition, quality characteristics (Dal Bosco *et al.*, 2004), and sensory properties of the meat (Dalle Zotte, 2002). On the other hand, a high PUFA content in rabbit meat could affect its suitability for processing and storage, as it becomes more susceptible to oxidation.

In particular, as reported by Dalle Zotte (2002), the inclusion of dietary fat in moderate concentrations (3-6%) can improve carcass yield, but carcass adiposity (perirenal fat or total dissectible fat) increases. In the same review, it emerged that the lipid content of the meat does not vary significantly if oil is added in a moderate quantity. However, when higher fat inclusions are carried out, the fat content of the meat increases (Christ et al., 1996), while the water and protein contents decrease (Pla and Cervera, 1997). Moreover, fat inclusion can affect some physical properties of rabbit meat, with an increase in the ultimate pH (pHu) and, in some cases, in the cooking losses (Raimondi et al., 1975). No relationship was found between the lipid content of the longissimus dorsi (l.d.) muscle and juiciness, but a positive correlation was observed for sensory tenderness (Gondret et al., 1998).

There has been an increasing interest in the use of antioxidants in rabbit feed formulas because the dietary manipulation of tissue lipid composition to produce meat with a high PUFA content could decrease meat oxidative stability (Hernández, 2008) and different natural ways to improve the oxidative stability of rabbit meat have also been studied (Lòpez-Bote et al., 1998; Coni et al., 2000).

Chia (Salvia hispanica L.) seeds (SHS) are rich in PUFAs (expressed per 100 g of total fatty acids: linolenic acid: 64.1 g; linoleic acid: 18.8 g) (Peiretti and Gai, 2009) and they contain some compounds, such as myricetin, quercetin, and kaempferol, which act as potent antioxidants (Reyes-Caudillo *et al.*, 2008).

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Ayerza *et al.* (2002) reported the nutritional advantage of chia in poultry nutrition. SHS supplementation in rabbit diets could contribute to the novel concept of "functional food" in human nutrition (Almeida *et al.*, 2006).

The aim of the present research was to study the effect of the intrinsic antioxidant activity of SHS on the physico-chemical characteristics, sensory quality and oxidative stability of the rabbit meat.

Materials and methods

Animals and diets

The study was carried out at the CISRA experimental rabbitry at the University of Turin, according to the guidelines for applied nutrition experiments in rabbits (Fernández-Carmona et al., 2005). A complete description of the performance and carcass characteristics (carcass yield and proportions of various parts and the rabbit organs) and the fatty acid composition of the l.d. and perirenal fat of thirty carcasses obtained from three groups of ten crossbred rabbits (five male and five female rabbits each) can be found in Peiretti and Meineri (2008). These groups were fed with an isocaloric and isonitrogenous diet ad libitum, enriched with different levels of SHS (0, 10, and 15%). Ingredients and composition of the experimental diets are reported in Table 1. All the diets were pelleted fresh and stored in





darkness in a temperature controlled room to avoid auto-oxidation of the lipid sources.

The animals were housed individually under standard conditions at a temperature of $22\pm2^{\circ}$ C in wire cages at a height of 90 cm from the concrete floor. At the end of the experimental period, which lasted 35 days, all the rabbits were slaughtered at a weight of 1433 ± 28 g in an experimental slaughterhouse without fasting, one by one.

Meat quality

Immediately after slaughtering, the carcasses were prepared singly by removing the skin, feet, paws, genital organs, urinary bladder and digestive tract (Blasco *et al.*, 1993).

The initial pH (pHi) was measured in duplicate within 60 minutes after the death of each rabbit in the l.d. and biceps femoris muscles, using a Knick 752 pHmeter with a Crison 52-32 electrode. The rabbit carcasses were then refrigerated at 4°C for 24 hours. After this hanging period, the ultimate pH (pHu) was measured on the same muscles and then each carcass was dissected as recommended by Blasco et al. (1993). The two l.d. muscles and the right hind legs were collected from each carcass. Drip loss (DL) was determined according to Lundström and Malmfors (1985) on a 1.5 cm thick slice of the l.d. muscle of each rabbit. The right l.d. muscle was lyophilized and the left l.d. muscle was vacuum packed and frozen at -20°C until the successive analysis (cooking losses and sensory analysis).

Chemical analyses (water, ash, protein and ether extract) were conducted in duplicate on lyophilized samples of the right *l.d.* muscle and expressed on a fresh basis (AOAC, 2000).

Cooking loss (CL) was evaluated on the thawed left *l.d.* muscle by cooking it in an electric oven (G-Therm 035, Galli, Italy) at 165°C until an internal temperature of 70°C was reached (Obuz *et al.*, 2003). The samples were immediately weighed and the CL was expressed as the ratio (%) of the difference in weight between the cooked and raw muscle relative to the weight of the raw muscle. After this measurement, the same cooked sample was used for sensory analysis.

The right hind legs were dissected, trimmed of all visible extramuscular fat, minced in a kitchen homogenizer (Multi moulinette, Moulinex, France) for 5 min, divided into four subsamples and singularly packed in a plastic bag: one meat sample was frozen at -20°C for two months and three meat samples were stored at 4°C for 1, 8 and 14 days, respectively. After the conservation period, the ground hind leg samples were analysed for susceptibility to lipid oxidation.

Susceptibility to lipid oxidation

The thiobarbituric acid reactive substance (TBARS) assay was modified from that of Witte et al. (1970) and was performed for each rabbit on a sample of 10 g of minced meat, prepared according to standard conditions (ground sample oxygenated for 30 minutes after opening each time), was homogenised for 30 sec at high speed with 20 mL of 10% trichloroacetic acid (TCA) using a Polytron tissue homogenizer (Type PT 10-35; Kinematica GmbH, Luzern, Switzerland). After centrifugation of the homogenate (600 rpm for 5 min at 4°C), the supernatant was filtered through Whatman #1 filter paper. One mL of filtrate was combined with 1 mL of 0.02M aqueous 2thiobarbituric acid solution (TBA), heated in a boiling water bath for 20 min together with a blank containing 1 mL of a TCA/water mix (1/1) and 1 mL of TBA reagent and subsequently cooled under running tap water. The samples were analysed in triplicate and the results were expressed as mM MDA kg-1 DM, using a standard curve that covered the concentration range of 1 mmol l-1 to 10 mmol l-1 1,1,3,3-tetramethoxypropane (Sigma-Aldrich, Steinheim, Germany). The absorbance was measured at 532 nm with a Helios spectrophotometer (Unicam Limited, Cambridge, UK) against a blank that contained all the reagents but no meat.

Sensory traits

The sensory analysis was performed on the roasted samples, without salt or spices, cooked in an electric oven at 165°C until an internal temperature of 70°C was reached. Just after the weighing for cooking loss determination, the samples were immediately sliced into pieces of uniform dimensions and randomly offered to the trained panel.

The trial consisted of six sessions, with five samples analysed per session by six panellists, selected and trained specifically on rabbit meat according to AMSA (1978). A descriptive test was used to assess: off odours, tenderness, juiciness, fibrousnesses and overall acceptability.

The evaluation was expressed according to a five-point scale: 1 referring to very disagreeable, very tough, very dry, very fibrous and 5 to very agreeable, extremely tender, very juicy, not fibrous (Cross *et al.*, 1986).

Statistical analyses

The statistical analyses were performed using the SPSS software package (version 11.5.1 for Windows, SPSS Inc., USA). An analysis of variance was used to evaluate the effects of different concentrations of SHS on the quality and sensory traits of the rabbit meat. A value of P<0.05 was viewed as statistically significant. The differences were tested using Duncan's Multiple Range Test. For the sensory

Table 1. Ingredients and composition of the experimental diets.

| ~O` | Chia seed | Chia seed (% of diet) | | | |
|--------------------------|-----------|-----------------------|------|------|--|
| <u>U</u> | | 0 | 10 | 15 | |
| Ingredients, % | | | | | |
| Corn | | 15 | 18 | 21 | |
| Barley | | 19 | 24 | 20 | |
| Dehydrated alfalfa meal | | 46 | 33 | 30 | |
| Soybean seed meal | | 12 | 11 | 10 | |
| Palm oil | | 4 | 0 | 0 | |
| Chia seed | | 0 | 10 | 15 | |
| Vitamin-mineral premix* | | 2 | 2 | 2 | |
| Lignosulphite | | 2 | 2 | 2 | |
| Chemical composition | | | | | |
| Dry matter, % | 94.9 | 92.3 | 91.1 | 91.3 | |
| Organic matter, % | 95.2 | 93.4 | 94.4 | 94.7 | |
| Crude protein, % | 23.5 | 15.9 | 15.6 | 15.7 | |
| Ether extract, % | 31.1 | 4.9 | 6.1 | 5.0 | |
| Crude fibre, % | 32.9 | 13.6 | 11.5 | 11.9 | |
| Crude ash, % | 4.8 | 6.6 | 5.6 | 5.3 | |
| Nitrogen free extract, % | 7.8 | 59.0 | 61.2 | 62.1 | |
| Acid detergent fibre, % | 34.5 | 16.1 | 14.2 | 14.1 | |
| Acid detergent lignin, % | 12.6 | 5.5 | 4.6 | 5.2 | |
| Gross energy, MJ/kg DM | 26.1 | 15.9 | 15.6 | 15.9 | |
| Linoleic acid, % of FA | 18.8 | 21.9 | 28.6 | 27.7 | |
| Linolenic acid, % of FA | 64.1 | 10.5 | 36.9 | 39.3 | |

*per kg of diet: Vit. A 200 U; α -tocopheryl acetate 16 mg; Niacine 72 mg; Vit. B $_6$ 16 mg; Choline 0.48 mg; DL-methionine 600 mg; Ca 500 mg; P 920 mg; K 500 mg; Na 1 g; Mg 60 mg; Mn 1.7 mg; Cu 0.6 mg.





traits, the random effect of panel member and session were also included in the model.

Results and discussion

Meat quality

The physico-chemical characteristics of the *l.d.* muscle are shown in Table 2. The pHi values measured within 60 minutes after the death of each rabbit resulted to be higher in the treated group, but this difference disappeared at 24 hours (pHu) after slaughtering and the value returned to a normal level, in agreement with bibliographic values (Pascual and Pla, 2007; Pla, 2008). Similarly, the observed pH on the *biceps femoris* muscle (Table 2) did not show any unusual reactivity to stress, and no difference was measured among the groups.

The chemical composition (Table 3) was not significantly affected by the dietary treatment (P>0.05). This confirms the leanness of the rabbit meat, which was maintained in the treated animals (*maximum* 10.4±1.0 g kg⁻¹ fresh basis) and the high nutritional properties due to the high protein and minerals content, of about 225 and 141 g kg⁻¹ fresh basis, respectively. The meat maintained a low lipid level and the high nutritional and dietetic properties resulted to be improved by the SHS dietary supplementation (Peiretti and Meineri, 2008).

No statistical differences among groups were observed for drip losses (Table 3; P>0.05), while cooking losses increased in the groups with SHS supplementation (P<0.05) in agreement with Raimondi *et al.* (1975), but in contrast with Oliver *et al.* (1997) and Maertens *et al.* (1998).

Susceptibility to lipid oxidation

The TBARS data (Table 4) already showed an evident effect of SHS inclusion on oxidation susceptibility of the ground hind leg muscle from the second post mortem day and this effect increased during the conservation period. Higher values were in particular found at 15% SHS inclusion between 8.02 on the first day and 48.23 mM MDA kg⁻¹ DM after 2 months of storage at -20°C. Furthermore, the compounds with the antioxidant effect of the Chia seeds resulted to be ineffective in preventing meat oxidation.

The rabbit meat is more susceptible to lipid oxidation because it is richer in PUFA than the red meat (Fernández-Esplá and O'Neill, 1993). Our TBARS data showed a greater susceptibil-

ity of the ground hind leg meat to oxidation during storage and the mincing treatment might have increased oxidation susceptibility. Hernández (2008) found that oxidation products evaluated by measuring peroxide value and TBARS were not very high in rabbit meat, although both oxidation parameters increase with storage time. However, when rabbits were fed with a diet enriched with 3% linseed oil,

rabbit meat for consumers. Furthermore, SHS can be fed to rabbits at levels of up to 15% of the diet without any adverse effects on growth performance or carcass characteristics. The enrichment of the diet with Chia seed results in a rabbit meat that contains more unsaturated lipids (Figure 1), which is mainly due to C18:2(n-6) and C18:3(n-3) (PUFA control 27.7 vs 5% Chia 45.6 vs 15% Chia 50.7). This very

Table 2. The pH of the longissimus dorsi and biceps femoris muscles (means±SE).

| | 0 | Chia seed (% of diet) | 15 |
|--------------------------|---------------------|------------------------------|-------------------|
| Longissimus dorsi muscle | | | |
| pHi (60 minutes) | 6.26 ± 0.11^{a} | 6.72 ± 0.06 ^b | 6.72 ± 0.07 b |
| pHu (24 hours) | 5.63 ± 0.02 | 5.70 ± 0.02 | 5.65 ± 0.02 |
| Biceps femoris muscle | | | |
| pHi (60 minutes) | 6.53 ± 0.08 | 6.55 ± 0.09 | 6.55 ± 0.08 |
| pHu (24 hours) | 5.98 ± 0.05 | 5.98 ± 0.05 | 5.99 ± 0.04 |
| | | | |

 $^{\mathrm{a,b}}$ Means in the same row with different superscripts differ (P<0.05).

Table 3. Physico-chemical characteristics (means±SE) of the longissimus dorsi muscle.

| . 0 | Chia seed (% of diet) | | |
|---|-----------------------|--------------------|--------------------|
| 7.0 | 0 | 10 | 15 |
| Chemical composition (g kg ⁻¹ fresh basis) | | | |
| Water | 747.6 ± 2.9 | 742.6 ± 3.5 | 742.0 ± 2.1 |
| Protein | 220.9 ± 2.9 | 224.9 ± 3.2 | 224.3 ± 1.5 |
| Lipid | 9.3 ± 1.3 | 9.7 ± 0.7 | 10.4 ± 1.0 |
| Ash | 14.1 ± 0.5 | 14.0 ± 0.2 | $1.4.2 \pm 0.3$ |
| Physical characteristics | | | |
| Drip losses, % | 2.30 ± 0.30 | 2.35 ± 0.50 | 2.99 ± 0.58 |
| Cooking losses, % | 13.34±1.50a | 17.66 ± 0.80 b | 16.63 ± 0.95 b |

a,bMeans in the same row with different superscripts differ (P<0.05).

Table 4. Oxidative status (TBARS, mM MDA kg $^{-1}$ DM) of the ground hind leg muscle (means \pm SE) after 1, 8, and 14 days of storage at 4°C and after 2 months of refrigeration at -20°C.

| | Chia seed (% of diet) | | |
|---------------|-----------------------|----------------------------|--------------------|
| | 0 | 10 | 15 |
| TBARS at 1 d | 6.06±0.41a | $6.79 \pm 0.62 ab$ | 8.02 ± 0.51 b |
| TBARS at 8 d | $9.86 \pm 0.77a$ | $12.69 \pm 1.10ab$ | $16.41 \pm 2.31b$ |
| TBARS at 14 d | $12.27 \pm 1.02a$ | $17.32 \pm 2.44ab$ | 19.03 ± 1.91 b |
| TBARS at 2 m | $11.59 \pm 2.22a$ | $30.90 \pm 5.97 \text{ b}$ | 48.23 ± 8.70 b |

a,bMeans in the same row with different superscripts differ (P<0.05).

rabbit meat showed higher TBARS values after 5 post-mortem days of refrigerated storage (Hernández et al., 2007). Peiretti and Meineri (2008) showed that SHS dietary supplementation is effective in improving the n-3 PUFA content, decreasing the n-6/n-3 ratio and reducing the saturation, atherogenic and thrombogenic indexes of meat, with consequent benefits on the nutritional quality of

high increase is probably responsible of the decrease of oxidative stability.

In order to counteract the oxidative processes found in the ground meat of the rabbits fed with SHS enriched diets, adequate protection, using high levels of various antioxidants, is required and this could improve tissue oxidation stability, as described by different studies (Dalle Zotte *et al.*, 2000; Castellini *et al.*, 2001;





Dal Bosco et al., 2001).

Sensory traits

Table 5 shows the means of the sensory characteristics. In all the studied characteristics, none of the statistical differences were found significant (P>0.05).

Ayerza and Coates (2002) used SHS in laying hen diets to produce eggs with a high n-3 PUFA content, a lower saturated fatty acid content, and a lower n-6/n-3 ratio without imparting off-flavors. Unacceptable off-flavors were not reported in the dark and white meats of poultry fed with a 10% SHS diet (Ayerza *et al.*, 2002).

It is very important that the odour and taste were not affected by the dietary treatment, since such an enrichment might result in off-flavours of the product (O'Keefe *et al.*, 1995).

The higher cooking losses measured in the groups fed with SHS could instead influence the juiciness sensory trait, since it has been demonstrated that there is a negative correlation between cooking loss and fat content (Hernández *et al.*, 2000).

Conclusions

SHS dietary supplementation did not cause any adverse effects on meat quality or consumer acceptability. The inclusion of 15% SHS in the rabbit diets increased the lipid oxidation in the ground hind leg meat during conservation at 4°C probably due to a concurrent enlargement of PUFA. Further research is needed to determine an improvement in tissue oxidative stability by feeding rabbits with high levels of antioxidants, since the compounds with the antioxidant effect of Chia seeds alone has resulted to be ineffective in preventing meat oxidation.

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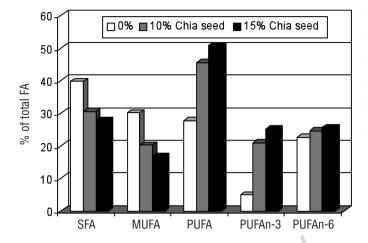


Figure 1. Fatty acid percentage in the longissimus dorsi muscle of rabbit fed experimental diets (Peiretti and Meineri, 2008).

Table 5. Sensory traits measured on the roasted longissimus dorsi muscle.

| | Chia seed (% of diet) | | |
|---------------|-----------------------|------|------|
| | 0 | 10 | 15 |
| Off odours | 3.40 | 3.47 | 3.28 |
| Tenderness | 3.72 | 3.33 | 3.60 |
| Juiciness | 3.15 | 2.59 | 2.66 |
| Fibrousnesses | 3.33 | 3.43 | 3.33 |
| Acceptability | 2.50 | 2.57 | 2.38 |

The evaluation is based on a five-point scale where 5 represents the highest score and 1 the lowest.

AOAC. AOAC, Arlington, VA, USA.

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