# Original article

# Effects of dietary inclusion of sunflower soap stocks on colour, oxidation and microbiological growth of meat from light fattening lambs

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**Summary**   Thirty-two lambs were finished on a total mixed ration (TMR) pelleted alone (00SS) or including sunflower soap stock (SS): 15SS (15 g SS per kg TMR), 30SS (30 g SS per kg TMR) and 60SS (60 g SS per kg TMR). Lambs (8 per group) were slaughtered at 27 kg live weight. Colour evolution, lipid oxidation, microbial growth and detection of diarrhoeagenic *Escherichia coli* were studied in meat samples. SS in the diet reduced meat lipid oxidation ($P < 0.05$), but had no significant effect $(P > 0.05)$ on the cholesterol oxidation products. Meat from 60SS and control animals (00SS) revealed greater discoloration ($P < 0.05$) than 15SS. SS supplementation did not affect the microbiological populations, whereas high doses of SS seemed to increase the proliferation of diarrhoeagenic *E. coli* on day 14 ($P < 0.10$). These results suggest that the rate of SS inclusion in the diet of fattening lambs should not be above 15 g SS per kg TMR.

## Introduction

Supplementing ruminant diets with vegetable oils is nowadays a common practice to improve animal performance, reduce methane emissions and modify meat fatty acid profile (Chikwanha *et al*., 2018). Soap stocks (SS), a commercial by-product from the vegetable oil industry, represent a suitable alternative used for feeds but not for foods (Blanco *et al*., 2012, 2014b) and can be used in the diet for fattening lambs to reduce the atherogenic and saturation indices of meat (Blanco *et al*., 2017). The high content of unsaturated fatty acids of SS could increase the susceptibility of meat to lipid oxidation, thereby impairing the meat shelf life. However, antioxidants are also included in this by-product (e.g. 0.1% butylated hydroxytoluene [BHT], to avoid rancidity of SS). Hence, these compounds could also be accumulated in the animal tissues and protect the meat against oxidation, but also show toxic effects for the consumer.

Notwithstanding the combination of unsaturated fatty acids and antioxidants may promote the growth of some human pathogen bacteria (Shin *et al*., 2007). Among others, Shiga-toxin-producing *Escherichia coli* (STEC, including O157 and non-O157 serogroups) and enteropathogenic *E. coli* (EPEC) are increasing in interest. Ruminants are considered important reservoirs of STEC, which are characterised by the *stxs* genes encoding the Shiga-like toxins (*stx-1* and *stx-2*). EPEC pathotype is one of the most important causes of diarrhoea (particularly in children). This pathotype is characterised by the presence of the locus of enterocyte effacement (LEE), which contains the *eae* gene (*eae +* ) responsible for producing an attaching and effacing lesion in intestinal cells, but does not produce Shiga-like toxins (*stx*–). This important virulence determinant, the LEE region, is also shared by some STEC strains (*eae*+).

Therefore, the present study investigated the meat shelf life (colour, microbial spoilage and oxidation) and safety (growth of human pathogens) when

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different doses of SS are included in the diet for light fattening lambs.

## Materials and methods

All handling practices followed the recommendations of the Directive 2010/63/EU of the European Parliament for the protection of animals used for experimental and other scientific purposes, and the IGM-CSIC Animal Experimentation Committee. All of the animals were able to see and hear other lambs.

### Animals and diets

After stratification on the basis of body weight (average BW = 15.9 ± 0.24 kg), 32 male Merino lambs were penned individually and randomly allocated to one of four different treatments (eight lambs per treatment), according to the amount of SS (Riosa, Jaen, Spain) offered in the diet: 00SS (0 g SS per kg TMR pellets), 15SS (15 g SS per kg TMR pellet), 30SS (30 g SS per kg TMR pellets) and 60SS (60 g SS per kg TMR pellet). Ingredients and chemical composition of the feeds are shown in Table S1. Further details can be found in Blanco *et al.* (2012).

Each lamb was fed the corresponding experimental diet *ad libitum* (TMR pellets, Blanco *et al.*, 2014a, 2015) as described previously in Blanco *et al.* (2014b), where all the data regarding dry matter intake, growth performance and feed efficiency can be found as described precisely. When animals reached 27 kg of BW, they were slaughtered by stunning and exsanguination as described elsewhere (Blanco *et al.*, 2014a,b). After 24 h at 4 °C, the *longissimus lumborum* (LL), *gluteus medius* (GM) and *quadriceps femoris* (QF) muscles were removed and analyses in duplicate were carried out as described below.

### Meat colour changes

The LL from the right and left carcass sides of each lamb was cut into 2.5-cm-thick slices; twelve slices from each animal were distributed in six impermeable polypropylene trays, which were wrapped with bags and immediately modified atmosphere packaged (MAP) with a commercial gas blend intended for red and poultry meat, consisting of 35% $CO_2$, 35% $O_2$ and 30% $N_2$ (Morán *et al.*, 2012a,b). All packages were stored under simulated retail display conditions (12-h daily fluorescent illumination [34 W] and 3 ± 1 °C). A tray with two slices of LL from each animal was opened each day, and both slices were measured (two measurements per slice) for colour parameters on days 0 (allowing ca. 2 h for blooming), 1, 3, 7, 9 and 14, as described by Morán *et al.* (2012a,b).

### Lipid peroxidation of meat

The trays to be opened after 0, 7 and 14 days of refrigerated storage also included another two slices of GM, one of them being frozen at −20 °C when sampling at each time. Thiobarbituric acid reactive substances (TBARS) were determined on 2.5 g of previously thawed, raw LL and GM samples aged for 0, 7 and 14 days under MAP, according to the procedure described by Bodas *et al.* (2012). This procedure can be found fully described as well in Appendix S1.

### Cholesterol oxidation products (COPs) in meat

After 7 days of refrigerated storage, the second slice of GM was weighed and cooked in a double-sided griddle (preheated at 220 °C) until a core temperature of 75 °C was reached, following the guidelines for cooking procedures of American Meat Science Association (AMSA, 1995). Then, they were cooled at 4 °C for 30 min, re-weighed, frozen and freeze-dried for oxysterol analysis. The COPs, also called oxysterols, were determined, according to the method described by Andrés *et al.* (2014). Further details have been included in Appendix S1.

### Microbiological analysis and extract release volume (ERV)

The trays for 0, 7 and 14 days also included slices of QF muscle. QF muscle (25 g) from each tray and at each storage time (0, 7 and 14 days) under MAP conditions was placed into sterile stomacher bags and rinsed with peptone water (1:5 dilution), and the recovered rinse liquid was then diluted 10-fold. The numbers of total viable bacteria (TVB; 4.5 °C/7 days) on plate count agar (Oxoid, Basingstoke, UK), *Pseudomonas* spp. (25 °C/2 days) on *Pseudomonas* agar base (Oxoid), and *Enterobacteriaceae* (EC; 37 °C/24 h) and lactic acid bacteria (LAB; 30 °C/3 days) using overlaid plates of violet red bile glucose agar (Oxoid) and MRS agar (Oxoid), respectively, were determined and confirmed as described elsewhere (Rodríguez-Calleja *et al.*, 2004).

The ERV of meat, which correlates with bacterial growth, was measured (on days 0, 7 and 14) in QF muscle (Rodríguez-Calleja *et al.*, 2004). Further details are included in Appendix S1.

### Detection of enteropathogenic bacterial groups in meat by polymerase chain reaction (PCR)

The $stx_1$, $stx_2$ and *eae* genes were selected to estimate the occurrence of diarrhoeagenic *E. coli* included in the STEC or EPEC pathogroups (Table S2). Thus, genomic DNA was extracted from both lamb meat

**Table 1** Mean values ($\mu$g malondialdehyde per g meat) of thiobarbituric acid reactive substances (TBARS) in raw meat samples (*Longissimus dorsi* and *Gluteus medius*) stored at 4 °C during 0, 7 and 14 days under modified atmosphere package (35% $CO_2$, 35% $O_2$ and 30% $N_2$) from lambs being fed different doses of sunflower soap stocks

| | Diets[†] | | | | Storage days | | | | P-values[‡] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 00SS | 15SS | 30SS | 60SS | 0 | 7 | 14 | RSD | Diet | Day | D*d |
| *Longissimus lumborum* | 2.93[a] | 1.84[b] | 1.63[b] | 1.87[b] | 0.94[c] | 1.78[b] | 3.16[a] | 0.901 | 0.019 | <0.001 | 0.415 |
| *Gluteus medius* | 2.24[a] | 1.80[b] | 1.53[b] | 1.40[b] | 1.39[b] | 1.81[ab] | 2.03[a] | 1.024 | 0.048 | 0.049 | 0.905 |

Means on the same row with different superscripts ([a, b, c]) indicate statistical differences between diets or days ($P < 0.05$). RSD, Residual standard deviation.

[†]00SS = TMR pellet without sunflower soap stock (SS); 15SS = TMR pellet containing 15 g SS per kg; 30SS = TMR pellet containing 30 g SS per kg; 60SS = TMR pellet containing 60 g SS per kg.

[‡]*P*-value for diet, storage day or the interaction between diet and storage day (D*d).

enrichments and isolates, according to the adapted method previously described (Rodríguez-Calleja *et al.*, 2006), taking into account ISO 22174 general requirements and ISO 20838 (ISO, 2005, 2006). Further details are included in Appendix S1.

## Statistical analysis

Microbiological counts were transformed and expressed as log CFU g$^{-1}$. Colour parameters ($L^*$, $a^*$, $b^*$, $C^*$ and $h^*$), TBARS, ERV and transformed microbial counts, were subjected to a two-way analysis of variance (ANOVA), with treatment and day as the main factors, using the MIXED procedure of SAS (SAS

**Table 2** Oxysterol content ($\mu$g g$^{-1}$ meat) in meat samples (*Gluteus medius*, from lambs being fed diets with doses of sunflower soap stocks) stored at 4 °C during 7 days under modified atmosphere package (35% $CO_2$, 35% $O_2$ and 30% $N_2$), and then cooked

| | Groups[†] | | | | | |
|---|---|---|---|---|---|---|
| | 00SS | 15SS | 30SS | 60SS | RSD | P-value |
| 7$\alpha$-HC | 0.690 | 0.667 | 0.825 | 0.900 | 0.476 | 0.766 |
| 7$\beta$-HC | 0.776 | 0.719 | 0.953 | 1.018 | 0.527 | 0.681 |
| 7-KC | 0.455 | 0.393 | 0.479 | 0.659 | 0.449 | 0.716 |
| $\beta$-CE | 0.369 | 0.358 | 0.410 | 0.524 | 0.292 | 0.694 |
| $\alpha$-CE | 0.130 | 0.109 | 0.125 | 0.169 | 0.105 | 0.742 |
| 25-HC | 0.012 | 0.010 | 0.011 | 0.019 | 0.010 | 0.311 |
| CT | 0.009 | 0.007 | 0.009 | 0.018 | 0.008 | 0.086 |
| $\sum$ COPs | 2.44 | 2.26 | 2.81 | 3.31 | 1.819 | 0.714 |

RSD, residual standard deviation; 7$\alpha$-HC, 7$\alpha$-hydroxycholesterol; 7$\beta$-HC, 7$\beta$-hydroxycholesterol; 7-KC, 7-ketocholesterol; $\beta$-CE, 5,6$\beta$-epoxycholesterol; $\alpha$-CE, 5,6$\alpha$-epoxycholesterol; 25-HC, 25-hydroxycholesterol; CT, cholestanetriol; $\sum$ COPs, Sum of total cholesterol oxidation products (detected).

[†]00SS = TMR pellet without sunflower soap stock (SS); 15SS = TMR pellet containing 15 g SS per kg; 30SS = TMR pellet containing 30 g SS per kg; 60SS = TMR pellet containing 60 g SS per kg.

Inst. Inc., Cary, NC, USA). Oxysterol content was subjected to one-way ANOVA, with diet as the only source of variation, using the GLM procedure of SAS. The proportion of positive and negative samples of PCR amplification of virulence genes was analysed by FREQ procedure, using a chi-square test (SAS Inst. Inc.).

## Results

### Lipid oxidation

Results of lipid oxidation of raw meat quantified by the TBARS assay are shown in Table 1. In the present study, no significant interactions between storage day and diet were found. However, differences between storage day and diet were observed in both muscles studied (LL and GM). As expected, the TBARS values increased as time progressed ($P < 0.05$). Likewise, meat from animals fed with SS diets showed lower TBARS values ($P < 0.05$) than meat from 00SS, in both muscles studied.

#### Cholesterol oxidation products

The contents of the different COPs in cooked meat samples are shown in Table 2. The dietary treatment did not significantly ($P > 0.05$) affect either the total or the individual oxysterol content (7$\alpha$-HC, 7$\beta$-HC, $\alpha$-CE, $\beta$-CE, 25-HC and 7-KC) in meat samples.

#### Colour attributes

Table 3 summarises the effects of dietary treatments on the colour stability of LL muscle after retail display under refrigerated MAP for 14 days. Meat from 15SS to 30SS animals presented lower $L^*$ values than control (00SS) and 60SS lambs ($P < 0.01$). Similar results were observed in $b^*$ and $h^*$ parameters, where the lowest values were recorded in 15SS compared to 00SS and 60SS animals ($P < 0.05$). The redness index ($a^*$) values showed significant differences among groups,

**Table 3** Evolution of mean values of colour parameters ($L^*$, $a^*$, $b^*$, $C^*$ $y$ $h^*$) for meat samples (*Longissimus lumborum*) stored at 4 °C during 14 days under modified atmosphere package (35% $CO_2$, 35% $O_2$ and 30% $N_2$) from lambs being fed different doses of sunflower soap stocks

| | Diets[†] | | | | Storage days | | | | | | | *P*-values[‡] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 00SS | 15SS | 30SS | 60SS | 0 | 1 | 3 | 7 | 9 | 14 | RSD | Diet | Day | *D*d* |
| $L^*$ | 45.2[a] | 44.2[b] | 44.3[b] | 45.4[a] | 45.6[ab] | 45.2[a] | 44.5[bc] | 43.6[c] | 43.9[c] | 45.2[ab] | 1.97 | 0.004 | 0.002 | 0.459 |
| $a^*$ | 10.3[ab] | 10.5[a] | 10.5[a] | 9.9[b] | 9.3[c] | 10.0[b] | 11.0[a] | 10.9[a] | 11.1[a] | 10.0[b] | 1.03 | 0.011 | <0.001 | 0.393 |
| $b^*$ | 5.7[a] | 5.3[b] | 5.5[ab] | 5.7[a] | 3.8[d] | 6.1[a] | 6.0[ab] | 5.5[c] | 5.6[bc] | 6.1[a] | 0.63 | 0.030 | <0.001 | 0.511 |
| $C^*$ | 11.7 | 11.8 | 11.9 | 11.5 | 10.1[d] | 11.7[c] | 12.5[a] | 12.2[abc] | 12.4[ab] | 11.7[bc] | 0.98 | 0.109 | <0.001 | 0.352 |
| $h^*$ | 28.9[ab] | 26.8[c] | 27.7[bc] | 29.8[a] | 22.5[c] | 31.3[a] | 28.7[b] | 26.9[b] | 27.0[b] | 31.3[a] | 3.54 | <0.001 | <0.001 | 0.603 |

Means with different superscripts ([a, b, c]) indicate statistical differences between diets or days ($P < 0.05$). RSD, residual standard deviation.
[†]00SS = TMR pellet without sunflower soap stock (SS); 15SS = TMR pellet containing 15 g SS per kg; 30SS = TMR pellet containing 30 g SS per kg; 60SS = TMR pellet containing 60 g SS per kg.
[‡]*P*-value for diet, storage day or the interaction between diet and storage day ($D^*d$).

**Table 4** Microbial population (log CFU per g) and extract release volume (ERV, ml) in meat samples (*Quadriceps femoris*) stored at 4 °C during 0, 7 and 14 days under modified atmosphere package (35% $CO_2$, 35% $O_2$ and 30% $N_2$) from lambs being fed different doses of sunflower soap stocks

| | Diets[†] | | | | Storage days | | | | *P*-values[‡] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 00SS | 15SS | 30SS | 60SS | 0 | 7 | 14 | RSD | Diet | Day | *D*d* |
| TVB | 3.18 | 3.55 | 2.78 | 3.19 | 2.00[c] | 2.65[b] | 4.89[a] | 0.647 | 0.064 | <0.001 | 0.697 |
| *Pseudomonas* | 2.94 | 3.08 | 2.73 | 3.38 | 2.28[c] | 2.63[b] | 4.19[a] | 0.481 | 0.074 | <0.001 | 0.562 |
| EC | 1.77 | 1.95 | 1.49 | 1.36 | 0.87[c] | 1.57[b] | 2.50[a] | 0.537 | 0.128 | <0.001 | 0.359 |
| LAB | 2.84 | 2.36 | 2.50 | 2.31 | 1.36[c] | 2.05[b] | 4.11[a] | 0.909 | 0.574 | <0.001 | 0.181 |
| ERV | 23.5 | 25.2 | 22.9 | 22.4 | 30.6[a] | 21.7[b] | 18.8[c] | 4.43 | 0.383 | <0.001 | 0.766 |

Means with different superscripts ([a, b, c]) indicate statistical differences between diets or days *(P < 0.05)*. EC, *Enterobacteriaceae*; ERV, extract release volume; LAB; lactic acid bacteria; RSD, residual standard deviation; TVB, total viable bacteria.
[†]00SS = TMR pellet without sunflower soap stock (SS); 15SS = TMR pellet containing 15 g SS per kg; 30SS = TMR pellet containing 30 g SS per kg; 60SS = TMR pellet containing 60 g SS per kg.
[‡]*P*-value for diet, storage day or the interaction between diet and storage day ($D^*d$).

with 15SS and 30SS showing the highest values, followed by 00SS and 60SS animals ($P < 0.05$). No differences between experimental groups were observed in $C^*$ ($P > 0.10$). As expected, time-related changes were observed in all colour parameters ($P < 0.01$), but no differences were attributed to time-by-treatment interaction ($P > 0.10$).

### Microbiological analysis

The effects of SS supplementation on microbial counts (TVB, *Pseudomonas* spp., EC and LAB) and ERV of meat samples after 0, 7 and 14 days under retail display in MAP conditions are summarised in Table 4. Regarding the effect of dietary treatments, no differences were found in EC and LAB counts ($P > 0.10$), whereas TVB and *Pseudomonas* counts in 30SS tended to be lower than in the other groups ($P < 0.10$). The ERV values became significantly reduced during the refrigerated storage period ($P < 0.001$) in all groups.

Table 5 shows the effect of SS on PCR amplification of *E. coli* virulence genes in the lamb meat samples. The initial values (day 0) of positive samples were very similar in all the groups ($P > 0.01$). However, after 14 days of refrigerated MAP storage, the ratio of positive samples tended to be higher in meat samples from lambs being fed the highest doses of SS (30SS and 60SS groups) when compared with those containing a low level of SS (15SS group) or control samples (00SS group) ($P < 0.10$).

### Discussion

A dietary supply of unsaturated fat may modify the fatty acid profile of meat, increasing its susceptibility to lipid oxidation (Campo *et al.*, 2006). However, previously, even though SS dietary supplementation had no effect on the intramuscular polyunsaturated fatty acid percentages, the inclusion of SS resulted in an average reduction of 6% of the saturated fatty acids

**Table 5** PCR amplifications of virulence genes from human diarrhoeagenic *E. coli* in meat samples stored at 4 °C during 0 and 14 days under modified atmosphere package (35% $CO_2$, 35% $O_2$ and 30% $N_2$) from lambs being fed different doses of sunflower soap stocks

| | | | Diets[†] | | | | P-value |
|---|---|---|---|---|---|---|---|
| | | | 00SS | 15SS | 30SS | 60SS | |
| Samples[‡] | Day 0 | + | 0 | 0 | 0 | 1 | 0.398 |
| | | − | 5 | 6 | 6 | 5 | |
| | Day 14 | + | 0 | 0 | 3 | 3 | 0.059 |
| | | − | 5 | 6 | 3 | 3 | |
| Enrichments[§] | Day 0 | + | 1 | 0 | 2 | 1 | 0.503 |
| | | − | 4 | 6 | 4 | 5 | |
| | Day 14 | + | 0 | 1 | 4 | 3 | 0.077 |
| | | − | 5 | 5 | 2 | 3 | |

[†]00SS = TMR pellet without sunflower soap stock (SS); 15SS = TMR pellet containing 15 g SS per kg; 30SS = TMR pellet containing 30 g SS per kg; 60SS = TMR pellet containing 60 g SS per kg.

[‡]Number of samples for which one (+) or none (−) *E. coli* strain harbouring $stx_1$ and/or $stx_2$ and/or *eae* genes was isolated by using the spread plate technique in each storage day.

[§]Number of lamb sample enrichments for which presence (+) or lack (−) of $stx_1$ and/or $stx_2$ and/or *eae* genes was detected in each storage day.

and the consequent increment (6%) of the monounsaturated fatty acids averaged percentage (Blanco *et al.*, 2017).

These changes in the intramuscular fat composition might have increased the susceptibly of SS samples to meat oxidation. Nevertheless, SS contains 0.01% BHT, a technological synthetic additive used to improve its antioxidant properties. Consequently, the presence of this compound in SS could result in a greater antioxidant concentration in meat, which together with the lower (albeit not statistically significant) levels of PUFA could explain the lower TBARS values in fresh meat samples from SS lambs, despite the lower saturation indices observed in the meat of these animals (Blanco *et al.*, 2017). In fact, the average TBARS values for SS-supplemented groups (but not for control ones) were below 2 µg malondialdehyde per g meat, which is the threshold for consumer perception of rancidity suggested by Campo *et al.* (2006).

Regarding oxysterol content in meat samples, these compounds can be absorbed through the human intestinal tract into the bloodstream, increasing the susceptibility of the consumer to coronary heart disease (Valenzuela *et al.*, 2003). Given that the main source of oxysterols in meat is heat processing, these substances were determined in cooked meat samples (cooked GM after 7 days of refrigerated storage of raw muscles, previously used to measure the water holding capacity by cooking loss). As expected, the highest concentrations in cooked meat samples

corresponded to 7β-HC, 7α-HC and 7-KC, which are the primary products of cholesterol oxidation (Guardiola *et al.*, 1995). The last product of the cholesterol oxidation, CT, presented comparatively lower amounts, thereby suggesting that cholesterol oxidation was in the initial stages. The values obtained were comparable to those reported by Bodas *et al.* (2012) and Morán *et al.* (2012a,b), in lambs with similar characteristics. In any case, it is clear that BHT supplied by the SS was not able to reduce COP formation during cooking in the present study, which suggests that the inclusion of other natural antioxidant (e.g. carnosic acid) in the diet of fattening lambs being fed SS would be required to achieve this goal (Morán *et al.*, 2012a,b).

Moreover, among the samples in the present study, numerically higher values in the 60SS group were detected for the COPs. In this sense, the oxysterol contents did not seem to be related to TBARS values, which is corroborated by earlier studies (Conchillo *et al.*, 2005). This apparent disparity of results between TBARS and oxysterol values might be caused by protein oxidation (Santé-Lhoutellier *et al.*, 2008). In this sense, aromatic hydrocarbons (benzene, toluene and quinones) detected in both the TMRs and meat from lambs being fed SS (Blanco *et al.*, 2017) and probably accumulated at higher levels in the meat of 60SS lambs might have induced firstly the generation of reactive oxygen species produced by protein oxidation (which are involved in cholesterol oxidation), and finally enhanced lipid peroxidation. Moreover, GM was cooked without previous mechanical rupture, so the intramuscular location of triacylglycerols might have provided temporary protection against oxidation.

Initial meat colour parameters were within the range of values reported for lambs of similar characteristics (Andrés *et al.*, 2013). Colour changes in meat samples stored under MAP conditions revealed lower *h** values in 15SS and 30SS groups than the controls. Hence, there was a delay in discoloration in those groups, which may also be indicative of lower and higher levels of metmyoglobin and oxymyoglobin, respectively (Young & West, 2001), when compared to 00SS and 60SS lambs. A higher oxidation of myoglobin in the 00SS group was in concordance with greater TBARS values. Nevertheless, according to TBARS values, no adverse effect of the highest dose of SS was observed in the 60SS meat samples. This result suggests that meat discoloration could be due to other factors in this group of samples, such as the oxidation of the non-lipid fraction, probably due to the accumulation of other unknown pro-oxidant substances contained in the SS (e.g. the aromatic hydrocarbons; Blanco *et al.*, 2017). In any case, the dose-dependent effect of lipid supplementation on colour stability observed in the current work agrees with the results reported by Mir

*et al.* (2003). In that study, a higher discoloration of meat was noticed when sunflower oil was included in the diet of fattening steers at 3 and 6%, with the latter undergoing the greatest impact, probably due to the decompensation achieved in the antioxidant status when high amounts of PUFA are supplied in the diet.

As expected, the microbial contamination of the meat by spoilage bacteria increased with the time of storage, whereas ERV values decreased in parallel, an effect that was independent of the diet administered to the lambs. Differences in meat fatty acid profile (Blanco *et al.*, 2017) or the eventual impact of SS supplementation on antioxidant compounds in meat did not trigger any inhibitory effect on the spoilage bacteria. These results concurred those reported on meat from lambs reared under similar conditions (Morán *et al.*, 2012b).

Our study provides an understanding of the effect of SS dietary supplementation on some human pathogenic *E. coli* (STEC and EPEC) on meat. This research is important because a lack of relationship between total *E. coli* numbers and pathogenic *E. coli* has been reported (Berg *et al.*, 2004). Hence, other factors, such as the diet received by the animal or some types of feedstuffs, could promote the growth of faecal *E. coli* populations and select for STEC in cattle or sheep (Díez-González *et al.*, 1998; Díez-González & Russell, 1999). Accordingly, acidotic conditions in ruminants could increase both the presence of virulence genes in *E. coli* (Khafipour *et al.*, 2011) and the population of these bacteria (Plaizier *et al.*, 2014). In this sense, the lambs fed SS showed lower ruminal pH values and greater parakeratosis of the ruminal mucosa (Blanco *et al.*, 2014a,b) than the 00SS lambs. Thus, probably the acidotic conditions promoted by the SS included in the diet could be responsible for the significantly greater growth of this pathogen in the meat of lambs being fed SS. Notably, we recovered *E. coli* isolates belonging to those *E. coli* pathotypes from 6 out of 23 meat samples stored for 14 days at refrigeration, and these positive samples were only obtained from lambs fed diets supplemented with both 30 and 60 g kg$^{-1}$ of SS. Therefore, our study reveals that lamb meat from animals fed 15 g kg$^{-1}$ SS supplements appears not to raise diarrhoeagenic *E. coli* (STEC and EPEC) risk compared to control samples.

## Conclusions

The effects of the inclusion of SS in the TMR for fattening lambs are dependent on the dose. All the doses have a positive impact on delaying lipid oxidation, whereas colour degradation seems to be reduced only at rates of 15–30 g SS per kg TMR. However, meat from 30SS to 60SS tended to show enhanced proliferation of diarrhoeagenic *E. coli* after 14 days of refrigerated storage under MAP conditions. Therefore, according to the results observed in this study, when adding SS to the diet of fattening lambs, inclusion rates should not be above 15 g SS per kg TMR.

## Conflict of interest

The authors have no conflicts of interest to declare.

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## Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Appendix S1**. Material and methods.

**Table S1**. Ingredients and chemical composition of the experimental diets[1].

**Table S2**. Target virulence genes and primer sequences for PCR detection of enteropathogenic bacterial groups in meat by PCR.