Food Research International 108 (2018) 42–47

Contents lists available at ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

Effect of hop (*Humulus lupulus* L.) inclusion in the diet for fattening lambs on animal performance, ruminal characteristics and meat quality

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ARTICLE INFO

Keywords:
Antioxidant
Colour
Growth rate
Muscle fibre
Peroxidation
Polyphenol
Rumen fermentation
Texture

ABSTRACT

Thirty male merino lambs were fed with a pelleted total mixed ration (TMR) alone or supplemented with hop (*Humulus lupulus* L.) cones at two different doses (1.5 and 3.0 g hop cones/kg pelleted TMR, respectively), to study the effects of this dietary source of antioxidants on animal performance, ruminal parameters and meat quality attributes. The results showed that dietary supplementation with hop cones decreased lambs' growth rate ($P < 0.05$) due to a shift in ruminal fermentation, towards a more acetic and less propionic acid production ($P < 0.05$). These changes in animal growth rate might have promoted microstructural modifications in the quantity and size of muscle fibres, thereby inducing the differences observed in meat chemical composition, colour and texture ($P < 0.05$), regardless of the lack of differences in meat antioxidant status ($P > 0.10$).

1. Introduction

Meat quality is deteriorated by two main causes, lipid oxidation and microbiological spoilage, leading to meat discolouration and off-flavours (Ahn, Grün, & Mustapha, 2007; Monahan, 2000). Several "non-meat ingredients" have been used for centuries to preserve the flavour and improve the taste of meats, whereas others have been identified as preservatives to prevent or inhibit food spoilage by displaying antioxidant or antimicrobial activities (Mitropoulou et al., 2015). Antioxidants are food additives that inhibit lipid oxidation. Natural antioxidants have received growing attention due to increasing consumer concerns about the possible biological activity and harmful effects of a variety of commonly used synthetic additives on human health (Jeong, Seol, Seong, Park, & Kim, 2015).

Hop (*Humulus lupulus* L.) is a plant rich in secondary compounds, including hop resins, phenolic compounds and volatile oils. The hop cones are widely utilized, primarily in brewery applications, as flavourings and preservatives. Different hop secondary compounds are responsible for bitterness, aroma and preservative attributes (Farnsworth, 2003; Van Cleemput et al., 2009). Hop resins contain between 5 and 20% bitter acids (α-acids and β-acids) (Chadwick, Pauli, & Farnsworth, 2006). These acids have antimicrobial properties, inhibiting the growth of most Gram-positive bacteria, albeit their activity against Gram-negative bacteria is limited (Wang, Chaves, Rigby, He, & McAllister, 2010). Within the phenolic compounds, which are present in comparatively smaller amounts, flavonoids (xanthohumol, quercetin and kaempferol) are the most important ones and responsible for the antioxidant properties of hops (Lermusieau, Liégeois, & Collin, 2001).

Although hop has been found to display antioxidant capacity when added as a powder infusion directly to the meat (Villalobos-Delgado et al., 2015), it might alternatively be included in the animals' diet (Axman, 2015; Narvaez et al., 2013; Schmidt & Nelson, 2006). This strategy is especially interesting because if antioxidants are deposited in the animal tissues during the life of the animal, the addition of exogenous products after slaughter would not be required, which is perceived by the consumer as a high-quality standard (Andrés, Huerga, et al., 2014; Andrés, Morán, et al., 2014; Brewer, 2011; Morán, Andrés, et al., 2012; Morán, Rodríguez-Calleja, et al., 2012).

To our knowledge, no *in vivo* studies have yet been carried out to examine hops as a feed additive for fattening lambs. Moreover, studies on the effect of dietary hop supplementation of ruminants on meat quality are scarce and inconclusive (Flythe, Kagan, Wang, & Narvaez, 2017). Therefore, the aim of the present study was to investigate the

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https://doi.org/10.1016/j.foodres.2018.03.030
Received 2 February 2018; Received in revised form 6 March 2018; Accepted 10 March 2018
Available online 12 March 2018
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effects of the addition of two different doses of hop cones (1.5 and 3.0 g per kg of total mixed ration (TMR)) to the diet of fattening lambs on animal performance parameters, ruminal fermentation profile and meat quality characteristics (chemical composition, texture, lipid peroxidation and shelf-life extension regarding colour stabilization).

2. Material and methods

2.1. Animals and diets

Thirty male merino lambs (6–8 weeks old and mean body weight (BW) 14.6 ± 1.2 kg at the beginning of the experiment) were used in this study. Lambs remained with their dams, with free access to commercial starter concentrate, barley straw and alfalfa hay. The animals were treated with Ivermectin (Ivomec, Merial Labs, Barcelona, Spain) and vaccinated against enterotoxaemia (Miloxan, Merial Labs, Barcelona, Spain) prior to the trial. After weaning, lambs were allocated by stratified randomization on the basis of BW to one of three experimental treatments (n = 10): control (pelleted TMR without hop cones), HOP15 (1.5 g hop cones/kg pelleted TMR) and HOP30 (3 g hop cones/kg pelleted TMR). Lambs were housed in individual concrete-floored pens (1.45, 1.40 and 1.30 m width, length and height, respectively, with individual feeding and watering troughs) with sawdust bedding during the entire experimental period.

Control pelleted TMR was comprised of (g/kg dry matter [DM]) barley grain (433), corn grain (150), soybean meal of 44% crude protein (CP, 237), barley straw (150) and mineral-vitamin premix (30). The chemical composition of these pellets was as follows: 912 g DM/kg fresh matter, 216 g neutral detergent fibre/kg DM, 110 g acid detergent fibre/kg DM, 197 g CP/kg DM and 71 g ash/kg DM. The remaining diets were prepared by adding the corresponding proportion of hop cones and homogenising before pelleting. Hop cones were provided by "S.A. Española de Fomento del Lúpulo" (Villanueva de Carrizo, Spain). The variety used was Nugget (117 g α-acids, 37 g β-acids and 17 g gallic acid equivalents [total phenolics] per kg DM).

After 5 days of adaptation to the diet, the corresponding experimental diet (control, HOP15 or HOP30) was individually offered to each lamb. The pelleted concentrate was provided *ad libitum* (*i.e.*, allowing refusals of 200 g/kg day feed offered). Animals were presented a fresh allowance at 10:00 h daily and did not have access to the previous days' allowance, while fresh drinking water was always available. 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 were approved by the IGM-CSIC Animal Experimentation Committee (protocol number 2012-E82); all the animals used were able to see and hear other lambs.

2.2. Slaughter procedure, packaging, storage and sampling

The BW was recorded twice a week, before feeding, until the lambs attained the intended slaughter BW (27 kg BW). When the target BW was reached, feed and water were withdrawn, and after 1 h, the lamb was re-weighed. The animal was immediately stunned and slaughtered by exsanguination from the jugular vein, eviscerated and skinned. Liver samples (5 g) were collected directly after slaughter, frozen in liquid nitrogen and kept at −80 °C for thiobarbituric acid reactive substances (TBARS) analysis.

The carcass obtained following the procedure described by Manso, Mantecón, Giráldez, Lavín, and Castro (1998) was weighed, chilled at 4 °C for 24 h and weighed again. Chilling losses were calculated as the difference between hot (HCW) and cold carcass weights (CCW) and expressed as a proportion of the initial HCW. Dressing percentage was calculated as CCW and expressed as a proportion of BW recorded just before slaughter. The pH of the *longissimus thoracis* (LT) was measured at the 6th rib at 0 h, 45 min and 24 h post-mortem (Metrohm 704 pH meter, Metrohm, Zofinger, Switzerland). The left side of each carcass was jointed into commercial cuts, which were weighed and grouped according to Colomer-Rocher, Morand-Fehr, Kirton, Delfa-Belenguer, and Sierra-Alfranca (1988): first quality (leg, loin-ribs, and best-end), second quality (shoulders) and third quality (breast-flank, scrag-end and tail).

Afterwards, the *longissimus lumborum* (LL), LT and *gluteus medius* (GM) muscles were removed from the right and left carcass sides. The LT samples were used for chemical analysis (AOAC, 2003). The LL and GM samples were cut into 2.5 cm thick slices, placed in impermeable polypropylene trays, over-wrapped with an oxygen-permeable polyvinylchloride film and stored under simulated retail display conditions (12 h daily fluorescent illumination [34 W] and 3 ± 1 °C). The LL samples were used for colour evolution (days 0, 1, 3, 6, 8 and 10 of storage), cooking losses and texture (days 0, 6 and 10 of storage). The LL and GM samples were used for TBARS analysis (days 0, 6 and 10 of storage). For the sampling day, samples for TBARS, texture and chemical composition were vacuum packed, frozen and stored (−20 °C) for subsequent analysis.

2.3. Ruminal fermentation parameters

Immediately after evisceration, ruminal fluid samples from each lamb were collected and strained through two layers of cheesecloth. Ruminal liquor pH was measured (Metrohm 704 pH meter). A 2 mL aliquot was acidified with 2 mL of 0.5 N hydrochloric acid (HCl) and another 0.8 mL aliquot was added to 0.5 mL of a deproteinising solution (5 g metaphosphoric acid and 1 g crotonic acid in 250 mL of 0.5 N HCl; García-Martínez, Ranilla, Tejido, & Carro, 2005). Both samples were centrifuged at 14,500 × *g*/4 °C for 15 min, and the resultant supernatants were used for ammonia (Weatherburn, 1967) and volatile fatty acid (VFA; Ottenstein & Bartley, 1971) analyses, respectively.

2.4. Lipid peroxidation of meat

The TBARS analyses were performed on pre-thawed, raw LL and GM samples at 0, 6 and 10 days of storage, whereas, liver TBARS was measured only on day 0. The TBARS analysis was conducted according to the procedure described in Morán, Andrés, et al. (2012).

2.5. Meat colour changes

During refrigerated storage, a same LL slice per animal was unpackaged and measured for colour parameters on days 0, 1, 3, 6, 8 and 10. After measuring, the sample was newly over-wrapped with the oxygen-permeable polyvinylchloride film. The colour parameters, L^* (lightness), a^* (redness) and b^* (yellowness) (Commission Internationale de l'Eclairage, 1986), were measured using a chromameter (Minolta® Chroma Meter 2002, Germany). The hue angle (h^*, which defines colour; 0° is red and 90° is yellow) and the chroma (C^*, a measure of colour intensity; 0 is dull and 60 is vivid) were also calculated (Young & West, 2001).

2.6. Texture profile analysis (TPA) and cooking losses of meat

The slices of LL after 0, 6 and 10 days of refrigerated storage under display conditions were weighed and cooked in a water bath (preheated at 75 °C) until a core temperature of 70 °C was reached (Digi-Sense®, Thermocouple Thermometer, Cole-Parmer Instrument Company, Chicago, IL), following the guidelines for cooking procedures of the AMSA (1995). After cooling at 4 °C for 30 min, the samples were weighed again and cook loss was expressed as a percentage of the initial sample weight (Honikel, 1998). Subsequently, TPA was performed according to the procedure described by Herrero et al. (2008) using 1 cm cubic meat specimens and a compression of 80% of the initial weight, at an axis perpendicular to the muscle fibre direction with a QTS texture analyser (CNS Farnell, UK).

2.7. Statistical analysis

Average daily gain (ADG) was estimated as the regression coefficient (slope) of BW against time, using the REG procedure of the SAS package (SAS, 1999). Dry matter intake (DMI), animal performance parameters, rumen fermentation, carcass characteristics and meat chemical composition data were subjected to one-way analysis of variance (ANOVA), with the inclusion of hop in the diet as the fixed effect, according to the model $y_{ij} = \mu + H_i + e_{ij}$; where y_{ij} is the observation j in group i, μ is the overall mean, H_i is the fixed effect of hop inclusion and e_{ij} is the random error.

The TBARS, cooking losses and TPA data were subjected to a two-way ANOVA, with hop inclusion and day as the main factors and the LL or GM slices considered as experimental units (model: $y_{ijk} = \mu + H_i + D_j + (H*D)_{ij} + e_{ijk}$; where y_{ijk} is the observation k in group i and day j, μ is the overall mean, H_i is the effect of hop inclusion, D_j is the effect of day, $(H*D)_{ij}$ is the effect of the interaction between hop inclusion and day, and e_{ijk} is the random error).

Meat colour data were analysed by repeated measures analysis, including in the statistical model the fixed effects of hop supplementation, day and their interaction. In this analysis, different covariance matrices were evaluated based on Schwarz's Bayesian information model fit criteria (model: $y_{ijk} = \mu + H_i + d_{ij} + T_j + (H*T)_{ik} + e_{ijk}$; where y_{ijk} is the observation k in group i and day j, μ is the overall mean, H_i is the effect of hop inclusion, T_j is the effect of time, $(H*T)_{ik}$ is the effect of the interaction between hop inclusion and time, d_{ij} is the random error between slices within treatment, and e_{ijk} is the random error between measurements within slices). In all the cases, the residual standard deviation (RSD) was estimated as the root of the corresponding residual (error) mean square.

All the analyses were performed using the MIXED procedure of SAS and means were separated using the LSMEANS/PDIFF option.

3. Results

The mean values of DMI, ADG, length of the fattening period, feed-to-gain ratio and ruminal characteristics are displayed in Table 1. Although feed intake and the feed-to-gain ratio were not affected by experimental treatments ($P > 0.10$), HOP30 lambs showed the lowest ADG values and longest fattening period ($P < 0.05$). Likewise, ruminal acetate concentration and the acetate-to-propionate ratio ($P < 0.05$) were higher for HOP30 lambs compared to the other groups. The opposite behaviour was observed for the ruminal propionate concentration ($P < 0.05$), whereas ruminal ammonia concentrations did not significantly differ among groups. No differences were noticed in carcass characteristics (Table 2) among the experimental treatments ($P > 0.10$).

Table 1
Mean values of DM intake, average daily gain, feed to gain ratio and ruminal characteristics of lambs fed on a total mixed ration without hop cones (control) or with 1.5 and 3.0 g of hop cones per kg (HOP15 and HOP30, respectively).

	Control	HOP15	HOP30	RSD[1]	*P*-value
Dry matter intake	978	914	918	103.1	0.315
Average daily gain (g/day)	351[a]	309[a,b]	273[b]	58.3	0.035
Feed: gain (g/g)	2.83	3.09	3.40	0.652	0.192
Fattening period (days)	35.8[a]	38.9[a]	46.5[b]	7.29	0.011
Ruminal characteristics					
pH	5.41	5.57	5.65	0.532	0.586
Total VFA (mmol/L)	143	141	139	43.5	0.848
Acetic (mmol/mmol VFA)	44.2[a]	44.0[a]	47.8[b]	2.34	0.003
Propionic (mmol/mmol VFA)	43.8[a]	42.7[a]	37.7[b]	3.12	0.001
Acetic/propionic (mmol/mmol)	1.02[b]	1.05[a,b]	1.29[a]	0.155	0.001
Ammonia (mg/L)	222	237	291	105.3	0.388

Different superscript letters ([a,b]) within the same row indicates significant ($P < 0.05$) differences between dietary treatments.
[1] Residual standard deviation.

Table 2
Mean values of carcass characteristics of lambs fed on a total mixed ration without hop cones (control) or with 1.5 and 3.0 g of hop cones per kg (HOP15 and HOP30, respectively).

	Control	HOP15	HOP30	RSD[1]	*P*-value
Cold carcass weight (kg)	12.3	12.1	12.1	0.62	0.460
Dressing percentage (%)	45.5	44.9	44.4	2.08	0.480
Chilling losses (%)	3.19	4.52	3.90	1.859	0.328
Longissimus thoracis muscle pH					
At slaughter	6.49	6.49	6.45	0.201	0.932
45 min after slaughter	6.15	6.09	6.15	0.220	0.963
24 h after slaughter	5.58	5.64	5.57	0.119	0.409
Carcass commercial cuts (%)[2]					
1st category	56.2	56.3	54.2	4.06	0.483
2nd category	22.0	21.7	22.6	6.21	0.740
3rd category	21.8	22.1	23.1	2.20	0.386

[1] Residual standard deviation.
[2] First category-higher priced joints (legs, ribs and fore ribs); Second category-medium priced joints (shoulders); Third category-lower priced joints (breasts, necks and tails).

Table 3
Mean values of chemical composition of *Longissimus thoracis* muscle (g kg^{-1} meat) of lambs fed on a total mixed ration without hop cones (control) or with 1.5 and 3.0 g of hop cones per kg (HOP15 and HOP30, respectively).

	Control	HOP15	HOP30	RSD[1]	*P*-value
Dry matter	220	228	217	16.5	0.378
Ash	14.3[a]	11.8[b]	11.9[b]	2.31	0.046
Crude protein	177	185	176	12.3	0.235
Ether extract	23.8	25.8	21.8	8.94	0.643

Different superscript letters ([a,b]) within the same row indicate significant ($P < 0.05$) differences between dietary treatments.
[1] Residual standard deviation.

The mean values of LT chemical composition are summarised in Table 3. No statistical differences between the experimental groups were observed for DM, CP and ether extract contents ($P > 0.10$). However, the ash content was lower ($P < 0.05$) for HOP15 and HOP30 than control lambs.

The mean values of LL, GM and liver TBARS are shown in Table 4. There was an increase in malondialdehyde (MDA) concentration over time (0, 6 and 10 days of refrigerated storage) in GM ($P < 0.05$). The same tendency was observed in LL ($P < 0.10$). No differences were attributed to diet or time by diet interaction ($P > 0.10$). Liver TBARS values were unaffected ($P > 0.10$) by hop supplementation.

Table 5 provides the results of the colorimetric parameters measured on the LL muscle after 0, 1, 3, 6, 8 and 10 days of refrigerated storage. As expected, there was an evolution in colour parameters over time ($P < 0.001$), but no differences were attributed to time by treatment interaction ($P > 0.10$). No differences were found between dietary groups in a^* and C^* parameters ($P > 0.10$). However, L^*, b^* and h^* values were deemed higher in control animals relative to those supplemented with hop cones ($P < 0.05$).

Table 6 summarises the cooking loss and TPA values measured on LL samples during refrigerated storage. A decrease in cooking losses ($P < 0.001$) was seen over time, but no differences between diets were identified ($P > 0.10$). Regarding TPA parameters, the lowest values for hardness and chewiness corresponded to the HOP30 LL samples ($P < 0.05$), with control samples showing the lowest cohesiveness values ($P < 0.05$).

Table 4
Mean values of thiobarbituric acid reactive substances (TBARS) (μg malondialdehyde g^{-1} of meat) in meat samples from *gluteus medius* and *longissimus lumborum* muscles after 0, 6 and 10 days of refrigerated storage at 4 °C and liver samples at slaughter from lambs fed on a total mixed ration without hop cones (control) or with 1.5 and 3.0 g of hop cones per kg (HOP15 and HOP30, respectively).

	Hop supplementation			Storage days			*P*-value[2]			
	Control	HOP15	HOP30	0	6	10	RSD[1]	H	d	H*d
Gluteus medius	1.99	1.81	2.39	1.66^{b}	1.87^{b}	2.67^{a}	1.400	0.311	0.025	0.988
Longissimus lumborum	1.40	1.31	1.47	1.16	1.31	1.71	0.854	0.794	0.092	0.331
Liver	3.34	2.89	3.50	–	–	–	1.901	0.815	–	–

Different superscript letters (^{a,b}) within the same row and main effect indicate significant differences ($P < 0.05$).
[1] Residual standard deviation.
[2] *P*-value for hop supplementation (H), storage day (d) or the interaction between dietary treatment and storage day (H*d).

4. Discussion

4.1. Rumen fermentation

Despite few *in vivo* studies, the effects of hop inclusion on ruminal parameters have been previously studied *in vitro*, with highly contradictory results. For instance, Schmidt and Nelson (2006) reported a decrease in the acetate-to-propionate ratio and total VFA production *in vitro* in response to hop inclusion, using steers rumen liquor. Moreover, Narvaez, Wang, Xu, and McAllister (2011) observed a decrease of acetate and an increase of propionate proportions in ruminal liquid from finishing cattle when 1.6 μg/mL of whole hop was added to the substrate (forage). Also, the magnitude of these differences was greater in high than in low forage diets, so the effects of hop inclusion in the diet seemed to be dependent on the diet (substrate). Accordingly, and in concurrence with the results observed in the present study, Wang et al. (2010) recorded an increase in the acetate-to-propionate ratio when including whole hop at 476 and 952 mg/kg incubated DM in a concentrate (grain-based) diet. However, contrasting results were reported by these authors when a forage-based diet (silage) was used instead. The reason for the observed changes in the ruminal parameters remains unclear, but it may be attributed to different tannins and β-acid contents in the diet. It has been stated that tannins may influence microbial fermentation, increasing the proportion of tannin-resistant Gram-negative bacteria and decreasing the total VFA production (Bodas et al., 2012; Smith & Mackie, 2004; Wang et al., 2010), whereas β-acids inhibit Gram-positive bacteria, leading to an increase in propionate and succinate production (Axman, 2015; Bergen & Bates, 1984; Kennelly, Doepel, & Lien, 1998).

Also, Flythe (2009) observed that hop inclusion inhibited ruminal ammonia production, probably due to the antimicrobial properties of different hop compounds, which would promote growth inhibition of hyper-ammonia-producing bacteria. Conversely, our results show no changes in rumen ammonia due to hop inclusion, which agrees with Narvaez et al. (2011), thus indicating a subtler effect of this ingredient at a ruminal level. These differences between studies on *in vitro* parameters could also be explained by the use of different doses and varieties of hop cones or extracts (Lermusieau & Collin, 2001). These factors might have modified the quantity and the type of different active compounds supplied by the diet. The proportions of hop compounds, their isomers and their potential effects depend not only on the amount of hop supplied, but also on the hop variety included in the diet (Lavrenčič, Levart, Košir, & Čerenak, 2014). This information explains why Narvaez et al. (2013) reported that rumen bacterial populations responded differently to hops varieties, demonstrating that the total amount of α-acids and β-acids in hops affects ruminal fermentation in different ways.

4.2. Animal performance

As stated above, most of the studies with whole hops or hop extract (β-acids) have been performed *in vitro*. Nonetheless, *in vitro* trials do not always reproduce what happens *in vivo* (Jayanegara, Leiber, & Kreuzer, 2012). Hence, Wang et al. (2010), feeding steers with whole hop, and Axman (2015), feeding heifers with different proportions of hop β-acids extracts, observed little impact on feedlot performance, regardless of the potential effects at ruminal level. However, in our experiment, ruminal fermentation towards a less efficient VFA profile (*i.e.*, increased acetate and decreased propionate proportion), together with a subtle but not statistically significant reduction in DMI, could partially contribute to reduce ADG values of hop-supplemented lambs (Bodas, Giráldez, López, Rodríguez, & Mantecón, 2007). The decreased weight gain is usually associated to an extended time for animals to reach the target slaughter weight (length of fattening period) (Blanco et al., 2014).

In spite of the differences observed in weight gain, no differences in carcass characteristics occurred between experimental diets. Moreover, the values obtained corroborated those reported in previous studies carried out with animals and under conditions similar to those in the present experiment (Andrés, Huerga, et al., 2014; Andrés, Morán, et al.,

Table 5
Mean values of colour parameters after 0, 1, 3, 6, 8 and 10 days of light-exposed refrigerated storage (4 °C) of meat (*longissimus lumborum*) samples from lambs fed on a total mixed ration without hop cones (control) or with 1.5 and 3.0 g of hop cones per kg (HOP15 and HOP30, respectively).

	Hop supplementation			Storage days						*P*-value[3]				
	Control	HOP15	HOP30	0	1	3	6	8	10	RSD[1]	RSD[2]	H	d	H*d
L*	44.9^{a}	43.7^{b}	43.2^{c}	$42.9^{a,b}$	45.2^{a}	44.7^{a}	43.8^{b}	43.0^{c}	44.0^{b}	2.21	1.16	0.028	< 0.001	0.857
a*	9.40	9.67	9.59	$9.52^{b,c}$	10.4^{a}	10.7^{a}	9.71^{b}	9.28^{c}	7.67^{d}	1.901	0.818	0.717	< 0.001	0.322
b*	5.96^{a}	5.35^{b}	4.84^{b}	4.15^{c}	6.57^{a}	6.39^{a}	6.18^{a}	4.97^{b}	4.04^{c}	1.574	0.854	0.003	< 0.001	0.714
h*	32.3^{a}	28.6^{b}	26.5^{b}	23.7^{c}	32.3^{a}	30.9^{a}	32.5^{a}	27.9^{b}	27.5^{b}	8.736	4.627	0.005	< 0.001	0.868
C*	19.8	19.7	19.1	18.5^{c}	21.8^{a}	22.2^{a}	20.5^{b}	18.8^{c}	15.5^{d}	3.240	1.501	0.484	< 0.001	0.151

Different superscript letters (^{a,b}) within the same row and main effect indicate significant differences ($P < 0.05$).
[1] Residual standard deviation to compare hop supplement groups.
[2] Residual standard deviation to compare storage days.
[3] *P*-value for hop supplementation (H), storage day (d) or the interaction between dietary treatment and storage day (H*d).

Table 6
Cooking losses (% of lost water) and texture profile analysis (hardness, cohesiveness, springiness, chewiness) of lamb meat samples (*longissimus lumborum*) after 0, 6 or 10 days of light-exposed refrigerated storage (raw) and subsequent cooking; lambs were fed on a total mixed ration without hop cones (Control) or with 1.5 and 3.0 g of hop cones per kg (HOP15 and HOP30, respectively).

	Hop supplementation			Storage day			*P*-value[2]			
	Control	HOP15	HOP30	0	6	10	RSD[1]	H	d	H*d
Cooking losses	19.3	18.6	20.3	21.7^{a}	20.4^{a}	16.1^{b}	3.724	0.233	< 0.001	0.816
Hardness	127^{a}	128^{a}	106^{b}	133^{a}	122^{b}	107^{c}	26.84	0.002	0.005	0.288
Springiness	0.420	0.453	0.420	0.429	0.441	0.421	0.078	0.584	0.159	0.761
Cohesiveness	0.439^{b}	0.453^{a}	0.443^{a,b}	0.466^{a}	0.444^{b}	0.439^{b}	0.021	0.024	0.022	0.007
Chewiness	23.6^{a,b}	26.2^{a}	20.4^{b}	26.2^{a}	24.3^{a}	19.8^{b}	7.28	0.009	0.023	0.522

Different superscript letters (^{a,b}) within the same row and main effect indicate significant differences ($P < 0.05$).
[1] Residual standard deviation.
[2] *P*-value for hop supplementation (H), storage day (d) or the interaction between dietary treatment and storage day (H*d).

2014; Blanco et al., 2014; Morán, Andrés, et al., 2012). Our results also agree with the findings of Wang et al. (2010), in which different proportions of hop cones (0, 238, 476 and 952 mg/kg) in the diet of steers had no effects on carcass characteristics. Some specific compounds present in hop (β-acids) can affect animal performance and carcass characteristics (Axman, 2015). In the present study, however, lambs were slaughtered at the same weight, which might have diluted differences in carcass characteristics promoted by differing growth rates (Huidobro & Cañeque, 1994).

4.3. Meat texture and colour

On the contrary, the effect of hop inclusion on lambs growth rate, due probably to changes in ruminal fermentation parameters, and thus, in animal growth rate, might have caused microstructural modifications in the muscles. Such alterations can modify the quantity and size of muscle fibres and, consequently, some meat characteristics, such as texture and colour (Lee, Joo, & Ryu, 2010; Rehfeldt & Kuhn, 2006). For example, Mlynek and Gulinski (2007) observed a lower number of white fibres (glycolytic properties) and a higher number of red fibres (higher oxidative activity) in the muscle of steers with a lower growth rate. These changes may have triggered a decrease in L^* and b^* values, and thereby h^* modifications similar to those observed in the hop-supplemented animals, which presented the lowest growth rate.

The decrease in hardness observed in the meat of HOP30 lambs could also be related to the growth rate and its implications on the nature of muscular fibres. Solomon and Lynch (1988) observed that higher growth rates in lambs might promote morphological changes, thereby inducing higher proportions of glycolytic fibres, a lower proteolytic potential in the muscles and, hence, an increase in meat hardness. Consequently, the lower hardness values of the meat in HOP30 lambs might be related to the reduced growth rates observed in this group of lambs, which might have increased the proteolytic potential due to a reduced proportion of glycolytic (white) fibres. In support of this theory, it has been previously described that differences in the mineral content of different fibre types might be related to tenderness. In this context, the sarcoplasmic reticulum is less developed in red than in white fibres (Schiaffino & Reggiani, 2011). Therefore, muscles with higher red fibre contents contain less Ca^{2+} (Whipple et al., 1990), which could contribute to explain the lower meat mineral content observed in hop-supplemented lambs and the changes in the texture parameters when compared with the control group.

4.4. Meat lipid peroxidation

The lack of changes in liver and meat TBARS values between groups suggest that the ingestion of hop did not result in the presence of sufficient quantities of hop-derived antioxidant compounds in meat, to retard lipid oxidation. It also indicates that the mechanisms of the changes observed in colour or texture due to feeding treatment could not be related to lipid oxidation. However, the antioxidant effect of hop on meat lipids was demonstrated in a previous study, where hop infusions were added directly to meat (Villalobos-Delgado et al., 2015). In the present study, hop was fed directly to the lambs, and its effect on meat would have been limited by the low content of polyphenols (in comparison with other hop varieties), which 17 mg of gallic acid equivalent per g of dry weight. Likewise, polyphenols and α- or β-acids could have been degraded in the digestive tract (Kowalczyck, Swieca, Cichocka, & Gawlik-Dziki, 2013; Lermusieau et al., 2001; Manach, Scalbert, Morand, Remezy, & Jiménez, 2004), probably at the ruminal level, where hop demonstrated a distinct effect on fermentation parameters. Therefore, the antioxidant components of hop would have either not been transferred to meat or they were not present in sufficient amounts to delay lipid oxidation. To the best of our knowledge, there are no literature studies conducted on ruminants that focus on the effect of dietary hop supplementation on meat lipid oxidation. However, some studies done on broilers and pigs using different hop varieties and doses have reported both detrimental and beneficial effects (Hanczakowska, Swiatkiewicz, & Grela, 2017; Rezar, Levart, & Salobir, 2015). It suggests that the influences of hop supplementation on oxidative stress and meat lipid oxidation seem to be dependent on hop variety and level of supplementation.

5. Conclusion

The results obtained from this experiment suggest that the inclusion of hop in the diet of fattening lambs reduces growth rates (thus extending the length of fattening period) and modifies ruminal fermentation, increasing acetate and decreasing propionate proportion in a dose-dependent manner. These changes in animal performance could have been responsible for the modifications observed in the studied meat quality parameters (chemical composition, colour and texture evolution), probably due to microstructural differences (quantity and size) in muscle fibres. Nevertheless, hop supplementation does not seem to affect antioxidant meat capacity.

Declaration of interest

The authors have no conflicts of interest to declare.

Authors' contributions

CB and LM performed the *in vivo* trial and the analyses of samples (ruminal fermentation, meat colour and TBARS). JM was responsible of the texture analyses. FJG, RB and SA were responsible of the design of the study, statistical analysis and revised the manuscript. All authors read and approved the final manuscript.

Acknowledgements

Financial support received from CSIC (Proyecto Intramural Especial; Project 201540E084) is gratefully acknowledged. Raúl Bodas was funded by the 'Doc-INIA' subprogramme. Carolina Blanco gratefully acknowledges receipt of a pre-doctoral grant (Junta de Castilla y León and European Social Fund).

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