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Modifying milk and meat fat quality through feed changes

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A B S T R A C T Feeding is the major factor affecting the quality of sheep products (milk and meat). The feeding strategies

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20 Sheep products (milk and meat) are considered to be highquality foods because of their high nutritive value and their 21 organoleptic characteristics. However, although they are tradi-22 tional products strongly established in society, consumption of 23 them has sometimes been questioned, partly because of the nega-24 tive image that the consumer has of the quantity and composition 25 of the fat that they contain. Fat in sheep milk and meat has a high 26 content of saturated fatty acids and low content of polyunsaturated 27 fatty acids and has been related to the incidence of cardiovascular 28 diseases. 29

However, this negative idea of ruminant fat has been changing 30 in recent years because it has been found that some saturated fatty 31 acids are atherogenic only if they are ingested in excessive guan-32 tities and that it contains some unsaturated fatty acids that are 33 potentially beneficial for human health (Parodi, 2009). The rumi-34 nant fatty acids that have bioactive properties include unsaturated 35 fatty acids such as vaccenic acid (VA), conjugated linoleic acid (CLA), 36 particularly its most abundant isomer, known as rumenic acid (RA, 37 cis-9 trans 11CLA), and the omega-3 fatty acids (n-3 PUFAs), so there 38 is great interest in increasing their levels in milk and meat (Lock and Bauman, 2004; Raes et al., 2004). 4003

Numerous biological, antic-carcinogenic, anti-obesity and
immune system enhancing properties, among others, have been
attributed to CLA, particularly to *cis*-9 *trans*-11CLA. The omega-3

fatty acids, which include α -linolenic acid (ALA, C18:3 n3), 22:6 n-3 (DHA) and 20:5 n-3 (EPA), have been related mainly to reductions in the risk of cardiovascular diseases, type-2 diabetes, cancer and neurological alterations (Lock and Bauman, 2004).

useful for increasing the levels of healthy fatty acids (FA), such as conjugated linoleic acid and omega-3

FA, in milk and meat in the human diet are reported. The addition of supplements rich in oils and the

level and quality of forage seem to be valuable tools for influencing the fatty acid composition of milk and

lamb meat. The use of alternative feed resources such as grape pomace, rich in phenolic compounds, in

sheep and lamb diets and their effects on meat FA composition and oxidative stability are also discussed.

Feeding is the major factor affecting the quality of sheep products. Consequently, nutritive strategies have been used most to modify the composition of fat and adapt it to consumer demands. The incorporation of fat in rations and the possibilities offered by microbial biohydrogenation in the rumen have been indicated as effective methods for increasing levels of functional fatty acids in milk and meat. These feeding changes must be made carefully because they sometimes lead to increases in *trans*-10 fatty acids associated with negative effects for human health (Shingfield et al., 2008).

An increase in the degree of unsaturation of fat also makes it more susceptible to oxidation and therefore shortens its shelf life. One of the strategies most commonly employed to prevent lipid oxidation of meat is the use of antioxidants in rations.

Vitamin E has been widely used in animal nutrition in order to preserve meat, but it have been questioned because of their synthetic origin and its limited bioefficiency when n-3 PUFA intake is too high. The increasingly demanding consumer preference for natural products and health benefits has intensified the search for alternative methods to retard lipid oxidation of meat. Supplementation of the diet with phenolic substances has been suggested as a feeding strategy to increase the functional characteristics of meat and milk of small ruminants, and also to improve the oxidative stability and colour of meat during storage (Nieto et al., 2010; Vasta and Luciano, 2011).

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Grape pomace is a winemaking by-product that has a high content of phenolic compounds with a high antioxidant capacity that can act on the quality of the products obtained (Makris et al., 2007; Spanghero et al., 2009). Therefore the use of this by-product is being studied as an interesting low-cost feed alternative to decrease the unhealthy fatty acids and to increase the functional fatty acids of fat and the oxidative stability of meat.

With these points in mind, in this paper we present some of the nutritional strategies based on the use of fat and aimed at increasing the level of fatty acids with bioactive effects in sheep milk and meat. Some of the results of our research group concerning the use of grape pomace in sheep rations as a possible strategy to improve meat and milk fatty acid profile and as an alternative to other antioxidants of synthetic origin that favour the oxidative stability of animal products are also presented.

2. Metabolism of lipids in the rumen

Fat in milk and meat differs considerably from the fat consumed by ruminants because the fatty acids that leave the rumen are different from those that are present in the diet. This is a consequence of the processes of digestion and metabolism of fat that take place in the rumen, in the mammary gland and other tissues.

Metabolism of fat in the rumen includes its hydrolysis and subsequent biohydrogenation. As the major polyunsaturated fatty acids present in food consumed by ruminants are linoleic acid (cis-9 cis-12C18:2) and α -linolenic acid (ALA, cis-9 cis-12 cis-15C18:3) n-3), their biohidrogenation routes are the most studied and their intermediate products the best identified.

As shown in Fig. 1, during the process of biohydrogenation 100 of linoleic acid to stearic acid in the rumen various intermediate 101 fatty acids are generated, such as conjugated linoleic acid (CLA), 102 whose major isomer is rumenic acid (RA, cis-9 trans-11CLA), and 103 vaccenic acid (VA, trans-11C18:1) (Shingfield et al., 2010). Biohy-104 105 drogenation of α -linolenic acid also involves the formation of VA; however, although the conversion of VA to stearic acid is identical 106 to linoleic acid biohydrogenation, what it generates is not RA but 107 different intermediates. RA and VA have been associated with ben-108 eficial effects on the health, and therefore there is great interest in 109 110 increasing their levels in milk.

One of the most commonly employed strategies for increasing the levels of VA and RA in meat and milk has been to increase their 112 levels in the rumen by the use of fats rich in linoleic acid and α -113 linolenic acid, such as vegetable oils and fats. 114

However, rations low in fibre and with an excess of rapidly fer-115 mentable starch, as in the case of the concentrates and rations used 116 in intensive systems, or with a small particle size, reduce ruminal 117 pH and have a negative effect on cellulolytic bacteria, which are 118 mainly responsible for the efficiency of biohydrogenation of linoleic 119 acid and/or produce alternative routes (see Fig. 1) with increases 120 in other trans fatty acids such as trans-10 cis-12C18:2 and trans-121 10C18:1. These fatty acids have been associated with a greater risk of suffering coronary diseases and sometimes with negative effects 123 on the productive yield of dairy animals associated with milk fat 124 depression syndrome, and therefore any feeding strategy should 125 avoid the formation of these trans-10 fatty acids. 126

127 The ruminal metabolism of other omega-3 fatty acids that are less frequent in ruminant diets, such as 20:5 n-3 (EPA) and 22:6 n-3 128 (DHA), is less well known. Some authors (Abughazaleh and Jenkins, 129 2004) have indicated that EPA and DHA are totally biohydrogenated 130 in the rumen, and, although the mechanisms responsible and the 131 intermediate products of their biohydrogenation are not known, 132 this might explain the low transfer of EPA and DHA from dietary 133 134 origin to milk and meat. Moreover, it has been verified that, as with 135 linoleic acid, these long-chain fatty acids contribute to the accumulation of VA in the rumen owing to the inhibition that they produce in the reduction of trans-11C18:1 to C18:0. Therefore the incorporation of very long chain fatty acids in rations has also been proposed as a strategy to increase the levels of RA in milk from VA (Lock and Bauman, 2004).

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However, the composition of the lipids in milk and meat does not depend solely on the fatty acids absorbed in the intestine, and there are descriptions of transformations of these fatty acids in the mammary gland and in tissues as a result of a complex enzymatic system of desaturases and elongases. Mammary cells and muscle tissue cells have a powerful Δ^9 -desaturase activity. In fact, it has been estimated that 64 and 97% of the cis-9 trans-11CLA in milk comes from endogenous synthesis from vaccenic acid in the mammary gland (Bauman et al., 2003). Therefore an increase in Δ^9 desaturase activity in tissues has also been proposed as a strategy to increase not the levels of RA in milk from VA (Lock and Bauman, 2004).

In view of the foregoing, various alternatives for feeding sheep have been proposed in order to modify the fatty acid profile of milk and meat in accordance with current trends.

3. Feeding strategies to modify the fatty acid profile of sheep milk

Of the feeding strategies employed, supplementation with fat and the consumption of a high-quality forage ration that favours a ruminal environment suitable for ruminal biohydrogenation are the alternatives that have been most studied and that have produced the most favourable results to increase the levels of fatty acids with beneficial effects on human health (RA, VA and n-3 PUFAs) in milk. Moreover, cheese and dairy products reflect the fatty acid profile of the milk used to make them, so any strategy for improving milk quality is applicable to the dairy products that are obtained (Nudda et al., 2005; Bodas et al., 2010).

3.1. Supplementation with fat

The effect of fat on the fatty acid profile of milk depends on its composition and on the form in which it is incorporated in rations. Fat can be added in the form of oils (bare oils), whole seeds and/or plants and processed seeds and also fat could be protected from ruminal biohydrogenation, calcium soap being the form of protection most commonly used.

Fig. 2 shows the effects of various commercially available fat sources tested by our research group on the levels of rumenic acid, omega-3 fatty acids (n-3 PUFAs) and very long chain omega-3 fatty acids (n-3 VLCFAs) in milk.

Free oils are the most accessible sources of fat for the microorganisms responsible for biohydrogenation in the rumen, and they can increase the contents of rumenic acid, vaccenic acid and polyunsaturated fatty acids in milk. In a study conducted by Bodas et al. (2010) on dairy sheep, the results of which are presented in Fig. 2, which compared the effect of rations that differed only in the type of oil incorporated (palm, olive, soy or linseed), it was found that, with an oil intake of 70 g per day, the oil with the highest linoleic acid content (soybean oil) was the one that was most effective in increasing the levels of RA, and linseed oil, which is high in α -linolenic acid (ALA, C18:3 n-3), not only increased the levels of ALA but also generated considerable increases in RA in the milk, although not as great as those produced by soybean oil. In the case of soybean oil, RA is the result of processes of biohydrogenation of linoleic acid in the rumen and is also generated by desaturation of VA in the mammary gland, whereas in the case of α -linolenic acid, which is predominant in linseed oil, RA is only generated in the mammary gland, from VA, as Fig. 1 shows.

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Fig. 1. Biohydrogenation of linoleic acid and α-linolenic acid in the rumen. Alternative route with concentrate rations (....). Adapted from Griinari et al. (1999).



Fig. 2. Percentage (% of total fatty acids) of rumenic acid (RA, *cis-9 trans-*11CLA), omega-3 fatty acids (n-3 PUFAs) and very long chain omega-3 fatty acids (n-3 VLCFAs) in milk obtained from sheep fed on diets supplemented with 3% of various fat sources (Control: without added fat; PALM: hydrogenated palm oil; OLI: olive oil; SOY: soybean oil; LIN: linseed oil; ELS: extruded linseed; FO: fish oil calcium soap) (Bodas et al., 2010; Manso et al., 2011; Gómez-Cortés et al., 2013; Gallardo et al., 2014).

Nudda et al. (2014) analysed data from various experiments and 197 indicated that there was a linear relationship ($R^2 = 0.78$) between 198 the quantity of soybean oil ingested and the RA concentration in 199 the milk; when the fat intake is about 70 g a day it is possible to 200 achieve CLA levels between 2 and 3% in the milk, whereas concen-201 trations greater than 3% are only obtained with very high doses 202 of soybean oil (140 g/day). It must be noticed that the incorpora-203 tion of high levels of fat, as well as high level of concentrate in 204 rations can have negative effects on ingestion and metabolism of 205 the cellulolytic bacteria responsible for digestion of fibre, reducing 206 production of acetic acid and, consequently, production of milk fat. 207 This causes significant increases in trans-10 fatty acids associated 208 with a greater risk of suffering coronary diseases, which might cast 209 doubt on the quality of the milk produced (Shingfield et al., 2008). 210 Fats and oils protected from ruminal biohydrogenation have been 211 used to avoid these negative effects, calcium soaps and seeds being 212 the ones most commonly employed. 213

When whole seeds of oil-bearing plants are incorporated, the oil that they contain is available in the rumen more gradually than when it is supplied in the free form. This is a strategy that overcomes the problem of *trans-10* fatty acids without increasing RA and VA.

Therefore, the inclusion of processed seeds (extruded, heat 218 treated, micronised or ground) is more effective than the use of 219 whole seeds for generating RA in milk because the treatments allow 220 the triglycerides in the seeds to be more accessible to microor-221 ganisms in the rumen for lipolysis and biohydrogenation than 222 untreated seeds (Doreau et al., 2009). Moreover, the use of fat in 223 224 the form of seeds also makes it possible to maintain low, stable levels of trans-10 fatty acids that are produced when free oils are 225 administered (Gómez-Cortés et al., 2013). 226

The processes of desaturation and elongation of α -linolenic acid (C18:3 n-3) in the mammary gland explain the increases in omegafatty acids (n-3 PUFAs and n-3 VLCFAs) observed in milk when linseed oil and extruded linseed are incorporated (Fig. 2). This aspect is of great interest because of the relationship of n-3 PUFAs, particularly long-chain ones, with prevention of various diseases.

Studies conducted with other fats low in linoleic acid and α linolenic acid have also shown increases in levels of RA, VA and n-3 PUFAs in milk. This is the case with fats of marine origin (fish and algae oils) rich in long-chain fatty acids (EPA and DHA), the interest of which lies in the fact that they cause changes in the proliferation of bacteria responsible for conversion of VA into stearic acid in the rumen, favouring post-ruminal flow of VA and increasing endogenous synthesis of RA through the action of the Δ^9 -desaturase enzyme in the mammary gland. Provided that there is a source of linoleic acid to promote production of VA in the rumen, inhibition of the last step of biohydrogenation and accumulation of VA in the rumen has been suggested as an alternative to achieve greater production of endogenous RA in the mammary gland and thus to increase the level of RA in milk (Abughazaleh and Jenkins, 2004; Toral et al., 2010).

With regard to the interest in using fats of marine origin (fish and algae oils) with considerable quantities of 20:5 n-3 (EPA) and C22:6 n-3 (DHA) to raise the levels of very long chain n-3 PUFAs (n-3 VLCFAs) in milk, the results obtained so far indicate that, although they increase these fatty acids in milk (Fig. 2), the transfer of EPA and DHA from rations to milk is low, even when the fat is supplied in the form of calcium soap (Lock and Bauman, 2004). The low level of transfer of these fatty acids might be due to the fact that long-chain n-3 PUFAs are highly biohydrogenated in the rumen (Wachira et al., 2000) and that, because of the form in which they are transported in plasma, they supply a smaller quantity of these FAs to the mammary gland (Kitessa et al., 2001).

As a result of the foregoing, the use of fats of marine origin as a nutritional strategy to increase levels of n-3 PUFAs in milk is very limited. Moreover, the high price of fats of marine origin and the fact that they are associated with marked decreases in the production

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and concentration of milk fat mean that now their use operationally
is not practical.

In general, maximum levels of 6% of added fat in rations are
recommended to ensure that the improvements in the fatty acid
profile are not accompanied by adverse effects at ruminal level and
on the production and composition of milk fat.

270 3.2. Level and type of forage

Keeping the rumen functioning in optimum conditions by
means of suitable consumption of high-quality forage is another
of the strategies that can be used to obtain higher levels of VA, RA
and n-3 PUFAs in milk.

Forage is an important source of polyunsaturated fatty acids in ruminant diets. Therefore pasture grazing sheep produces higher levels of unsaturated fatty acids, VA, RA and n-3 PUFAs, in the milk than when concentrate or preserved forage are employed (Nudda et al., 2005).

The effects of pasture on the fat content of milk are related to the 280 high concentration of α -linolenic acid (ALA) in green pasture. Con-281 sequently, as intake of pasture increases, the concentrations of ALA 282 (r=0.69) and CLA (r=0.79) increase (De Renobales et al., 2012). In 283 general, fresh pasture gives rise to higher levels of RA, VA and ALA 284 285 in milk fat than hay and silage, and increases in RA decrease as the pasture matures (Nudda et al., 2005; Joy et al., 2012). It has been 286 pointed out that this effect is not due solely to differences in the 287 composition of forage and that it might be related to the presence 288 of fresh pasture compounds that stimulate production of VA, its 289 concentration being reduced in mature pasture. These compounds 290 might also inhibit complete biohydrogenation of VA to stearic acid 291 in the same way that other very long chain fatty acids, such as EPA 292 and DHA, act, as indicated earlier (Lock and Bauman, 2004). In gen-293 eral, some phenolic compounds in plants, such as tannins, affect 294 ruminal fermentation, and it has also been pointed out that they 295 might alter the fatty acid profile of milk (Vasta et al., 2008). 296

297 Although there are few data concerning the use of different types of forage on the fatty acid profile of sheep milk, Reynolds et al. 298 299 (2006) showed that the RA concentration was higher and the ALA concentration lower in milk of sheep fed on corn silage than on 300 alfalfa pellets, and that alfalfa haylage was more likely to increase 301 levels of RA in milk. In general, diets with a low forage:concentrate 302 ratio and associated with a ruminal pH below 6 reduce biohydro-303 304 genation of fatty acids and give rise to alternative routes and to changes in levels of CLA. However, sheep seem to be less sensitive 305 than cows to changes in the forage:concentrate ratio in the ration 306 (Nudda et al., 2014), and in fact ewes fed on increasing levels of 307 forage (30:70, 50:50 and 70:30) did not present changes in the RA 308 concentration in the milk (Gómez-Cortés et al., 2013). 309

4. Feeding strategies to modify the fatty acid profile of lamb meat

312 4.1. Supplementation with fat

The composition of the fat of lambs fed by means of natural suckling is influenced by the composition of the ration consumed by the ewes (Scerra et al., 2007; Joy et al., 2012). Consequently, the strategies indicated for improving the quality of milk fat are also useful for improving the fatty acid profile of the meat of the suckling lambs produced.

Fig. 3 shows the effects of various commercially available fat sources in the ration of Churra ewes used in studies conducted by our research group on the levels of rumenic acid (*cis-9 trans-*11CLA), omega-3 fatty acids (n-3 PUFAs) and very long chain omega-3 fatty acids (n-3 VLCFAs) in intramuscular fat of suckling lambs. In studies conducted by Manso et al. (2011) and Gallardo et al. (2014), as Fig. 3 shows, it was found that the composition of fat in suckling lamb meat depends on the fatty acid profile of the fat source supplied in the feed given to the ewes, and on the way in which it is incorporated. The VA and RA concentrations were reflected in the fat deposits of the sucklings as they were incorporated mainly in the triglycerides. The variations in n-3 PUFA levels were reflected mainly in the intramuscular fat of the suckling lambs owing to the phospholipid content of the muscle fibre membranes, reaching n-3 PUFA values much higher than those of the milk (Raes et al., 2004).

Feeding ewes on pasture produces suckling lambs with less saturated fat and with higher levels of RA, VA and n-3 PUFAs, and with a more favourable n-3/n-6 ratio, than when the lambs come from dams penned indoors and fed on preserved forage and/or a high proportion of concentrate (Scerra et al., 2007; Joy et al., 2012).

In the Mediterranean countries fattening lambs are usually slaughtered at live weights close to 25 kg and fed indoor with concentrate and cereal straw. In these feeding system vegetable oils have also been studied as alternatives to improve the fatty acid profile and increase levels of CLA and unsaturated fatty acids in fattening lamb meat.

As Fig. 4 shows, despite its high linoleic acid content, 3% of sunflower oil in the lamb feed did not improve levels of rumenic acid (*cis*-9, *trans*-11CLA) in the meat, but it increased the levels of *trans* fatty acids (Manso el al., 2009). Moreover, although the differences in the saturated/unsaturated ratio were not significant, the intramuscular fat of the lambs that received sunflower oil had a higher atherogenicity index than the fat of lambs fed without added fat or with hydrogenated palm oil. Some authors, such as Bessa et al. (2009), have also reported increases in the proportion of certain fatty acids associated with negative effects on human health (*trans*-10C18:1) in meat when oil was supplied in rations with a low forage:concentrate ratio. Therefore this is an aspect to be taken into account when making recommendations about the use of oils in lamb feed rations. Similar results were found when soybean oil and linseed oil were used in lamb rations (Francisco et al., 2015).

The use of oils and seeds rich in n-3 PUFAs, such as linseed oil, extruded linseed or chia seed, in lamb concentrates has proved to be effective in increasing the levels of n-3 PUFAs in the meat, which is favourable from the point of view of human health, despite the fact that the level of *cis*-9 *trans*-11CLA is not modified (Urrutia et al., 2015). Also, the use of fish oils seems to be the most effective way of increasing the levels of EPA and DHA in lamb muscle (Raes et al., 2004).

4.2. Level and type of forage

In lambs weaned and fed on pasture and forage, as the grass has a high concentration of n-3 polyunsaturated fatty acids, the meat has higher concentrations of RA and a healthier fatty acid profile (Raes et al., 2004). However, when the lambs are fed intensively on concentrate and cereal straw *ad libitum*, as is the case with most lambs that are fattened in Mediterranean countries, the levels of RA and other n-3 PUFA polyunsaturated fatty acids are more limited.

5. Use of grape pomace in the feeding of sheep

The increase in the degree of unsaturation of fat also makes it more susceptible to oxidation and therefore shortens its shelf life. One of the strategies most commonly employed to prevent lipid oxidation of meat is the use of antioxidants in the rations. Synthetic antioxidants are often employed, but their use is considerably restricted in some countries because of their potential toxic and carcinogenic effects, and therefore the development of natu-

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Fig. 3. Percentage (% of total fatty acids) of rumenic acid (*cis-9 trans-*11CLA), omega-3 fatty acids (n-3 PUFAs) and omega-3 very long chain fatty acids (n-3 VLCFAs) in the intramuscular fat of suckling lambs obtained from ewes fed on a diet supplemented with 3% of various fat sources (Control: without added fat; PALM: hydrogenated palm oil; OLI: olive oil; SOY: soybean oil; LIN: linseed oil; ELS: extruded linseed; FO: fish oil calcium soap). (Manso et al., 2011; Gómez-Cortés et al., 2013; Gallardo et al., 2014).



Fig. 4. Effect of 3% of hydrogenated palm oil and 3% of sunflower oil on the concentration (percentage of total fatty acids) of *trans* C18:1 and *cis*-9 *trans*-11CLA fatty acids in the intramuscular fat of lambs during the period of fattening growth (Manso et al., 2009).

ral antioxidants, including phenolic compounds, has aroused great
interest.

In the feeding of ruminants in general and sheep in particular it
is very common to use agriculture and food industry by-products,
because in this way it is possible not only to make use of by products that would otherwise be merely waste but also to reduce
the cost of rations and/or to provide bioactive substances with ben eficial effects on product quality and consumer health.

Grape pomace is a winemaking by-product that is very rich in phenolic compounds with a high antioxidant capacity, and therefore it has been proposed as an interesting, low-cost feeding alternative that increases the oxidative stability of the products and the functional fatty acid content of the fat of small ruminants.

In this connection, some authors have related the ingestion 398 of polyphenols and condensed tannins to changes in the bacte-399 rial flora involved in ruminal biohydrogenation processes, causing 400 inhibition of the conversion of VA in the rumen. Furthermore, the 401 Δ^9 -desaturase enzyme may also be influenced by the presence 402 of some polyphenols, favouring endogenous synthesis of RA from 403 VA in animal tissues (Vasta et al., 2009). Consequently, the use of 404 polyphenols in general, and of grape pomace in particular, is being 405 proposed, not only for its antioxidant effect but also to improve the 406 quality of milk and meat fat. 407

Our research group has recently conducted a study (Guerra-408 Rivas et al., 2015a) with sheep at the beginning of lactation, 409 receiving either two levels of grape pomace (5% and 10% of ration 410 dry matter) or the antioxidant customarily used (vitamin E), .It 411 could be observed that the CLA and VA content in the intramuscu-412 lar fat was higher in the lambs whose dams ingested grape pomace, 413 which might corroborate the effect of grape pomace polyphenols 414 415 on fat biohydrogenation processes.

Similarly, in another experiment (Guerra-Rivas et al., 2013),
when 5% of grape pomace was added to the concentrate for fat tening lambs, although the effects of the treatments were minimal,

a tendency towards a higher polyunsaturated fatty acid content in the meat of the lambs that received grape pomace was also detected.

Despite the low level of fat present in grape pomace (73.3 g per kg of dry matter), its high degree of unsaturation, with a level of linoleic acid greater than 60%, and the inhibitory effect of some of its phenolic compounds on biohydrogenation processes in the rumen and their interactions with the microbe population and with the desaturase enzymes that intervene in the processes of ruminal biohydrogenation might explain these results.

Some authors, using different plant extracts, have observed positive linear correlations between the content of phenolic compounds and their antioxidant capacity (Zheng and Wang, 2011). Moreover, it has been found that supplementation of the diet with substances of a phenolic nature improves the stability of meat colour during storage (Vasta and Luciano, 2011). In this regard, the high content of phenolic compounds in winemaking waste might be interesting as a source of antioxidants in sheep rations.

When grape pomace was incorporated in the ration of lactating ewes at levels of 5 and 10% of dry matter, as shown in Fig. 5a, Guerra-Rivas et al. (2015b) found that the content of malondialdehyde (MDA), an indicator of the degree of oxidation of the meat, increased with refrigeration time, and that the level of MDA tended to be lower from day 3 onwards of storage of meat of suckling lambs whose dams received grape pomace in the ration, showing an effect similar to that produced by vitamin E.

Although the colour parameters did not show great differences due to the experimental treatment, MDA levels were positively correlated with the levels of metmyoglobin, and the results found in the suckling lamb meat seem to indicate a possible transfer of polyphenols from grape pomace to milk. A tend to lower level of MDA in meat was also observed when grape pomace was added to the concentrate for fattening lambs.

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meat of suckling lambs compared to ewes that were not supplemented (Control) or supplemented with 500 mg of vitamin E (VIT-E). Lowercase letters indicate significant differences between treatments (*P* < 0.05) and uppercase letters indicate significant differences between days of storage (Guerra-Rivas et al., 2015b).

452 6. Conclusions

The type and amount of fat and forage in sheep rations are the 453 most important factors to be taken into account in order to produce 454 milk and meat of lamb and suckling lamb with a fatty acid profile 455 adapted to consumer demands from the point of view of human 456 health. Also, the use of agriculture and food industry by-products, 457 such as grape pomace, mainly due to their abundance of polyphe-458 nols, might be considered as a sustainable, economical strategy for 459 increasing vaccenic acid and CLA in milk and for improving the 460 oxidative stability of lamb meat. However, further research is need 461 to establish precisely the most suitable level of pomace in sheep 462 463 rations during the various phases of the period of production.

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