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Use of natural products to improve meat quality of sheep reared in the Mediterranean environment

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Abstract. Recently, research in small ruminant feeding has been focusing on the use of natural plant-derived molecules, such as phenolic compounds (PCs) and essential oils (EOs). While it is necessary that such strategies do not compromise animal productivity, evidence has been provided for potential positive effects of feeding animals with these plant bioactive compounds on meat quality traits, such as intramuscular fatty acid composition, sensory properties and oxidative stability. The potential mechanisms of action of these bioactive molecules have been suggested by a number of studies in which selected plant extracts or purified compounds were studied *in vitro* or were fed to animals. The results of these studies highlighted, for instance, a possible ability of dietary PCs and EOs to reduce the extent of the ruminal biohydrogenation of polyunsaturated fatty acids, with a consequent increase in the content of desirable unsaturated fatty acids in meat. Dietary PCs can exert other effects upon the ruminal metabolism leading, for instance, to the reduction of the production of odour-active compounds, such as skatole and indole, responsible for conferring on the meat unpleasant flavours when present at high concentration. Also, the strong antioxidant properties of both PCs and EOs make them good candidates to replace synthetic antioxidants in ruminant diets, with positive effects being observed on the reduction of the oxidative deterioration of meat nutritional and sensory quality. However, it is important to stress that these potential activities need to be regarded in the light of the great variability in the chemical nature of these compounds and of the concentration at which they actually exert effects when ingested by small ruminants. These considerations are of importance because the practical interest in the dietary administration of such compounds to ruminants lies on the fact that they are naturally occurring in plant biomasses typically produced in the Mediterranean areas, such as agro-industrial wastes and by-products. Nowadays, the replacement of conventional feedstuffs with these low-cost and locally available feeds might represent an effective strategy to promote low input production systems and to cope with the increasing costs of conventional systems. Some of these feed resources are known for containing PCs and EOs (e.g. olive, citrus and aromatic plant by-products, carob pulp and others) and research has demonstrated that their inclusion into small ruminant diets positively affects meat quality. However, the direct attribution of such effects to the presence of PCs and EOs should be made with caution, because the chemical nature of these compounds, their concentration and the presence of other molecules (such as unsaturated fatty acids and antioxidant vitamins) need to be taken into account.

Keywords. Meat quality – Small ruminants – Phenolic compounds – Essential oils – Alternative feed resources.

Les composés naturels et l'amélioration de la qualité de la viande des petits ruminants élevés dans l'environnement Méditerranéen

Résumé. Récemment, la quasi-totalité des recherches dans le domaine de l'alimentation des petits ruminants se sont focalisées sur l'utilisation de molécules naturelles d'origine végétale (ou issues d'extraits de plantes), comme les composés phénoliques (CP) et les huiles essentielles (HE). Bien qu'il soit nécessaire que de telles stratégies ne compromettent pas la productivité animale, des effets positifs ont été mis en évidence chez les animaux recevant ces types de composés, et notamment sur la qualité de la viande, tels que la composition en acides gras des lipides intramusculaires, les propriétés sensorielles et la stabilité oxydative. Les mécanismes d'action de ces molécules bioactives ont été suggérés par un certain nombre d'études dans lequel des extraits de plantes choisis ou des composés purifiés ont été étudiés *in vitro* ou directement sur les animaux. Les résultats de ces études mettent en évidence la capacité des CP et des HE à réduire la biohydrogénation ruminale des acides gras polyinsaturés, ce qui se traduit par une augmentation de la teneur en acides gras insaturés qui sont des acides gras dont la présence est souhaitable dans la viande. Les CP peuvent exercer d'autres effets sur le métabolisme ruminal, particulièrement dans la réduction de la production des composés actifs de l'odeur, notamment le scatole et les indoles, qui confèrent à la viande une saveur

désagréable quand ils sont présents à des fortes concentrations. De plus, grâce à leurs propriétés antioxydantes, les CP et les HE pourront être la meilleure alternative pour remplacer les antioxydants synthétiques utilisés dans la nutrition des ruminants, avec des effets positifs sur la réduction de la détérioration oxydative des qualités nutritionnelles et sensorielles. Cependant, il est important de souligner que cette activité potentielle doit être considérée avec une grande précaution en tenant compte de la grande variabilité chimique de ces composés et de la concentration optimale à laquelle sont actifs lorsqu'ils sont ingérés par les petits ruminants. Ces considérations sont très importantes parce que l'intérêt pratique dans l'utilisation de tels composés tient du fait qu'ils sont présents d'une façon abondante dans les biomasses végétales des zones méditerranéennes, telles que les déchets agro-industriels et sous-produits. Le remplacement des ressources nutritionnelles conventionnelles avec ces aliments à faible coût localement disponibles pourrait représenter une stratégie efficace pour promouvoir les systèmes de production à faibles intrants et faire face aux coûts croissants des systèmes conventionnels. Certaines de ces ressources sont connues pour leurs teneurs considérables en CP et HE (par exemple l'olive, les agrumes, les plantes aromatiques, la pulpe de caroube...) et les recherches ont démontré que leur incorporation dans les régimes alimentaires des petits ruminants améliore la qualité de la viande. Cependant, l'attribution directe de tels effets à la présence de CP et de HE doit être interprétée avec prudence, parce que la nature chimique de ces composés, leur concentration et la présence d'autres molécules (comme des acides gras insaturés et les vitamines antioxydantes) doivent être pris en compte.

Mots-clés. Qualité de la viande – Petit ruminant – Composés phénoliques – Huiles essentielles – Ressources alimentaires alternatives

I – Introduction

Consumers' safety concerns have led to an increased attention to the "green" image of animal products, with low input animal rearing systems being deemed safe and respectful of animal welfare. In this context, research is currently investigating on the use of natural products, such as bioactive plant-derived molecules, in livestock feeding to potentially replace synthetic growth promoters, anti-parasitic drugs and antioxidants. Among the most studied plant bioactive compounds, phenolic compounds (PC) and essential oils (EO) have received a special attention as their use in small ruminant feeding has been shown to positively affect animal welfare and product quality, especially with respect to meat (Vasta and Luciano, 2011). However, the mechanisms behind the effects of these compounds *in vivo* are still under debate and, especially in the case of PC, negative effects resulting from high levels of these compounds in the diet must be considered (Makkar, 2003). Research has often focused on the dietary administration of purified molecules or of selected plant extracts rich in particular classes of PC and EO. These studies are certainly of importance to unravel the effects of dietary plant bioactive compounds on the quality traits of meat from small ruminants. Nevertheless, it is unpractical to propose some of these feed supplements in the real farming conditions. Additionally, in the case of PC, most of the studies conducted so far have evaluated extracts from a limited number of plant species, such as the tannin-rich quebracho, chestnut and oak extracts, with the aim of generalizing the effects of the most represented classes of PC. It should be noticed that, within the same class of compounds, the chemical nature of PC is greatly variable and extrapolations from the specific experimental conditions of each study should be made with caution (Mueller-Harvey, 2006).

This short review is intended to provide a view on the ongoing research focusing on the use of natural compounds (mainly PC and EO) in small ruminant feeding to manipulate some main meat quality traits, including intramuscular fatty acid composition, sensory properties and stability to oxidative deterioration. The literature cited is not exhaustive and relevant studies and reviews in the area are provided. The main aim of this review is to highlight the need for further investigation on the effects feeding PC and EO to small ruminants on meat quality. The main considerations will be based on results provided by using selected plant extracts.

II – Effect of phenolic compounds and essential oils on meat quality

Attempts have been made by researchers to modify the nutritional quality of meat by increasing, for instance, the concentration of polyunsaturated fatty acids (PUFA) and of specific beneficial fatty acids. This is of special importance when animals are raised under intensive feeding systems, such as during the finishing phase or, generally, in seasons in which fresh forages are scarcely available. Unlike monogastric animals, ruminants operate a peculiar lipid metabolism in the rumen, known as biohydrogenation (BH), in which the ingested PUFA are converted to saturated fatty acids (SFA). Additionally, during the BH of linoleic acid (LA; *cis*-9 *cis*-12 C18:2), and of linolenic acid (LNA, *cis*-9 *cis*-12 *cis*-15 C18:3), a number of intermediate 18-carbon-chain fatty acids originate before the formation of stearic acid (SA; C18:0) as final product. In the classical BH pathways described, rumenic acid (RA; *cis*-9 *trans*-11 C18:2) originates from the BH of LA and it has received a special attention since early evidences supporting its health-promoting activities (Ha *et al.*, 1990). Additionally, the BH of LA and LNA results in the formation of vaccenic acid (VA; *trans*-11 C18:1), which is the substrate for the endogenous synthesis of RA in the mammary gland and muscle. Years of research have later demonstrated several different pathways of the ruminal BH and the reader is invited to refer to broader studies and reviews on the topic (Jenkins *et al.*, 2008).

In the light of the above, the modification of the fatty acid profile of meat from ruminants is largely dependent on the possibility of overcoming and/or modulating the ruminal BH. Possible approaches could be related to feeding fat sources which are protected from ruminal metabolism, or to feeding lipid-enriched diets in order to ensure a substantial amount of PUFA to escape the BH (Raes *et al.*, 2004; Bessa *et al.*, 2007). Nevertheless, this topic, while important, is out of the scope of the present article and will not be discussed. Furthermore, it should be also considered that such approaches need to be applied to a rather large scale and to high-value productions in order to overcome their costs. Additionally, increasing the PUFA content in meat through the dietary administration of PUFA-supplemented concentrate diets might result in the drawback of decreasing meat sensory acceptability and the resistance of meat to oxidative deterioration. Alternative approaches to try to increase the deposition of desirable fatty acids in meat have been more recently based on the use of plant-derived compounds able to impair the ruminal BH, thus resulting in a higher outflow of PUFA and BH intermediates from the rumen. Among these compounds, particular emphasis has been given to PC, while additional results suggested a potential positive effect of EO. The interest in the use of these compounds also lies on the fact that they possess antioxidant properties and could, therefore, increase the stability of meat to oxidative deterioration. Lastly, the ability of PC and EO to interfere with ruminal microbial ecosystem could reduce the ruminal production of odour-active compounds which can confer on the meat unpleasant flavour notes if present at high concentration.

1. Manipulation of meat fatty acid composition

Several studies have investigated on the effects of plant metabolites as potential modulators of the ruminal BH. In the case of PC, most of these studies were performed using selected compounds and plant extracts known for their high content in different classes of PC, such as condensed or hydrolysable tannins. Indeed, it has been demonstrated that tannins can strongly affect the ruminal microbial community (McSweeney *et al.*, 2001). Among the effects of tannins in the rumen, research has rather recently focused on the potential inhibitory activity of these compounds upon the BH of dietary PUFA which could represent a strategy to increase the amount of PUFA and BH intermediates escaping the complete saturation. More comprehensive overviews on the topic can be consulted elsewhere (Vasta and Luciano 2011; Vasta and Bessa, 2012).

Most of the studies conducted in the last 10 years have evaluated the effects of tannins on the ruminal BH *in vitro* through the determination of the fatty acid composition of ruminal fluid incubated with diets supplemented with different doses and classes of tannins. The results of some studies have

suggested that tannins-rich extracts from acacia and quebracho are able to impair the last step of the BH process (the conversion of VA to SA), with a consequent accumulation of VA at the expenses of SA in the ruminal liquor (Khiaosa-Ard *et al.*, 2009, Vasta *et al.*, 2009a). Furthermore, Buccioni *et al.* (2011) found that the inclusion of quebracho and chestnut extracts (as sources of condensed and hydrolysable tannins, respectively) to a concentrate-based diet fermented *in vitro* resulted in an accumulation of VA and decreased the concentration of SA. If confirmed and reproducible, all the above results would be quite interesting for proposing dietary tannins as a strategy to increase the content of RA in meat. Indeed, it has been demonstrated that, in lamb muscle, RA can be actively formed endogenously from VA through the action of the Δ^9 -desaturase enzyme (Palmquist *et al.*, 2004). Nevertheless, the results of other studies do not fully support the hypothesis of such a specific effect of tannins on precise steps of the BH process. Rather, these studies indicated that, if an effect of tannins on the BH was detected, general rather than specific deviations of the ruminal BH were detected and that this effect was linked to both the concentration and to the type of tannins used (Carreño *et al.*, 2015). A further issue with certainly needs to be elucidated is related to the identification of those ruminal microbes which are possibly affected by tannins. Buccioni *et al.* (2011) demonstrated *in vitro* that chestnut and quebracho extracts were able to increase the levels of fatty acids produced during the BH and that these fatty acids were accumulated preferentially in the solid-associated bacteria (SAB), confirming that these bacteria are among the ones more involved in the ruminal BH. Furthermore, Khiaosa-Ard *et al.* (2009) and Vasta *et al.* (2009a), reported that the acetate:propionate ratio was reduced in the ruminal fluid incubated with tanniferous extracts, which could suggest that cellulolytic bacteria (with a preminent role in the BH) are impaired by tannins. Nevertheless, the results of other studies suggested that the variability of the chemical nature between different sources of tannins and the dose-dependent efficacy of different tannin extracts do not allow straightforward conclusions on the effects of tannins on specific bacterial strains (Carreño *et al.*, 2015). Indeed, it should be stressed that characterized and cultivated microorganisms to date only represent a minor component of the whole microbial ecosystem in the rumen and, therefore, it cannot be excluded that other microbes might play an important role on the ruminal BH.

All the *in vitro* experiments conducted, including the above cited studies, are of special importance for the understanding of the effect of PC and tannins, in particular, on the lipid metabolism in ruminants. Nevertheless, the trends highlighted in these investigations need to be validated by *in vivo* experiments in which possible effects of plant secondary compounds on the ruminal BH should be confirmed and should also result in appreciable modifications of the fatty acid composition of animal products, such as meat and milk. In this regard, *in vivo* studies designed to specifically study the effects of selected tannins and tannin-rich extracts were rather recently performed. Additionally, looking at meat production, only few studies are available. Some of these studies demonstrated that the supplementation of concentrate-based diets with 4% and 6.4% (dry matter basis) of condensed tannins from quebracho appeared to be able to impair the ruminal BH, leading to the accumulation of VA at the expenses of SA in the rumen, thus confirming the observations from some of the *in vitro* studies (Vasta *et al.*, 2009b; Vasta *et al.*, 2010). Additionally, in agreement with the modification of the ruminal fatty acids, it was reported that quebracho tannins were able to interfere with microbes involved in the ruminal BH by decreasing the population of *Butyrivibrio proteoclasticus* (converting VA to SA; Vasta *et al.*, 2010). These effects of quebracho tannins in the rumen partially corresponded to modifications of the fatty acid composition in meat, with higher concentrations of PUFA and RA and lower levels of SFA in muscle from lambs receiving dietary tannins (Vasta *et al.*, 2009b). Only few studies were subsequently conducted to confirm these results and a complete overview of the fatty acid metabolism in the rumen and muscle is often missing. For example, Jerónimo *et al.* (2010) reported no effect of supplementing the diet with 2.5% grape seed extract on the fatty acid composition of ruminal digesta and of muscle in lambs. In another study, the supplementation of a total mixed ration with 2g / kg of a flavonol (quercetin), in combination with linseed oil, resulted in an increase of RA in muscle, suggesting a role of this phenolic

compound on the ruminal BH (Andrés *et al.*, 2014). Moreover, the issue of the dose-dependent effect of dietary tannins should be taken into account. Indeed, if present at high levels in the diet, tannins can exert negative effects on animal performances (Makkar, 2003). Therefore tannins-based feeding strategies to improve meat fatty acid profile should not compromise productivity. For example in the study from Vasta *et al.* (2010) the authors reported that 6.4% quebracho tannins in the diet reduced the dry matter intake and growth performances of the lambs. Lastly, it should be stressed that most of the studies so far available have used a limited range of compounds or plant extract, such as quebracho and chestnut extracts, with varying degree of tannins concentrations and purity. Considering that phenolic compounds occur in plant extracts with greatly variable chemical composition and relative proportion of the different classes of phytochemicals, it is rather difficult to extrapolate the results of the different studies to a broader field. Most of the plants extracts used so far are commercially available and some are already used as feed supplements for animals (such as quebracho and chestnut). Nevertheless, a number of other plant extracts are commercially available and used, for example, as tanning agents in the leather industry (to give some examples: tara, sumac, myrabolan, mimosa, gambier and others). All these extracts cover a wide selection of different classes of tannins, such as gallotannins, ellagitannins, catechin-type and proflisetinidin-type tannins (Kardel *et al.*, 2013). Therefore, experiments conducted with different available extracts would probably contribute to a better understanding on the possible use of PC as means for improving meat nutritional quality traits.

Apart from phenolic compounds, essential oils (EO) are phytochemicals, often naturally occurring at high levels in many plants, which have received attention for their possible application as feed supplements. Essential oils are basically blends of a number of compounds that can be tentatively classified as terpenoids and phenylpropanoids. These compounds bear biological properties, such as antimicrobial activities, which have inspired researchers to investigate, for example, their possible role as natural antibiotic and antiparasitic remedies for livestock (Franz *et al.*, 2010). The specific potent antimicrobial activity of most of these compounds has motivated the study of the effect of EO as modulators of the ruminal BH. Several *in vitro* studies have therefore tested the efficacy of selected terpenoids or of EO extracted from different plants on the ruminal microbial community and on the BH. Most of these studies provided encouraging results. For example, it was demonstrated that bacteria involved in the ruminal BH, such as *Butyrivibrio fibrisolvens* and *Clostridium proteoclasticum*, were particularly sensitive to various EO tested and that this sensitivity was dependent on the type and dose of EO and on the specific microorganism (McIntosh *et al.*, 2003; Durmic *et al.*, 2008). Other authors demonstrated a clear effect of EO on the microbial ruminal fermentation (Calsamiglia *et al.*, 2007). Regarding the ruminal BH, Lourenço *et al.* (2008) demonstrated *in vitro* that, while eugenol exerted only minor effects on the ruminal BH, cinnamaldehyde strongly impaired the BH and resulted in an increase of those fatty acids produced during the BH. The variable effect of different terpenoid compounds on the ruminal BH can be explained considering that ruminal microorganisms can adapt to EO and that some compounds can be degraded in the rumen (Brodiscou *et al.*, 2007). Therefore, it is likely that different blends of naturally occurring EO could differently affect the lipid metabolism in the rumen.

The results obtained with the *in vitro* studies encouraged to test *in vivo* if dietary EO could improve the deposition of desirable fatty acids in the animal products through the impairment of the ruminal BH. Unfortunately, only few studies were performed and controversial results were provided. Additionally most of the studies performed tested the efficacy of EO supplementation on milk fatty acid composition, while very limited information is available on meat. For example, it has been demonstrated that the dietary administration of cinnamaldehyde to cows or of a blend of terpenes to goats did not modify milk fatty acid composition (Benchaar and Chouinard, 2009; Malecky *et al.*, 2009). Conversely, it was demonstrated that the dietary administration of 400 ppm (dry matter) of EO from *Artemisia herba alba* to lambs was able to increase the deposition of VA, RA and PUFA

in meat, while EO from rosemary did not affect meat fatty acid composition (Vasta *et al.*, 2013). Several plants in the Mediterranean environment are rich in EO and purified EO are being proposed as feed additives for livestock. In this context, further research is needed to clarify the effects of different EO on the nutritional quality of meat from ruminants and to understand which is the optimal blend of compounds to be used and the ideal conditions for treating the animals.

2. Improvement of meat sensory properties and quality preservation

Among the main meat quality traits, flavour certainly plays a major role and this is especially true for sheep meat, which is often associated to typical species flavours, not appreciated by consumers. Additionally, pasture feeding may amplify these distinctive flavours and can confer on the meat the so-called “pastoral” flavour which could result too strong for not accustomed consumers (Sañudo *et al.*, 2000). These flavours in lamb meat are believed to be linked to the presence of specific branched-chain fatty acids (4-methyl octanoic and nonanoic acids) and of indolic compounds, such as indole and skatole (Young *et al.*, 2003). The latter compounds has been particularly studied and researchers have investigated the possibility of reducing its occurrence in meat by opportune feeding strategies. In ruminants, skatole originates from the metabolism of tryptophan, operated by the ruminal microorganisms, and accumulates in the tissues. Skatole production is enhanced by pasture feeding compared to concentrate-based diets (Young *et al.*, 2003) and, therefore, strategies to reduce its production are of special importance in the case of extensive pasture-based feeding systems. Phenolic compounds, being able to interfere with the ruminal microorganisms, could be regarded as potential means for reducing the production of skatole in ruminants. Studies conducted *in vitro* have clearly demonstrated that tannins are able to reduce the formation of skatole (Schreurs *et al.*, 2007a; 2007b). Most of the *in vivo* studies conducted to validate the ability of tannins to reduce skatole accumulation in meat were conducted comparing plants naturally containing different levels of tannins (Vasta and Luciano, 2011). Only few studies are available on the potential use of selected compounds or of plant extracts. For example, Schreurs *et al.* (2007c) demonstrated that the addition of condensed tannins from grape seeds to grass or legume forages for lambs decreased the concentration of skatole in ruminal fluid and plasma, but did not affect its deposition in muscle and slightly affected the consumer appreciation of meat flavour. In another study, Priolo *et al.* (2009) demonstrated that adding quebracho tannins to a concentrate-based diet for lambs reduced the concentration of skatole in the rumen and in the muscle, while milder effect of tannins were observed when quebracho extract was added to a legume forage. Additionally, the authors reported an overall positive effect of tannins on the sensory appreciation of meat flavour. The use of plant extracts rich in PC could be a promising strategy to improve sheep meat sensory properties. Therefore, studies should focus on the effects of different classes of PC on the deposition of skatole in meat and on the interaction of PC supplementation with the basal diet. Also, another interesting challenge would be to test the effects of supplementing EO to sheep diet on the accumulation of skatole in meat. Indeed, considering their strong antimicrobial activity, EO could be effective in impairing the production of skatole in the rumen. No studies have so far tested this hypothesis.

In red meats, such as sheep meat, sensory properties can be strongly affected by oxidative reactions involving muscle lipids and proteins. Indeed, the oxidation of intramuscular fatty acids results in the production of unpleasant rancidity flavours, due to the accumulation of specific volatile organic compounds (VOC) as breakdown products of PUFA oxidation. Moreover, the oxidation of the haem-protein myoglobin and the consequent accumulation of metmyoglobin (MMb) in meat is responsible for the deterioration of meat colour. These deteriorative processes are not independent and strategies to reduce lipid oxidation in meat generally extend meat colour stability (Faustman *et al.*, 2010). Additionally, more recently, researches have focused on the oxidative reactions involving myofibrillar proteins, as it has been shown that these processes negatively affect meat sensory properties such as tenderness (Lund *et al.*, 2011). Antioxidant compounds of dietary origin can

effectively increase the resistance of muscle to the oxidative reactions, with an ultimate positive impact on meat sensory and nutritional quality. Among the most studied dietary antioxidants, vitamin E has been probably the most studied (Descalzo and Sancho, 2008). Nevertheless, in the case in which animals do not have access to fresh forages, vitamin E is always added to the diet as a synthetic supplement (α -tocopheryl acetate). Therefore, looking for other natural antioxidant supplements has been a priority goal for animal and meat scientists.

Plant secondary compounds, such as PC and EO possess strong antioxidant properties and studies were conducted to assess the efficacy of the dietary administration of these compounds to ruminants in order to improve the resistance of meat sensory and nutritional quality to oxidative deterioration. In particular, the dietary administration of selected compounds or plant extracts would allow to increase the intake of antioxidants for animals fed concentrate-based diets, such as in the case in which pasture is not available or during the finishing phase in stall. The reader is invited to consult more comprehensive documents on the topic (Vasta and Luciano, 2011 for a review). In the context of the present work, emphasis will be given to issues which need to be investigated and clarified for proposing PC and EO as effective antioxidant supplements in small ruminant diets.

Certainly, one of the main issues which needs to be accounted for is related to the antioxidant mechanisms of action *in vivo* of these compounds when ingested by ruminants. Dietary antioxidants, such as vitamin E, are bioavailable compounds which are absorbed in the gastrointestinal tract and are deposited in muscle where they can exert antioxidant protection. In the case of PC, the issue of their metabolic fate in animals is a *condurum* which has not been yet unravelled. Additionally, as already commented above, most of the studies conducted so far have used a limited number of compounds and of plant extracts. For example, Luciano *et al.* (2009; 2011) found that the inclusion of quebracho extract into a concentrate-based diet for lambs extended meat colour stability, increased the resistance of myoglobin to oxidation and improved the overall antioxidant status of muscle. Nevertheless, López-Andrés *et al.* (2013) found that the improvement of muscle antioxidant status consequent to the dietary administration of quebracho extract was not associated to the presence of phenolic compounds in the muscle. The authors speculated that the profisetinidin compounds, identified as the main PC in quebracho, are particularly resistant to digestive metabolism and, therefore, that their absorption and deposition in the animal tissues is unlikely. In another study, Zhong *et al.* (2009) reported that feeding purified tea catechins to goats produced an overall improvement of meat lipid and colour stability. Although in that study the author did not investigate the bioavailability of PC, it could be speculated that catechins were partially absorbed in the digestive tract and reached the animal tissues, thus exerting antioxidant protection *in situ*. Indeed, studies conducted with humans and rats have demonstrated that these PC can be bioavailable. Furthermore, Gladine *et al.* (2007) found that the administration of grape seed extract to sheep produced an increase in the resistance of blood plasma to lipid oxidation and that epicatechin could be detected in the plasma from sheep receiving the extract. Therefore, different PC can undergo different metabolic pathways in animals. Many authors suggest that the bioavailability of PC in animals should not be regarded as the sole possible antioxidant mechanism *in vivo* and that, for instance, possible antioxidant effects exerted exclusively in the gastrointestinal tract would then result in and overall improvement of the antioxidant status of the animal organism (Halliwell *et al.*, 2005). Most of the clinical studies suggesting this hypothesis were conducted on monogastric animals, while no studies have specifically tested this hypothesis in ruminants. Additionally, as commented above, different classes of PC can have different propensity to be degraded in the digestive tract. Therefore, it would be of interest to assess the antioxidant effects of PC-supplemented diets using a wider range of plant extracts, in order to take into account the variable occurrence of PC in different available sources.

Similarly to PC, EO possess antioxidant properties and recent studies have tested the hypothesis that supplementing ruminant diets with EO could improve meat oxidative stability. As commented above, some terpenoid compounds can be degraded by ruminal microorganisms. Nevertheless, some

compounds typically present in EO blends has been shown to be bioavailable when ingested by animals (Michiels *et al.*, 2008). In agreement with these observations, Vasta *et al.* (2013) found that the muscle from lambs receiving dietary supplementation of artemisia and rosemary EO contained several terpenoid compounds originally present in the respective EO. Nevertheless, using the same animals, Aouadi *et al.* (2014) found that both rosemary and artemisia EO increased the overall antioxidant capacity of muscle, but reported no effect of EO supplementation on meat lipid and colour stability. These results agree with those reported by Smeti *et al.* (2013) who showed that supplementation of rosemary EO to lambs did not affect meat oxidative stability nor the sensory appreciation of meat eating quality. Conversely, Simitzis *et al.* (2008) reported that feeding oregano EO to lambs improved meat oxidative resistance. Actually, information on the effect of the dietary administration of EO to small ruminants on meat quality is very limited. Additionally, studies conducted with other species provided controversial results, with some suggesting positive effects of dietary EO on meat quality preservation (López-Bote *et al.*, 1998), while slight or no effects were reported in other instances (Botsoglou *et al.*, 2002). Considering the interest in the use of dietary EO as natural additives for various purposes, it would be interesting to keep studying their effects on meat quality.

III – Conclusions

Searching for natural compounds to be used as feed supplements in diets for ruminants is progressively catching attention. Among the most promising compounds, PC and EO are the most studied as it is being demonstrated that their dietary administration to animals could serve to achieve different objectives. Studies focusing on the effects on meat quality traits have been especially focusing on the ability of these compounds to improve meat nutritional and sensory properties. Nevertheless, a number of issues still need to be clarified, such as the ability of different classes of molecules to affect the ruminal metabolism of fatty acids and the production of odour-active compounds, as well as their metabolic fate and antioxidant activity *in vivo*. Additionally, several feedstuffs, such as agro-industrial wastes and byproducts, are being evaluated as feed resources to potentially replace to conventional feeds in low-input production systems. Most of these biomasses contain remarkable concentrations of plant bioactive compounds and, often, a variety of different PC and EO occur together. Nevertheless, given the high variability in the chemical composition of these biomasses and the presence of other compounds (such as antioxidant vitamins and unsaturated fatty acids), research should certainly devote attention to better characterize these potential feeding resources and to clarify the specific effect of the different compounds on meat quality.

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