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Dietary inclusion of whole pomegranate by-product to improve oxidative stability of lamb meat

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Abstract. This study evaluated the effect of dietary whole pomegranate by-product (WPB) on the oxidative stability of lamb meat. Seventeen Comisana lambs were divided into two groups and fed for 36 days: a barley-corn based concentrate diet (CON) or the same concentrate as the CON diet containing 20% of WPB to partially replace barley and corn (WPB). The dietary treatment did not affect the growth performance of lambs. Colour descriptors and metmyoglobin percentages were measured on *longissimus dorsi* (*LD*) muscle over 7 days of refrigerated storage. Lipid oxidation (TBARS values) was measured over time of refrigerated storage in raw and cooked muscle slices (7 days and 4 days, respectively). The dietary WPB increased the concentration of α-Tocopherol in *LD* ($P < 0.001$). Colour stability descriptors (*a**, *b** and *H**) were affected by storage time, but not by dietary treatment. Metmyoglobin accumulated over time ($P < 0.001$), but WPB diet tended to decrease the overall metmyoglobin percentages ($P = 0.051$). TBARS values increased with time of storage ($P < 0.001$). WPB group showed lower TBARS values in cooked meat ($P < 0.05$). A significant interaction between dietary treatment and storage time was found for the TBARS value in raw meat ($P = 0.013$), with lower values found for WPB at 7 days of storage. The results highlight the positive effect of feeding lambs with WPB on meat shelf-life and demonstrate, for the first time, the contribution of α-Tocopherol (vitamin E) to this effect.

Keywords. Lamb – Oxidative stability – Pomegranate – Meat.

Inclusion alimentaire du sous-produit de grenade entière pour améliorer la stabilité à l'oxydation de la viande d'agneau

Résumé. *Cette étude a évalué l'effet du sous-produit alimentaire de grenade entière (WPB) sur la stabilité à l'oxydation de la viande d'agneau. Dix-sept agneaux Comisana ont été divisés en deux groupes et nourris pendant 36 jours: un régime concentré à base d'orge-maïs (CON) ou le même concentré contenant 20% de WPB pour remplacer partiellement l'orge et le maïs (WPB). Le traitement diététique n'a pas affecté les performances de croissance. La couleur et les pourcentages de métmyoglobine ont été mesurés sur le muscle longissimus dorsi (LD) pendant 7 jours de stockage au réfrigérateur. L'oxydation des lipides (TBARS) a été mesurée en fonction du temps de stockage au réfrigérateur dans des tranches de muscle crues et cuites (7 jours et 4 jours, respectivement). Le WPB alimentaire a augmenté la concentration d'α-tocophérol dans le LD (P <0,001). Les descripteurs de stabilité de la couleur (a *, b * et H *) ont été affectés par la durée de conservation, mais non par le traitement alimentaire. La métmyoglobine s'est accumulée au fil du temps (P <0,001), mais le régime alimentaire du WPB avait tendance à diminuer les pourcentages globaux de la métmyoglobine (P = 0,051). Les valeurs TBARS ont augmenté avec la durée de stockage (P <0,001). Le groupe WPB a montré des valeurs TBARS plus faibles dans la viande cuite (P <0,05). Une interaction significative entre le traitement diététique et la durée de conservation a été observée pour la valeur TBARS dans la viande crue (p = 0,013), les valeurs les plus faibles ayant été trouvées pour la gale blanche à 7 jours de stockage. Les résultats mettent en évidence l'effet positif de l'alimentation des agneaux avec du WPB sur la durée de conservation de la viande et démontrent, pour la première fois, la contribution de l'α-tocophérol (vitamine E) à cet effet.*

Mots-clés. *Agneau – Stabilité à l'oxydation – Grenade – Viande.*

I – Introduction

The use of *ecological leftovers*, such as by-products from the agro-industry, for ruminant feeding could generate several advantages in terms of sustainability, and it has also been demonstrated that most of these biomasses improve important quality traits of meat when fed to animals (Salami *et al*., 2019). This applies, for instance, to the wastes from the industrial production of pomegranate juice, which has remarkably increased in recent years. Residual portions of the fruits (peels, seeds and part of the arils) compose the "whole pomegranate by-product" (WPB), which is therefore rich in bioactive compounds among which polyphenols, other antioxidant compounds and polyunsaturated fatty acids. We recently demonstrated that the partial replacement of cereals with 20% WPB in diets for lambs increased the content of desirable health promoting PUFA in meat (Natalello *et al*., 2019). Other authors reported positive effects of feeding different pomegranate by-products to ruminants on meat oxidative stability and mainly attributed these effects to the polyphenols (Emami *et al*., 2015a; 2015b). Nevertheless, pomegranate seed oil contains fat-soluble antioxidants which could contribute to the antioxidant effects of WPB. Therefore, the objective of this study was to assess the effects of dietary WPB on the oxidative stability of lamb meat, with a focus on antioxidant compounds other than polyphenols.

II – Materials and methods

1. Animals and diets

The experiment was conducted at the experimental farm of the University of Catania. Comisana male lambs (n=17, 14.82 kg ± 2 kg body weight) were individually housed indoors. After an 8-day adaptation to the experimental diets, lambs were fed *ad libitum* for 36 days: a pelleted barley/corn-based diet (CON, n=8) or the same diet in which 200 g/kg DM of WPB partially replaced barley and corn (WPB, n=9). The fresh WPB containing peels, seeds and residual aril pulp, was dried at 40°C. The amount of offered and refused diet was recorded daily, while bodyweight was measured weekly.

2. Samplings and analyses

Lambs were slaughtered at a commercial abattoir according to the European guidelines. Each carcass was immediately weighted and stored at 4° C for 24 h. The *longissimus dorsi* muscle (LD) was excised and one portion was immediately vacuum-packed and stored at ! 80 °C for vitamin E analysis, while the other was aged vacuum-packaged for 3 days at 4 °C and used for oxidative stability measurements. Vitamin E (α-Tocopherol) in feedstuffs and muscle was analysed as described in detail by Valenti *et al*. (2018), using an HPLC system equipped with a fluorescence detector. The oxidative stability was monitored in raw and cooked LD slices using the storage conditions and analyses detailed by Valenti *et al*. (2018). Briefly, three slices were cooked vacuum-packaged for 30 min at 70°C in a water bath. One of these was used immediately for measurement of lipid oxidation (day 0), whereas the other two slices were placed in polystyrene trays, over-wrapped with PVC film and stored at 4 °C for lipid oxidation analysed after 2 and 4 days. The other three slices were stored raw in the same conditions as cooked meat for 0 (after 2 hours of blooming), 4 and 7 days. At the end of respective storage time, colour was measured in duplicate on each raw meat slice by a Minolta spectrophotometer. The colour descriptors L* (lightness), a* (redness), b* (yellowness), C* (saturation) and H* (hue angle) were measured in the, while the reflectance spectra from 400 to 700 nm wavelength were recorded for calculation of metmyoglobin (MMb, % of total myoglobin). For both raw and cooked meat, lipid oxidation was measured as TBARS values (mg/kg of meat).

Data on muscle vitamin E concentration were analysed using a GLM model to test the effect of the Diet (CON *vs*. WPB). Oxidative stability parameters were analysed using a GLM model to test the

fixed effects of Diet, Time of storage (days 0, 4, 7 or 0, 2, 4) and Diet × Time interaction. The model included the individual animals as random effect nested with the Diet. The Tukey's test was used for multiple comparisons.

III – Results and discussion

For the same animals used in the present study, Natalello *et al*. (2019) reported no effect of the feeding the WPB diet on the main performance parameters (daily bodyweight gain, carcass weight and feed intake). Also, as commented above, the WPB treatment resulted in a higher concentration of total polyunsaturated fatty acids (PUFA) in meat, with a specific effect on some individual health-promoting compounds (Natalello *et al*., 2019). If a higher content of PUFA in meat is desirable from a nutritional perspective, a possible drawback may be represented by the greater susceptibility of PUFA to oxidation, which in turn reduces the resistance of meat to lipid oxidation and colour deterioration. This applies to conditions in which PUFA increase in muscle without a concurrent greater deposition of antioxidants, among which vitamin E plays a major role (Ponnampalam *et al.*, 2014). Nevertheless, in the present study, we found that the colour stability descriptors were not affected by the dietary treatment, but were only subjected to the changes usually observed in meat over time of storage or display (Table 1). Specifically, a* values decreased over the 7-day storage time indicating the loss of redness, while increases in b* and H* reflected meat browning, consistently with the increase in the percentage of metmyoglobin (MMb%) with time. Additionally, the WPB diet tended to reduce the overall MMb% (lower oxidation of myoglobin) in meat, which suggests an antioxidant effect of pomegranate by-product. However, the differences in MMb% were numerically minimal and, probably, not sufficient to produce appreciable effects on the colour descriptors.

Table 1. Effect of the diet and time of storage on meat oxidate stability

	Diet (D)		Time of storage (T)[1]			SEM	*P*-values		
	CON	WPB	0	1	2		D	T	D × T
Colour descriptors and Metmyoglobin of raw meat									
a*	11.6	11.6	12.2^{a}	11.6^{ab}	10.9^{b}	0.166	0.929	<0.00	10.181
b*	11.2	11.4	10.2^{b}	11.9^{a}	11.9^{a}	0.194	0.663	<0.00	10.159
H*	44.1	44.3	39.6^{c}	45.6^{b}	47.5^{a}	0.494	0.620	<0.00	10.087
MMb %	47.6	46.0	39.5^{c}	49.2^{b}	51.3^{a}	0.762	0.051	<0.00	10.498
TBARS values in raw and cooked meat, mg/kg meat									
Raw	0.95	0.56	0.25^{b}	0.80^{a}	1.08^{a}	0.080	0.024	<0.00	10.013
Cooked	3.74	3.07	1.69^{c}	3.60^{b}	4.70^{a}	0.192	0.006	<0.00	10.266

[1]Times 0, 1, 2 indicate respectively: days 0, 4, 7 (raw meat), or 0, 2, 4 (cooked meat).

The results of the present study clearly showed that feeding the WPB diet improved the resistance of meat to lipid peroxidation, with lower TBARS values being found overall in meat from WPB-fed lambs over storage duration in both raw and cooked meat ($P < 0.05$; Table 1). Also, the Diet × Time interaction found for TBARS values of raw meat revealed a lower rate of lipid oxidation development in meat from lambs fed the WPB diet, which brought to lower TBARS values after 7 days of storage compared to CON ($P < 0.05$; Fig. 1a). These results are consistent with previous reports demonstrating that feeding different pomegranate by-products to ruminants improved the antioxidant status and oxidative stability of meat (Kotsampasi *et al*., 2014; Emami *et al*., 2015a; 2015b). However, the authors mainly attributed these effects to the occurrence of phenolic compounds in pomegranate fruits and did not determine the content of vitamin E and other fat-soluble antioxidants in feeds and muscle. Only Shabtay *et al*. (2008) reported greater content of vitamin E in the blood of calves, but meat oxidative stability was not studied. In the present study, feeding the WPB diet produced a greater deposition of α-tocopherol in muscle (Fig. 1b). This result can be explained to

the greater content of this molecule in the WPB diet compared to CON (16.8 vs 7.8 mg/kg DM, respectively; data not shown), which was likely due to the occurrence of vitamin E in pomegranate seeds. Therefore, the higher concentration of vitamin E in meat from the WPB-fed lambs may have at least partially increased the oxidative stability of meat compared to CON.

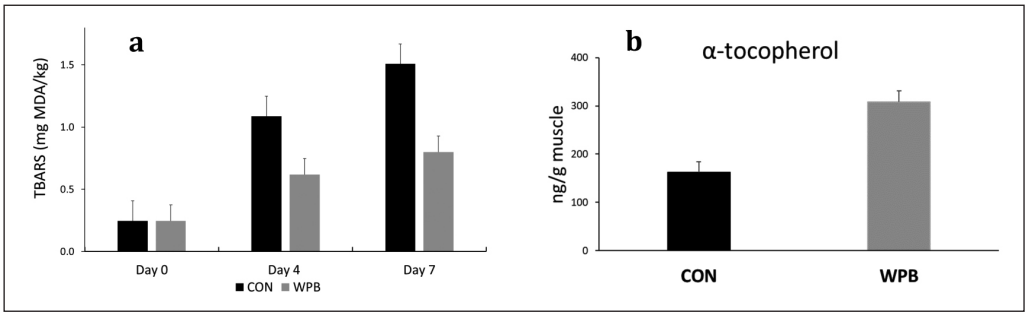

Fig. 1. Lipid oxidation (a) and α-tocopherol content (b) in raw meat.

IV – Conclusions

The results of this study demonstrated that replacing conventional feedstuffs with whole pomegranate by-product in diets for lambs allows improving meat quality traits without compromising productive performances. Specifically, here we found that meat from lambs fed the WPB diet was more resistant to oxidation, despite the greater content of PUFA previously reported. Also, our results suggest, for the first time, that α-tocopherol contributes to the antioxidant capacity of meat from animals fed pomegranate by-product.

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