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Dietary pomegranate pulp to improve meat fatty acid composition in lambs

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Abstract. The aim of this study was to evaluate the effect of dietary pomegranate pulp (PP) on the intramuscular fatty acid (FA) composition in lambs. Seventeen Comisana lambs were individually stalled, divided into two groups and fed for 44 days: a barley-corn based concentrate diet (CON-8 lambs) or a concentrate diet containing 20% of PP to partially replace barley and corn (POM-9 lambs). The inclusion of PP in the diet produced an overall improvement of the fatty acid composition of LTL. In particular, percentages of C18:1 t11 (0.73 *vs* 1.38, P < 0.001), C18:2 c9t11 (0.35 *vs* 0.94, P < 0.001), C18:3 c9c12c15 (0.37 *vs* 0.51, P < 0.001) and C18:2 c9c12 (5.37 *vs* 7.09, P = 0.035) were higher in the meat of POM group in comparison with CON lambs. All the mentioned FAs are involved in the ruminal metabolism of lipids, suggesting an effect of the bioactive compounds contained in PP on the FA biohydrogenation. In addition, C18:3 c9t11c13 (punicic acid), the peculiar FA of pomegranate seeds, was detected only in the muscle of POM lambs, representing 0.48% of the total fatty acids. The percentage of PUFA was significantly higher in meat from POM group compared to CON. In conclusion, a diet containing 20% of PP increased the proportion of healthy fatty acids in meat without compromising animal growth.

Keywords. Pomegranate – Lamb – Fatty acids – Conjugated fatty acids.

Régime à base de pulpe de grenade pour améliorer la composition en acide gras dans la viande d'agneaux

Résumé. *Le but de cette étude est d'évaluer l'effet sur la composition en acide gras intramusculaire des agneaux exposés à une alimentation basée sur l'utilisation de pulpe de grenade. Dix-sept agneaux d'espèce Comisana ont été séparés dans des box, classés en deux groupes et nourris pour 44 jours : un régime concentré à base d'orge et de maïs (8 agneaux) ou un régime concentré composé à 20% de pulpe de grenade qui remplace, en partie, l'orge et le maïs (9 agneaux). L'inclusion dans le régime de pulpe de grenade a produit, en général, une amélioration de la composition en acide gras du muscle LTL. En particulier, les pourcentages de C18 :1 t11 (0,73 vs 1,38, P < 0,001), C18 :2 c9t11 (0,35 vs 0,94, P < 0,001), C18 :3 c9c12c15 (0,37 vs 0,51, P < 0,001) et C18 :2 c9c12 (5,37 vs 7,09, P = 0,035) ont été plus élevés dans les viandes du groupe alimenté avec la pulpe de grenade (POM) en comparaison avec les agneaux du groupe alimenté avec l'orge et le maïs (CON). Tous les acides gras mentionnés sont impliqués dans le métabolisme des lipides des ruminants ce qui suggère un effet des composants bioactifs contenus dans la pulpe de grenade et dans la bio-hydrogénation des acides gras. En outre, C18 :3 c9t11c13 (acide punicique), acide gras spécifique de la pulpe de grenade ; a été détecté seulement dans le muscle des agneaux POM, représentant' 0,48% du total des acides gras. Les pourcentages de AGPI et AGMI ont considérablement augmenté dans la viande du groupe (POM) par rapport à celle du groupe (CON). En conclusion, un régime contenant 20% de pulpe de grenade a permis d'augmenter la proportion de bons acides gras dans la viande, sans compromettre la croissance des animaux.*

Mots-clés. *Grenade – Agneau – Acides gras – Acides gras combinés.*

I – Introduction

Pomegranate (*Punica granatum* L.) is widely consumed as fresh fruit and juice and is appreciated for its health-promoting potential. In the last years, the increased production of pomegranate juice has led to an increase in the amount of its by-products. Pomegranate pulp (PP) is the residue of pomegranate juice extraction, and consists of peels, membranes, seeds and residual arils. All the components of PP have been reported to contain bioactive compounds in higher proportion than the edible fractions (Goula & Lazarides, 2015). PP contains phenolic compounds such as flavonoids, anthocyanidins, ellagitannins, ellagic acid, punicalagin and gallic acid, which possess antimicrobial, antioxidant, antinflammatory, and antitumoral properties (Viuda-Martos *et al.,* 2010). Pomegranate seeds contain about 12-20% oil (Lansky & Newman, 2007), with punicic acid (C18:3 c9t11c13) accounting for approximately 60-80% of total fatty acids. Punicic acid is one of the few naturally occurring conjugated linolenic acids (CLnA). The CLnA isomers are known to directly exhibit several health benefits that are largely based on animal and *in vitro* studies (Yuan *et al.,* 2014). Furthermore, studies have shown that CLnA isomers can be metabolized into conjugated linoleic acid (CLA) *in vivo* (Tsuzuki *et al.,* 2006) and *in vitro* (Schneider *et al.,* 2013), with the consequent health benefits of CLA. The aim of the present study was to investigate the effects of dietary pomegranate pulp on the intramuscular fatty acid composition of lambs.

II – Materials and methods

Seventeen male Comisana lambs (60 days of age) were individually housed and randomly assigned to one of two dietary treatments. After 8 days of gradual adaptation to the experimental diets, the following diets were offered *ad libitum*: a pelleted diet composed of 20% wheat bran, 20% alfalfa hay, 12% soybean meal, 22.5% barley, 22.5% corn, 1% molasses and 2% mineral premix (CON-8 lambs) and the same diet containing 20% pomegranate pulp (PP) to replace 50% barley and 50% corn (POM-9 lambs). Both diets were isoenergetic and isonitrogenous. Individual intakes and live weight were recorded over the trial. After 36-days of experimental feeding, lambs were slaughtered. The *longissimus thoracis et lumborum* muscle (LTL) was removed, vacuum packed and stored at -80 °C until analysis. Phenolic compounds (Makkar *et al.,* 1993) and fatty acids (Sukhija & Palmquist, 1988) were analysed in the feeds.

Table 1. Phenolic compounds and fatty acids of the experimental diets

Chemical composition	CON	POM
Total phenols†	3.04	18.91
Total tannins†	1.41	16.96
Individual fatty acids,% total fatty acids		
C16:0	14.27	10.90
C18:0	2.27	2.11
C18:1 c9	18.51	13.13
C18:2 c9c12 (LA)	46.35	32.74
C18:3 c9c12c15 (ALA)	5.32	4.01
C18:3 c9t11c13 (PA)	0.00	21.68

† Expressed as gram tannic acid equivalent per kg DM.

The CON diet contained higher levels of C16:0, C18:1c9, C18:2 c9c12 and slightly higher C18:3 c9c12c15 than POM diet. A remarkable amount of C18:3 c9t11c13 (punicic acid, PA) was found only in the POM diet (21.68%; Table 1). The intramuscular fat of LTL sample was extracted (Folch *et al.,* 1957) and fatty acid methyl esters (FAME) were prepared (Christie, 1982). Gas chromatographic analyses was carried out with a GC 8000 Top (Thermo Fisher Scientific Inc.), equipped with

a flame ionization detector (FID) and with a 100-m high-polar fused silica capillary column. Data were analysed using a GLM model to test the effect of the dietary treatment (diet: CON or POM). The individual lamb was considered the experimental unit.

III – Results and discussion

The inclusion of PP in the diet did not affect the performance parameters of lambs: animals in the two treatments had comparable dry matter intake, final body weight and average daily gain (data not shown).

Table 2. Effects of dietary treatment on some selected fatty acid composition of *longissimus thoracis et lumborum* (g/100 g of fatty acids)

	Dietary treatment			
Chemical composition	**CON**	**POM**	**SEM**	**P value**
C18:1 t11 (VA)	0.73	1.38	0.133	0.012
C18:2 c9, c12 (LA)	5.37	7.09	0.409	0.035
C18:2 c9, t11 (RA)	0.35	0.94	0.084	<0.001
C18:3 c9, c12, c15 (ALA)	0.37	0.51	0.022	<0.001
C18:3 c9, t11, c13 (PA)	0.00	0.48	0.062	<0.001
SFA	41.43	41.24	0.770	0.910
MUFA	43.98	41.46	0.532	0.016
PUFA	8.50	12.28	0.827	0.020
OBCFA	3.32	2.12	0.190	<0.001
PUFA n-3	0.86	1.19	0.087	0.062
PUFA n-6	7.27	9.64	0.662	0.080
n-6/n-3	8.51	8.18	0.138	0.244

Despite the greater amounts of linoleic acid (LA, C18:2c9c12) and alpha-linoleic acid (ALA, C18:3 c9c12c15) in CON diet as compared to POM diet, these fatty acids were present in significantly higher concentrations in the muscle of POM fed lambs ($P < 0.05$; Table 2). Considering that in ruminant meat these fatty acids can only be derived from the diet, the results suggest that biohydrogenation was inhibited in the lambs fed POM diet. It has been widely demonstrated that ruminal biohydrogenation may be inhibited by tannins (Vasta & Luciano, 2011), which were abundantly present in POM diet. In fact, there were 6-fold higher total phenols and 12-fold higher total tannins in POM diet in comparison to CON diet (Table 1), which could have exerted inhibitory effects on the microorganisms in the rumen that participate in the biohydrogenation. Furthermore, a higher concentration of OBCFA, which originated from microbial rumen population, in muscle of lambs fed the CON diet in comparison to muscle of lambs fed POM supports our above suggestion. Moreover, the increase of VA in the muscle of POM lambs (1.38%) than in the muscle of CON group (0.73%) could lead to hypothesis that the last step of ruminal BH was mostly inhibited. Similar results were found by Emami *et al.* (2015) using 5-10-15% pomegranate seed pulp in the diets of kids.

The significantly higher amount of rumenic acid (RA) in POM fed lamb muscle (0.94%) as compared to that in CON fed lamb muscle (0.35%), could have been derived from both the isomerization of LA in rumen and from synthesis in the tissues by the action of Δ^9-desaurase on vaccenic acid (VA) (Palmquist*, et al.,* 2004). Moreover, the RA may also be derived from metabolism of PA in liver and small intestine (Tsuzuki *et al.,* 2006). The punicic acid (PA) was detected only in the muscle of POM lambs, likely because it partially escaped from ruminal biohydrogenation. Lastly, it may be supposed that RA may also derive from the direct ruminal BH of PA. Indeed, the saturation of the c13 double bond of PA would directly produce RA.

The inhibition of biohydrogenation has resulted in a greater amount of PUFA (*P = 0.020*) in meat of POM fed lambs (12.28%) than of CON fed lambs (8.50%). Unexpectedly, no effect of the dietary treatment was found for the sum of SFA. If the ruminal BH was inhibited by tannins, the muscle from POM animals would have been expected to possess a lower content of SFA than the CON treatment, which was not the case in the present study (*P = 0.910*). A possible explanation could be that the SFA were produced in greater amount in the rumen of CON lambs than POM animal, but the higher concentration of PUFA in POM muscles had decreased the Δ^9-desaurase activity (Gillis *et al.,* 2004) in lambs fed POM diet than CON group. As a consequence, the major amount of SFA and Δ^9-desaurase activity generated higher level of MUFA in CON muscles compared to POM muscles.

IV – Conclusions

The fatty acid composition of lamb meat could be improved by feeding a diet containing 20% pomegranate pulp, with no adverse effects on animal performances. The presence of pomegranate in the diet resulted in increased concentrations of conjugated linoleic acid (rumenic acid), vaccenic acid, " -linolenic acid and punicic acid in the meat. Specific studies are needed to clarify which specific bioactive components of pomegranate pulp could affect fatty acid metabolism in ruminants. In conclusion, the pomegranate pulp as a low-cost feedstuff source could be used successfully as a replacement for cereal grains in ruminant nutrition.

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