CAN THE INCLUSION OF POMEGRANATE POMACE IN RABBITS DIETS IMPROVE THE FATTY ACID PROFILE OF THEIR MEAT?

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ABSTRACT

The present research studied the effect of the inclusion of pomegranate pomace in fattening rabbit diets on the fatty acid profile of the intramuscular fat. Thirty-six New Zealand white rabbits were weaned at 35 d old, allotted in two groups and fattened in cages with 6 animals each. Both groups were fed *ad libitum*, either the medicated control diet (CON) or the experimental diet: a non-medicated control supplemented with 20% of pomegranate pomace (POM). At 65 days of age, rabbits were slaughtered and after 24h samples of *Longissimus dorsi* were obtained. Meat from rabbits from POM group reached the same intramuscular fat but a distinctive fatty acid profile compared to CON. The bioactive compound punicic acid was only present in the meat from animals fed the by-product tested, achieving 5.40% of the total fatty acids. The inclusion of pomegranate pomace in the fattening rabbit diets increased the intramuscular percentage of polyunsaturated, saturated, total *n*-3 fatty acids, thrombogenic and peroxidability indices, and decreased the monounsaturated and the *n*-6/*n*-3 ratio.

Key words: Meat quality, Feeding, By-product, Intramuscular fat, Punicic acid.

INTRODUCTION

The rabbit is able to use fodder not consumed by humans, to produce a food of high nutritional quality, favoring the sustainability of agricultural systems. The excellent dietetic and nutritive properties of rabbit meat as food is usually recognised, between other characteristics, due to its low fat content and high percentage of *n*-3 fatty acids, but the fatty acid composition can change through animal feeding (Dalle Zotte and Szendro, 2011).

During the industrial production of pomegranate juice, important amounts of pomace (composed mainly of non-edible rind, residual arils pulp and carpelar membranes) are produced (Cano-Lamadrid *et al.*, 2018). Previous studies have tested the use of pomegranate by-products in ruminants and broilers diets, showing promising results (Sharifian *et al*., 2019). In lambs, the use of pomegranate by-products improved the fatty acid profile of the meat, not only due to the contribution of specific fatty acids derived from them, but also to the indirect effect of compounds such tannins, that can affect the biohydrogenation of fatty acids (Natalello *et al.*, 2019). In rabbits, pomegranate by products were tested in terms of animal performance and meat quality (María *et al.*, 2019; 2020). However, their effect on the nutritional composition of the meat was not reported in this specie.

Thus, the aim of this experiment was to study the effect of the inclusion of 20% of pomegranate pomace in fattening rabbit diet on the profile of intramuscular fatty acids.

MATERIALS AND METHODS

Animals and experimental design

Thirty-six New Zealand white rabbits were weaned at 35d old, allotted in two groups (balanced by sex) and fattened in cages with 6 animals each until reaching 65d old. Weaned rabbits were fed *ad libitum* one of the two diets: a medicated commercial control diet (CON) and the experimental diet: a non-medicated control supplemented with 20% of pomegranate pomace (POM). The commercial pellets contained alfalfa forage flour, vetch, fescue, ray-grass, barley, sunflower seed meal, corn gluten feed, soybean hulls, palm kernel pressure cake, sugar cane molasses, wheat bran, calcium carbonate, palm vegetable oil, sodium chloride, vitamins and minerals. The "withdrawal food" replaces the CON diet a week before slaughter. The CON diet has robenidine hydrochloride as coccidiostat. Housing conditions were set at 20°C and 16 L:8 D was the lightening schedule and they had free access to water. Diet samples were taken during the experiment to analyse the chemical and fatty acid compositions (table 1).

The same day, all rabbits were slaughtered without fasting in a commercial slaughterhouse located at 28 km. After slaughter, carcasses were immediately transported to the Laboratory of the Animal Production Unit from the University of Zaragoza and refrigerated at 4 ºC for 24 h. The right cranial *longissimus dorsi* were minced, obtaining two replicates and freeze-dried.

Chemical Analyses

Chemical composition of the diets and fat percentage of the meat were performed at CITA (Zaragoza, Spain) at the Laboratory of Physical-Chemical and Instrumental Analysis. The fatty acid profile was carried out on diets and *longissimus dorsi* muscle samples according to Lee *et al*. (2012), at the Institute of Science and Technology of Food and Nutrition (ICTAN) of Madrid. Indexes of atherogenicity (AI) and thrombogenicity (TI) were calculated (Ulbricht and Southgate, 1991): AI = (C12:0 + 4 × C14:0 + C16:0)/ [(MUFA + *n*-6) + *n*-3)]; TI= (C14:0 + C16:0 + C18:0)/ [(0.5 × MUFA + 0.5 × *n*-6+ 3 × *n*-3) + (*n*-3/*n*-6)]. And also the peroxidability index (PI), according to Arakawa and Sagai (1986): PI= (% monoenoic × 0.025) + (% dienoic × 1) + (% trienoic × 2) +(% tetraenoic × 4) + (% pentaenoic × 6)+ (% hexaenoic × 8).

Table 1: Chemical composition (%) and fatty acid (FA) composition (% of total fatty acids) of the experimental diets

	CON	POM
Chemical composition (%)		
Dry matter	91.54	91.75
Ether extract	4.61	5.10
Crude protein	14.08	13.64
Neutral detergent fibre	53.46	40.91
Acid detergent fibre	33.60	20.36
Acid detergent lignin	7.84	4.11
FA composition (% of total fatty acids)		
C12:0	7.91	7.28
C14:0	3.28	2.72
C16:0	22.25	16.68
C18:0	3.03	2.82
C18:1*n*-7	0.82	0.75
C18:1*n*-9	33.22	18.77
C18:2*n*-6	23.59	23.36
C18:3*n*-3	3.21	2.22
C18:3*n*-5	n.d.	23.13

CON= control diet; POM= experimental diet supplemented with 20% of pomegranate pomace; SFA: sum of saturated fatty acids; MUFA: sum of monounsaturated fatty acids; PUFA: sum of polyunsaturated fatty acids; n.d.: no detected.

Statistical Analysis

ANOVA was used to evaluate the effect of pomegranate pomace dietary on the fat percentage and fatty acid profile, using the SPSS software (v22.0).

RESULTS AND DISCUSSION

The fat percentage and the fatty acid composition of the intramuscular fat of rabbits fed on control (CON) or the experimental diet containing 20% of pomegranate pomace (POM) is reported in table 2. Overall, the intramuscular fat of the *Longissimus dorsi* muscle of rabbits fed CON showed a SFA, MUFA, and PUFA percentages in line with what found by other authors (Dalle Zotte and Szendro, 2011; Mattioli *et al.,* 2018). The POM group decrease the percentage of MUFA, mainly due to lower proportion of oleic acid, and increased PUFA for the higher proportion of punicic, linoleic and eicosapentaenoic (EPA) acids. Consequently, total *n*-3 and *n*-6 fatty acids in the intramuscular fat of the experimental group increased by 90.70% and 10.97%, respectively, compared to the control.

The percentage of punicic acid found in POM is not negligible, since it is considered an anticarcinogenic compound and comes from the pomegranate by-product tested (Grossmann *et al.*, 2009). Furthermore, the *n*-6/*n*-3 ratio showed an advantageous decrease in the experimental group compared to the control. However, the results of the control group are higher compared to other authors (Dalle Zotte *et al.*, 2018; Peiretti *et al.*, 2011). The *n*-6/*n*-3 ratio in rabbit meat generally exceed the recommended value (4) given by the Department of Heath (1994), because of the high linoleic acid percentage (Dabbou *et al.,* 2014).

Table 2: Fatty acid profile (% of total fatty acids), fatty acid ratios and nutritional indices of intramuscular fat of rabbits fed different diets

	CON	POM	*P* value
Fat	1.54 (0.39)	1.28 (0.59)	0.130
C16:0	28.19 (1.36)	27.28 (1.32)	0.050
C18:0	6.66 (0.58)	9.31 (0.65)	<0.001
SFA	42.37 (1.36)	43.42 (1.53)	0.036
C18:1*n*-9	24.96 (1.34)	18.03 (1.06)	<0.001
MUFA	29.30 (1.87)	19.90 (1.15)	<0.001
C18:2*n*-6	20.71 (1.49)	22.27 (1.23)	0.002
C18:3*n*-3	1.12 (0.15)	1.04 (0.15)	0.102
C18:3*n*-5	n.d.	5.40 (1.04)	<0.001
PUFA	27.83 (2.89)	36.68 (2.32)	<0.001
n-3	1.29 (0.12)	2.46 (0.15)	<0.001
n-6	25.97 (2.76)	28.82 (2.93)	0.005
n-6/*n*-3	20.31 (3.15)	11.70 (0.92)	<0.001
PUFA/SFA	0.66 (0.09)	0.85 (0.08)	<0.001
C18:2*n*-6/ C18:3*n*-3	18.85 (3.07)	22.12 (4.48)	0.015
PI	47.23 (7.62)	67.66 (7.83)	<0.001
AI	0.83 (0.08)	0.88 (0.11)	0.099
TI	0.76 (0.07)	0.94 (0.07)	<0.001

CON= control diet; POM= experimental diet supplemented with 20% of Pomegranate pulp; *P* value is significant at $P < 0.05$; SFA: sum of saturated fatty acids; MUFA: sum of monounsaturated fatty acids; PUFA: sum of polyunsaturated fatty acids; PI: peroxidability index; AI: atherogenicity index; TI: thrombogenicity index; n.d.: no detected.

The atherogenic (AI) and thrombogenic (TI) indices indicate the potential effects that the fatty acids might have on human health: AI assesses the risk of atherosclerosis, while TI evaluates the potential aggregation of blood platelets, which must be as low as possible and still <1 (Perna *et al.,* 2019). In this study, the TI value in the intramuscular fat of the experimental group were increased by 27.21% compared to the control group, but remained lower than 1. This was due to the higher percentage of SFA.

Due to the high PUFA percentage, the meat from POM can be considered more susceptible to lipid peroxidation, as showed the peroxidability index compared to CON.

CONCLUSIONS

In terms of human health, while the intramuscular fat from rabbits fed diets supplemented with 20% of pomegranate pomace accumulate punicic acid and improved the polyunsaturated fatty acids, total *n*-3 and the *n*-6/*n*-3 ratio; oleic, saturated fatty acids and the thrombogenicity index were less favourable in comparison to a conventional feeding. However, since total fat is very low, the amount of fatty acids delivered by eating rabbit meat is minimal. Furthermore, susceptibility to oxidation might be higher in the tested diet. Other considerations, such as sustainability and feeding cost should be considered.

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