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Dietary pomegranate seed pulp increases conjugated-linoleic and -linolenic acids in muscle and adipose tissues of kid

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ABSTRACT

The effect of level of dried pomegranate seed pulp (PSP) in the diet of kids on meat quality and fatty acid profiles of intramuscular and subcutaneous fat was studied. Thirty two *Mahabadi* kids were randomly allocated to four dietary treatments: without PSP (control), containing 50 g PSP/kg DM (PSP5), containing 100 g PSP/kg DM (PSP10), and containing 150 g PSP/kg DM (PSP15). At the end of the 84-day feeding trial, the kids were slaughtered and *m. longissimus lumborum* (LL) and subcutaneous adipose tissues were sampled. Addition of PSP linearly increased ($P = 0.01$) fat content and decreased ($P < 0.01$) shear force, drip loss, total aerobic bacterial count and lipid oxidation of LL muscle. Feeding PSP diets linearly increased the concentrations of C18:2 n-6 ($P < 0.01$), C18:3 n-3 ($P < 0.001$), n-6 polyunsaturated fatty acids (PUFA; $P < 0.01$) and n-3 PUFA ($P < 0.001$) and decreased ($P < 0.05$) the ratio of n-6/n-3 in both muscle and adipose tissues. A linear increase was observed in vaccenic acid (VA, $P < 0.01$), conjugated linoleic acid (CLA; $P < 0.001$) and punicic acid (PUA; $P < 0.001$) concentration in subcutaneous and intramuscular fat, with increasing PSP level in diet. In conclusion, PSP supplementation of kid's diet up to 150 g/kg DM can improve the nutritional and functional properties of meat.

1. Introduction

There has been an increased interest in food containing high amount of polyunsaturated fatty acids (PUFA) because these fatty acids (FA) are considered as functional ingredients to prevent coronary heart disease and other chronic diseases in human (Krauss et al., 2001; Russo, 2009). Thus, a lower saturated fatty acids (SFA) and a higher PUFA intake, especially of n-3 PUFA to achieve an appropriate n-6/n-3 ratio (<5:1, World Health Organization, 2003) are recommended in order to avoid cardiovascular-type diseases. Conjugated fatty acid (CFA) is the general term of positional and geometric isomers of PUFA with conjugated double bonds. It has been reported that conjugated linoleic acid (CLA), the CFA form of linoleic acid, has favorable physiological effects, such as anti-atherosclerosis, anti-obesity, anti-tumor, and anti-hypertension (Lee et al.,

Abbreviations: ADF, acid detergent fiber; CFA, conjugated fatty acid; CFU, colony forming units; CLA, conjugated linoleic acid; CLnA, conjugated linolenic acid; CP, crude protein; DM, dry matter; EE, ether extract; FA, fatty acids; FAME, fatty acid methyl esters; GC, gas chromatograph; LL, *m. longissimus lumborum*; MUFA, mono-unsaturated fatty acids; NDF, neutral detergent fiber; NFC, non-fibrous carbohydrates; PSP, pomegranate seed pulp; PUA, punicic acid; PUFA, polyunsaturated fatty acids; SEM, standard error of mean; SFA, saturated fatty acids; TBARS, thiobarbituric acid reactive substances; VA, vaccenic acid.

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1994; Nagao et al., 2003). The specific CLA isomer(s) responsible for these various biological effects has not been clearly established, although *cis*-9, *trans*-11 CLA is thought to be the key in the anti-carcinogenic effects (Ha et al., 1990; Ip et al., 1991).

Conjugated linolenic acid (CLnA) occurs abundantly in some specific seed oils, such as karela oil (Dhar and Bhattacharyya, 1998), tung oil (Igarashi and Miyazawa, 2000; Suzuki et al., 2001), and pomegranate oil (Suzuki et al., 2001). Many studies have been conducted in order to manipulate diets of ruminants to increase the PUFA levels of meat, thereby improving its potential health benefits (Manso et al., 2009; Ponnampalam et al., 2001; Scollan et al., 2001). However, increased PUFA level may limit the shelf-life of meat, because they are more prone to oxidation. Use of dietary antioxidants is recommended to limit lipid peroxidation and preserve animal health and product quality (Wood and Enser, 1997). Many synthetic and natural substances have been investigated as potential antioxidants to prevent lipid oxidation. Recent investigations have focused on naturally occurring molecules to satisfy consumer concerns over safety and toxicity of food additives. The use of agro-industrial by-products as alternative feed resources may be a strategy to improve the FA composition and the antioxidant content of ruminant's meat and also to reduce the cost of production (Vasta and Luciano, 2011).

Pomegranate seed pulp (PSP) is by-product of the pomegranate juice industries and has powerful antioxidants, anti-inflammatory compounds, vitamin E, sterols, phenols and natural estrogens (Fadavi et al., 2006; Lansky and Newman, 2007). The PSP contains large amounts of oil, with some varieties having total lipid contents ranging from 6.6 to 19.3 g/kg DM (Fadavi et al., 2006). Recent studies have found that pomegranate by-products may have the potential to be a good source of nutrients and antioxidants for livestock nutrition. At present, research on the effect of pomegranate by-products in animal nutrition is restricted to the inclusion of pomegranate by-products as fresh peel (Shabtay et al., 2008), seeds (Modaresi et al., 2011), extract additives (Oliveira et al., 2010; Shabtay et al., 2012) or peel and seed silage (Kotsampasi et al., 2014) in ruminant's diet. In addition, as far as we know, no published data have evaluated the effects of feeding PSP on meat quality of fattening kids. Thus, the objective of the current study was to evaluate the effect of replacing part of the cereal grains in the diet with PSP on meat quality and specifically FA profile of subcutaneous and intramuscular lipids of *Mahabadi* kids.

2. Materials and methods

2.1. Design, animal and diet

Animals were cared for according to the guidelines of the Iranian Council of Animal Care (1995). Thirty-two growing *Mahabadi* goat kids with an average initial body weight of 16.5 ± 2.8 kg were randomly assigned to one of four treatments (eight kids per treatment): a control group fed a basal diet (control) and three groups fed with diets contained dried PSP at 50 g/kg DM (PSP5), 100 g/kg DM (PSP10) and 150 g/kg DM (PSP15), which PSP replaced barley and corn grains. All of the experimental diets (Table 1) were formulated to meet the requirements recommended by NRC (2007). The PSP (Table 2) used for this study originated from the Yazd variety of pomegranate, and was obtained from the "Anariyan" factory in Ferdows, Iran. Fresh seed pulp (containing 47.5% DM) was dried for 48 h at 65 °C. The diets were based on 700 g concentrates/kg DM and 300 g forage/kg DM. Rations were mixed and fed ad libitum with each kid penned separately. Fresh water was provided. Kids were allowed a 2 weeks adjustment period and were then fed for 12 weeks. Feed offered and refused was recorded daily. At the end of the 84 days feeding period, kids had a 14 h fasting period and were harvested. After harvest, non-carcass components were removed, and then carcasses were chilled at 4 °C for 24 h.

Samples from *m. longissimus lumborum* (LL; between the 12th thoracic and 5th lumbar of the left half carcass), liver and adjacent subcutaneous fat layer were collected. Fresh LL muscle samples were divided into two portions. One portion was vacuum-packed, and stored immediately at −20 °C and then analyzed for chemical composition, thawing loss, FA profile and lipid stability during 1 and 2 months after slaughter. The remaining portion was stored at 4 °C and used to measure pH, drip loss, cooking loss, shear force and total aerobic bacteria counts. Subcutaneous fat and liver samples were stored at −20 °C for determining the FA profile and chemical composition, respectively.

2.2. Analysis of PSP and diets

During the trial, at weeks 4, 8 and 12 the feed samples (diets and PSP) were collected, then were pooled with the same weight ratio and these final samples were ground in a hammer mill with a 1 mm screen (Arthur Hill Thomas Co., Philadelphia, PA), and analyzed (three replicates) for ash (967.05), crude protein (CP, Kjeldahl N × 6.25, 990.03), ether extract (EE, 945.16) according to AOAC (1990). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) content of samples were analyzed (Fibertec 1010, Tecator, Sweden) according to Van Soest et al. (1991). Non-fibrous carbohydrates (NFC) were estimated according to the equation: NFC = 100 − (NDF + CP + EE + Ash). To determine the metabolizable energy (ME) content of PSP, first its gross energy content was measured (16.63 MJ/kg of DM). Then the DM digestibility of PSP was measured by in situ and in vitro (Feng et al., 1996) techniques (the mean value was 81%) and digestible energy (DE) content was calculated (16.63 × 0.81 = 13.47 MJ/kg of DM). The ME content was calculated using the equation of McDonald et al. (1995), ME = 0.80 DE (13.47 × 0.80 = 10.78 MJ/kg of DM).

Samples of PSP were extracted by shaking at room temperature with methanol–water (80:20 vol/vol, 50 ml/g of PSP flour) for 60 min. After centrifugation (15 min, 3000 × *g*), supernatants were collected and kept in the dark at 4 °C until