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Use of apple pomace in animal feed as an antioxidant of meat

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Abstract

The experiment was aimed to evaluate the effects of dietary inclusion of fermented apple pomace (FAP) in lamb diet on physicochemical and lipid oxidation of meat during storage at 4 °C. Twenty-four crossbred sheep were randomly assigned to two experimental diets with or without 11% (dry matter basis) FAP for 56 d. The lambs were then slaughtered, and the *longissimus dorsi* muscle was removed 24 hours after slaughter to measure meat quality. The inclusion of FAP in the diet of sheep did not change lightness and redness of meat, but greater yellowness was observed with increased storage time. Diet did not have any influence on the pH, water-holding capacity (WHC), drip loss (DL) and shear force of meat. Storage time increased yellowness and tenderization of meat. Oxidation of lipids in stored meat from sheep that were fed FAP was lower than that in the control group. It is concluded that the use of fermented apple pomace in the diet of sheep decreased meat oxidation during storage, without affecting other quality characteristics of the meat.

Keywords: Diet, fermented, *longissimus dorsi*, sheep, storage
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Introduction

Agribusinesses related to fruits and vegetables produce considerable amounts of by-products with potential for use in livestock diets (Mirzaei-Aghsaghali & Maheri-Sis, 2008; Taasoli & Kafilzadeh, 2008). In Mexico, the state of Chihuahua is the main producer of apples. Apple waste, as a by-product from the juice industry, represents a potential food source for animals owing to its low cost and highly fermentable nutrients. Yeasts and bacteria play a significant role in the fermentation of apple by-products (Rodriguez *et al*., 2017). One of these by-products is apple pomace (FAP), which has 2.37% protein and 84.7% carbohydrates (O'Shea *et al*., 2015), including polyphenols with antioxidant effects (Lu & Yeap, 2000; Sudha *et al*., 2007; Brenes *et al*., 2008; Ćetković *et al*., 2008). FAP is obtained through a process of aerobic fermentation of apple pomace with the addition of urea, ammonium sulfate and minerals. As a result, a 25% - 28% increase in crude protein is obtained (Alibes *et al*., 1984). This product has been used as an additive to animal feed (Díaz-Plascencia *et al*., 2013). FAP could be an important supplement for sheep, particularly for Katahdin, Charollais and Dorper ovine breeds, which are used for meat production in Mexico owing to their growth rate and carcass characteristics (Vázquez *et al*., 2011).

Many agro-industrial by-products have been added to animal diets to improve meat quality. For example, grapeseed extract improves the oxidative stability of meat (Kim *et al*., 2002), oregano oil delays lipid oxidation during storage (Simitzis *et al*., 2008), beet pulp increases meat lightness and decreases the final pH and cooking weight loss (Olfaz *et al*., 2005), olive pomace increases meat lightness without affecting redness, quebracho *(Schinopsis lorentzii)* increases the redness of fresh meat (Luciano *et al*., 2009) and fermented persimmon peel increases the lightness and redness of meat (Kim *et al*., 2006). However, in apple-producing areas of Mexico, apple pomace is not commonly used in the production of sheep meat, and there are no studies that have examined the effects of FAP on lamb meat quality. The hypothesis was that fermented apple pomace can be used as an ingredient in sheep diets because it improves meat

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characteristics. Therefore, this study was aimed to evaluate the effects of dietary inclusion of FAP in lamb diet on the quality parameters of meat.

Materials and Methods

A fermentation bed (1.5 m wide and 15 m long) was prepared for the production of FAP. To this bed, 1.5% urea, 0.5% of a mineral mixture and 0.4% ammonium sulfate (based on the total wet weight) were added to the apple pomace and mixed with a rototiller to allow fermentation. The bed was stirred four times during the first day of mixing and three times daily from day 2 to day 5 of fermentation to allow ventilation. On day 6, the fermentation bed was spread on the ground to dry in the sun until it reached a dry matter content that permitted storage. The FAP that was prepared had 26% crude protein content and 13% true protein (Rodriguez *et al*., 2017).

Twenty-four sheep were used in this study with an average initial weight of 25.37 ± 2.9 kg and final weight of 42.42 ± 3.1 kg, of 147 ± 6.2 days old, with an average cold carcass weight of 20.5 kg. The slaughter weight was chosen to represent a range of the weight at which local animals are traditionally slaughtered. All animals were fed ad libitum a diet of alfalfa hay and 300 g of concentrate per animal. The sheep were randomly housed in individual cages for a 56-day feeding test. Half of the animals (12) were fed an elemental isoenergetic diet as the control, and the other half were fed diets supplemented with FAP. The diets were formulated according to the NRC (1985) for growing sheep (Gómez-Vázquez *et al*., 2011). The ingredients were the same in the two diets, and only the proportion changed when including FAP in the treatment diet (Table 1).

After the fattening period, animals were fasted for 24 hours and slaughtered by conventional techniques using electrical stunning. The carcasses were stored at 4 °C, and 24 hours later, the *longissimus dorsi* muscle was obtained from the right half of the carcass. This meat portion was divided into two parts: one was stored at -25 °C and was used for the shear force (tenderness) analysis, and the other was used for the remaining physicochemical tests.

Table 1 Ingredients and chemical composition of experimental diets with fermented apple pomace (FAP) for the sheep used in the present experiment

	Diet, g/kg DM	
	Control	**FAP**
Ingredient, g/kg DM		
Alfalfa hay	381	286
Soybean meal	124	94
Corn grain, rolled	425	430
Fermented apple pomace		110
Animal Fat	20	20
Molasses cane	30	40
Salt	10	10
Mineral premix	10	10
Proximal analysis[1]		
Dry matter	905	913
Crude protein	114	116

[1] According to Van Soest *et al*. (1991)

The pH value was measured 24 hours after slaughter using a potentiometer (Thermo Scientific Orion 210A) in a homogenised sample of 1 g meat with 9 mL distilled water for 1 min (Antonomanolaki *et al*., 1999). WHC was determined 24 hours after slaughter using the methods reported by Tsai & Ockerman (1981) with a modification of 0.3 g for the weight of the sample.

Drip loss was determined 24 hours after slaughter, using 3 g (Q1) of meat suspended inside a plastic container at 4 °C for 48 hours. Subsequently, the weight of the sample was recorded one more time (Q2) (Honikel & Jim, 1986). DL was determined by calculating the weight difference before and after refrigeration.

Colour was evaluated on days 1, 4 and 7 of storage after slaughter using a HunterLab MS-S colourimeter, and the coordinates L*, a* and b* were obtained (Garrido *et al*., 1994). Shear force was evaluated in cooked meat samples using a Lloyd Instruments TA1 Texture Analyzer Chatillon texturometer.

The degree of lipid oxidation was determined by measuring thiobarbituric acid (TBA)-reactive substances (TBARS) according to the technique described by Piccini *et al*. (1986). Ten g of muscle were homogenized (ESGE bio homogenizer model M133/1281-0; Bio Spec products Inc., Bartlesville, OK, USA) with a 10% solution of 6N HCl solution for 40 seconds, the resulting mixture was subjected to distillation, and 50 mL aliquots were collected. Afterwards, 2.5 mL distillate was taken and mixed with 2.5 mL 0.02M TBA. The mixture was incubated in a boiling water bath (100 °C) for 40 min. A sample containing 2.5 mL distilled water and 2.5 mL TBA was used as a blank. Both were cooled for 10 min in running tap water and absorbance was measured at 535 nm on a spectrophotometer (Genesys 20, model 4001/4, Thermo Spectronic, Waltham, MA, USA). The results from the samples were plotted against a standard curve prepared with known concentrations of tetraethoxypropane. This procedure was performed in triplicate, and the results were expressed as mg malondialdehyde (MDA) per kg meat (mg MDA/kg meat). The process was carried out in duplicate on days 1, 3, 7 and 10 after slaughter.

Data of shear force and lipid oxidation were analysed as a completely randomized design with two factors (diet and storage time) and 12 experimental units each, using SAS System (2012), whereas data of pH, WHC and DL were analysed as a completely randomized design to evaluate only the effect of diet (FAP) with a total of 24 experimental units. Lambs were considered the random component in the model. Comparisons of means for the group of variables were evaluated using Tukey tests (P <0.05).

Results and Discussion

Meat quality and acceptability are determined by its physicochemical characteristics, especially colour, appearance, flavour and tenderness. Table 2 shows the results for colour of meat. Meat lightness (L*) and redness (a*) did not change with diet (P >0.05), or with storage of meat but yellowness (b*) of meat increased with storage time (P <0.01). (P >0.05)

Table 2 Effect of fermented apple pomace (FAP) added to diet of sheep on the colour[1] of meat stored at 4 °C (mean ± SD)

	Diet		Time (day)			*P* value		
	Control	**FAP**	**1**	**4**	**7**	**D[2]**	**S[3]**	**DxS[4]**
L*	$33.3^{a} \pm 1.3$	$33.2^{a} \pm 1.6$	$33.0^{a} \pm 1.4$	$33.4^{a} \pm 1.3$	$33.2^{a} \pm 1.5$	0.81	0.77	0.94
a*	$6.9^{a} \pm 0.4$	$7.0^{a} \pm 0.4$	$6.9^{a} \pm 0.4$	$7.0^{a} \pm 0.4$	$6.9^{a} \pm 0.4$	0.35	0.62	0.71
b*	$9.5^{a} \pm 0.6$	$9.5^{a} \pm 0.5$	$9.2^{b} \pm 0.6b$	$9.6^{b} \pm 0.4$	$9.8^{a} \pm 0.5$	0.57	0.01	0.57

Row means with different superscripts differ significantly at P <0.05
[1] L*: luminosity, a*: redness, b*: yellowness
[2] D: diet
[3] S: storage
[4] DxS: diet x storage interaction

No interactions were observed between diet and storage (P >0.05) for any colour parameter of the meat. These observations indicate that FAP added to sheep diet does not cause changes in the main colour parameters of meat. In addition, L* and a* values remained constant through days 1 to 7, since differences between values were not significant. However, the colour of the meat showed a tendency to change with time of storage at 4 °C, the yellowness (b*) of meat varied significantly through measured days. Although it remained constant until day 4, it increased at day 7 of storage (P <0.01). The typical colour of lamb or mutton varies from light red to brick red. This optimum surface colour of fresh meat is highly unstable and short-lived. it can change during storage, but this colour change alone does not mean the product is spoiled. It is well known that meat colour changes during display as myoglobin pigments in the meat surface transform on exposure to oxygen from primarily purple deoxymyoglobin to red oxymyoglobin and finally to brown metmyoglobin (Moore *et al*., 2003). In the present study, meat yellowness (b*) increased during storage

might be due to the oxidation of lipids of meat, since lipid oxidation precedes oxymyoglobin oxidation with the consequent change in colour of meat.

Post-mortem biochemical processes influence the colour of muscle foods. As the proportion of metmyoglobin increases, the meat colour turns to brown. Changes in colour during chilling storage have been observed by other authors. Vieira & Fernandez (2014) reported an increase in the b* value of lamb when it was aged for five days at (2 ± 2 °C). Similarly, Fregonesi *et al.* (2014) observed that b* showed an increase over time (P <0.05) up to 28 days when vacuum-packed lamb meat was stored under refrigeration. These authors found the highest b* value at 14 day of storage. In another study, Ponnampalam *et al.* (2013) analysed the surface brownness of meat during retail display from loin cuts of lamb stored for shorter or longer duration. They found that the aged meat was yellower than the fresh meat. These authors did not report changes in L* values of aged meat, but observed an increase of a* values in aged lamb.

Meat redness was not modified because of storage (P >0.05). It has been reported that the organic acids in apples activate the secretion of gastric juices, which enhance iron absorption. Such absorption combined with myoglobin increases redness in meat (Dae *et al*., 2009). Moreover, the concentration of haemoproteins has been shown to increase in pigs fed apples (Muriel *et al*., 2002). However, in the present study, meat redness was not affected by diet (P >0.05). According to Kerry *et al*. (2000), colour is the single most important sensory characteristic that affects consumer purchasing decisions for red meats, because consumers associate red colour with freshness (Morrissey *et al*., 1994). Interactions between diet and storage did not affect the colour coordinates of lamb (P >0.05). In accordance with a study by Mancini & Hunt (2005) supplementation may reduce meat colour deterioration from red to brown and reflect the myoglobin concentration and its redox state in meat.

No differences were observed between treatments (P >0.05) for pH, WHC, and DL (Table 3), demonstrating that fermented apple pomace did not affect those quality parameters. The pH changes occurring in a carcass during the first 24 hours after slaughter are important for the quality of the final meat or meat products. The pH values obtained in the present study (5.19) are close to those reported for lamb 24 hours after slaughter, which normally reaches an average final pH between 5.2 and 5.8 (Lawrie & Ledward, 2006). Abdullah & Qudsieh (2009) observed higher pH values (5.35) in lambs of the same weight (40 kg live weight) than those of the present experiment. It is known that the pH of the *longissimus* muscle is higher in lambs fed grass (5.62) than lambs fed concentrate (5.57) (Brito *et al*., 2016). Although lambs of the present study were fed concentrate, the pH of their meat was even lower. Many factors can affect the final pH of lamb. Reported values of lamb pH are wide. They vary from 5.2 to 5.8 and there are even changes between individual animals (McGeehin *et al*., 2001; Texeira *et al*., 2005). Glycogen content in meat affects the ultimate pH of meat. However, Van Laack *et al.* (2001) mentioned that only 37% of the variation in ultimate pH can account for glycogen concentration, and that other factors affecting ultimate pH variation were unknown.

Water holding capacity was not affected by diet (P <0.05) (Table 3). Similar results were observed by Bianchi *et al*. (2006) who reported that diet does not affect the WHC of lamb. WHC is one of the most important indicators of meat quality. It is defined as the ability of fresh meat to retain its own water during cutting, heating, grinding and pressing, and during transport, storage and cooking. The water released can be described as drip, purge, weep, exudate or cook loss (Huges *et al*., 2014). Poor WHC results in high drip and purge loss, which can represent significant loss of weight from carcasses and cuts, and may affect the yield and quality of processed meat (Aaslyng, 2002). Moreover, the presence of water in meat significantly affects the sensory properties of meat and products made of it.

Table 3 Effect of fermented apple pomace added to diet of sheep on the pH, water-holding capacity and drip loss of meat stored at 4 °C (mean ± SD)

	Diet		*P* value
	Control	FAP	
pH	$5.14^{a} \pm 0.13$	$5.24^{a} \pm 0.17$	0.18
WHC (%)[1]	$64.04^{a} \pm 2.41$	$64.46^{a} \pm 2.25$	0.70
DL (%)[2]	$4.33^{a} \pm 0.65$	$4.74^{a} \pm 0.88$	0.28

Row means with different superscripts differ significantly at P <0.05
[1]WHC: water holding capacity
[2]DL: drip loss

Drip loss was not affected by diet (P >0.05) (Table 3). The observed values for DL (4.53%) were similar to those reported as normal (Offer & Knight, 1988). When the meat is chopped, DL is increased from 2% to 6% of the weight of the lean meat under refrigeration (Offer & Knight, 1988). Sheridan *et al*. (2003) evaluated meat quality in Boer goat kids and Mutton Merino lambs and found that diet did not influence DL in the 8-9-10-rib cut within a species. D'Alesandro *et al.* (2013) found 2.43% of shrinkage loss in 60-day-old lambs, being lower than those of the present study. Recently, it was reported by Budimir *et al.* (2018) that lamb of heavier carcasses (more than 10 kg) have better WHC for raw meat and lower DL than light carcasses.

Meat tenderness (shear force) was affected by storage time (P <0.05) and by diet (P <0.05) (Figure 1). The initial shear force (toughness) of FAB meat was higher (3.08 kg) than that of control meat (2.80 kg). However, after 6 days of storage at 4 °C, the tenderness of meat from both diets was similar (2.00 kg; P >0.05). Although there was a significant difference in the initial meat tenderness between control and FAB treatments, all values observed were within the acceptable range for lambs. Shorthose *et al.* (1986) reported that samples of lamb meat with shear force values of 5 kg or less were considered acceptably tender by Australian consumers. Therefore, the meat of the present study could be considered tender.

Tenderness appears to be the most important sensory characteristic of meat and a predominant quality determinant. Factors that affect meat tenderness include breed, nutrition, age and muscle location. Nutrition influences tenderness principally through its effects on the amount and type of fat in the meat. Post-mortem storage or ageing of meat is the conventional practice used to tenderize meat. Vieira & Fernandez (2014) observed a decrease of 1 kg in shear force of lamb aged for five days at 2 °C. Increasing ageing time was reported to cause a reduction in meat toughness. Physical weakening of structures in the meat, cellular and sub-cellular damage, the release of lysosomes and other proteases, and an increase in protease activity, as well as the muscle protein denaturation at elevated temperatures and low pH, could account for the tenderness of meat. Shear force values of lamb are between 1.84 to 5.0 kg on concentrate, but shear force can go up to 8.7 kg if sheep are on grasslands (Ramirez-Retamal & Morales, 2014). Clelland *et al.* (2018) carried out research with 377 purebred Texel, males and females, and observed shear force values between 2.82 and 3.73 kg, whereas Monteschio *et al.* (2018) reported a mean of shear value of 3.65 kg for wethers of similar weight (40 kg) to those used in the present study.

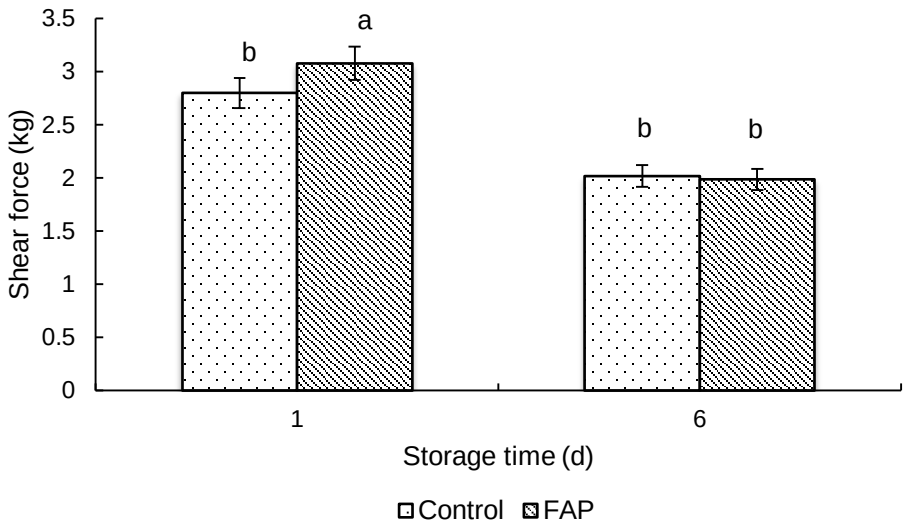

Figure 1 Effect of fermented apple pomace (FAP) added to diet of sheep on the shear force of meat stored at 4 °C (mean ± SD)

Oxidation of meat lipids or TBARS level was measured as malonaldehyde content (MAD) (µg/kg) of the meat (Figure 2). Diet and storage time affected (P <0.05) MAD content. The interaction of diet and days of storage was significant. Similar values of MDA were observed at day 1 of storage for meat from both diets (FAP and control). However, from day 3 onwards, animals fed FAP showed lower oxidation values than those of meat from animals fed the control diet (P <0.05). Natural antioxidants are commonly used in the animal industry and have positive effects on meat oxidation.

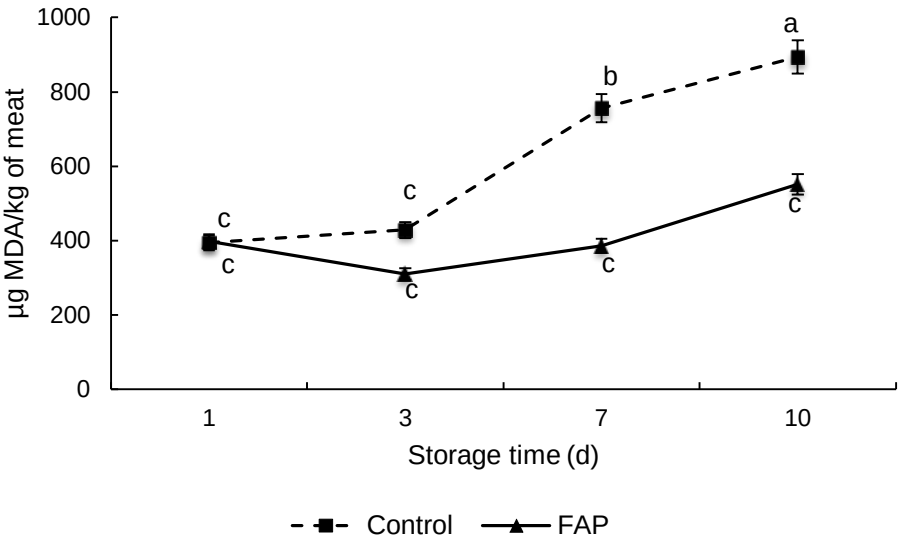

Figure 2 Effect of fermented apple pomace (FAP) added to diet of sheep on lipid oxidation (µg MDA/kg) of meat stored at 4 °C (mean ± SD)
MDA: malonaldehyde
Means with different letters are statistically different (Tukey, P <0.05)

Luciano *et al*. (2009) found that the tannins (from *Schinopsis lorentzii*, a hard wood tree from Argentina), had no significant effect on lipid oxidation of meat from lambs fed this diet. However, during storage, the lipid oxidation of meat from lambs fed the tannin-rich diet decreased. Lipid oxidation products accelerate haemeprotein oxidation, resulting in a positive correlation between metmyoglobin formation and lipid oxidation (Faustman *et al*., 1999). Long ageing also leads to high TBARS levels (measured as MDA), suggesting a product that is likely to exhibit rancidity and off flavours from lipid oxidation (Ponnampalam *et al*., 2013).

Post-mortem biochemical processes are active during retail and storage, and they influence the colour of muscle foods. As the proportion of metmyoglobin increases, meat colour turns to brown. This induces a negative response in consumers. When the proportion of metmyoglobin reaches 20%, it has been found that the product is rejected by half of the potential consumers (Renerre & Mazuel, 1985). After the intake of plant-food by-products, such as apple pomace, the antioxidant activity in muscle increases (Guil-Guerrero *et al*., 2016). As well as the antioxidant effect of apple pomace (Lu & Foo, 2000), it has antibacterial, antiviral and anti-inflammatory properties (Yue *et al*., 2012).

Numerous studies have reported that the polyphenols responsible for the antioxidant activity in apple are still present in the pomace (Gazalli *et al.*, 2014) and in apple peel (Shanmugam *et al.*, 2017). These activities can be up to 30 times better than those of the antioxidant vitamins C and E. Therefore, apple pomace can provide an inexpensive source for food fortification (Foo & Loo, 1999). Several minerals and vitamins are antioxidative, and their roles in protecting the muscle tissue from structural damage are well established (Hercberg *et al.*, 2004). Apple pomace has already been included in the formulation of several meat products (Verma *et al.*, 2010) without changes in overall palatability (Rather *et al.*, 2015).

Conclusion

Based on the results in the present study, it can be concluded that fermented apple pomace (FAP) can replace 11% (DM) of alfalfa hay and soybean meal in sheep diet with an improvement in meat quality. Loin meat from sheep fed FAP will exhibit less oxidation of lipids after storage at 4 °C, while it retains good quality in terms of colour, pH, WHC, DL and tenderness. The decrease of lipid oxidation in meat reflects the benefits of the antioxidant potential of FAB components. Utilization of apple juice by-products in sheep nutrition could improve profitability and valorization of local agricultural wastes since feeding FAP to sheep is an efficient way to produce high-quality meat with longer shelf life.

Authors' Contributions

VL is a master's student, who conducted the experiment. ADAR is the mentor, who was in charge of conception and design, and LCL & HJ did chemical and data analysis and prepared the manuscript.

Conflict of Interest Declaration

The authors have declared no conflict of interest.

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