



UNIVERSIDADE
ESTADUAL DE LONDRINA

FRANCINE MEZZOMO GIOTTO

**PECAN BY-PRODUCTS AS FEEDSTUFF IN LAMB DIETS:
CARCASS CHARACTERISTICS, AND MEAT QUALITY
ATTRIBUTES**

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Tese apresentada ao Programa de Pós-Graduação em
Ciência Animal da Universidade Estadual de
Londrina, como requisito parcial à obtenção do
título de Doutora em Ciência Animal (Área de
Concentração: Produção Animal).

Orientador: Prof. Dr. Edson Luis de Azambuja
Ribeiro.

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BANCA EXAMINADORA

Prof^o. Dr. Edson Luis de Azabuja Ribeiro
Universidade Estadual de Londrina - UEL

Prof^a. Dra. Adriana Lourenço Soares
Universidade Estadual de Londrina - UEL

Prof^a. Dra. Ana Maria Bridi
Universidade Estadual de Londrina - UEL

Prof^a. Dra. Fabíola Cristine de Almeida Rego
Grecco
Universidade Norte do Paraná – UNOPAR

Prof^a. Dra. Sandra Galbeiro
Universidade Estadual de Londrina - UEL

Londrina, 16 de Outubro de 2019.

DEDICATION

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To my family,
my base,
my everything.

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God is always helping me to achieve all of my dreams and goals. He supports me during hard and difficult days, and blesses me with many good days. He showed me that everything is easier when you trust in Him. Specially, He blessed me during my academic journey with many special people that, and I am very thankful for all that they have done for me. Specially...

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*“The greatest obstacle to progress is not
ignorance, but the illusion of knowledge”*

DANIEL BOORSTIN

RESUMO

GIOTTO, Francine Mezzomo. **Pecan by-products as feedstuff in lamb diets: carcass characteristics, and meat quality attributes.** 2019. 112 f. Tese (Doutorado em Ciência Animal) – Universidade Estadual de Londrina, Londrina. 2019.

Este estudo avaliou os efeitos da inclusão do coproduto da noz pecan na dieta de cordeiros. Quarenta e quatro ovinos Santa Inês (24 machos e 20 fêmeas) com aproximadamente $23,24 \pm 3,25$ kg, de peso corporal, $3,56 \pm 0,55$ meses de idade, foram divididos aleatoriamente entre 1 a 4 tratamentos dietéticos (n = 11 por tratamento). As dietas foram formuladas com silagem de sorgo, arroz quebrado, farelo de soja, mistura mineral comercial, calcário calcítico, óleo de soja e quatro níveis de coproduto da noz pecan: 0%, 3%, 6% e 9% (base da MS). Os cordeiros foram alimentados na proporção volumoso:concentrado de 40:60 e, após 52 dias de alimentação, com peso final de $37,17 \pm 4,67$ e $5,28 \pm 0,45$ meses, os animais foram abatidos em frigorífico comercial. Foram calculados peso ao abate, pesos de carcaça quente e fria, rendimento, índice de compactidade, conformação, acabamento, perda na refrigeração, medidas de pernil e da paleta, área de olho de lombo, medidas de lombo, marmoreio, pesos e rendimentos dos cortes comerciais e composição tecidual. As amostras de carne foram submetidas ao perfil de ácidos graxos, oxidação lipídica, força de cisalhamento, análise sensorial, perda por cocção e análises centesimais. Observou-se efeito de gênero para peso da carcaça ao abate (40,20a e 33,95b), peso da carcaça quente (19,99a e 16,68b) e fria (19,19a e 16,05b), perda na refrigeração (4,02a e 3,77b), índice de compactidade da carcaça (0,29a e 0,25b), comprimento de carcaça (66,00a e 62,39b), profundidade torácica (27,45a e 25,64b), comprimento da perna (42,91a e 40,97b), perímetro do braço (18,00a e 16,47b), área do olho de lombo (17,36a e 15,82b), peso dos cortes comerciais, rendimento do pescoço (7,69a e 6,83b) e proporção dos ossos (21,15a e 18,74b) onde os machos apresentaram maiores valores em relação as fêmeas. Fêmeas apresentaram maiores valores de porcentagem de gordura (3,72a e 2,98b), espessura de gordura (1,63a e 0,58b) e rendimento de pernil (31,54a e 30,47b) em comparação aos machos. A inclusão do coproduto da noz pecan não afetou umidade, proteína, pH, perda por cocção, oxidação lipídica e força de cisalhamento. Os tratamentos dietéticos não afetaram os parâmetros objetivos de cor, no entanto, a carne das borregas apresentou-se mais avermelhada e mais amarelada quando comparada à carne dos cordeiros. Observou-se interação entre os tratamentos dietéticos e os gêneros sexuais para a porcentagem de cinzas na carne. A carne do tratamento controle apresentou escores mais altos de maciez e suculência. Maiores níveis de ácido margarico e oleico foram observados na carne das fêmeas, enquanto a carne dos machos apresentou maiores valores de ácido linoleico. A inclusão de 6% das nozes aumentou a deposição de DHA (0,68bc, 0,60c, 1,12a e 1,02ab, controle, 3%, 6% e 9%, respectivamente) enquanto a inclusão de 9% levou a níveis mais altos de EPA (0,04b, 0,01b, 0,09ab e 0,16a, controle, 3%, 6% e 9%, respectivamente). Os resultados sugerem que o coproduto da noz-pecan pode ser utilizado em até 9% como fonte de alimentação para produtores que tem acesso a esse alimento.

Palavras-chave: ácidos graxos, *Carya illinoensis*, painel sensorial, força de cisalhamento, ovinos

ABSTRACT

GIOTTO, Francine Mezzomo. **Pecan by-products as feedstuff in lamb diets: carcass characteristics, and meat quality attributes.** 2019. 112 p. Thesis (Philosophy Doctor in Animal Science) – Londrina State University, Londrina. 2019.

This study evaluated the effects of including pecan by-products into lambs' diet. Forty-four Santa Ines lambs (24 males and 20 females) with approximately 23.24 ± 3.22 kg, of body weight, 3.56 ± 0.55 months old, were randomly assigned to 1 of 4 dietary treatments (n=11 per treatment). Diets were formulated with sorghum silage, broken rice, soybean meal, a commercial mineral mix, calcitic limestone, soybean oil, and four different levels of pecan by-product: 0%, 3%, 6% and 9% (DM basis). Lambs were fed in a roughage:concentrate ratio of 40:60, and after 52 days of feeding, with final body weight of 37.17 ± 4.67 kg, and 5.28 ± 0.45 months old, animals were slaughtered in a commercial slaughter plant. Carcass weight at slaughter, hot and cold carcasses weights, yields and carcass index, conformation, fattening degree, cooling loss, leg and shoulder measurements, loin eye area, loin measurements, marbling, commercial cuts weight and yields, and tissue composition were calculated. Meat samples were subjected to fatty acid profile, lipid oxidation, Warner Braztler Shear Force (WBSF), sensory analysis, cooking loss, and proximate analyses. Gender effect was observed for carcass weight at slaughter (40.20a and 33.95b), hot (19.99a and 16.68b) and cold carcass weight (19.19a and 16.05b), cooling loss (4.02a and 3.77b), carcass index (0.29a and 0.25b), carcass length (66.00a and 62.39b), thoracic depth (27.45a and 25.64b), leg length (42.91a and 40.97b), shoulder perimeter (18.00a and 16.47b), loin eye area (17.36a and 15.82b), commercial cuts weight, neck yield (7.69a and 6.83b), and bones proportion (21.15a and 18.74b) where male lambs showed higher values than ewe lambs. Females had higher values for percentage of fat (3.72a and 2.98b), fat thickness (1.63a and 0.58b), and leg yield (31.54a and 30.47b) when compared to males. Inclusion of pecans did not affect moisture, protein, pH, cooking loss, lipid oxidation, and WBSF. Dietary treatments did not affect objective color parameters, however, meat from ewe lambs were redder and yellower when compared to meat from ram lambs. It was observed an interaction between dietary treatments and sexual gender for ash percentage on meat. Meat from the control treatment had higher scores for tenderness and juiciness. Greater levels of margaric and oleic acids were observed in the lean of ewe lambs, whereas meat from ram lambs had greater values of linoleic acid. Inclusion of 6% of pecans increased deposition of DHA (0.68bc, 0.60c, 1.12a and 1.02ab, control, 3%, 6% e 9%, respectively), whereas a 9% inclusion led to higher levels of EPA (0.04b, 0.01b, 0.09ab and 0.16a, control, 3%, 6% e 9%, respectively). Results suggest that pecan by-products can be used up to 9% as a feeding source for producers who may have access to this feedstuff.

Keywords: *Carya illinoensis*, fatty acids, sensory panel, shear force, sheep

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1. INTRODUCTION

Currently, Brazil has an estimated flock size of approximately 18,948,934 million heads of sheep, with 511,768 producers. Brazilian sheep are primarily found in the Northeast region that has 64.2% of the animals, while the South has 24%, the Midwest has 5%, North has 4%, and the Southeast has 3% (IBGE, 2018).

The production of sheep meat in Brazil has progressively increased due to higher consumption and market demands (FIRETTI *et al.*, 2017). However, consumption is still low and current domestic production is not sufficient to meet demands, which sustains good prices for slaughtered lambs (ARCO, 2018).

According to Ribeiro *et al.* (2004), the success of sheep farming for meat is closely linked to the production of quality carcasses, meat acceptability by consumers, and the use of management practices that make the activity economically viable. As production intensifies, a number of factors can be controlled to achieve a better quality product (HERMUCHE *et al.*, 2013).

The use of feedlots by sheep producers generates positive results by increasing carcass standardization and availability of meat in the off season, while reducing slaughter time of the animals and mortality rates; however, the higher feeding costs can become expensive (SÉRGIO *et al.*, 2007). In this context, studies have been performed with the objective of enabling nutrition with high production rates and reduced costs using agro-industrial byproducts.

Pecans are discarded yearly due to diseases, discoloration, and changes in size or shape generating a by-product rich in fatty acids, fat, protein, fibers, and antioxidants (ORTIZ-QUEZADA; LOMBARDINI; CISNEROS-ZEVALLOS, 2011; ATANASOV *et al.*, 2018; RIVERA-RANGEL *et al.*, 2018) with potential to be used as a feedstuff in animal nutrition.

Very few studies have reported the use of pecan or pecan by-products in animal nutrition. Ramirez *et al.* (1986) evaluated the effect of diets containing 0, 5, or 10% of pecan shells or hulls on milk production in Holstein cows, digestion and N balance in sheep, and rumen fermentation, digestion and passage rates in beef steers. Reyes-Padilla *et al.* (2018) developed a bologna-type meat product using cranberries, prunes, pecan nuts, and flaxseed as ingredients and evaluated the physiochemical, sensory, and nutritional qualities of the

product. No studies have been carried out using pecan by-product in lamb nutrition evaluating carcass characteristics and meat quality attributes.

2. LITERATURE REVIEW

2.1 PECAN

The pecan tree (*Carya illinoensis* (Wangenh.) K. Koch) belongs to the family Juglandaceae, being the specie *Carya illinoensis* known worldwide as pecan, is a fruitful cultivar predominantly in temperate regions of the Northern Hemisphere. Native from United States and Mexico, nowadays it is the most valuable nut tree native to North America. Pecans are commercially produced in Alabama, Arkansas, Arizona, California, Florida, Georgia, Kansas, Louisiana, Missouri, Mississippi, North Carolina, New Mexico, Oklahoma, South Carolina, and Texas (SAGARAM; LOMBARDINI; GRAUKE, 2007). The United States is the world leader in pecan nut production, accounting for about 50% of the total production (FABRIZIO; ELMARIE; GESINE, 2018). In past decades, pecans have been introduced to many other countries including South Africa, Australia, Argentina, and China (ZHANG; PENG; LI, 2015), and the world production increases every year (Figure 1).

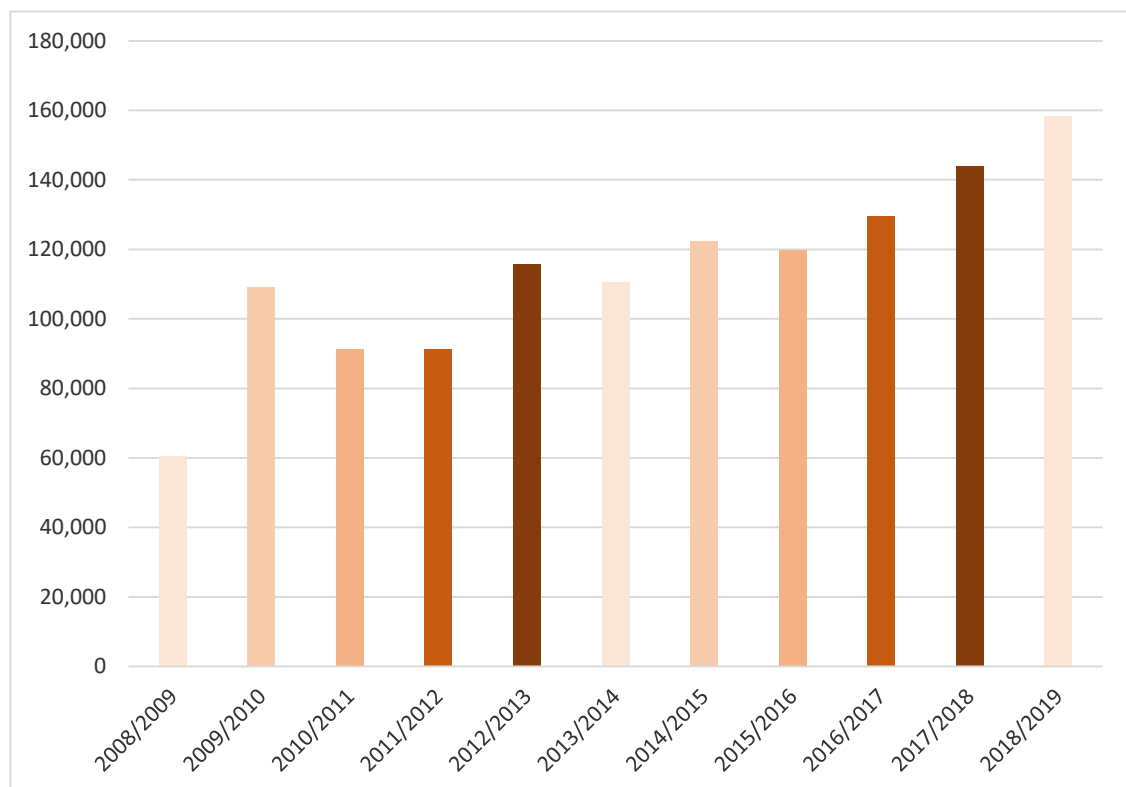


Figure 1. World pecan production (tons).
Source: International Nut and Dried Fruit Council (2018)

In Brazil, pecans were introduced in 1910 in Santa Bárbara D'Oeste, Piracicaba, and Americana, in the state of Sao Paulo. In 1915, they were introduced to Nova Friburgo, and Rio de Janeiro. Around 1943, Armínio Miotto, mayor of Anta Gorda, introduced the first seedlings of pecans in Rio Grande do Sul. Due to the good adaptability, Armínio Miotto started to produce seedlings and commercialize it in the different regions of Southern Brazil (MARTINS *et al.*, 2018).

The great evolution of pecan was through public policies to encourage the planting of forests in the 1960s and 1970s. Law No.5.106 / 66, regulated by Decree 59.615/66 (BRASIL, 1966), encouraged the tax-free planting of forests, enabling the planting of some fruit species, among them being pecan. At this time, the crop began to be commercially exploited, being cultivated from the State of Minas Gerais to Rio Grande do Sul. During this period, several orchards were established, reaching about 17 thousand ha, mainly concentrated in the southern region of Brazil. However, its continuity has been compromised by phytosanitary problems and lack of support, technical information, and research to support its cultivation (RASEIRA, 1990; HAMANN *et al.*, 2018).

Pecan nut cultivation has been growing in recent years, especially in the southern region of Brazil, supported by the growing interest of the Brazilian and world markets for nuts. Allied to the increase in demand, other factors contributed to the Brazilian expansion such as the harvest period not coinciding with the traditional harvest of grains and other fruits; poor perishability of nuts; search for productive diversification; chance of cultivation intercropped with livestock and crops, among others, which together has provided not only an expansion of pecan planting, but a revitalization of abandoned orchards (BILHARVA *et al.*, 2018; HAMANN *et al.*, 2018). It is estimated that there are about 10 thousand ha of pecan trees, mostly cultivated by small farmers and their families, whose properties range from 4 to 15 ha (MARTINS *et al.*, 2017a). Currently, Rio Grande do Sul is the largest producer of pecans, followed by the states of Parana and Santa Catarina (Figure 2).

The pecan tree also provides fruits that are important raw material for agro-industry. The nuts can be used in the food industry and for the production of oil. Shells can be used to produce tea and fertilizers, and its wood can be used for furniture. The species also enables a consortium with other cultures and the integration of with livestock (MOKOCHINSKI *et al.*, 2017).



Figure 2. Pecan production in Brazil.
Source: Martins *et al.* (2018)

Pecans are good sources of lipids and proteins (Table 1), unsaturated fatty acids (UFA) containing primarily monounsaturated fatty acids (MUFA), a good quantity of polyunsaturated fatty acids (PUFA), and a low content of saturated fatty acid (SFA) (Table 2). The content of oleic acid (C18:1n-9) is the highest followed by linoleic acid (C18:2n-6), lower concentrations of palmitic acid (C16:0), stearic acid (C18:0), and alfa-linolenic acid (C18:3n-3) (RIVERA-RANGEL *et al.*, 2018).

Phytochemicals and other bioactive compounds, including tocopherols and phytosterols are present in pecan oil. Phenolic compounds such as phenolic acids and condensed tannins, in addition to the tocopherols and phytosterols in the pecan oil, have been reported in pecan nut and nutshells using colorimetric assays (Folin-Ciocalteu and Vanillin) and of antioxidant activity assays (ABTS, DPPH, ORAC system and β -carotene linoleic acid) performed *in vitro* (Prado *et al.*, 2013).

Healthy MUFAs, PUFAs, polyphenolic compounds, and other constituents from pecans offer significant benefits for human health in the context of adiposity management and cardiovascular disease prevention (RIVERA-RANGEL *et al.*, 2018).

Table 1. Chemical composition of pecans ($\text{g} \cdot 100\text{g}^{-1}$).

Moisture	2.1 - 7.4
Energy (Kcal)	673.5 – 690.0
Protein	6.0 – 11.3
Nitrogen	1.0 - 1.4
Lipid	65.9 – 78.0
Ash	1.2 - 1.8
Carbohydrates	21.0 – 21.8
Starch	0.0 - 0.4
Tannins	0.7 – 2.7
Sugars, total	3.3 – 5.3
Sucrose	3.5 - 3.9
Glucose	0.00 - 0.04
Fructose	0.00 - 0.04
$\mu\text{g g}^{-1}$	
Arsenic (As)	0.019 ± 0.001
Calcium (Ca)	2088.4 ± 32.7
Chromium (Cr)	2.02 ± 0.07
Copper (Cu)	35.5 ± 0.05
Iron (Fe)	105.86 ± 1.68
Magnesium (Mg)	4197.0 ± 60.8
Manganese (Mn)	192.60 ± 3.05
Zinc (Zn)	137.86 ± 0.39

Source: Venkatachalam, 2004; Moodley; Kindness; Jonnalagadda, 2007; Freitas; Naves, 2010

Table 2. Fatty acid profile of pecans (% of FAME).

C4:0	0.29
C8:0	0.01
C12:0	0.01
C14:0	0.05
C14:1 n5	0.01
C15:0	0.01
C16:0	5.70
C16:1 n7	0.03
C17:0	0.06
C17:1 n7	0.05
C18:0	2.35
C18:1 c9	67.49
C18:2 n6	21.26
C20:0	0.13
C18:3 n6	0.00
C20:1 n9	0.30
C18:3 n3	0.92
C20:2 n6	0.01
C22:1 n9	0.03
C22:2 n6	0.03
C20:4 n6	0.01
C20:5 n3	0.04
C22:4 n6	0.01
C22:5 n3	0.26
C22:6 n3	0.29

2.2 BY-PRODUCTS IN LAMB NUTRITION

The production and consumption of food requires large amounts of resources such as land, water, materials, and energy. This is expected to increase due to population growth and a more luxurious consumption (ELFERINK; NONHEBEL; MOLL, 2008). Due to political and social pressure to reduce the pollution arising from industrial activities, almost all developed and underdeveloped countries are modifying their processes so that their residues can be recycled (MIRZAEI-AGHSAGHALI; MAHERI-SIS, 2008). According to Tufarelli et al., (2013), most by-products are environmental waste management problems.

Ruminant species play an important role in the country's economy, being able to digest fibrous material and by-products. They are well suited for recycling such material and providing an additional source of income (TUFARELLI *et al.*, 2013). Utilization of agro-industry by-products in farm animal nutrition reduces the environmental impact of the food industry, improves profitability, and increases valorization of the agricultural by-products since feeding food residue to livestock is an efficient way to upgrade low quality materials into high quality feed (KASAPIDOU; SOSSIDOU; MITLIANGA, 2015).

According to normative instruction 81 (BRASIL, 2018), by-products are products intended for animal feed obtained from solid waste from the food industries. Product or substances that result from a production process whose main purpose is not its production. It can be used directly in animal feed without any other processing besides normal industrial practice.

The use of alternative regional foods (co-products or by-products) from agribusiness including grain farming, fruit and fruit processing companies, and from biofuel (alcohol and mainly biodiesel) industries in ruminant feeding has been widely studied. Aspects of nutritional value and digestibility of food, as well as performance (consumption, weight gain, and feed conversion), ruminal and blood parameters of animals, meat and milk production and quality, and the economic viability have all been studied. The use of by-products in animal feed, especially in ruminant nutrition, will result in increased demand with consequent reduction in the differential price advantage of traditional ingredients. When the producer includes these by-products into feed, they must be aware of their availability, nutritional quality, and cost compared to traditional feed. Although, in some cases, a drop in productivity is offset by lower production costs, without harming the profitability of the

activity. Thus, the inclusion of co-products are more suitable for those who can purchase them at lower prices close to their property, otherwise it may result in reduced profit margins.

A large volume of agro-industry by-products is produced annually in Brazil, from processing a wide variety of crops cultivated for food or fiber. Some are restricted to certain regions, while others are found all over the country. Successful use of by-products is often limited by poor knowledge of their nutritional characteristics and economic value as feed ingredients, such as the lack of performance of animals fed with this type of food. Looking at another approach, the use of agro-industry by-products meets the aspirations of current environmental policies that, increasingly growing stronger, have been closely following the elimination of potentially polluting products by industries. Demographic growth combined with supply crises, especially in developing countries, increases the discussion about competition between humans and domestic animals for valuable foods. In this sense, the study and use of alternative food sources are fundamentally important (MENEGHETTI; DOMINGUES, 2008).

Processing of raw material generates several by-products with great potential for use in animal feed, and knowledge of their composition and levels of use by ruminant animals is fundamental for the generation of additional income in the chain (BOMFIM; SILVA; SANTOS, 2009).

According to Meneghetti; Domingues (2008), and Pedroso; Santos; Bittar (2009) the pros and cons of using by-product in animal nutrition are:

- Pros
 - Usually by-products enter the diet in place of other food, more traditionally like corn or soy, and the main factor considered in the evaluation is possible economic advantage, either by a direct reduction in the cost of food or by better animal performance resulting from increased feed efficiency;
 - Greater flexibility in diet formulation by availability of greater food diversity; in addition, some by-products may contain special or complementary ingredients to existing ingredients which can provide an improved composition of the diet, allowing for better performance by the animal.
 - Most by-products do not require any kind of processing such as grinding because they are marketed in forms suitable for use (crushed or pelleted). This also represents economic use of labor and energy, and

is most evident in situations with complete feed, which do not premix the ingredients.

- Reduction in starch content of the diets, with concomitant increase of digestible fiber content, contributes to the improvement of the rumen environment.
- Cons
 - In connection with possible advantages, several risk factors must be evaluated before introducing by-products into the diet. The first of these refers to the decision itself of which products to buy. It is necessary to formulate the whole diet on the farm, which requires knowledge or specialized technical advice.
 - By-product marketing rules may also differ from conventional products. Usually the great advantage of getting by-products comes from negotiating directly with the producing company or its representative direct. This, however, has limitations. In most cases, only large closed volumes or loads may be provided, which may be an impediment for small properties, especially when it comes to easily spoiled foods which require fast consumption. In this situation, the producer is forced to buy smaller quantities more often, even at a higher cost, so that payment is better distributed.
 - Lack of quality control leads to another problem, which is the correct establishment of nutritional value of the by-product. When the variation in composition is large, which is common to some by-products, it is difficult to balance the diet because the real nutritional value of the food is unknown. Due to this, many by-products do not have sufficient researched data for a recommendation of consistent use, which may lead to increasing the frequency of the bromatological analyzes. This is an additional cost factor and increases the risk for unexpected results in the process. In this case the nutritionist's experience with the product has an important role.
 - Success in using by-products depends on good planning, storage, and handling. Farm facilities should allow for easy reception, discharge and maintenance of product quality. Ideally, strict management of losses

and quantities must be used so cost and inventory can be controlled. Some products do not allow prolonged storage due to its high fat content as it becomes rancid in a few days and, therefore, is not recommended for feed. This type of consideration should be made when choosing each type of food.

2.2.1 Pecan By-Product

Pecan by-products are usually generated due to defects in color, shape, size, and yield (Figure 3). According to Arena *et al.* (2014), Glen (2015) and Arena; Polomski (2019), the most important factors contributing to losses are:

- Pollination causing low nut production or poorly developed nuts;
- Drought causing poorly filled pecans;
- Diseases caused by fungi or bacterial pathogens;
- Pests causing damages to the shell;
- Zinc deficiency, producing abnormally small nuts;
- Failure to fill caused mainly by insects, disease, or low nutrients;
- Premature loss is mainly caused by pest damage, disease and drought.



Figure 3. Defect in pecans.
Source: Glen, 2015; Reid, 2015

With the higher demand for feed for the composition of the concentrated formulated for the different animal categories of sheep farming, the demand for products that allow good and economic animal performance is increasing. As a result, the adoption of alternative foods has emerged as an excellent alternative to compose ruminant diets (MADRUGA *et al.*, 2005).

The by-product generated by pecans (Table 1) is rich in fatty acids, fat, protein, fibers, antioxidants, and minerals including calcium, iron, zinc, selenium, and potassium, and are good sources of dietary fiber (MARTINS *et al.*, 2017b). The most important, it is the potential to be used as a feedstuff in animal nutrition.

Another benefit is that due to the sheep oral anatomy, characterized by the extreme mobility of the lips and the form of apprehension of the food using lips, teeth and tongue, they can be very efficient in separating and choosing the food to be eaten (Santos *et al.*, 2009). Pecan by-product can be used in lamb nutrition in halves, large, medium or small pieces, or as a meal (Figure 4).



Figure: 4. Pecan varieties that can be introduced into animal diets.
Source: Millican, 2017

2.3 CARCASS AND MEAT QUALITY ATTRIBUTES

Many aspects of animal production are involved in the attempt to produce quality carcasses and lean meat, including type of rearing (grain or grass fed), feeding, management, sex (male, female or castrated male), animal age (young or adult), genetics, and all of these interactions (PAULO *et al.*, 2010).

Carcass quality parameters are mainly divided on those that affect morphology, as conformation and morphological measurements, and those related to composition as commercial cuts, fattening score degree, tissular or chemical composition. Meat quality can be defined as a set of properties that together identify what we appreciate about meat when we purchase it, eat it, or select it for use as a raw material for processing into meat products (PURSLOW, 2017).

The carcass can be analyzed from several points of view: nutritional, pre-established image, presentation and sensory (OSÓRIO *et al.*, 2012). The carcass contains the edible portion, muscles and fat, and the inedible portion consisting predominantly of bones. The carcasses should have a high percentage of muscles, with satisfactory fattening degree and conformation. Carcass measurements allow comparisons between weights, slaughter ages and feeding systems (SILVA; PIRES, 2000). Whereas meat is composed of a complex organization of muscle, connective tissue, adipose and blood tissues, and the reactions that occur in these tissues before and after slaughter will determine their nutritional and sensory qualities, directly impacting their acceptability (RAMOS; GOMIDE, 2007).

Lamb is the animal category that offers the most acceptable meat in the consumer market, characterized by being soft and pink, with a smooth texture, firm consistency and satisfactory fat content. The meat of the yearling sheep is still soft, but the color is already stronger, reddish. The meat of adult sheep (mutton) is no longer so attractive because it is harder, has a yellowish fat and a stronger flavor (OSÓRIO; OSÓRIO; SAÑUDO, 2009).

The Santa Inês breed is pointed as a promising alternative in crossbreeding for the production of lambs for slaughter, because it has easy adaptability, rusticity and reproductive efficiency, low susceptibility to endo and ectoparasites. In addition to these advantages, it has no seasonal reproductive behavior, playing an important role in protein production (MADRUGA *et al.*, 2005).

Lamb meat quality and acceptability is primarily determined by its physico-chemical characteristics (pH, color, tenderness, water holding capacity) (TEJEDA *et al.*, 2008), nutritional (proximate composition, fatty acid profile) and sensory (odor, juiciness, tenderness, taste) (LEÃO *et al.*, 2011; MADRUGA *et al.*, 2005).

The organoleptic characteristics of meat may be modified by the feed that the animal receives due to changes in fat content and composition. Fatty acids may alter the firmness of fat tissue, shelf life (lipid oxidation and pigment), taste and aroma (MADRUGA *et al.*, 2002). Thus, slaughter weight is often the variable to be considered by producers as an indicator that animals are ready to be slaughtered.

Colour, flavour and texture are affected not only by preslaughtering management, but also by processes of proteolysis and lipid oxidation occurring during post-mortem storage (JACOB *et al.*, 2014). The oxidation of PUFA in meat causes the rapid development of meat rancidity (KANNER, 1994). Lipid oxidation in muscle initiates at a cellular membrane level, specifically in the phospholipid fractions, as a free-radical

autocatalytic chain mechanism in which prooxidants interact with unsaturated fatty acids. The result of the interaction generates free radicals and propagation of the oxidative chain (O'SULLIVAN; KERRY, 2012). Oxidation of unsaturated fatty acids is generally thought to occur in three stages: (1) initiation: the formation of free radicals; (2) propagation: the free-radical chain reactions; (3) termination: the formation of non-radical products.

Consumers perceive meat color to be a cue for freshness and prefer lamb meat to be red in color for this reason. On the other hand, meat has a tendency to change from red to brown after slicing and in the context of retail display this characteristic is known as colour stability. Meat that remains red during display is stable in color, and meat from lambs is regarded as less stable than beef (GUTZKE; TROUT, 2002). This has been attributed at least in part, to subtle differences in the sequence of amino acids in the globin heme moiety of myoglobin that interact with aldehyde compounds produced during oxidation (JACOB *et al.*, 2014).

Generally, for the consumer, sensory parameters are more important than nutritional ones. Sensory analysis is the set of techniques for reproducibly measuring the characteristics of a product through the senses. Sensory properties are the characteristics of food perceived by the senses, intervening to a greater or lesser extent all the sense organs (OSÓRIO; OSÓRIO; SAÑUDO, 2009).

Within the set of sensations obtained by the consumer, three aspects can be distinguished: qualitative, which allows to describe the sensation of hard, soft, odor, dark, light, among others; quantitative, which values the intensity of this perception, and hedonic aspects, which are related to the consumer's response to these characteristics, such as unpleasant or pleasant. The third aspect characterizes the subjectivity of sensory analysis (SAÑUDO; OSÓRIO, 2004). Therefore, the central objective of meat production should be to provide a product with adequate qualitative and quantitative characteristics in order to provide better consumer responses to these characteristics.

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3. OBJECTIVES

3.1. General Objective

- The effect of gender and pecan by-product at different levels of inclusion on carcass characteristics and meat quality parameters of Santa Ines lambs.

3.2 Specific Objectives

Paper I

- Morphometric measurements of carcass;
- Morphometric measurements of *longissimus dorsi* muscle;
- Measurements of commercial cuts;
- Determination of tissue composition.

Paper II

- Evaluation of fresh meat attributes;
- Evaluation of physical, and chemical composition of meat;
- Sensory evaluation of meat;
- Fatty acid profile of meat.

4. PAPER I

Paper submitted to Small Ruminant Research

Carcass characteristics, loin measurements, and tissue composition of Santa Ines lambs fed pecan by-product

F.M. Giotto^{a, b}, M.I. Custódio^b, L.J. Guimarães^b, A. de Mello^a, E.L.A. Ribeiro^b

^aDepartment of Agriculture, Veterinary, and Rangeland Science, University of Nevada, Reno. 1664 N. Virginia St. Mail Stop 202, Reno, NV, United States, 89557.

^bDepartment of Animal Science, Londrina State University. Rodovia Celso Garcia Cid, Pr 445, Km 380. Londrina, Parana, Brazil, 86057-970.

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Highlights

- Pecan by-product does not affect carcass characteristics, loin measurements and tissue composition
- Males have higher carcass weights, whereas females have higher leg yield
- Males have higher loin eye area, whereas females have higher fat thickness

Abstract

This study evaluated the effects of the inclusion of pecan by-products in lamb diets. Forty-four Santa Ines lambs, intact males and females were used. Carcasses weights, index and yields, conformation, fattening degree, refrigeration loss, leg and shoulder measurements, loin eye area, loin measurements, marbling, commercial cuts weight and yields, and tissue composition were calculated. No effects of dietary treatments were observed on any parameters. Gender effect was observed for weight at slaughter, hot and cold carcass weight, carcass index, length, and depth, leg length, shoulder perimeter, refrigeration loss, loin eye area, commercial cuts weight, neck yield, and weight of bones were higher in males, whereas females had higher values for fat thickness, and leg yield. Results suggest that up to 9% of pecan by-products may be used as an alternative feedstuff in lamb diets without compromising carcass characteristics.

Keywords: commercial cuts, nuts, sheep

1. Introduction

Due to good marketing prospects of sheep meat, it is necessary to intensify the lamb termination process to reduce the production cycle and improve carcass quality (Costa et al., 2011). The use of feedlots by sheep producers generates positive results by shortening the time animals stay on feeding due to faster weight gain. Due to consistent and balanced feeding managements, feedlot systems also allow carcass standardization, availability of meat in the off season, and reduced mortality rates. However, when compared to extensive systems, feedlots have higher feeding costs (Ribeiro et al., 2011; Sérgio et al., 2007).

In order to minimize feeding costs, studies have been performed using agro-industrial byproducts from fruits processing (da Silva et al., 2014; Almeida et al., 2015), coffee (Souza et al., 2004), byofuels (D'Aurea et al., 2018), among others (Nunes et al., 2006), as a replacement for standard feedstuffs such as corn and soybean meal.

Pecans are available in many countries, including Australia, Argentina, Brazil, China, Israel, Mexico, Peru, South Africa, and United States (Zhang et al., 2015; Blayney and Gutierrez, 2017). During processing for human consumption, nuts may be discarded due to quality issues such as diseases, defects in size, shape, or color (Glen, 2015). Discarded nuts become by-products that are rich in oleic and linoleic fatty acids, fat, protein, fibers, and antioxidants (Ortiz-Quezada et al., 2011; Atanasov et al., 2018; Rivera-Rangel et al., 2018), with potential to be used in animal nutrition.

In this study we evaluated the effects of diets with different inclusion levels of pecan by-products on carcass composition and measurements of ewe lambs and ram lambs fed pecan by-product in feedlot.

2. Study Area

The experiment was carried out at the Laboratory of Feed Analysis and Animal Nutrition, located on the campus of Londrina State University. The laboratory is located in Londrina,

Parana, Brazil (23° 17' 34" south latitude, 51° 10' 24" west longitude), and approximately 610 meters of altitude. According to Köppen (1948), the climate is Cfa (humid subtropical climate).

This study was approved by the Ethics Committee on Animal Experimentation of the Londrina State University under the number 11821.2017.25.

3. Material and Methods

3.1 Animals and diet composition

Forty-four Santa Ines lambs (24 males and 20 females) with approximately 23.24 ± 3.22 kg, of body weight, 3.56 ± 0.55 months old, were randomly assigned to 1 of 4 dietary treatments (n=11 per treatment). Diets were formulated with sorghum silage, broken rice, soybean meal, a commercial mineral mix, calcitic limestone, soybean oil, and four different levels of pecan by-product: 0%, 3%, 6% and 9% (DM basis) (Table 1), to meet the nutritional requirements according to NRC (2007) for late maturing lambs and potential daily gain of 250 g. Pecan by-products were ground and added to the diets as flour. Lambs were fed in a roughage:concentrate ratio of 40:60, and after 52 days of feeding, animals were weighted after fasting for 16 hours to obtain slaughter weight (SW). Animals were harvested in a commercial meat plant, with final body weight of 37.17 ± 4.67 kg, and 5.28 ± 0.45 months old. Pre-harvest handling was in accordance with good animal welfare practices, and slaughtering procedures followed the Sanitary and Industrial Inspection Regulation for Animal Origin Products (Brasil, 2017). After slaughtered, hot carcass weight (HCW) was recorded, carcasses were chilled, and after 24 h cold carcass weight (CCW) was recorded. Hot carcass yield ($HCY = (HCW/SW)*100$), cold carcass yield ($CCY = (CCW/SW)*100$), and cooling loss ($RL = 100 - (CCW/HCW)*100$) were calculated.

3.2 Carcass Measurements

Carcass fattening degree (1 - cover fat absent to 5 - fat abundant covering), conformation (1 - concave to 5 - convex), and fat flank streaking were performed based on photographic standards (Cañeque and Sañudo, 2000). The carcass index (CI) was calculated ($CI = CCW/\text{carcass length}$).

The length of the carcass was measured from between the hind of the legs to the front of the neck; thoracic depth from the distance between the vertebral column starting from the first thoracic vertebra to the sternum.

Shoulder measurements were taken for length, distance between the olecranon and the portion of carpal bones; perimeter was calculated based on the largest measurement on the top of the shoulder; and depth taken in the average portion of the length of the shoulder. The length of the leg was measured from the tubercle on the proximal end of the tibia to the distal end of the tarsus; leg perimeter measured in the wider base of the leg; and leg depth from the maximum width between the most caudal point on the median line between the legs to the distal edge of the *biceps femoris*.

3.3 Loin Measurements

Loin eye area was obtained by exposing the *longissimus dorsi* muscle after a transverse section on the carcass, between the 12th and 13th ribs, and the area was measured through plastic grid over the loin eye and counted the dots (0.25cm^2). Also, transverse measurements of maximum width and depth of the loin were measured using a digital caliper.

Subcutaneous fat thickness was measured at the final third of the muscle, from the backbone, perpendicularly to the muscle, with a digital caliper, and the degree of marbling was subjectively assessed using photographic standards (1 - traces of marbling and 10 - abundant marbling) (AMSA, 2001).

3.4 Commercial cuts, and tissue composition

The left side of the carcass was weighed and fabricated into the following commercial cuts: neck, shoulder/fore shank, loin, rack/breast, and leg. After splitted, individual weights of each cut were recorded, and the yield of the commercial cuts were calculated based on the weight of each cut, and the weight of each carcass half.

The tissue composition was determined using the methodology described by Fisher and de Boer (1994) in which the 44 shoulders, which had been previously stored and were then gradually thawed while being kept at a temperature of approximately 4 °C for 24 hours, were dissected.

3.5 Statistics

Data were analyzed as a complete randomized design using the GLIMMIX procedure of SAS, whereas dietary treatment (control, 3%, 6%, and 9% of pecan inclusion) and gender (male and female) were considered the main effects. Data were arranged as a 4x2 factorial, and means separations by using the LSMEANS and DIFF functions when means were significant at $P \leq 0.05$.

4. Results

4.1 Carcass characteristics

No treatment effect ($P > 0.05$) was observed for any of the variables (Table 2). Table 2 shows differences ($P < 0.05$) observed for gender. Males had higher weights at slaughter ($P < 0.01$) than females, and also heavier hot and cold carcasses ($P < 0.01$) than females. No treatment or gender ($P > 0.05$) effect was observed for hot or cold carcass yielder. It was observed gender effect ($P < 0.05$) for carcass index, carcass length, thoracic depth, leg length, shoulder perimeter, and refrigeration loss, with males having higher values than females. No gender effect ($P > 0.05$) was observed for carcass fattening degree, conformation, fat flank streaking, leg perimeter, leg depth, shoulder length, and should depth.

4.2 Loin measurements

No treatment effect ($P>0.05$) was observed for any of the variables (Table 3). Table 3 shows that no gender effect ($P>0.05$) was observed for *longissimus dorsi* depth, width, and marbling. Fat thickness was higher ($P<0.05$) for females than males, and loin eye area was higher for males than females (Table 3).

4.3 Commercial cuts and tissue composition

No treatment effect ($P>0.05$) was observed for any of the variables (Table 4, Table 5). Gender effect ($P<0.05$) was observed for the weight of commercial cuts with males having higher weights for all cuts (Table 4). Higher yield was observed for female legs ($P<0.05$) than for males, whereas male necks had a higher yield than females (Table 4). Table 5 shows that for tissue composition, males had higher values for bones than females ($P<0.05$), and no gender effect was observed for muscle and fat composition ($P>0.05$).

5. Discussion

5.1 Carcass characteristics

Males showed higher growth rates when compared to females (Geraldo et al., 2016) due to effects of androgens hormones, particularly testosterone (Schanbacher et al., 1980), which stimulate growth in muscle by increasing protein synthesis (Aberle et al., 2012). Consequently, intact males had higher SW, HCW, and CCW than females. Values for hot and cold carcass yields are adequate for Santa Ines lambs slaughtered at 40 and 34 kg and did not differ between genders probably due to the heavy weight of the males, and the precocity of females.

Sexual dimorphism may result in differentiation of carcass fattening degree, in which females have higher fat deposition than males (Homem et al., 2015). However, no difference ($P> 0.05$) was obtained between genders in the visual evaluation of the carcass fattening degree. According to Osório et al. (2012) carcasses from this study were classified as poor for

fattening degree, and thin to slightly thin for conformation. For Cañeque and Sañudo (2000) the subjective conformation assessment values indicate that all animals were with a conformation below 2 (rectilinear carcasses with reasonable muscle coverage), and for the fattening degree the animals were in a standard 2 (reasonable fat coverage with portions of apparent muscle). The young age of the animals and the late maturation for the genetic group of animal used in this study, probably contributed to these results, once fat is the last tissue to be deposited (Osório et al., 1995) probably not allowing the carcass to have a proper fat deposition.

The higher cooling loss for males was due to low fat thickness observed in these animals, since fat is an important carcass protection against the adverse effects of the cold, low temperature of the cooling and freezing, ventilation, humidity, as well as the excessive loss of water by the formation of ice crystals within the cells (Dallantonia et al., 2015). For Cunha et al. (2008) it varies from 1 to 7%. Results obtained in this study are in accordance to results obtained by Grandis et al. (2016), for Santa Ines intact males slaughtered with more than 38 kg.

Carcass index is the amount of tissue deposited per unit of carcass length, and according to Simela et al. (1999) is the best predictor for meat content of male carcasses as well as the fat in female carcasses. Grandis et al. (2016) also conducted a trial with Santa Ines lambs and showed that all intact males, slaughtered with an average weight of 38.58 kg, obtained 0.28 for carcass index. Similar values were observed in this study.

Regarding the carcass length, thoracic depth, leg and shoulder measurements, in general, Grandis et al. (2016), and Fernandes Júnior et al. (2013) observed greater influence of genetics, age of animals and slaughter weight than nutritional effects. The same occurred in this study, where no effect of the diet was observed on the parameters evaluated. Male carcasses were longer, heavier, and had deeper thoracic depth due to the higher muscle

deposition in the neck of the males (Butterfiel, 1988). Leg length is usually higher in males than females, and occurs in response to the greater bone elongation compared to females (Siqueira et al., 2001). However, no differences ($P>0.05$) were observed for shoulder length.

The values obtained for Santa Ines intact males for all the measurements are in agreement with those obtained by Grandis et al. (2016) with animals slaughtered closer to 40kg, and higher than the ones observed by Fernandes Júnior et al. (2013) who slaughtered animals with a final weight of 36.65 kg.

5.2 Loin measurements

Fat ratio is higher in females, intermediate in castrated males and minor in intact, happening the opposite with the proportion of muscle in the carcass (Cezar and Souza, 2007). For these authors, when the final phase of growth occurs in feedlot, based on high feed energy content diets, mature animals especially females, soon reach growth stage in which the largest proportion of energy is deposited in the form of fat. On the other hand, intact males may hold smaller content of fat even with higher weights (Silva et al., 2009). The fat thickness in this study was higher in females and minimum in males, and For both genders, it was lower than the ideal fat thickness (3.0 mm) recommended by Queiroz et al. (2015) and Fernandes Júnior (2017) for Santa Ines lambs. However, considering the current consumer preference for lean or low fat meat (Firetti et al., 2017), the values observed can be considered satisfactory.

The loin eye area according to Macedo et al. (2008) is mostly affected by the slaughter weight, in which animals slaughtered with heavier carcasses weight usually present larger loin eye area. This finding suggests the higher value for loin eye area in males observed in this study.

In general, Santa Ines breed has a late development when compared to other breeds specialized in meat production (Grandis et al., 2016), and this suggests the lower marbling score presented in this study, for both males and females.

5.3 Commercial cuts and tissue composition

Heavier weights for commercial cuts were expected for males since their SW and carcass weights were heavier than the female ones. For Osorio et al. (2012) animals' body weight has a high correlation with carcass weight, commercial cuts weight, and tissue composition. The higher leg yield for females possibly occurred due to the lower slaughter weight, since for Osório et al. (1995), the legs are of early development, causing a reduction in the percentages of these cuts, when there is an increase in carcass weight. Regarding the highest proportion of neck in males, Beermann et al. (1995) reported that increased neck development is one of the disadvantages of using intact males, and according to Aberle et al. (2012) this happens because certain muscles are more sensitive to androgens, in particular muscles of the forequarter of the male, especially the ones in the neck and crest region, show greater development than in females or castrates. For Butterfiel (1988) in rams, the achievement of adulthood embraces the biological need to fight with other rams for the right to mate with the available females, and this contest demands, in addition to total mass, robust muscles of the neck.

The most valued cuts in a sheep carcass correspond to leg, shoulder and loin, so the higher the percentage of these in the carcass, greater the appreciation of it (Alves et al., 2015). In this work, the percentage of the value added cuts of the carcass remained around 72%.

Tissue composition highly affects commercial quality of the carcass, and consumers pay the same price for fat, muscle, and bone, all-inclusive in the commercial cuts (Guerrero et al., 2017). According to Osório (1992) complete dissection of the carcass is the most accurate method to evaluate its tissue composition, but because it's a slow and costly technique, a representative part of the carcass has been used. Leg and the shoulder present great representativeness and correlation with the whole carcass. For this reason, these cuts are usually used for quantitative and qualitative analyzes (Cañeque and Sañudo, 2005, Cezar and

Sousa, 2007). Also, according to Osório et al. (2012) these compositions are the result of biological processes determined by race and gender and may vary with slaughter weight and age and, modified by environmental factors, especially by diet. The higher percentage of bones obtained in the tissue composition for males can be explained by the stimuli of bone salts deposition caused by androgens, which will increase bone growth in males when compared to females, or castrates (Aberle et al., 2012).

6. Conclusion

Pecans included in up to 9% in lamb diets did not cause any detriment to carcass characteristics, loin measurements, value-added cuts, and tissue composition which validates their use in lamb nutrition.

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Table 1. Ingredients and chemical composition of dietary treatments (% of dry matter)

	Ingredients			
	Control	3%	6%	9%
Sorghum Silage	40.00	40.00	40.00	40.00
Soybean meal	35.00	33.72	32.40	31.45
Broken rice	21.00	19.95	18.90	17.85
Commercial mineral mix	1.00	0.95	0.90	0.85
Calcitic limestone	1.00	0.95	0.90	0.85
Soybean oil	2.00	1.42	0.90	0.42
Pecan	0.00	3.00	6.00	9.00
	Chemical composition			
	Control	3%	6%	9%
Dry Matter	62.95	62.97	62.92	63.00
Protein	22.60	22.17	22.00	22.10
Ether Extract	3.70	4.40	5.12	5.47
Ash	4.56	4.50	4.08	4.14
Acid Detergent Fiber	15.40	15.43	15.42	15.43
Neutral Detergent Fiber	30.12	30.10	30.10	30.09
NDT	72.50	73.12	73.30	73.03

Table 2. Carcass characteristics and measurements of Santa Ines lambs fed pecan by-product.

	Dietary Treatments				Gender		P Value	
	Control	3%	6%	9%	Male	Female	Treatment	Gender
SW (kg)	36.83	38.33	36.66	36.50	40.20 ^a	33.95 ^b	0.7268	0.0002
HCW (kg)	18.15	18.87	18.33	18.01	19.99 ^a	16.68 ^b	0.8444	0.0003
CCW (kg)	17.42	18.16	17.61	17.32	19.19 ^a	16.05 ^b	0.8385	0.0004
HCY (%)	49.31	49.17	49.90	49.38	49.71	49.16	0.9059	0.4685
CCY (%)	47.33	47.30	47.94	47.48	47.72	47.30	0.9198	0.5850
CI (kg/cm)	0.28	0.28	0.27	0.27	0.29 ^a	0.25 ^b	0.9620	0.0027
COL (%)	4.03	3.79	3.93	3.85	4.02 ^a	3.77 ^b	0.4874	0.0434
CON	1.58	1.33	1.41	1.33	1.45	1.37	0.7070	0.6342
FD	2.00	2.16	2.42	2.16	2.25	2.12	0.4653	0.5012
FFS	2.40	2.50	2.42	2.25	2.50	2.29	0.7739	0.2429
CL (cm)	62.67	65.66	64.79	63.66	66.00 ^a	62.39 ^b	0.2653	0.0044
TD (cm)	25.79	26.79	26.71	26.92	27.45 ^a	25.64 ^b	0.2030	0.0003
LL (cm)	42.08	42.21	41.58	41.92	42.91 ^a	40.97 ^b	0.8158	0.0010
LP (cm)	39.79	40.29	39.58	40.37	40.52	39.50	0.7272	0.0967
LD (cm)	10.04	10.00	10.00	10.13	10.04	10.04	0.9942	1.0000
SL (cm)	20.33	20.71	20.25	20.08	20.60	20.08	0.7149	0.2008
SP (cm)	17.00	17.46	17.37	17.13	18.00 ^a	16.47 ^b	0.6952	0.0001
SD (cm)	4.37	4.50	4.33	4.17	4.33	4.35	0.4403	0.8846

SW: Slaughter weight; HCW: hot carcass weight; CCW: cold carcass weight; HCY: hot carcass yield; CCY: cold carcass yield; CI: carcass index; COL: cooling loss; CON: conformation; FD: fattening degree; FFS: fat flank streaking; CL: carcass length; TD: thoracic depth; LL: leg length; LP: leg perimeter; LD: leg depth; SL: shoulder length; SP: shoulder perimeter; SD: shoulder depth.

^{a,b}Means in the same row having different superscripts are significant at $P \leq 0.05$ level.

Table 3. Loin eye area, loin measurements, fat thickness, and marbling of *Longissimus dorsi* muscle from Santa Ines lambs fed pecan by-product.

	Dietary Treatments				Gender		P Value	
	Control	3%	6%	9%	Male	Female	Treatment	Gender
LEA (cm ²)	16.52	17.38	16.29	16.19	17.36 ^a	15.82 ^b	0.4780	0.0169
LD (mm)	25.21	26.92	24.67	25.41	25.67	25.42	0.4576	0.8068
LW (mm)	53.82	50.86	51.46	53.12	53.51	51.10	0.2667	0.0529
FT (mm)	0.90	1.20	1.15	1.18	0.58 ^b	1.63 ^a	0.9335	0.0108
Marbling	1.92	1.92	1.67	1.75	1.87	1.75	0.7609	0.5404

LD: loin depth; LW: loin width; FT: fat thickness; LEA: loin eye area.

^{a,b}Means in the same row having different superscripts are significant at $P \leq 0.05$ level.

Table 4. Commercial cuts weight and yield of Santa Ines lambs fed pecan by-product.

	Dietary Treatments				Gender		P Value	
	Control	3%	6%	9%	Male	Female	Treatment	Gender
Leg (kg)	2.66	2.82	2.72	2.70	2.92 ^a	2.53 ^b	0.7204	0.0011
Shoulder (kg)	1.76	1.90	1.84	1.80	1.99 ^a	1.65 ^b	0.5603	0.0002
Loin (kg)	1.67	1.75	1.72	1.68	1.84 ^a	1.57 ^b	0.9054	0.0044
Rib (kg)	1.85	1.92	1.87	1.74	2.01 ^a	1.67 ^b	0.6496	0.0031
Neck (kg)	0.63	0.62	0.70	0.62	0.73 ^a	0.55 ^b	0.3922	0.0002
Leg %	30.67	31.07	31.04	31.26	30.47 ^b	31.54 ^a	0.4483	0.0007
Shoulder %	20.26	20.96	20.88	20.82	20.77	20.68	0.5172	0.8048
Loin %	19.14	19.24	19.59	19.48	19.15	19.57	0.8333	0.3055
Rib %	21.11	21.14	21.25	20.14	21.00	20.81	0.7065	0.7985
Neck %	7.19	6.80	7.91	7.15	7.69 ^a	6.83 ^b	0.1700	0.0224

^{a,b}Means in the same row having different superscripts are significant at $P \leq 0.05$ level.

Table 5. Tissue composition of Santa Ines lambs fed pecan by-product.

	Dietary Treatments				Gender		P Value	
	Control	3%	6%	9%	Male	Female	Treatment	Gender
Fat %	18.67	20.02	18.38	21.57	18.45	20.87	0.4548	0.1318
Muscle %	60.25	58.38	59.71	58.08	59.71	58.51	0.6869	0.4296
Bone %	20.17	20.86	19.79	18.95	21.15 ^a	18.74 ^b	0.5267	0.0170

^{a,b}Means in the same row having different superscripts are significant at $P \leq 0.05$ level.

5. PAPER II

Paper submitted to Meat Science

Effects of feeding pecan by-products in fatty acids and quality attributes of Lambs meat

Francine Mezzomo Giotto^{ab}, Fernando Augusto Grandis^b, Amilton de Mello^a, Edson Luis de Azambuja Ribeiro^b

^aDepartment of Agriculture, Veterinary & Rangeland Sciences, University of Nevada, Reno.

^bDepartment of Animal Science, University of Londrina, Parana, Brazil.

Abstract

The aim of this study was to evaluate the effects of the inclusion of pecan by-products in lambs' diet. Meat samples were subjected to fatty acid profile, lipid oxidation, Warner Braztler Shear Force (WBSF), sensory, cooking loss, and proximate analysis. Inclusion of pecans did not affect moisture, protein, pH, cooking loss, lipid oxidation, and WBSF. Fat percentage was significant higher in ewes. Dietary treatments did not affect objective color parameters, however, meat from ewes were redder and yellower when compared to meat from rams. Meat from lambs fed a control diet without pecan inclusion had higher scores for tenderness, and juiciness. Greater levels of margaric and oleic acids were observed in the lean of ewes, whereas meat from rams had greater values of linoleic acid. Inclusion of 6% of pecans increased deposition of DHA, whereas a 9% inclusion led to higher levels of EPA. Results suggest that pecan byproducts can be used as a feeding source for producers who may have access to this feedstuff.

Keywords: DHA, EPA, fatty acid, sheep

1. Introduction

Feedstuffs used for livestock are fundamentally important in terms of final meat quality and nutritional values (Sapkota, Lefferts, McKenzie, & Walker, 2007). In recent decades, there has been an increased demand for healthier foods that have proper nutritional elements recommended for health maintenance, such as nuts (Souza, Schincaglia, Pimentel, & Mota, 2017; Shah, Murthy, & Freedman, 2019).

The pecan tree (*Carya illinoensis* (Wangenh.) K. Koch) provides fruits that are considered an important raw material for the agro-industry. Its almonds can be used as food

and for the production of pecan oil. Shells can be used to obtain tea and fertilizers, and its wood can be used for furniture. The species also enables a consortium with other cultures and the integration of livestock (Mokochinski, Watzlawick, Botelho, & Moreira, 2017). Nowadays, the *Carya illinoensis* is the most valuable North America-native nut tree, with the United States being the world's largest pecan-producing country (Blayney & Gutierrez, 2017), and in the past decades, it has been introduced to many other countries, including Australia, Argentina, Brazil, China, Israel, Peru and South Africa (Zhang, Peng, & Li, 2015; Blayney & Gutierrez, 2017).

Pecans are discarded due to diseases, defects in size, shape, or color (Glen, 2015), generating a by-product rich in oleic and linoleic fatty acids, fat, protein, fibers, and antioxidants (Ortiz-Quezada, Lombardini, & Cisneros-Zevallos, 2011; Atanasova et al., 2018; Rivera-Rangel et al., 2018) that may be potentially used as a feedstuff in animal nutrition.

In addition to the effects of diet on production parameters, dietary constituents may also have a considerable effect on meat quality (De Brito, Ponnampalam, & Hopkins, 2017). Changes in finishing diets may alter the lipid profile and improve the nutritional composition of edible tissues. Furthermore, it is possible to improve nutritional values of lamb meat by increasing the deposition of compounds that are beneficial to human health, such as long-chain n-3 polyunsaturated fatty acids (PUFA), including eicosapentaenoic acid (EPA, C20:5 n-3) and docosahexaenoic acid (DHA, C22:6 n-3) (Cheng et al., 2015; De Brito, Ponnampalam, & Hopkins, 2017).

Therefore, the objective of this study was to evaluate the inclusion of pecan by-product on fresh lamb attributes, sensory, and fatty acid profile of the lean meat.

2. Materials and methods

This study was approved by the Ethics Committee on Animal Experimentation of the Londrina State University under the number 11821.2017.25.

2.1 Animals, diets, and sample collection

Forty-four Santa Ines lambs (24 intact males and 20 females) with approximately 23.24 ± 3.22 kg of body weight were randomly assigned to 1 of 4 dietary treatments (n=11 per treatment). Diets were formulated with sorghum silage, broken rice, soybean meal, a commercial mineral mix, calcitic limestone, soybean oil, and four different levels of pecans: 0%, 3%, 6% and 9% (DM basis) (Table 1), to meet the nutritional requirements according to NRC (2007) for late maturing lambs and potential daily gain of 250 g. Pecan by-products were ground and added to the diets as flour. Overall, the final diet had a roughage:concentrate ratio of 40:60. Lambs were fed for 52 days and slaughtered around 5 months old with an average body weight of $37.17 \text{ kg} \pm 4.68$. After slaughtered, carcasses were chilled, and the *M. longissimus dorsi et lumborum* was excised from loins 24 h *post-mortem*.

2.2 Sample analyses

2.2.1 pH and proximate composition

Loin chops (2.54 cm) were cut and pH was assessed by using a Hanna® pH meter. Moisture, ash, protein ($\text{N} \times 6.25$) and fat content were determined according to AOAC methods (AOAC, 2000). Moisture was determined according to AOAC Method 950.46. Protein content was determined by estimating the nitrogen content using the Kjeldahl method (AOAC Method 920.152). Ash content was determined by incineration at 525 °C (AOAC Method 940.26) while fat was determined by the Soxhlet method (AOAC Method 963.15).

2.2.2 Objective color and lipid oxidation

Objective color (L^* , a^* , b^*) was recorded by using a CR-10 Konika Minolta® color reader after 30 minutes of blooming. For lipid oxidation, the methodology described by Pikul, Leszczynski, & Kummerow (1989) was used to perform the Thiobarbituric Acid Assay (TBA). Lipid oxidation (TBA values) was expressed as malonaldehyde concentration (mg/kg) and the quantification was realized comparing samples to standards absorbance.

2.2.3 Cooking loss, sensory analysis and objective tenderness

Cooking for sensory and WBSF (N) analysis was performed by thawing the lamb chops for 24 h at 5 °C and grilling to 71 °C (AMSA, 2016). During cooking, chops were flipped after reached 35 °C at the geometric center and grilled until temperature reached 71 °C. After being removed from the grill, chops rested for 30 min prior to final weighing. For WBSF, chops were cooled for 24 h at 4 °C and 6 cores (1.27 cm in diameter) were removed from each sample with a drill press parallel to muscle fiber orientation. Cores were sheared with a C3 Texture Analyzer Brookfield® (Brookfield Engineering, Middleboro, MA), with a Warner-Bratzler blade.

Cooking loss was calculated from chops used for WBSF by using the following formula:

$$\text{Cooking loss \%} = 100 - \left(\frac{\text{Grilled weight of the chop} \times 100}{\text{Raw weight of the chop}} \right)$$

For sensory analysis, a total of 6 panelists were trained by following the AMSA (2016) guidelines, and to be eligible for participation, consumers had to be regular or occasional lamb consumers. A total of 9 chops were served in 4 sessions, and 8 chops were served in one session. Chops were randomly assigned to each panelist according to sexual gender and dietary treatment. Chops were cooked, trimmed of subcutaneous fat cover, and 6 cubes (2.54 cm × 1.27 cm × 1.27 cm) from each chop were served to the six-member panel.

Each panelist received 1 cube, totaling nine samples per session, and one session with 8 samples. Samples were served to panelists individually and unsalted crackers and water were available to the panelists to cleanse their palates between samples. Panelists evaluated juiciness from 1 = extremely dry to 8 = extremely juicy; tenderness from 1 = extremely tough to 8 = extremely tender; off-flavor intensity from 1 = extremely mild to 8 = extremely intense; and global acceptability from 1 = extremely liked to 8 = extremely disliked. Off-Flavor descriptors were also identified as: sweet, bloody, sour, oxidized, liver, and sheep meat.

2.2.4 Fatty acids

The lipids were extracted from lamb chops according to the method described by Bligh and Dyer (1959), in which 40 g of meat was homogenized in chloroform:methanol (80:40 v/v). After being stirred for 1 h, 40 ml of chloroform and 40 ml of distilled water were added and the homogenate was stirred for an additional 20 min. The homogenate was then filtered through a Whatman filter paper N^o. 1 held in a Buchner funnel using vacuum pressure. The filtrate was transferred to a separator funnel and 40 ml of an aqueous solution of 0.9% NaCl was added. After the phase separation was complete, the chloroform phase and all of the lipid was collected in a round-bottom volumetric flask and the solvent was evaporated using a rotary evaporator with the temperature held at 33–34 °C. The hydrolytic and transesterification processes were performed according to ISO method 5509 (1978). Two milliliters of n-heptane was added to 200 mg of lipid and the mixture was vigorously stirred until the lipids were completely solubilized. Then, 2 ml of 2 M NaOH in methanol were added and the mixture was stirred. After the phase separation was complete, the upper phase that contained n-heptane and the fatty acid methyl esters was removed using an automated pipette, transferred to an amber vial, and stored at –18 °C until gas-chromatographic analysis was performed. The fatty acid methyl esters were analyzed using a Shimadzu 17A gas

chromatograph equipped with a flame-ionization detector and a capillary column (100 m × 0.25 mm) containing 0.25 µm particles in IBS CP 88 cyanopropyl polysiloxane. The column temperature program was as follows: 65 °C for 15 min; 10 °C min⁻¹ to 165 °C and held for 2 min; 4 °C min⁻¹ to 185 °C and maintained for 8 min; 4 °C min⁻¹ to 235 °C and maintained for 5 min. The detector and the injector were maintained at 260 °C, and the split ratio was 1/100. The gas flow rates were 1.2 ml min⁻¹ for the carrier gas (H₂), 30 ml min⁻¹ for the auxiliary gas (N₂), 30 and 300 ml min⁻¹ for the flame gases, H₂ and synthetic air, respectively. To identify the fatty acids, the relative retention times of the peaks of the samples were compared with those of fatty acid methyl ester standards (Sigma). The results were expressed as the percentage of the normalized area of the fatty acid peak.

2.2.5 Statistical analysis

Data were analyzed as a complete randomized design using the GLIMMIX procedure of SAS, whereas dietary treatment and gender was considered main effects. For sensory evaluation, the experiment was arranged as a 4x2 factorial with fixed effects of dietary treatment (control, 3%, 6%, and 9% of pecan inclusion) and gender (male and female), whereas panelist was the random effect. Means separations were performed using the LSMEANS and DIFF functions when means were significant at $P \leq 0.05$. For Chi-square analysis, the FREQ procedure was used to test the frequency distribution of off-flavor descriptors.

3. Results and discussion

3.1 pH and proximate composition

Inclusion of pecans by-products in lamb diets did not affect ($P > 0.05$) pH, moisture, and protein (Table 2). The final pH values observed in this study was 5.48. The value is in

accordance to values reported by Landim et al. (2011) and Andrade et al. (2015) for Santa Ines lambs, and indicates a proper resolution of the *rigor mortis*. Moisture and protein values are also in accordance with the values reported by Monaco et al. (2014) for Santa Ines lambs raised in feedlot.

Fat % in the lean was significant higher in meat from females when compared to males (Table 2). Previous research showed that carcass weight and fat content is lower in meat from non-castrated males and highest in females (O’Riordan & Hanrahan, 1992). According to Aberle, Forrest, Gerrard, & Mills (2012) this variation occurs due to estrogens produced by ovaries in females, promoting deposition of body fat, where females fatten at younger ages and lighter weights than males.

An interaction between the two fixed effects led to higher levels of ash in meat from males fed control when compared to diets containing 9% of pecans (Table 3). In a study conducted by Gkarane et al. (2019) the authors observed an interaction between diet x feeding duration where animals fed 54 d had higher ash content in muscle, receiving a 50:50 concentrate:roughage diet, than animals receiving only roughage, or only concentrate.

3.2 Objective color and lipid oxidation

Dietary treatments did not affect objective color parameters (Table 4). However, meat from females were significant redder (a*) and yellower (b*) when compared to meat from males. According to Warner et al. (2007), and Pinheiro, Sobrinho, Souza, & Yamamoto (2009) animals with higher fat content can present darker and redder flesh, which determines lower capillary permeability, inducing difficulties in oxygen transfer between muscle fibers, causing an increase in the amount of myoglobin to supply the adequate oxygen needed. It is possible that greater amount of fat in the muscle is likely to be the reason for the yellower values in the meat (Mashele, Parker, & Schreurs, 2017). In addition, higher yellowness may

be correlated with carotenoid content in adipose tissue (Röhrle et al., 2011), which is usually higher in females when compared to males (Barrón et al., 2012).

Lipid oxidation was not affected by inclusion of pecans ($P>0.05$). (Table 4). Overall there was no significant difference between treatments for pH, unsaturated, and polyunsaturated fatty acids, which contributed to these findings for lipid oxidation (Ayala, Muñoz, & Argüelles, 2014; Amaral, Silva, & Lannes, 2018).

3.3 Cooking loss, sensory analysis, and objective tenderness

Inclusion of pecans in lamb diets did not affect cooking loss, and WBSF (Table 2). Lamb meat in this study was classified as mid soft (33.53 N). Sheep meat is classified as soft (22.26 N), mid soft (22.36 to 35.60 N), tough (22.36 to 35.60 N) and extremely hard (above 53.35 N) (Cezar & Sousa, 2007).

For tenderness it was observed an interaction between treatment and gender ($P=0.0017$) (Table 5). Meat from the control diet from males received the highest tenderness ratings followed by 9% and 3%, and treatment 6% being the less tender. For females, meat from animals fed the control diet also had the highest ratings and was not different from treatment 3%. For gender, treatment 6% females were more tender than meat from males ($P=0.0012$).

A diet effect was observed for juiciness ($P<0.0001$) where meat from control treatment had the highest ratings (Table 6). The adipose tissue influences the tenderness of meat (Nishimura, Hattori, & Takahashi, 1999), whereas higher intermuscular fat increases succulence perception (Osório, Osório, & Sañudo, 2009). In this study, authors did not expect to detect difference in tenderness and juiciness when comparing dietary treatments since different diets did not affect WBSF or fat content in the lean. The results observed for gender suggest that fat is not the only attribute for tenderness where treatment 6% received the

highest score, but it was the treatment with less fat, even not being significant. According to Osório, Osório, & Sañudo (2009) other tenderness characteristics are related to water holding capacity, pH, marbling, and the characteristics of the connective tissue and muscle fiber.

Flavor intensity was higher in meat from males than females ($P=0.0217$), and for treatments, intensity ratings were higher ($P=0.0132$) for treatment control, and similar to treatment 6%, while no differences were observed between treatments 3%, and 9% (Table 6). A gender effect was expected where it is known that intact males show more intense flavour, both in meat and fat, than castrated males, or females (Osório, Osório, & Sañudo, 2009; Gkarane et al., 2017)

For overall acceptability treatment control had the highest ratings ($P>0.0346$), and 6% receiving the lowest ratings (Table 6). Treatments 3%, and 9% were similar and received an intermediary classification. The higher overall acceptability for meat from animals fed the control diet can be related to higher ratings for tenderness, and juiciness. A gender effect was observed ($P=0.0350$) with meat from males receiving higher scores for overall acceptability than meat from females. According to Rousset-Akrim, Young, & Berdagué (1997) and Arshad et al. (2018), several researches have explored the biochemical and chemical origin that cause sheep odors, there is an understanding that fat is the main source of odor. This suggests the highest overall acceptability for gender may be associated to less fat amount in meat from males (2.98), than in meat from females (3.72).

Dietary treatments did not affect the frequency of different off-flavors (Figure 1). However, meat from females were sweeter ($P<0.0001$), and bloodier ($P=0.0249$) than meat from males, and meat from males were higher ($P<0.0001$) in sheep meat flavor than meat from females (Figure 2). Sweet odor and taste are traits often considered to be positive in meat according to Prescott, Young, & Neil (2001), and are related to specific volatile compounds, amino acids, organic acids, and sugars (Brewer, 2006). Young, Lane, Priolo, &

Fraser (2003) noted a sweet note in lamb meat that was very easily detectable by their panelists, yet no volatiles compounds were associated with this descriptor as a result of their analyses. Results from this study showed that meat from females had a higher intensity of sweet flavor, which is in agreement with data published by Lind, Berg, Eilertsen, Hersleth, Eik (2011). According to Arshad et al. (2018) meat from intact males may be different in flavor characteristics when compared to castrated males or females. For Caporaso, Sink, Dimick, Mussinan, & Sanderson (1977) fourteen “key” compounds (aldehydes, ketones, and lactones) in the neutral fraction of cooked fat volatiles are significant contributors to lamb/mutton flavor.

3.4 Fatty acids

The fatty acid composition of meat will vary according to animal's age, sex, breed, diet, and within muscles (Wood & Enser, 1997). Triglycerides are biohydrogenated in the rumen by microbial lipases produced by bacteria releasing the fatty acids (Jenkins, 1993), and the fatty acids reaching the duodenum originate directly from diets and microbial transformation (Jenkins, Wallace, Moate, & Mosley, 2008).

Greater levels of margaric (C17:1n7 – Heptadecenoic acid) and oleic (C18:1n9 – *cis*-9-Octadecenoic acid) acids were observed in the lean of females (P=0.03 and 0.01, respectively), whereas meat from males had greater values of linoleic acid (C18:2n6 – *cis*-9,*cis*-12-Octadecadienoic acid) (P<0.01) (Table 7). Fatty acids with an odd number of carbon atoms such as pentadecanoic (C15:0) and heptadecanoic acid (C17:0) are produced primarily from accumulation of a 3-carbon fatty acid end product that occurs due to the lack of vitamin B12 (Abbas, Mohamed, & Jamilah, 2009). Linoleic and linolenic acids are the main unsaturated fatty acids in ruminant diets (Woods & Fearon, 2009), and must be provided in the diet because *de novo* synthesis requires desaturase enzymes that are absent in mammals (Nakamura, & Nara, 2003). For most diets, ruminal biohydrogenation of 18:1 *cis*-9, 18:2n-6

and 18:3n-3 varies between 58% to 87%, 70% to 95% and 85% to 100%, respectively (Glasser, Schmidely, Sauvant, & Doreau, 2008; Shingfield, Bernard, Leroux, & Chilliard, 2010). According to Wood, Bell, Grainger, & Teekel (1963), after 48 hours in the rumen, 3 to 6% of linoleic acid remain unaltered by biohydrogenation, and 46% is converted to stearic acid, 33 to 50% in oleic acid, or 18:1 trans. Additionally, Ward, Scott, Dawson (1964) reported that linolenic acid is rapidly hydrogenated in the rumen environment, generating linoleic, oleic, and stearic acids.

The present study showed that dietary treatment significantly affected the intramuscular concentration of individual fatty acids (Table 7). Feeding 9% of pecans significantly increased deposition of Eicosapentaenoic acid (EPA, C20:5n3) when compared to 0% and 3% (0.15^a, 0.08^{ab}, 0.03^b, and 0.01^b for 9%, 6%, 0%, and 3%, respectively). Additionally, the inclusion of 6% of pecans in diets led to greater values of Docosahexaenoic acid (DHA, C22:6n3) when compared to lamb fed 0% and 3% diets (1.12^a, 1.02^{ab}, 0.68^{bc}, and 0.60^c for 6%, 9%, 0%, and 3%, respectively).

Phospholipids in muscle contain a high PUFA content (20–50% of the total fatty acids in the phospholipids), which includes long-chain fatty acids with 18, 20, and 22 carbons and two to six double bonds. The PUFA proportion of the phospholipids is controlled by a complex enzymatic system, including desaturases and elongases, which are responsible for the conversion of both the precursor's linoleic and α -linolenic acid to arachidonic acid (AA), EPA, docosapentaenoic acid (DPA), and DHA (Raes, De Smet, & Demeyer, 2004).

In an attempt to lower the economic and social load of chronic diseases, public health policies in most developed countries recommend population to decrease the intake of total fat, SFA and TFA and increase the consumption of the long-chain n-3 polyunsaturated fatty acids (PUFA), 20:5n-3 and 22:6n-3 (Shingfield, Bonnet, & Scollan, 2013; Alvarenga, Chen,

Furusho-Garcia, Perez, & Hopkins, 2015), which are the n-3 fatty acids involved in decreasing the thrombotic tendency of blood in humans (Wood & Enser, 1997).

4. Conclusion

In this study, feeding up to 9% of pecans positively affect nutritional value of lamb by increasing deposition of desirable fatty acids such as EPA and DHA, without compromising carcass weight, color, lipid stability, and sensory. Utilizing pecan byproducts is an alternative for producers who may have access to this feedstuff.

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Table 1. Ingredients and chemical composition of dietary treatments

Ingredient	Ingredients (% of dry matter)			
	Control	3%	6%	9%
Sorghum Silage	40.00	40.00	40.00	40.00
Soybean meal	35.00	33.72	32.40	31.45
Broken rice	21.00	19.95	18.90	17.85
Commercial mineral mix	1.00	0.95	0.90	0.85
Calcitic limestone	1.00	0.95	0.90	0.85
Soybean oil	2.00	1.42	0.90	0.42
Pecan	0.00	3.00	6.00	9.00
	Chemical composition (% of dry matter)			
	Control	3%	6%	9%
Dry Matter	62.95	62.97	62.92	63.00
Protein	22.60	22.17	22.00	22.10
Ether Extract	3.70	4.40	5.12	5.47
Ash	4.56	4.50	4.08	4.14
Acid Detergent Fiber	15.40	15.43	15.42	15.43
Neutral Detergent Fiber	30.12	30.10	30.10	30.09
NDT	72.50	73.12	73.30	73.03

Table 2. Fatty acid profile of dietary treatments (% of FAME).

	Dietary Treatments			
	Control	3%	6%	9%
C4:0	0.09	0.11	0.06	0.04
C5:0	0.08	0.05	0.04	0.03
C12:0	0.13	0.08	0.05	0.04
C13:0	0.08	0.04	0.00	0.00
C14:0	0.18	0.13	0.11	0.10
C14:1 n5	0.00	0.04	0.01	0.00
C15:0	0.07	0.03	0.04	0.03
C15:1 n5	0.05	0.15	0.20	0.07
C16:0	13.23	10.54	9.33	8.40
C16:1 n7	0.13	0.10	0.09	0.08
C17:0	0.21	0.14	0.12	0.10
C17:1 n7	0.05	0.03	0.05	0.05
C18:0	3.96	3.19	2.88	2.67
C18:1 t6	0.09	0.06	0.04	0.03
C18:1 t9	0.02	0.00	0.00	0.00
C18:1 t11	0.00	0.03	0.01	0.02
C18:1 c9	19.36	35.41	41.71	46.68
C18:1 c11	1.40	1.31	1.28	1.27
C18:2 n6	48.01	40.49	37.85	35.61
C18:3 n6	0.50	0.33	0.27	0.20
C20:0	0.25	0.11	0.06	0.03
C18:3 n3	7.73	4.57	3.43	2.45
C20:1 n9	0.21	0.24	0.26	0.26
CLA c9, t11	0.00	0.00	0.00	0.02
C20:2 n6	0.04	0.08	0.01	0.02
C20:3 n6	0.55	0.30	0.21	0.15
C20:5 n3	0.02	0.03	0.04	0.02
C24:0	0.56	0.32	0.22	0.16
C22:3 n3	0.11	0.08	0.09	0.06
C22:5 n3	0.03	0.05	0.00	0.00
C22:6 n3	0.09	0.05	0.07	0.08
Total	97.20	98.10	98.53	98.67
SFA	18.82	14.74	12.92	11.59
PUFA	57.07	45.98	41.97	38.61
MUFA	21.31	37.38	43.64	48.47
Omega 6	49.09	41.20	38.34	35.98
Omega 3	7.98	4.78	3.64	2.61
n6:n3	6.15	8.61	10.55	13.79
Others	2.80	1.90	1.47	1.33

Table 3. Proximate composition, pH, cooking loss, and WBSF of *longissimus dorsi et lumborum* from lambs fed pecan by-products.

	Dietary Treatment				Gender		P Value	
	Control	3%	6%	9%	Male	Female	Treatment	Gender
FAT (%)	3.40	3.41	3.20	3.41	2.98 ^b	3.72 ^a	0.9559	0.0303
MOIS (%)	75.31	75.04	74.61	74.60	24.82	25.25	0.3385	0.1312
PROT (%)	19.98	19.86	20.49	20.24	20.26	20.02	0.6360	0.5414
pH	5.54	5.44	5.49	5.47	5.48	5.48	0.3004	0.9823
CKL (%)	25.85	26.97	26.49	26.76	26.18	26.84	0.8128	0.4592
WBSF (N)	32.35	32.66	35.79	33.34	35.40	31.67	0.6572	0.0987

^{a,b}Means in the same row having different superscripts are significant at $P < 0.05$.

Table 4. Ash interactions of *longissimus dorsi et lumborum* from lambs fed pecan by-products.

	Control	3%	6%	9%
Male	1.05 ^A	1.02 ^{AB}	0.97 ^{AB}	0.93 ^B
Female	0.97	0.93	1.00	1.03

^{A,B}Means in the same row having different superscripts are significant at $P = 0.0499$ within ash.

Table 5. Objective color and lipid oxidation of *longissimus dorsi et lumborum* from lambs fed pecan by-products.

	Dietary Treatment				Gender		P Value	
	Control	3%	6%	9%	Male	Female	Treatment	Gender
L*	38.68	37.71	37.90	38.55	37.91	38.51	0.2364	0.1345
a*	15.70	15.80	15.32	15.73	15.13 ^b	16.14 ^a	0.8536	0.0292
b*	10.83	10.68	10.53	10.72	10.41 ^b	10.97 ^a	0.6436	0.0038
TBA	1.31	1.45	1.63	1.45	3.22	3.37	0.3892	0.5810

^{a,b}Means in the same row having different superscripts are significant at $P < 0.05$.

Table 6. Interactions of sensory attribute for tenderness of *longissimus dorsi et lumborum* from lambs fed pecan by-products.

	Control	3%	6%	9%
Male	7.53 ^A	6.48 ^B	5.61 ^{Cb}	6.55 ^B
Female	7.11 ^A	6.69 ^{AB}	6.39 ^{Ba}	6.28 ^B

^{A,B}Means in the same row having different superscripts are significant at $P = 0.0017$ within tenderness.

^{a,b}Means in the same column having different superscripts are significant at $P = 0.0017$ within tenderness.

Table 7. Sensory attributes of *longissimus dorsi et lumborum* from lambs fed pecan by-products.

Attribute	Dietary Treatment				Gender		P Value	
	Control	3%	6%	9%	Male	Female	Treatment	Gender
Juiciness	6.72 ^a	6.07 ^b	5.84 ^b	5.76 ^b	6.06	6.14	<0.001	0.5083
Flavour Intensity	5.07 ^a	4.35 ^b	4.64 ^{ab}	4.40 ^b	4.81 ^a	4.42 ^b	0.0132	0.0217
Overall Acceptability	5.18 ^a	4.93 ^{ab}	4.49 ^b	4.76 ^{ab}	5.02 ^a	4.66 ^b	0.0346	0.0350

^{a,b}Means in the same row having different superscripts are significant at $P < 0.05$.

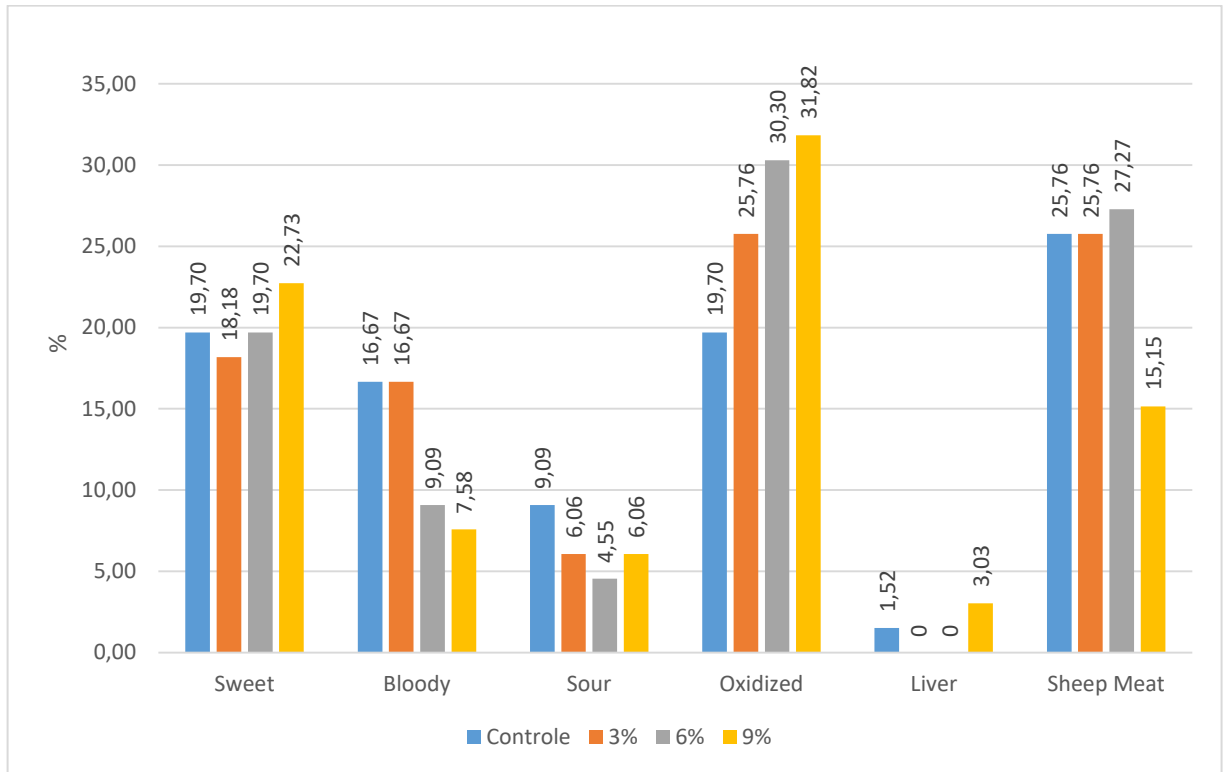


Figure 1. Sensory off-flavor descriptor Chi-square frequency distribution per treatments. Descriptors were equally distributed across muscle ($P>0.05$).

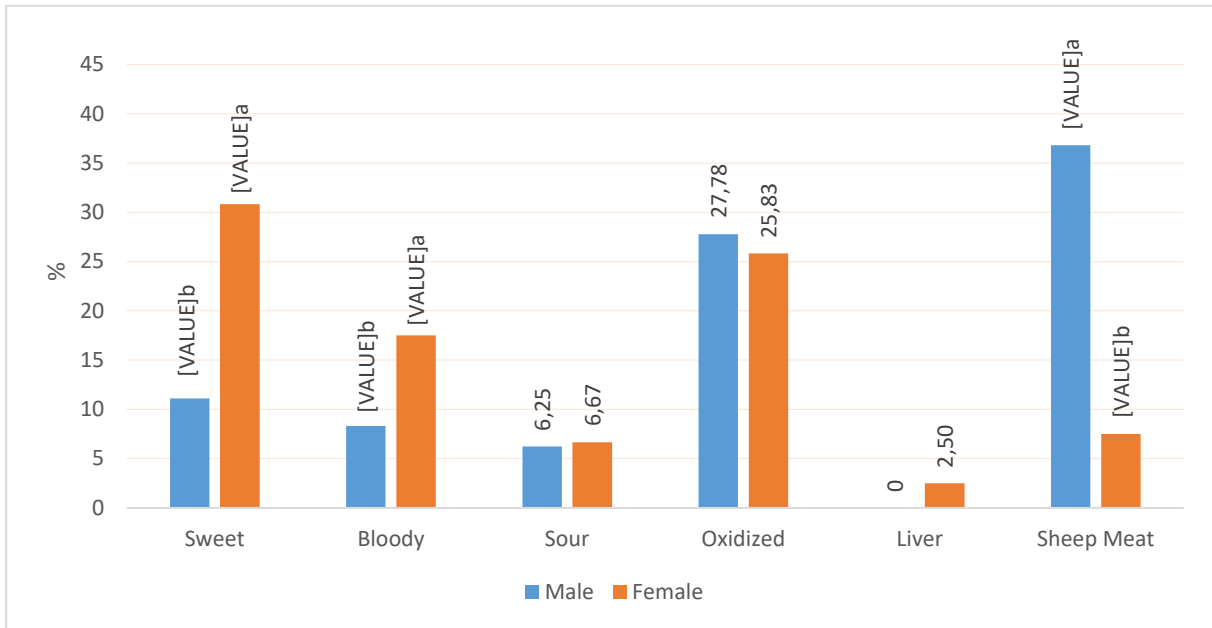


Figure 2. Sensory off-flavor descriptor Chi-square frequency distribution for gender. Sweet, and bloody were not equally distributed across muscle ($P \leq 0.05$) for females, and sheep meat for males.

Table 8. Weight percentage of fatty acids of *longissimus dorsi et lumborum* from lambs fed pecan by-products (% of FAME).

	Dietary Treatments				Gender		P Value	
	Control	3%	6%	9%	Male	Female	Treatment	Gender
C14:0	2.19	2.07	2.03	2.22	2.08	2.18	0.6296	0.4321
C14:1 n5	0.09	0.15	0.10	0.13	0.10	0.13	0.4269	0.2013
C16:0	24.08	23.26	22.34	22.89	22.98	23.30	0.3480	0.6456
C16:1 n7	1.72	1.84	1.64	1.70	1.70	1.75	0.1665	0.4224
C17:0	0.65	0.61	0.54	0.61	0.57	0.63	0.1489	0.0611
C17:1 n7	0.41	0.40	0.35	0.38	0.34 ^b	0.43 ^a	0.7143	0.0319
C18:0	15.62	14.47	15.58	15.21	15.69	14.75	0.3953	0.0890
C18:1 t11	1.76	1.56	1.66	1.55	1.72	1.54	0.7227	0.2385
C18:1 c9	44.97	47.46	45.13	44.04	43.82 ^b	46.98 ^a	0.1985	0.0110
C18:2 n6	3.15	3.42	3.23	3.23	3.90 ^a	2.62 ^b	0.9658	0.0050
C18:3 n3	1.25	1.06	1.34	1.11	1.30	1.09	0.3189	0.0863
CLA c9, t11	0.24	0.17	0.26	0.33	0.28	0.21	0.4740	0.3286
C20:0	0.00	0.01	0.05	0.03	0.04	0.01	0.4938	0.3498
C20:3 n6	0.19	0.15	0.27	0.36	0.26	0.23	0.4154	0.7640
C20:4 n6	0.33	0.17	0.28	0.47	0.26	0.23	0.3829	0.6822
C20:5 n3	0.04 ^b	0.01 ^b	0.09 ^{ab}	0.16 ^a	0.07	0.07	0.0300	0.9195
C22:5n3	0.25	0.09	0.21	0.23	0.23	0.16	0.6324	0.4349
C22:6 n3	0.68b ^c	0.60 ^c	1.12 ^a	1.02 ^{ab}	0.96	0.74	0.0187	0.0925
Total	97.62	97.51	96.22	95.65	96.37	97.12	0.5182	0.5071
SFA	42.54	40.42	40.54	40.96	41.36	40.87	0.3895	0.6115
PUFA	6.13	5.67	6.80	6.91	7.26 ^a	5.35 ^b	0.5578	0.0120
MUFA	48.95	51.41	48.88	47.80	47.68 ^b	50.83 ^a	0.1782	0.0126
Omega 6	3.91	3.91	4.04	4.39	4.70 ^a	3.29 ^b	0.9352	0.0078
Omega 3	2.22	1.76	2.76	2.52	2.56	2.06	0.1639	0.1205
n6:n3	1.76	2.22	1.46	1.74	1.84	1.60	0.3758	0.2499
Others	2.38	2.50	3.78	4.33	3.70	2.95	0.5178	0.5075

^{a,b}Means in the same row having different superscripts are significant at $P < 0.05$.

6. CONCLUSIONS

Pecan by-products can be successfully incorporated in lamb diets in up to 9% without leading to any detrimental effects on carcass, value-added cuts, and meat quality attributes. Feeding 9% of pecan by-products increased deposition of desirable fatty acids such as eicosapentanoic acid (EPA) and feeding 6% increased docosahexaenoic acid (DHA).

7. ATTACHEMENTS

ATTACHMENT A – GUIDELINES SMALL RUMINANT RESEARCH



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ATTACHMENT B – GUIDELINES FOR MEAT SCIENCE



MEAT SCIENCE



ISSN: 0309-1740

DESCRIPTION

Meat Science has been the leading journal in its field now for more than 40 years.

The qualities of **meat** – its **composition**, **nutritional value**, wholesomeness and **consumer** acceptability – are largely determined by the events and conditions encountered by the embryo, the live animal and the postmortem musculature. The control of these qualities, and their further enhancement, are thus dependent on a fuller understanding of the commodity at all stages of its existence – from the initial conception, growth and development of the organism to the time of slaughter and to the ultimate **processing**, preparation, distribution, cooking and consumption of its meat.

It is the purpose of *Meat Science* to provide an appropriate medium for the dissemination of interdisciplinary and international knowledge on all the factors which influence the **properties** of meat. The journal is predominantly concerned with the flesh of **mammals**; however, contributions on poultry will only be considered, if they demonstrate that they would increase the overall understanding of the relationship between the nature of muscle and the quality of the meat which muscles become *post mortem*. Papers on large birds (e.g. emus, ostriches) and wild capture mammals and crocodiles will be considered.

AUDIENCE

Meat scientists, food technologists, food manufacturers, agricultural chemists and research workers.

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 Chemical Abstracts
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Introduction

The qualities of meat - its composition, nutritional value, wholesomeness and consumer acceptability - are largely determined by the events and conditions encountered by the embryo, the live animal and the postmortem musculature. The control of these qualities, and their further enhancement, are thus dependent on a fuller understanding of the commodity at all stages of its existence – from the initial conception, growth and development of the organism to the time of slaughter and to the ultimate processing, preparation, distribution, cooking and consumption of its meat.

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Introduction

State the objectives of the work and provide an adequate background, avoiding a detailed literature survey or a summary of the results.

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Statistical Analysis

Prior to conducting an experiment, due consideration needs to be given to the design of the experiment. This is so that after analysis of the data, some confidence can be given to the conclusions. For example if a study is designed to compare different breeds of cattle it is important that the animals selected are representative of the breed, not from a small number of sires and that individual animals sampled in the study can be linked back to their sire. If this condition isn't applied then the results may well reflect sire effects more than breed effects and the difference impossible to determine.

Another common problem in meat and food science is the lack of replication and also confounding. This is illustrated with two examples below taken from submitted papers:

Example 1

A total of thirty crossbred male lambs, single born in June were used in an experiment to compare three production systems (12 lambs allocated per system) and the subsequent effects not only on growth and carcass traits, but also meat quality traits. Lambs of the three production systems were weighed fortnightly. When a 35kg live weight target was achieved the lambs weighing >35kg were transported to an abattoir. Lambs were slaughtered after an overnight lairage without feed, but free access to water.

There are a number of issues with the design.

1. No mention was included in the paper as to whether the 36 lambs used in the study (a) were randomly selected from a population; or (b) were randomly assigned to the three treatment groups. It was assumed by the reviewer that they were randomly selected and assigned.
2. The animals within each group were run together, but separately from the other two groups. Hence there is no replication of treatment group.
3. Each lamb in a treatment group in the study is subjected to a specific production system and this may not be representative of other lambs grown under that specific treatment at a different establishment. Thus treatment group is not replicated which is necessary to assess the variability of a particular production system under different conditions.
4. The other major issue with the design is that, at fortnightly intervals, lambs were weighed and lambs exceeding 35 kg were slaughtered. Hence not only were the treatment groups not replicated, they were also confounded with slaughter age/day and for meat quality traits like pH and colour it meant slaughter day effects could arise. With such small numbers per treatment group slaughter day could not be effectively accounted for in the analysis.

Example 2

Hams were produced with five decreasing levels of phosphate in combination with 5 increasing levels of thyme. All formulations were applied to a **single batch** of pig meat. Each formulation produced one mixture which was vacuum stuffed into plastic casings to produce four ham 'replicates'. These were cooked in a water bath.

This method produced pseudo replicates (Hurlbert 1984, 2009; Maindonald 1992). The cooked hams are subsamples of the pig mixtures of each formulation. The ham to ham (sub-sample) variability does not represent the mixture to mixture (treatment) variability. To get the correct measure of variability to compare treatments the mixing process for each formulation would need to be replicated. The hams produced from each mixing of the formulation would give true replication of that formulation.

Relevant references:

Granato, D., Calado, V., & Jarvis, B. (2013). Observations on the use of statistical methods in Food Science and Technology. *Food Research International*, 55, 137-145.

<http://www.sciencedirect.com/science/article/pii/S0963996913005723>

Hill, T. & Lewicki, P. (2007). *STATISTICS: Methods and Applications*. StatSoft, Tulsa, OK.

Hassleer & Thadewald (2003) - *The Statistician* 52(3) 367-379 for detail on multivariate linear modelling. Some other papers to consider in this area - Starkey, C.P., et al. (2017).

The relationship between shear force, compression, collagen characteristics, desmin degradation and sarcomere length in lamb biceps femoris. *Meat Science*, 126, 18-21.

Starkey, C.P., et al. (2015). Explaining the variation in lamb longissimus shear force (tenderness) across and within ageing periods using protein degradation, sarcomere length and collagen characteristics. *Meat Science*, 105, 32-37.

Experimental

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[dataset] Oguro, M., Imahiro, S., Saito, S., Nakashizuka, T. (2015). Mortality data for Japanese oak wilt disease and surrounding forest compositions. Mendeley Data, v1. <http://dx.doi.org/10.17632/xwj98nb39r.1>.

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