UNIVERSIDAD POLITÉCNICA DE MADRID
ESCUELA TÉCNICA SUPERIOR DE INGENIERÍA AGRONÓMICA, ALIMENTARIA Y DE BIOSISTEMAS

EVALUATION OF ALMOND HULLS AS AN ALTERNATIVE INGREDIENT FOR RUMINANT FEEDING

DOCTORAL THESIS

Submitted for the degree of Doctor by:

Adriana Recalde Garcete
M. Sc in Animal Production and Health

Madrid, 2025

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ABSTRACT

The objective of this Doctoral Thesis was to investigate the nutritional value for ruminants of Spanish almond hulls (AH). Experiment 1 analysed the variability in chemical composition and energy content of 38 AH samples and the influence of the almond variety (Guara and Soleta) on AH characteristics, and studied the 24-h *in vitro* ruminal fermentation of diets including dried AH (0, 8, 16 and 24% of the diet; fresh matter basis). Samples had low content (dry matter (DM) basis) in crude protein (CP; average 7.29%) and ether extract (EE; 4.01%), medium in neutral detergent fibre (NDF; 33.9%) and acid detergent fibre (ADF; 24.7%), and high in total sugars (26.8%). Coefficient of variation was above 21% for all chemical fractions, except for fibre fractions (< 17%). The estimated net energy ranged from 0.60 to 0.82 UFL (forage units for milk production)/kg DM and from 0.52 to 0.71 UFV (forage units for meat production)/kg DM. Guara-AH had greater ($P \leq 0.015$) content in ash, CP, EE and energy, but lower ($P \leq 0.001$) in NDF and ADF than Soleta-AH. In the *in vitro* trial, including up to 16% of AH partially substituting corn, wheat bran and sugar beet pulp had no negative effects on volatile fatty acid (VFA) production, but VFA production was reduced at all AH levels when AH replaced alfalfa hay and corn. No antimethanogenic effects of AH were detected *in vitro*.

Experiment 2 investigated the effects of including dried AH (0, 6 and 12%) in isonitrogenous and isoenergetic concentrates for light lambs on animal performance, and carcass and meat characteristics. Thirty Manchega lambs (15 males and 15 females; 12.3 ± 0.18 kg body weight (BW)) were distributed into 3 homogeneous groups according to BW and sex, and each group was randomly assigned to one concentrate. Lambs were fed concentrate and barley straw *ad libitum* for 47 days until reaching about 23.0 kg BW. There were no differences ($P \geq 0.182$) between groups in final BW, feed intake, and feed conversion rate. Average daily gain and digestibility of DM and CP tended ($P < 0.10$) to decrease by feeding the AH-

concentrates, but concentrations of plasma metabolites and hepatic enzymes were similar ($P \geq 0.213$) in all groups. Weights, yield, cooling losses and most conformation measurements of carcass and colour, pH and chemical composition of meat were unaffected ($P \geq 0.175$) by the concentrate. Concentration of thiobarbituric acid reactive substances after 6 days of meat storage tended to increase ($P = 0.056$) by feeding AH, which agrees with the greater ($P < 0.05$) polyunsaturated fatty acid (FA) content in the meat of lambs fed AH concentrates compared with control lambs. The results indicate that including up to 12% of AH in the concentrate of light lambs can improve the meat FA profile without impairing lamb growth, but can increase meat lipid oxidation.

Experiment 3 analysed the effects of different additives for ensiling AH. Silages were prepared in vacuum bags (500 g AH/bag; 5 bags/treatment) without additives (control) and with formic acid (4 mL/kg), a 1:1 acetic:lactic acids mixture (4 mL/kg), or a *Lactobacillus buchneri* inoculum (5×10^9 CFU (colony forming units)/kg). After 90 days of storage, all silages were well preserved and had pH < 5.05. Compared with control silage, all additives decreased ($P < 0.05$) final pH and reduced ($P < 0.05$) yeasts and molds counts. No *Escherichia coli*, *Salmonella* spp., *Listeria* spp., *Clostridium perfringens* or *Staphylococcus coagulase+* were detected in any silage. No propionic were detected in any silage and butyric was only detected in silages with *L. buchneri*. All additives resulted in only minor differences in chemical composition of silages and did not affect ($P = 0.131$) their energy content.

The results of this Doctoral Thesis could contribute to promoting the use of AH in ruminant feeding, minimizing the environmental effects produced by their management and disposal, and to foster circular bioeconomy, especially when used locally.

RESUMEN

El objetivo de esta Tesis Doctoral fue determinar el valor nutritivo para rumiantes de la capota de almendra (CA) generada en España. En el Experimento 1 se analizó la composición química y el contenido energético de 38 muestras de CA, se compararon muestras de CA de dos variedades de almendra (Guara y Soleta), y se estudió la fermentación ruminal *in vitro* (24 h) de dietas con CA seca (0, 8, 16 y 24%, en materia seca (MS)). La CA presentó bajo contenido en proteína bruta (PB; 7,29%) y extracto etéreo (EE; 4,01%), medio en fibra neutro detergente (FND; 33,9%) y fibra ácido detergente (FAD; 24,7%), y elevado contenido en azúcares totales (26,8%). Todas las fracciones químicas tuvieron altos coeficientes de variación (> 21%) excepto las fracciones fibrosas (< 17%). El contenido energético varió de 0,60 a 0,82 unidades forrajeras leche (UFL)/kg MS y de 0,52 a 0,71 unidades forrajeras carne (UFC)/kg MS. La CA Guara tuvo mayor ($P \leq 0,015$) contenido en cenizas, PB, EE y energía, pero menor ($P \leq 0,001$) en FND y FAD que la CA Soleta. La sustitución parcial de maíz, salvado de trigo y pulpa de remolacha por CA hasta el 16% de la dieta no redujo la producción de ácidos grasos volátiles (AGV) *in vitro*, pero la producción se redujo cuando la CA reemplazó al heno de alfalfa y maíz. No se detectaron efectos antimetanogénicos de la CA *in vitro*.

En el Experimento 2 se evaluaron los efectos de incluir CA seca (0, 6 y 12%) en concentrados isonitrogénicos e isoenergéticos para corderos ligeros sobre el rendimiento productivo y la calidad de la canal y la carne. Treinta corderos manchegos (15 machos y 15 hembras; peso vivo (PV) 12,3 ± 0,18 kg) se distribuyeron en 3 grupos (10 corderos/tratamiento) y cada grupo recibió un concentrado y paja de cebada *ad libitum* durante 47 días, hasta alcanzar unos 23 kg de PV. No hubo diferencias ($P \geq 0,182$) en PV final, ingestión de alimento, índice de conversión ni metabolitos plasmáticos. La ganancia media diaria y digestibilidad de MS y PB tendieron ($P < 0,10$) a disminuir con los concentrados con CA. No hubo efectos ($P \geq 0,175$)

en el PV final, en el rendimiento, las pérdidas por enfriamiento y la conformación de la canal, ni en el color, el pH o la composición química de la carne. La concentración de sustancias reactivas al ácido tiobarbitúrico (TBARS) tras 6 días de almacenamiento de la carne tendió (P = 0,056) a aumentar con los piensos con CA, concordando con una mayor proporción (P < 0,05) de ácidos grasos (AG) poliinsaturados en la carne de estos animales. Los resultados indican que incluir hasta un 12% de CA en el concentrado de corderos ligeros puede mejorar el perfil de AG de la carne sin reducir el crecimiento de los corderos, pero puede aumentar la oxidación lipídica de la carne.

En el Experimento 3 se analizaron 4 tratamientos para ensilar la CA: sin aditivos (control), ácido fórmico (4 mL/kg), mezcla 1:1 ácido acético:láctico (4 mL/kg), o *Lactobacillus buchneri* (5×10^9 unidades formadoras de colonias (UFC)/kg). La CA se ensiló en bolsas de vacío (500 g CA/bolsa) y se prepararon 5 bolsas para cada tratamiento. Tras 90 días, todos los ensilados estaban bien conservados y tenían un pH menor de 5,05. Los tres aditivos redujeron (P < 0,05) el pH final y los recuentos de levaduras y mohos en comparación con el ensilado control. No se detectaron ácido propiónico y butírico (excepto con *L. buchneri*) ni bacterias patógenas (*Escherichia coli*, *Salmonella* spp., *Listeria* spp., *Clostridium perfringens*, *Staphylococcus coagulasa+*) en ningún ensilado. Hubo pequeñas diferencias en la composición química de los ensilados, sin verse afectado (P = 0,131) su contenido energético.

Los resultados de esta Tesis Doctoral pueden contribuir a promover el uso de la CA en la alimentación de rumiantes, minimizando los efectos ambientales de su manejo y eliminación, y a fomentar la economía circular en el sector agropecuario, en especial si la CA se utiliza localmente.

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Abbreviations and Acronyms

A	Absorbance
a*	Red – green index
ADF	Acid detergent fibre
ADG	Average daily gain
AFOM	Amount of apparently fermented organic matter
AGPR	Average gas production rate
AH	Almond Hulls
ALA	Acetic and lactic acid – Experiment 3
ALP	Alkaline phosphatase
ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemists
b*	Yellow – blue index
BUP	Buttock perimeter
BUW	Buttock width
BW	Body weight
C	Fractional rate of gas production
C*	Chroma
CF	Crude fibre
CFU	Colony forming units
CIE	International Commission on Illumination
CIELAB	CIE L* a* b* colour system
CON	Silage treatment without additives – Experiment 3
CP	Crude protein
CTD	Thoracic depth
CV	Coefficient of variation
CWD	Carcass width
DM	Dry matter
DMED	Effective dry matter degradability
EE	Ether extract

EU	European Union
FA	Fatty acid
FA	Formic acid– Experiment 3
FAO	Food and Agriculture Organization
FAOSTAT	Food and Agriculture Organization Statistics
FCR	Feed conversion rate
GOT	Glutamic oxaloacetic transaminase
GPT	Glutamic pyruvic transaminase
h*	Hue
HLL	Hind limb length
HP	High production diet – Experiment 1
ICL	Carcass internal length
INC	International Nut Council
INRA	National Institute for Agricultural Research
L*	Brightness
LAB	Lactic acid bacteria – Experiment 3
Lag	Time before starting gas production
MAPA	Ministry of Agriculture, Fisheries and Food of Spain
MDA	Malondialdehyde
ME	Metabolizable energy
MP	Medium production diet – Experiment 1
MUFA	Monounsaturated Fatty Acid
NDF	Neutral detergent fibre
NEf	Net Energy for meat production
NEl	Net Energy for milk production
NEm	Net Energy for maintenance
NRC	National Research Council
OM	Organic matter
PDMD	Potential dry matter degradability
PGP	Potential gas production
PUFA	Polyunsaturated Fatty Acid
SEM	Standard Error of the Mean

SEMc	Standard Error of the Mean of concentrate
SEMs	Standard Error of the Mean of sex
SFA	Saturated Fatty Acid
T	Time of gas measurement
TBARS	Thiobarbituric acid reactive substances
UFL	Forage units for milk production
UFV	Forage units for meat production
USA	United States of America
VFA	Volatile fatty acids

1. INTRODUCTION

The use of agroindustrial by-products in animal feeding has gained renewed interest in recent years due to its multiple benefits, such as a reduction of feeding costs and the use of feeds that do not compete directly with human food, and a decrease of the environmental burden produced by their accumulation (De Evan et al., 2020). Moreover, recycling agroindustrial by-products in animal feeding contributes to circular economy and sustainability in livestock farming (Rad et al., 2016). The global demand of almonds (*Prunus dulcis L.*) has increased worldwide above 150% over the past two decades and according to the Food and Agriculture Organization, the annual production reaches approximately 4 million tons (FAOSTAT, 2024). Spain has the largest area of almond orchards in the world, although currently ranks as the third country in terms of almond production, just behind USA and Australia (FAOSTAT, 2024). The processing of almond fruits for human consumption generates several products, being the almond hulls (AH) the most abundant. The AH are the outer covering of the seed and the hard shell, representing about half of the weight of the total fruit on fresh matter basis (Jafari et al., 2015; Barral-Martínez et al., 2021), although this value can range from 41.5 to 70.0% for different almond varieties (Godini, 1984). Almond hulls are rich in carbohydrates, including high proportions of sugars, and are widely used as feed for dairy cows in the USA and Australia. In contrast, AH are not regularly used in animal feeding in European countries, and they are traditionally left in the fields or used for biomass production (Barral-Martínez et al., 2021). Chemical composition and nutritive value of AH are influenced by several factors such as cultivation conditions, growing region, harvesting methods, dehulling procedures, and almond variety, among others (DePeters et al., 2020). Although some studies have compared different varieties of AH in the USA (DePeters et al., 2020; Aguilar et al., 1984) and Iran (Jafari et al., 2015), samples were collected from different growing regions, and this may have influenced the comparison.

Several studies have investigated the effects of replacing conventional feeds with AH in the diet of dairy cows (Aquilar et al., 1984; Williams et al., 20214), dairy goats (Reed et al., 1988), and lambs (Phillips et al., 2015; Rad et al., 2016; Scerra et al., 2022) on animal performance and quality of both milk and meat. However, there is limited information on the influence of AH on ruminal fermentation, and results on its potential effect on CH_4 production are controversial. Durmic et al. (2014) observed that AH could reduce *in vitro* CH_4 production without negatively affecting volatile fatty acids (VFA) production, which was attributed to the presence of bioactive compounds such as phenolics and triterpenoids in AH. In contrast, Williams et al. (2014) observed that replacing alfalfa cubes with AH in the diet of dairy cows did not affect CH_4 emissions. Therefore the potential antimethanogenic effect of AH is worth to be further investigated.

Spain has currently about 13.5 million heads of sheep, being the largest sheep producer in the European Union (EU) and producing about one quarter of the European sheep meat in 2023 (MAPA, 2023). In European Mediterranean countries, lambs are usually slaughtered at low body weight (BW), either as suckling lambs (10.0 – 12.5 kg BW) or light lambs (23 – 26 kg BW), because consumers prefer low-fat meat with mild flavor (Gutiérrez-Peña et al., 2022). The traditional feeding system for weaned light lambs in Mediterranean countries is based on *ad libitum* supply of high-cereal concentrates and straw to promote high growth rates. The potential of AH as feed ingredient for lamb feeding has been scarcely explored, and in previous studies AH replaced partly the forage (alfalfa) in the diet of lambs (Phillips et al., 2015; Rad et al., 2016) and in some cases, barley and maize were partially replaced by AH (Scerra et al., 2022). Moreover, these studies were conducted with heavy lambs with initial BW ranging from 27.8 kg (Scerra et al., 2022) to 40.0 kg (Phillips et al., 2015), and to our best knowledge there is no information on potential use of AH in the diet of light lambs. Moreover, there is limited information on the potential effects of AH on lamb meat quality. Scerra et al. (2022) reported

that replacing part of cereals (barley and maize) in the diet of lambs with AH did not affect the fatty acid (FA) profile of meat, but lipid oxidation after 5 and 7 days of refrigerated storage was reduced by AH for both cooked and raw meat. However, the effects of AH could be different in lambs slaughtered at lower weights.

Almond hulls have high content of moisture and are easily putrescible, making their preservation difficult. Therefore, the developing of low-cost effective preservation techniques to overcome this limitation would greatly contribute to facilitating their use in practical livestock feeding. Ensiling is a controlled microbial fermentation process that has been traditionally used to preserve high-moisture feeds for ruminants, but the ensiling potential of AH is still unexplored and to our knowledge only Sirakaya (2023) has reported values on composition and characteristic of AH silages. While ensiling of AH seems to be challenging due to their characteristics, the ensiling process can be enhanced by using additives such as acidifiers (propionic acid, acetic acid, formic acid) and bacterial inoculants (lactic acid bacteria; Yitbarek and Tamir, 2014).

2. STATE OF ART

2.1. Almond tree characteristics

The almond tree (*Prunus dulcis*) is a deciduous fruit tree from a temperate climate that, unlike other species of this genus whose commercial interest lies mainly in its juicy pulp or mesocarp, the commercial interest of the almond tree focuses on the seeds (Aguilar et al., 1984). In recent years, it has become a very profitable alternative fruit tree in fertile soils, due to the use of new varieties more appropriate to the climate and improvements in cultivation work (Malagón, 2020).

Regarding its climatic requirements, almond trees grow with low relative humidity, fertile and deep soils, with a loamy texture. Almond trees tolerate drought, but production can be multiplied by 20 if they are irrigated with sufficient water, going from 120 kg/ha to 2,500 kg/ha of seeds (Malagón, 2020). The optimal temperature range for photosynthetic activity is between 25-30ºC, but the almond tree is one of the most cold-resistant fruit species during the winter vegetative shutdown period, being able to withstand temperatures below -15ºC. The most determining climatic factors for the adaptation and productivity of almond trees cultivation are temperature, rainfall, relative humidity and wind (Felipe, 2000).

The almond fruit is a drupe that is composed of three different parts (King and Schade, 1986; Figure 2.1): the inner almond or kernel (seed), the central part or hard shell (endocarp), and the outer covering or greenish shell (mesocarp) that is known as almond hull (AH). As fruit ripening progresses, the AH undergoes dehiscence due to the ventral suture separating to show the lignified central shell that covers the almond, which is the part with the greatest commercial importance (Socias, 2017).

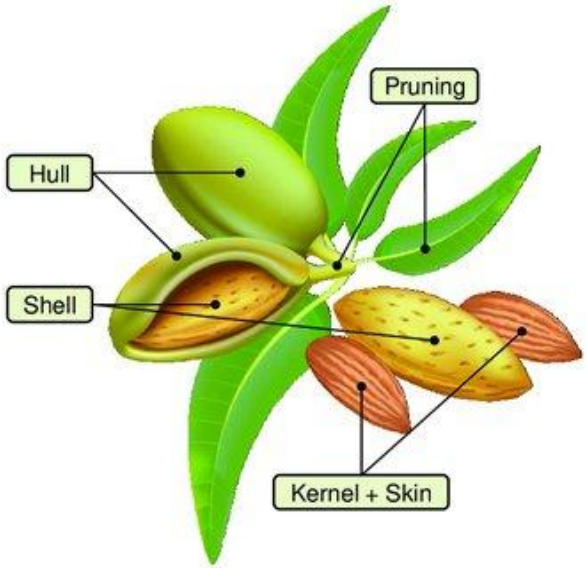

Figure 2.1 Almond fruit anatomy (Aguado, et al. 2021).

The external part of the AH is greenish in colour, although at maturity it changes to reddish tones, while the inner part of the AH in its youngest stages is generally yellowish-white (Esfahlan et al., 2010). The harvest of the almond is carried out normally when the first fruits begin to open, but the mesocarp remains completely attached to the inner shell. The thickness of the AH is very variable, ranging from 0.5 to 1.5 cm, and can represent between 50 to 65% of the total weight of the fruit (Jafari et al., 2015).

2.2. Almond production

In Europe, almond production is distributed in the Mediterranean region from Greece and the Balkans to Spain and Portugal. In the Middle East, Israel and Turkey are the greatest producer countries, while within North African countries Tunisia, Algeria and Morocco occupy the top positions. In the American continent, the production is mainly in the west coast of the United States of America (USA). Australia has also a significant almond production. Since 2012/13, almond crops in California, Australia and Spain have increased their volume by a factor of 1.3, 2.9 and 1.3, respectively (Kester and Asay, 1975; International Nut Council, 2023).

The USA is the main almond producer in the world with about 76% of the total almond production according to the INC (International Nut Council, 2023), Australia (10%) and Spain (4%) are next in importance, and they are followed by other countries such as Turkey, Tunisia, Chile, Iran, Morocco, Greece and Italy.

Spain produced approximately 317,907 t according to the data collected in the Ministry of Agriculture, Fisheries and Food of Spain (MAPA) Surface and Production Advance for 2023/2024 (MAPA, 2024). According to the MAPA estimates, almond production is expected to increase in the 2024/2025 campaign reaching 373,558 t, a significant 17.5% recovery from the previous year and about 9.1% above the average production of the last five campaigns (2019-2023). This increase in the almond production also means an increase of AH, as it is estimated that for each kg of almonds produced, two kg of AH are generated (Villagrasa and Díaz, 2021).

Almond production in Spain represents 73% of the total production of nuts and in the last years has continued to expand with an interannual average increase of 5%. According to the MAPA in the first quarter of the 2022/2023 campaign, nut exports were around 554 million of euros of value, of which 509 million of euros (about 92%) corresponded to almonds exportation.

The majority of almond production is concentrated in the Mediterranean region, where Andalusia is the region with the largest area of almond trees, followed by Castilla La Mancha, Murcia, Aragón, Valencian Community, Extremadura, and Catalonia (MAPA, 2023a). Most almond varieties grown in Spain have hard or very hard shells, and the most abundant are Guara and Lauranne, but they also coexist with Comuna, Marcona, Largueta, Soleta, Penta and Marta, among others (MAPA, 2023a). The main varieties in California are Nonpareil, California and Mission, representing 90% of the total production in the USA (Almond Board of California, 2023).

Almond world production is expected to continue increasing and the rapid and significant development is linked to the specific characteristics of the varieties grown. Thus the use of soft-shelled almond cultivars has a high yield at shelling, and this is one of the main factors that differ between California, Australia and the Mediterranean countries in terms of production.

Malagón (2020) classifies the almond varieties cultivated in Spain into two different groups:

1. Self-sterile varieties, which need at least 1/3 of another pollinating variety. They have good grain quality and are early to medium flowering and late to semi-late maturing. Within this group are:
    - Marcona
    - Desmayo Largueta
    - Masbovera
    - Glorieta
    - Ferragnes
    - Ferraduel
2. Self-fertile varieties, which are late and extra-late flowering and early maturing or mid-season, with a shorter cycle. These are divided into three further subgroups:
    - IRTA varieties in Catalonia: Francolí, Vayro, Constantí and Marinada.
    - CITA varieties in Aragon: Belona, Soleta and Mardía.
    - CEBAS varieties in Murcia: Antoñeta, Marta, Makako and Penta.
    - Others: Guara and Avijor Lauranne.

2.3. Almond harvesting processes

The almond harvesting period is between 30 and 45 days following the dehiscence of the first fruits, and depends on almond variety and climate, but in Spain it usually takes place during August and September. Almonds are considered to be ready for harvesting when 100% of the hulls have begun dehiscing. By this time, kernel components are fully accumulated, even if the hulls of different fruits on the same tree are in various stages of hydration.

Throughout this period, the opening of the ventral part of the outer shell becomes increasingly pronounced, leaving the endocarp visible, and simultaneously producing a general dehydration of the entire fruit. The optimum humidity in shelled almonds after harvesting and dehulling is usually 8% (Arquero et al., 2013). Another method used by producers to identify the ideal harvest time is the different stages of AH split that correspond to the degree of fruit development (Figure 2.2). These stages are critical management periods because as AH split progresses, the risk of pest pressure increases, which can compromise the yield and quality of the almond (Smith et al., 2022).

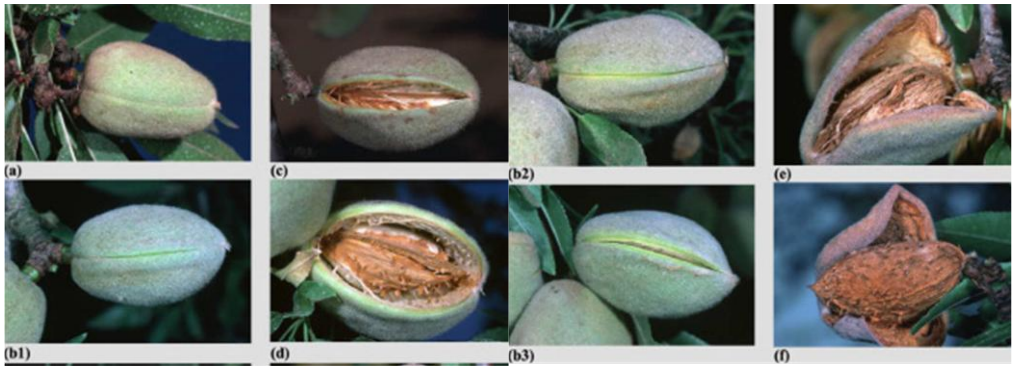

Figure 2.2 Standard almond hull split stages from (a) to (f) (Flint, 2002).

There are different almond harvesting methods, but one of the most common is the American system, which involves the use of tree trunk vibrators (*buggy*) that make the whole fruits fall to the ground. This procedure allows the almonds to air-dry and once their optimum humidity level is reached, the fruits are swept and stacked in rows in the center of the aisles between the trees for collection and transport to the factory, where dehulling is carried out.

Another conventional method that is frequently used involves the use of tarps that are placed on the ground and the fruits fall on them, being dried without having contact with the soil. There are also trunk vibrators equipped with a tarp or inverted umbrella that allows the fruits to be collected without them falling to the ground, and are then transported to the processing plant (Malagón, 2020). Another option is the use of specific machinery for collecting almonds (named Tenia), which vibrates the tree, collects the fruits and dehulls them, thus generating dehulled almonds in the crop field (Carro, 2023).

It is important to highlight that the almond harvesting and post-harvesting processes generate a considerable amount of wastes and by-products. The programs currently carried out to reduce the carbon footprint of agroindustrial activity, within a global strategy to obtain sustainable products, promote the use of the generated by-products in a circular economy model (Almendrave, 2019). Besides the AH, another by-product generated in the almond marketing processes is the hard shell or endocarp, which has a highly lignified structure and tends to break

into sharp pieces, and therefore its use in animal feeding is not viable. However, due to its high energy value, it is used for biomass production, for agglomerates manufacture such as active carbon, and it can also be reduced to powder for polishing some metals (Kodad et al., 2013).

2.4. Almond hulls

The AH structure includes the exocarp and the mesocarp of the fruit and their main role is to protect the seed. During the ripening period the AH accumulate a high concentration of flavonoids, becoming dry, leathery (hard with some flexibility) and astringent, as a protective method against intense heat, ultraviolet radiation and sometimes pests that the fruit can suffer during this period (Esfahlan et al., 2010).

The AH can represent between 50 and 65% of the total weight of the fruit, and depending on the variety and the harvesting period, can be soft or semi-soft in texture (Jafari et al., 2015). The process used for almond harvesting has a marked effect on AH quality. Thus, the main drawback of the American system is that fruits are usually contamined with soil particles, small stones and dust that contaminate the AH. The use of tarps can partly prevent soil contamination, but the AH can still contain small almonds, pieces of hard shell, small stems, and leaves, although the proportion of these materials will depend on the effectiveness of the cleaning and dehulling process.

Almond hulls can have different applications:

a) Biomass. When used as biomass, AH have been shown to have advantages over petroleum-derived fuels, both in economic and environmental terms, and even over other biofuels currently marketed. For industrial combustion, due to the large quantities required, it would be more advantageous to blend AH with other biomass sources having poorer thermal properties and thus obtain an improved fuel mix. Another alternative with considerable technological development, but still less developed, is the gasification of AH

for heat and power, which has been shown to improve energy efficiency and environmental impact over combustion (Espinosa, 2020).

b) Particle chipboard. Ferrández-García et al. (2019) showed that it is technically possible to develop panels using AH, as the high hardness obtained in the process makes its use viable for wooden flooring in the interior of buildings.

c) Phytoceuticals in food supplements. Barral-Martínez et al. (2021) reported that AH and almond shells have antimicrobial and antibacterial properties, anti-inflammatory, photoprotective and prebiotic effects, as well as antitumor properties and antiviral activity.

d) Organic amendment. It has been shown that AH can improve soil structure and increase its organic matter content, as well as improve soil aeration when used as a mulch (Arquero et al., 2013).

In Spain the use of AH in animal feeding is not currently widespread, and their use is almost entirely limited to biomass for heat and electricity generation. The AH are converted into biomass briquettes or pellets that are an efficient and versatile option for heating and power generation systems, both at residential and industrial level. In recent years, some farmers have been offering this by-product directly to their animals, mainly ruminants. However, some companies are working on creating a standardized product in pellet form, in order to promote and facilitate its use in animal feeding (Ortíz, 2024).

2.4.1. Chemical composition of almond hulls and use in animal feeding

In recent years, the use of by-products from agri-food industries in animal feeding has increased as a strategy to contribute to the circular economy. Feeding costs in a livestock farm can account for up to 70% of the total costs, and the use of these by-products can be a great saving, improving farm profitability (Reed and Brown, 1988).

In California, the official regulations establish that to be marketed as “almond hulls” a sample cannot contain more than 13% of moisture, 15% of crude fibre (CF) and 9% of ash, being the last two fractions expressed on fresh matter basis (DePeters et al., 2020).

The AH has a variable dry matter (DM) content, which is greatly influenced by harvest time and the drying time of the fruits on the ground before harvesting. As AH are a highly hygroscopic material, they tend to get damp and mold easily, which is a limiting factor for their storage.

On the other hand, AH have a low content of crude protein (CP) and starch, but a high content of total sugars that are rapidly degraded in the rumen providing energy for microbial growth. Sucrose, glucose and fructose are the main sugars characterized in AH (Holtman et al., 2015), being the last two accounting for between 35-44% and 30-40% of the total sugars, respectively, although the values depend on the almond variety (Offeman et al., 2014). These characteristics make AH a good source of energy for ruminants and due to their high fibre content and energetic value, AH can be considered as a substitute for both forages and concentrates.

The low CP content, together with the high proportion of CP linked to the acid detergent fibre (ADF), makes the protein value of the almond hull very low. In addition, AH have low ether extract (EE) content, with a fundamentally unsaturated profile. Oleic and linoleic acids constitute on average 50 and 25% of the total fatty acids (FA), while palmitic acid is usually the third most abundant FA, representing approximately 13% of total FA, but there is still scarce data available (Williams et al., 2018).

Although there are several studies evaluating the effect of including AH in animal feeding, it is important to emphasize that both physical and chemical characteristics of the AH used in the different studies are variable (see Table 2.1), difficulting a direct comparison of results. Differences in AH composition are attributed to different factors, such as genetics,

variety, humidity, fertilization, ripening stage or harvesting method, and these factors also produce a great variability in nutritional value. For example, rain can cause a loss of sugars through leaching, which in turn can cause a decrease in nutritional value and changes in chemical composition (VQP Group, 2021).

The cost of traditional raw materials for feed formulation is constantly increasing, which is a challenge for livestock producers. On the other hand, agri-food industries continue to generate wastes, making the use of these low-cost by-products not only economical but also sustainable (Barral-Martinez et al., 2021; Wang et al., 2021a). The AH have been investigated as an alternative ingredient to conventional raw materials to formulate animal diets but results have been variable (Table 2.2).

In California AH have become a very important feed ingredient in dairy cows diets, especially in farms located near to almond processing plants (Robinson, 2017). The dry AH generally used in these dairy farms have between 88 and 91% of DM, and when they are included in a totally mixed ration (TMR) with 40 to 50% moisture AH are readily consumed by cattle with no feed selection.

Table 2.1 Chemical composition (g/100 g dry matter (DM) unless otherwise stated) of almond hulls reported in the literature.

Reference	Year/variety	DM (%)	Ash	Crude protein	Ether extract	Crude fibre	NDF[1]	ADF[1]	Lignin	Sugars
Alibés et al., 1983	1975	-	-	5.0	-	11.3	-	-	-	-
	1976	-	-	6.0	2.4	13.2	27.5	25.7	10.6	-
	1977	-	-	6.6	3.3	13.3	31.3	21.7	7.9	-
	1978	-	-	5.7	2.7	13.3	27.1	17.9	6.6	-
	1979	-	-	5.7	-	12.2	26.7	18.6	6.3	-
Aguilar et al., 1984	Merced	-	7.3	5.4	-	14.4	-	21.5	7.9	26.4
	Nonpareil	-	6.1	6.7	-	14.3	-	27.3	12.1	31.7
	Neplus	-	7.6	6.1	-	21.1	-	29.9	11.7	23.9
	Commercial mix	-	7.4	6.2	-	15.1	-	29.2	11.9	30.2
Norollahi et al., 2005		-	12.0	7.0	2.5	13.5	21.1	13.7	4.1	-
Yalchi and Kargar., 2010	Commercial mix	89.4	6.47	2.86	2.62	-	36.0	24.3	11.8	-
	Stone shell	90.1	7.66	2.72	1.63	-	36.2	24	11.2	-
	Papel shell	90.2	5.32	2.18	3.35	-	34.7	23.8	10.6	-
Yalchi, 2011	Collected from several almond cultivars	89.5	6.47	2.86	1.65	-	37.1	24.3	11.8	-
Delavar et al., 2014	Collected from several almond cultivars	95.5	6.4	6.4	3.9	-	58.6	27.6	-	-
Elahi et al., 2017	Green AH	96.8	11.5	8.1	3.0	-	59.8	29.7	-	12.6
	Dry AH	97.1	9.9	10.3	2.7	-	62	30.4	-	14.1
DePeters et al., 2020	Nonpareil total AH	86.8	7.03	5.08	-	14.6	21.4	15.4	8.59	32.9
	Nonpareil pure AH	85.2	7.46	5.14	-	13.0	19.3	13.4	7.63	34.0
	Other varieties, total AH	88.1	7.56	5.04	-	18.1	25.6	18.1	9.7	28.2
	Other varieties, pure AH	87.3	8.46	4.87	-	15.1	22.1	15.6	8.7	29.7

[1]NDF: neutral detergent fibre; ADF: acid detergent fibre.

Aguilar et al. (1984) conducted an experiment with dairy cows to analyse the effects of replacing alfalfa hay with AH. In this study, 24 lactating Holstein dairy cows were fed 3 different diets: a control diet without AH and two experimental diets with either 12 or 25% of AH. The inclusion of AH showed no significant effect on milk production and milk fat and protein content remained constant for all diets. In contrast, Williams et al. (2018) observed that the inclusion of 17.6% of AH (replacing alfalfa pellets) in the diet of dairy cows significantly reduced DM intake and milk production, although it did not change the fat and protein content of the milk. In that study, no effect of AH on cows' methane emission levels was observed. In contrast, Durmic et al. (2013) found that AH reduced the *in vitro* methane production without negatively affecting volatile fatty acids (VFA) production and attributed this effect to the presence of bioactive compounds such as phenolics and triterpenoids in the AH.

In a more recent study, Swanson et al. (2021) examined the effects of including 7, 13 and 20% AH in the diet of dairy cows (replacing corn flakes and soybean hulls) and simultaneously increasing the amount of soybean cake to make the diets isoproteic. The use of AH increased the digestibility of the diet without affecting DM intake and milk production. Milk from cows consuming the higher levels of AH (13 and 20%) had higher fat content and lower protein content than the milk from control cows. The results of that study indicate that AH could be included in the diet of dairy cows up to 20% without impairing milk production.

Duncan et al. (2024) observed that replacing prairie hay with a mix of AH and almond shells, either ground or non-ground, at 13% of the diet DM of beef cattle resulted in similar final body weight (BW), average daily gain (ADG), feed conversion rate (FCR), and apparent diet digestibility but reduced growth performance and ruminal concentrations of VFA and ammonia (NH_3-N). Overall, these data suggest that ground or non-ground AH

and almond shells can be used as an alternative to prairie hay in growing diets for beef cattle.

Alibés et al. (1983) conducted a study with sheep to assess the nutritional value of AH when they were included in the diet at two particle sizes: coarse and ground to 3 mm diameter. During the study, the animals received daily 200 g of alfalfa hay and AH were fed *ad libitum* mixed with molasses for a period of 8 weeks. The results indicated that the particle size of the AH did not influence either diet digestibility or feed intake. That study concluded that AH are a reasonable quality by-product that could be used as an energy source for adult ewes, but contain little CP with low availability, probably due to the high tannin content (Alibés et al., 1983).

Other studies have shown that the addition of 10% of AH in the diet of fattening lambs in replacement of alfalfa did not affect productive performance and meat and carcass yields (Phillips et al., 2015; Rad et al., 2016). Likewise, Omar and Abdallah (2019) observed positive effects of feeding AH on lamb growth performance.

In a study conducted with Alpine dairy goats, Reed and Brown (1988) analysed the effects of different percentages of AH inclusion (0, 15, 25 and 35%) in the diet replacing alfalfa hay and supplementing with urea to achieve isoproteic diets. The inclusion of AH at 25 and 35% of the diet reduced digestibility, but increased DM intake, with no effects in milk yield and composition. The results of this study indicate that AH can be used as a substitute of alfalfa hay in dairy goat's diets without negatively affecting milk production.

Some studies with AH have also been carried out in non-ruminant species. In broilers, AH has been used as a source of fibre and energy at 6% (California type AH) and 9% (prime quality AH with less than 15% CF) of the diet without negatively affecting growth until 19 days of age (Wang et al., 2021a). These two types of AH were also tested in layers at 7.5 and 15% of the diet (isoprotein and isoenergetic diets) and no changes in

feed intake, laying rate, FCR and egg characteristics were observed, although there was a reduction in fat content in the body composition of the hens (Wang et al., 2021b). In a recent study, Ahammad et al. (2024) showed that incorporating up to 2% of AH into broiler diets could enhance growth performance, improve BW gain, increase nutrient digestibility and reduce both microbial counts and gas emissions by decreasing NH_3-N generation from poultry litter.

In pigs, feeding diets containing 5, 10 or 15% of AH as a replacement for barley grains during the fattening period (from 38.1 to 100 kg BW) resulted in lower fat content in the carcass with no effects on feed intake or productive performance (Calvert and Parker, 1985). Homedes et al. (1983) observed similar results in a short-duration study (35 days) with pigs (40-50 kg BW), in which the animals that were fed a control corn/soybean basal diet and a diet consisting 85% of the basal diet plus 15% of AH showed less body fat than the control group. Ahammad et al. (2024) reached similar conclusions when including 6% of AH in the diet of weaning pigs, as they observed not only a positive impact on ADG and FCR but also a reduction of environmental pollution by decreasing NH_3-N emissions. Therefore, the inclusion of AH in swine diets could potentially offer both productive and economic advantages in the pig industry.

Similar effects have been observed in castrated horses, as the inclusion of 15, 30 or 45% of Nonpareil bonnet AH in isonitrogenous diets did not affect feed intake or diet digestibility (Clutter and Rodiek, 1992).

Table 2.2 Effects of feeding almond hulls (AH) on different animal species and animal performance reported in the literature.

Reference	% AH inclusion	Animal species	Results
Alibés et al. 1983	25%	Adult ewes	AH did not influence diet digestibility and feed intake.
Aguilar et al., 1984	12 and 25%	Dairy cows	No significant effect on milk production and composition (fat and protein content).
Reed and Brown, 1988	15, 25 and 35%	Dairy goats	Inclusion of AH at 25 and 35% of the diet reduced digestibility, but increased dry matter intake, with no differences in milk yield and composition.
Durmic et al., 2014	50%	*In vitro* - Sheep	AH reduced methane production at 30% and also acetate:propionate ratio and ruminal NH_3-N concentrations.
Phillips et al., 2015	5 and 10 %	Lambs	No effects on growth performance or carcass yield.
Rad et al., 2016	20 and 40%	Lambs	No negative effects on animal performance.
Williams et al., 2018	17.6%	Dairy cows	Reduced dry matter intake and milk production, although it did not change the fat and protein content of the milk.
Omar and Abdallah, 2019	20%	Lambs	Positive effects on feed conversion ratio.
Wang et al., 2021	3, 6 and 9%	Broiler	Prime AH can be used at up to 9% without a negative effect on body weight gain.
Swanson et al., 2021	7, 13 and 20%	Dairy cows	AH up to 20% of the diet did not affect milk production, improved digestibility and milk fat content, but decreased milk protein production.

2.5. Sheep meat production and consumption

The EU has more than 70 million sheep and goats (about 85% sheep and 15% goats), many being reared in mountainous and generally economically vulnerable regions. The system of sheep rearing and production varies between countries and is often linked to consumer preference and demand, with heavy lambs being reared in Ireland and light lambs in southern countries such as Greece and Italy, while production in Spain and France is mixed (heavy and light; European Commission, 2024).

Spain is the country with the largest sheep census in the EU, with more than 13.5 million heads of which almost 80% are destined for meat production, thus reflecting an important meat production. Sheep meat production ranks fifth in economic importance, behind the pork, poultry, beef and dairy cattle sectors (MAPA, 2023b). Within the Autonomous Communities, Extremadura, Castilla y León, Castilla La Mancha, Andalusia and Aragón have the largest number of sheep herds, accounting for 80% of the total. In economic terms, the sheep and goat meat production reached a value of 1,165 million of euros, representing about 4.2% of the livestock production and 1.8% of the agricultural production in Spain (MAPA, 2023b).

Sheep meat production in Spain is a vital activity for many rural regions. Native breeds, such as Merina and Churra, are valued for the quality of their meat. The new breeding methods adopted are much more efficient and sustainable, and have considerably reduced the environmental impact and improved animal welfare. The quality of the Spanish products is a fundamental factor in the increase in sheep meat exports in recent years, mainly reaching markets in Europe and Asia. Quality certificates such as Protected Geographical Indications (PGI), as well as some organic certifications, seek to guarantee the excellent quality of the

meat. Some examples of these high-quality products are *Ternasco de Aragón*, *Lechazo de Castilla y León* and *Cordero de Extremadura*, all of them covered by PGI certification.

The consumption of lamb meat in Spain is very variable, and it is subject to a high seasonal component, with December being the month when the highest volume of purchases due to the impact of Christmas celebrations. During 2023, the high inflation contributed to the decline in sheep meat consumption, which stood at 0.84 kg per person per year (5.6% lower than in 2022), reaching 39,684 tonnes. Factors such as changes in dietary habits, sociological and demographic circumstances, as well as market competition with products from other livestock species that are cheaper and more affordable for the consumer have led to the fall in lamb consumption (MAPA, 2023b).

According to various surveys carried out by Bernabeu and Tendero (2004), the main attributes valued in lamb meat are the origin, the type, the price, and the fact that the meat has a specific designation as a guarantee of quality. Regarding the commercial types of lamb meat that we can find in the Mediterranean area, the most common are the suckling lamb (lambs fed only milk until they are 25-45 days old; Bernabeu and Tendero, 2004), the light lambs (carcass weight of 10-13 kg), and the *Pascual* lambs (carcass weight of more than 13 kg).

The future of the sheep meat sector in Spain depends on companies involved in sheep meat production adopting sustainable practices that address environmental, economic, and social challenges. Sustainability is a reality that challenges all sectors to innovate and diversify, not only as an environmental necessity but also as an opportunity to revitalize and strengthen a sector that is an integral part of Spain's heritage and economy.

2.6. Ensiling as feed preservation method

Livestock farms aim to obtain high production at a low cost. One of the strategies to achieve this is to ensure feed availability for livestock throughout the year. As is well known, both the quantity and quality of feed are seasonal, and usually during winter periods feed availability can be scarce. Therefore, to preserve crops at their optimal stage of growth for use during periods of feed scarcity, it is essential to develop conservation methods that ensure the nutritional needs of animals are met during these challenging times.

According to Callejo Ramos and Matesanz (2005), feed conservation for livestock has become a necessary process on livestock farms for several reasons:

- Fodder production is seasonal, generating a surplus in the spring and, depending on the rainfall each year, in the autumn.
- The supply of fibrous feed to many farms without sufficient land base for the fodder production needed by the animals. This fact generates a forage market of great economic importance.
- The different harvesting and preservation procedures currently available allow the selection of the optimum vegetative stage for harvesting.
- The conservation of by-products from the agri-food industry allows their use in animal feeding; taking advantage of a material that would otherwise have no use and would become a waste that is sometimes difficult to dispose of properly.

While high-moisture materials can be left to sun-dry in the field, like in the haymaking process, this method is subject to climatic variability and usually results in high losses during the drying process, leading to feed with variable composition and possibly low nutritional value. The initial interest of farmers in conserving crops by natural fermentation, such as silage, stemmed from the realization that this process was less dependent on both

the weather and the need to harvest crops at an advanced stage of growth (McDonald et al. 1991).

The essential objective of silage is to achieve anaerobic conditions. Jones et al. (2004) described the phases of normal fermentation over 21 days of ensiling (Figure 2.3). In an anaerobic environment, the fermentation of soluble carbohydrates in forages or other high-moisture plants originates a variety of products, ultimately resulting in the preservation of the material as silage. There are several parameters to identify the quality of the silage: pH, concentration of organic acids, alcohols, and NH_3-N, and the microorganisms present.

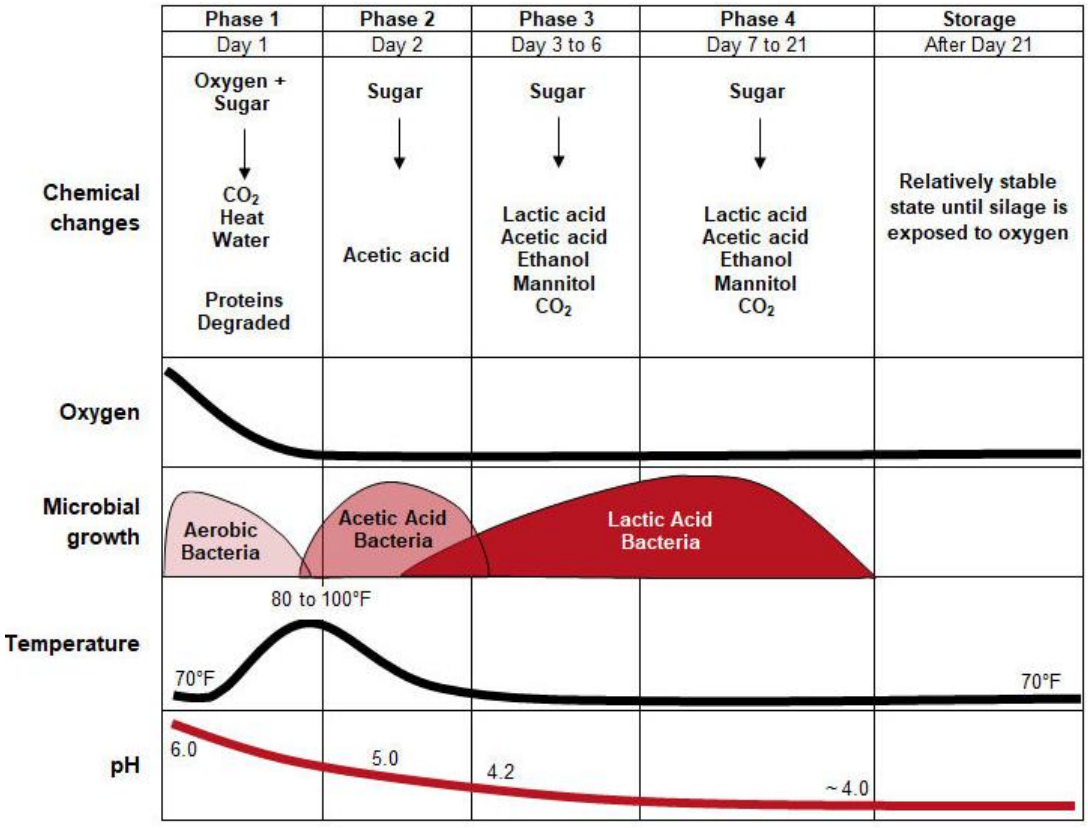

Figure 2.3 Phases of normal fermentation in silages (Jones et al., 2004).

Kung et al. (2018) reported that in an ideal fermentation process, homolactic bacteria use water-soluble carbohydrates to produce lactic acid, resulting in a relatively high recovery of DM and energy in the silage. However, the fermentation process is highly complex and involves many types of microorganisms, many of them undesirable.

Therefore, the second main objective of ensiling is to inhibit the activity of these undesirable microorganisms, such as *Clostridium* and *Enterobacter*, as they produce butyric acid and degrade amino acids into low nutritional value products.

As mentioned above, the fermentation process results in several end-products, and the proportion of these products determines the quality of the silage. Based on the analysis of silage fermentation from the most frequent types of silages in the United States, Kung and Shaver (2001) recommended a range of values for the end-products (Table 2.3).

Table 2.3 Suggested concentrations of common fermentation end-products in various types of silages with different dry matter (DM) content (Kung and Shaver, 2001).

Item	Legume silage <30-35% DM	Legume silage 45-55% DM	Grass silage 25-35% DM	Corn silage 30-40% DM	High-moisture corn 70-75% DM
pH	4.3-4.5	4.7-5.0	4.3-4.7	3.7-4.0	4.0-4.5
Lactic acid, %	6-8	2-4	6-10	3-6	0.5-2.0
Acetic acid, %	2-3	0.5-2.0	1-3	1-3	<0.5
Propionic acid, %	<0.5	<0.1	<0.1	<0.1	<0.1
Butyric acid, %	<0.5	0	<0.5–1.0	0	0
Ethanol, %	0.5-1.0	0.5	0.5-1.0	1-3	0.2-2.0
NH_3-N, % of total N	10-15	<12	8-12	5-7	<10

Although chemical analysis and nutrient profile are parameters that certainly indicate the quality of silage, the organoleptic characteristics, such as odour, colour, and temperature could serve as indicators of the fermentation process.

- Odour. Well-fermented silages should not have a strong particular odour because lactic acid should be the main organic acid from the fermentation, and it is nearly odourless. However, most silages tend to have a mild odour of vinegar because acetic acid usually is the second most abundant acid and it is very volatile (Kung et al., 2018). High ethanol production may give a fruity, even sweet, odour that could be associated with a well-fermented silage of raw materials rich in fermentable sugars, but could be also related to the presence

of an anomalous microbial population. For example, an earthy odour could be related with *Bacillus* growth, a musty or moldy smell may indicate the presence of mold that might lead to mycotoxins contamination, and a strong foul rancid-butter smell could suggest the presence of *Clostridium*. Excessive proteolysis can cause a fishy or ammonia-like odour, while the excess of heat in a silage may cause a burnt smell.

- Colour. Silages with high humidity may produce excessive acetic acid, which gives a yellow colour usually at the bottom of the silage. Some authors associated an olive-green colour with an excellent silage, while others qualify a dark-green colour as a regular silage and a yellowish-green as a good silage (Romero-Márquez, 2017; Morales-Querol et al., 2022). In addition, a dark brown or black colour could indicate a silage fermented under excessive heat conditions.
- Temperature. Heat is expected to be produced in the silage as part of the fermentation process. However, well-packed and sealed silage should have an average temperature no greater than 5 to 8°C above the ambient temperature at the time of filling. If the silage is left overnight unsealed in warm weather, the uppermost layers (5-10 cm) can reach temperatures of 45 to 60°C as a result of excessive air trapped in the material. This situation could consequently cause heat-damage to the protein and may also affect negatively the growth of the lactic acid bacteria population necessary for good ensiling process. The temperature in the core of the silo should decrease after the active phase of fermentation to 25 to 30°C. Dry silages tend to be more porous when packed, which slows down the fermentation process, decreasing microbial activity and

consequently hindering the control of the growth of yeasts and fungi due to the low production of antifungal acids and high pH (Kung et al., 2018).

2.6.1. Silage additives

A silage made with the appropriate techniques should maintain the nutritional value of the ensiled material and avoid the growth of undesirable microorganisms. However, in practice sometimes the conditions required for a good fermentation process are not met. In these cases, silage additives (natural or industrial products) could be added to the material ensiled to encourage desirable fermentation, limit undesirable fermentation, or improve the nutritional quality of silage. The purpose of silage additives is to control the ensiling process so that the ensiled material retains as much as possible the nutrients originally present, and to ensure that the growth of lactic bacteria predominates during the fermentation process, producing lactic acid in quantities high enough to guarantee a good silage (Yitbarek and Tamir, 2014).

Silage additives can be classified into 5 different categories (Yitbarek and Tamir, 2014; Muck et al., 2018):

1. Fermentation stimulants. These additives are widely used in practice since they are considered natural products, safe to handle, noncorrosive to machinery, and environmentally friendly. They can be classified into the following categories:

a. Microbial inoculants. The microorganisms are added to the silage to improve the population of beneficial bacteria that promote an adequate fermentation. The most common microbial inoculants are lactic acid bacteria (LAB) such as *Lactobacillus, Pediococcus,* and *Enterococcus* species. These bacteria enhance the production of lactic acid to achieve a more efficient fermentation by lowering the pH and limiting the growth of undesirable organisms. The homolactic bacteria produce a combination of lactic acid, acetic acid, ethanol, mannitol, and carbon dioxide, so that DM loss is expected to be low. The sugars content of the ensiled material is crucial since it directly influences the

fermentation process and silage quality. Soluble sugars are the primary substrate for LAB and low sugars levels can reduce the microbial inoculant effectiveness. Wang et al. (2022) conducted a trial ensiling whole corn crops and reported that the addition of mixed inoculants of 60% *L. plantarum*, 10% *P. pentosaceus,* and 30% *L. buchneri*, improved silage quality by drastically reducing pH values and increasing lactate production. Moreover, they found that the inoculant application facilitated the release of ester-linked phenolic acids from plant cell walls, significantly improving the microbial digestion of lignified fibre, and shifted rumen fermentation towards greater propionate and lower CH_4 production.

b. Enzymes. Treating forages before ensiling with enzymes may improve nutrient digestion and utilization by the animal, thus helping to enhance animal productivity and reduce animal faecal material and pollution. The main function of these enzymes is to accelerate the rate and extent of the initial pH decline by degrading complex compounds into simple sugars, encouraging bacterial growth in the silage. The most common enzymes are amylases, which degrades starch into sugars, and cellulases and xylanases, which degrade the cell wall components into sugars and in some cases can increase forage digestibility.

c. Fermentable carbohydrates. Adding these substrates (i.e. molasses) could be most beneficial in forages with low fermentable carbohydrate content, such as alfalfa, since the objective of molasses is to provide readily fermentable carbohydrates that promote the growth of LAB and lactic fermentation. The aim of using these additives is to reduce rapidly the pH of the silage, thus preventing clostridial growth and proteolysis, and to decrease the loss of organic matter.

2. Fermentation inhibitors. The aim of these compounds is to inhibit unwanted fermentation and they are generally a combination of acids, including benzoic, sorbic, acetic, formic, or citric acids among others, although there are also other types of fermentation inhibitors.

a. Acids and organic acid salts. Formic acid and other inorganic acids (sulfuric and hydrochloric) cause an immediate drop in pH and often increase bunk life and limit fermentation losses of proteins and carbohydrates. Acidity may arrest plant respiration, thus reducing heat production and nutrient loss, and can inhibit the production of butyric acid by clostridia activity (Nadeau and Auerbach, 2014). However, the concentration used must be carefully determined, as they are considered corrosive to machinery and irritant for people. The use of formic acid as a silage additive can contribute effectively to reduce the pH and to increase the ruminal degradability of silages (Novoa-Garrido et al. 2020) and can also increase the silage DM intake in dairy cows (Mayne, 1993). Propionic acid is one organic acid commonly used due to its high ability to inhibit the growth of yeasts and fungi. Several studies have shown that applying propionic acid to silage improves DM and organic matter digestibility (Johnson et al., 1979; Michelena et al., 2002).

b. Other chemical inhibitors. Formalin (35-40% formaldehyde solution) has also been used as a silage preservative, especially for reducing protein degradation in the silage and increasing the amount of non-degradable protein in the rumen reaching the duodenum of the silage-fed animals.

3. Aerobic deterioration inhibitors. Propionic acid has a high antimycotic activity and is an ideal candidate for improving the aerobic stability of corn silage with low pH. Due to the corrosive nature of propionic acid, acid salts (i.e. calcium, sodium and ammonium propionate) are used in commercial products, but their effectiveness is related to water solubility, as low-solubility products are less effective in inhibiting fungi.

4. Nutrients. Anhydrous ammonia and water- or molasses-ammonia mixtures can be used as silage additives with the aim of increasing the N content of the silages. Both ammonia and urea are an economical source of N, increase bunk life during feeding, and can decrease mould and heating during ensiling, and protein degradation, although the effect of urea on improving bunk life has not been totally justified (Britt, et al., 1975; Huber, et al., 1979; Johnson, et al., 1982). Special attention must be paid to the diet of the animals when adding ammonia or urea to silages, as the balance between degradable and non-degradable protein in the ruminant must be ensured. In addition, ammonia is a hazardous gas and should be handled with care. Other nutrients as minerals (calcium, phosphorous, sulfur and magnesium) have been added to forage to make the silage more nutritionally complete.
5. Absorbents. Feeds as grains and processed by-products such as corn and sorghum meal, rice bran, cassava meal and dried pulps (i.e. sugar beet pulp, citrus pulp) have been included in the silages with good results, not only because they provide fermentable substrates but also because they act as moisture absorbers. These compounds remove excessive moisture, can avoid effluent losses, and can be used in relatively high rates (aiming a DM content greater than 25% in the mixture), but they must be adequately mixed with the chopped forage in order to optimize their effectiveness.

2.6.2. Almond hull silage

Some studies, conducted mainly in non-European countries, indicate that AH can be dried after harvesting, thus allowing their long-term storage. However, the use of fossil energy to dry AH would increase their price preventing their use in practical feeding. Moreover, when AH are stored outside, they act like a sponge absorbing moisture and can quickly get moldy and lose their nutritional value (Jafari et al., 2011; Sirakaya, 2023). Ensiling AH would avoid these problems and could reduce nutrient losses through leaching

by avoiding exposure to rainfall. However, the available information on the ensilability of AH is still scarce.

Sirakaya (2023) analysed the characteristics and nutritive value of AH ensiled without additives and reported a final pH of 5.75 and a silage of medium quality. The ME content decreased significantly ($P < 0.001$) from 1.96 in the pre-ensiled AH to 1.85 Mcal/kg in the AH silage. Sechrist (2022) inoculated AH silages with *Saccharomyces cerevisiae* and *Lactobacillus plantarum* and observed a remarkable improvement in the characteristics of the silage with a final pH below 4.5, high concentrations of lactic acid and low concentrations of acetic acid. An *in vitro* trial (72 h incubation) indicated that CH_4 production was reduced by 96% when almond hulls were fermented with *S. cerevisiae* for 14 days and this fermented feed was included at 20% in a cattle diet (Sechrist 2022).

In other studies, AH has been mixed with other feeds. Aydin (2023) observed that, the addition of different levels of AH (0.5, 1, 1.5, 2 and 2.5%) to corn silage increased the *in vitro* organic matter digestibility and the ME content of the silages and reduced the CH_4 emission. In addition, there was a decrease in yeast content and in CO_2 production.

Although it has been proven that AH can be stored as silage when moisture content is appropriate, more studies are needed to investigate the most adequate conditions for a successful AH silage.

3. OBJECTIVES

3.1. General objective

The main objective of this Doctoral Thesis was to investigate the nutritional value of the AH produced in Spain to be used in ruminant feeding ensuring the quality and safety of the produced food. Spain is the third largest almond producer worldwide, generating large quantities of AH, which are the external covering surrounding the fruit and the hard shell. Previous studies indicate that this by-product could be an alternative to conventional raw materials in ruminant feeding, but there is no nutritional assessment of the AH produced in our country.

3.2. Specific objectives

1. To compare the chemical composition and nutritive value of samples of two varieties of AH (Guara and Soleta) collected from a single region and from cultivars grown under the same conditions.
2. To assess the influence of including increasing levels of AH in dairy sheep diets on *in vitro* ruminal fermentation and CH_4 production.
3. To investigate the effects of including two levels of AH in the concentrate of light lambs on feed intake, growth performance, diet digestibility, *post-mortem* ruminal and caecal fermentation, blood parameters, and meat quality.
4. To assess different silage treatments in order to identify the optimal silage treatment for AH preservation during long-term periods.

The results of this Doctoral Thesis could contribute to promote the use of AH in ruminant feeding, to minimize the environmental effects produced by their management and disposal, to reduce feeding costs, and to foster circular bioeconomy, especially when used locally. In addition, the development of low-cost effective preservation techniques to overcome the limitation of the high moisture content in AH would contribute to facilitate their use in the practice. Finally, feeding AH may potentially increase the quality of animal products, reduce

CH_4 emissions and be a valuable alternative to other polluting practices (burning in the field, landfilling, etc.) for their disposal. In order to fulfill the objectives of this research, three different experiments were conducted.

4. MATERIALS AND METHODS

Care of all animals used in this Doctoral Thesis was conducted according to European laws for experimental animal protection, and all the experimental protocols of the different experiments were approved by the Committee for the Care and Use of Experimental Animals of the Autonomous Community of Madrid (PROEX 212.2/22).

4.1. Experiment 1. Chemical composition and nutritive value of almond hulls from two almond varieties and influence of including almond hulls in the diet on *in vitro* ruminal fermentation and CH_4 production

4.1.1. Almond hulls samples and in vitro *incubations*

Samples of AH were obtained at almond orchards located at Écija in the South of Spain (O5°4'57.36" N37°32'31.92"). Five contiguous plots from each almond variety (Guara and Soleta) were harvested in both the 2020 and 2021 campaigns using the most common harvesting procedure in Spain, making a total of 10 samples of Guara and 10 of Soleta AH (5 of each almond variety per harvesting year). Briefly, almond fruits were dropped off the tree using shaking machines and left on the floor until almonds reached the desired humidity level. Then, the fruits were swept into a windrow and harvested. About 5 kg of AH were sampled, frozen (-20 °C), and sent to the laboratory for analysis. Samples were cleaned to eliminate debris (almond shells, sticks, leaves, …) and dried at low temperature (40°C; 72 h) before conducting the analyses of chemical composition and *in vitro* incubations.

Samples of AH were ground to pass a 1-mm screen and incubated *in vitro* with ruminal fluid to measure both gas production kinetics and ruminal parameters. Four rumen-fistulated Lacaune sheep (65.0 ± 1.32 kg body weight) were used as donors of ruminal content. Sheep were housed in floor pens with straw bedding and free access to fresh water. A diet composed of 80% grass hay and 20% concentrate (fresh matter basis) was fed twice daily at a daily rate of 45 g DM/kg body weight$^{0.75}$ to avoid diet selection. About 500 g of rumen content was sampled from each sheep via the rumen cannula immediately before the morning feeding and

strained through 4 layers of cheesecloth. The fluid was transported to the laboratory into thermal flasks within 20 min after collection, where it was mixed with the buffer solution of Goering and Van Soest (1970) in 1:4 ratio and the mixture was maintained at 39°C and flushed with CO_2.

In vitro incubations were performed as described by De Evan et al. (2020). Briefly, samples of AH (500 mg of DM) were accurately weighed into glass vials of 120 mL volume. Fifty mL of the ruminal fluid and culture medium mixture were added to each vial using a peristaltic pump (520UIP31 model; Watson-Marlow Fluid Technology Group, Barcelona, Spain), and the vials were capped with rubber stops and incubated at 39°C. In all *in vitro* runs, the ruminal fluid from each sheep was independently mixed with the culture medium to obtain 4 replicates for each sample, and one vial per sample was incubated.

The incubations for measuring gas production kinetics lasted for 120 h and the amount of gas produced was measured at 2, 4, 6, 9, 12, 24, 30, 36, 48, 60, 72, 96, and 120 h using a pressure transducer (Delta Ohm DTP704-2BGI; Herter Instruments SL, Barcelona, Spain) and a plastic syringe. The gas produced at each sampling time was released after measurement. Two *in vitro* runs were conducted, one for the AH from each harvesting year. In each run, 2 blanks (vials without substrate) were incubated for each mixture of ruminal fluid and culture medium (8 blanks in total) to correct gas production values for the gas generated in the fermentation of the substrates added with the ruminal fluid from each sheep.

The incubations for measuring ruminal parameters followed the same methodology as described above for gas production kinetics (including the use of blanks), but in this case, lasted for 24 h. In order to detect possible differences among samples at short incubation times, after 6 h of incubation a sample (1 mL) of each vial content was taken using an insulin syringe, which was mixed with 60 µL of H_2SO_4 and immediately frozen (-20°C) until analysis of volatile fatty acids (VFA) and NH_3-N concentrations. After 24 h of incubation, the gas production was

measured, vials were hand-shaken, uncapped, the pH of their content was measured (Crison Basic 20 pH meter; Crison Instruments, Barcelona, Spain), and 1 mL of content was taken and processed as described, for analysis of VFA and NH_3-N concentrations. Two *in vitro* runs were conducted, one for the AH samples collected at each harvesting year.

4.1.2. Experimental diets and **in vitro** *incubations*

The objective of these incubations was to assess the effects of including increasing amounts of dried AH in diets for dairy sheep on *in vitro* rumen fermentation and CH_4 production. Two diets for dairy sheep were formulated without AH (control diets): one for high-producing sheep (HP0) and the other for medium-producing sheep (MP0). For each type of diet, 3 additional diets were formulated by replacing different amounts of feed ingredients with 8, 16, or 24% (fresh matter basis) of dried AH. The AH used in the diet was from Soleta variety and contained 92.3, 6.83, 36.7, 26.6, 4.58, and 23.8 g of organic matter (OM), crude protein (CP), neutral detergent fibre (NDF), acid detergent fibre (ADF), ether extract (EE) and total sugars per 100 g of DM, respectively.

Diets were formulated to meet the nutritive requirements of high and medium-production dairy sheep according to INRA Feeding System (2018). Within HP and MP diets, all diets were formulated to have similar CP and NDF content. Feed ingredients and chemical composition of the experimental diets formulated for high-producing sheep (HP diets) are shown in Table 4.1. In this diet, the AH partly replaced corn, wheat bran, and sugar beet pulp.

Table 4.1 Ingredients and chemical composition of diets for high-producing dairy sheep without almond hulls (HP0) and with almond hulls at 8 (HP8), 16 (HP16), and 24% (HP24) of fresh matter.

Item	HP0	HP8	HP16	HP24
Ingredients (% fresh matter)				
Alfalfa hay	40.0	40.0	40.0	40.0
Almond hulls	-	8.00	16.0	24.0
Corn	14.0	10.2	6.28	2.41
Wheat	6.00	6.00	6.00	6.00
Barley	10.5	10.5	10.5	10.5
Soybean meal 46.5	7.00	7.00	7.00	7.00
Rapeseed meal 00	5.50	5.50	5.50	5.50
Wheat bran	5.00	3.62	2.24	0.86
Sugar beet pulp	10.0	7.24	4.48	1.72
Calcium soap	1.00	1.00	1.00	1.00
Calcium carbonate	0.50	0.50	0.50	0.50
Mineral/vitamin premix	0.50	0.50	0.50	0.50
Chemical composition[1]				
Dry matter (DM; %)	91.3	91.3	91.2	91.3
Organic matter (% DM)	92.6	92.3	91.9	91.6
Crude protein (% DM)	16.6	16.6	16.5	16.4
Neutral detergent fibre (% DM)	36.5	37.1	37.7	38.3
Acid detergent fibre (% DM)	21.1	22.3	23.5	24.7
Lignin (% DM)	4.26	5.25	6.18	7.15
Ether extract (% DM)	3.44	3.79	4.13	4.48

[1] Calculated from chemical analysis of individual feed ingredients.

As shown in Table 4.2, in the diet formulated for medium-producing dairy sheep (MP diets), the AH partly replaced alfalfa hay and corn, and urea was included in the diets containing AH to achieve isonitrogenous diets.

The *in vitro* incubations of experimental diets were conducted following the same methodology described for AH samples, with the exception that the composition of the culture medium of Goering and Van Soest (1979) was modified to exclude all N-containing chemicals, as the CP content of the diets was adequate to allow optimal microbial growth. In the 24-h incubations, gas production was measured at 9 and 24 h, and a 15 mL sample of the gas was collected in vacuum tubes and stored at room temperature until CH_4 analysis. Sampling at 9 h of incubation was chosen because previous studies from our group (Kamel et al., 2008; Mateos et al., 2016) showed that *in vitro* CH_4 production is very low at shorter incubation times.

Samples of all experimental diets were incubated in one run using one vial per sample and the ruminal fluid of the 4 sheep to get 4 replicates per diet.

Table 4.2 Ingredients and chemical composition of diets for medium-producing dairy sheep without almond hulls (MP0) and with almond hulls at 8 (MP8), 16 (MP16), and 24% (MP24) of fresh matter.

Item	MP0	MP8	MP16	MP24
Ingredients (% fresh matter)				
Alfalfa hay	60.0	53.4	46.9	40.3
Almond hulls	-	8.00	16.0	24.0
Urea	-	0.18	0.35	0.55
Corn	15.0	13.4	11.8	10.2
Barley	8.50	8.50	8.50	8.50
Soybean meal 46.5	3.00	3.00	3.00	3.00
Wheat bran	2.50	2.50	2.50	2.50
Sugar beet pulp	9.00	9.00	9.00	9.00
Calcium soap	1.00	1.00	1.00	1.00
Calcium carbonate	0.50	0.50	0.50	0.50
Mineral/vitamin premix	0.50	0.50	0.50	0.50
Chemical composition[1]				
Dry matter (DM; %)	91.8	91.8	90.9	90.5
Organic matter (% DM)	91.4	91.5	91.5	91.6
Crude protein (% DM)	14.7	14.7	14.7	14.8
Neutral detergent fibre (% DM)	40.9	40.9	40.5	40.1
Acid detergent fibre (% DM)	26.1	26.1	26.0	25.8
Lignin (% DM)	5.18	5.84	6.46	7.11
Ether extract (% DM)	3.22	3.58	3.94	4.30

[1] Calculated from chemical analysis of individual feed ingredients.

In addition, the potential DM degradability (PDMD) of each diet was determined by weighing 300 mg of each diet into Ankom Corp #57 polyester bags (30 µm pore size; Ankom Technology Corp., Fairport, NY, USA) in duplicate. Bags were heat-sealed and incubated in an Ankom Daisy II incubator (Ankom Technology Corp., Fairport, NY, USA) at 39°C in the 1:4 mixture of ruminal fluid and the incubation medium of Goering and Van Soest (France et al., 2000) previously described under continuous rotation. After 120 h, the bags were washed with cold water, dried at 60°C for 48 h, and weighed to calculate the PDMD. This value was used to estimate the effective DM degradability (DMED) as described below.

4.1.3. Chemical analyses

Chemical composition of AH samples and of the feed ingredients of the experimental diets was analysed as described by Marcos et al. (2019). Briefly, the content in DM, ash, CF

and EE was analysed using the Association of Official Analytical Chemists (AOAC, 2015) procedures, whereas NDF, ADF, and lignin content was determined according to Van Soest et al. (1991). The Dumas combustion method was used to measure N content, and total sugars content was determined by spectrophotometry according to the Folin–Ciocalteu assay (Yemm, et al., 1954). The concentration of Ca, Mg, K, Na, and Cu in the AH samples were determined by atomic absorption spectrometry according to the ISO (International Organization for Standardization) 6869 using a Varian SpectrAA 220FS atomic absorption spectrophotometer (Varian Medical System Ibérica, Madrid, Spain) (ISO 6869:2000, 2000). Phosphorus concentration was determined by a colorimetric method (Commission Regulation (EC) No 152/2009) using an ESPECORD® 200 plus (Analytik Jena GmbH, Jena, Germany) spectrophotometer.

The analysis of volatile fatty acids (VFA) was performed by gas chromatography as described by Tejido et al. (2005). Briefly, samples were centrifuged (13,000 x g; 15 min; 4ºC) and 0.8 mL of the supernatant were mixed with 0.5 mL of a deproteinizing solution (100 g metaphosphoric acid per L) including crotonic acid (0.6 g per L) as internal standard. The mixture was left overnight at 4°C before being centrifugated again, (same conditions described before) and the supernatants were transferred to chromatography vials. Samples were analysed using a Shimadzu GC 2010 (Shimadzu Europe GmbH, Duisburg, Germany) gas chromatograph provided with a TR-FFAP column (30 m x 0.53 mm x 1 μm; Supelco, Madrid, Spain).

Concentrations of NH_3-N in vials content were determined by the phenol-hypochlorite method (Weatherburn, 1967). For this, 60 μL of supernatant was taken in 10 mL tubes containing 5 mL of a phenolnitroprusside solution (10 g phenol + 50 mg sodium nitroprusside diluted in 1000 mL distilled water) and 5 mL sodium hypochlorite solution (5 g NaOH + 8.4 mL sodium hypochlorite diluted in 1000 mL distilled water). To obtain the standard curve, a 0.02143 M $(NH_4)_2SO4$ solution was prepared and diluted with distilled water to obtain different

NH_3-N concentrations. The tubes were vortexed and incubated in a water bath at 37°C for 20 min. For each sample the absorbance at 625 nm was measured in an Epoch spectrophotometer (Bioteck Instruments, Swindon, UK).

The analysis of CH_4 concentrations in the gas produced was performed by gas chromatography as described by Martinez et al. (2010) using a Shimazdu GC 14B (Shimadzu Europe GmbH, Duisburg, Germany) equipped with a flame ionization detector and a column packed with Carboxen 1000 (45-60 mesh, Supelco, Madrid, Spain). The carrier gas was He and peaks were identified by comparison with a standard containing 10% of CH_4. Each gas sample (0.6 mL) was injected in duplicate into the chromatograph.

4.1.4. Calculations and statistical analyses

All analyses in this Doctoral Thesis were conducted using the SAS package (SAS Institute, 2017). Data on gas production for each sample (AH and experimental diets) were fitted over time according to the model described by Krishnamoorthy et al. (1991): Gas = PGP x (1 - e $^{(-c \text{ x } (t - Lag))}$). In this model, PGP is the potential gas production, *c* is the fractional rate of gas production, *Lag* is the time before starting gas production, and t is the time of gas measurement. Data fitting to the model was achieved with the NLIN PROC of the statistical package SAS (SAS Institute, 2017) using an iterative least squares procedure. In addition, the average gas production rate (AGPR) was calculated using the following equation proposed by France et al. (2000): AGPR = PGP x c / [2 x (ln (2) + c x Lag)]. The AGPR is the rate of gas production between the incubation starts and the time at which gas production reaches 50% of PGP. The DMED was estimated for a rumen particulate outflow (Kp) of 6% per h from PDMD and gas production parameters as: DMED = [(PDMD x c)/(c + Kp)] e $^{(-c \text{ x } Lag)}$. Finally, the amount of apparently fermented OM (AFOM) for each experimental diet was estimated from the amount of acetate, propionate, and butyrate produced in each vial as proposed by Demeyer (1991).

Data on chemical composition and energy value of AH were analysed by analysis of variance (ANOVA) as a mixed model with repeated measures over time using the PROC MIXED of SAS. The model included the almond variety, harvesting year, and their interaction as fixed effects, and the cultivation plot as random effect. Gas production and ruminal parameters were analysed using the same model but additionally including the rumen inoculum (mixture of rumen fluid and culture medium) as random effect.

Data on gas production and fermentation parameters of experimental diets were analysed independently for each type of diet (HP or MP) and sampling time (9 and 24 h of incubation). Data were analysed by ANOVA as a mixed model, with the fixed effect of the diet and the random effect of the rumen inoculum. Non-orthogonal polynomial contrasts were used to test for linear and quadratic effects of the inclusion of AH. When a significant effect of AH inclusion was detected, means were compared by Tukey's test. For all statistical analyses, significance was established at values of $P < 0.05$, and values of $P < 0.10$ were treated as trends.

Finally, relationships between chemical fractions of AH samples and either gas production or fermentation parameters were assessed by Pearson correlation using the PROC CORR of SAS (SAS Institute, 2017).

4.2. Experiment 2. Effects of including two levels of AH in the concentrate of light lambs on feed intake, growth performance, diet digestibility, post-mortem ruminal and caecal fermentation, blood parameters, and meat quality

4.2.1. Experimental concentrates

The AH sample used for the formulation of the experimental concentrates in the *in vivo* lamb fattening trial had the same origin as those used in the first experiment. Before preparing the experimental concentrates, the sample of AH was analysed in an external laboratory (Agrolab Ibérica S.L.U., Tarragona, Spain) for aerobic microorganisms (ISO 4833-1:2013), *Enterobacteriaceae* spp. (ISO 21528-2:2017), coliforms (ISO 4832-2006), yeasts and moulds

(NF V 08-059-2002), *Escherichia coli* (ISO 16649-2:2001), *Staphylococcus coagulase+* (QMP_51_10_x), *Salmonella* spp. (ISO 6579-1:2017) and *Listeria monocytogenes* (QMP_504_AI_51_24_x) using colony-count methods. *Salmonella* spp., *E. coli* and *Listeria monocytogenes* were undetected. Counts of *Enterobacteriaceae* spp. and coliforms were below 1 x 10^1 log CFU (colony forming unit)/g, and those of aerobic microorganisms, yeasts and moulds, and *Staphylococcus coagulase+* were 5.55 x 10^3, 1.4 x 10^4, and below 1.0 x 10^2 log CFU/g, respectively. These microbial counts revealed that AH were safe as feed for lambs (Nutrofeed, 2024).

Three experimental concentrates were formulated: a high-cereal concentrate without AH (control) and two concentrates including either 6 (AH6) or 12 (AH12) g of AH per 100 g (fresh matter basis). Barley grains and wheat bran were partially replaced with AH in AH6 and AH12 concentrates, which also included urea and increased amounts of calcium soap to obtain isonitrogenous and isoenergetic concentrates. Feed ingredients were ground and pelleted (4-mm size). All concentrates were formulated to meet the nutritive requirements of fattening light lambs according to the INRA feeding system (INRA, 2018). Ingredients and chemical composition of concentrates are shown in Table 4.3.

Table 4.3 Feed ingredients of experimental concentrates and chemical composition of concentrates, barley straw and the almond hulls (AH) included in AH6 and AH12 concentrates.

Item	Concentrate			Barley straw	AH
	Control	AH6	AH12		
Ingredient (g/100 g fresh matter)					
Corn	33.0	33.0	33.0		
Wheat	10.0	10.0	10.0		
Barley	22.0	18.8	15.6		
Soybean meal 47%	11.0	11.0	11.0		
Sunflower meal	8.00	8.00	8.00		
Wheat bran	12.0	8.00	4.00		
Almond hulls	-	6.00	12.0		
Urea	-	0.17	0.35		
Calcium soap	1.40	2.40	3.40		
Calcium carbonate	1.30	1.30	1.30		
Sodium bicarbonate	0.80	0.80	0.80		
Salt	0.30	0.30	0.30		
Mineral-vitamin premix	0.20	0.20	0.20		
Chemical composition					
Dry matter (DM; g/kg fresh matter)	890	886	880	921	847
Ash (g/kg DM)	49.1	57.8	67.7	78.2	162
Crude protein (g/kg DM)	145	144	142	57.2	64.5
Ether extract (g/kg DM)	34.8	46.4	55.3	18.0	54.8
NDF (g/kg DM)	199	202	202	686	347
ADF (g/kg DM)	69.6	84.7	95.1	422	279
Lignin (sa) (g/kg DM)	15.1	25.8	35.4	75.3	167
Non-structural carbohydrates (g/kg DM)[1]	572	550	533	161	372
Forage units for meat production (UFV/kg DM)[2]	1.11	1.11	1.10	0.38	0.55

[1] Calculated as 1000 – (ash + crude protein + ether extract + NDF).
[2] Calculated according to INRA feeding system (INRA, 2018).

4.2.2. In vivo *trial*

Thirty Manchega lambs, 15 males (12.2 ± 0.26 kg BW) and 15 females (12.5 ± 0.24 kg BW), were distributed within sex in 3 homogeneous groups according to BW, and one group of each sex was randomly allocated to one of the experimental treatments (Control, AH6 and AH12). Lambs were housed in individual pens (1 m x 1 m) provided with slatted floor and placed 1 m above the floor. Each pen was equipped with two independent feeders for forage and concentrate and an automatic drinker. The room was temperature controlled (20-22°C) over the trial. Lambs were fed daily concentrate and barley straw *ad libitum* and had continuous free access to fresh water.

During the trial, concentrate and straw intake was monitored twice weekly, and all lambs were weighed once a week. Blood samples were collected from each lamb at the beginning and end of the trial via jugular venipuncture into tubes containing EDTA. Blood sampling was performed immediately before feeding in the morning, the samples were centrifuged (5,000 × g, 10 min, 4°C), and the plasma was immediately frozen (-20°C) until analysis of the concentrations of albumin, globulins, total proteins, urea, creatinine, glucose, total bilirubin, and cholesterol. The activities of the enzymes alkaline phosphatase (ALP), glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT) and alfa-amylase were also determined in plasma samples.

Diet digestibility and nitrogen (N) balance were measured in 8 lambs per treatment (4 males and 4 females) during the third week of the trial. A plastic tray (1 m x 1 m) was placed under the slatted floor of each pen for faeces collection. A bucket containing 80 mL of diluted H_2SO_4 (10 mL H_2SO_4 and 90 mL of distilled water) was used to collect the urine and to keep the pH below 3.0. Both the faeces and the urine produced by each lamb were quantitatively collected for 6 days, and a representative sample of both (10 g per 100 g of total weight) was taken each day to form pooled samples of faeces and urine for each lamb. Samples were stored frozen (-20°C) until analysis.

4.2.3. Slaughter, carcass measurements and sampling

Lambs were slaughtered at a commercial slaughterhouse located in Colmenar Viejo, approximately 30 km from the experimental farm after 46 and 48 days of starting the trial (5 lambs of each treatment per day) with approximately 23 kg of BW. The slaughter procedure followed commercial practices in Spain and involved electrical stunning, followed by the cutting of the carotid arteries and jugular veins. At the slaughterhouse, each lamb's liver, spleen and kidneys were collected and weighed. The pH of the *Longissimus dorsi* (T13 rib level) and *Semitendinosus* muscles was measured immediately after sacrifice, using a penetrating

electrode adapted to a portable pH-meter with a temperature probe (Hanna Instruments pH meter HI-9025; Hanna Instruments SL, Éibar, Spain) (Figure 4.1). These measurements were repeated after 24 h of refrigeration in a 4°C chamber and 2 measurements were made for each muscle at each time.

In addition, samples of rumen and caecal contents of each lamb were taken after hand-homogenization of their content. The ruminal content was filtered through four layers of cheesecloth and the pH of the fluid was immediately measured (Crison GPL 21 pHmeter, Crisson Instruments, Barcelona, Spain). Then, 2 mL of fluid were mixed with 2 mL of 0.5 N HCl and the samples were frozen (-20ºC) until analyses of VFA and NH_3-N. The same procedure was repeated for the caecal content, with the exception that the samples were weighed (2 g). Finally, a sample of rumen wall was taken from the ventral area of the rumen to evaluate rumen epithelium colour. Samples were washed with saline solution (0.9 g NaCl in 100 mL of distilled water), dried gently with a tissue and placed on a white surface. Samples were scored from 0 to 5, being 5 the darkest colour and 0 the lightest one. Scoring was performed by 4 people (blind to treatment allocation) and the average score was used for statistical analysis.

Figure 4.1 pH measurements after sacrifice.

Measurements of the carcasses were also recorded as described in other studies (Cañeque et al., 2004; Haro et al., 2020). Hind limb length (HLL) was the length from the

perineum to the most distal point of the medial edge of the tarsal-metatarsal articular surface; thoracic depth (CTD) was the maximum distance between the sternum and the back of the carcass at the level of the sixth thoracic vertebra; carcass width (CWD) was the maximum width of the carcass at the level of the ribs; buttock width (BUW) was the widest buttock measurement in a horizontal plane on the hanging carcass; buttock perimeter (BUP) was measured at the level of the trochanters of both femurs; and carcass internal length (ICL) was the distance between the anterior edge of the ischiopubic symphysis and the anterior edge of the first rib at its midpoint (Figure 4.2).

Figure 4.2 Measurements taken in lamb carcasses.

Classification of fatness degree was carried out following Regulations (EEC) No. 2137/92 and 461/93 for light carcasses of less than 13 kg, on a scale from 1 (low), 2 (slight), 3 (average) to 4 (high) (Figure 4.3). Pelvic-renal fat evaluation was performed according to the method proposed by Colomer-Rocher et al. (1988), using a 3-point scale with 3 subdivisions within each point (-1, 1, 1+, -2, 2, 2+, -3, 3, 3+) (Figure 4.4).

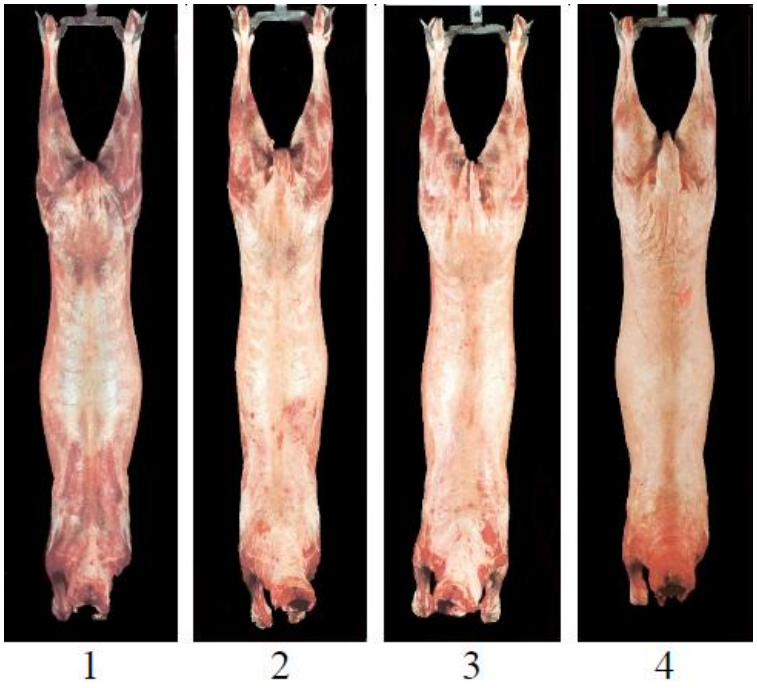

Figure 4.3 Photographic standards adopted by the EU for the classification of carcasses of light lambs according to their fat cover (Colomer-Rocher et al, 1988).

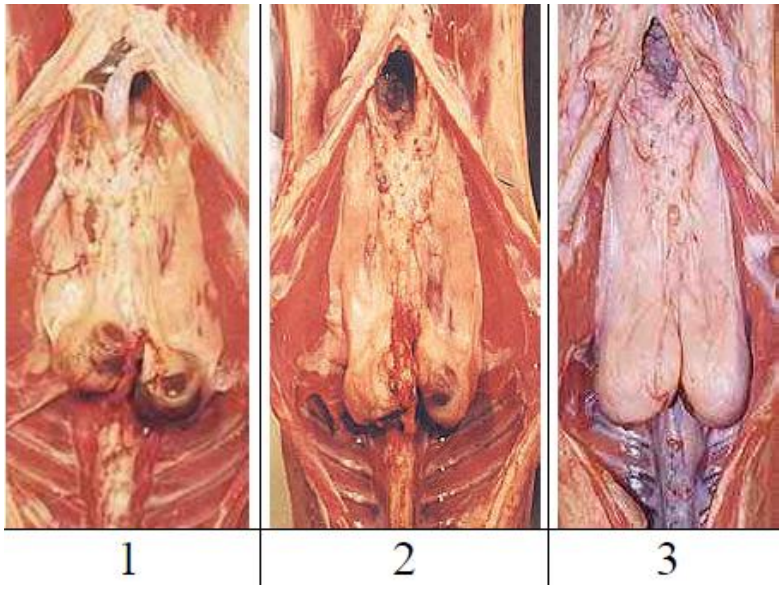

Figure 4.4 Photographic patterns for grading the amount of pelvic-renal fat according to Colomer-Rocher et al. (1988).

The *Longissimus dorsi* muscles of each lamb´s left and right side (L1–L4) were dissected and divided into several pieces. A sample from right side of *Longissimus dorsi* was taken and both the external fat and the connective tissue were removed before being frozen and freeze-dried for analysis of chemical composition and fatty acid (FA) profile, while the left side was divided into 3 equal portions to determine the evolution of lipid oxidation and colour over

a 6 days period. Meat samples were placed on styrofoam trays, overwrapped with an oxygen-permeable polyvinyl chloride film and stored in darkness at 2ºC. On days 0 and 6, subsamples for the analysis of lipid oxidation were collected and frozen at -20ºC. The samples for colour analysis were stored in the same conditions described before and colour was measured on days 0 and 6 (Figure 4.5).

Figure 4.5 Colour measurement on the cut surface of the *Longissimus dorsi* muscle.

In addition, the colour was also measured 24 h after slaughter on the subcutaneous fat of the tail root (Figure 4.6), and a sample of fat was taken for analysis of FA profile. All colour measurements were made at 3 different locations of each sample using a Konica Minolta Spectophotometer CM-2600d (Minolta, Osaka, Japan) following the procedure and equipment calibration described by de la Fuente-Vázquez et al. (2014) and de Evan et al. (2020). The colour coordinates were expressed following the *CIELAB Colour space* system (Commission on Illumination, 2004) as L*(brightness), a* (red-green index) and b* (yellow-blue index). Chroma (C*) and hue (h*) values were calculated as $C^* = (a^{*2}+b^{*2})^{0.5}$ and $h^* = \tan^{-1}(b^*/a^*)$, respectively. Furthermore, the proportions of metmyoglobin, myoglobin, and oxymyoglobin on the meat surface were estimated by measuring absorbances (A) at 470, 520, 530, 570, and 690 nm according to the procedure of Krzywicki (1979). The values were calculated as myoglobin (%) = $2.375 \times (1 - ((A_{470} - A_{690})/(A_{520} - A_{690}))) \times 100$; metmyoglobin (%) = $(1.395 - ((A_{570} - A_{690})/(A_{520} - A_{690}))) \times 100$; and oxymyoglobin (%) = 100 − (myoglobin + metmyoglobin).

Figure 4.6 Colour measurement of subcutaneous fat at the tail root.

4.2.4. In vitro *trial*

The 3 concentrates were fermented *in vitro* with ruminal fluid from sheep to analyse both the gas production kinetics and the main fermentation parameters. Two *in vitro* incubation runs were conducted on different days following the same methodology as mentioned in section *4.1.1* for measuring the gas production kinetics (120 h incubations) and the main fermentation parameters (24 h incubations). Samples of 200 mg of DM of each concentrate were accurately weighed into 60-mL glass vials, and the incubations were performed as described before.

4.2.5. Chemical analysis

The chemical composition of dietary feeds (both concentrates and barley straw), faeces, and meat was determined following the procedures described in section *4.1.3.* Meat chemical analyses (DM, ash, N, and EE) were performed in *Longissimus dorsi* muscle in duplicate.

For VFA and NH_3-N analysis, the samples of ruminal and caecal content and those taken from the vials content in the *in vitro* trial were thawed and processed as described in section *4.1.3.* Concentrations of metabolites in the plasma of lambs were determined using an automatic biochemistry analyser (Hitachi 7020; Hitachi High Technologies, Inc., Ibaraki, Japan).

The FA profile in meat and feeds (concentrates and barley straw) was analysed in duplicate from 250 mg of previously lyophilized muscle, 250 mg of concentrate, or 400 g of barley straw homogenates. The samples were submitted to *in situ* transesterification of the FA

according to Carrapiso et al. (2000). Analysis of FA was then carried out with a 7890A gas chromatograph coupled to a 5975C mass spectrometer (Agilent Technologies; Palo Alto, CA, USA) using a HP 88 column (100 m × 0.25 mm × 0.20 mm film thickness) according to Liu et al. (2007). Helium (3 mL/min) was the carrier gas, and the injector, transfer line, and detector were set at 200ºC, 230ºC, and 300ºC, respectively. The sample extract volume injected was 2 μL in a 30:1 split ratio. The oven program was 170ºC (maintained for 24 min), increased to 220ºC at 7.5ºC/min, and to 230ºC at 10ºC/min (maintained for 5 min). The concentration of the FA methyl esters (FAME) was calculated as g FA/100 g total FA.

Lipid oxidation was assessed by measuring the concentrations of thiobarbituric acid reactive substances (TBARS) in *Longissimus dorsi* samples at 0 and 6 days of storage. This method is based on the reaction of thiobarbituric acid with malondialdehyde (MDA) and was performed as described by Maraschiello et al. (1999). The extracts absorbance was measured using a Thermo Scientific spectrophotometer (Thermo Fisher Scientific, Madrid, Spain) model Evolution 220 (UV/Visible) at 532 nm, and the results were expressed as g MDA/kg meat.

4.2.6. Calculations and statistical analyses

Nutrient digestibility was calculated as the difference between the amount of nutrient ingested minus the amount excreted in the faeces. Nitrogen balance was estimated as the difference between the amount of N ingested and that excreted in faeces and urine. Data of gas production in the *in vitro* trial were analysed as mentioned in section *4.1.4* to estimate gas production parameters.

All *in vivo* data excepting plasma metabolites and meat pH and lipid oxidation data were analysed by ANOVA with the fixed effects of concentrate, sex, and their interaction. Plasma metabolites and meat pH and lipid oxidation data were analysed as a mixed model with repeated measures, in which the effects of concentrate, sex, time, and their interactions were considered fixed, and lamb was considered a random effect. *In vitro* data were analysed by a mixed model

in which the concentrate was a fixed effect and the donor sheep (inoculum) was a random effect. For all *in vivo* and *in vitro* data, when a significant effect of concentrate was detected ($P < 0.05$), means were compared using the Tukey test and differences were considered significant at $P < 0.05$.

4.3. Experiment 3. Effect of different additives on characteristics and nutritive value of almond hull silage

4.3.1. Almond hulls samples collection, silage preparation and sampling

The AH used in this study was obtained after collecting the almonds using a tree shaker (buggy system) coupled to a tractor equipped with a cone-shaped mantle, similar to an inverted umbrella. Using this system the whole fruits fell on the mantle avoiding contact with the ground and contamination. The fruits were transported directly to a processing plant, where almonds were pre-cleaned and dehulled. The obtained AH were immediately transported to the laboratory, kept at 4ºC overnight and the silages were prepared the next day. The AH were chopped by hand into pieces of approximately 1 x 1 cm, thoroughly mixed, and divided into 4 homogeneous portions. The experimental design included 4 different silage types, with 5 replicates per treatment that were stored for 90 days (20 bags in total). The AH were ensiled without any additives (Control; CON); with formic acid (FA; 4 mL/kg AH); with acetic and lactic acids in the same proportion (2 mL/kg of each acid; ALA); and with a bacterial inoculum of *Lactobacillus buchneri* (5×10^9 CFU (colony forming units)/kg; LAB). The amount of each additive was selected from previous studies reported in the literature (Yitbarek and Tamir, 2014; Kung et al., 2018; Okoye et al., 2023). The additives were homogenized in water before being applied to the AH and the mixture was thoroughly mixed. The amount of water was calculated to achieve about 50% in the material to be ensiled.

Once the additives were applied, the AH were weighed into plastic bags (500 g/bag) suitable for vacuum packaging (40 x 30 cm). The air inside the bags was removed using a

vacuum packaging machine, bags were heat sealed and stored in the dark for 90 days at room temperature (20°C) before being processed.

In addition, 9 additional bags per treatment were prepared to evaluate the timely evolution of AH silage characteristics and chemical composition over the storage period, and 3 bags per treatment were opened at 0 (the day of preparing the silages), 4 and 21 days of storage.

At each opening time (0, 4, 21 and 90 days of storage), the bags were opened, their content was evaluated organoleptically and was further processed as described by Samarasinghe et al. (2019). Briefly, the content of each bag was weighed, thoroughly mixed, and a representative sample of 50 g was taken and mixed with 500 mL of deionized water. The mixture was homogenized twice with a kitchen blender (40 seconds each time) with 1 min rest to prevent heating. Then, the content was filtered through 4 layers of cheesecloth and the pH of the resulting liquid (silage extract) was immediately measured measured using a Crison 20 pHmeter (Crison Instruments, Barcelona, Spain). Two samples (1.7 mL) were taken, mixed with 80 µL H_2SO_4 (10%; vol/vol) and immediately stored at -20°C until subsequent analysis of VFA, NH_3-N and lactic acid concentration. A sample of about 100 g of each silage bag were weighed and dried at 60°C for 48 h for determining the DM content. The rest of the content of the silage bags was frozen (-20°C), lyophilized and ground to 1 mm before analysis of chemical composition.

Additionally, only in the bags stored for 90 days 100 g of bags content was taken in a sterile container and sent under refrigerated conditions to an external laboratory (Finca Mouriscade Laboratory - Lalín, Pontevedra, Spain) for microbiological analysis by classical plate culture techniques within 24 h of receipt. The concentration of the following microorganisms was determined: *Clostridium perfringens*, *Enterobacteriaceae* spp, *Escherichia coli*, yeasts, *Listeria monocytogenes*, moulds, *Salmonella* spp and *Staphylococcus coagulase+*.

4.3.2. **In vitro** *trial, data calculations and chemical analyses.*

Samples from the bags opened after 90 days of storage were fermented *in vitro* with ruminal fluid from 4 sheep. *In vitro* incubations were conducted on different days following the methodology described in section *4.1.1* to measure both the gas production kinetics (120 h incubations) and the main fermentation parameters (24 h incubations). Gas production parameters and ME content of the 90-days silages were estimated as described in section *4.1.4.*

The DM, ash, EE, NDF, ADF, and lignin content in pre-ensiling AH (0 days silages) and silage samples (4, 21, and 90 days), as well as NH_3-N and VFA concentrations in all silages extracts and in the content of the *in vitro* incubation vials were analysed as described in Experiment 1. The lactic acid concentrations in the silage's extracts were analysed using the colorimetric method described by Taylor (1996). Briefly, the samples were centrifuged (13,000 x g; 15 min; 4ºC) and 50 µL of the supernatant were added to 450 µL of distilled water and 3 mL of H_2SO_4 96% (final volume of 3.5 mL). After stirring for 30 seconds, the samples were incubated for 10 min in an oil bath at 100°C and then allowed to cool at room temperature. Then, 50 µL of 4% $CuSO_4 \times 5H_2O$, and 100 µL of 1.5% p-phenyl phenol (in 95% ethanol) were added and the samples were vortexed. For the standard curve, a solution of 100 µg lactic acid/mL was prepared. This solution was diluted to obtain different concentrations of lactic acid, and 2 samples of each dilution followed the same process described for the samples. Finally, the tubes were vortexed and incubated in a water bath at 30°C for 60 min. For each sample, absorbance was measured at 570 nm in an Epoch spectrophotometer (Bioteck Instruments, Swindon, UK). All of the analyses were performed in duplicate.

4.3.3. Calculations and statistical analyses

At each sampling time, the DM recovery in the silages was calculated independently for each bag silage as the DM weight of the bag content divided by the amount of DM initially weighed in the bag, and it was expressed as the percentage of initial DM. Data of gas production

in the *in vitro* trial were fitted to the statistical model described in section *4.1.4* to estimate the gas production parameters. The metabolizable energy (ME) content of the silages was estimated from CP and EE content (expressed as g/kg DM) and the gas produced after 24 h of incubation from 300 mg of DM incubated (G24) using the following equation proposed by Menke and Steingass (1988): ME = 2.43 + 0.1206 x G24 + 0.0069 x CP + 0.0187 x EE.

The chemical composition and characteristics of the silages after 90 days of storage were analysed as one-way ANOVA with the silage treatment (Control, FA, ALA, and LAB) as the main effect. Data on gas production, ME content, and *in vitro* fermentation parameters were analysed as a mixed model, with the fixed effect of the silage treatment and the random effect of the inoculum (ruminal fluid) used in the *in vitro* trials. In addition, the timely evolution of silage characteristics and chemical composition data were analysed by ANOVA with the silage treatment, the storage time (0, 4, 21, and 90 days), and their interaction as main effects. Significance was declared at $P < 0.05$, and P values between 0.05 and 0.10 were considered as a trend. When a significant effect of the silage treatment or the time was detected, differences between means were assessed using the Tukey's test.

5. RESULTS AND DISCUSSION

5.1. Experiment 1. Influence of Almond Variety on Nutritive Value of Almond Hulls

5.1.1. Chemical composition and fermentative parameters of AH samples

Table 5.1 shows the chemical composition and energy content of AH samples from Soleta and Guara varieties harvested at 2 different years. There were significant differences ($P < 0.05$) between almond varieties in all chemical fractions analysed except EE and total sugars content. Almond hulls from Guara variety had greater ($P \leq 0.015$) DM, ash, CP and CF content, but lower ($P \leq 0.001$) NDF, ADF and lignin content and lignin/NDF ratio than AH from Soleta variety. Consequently, the estimated ME content and UFL and UFV values were greater ($P \leq 0.003$) for Guara compared with Soleta AH. Previous studies have also reported significant differences in the chemical composition of AH due to almond variety both in California (Merced, Nonpareil, and Neplus varieties; Aguilar et al., 1984) and in Iran (Rabbi, Mamaii, Shahrud, and Shokufe varieties; Jafari et al., 2011). Our data indicate that differences in chemical composition were maintained in the 2 harvesting years, as there were no variety x harvesting year interactions ($P \geq 0.05$) for any chemical fraction except for EE content ($P < 0.001$).

The chemical composition of AH is highly variable and is affected by multiple factors, such as the variety of almond, fertilizer treatment, cultivation conditions, maturity, weather conditions at harvesting, and procedure of almond harvesting, among others (DePeters et al., 2020). In the present study, harvesting year affected all chemical fractions excepting EE and total sugars content and lignin/NDF ratio. For both almond varieties, samples collected in 2020 contained more DM, ash and CP and less NDF, ADF and lignin than those harvested in 2021. In contrast, EE of Guara-AH was greater in 2021 than in 2020 samples, but for Soleta-AH was greater in 2020 compared with 2021 samples. In general, chemical composition of both AH varieties was in the range of values reported by Feedipedia (2020) and in the Tables of INRA

(2018) and NRC (2021), except for CP content that was slightly higher in our study, especially for Guara samples.

The greater ($P < 0.001$) ME content of Guara-AH compared with Soleta-AH is consistent with the lower content in fibre (NDF, ADF and CF fractions) and lignin and the greater CP content of Guara (Table 5.1). In fact, the ME level of the 20 AH samples was negatively correlated with their content in NDF (r = - 0.839; $P < 0.001$), ADF (r = - 0.982; $P < 0.001$), CF (r = - 0.515; $P = 0.020$), and lignin (r = - 0.776; $P < 0.001$), and positively correlated with CP content (r = 0.666; $P = 0.001$). For both AH varieties, samples harvested in 2021 had lower ME content ($P < 0.001$) than those collected in 2020, which agrees well with the greater fibre and lignin content of the AH samples harvested in 2021.

The average ME content of all samples (2096 kcal/kg DM) was greater than that reported by NRC (2021) but agreed well with the value of 2078 kcal ME/kg DM reported by Feedipedia (2020). Similarly, the average values of UFL and UFV for the 20 AH samples analysed in the present study (0.74 and 0.66 per kg DM, respectively; Table 5.1) are comparable to the 0.75 UFL and 0.66 UFV values reported by INRA (2018). These results indicate that AH collected in Spain have similar energy value for ruminants than those produced in other Mediterranean countries, and that AH are a medium-quality energy feed for ruminants.

Table 5.1 Influence of harvesting year (HY) and almond variety (VAR) on chemical composition (g/kg DM) and energy and mineral content of Guara and Soleta almond hulls.

Harvesting year	2020		2021			*P* =		
Almond variety	Guara	Soleta	Guara	Soleta	SEM[3]	VAR	HY	VAR*HY
Chemical composition								
Dry matter (DM; %)	75.4	69.6	67.2	60.6	2.41	0.015	0.007	0.885
Ash	10.9	7.57	9.92	7.15	0.292	<0.001	0.005	0.200
Crude protein	10.1	7.25	7.04	6.45	0.596	<0.001	0.029	0.176
Neutral detergent fibre (NDF)	29.9	34.6	37.9	39.8	0.67	<0.001	<0.001	0.069
Acid detergent fibre	20.9	25.6	27.3	30.0	0.41	<0.001	<0.001	0.071
Lignin	8.40	12.8	10.7	14.4	0.32	<0.001	<0.001	0.277
Lignin/NDF (%)	28.2	36.9	28.4	36.1	0.72	<0.001	0.636	0.323
Crude fibre	15.1	14.5	18.9	15.5	0.65	0.009	0.003	0.097
Total sugars	23.1	23.2	23.1	24.9	1.33	0.527	0.487	0.487
Ether extract	3.26	5.00	5.01	3.80	0.330	0.502	0.332	<0.001
Energy content (Kcal/kg DM)								
ME (kcal/kg DM)[1]	2275	2115	2088	1907	21.3	<0.001	<0.001	0.678
UFL (per kg DM)[2]	0.82	0.74	0.73	0.65	0.009	<0.001	<0.001	0.921
UFV (per kg DM)[2]	0.76	0.66	0.65	0.56	0.010	<0.001	<0.001	0.629
Minerals								
Calcium (g/kg DM)	4.44	2.36	5.15	2.34	0.241	<0.001	0.025	0.021
Phosphorus (g/kg DM)	1.58	1.74	1.13	1.44	0.107	0.010	0.015	0.600
Magnesium (g/kg DM)	2.04	1.19	2.11	1.18	0.055	<0.001	0.529	0.378
Potassium (g/kg DM)	45.7	33.0	36.0	27.9	1.58	<0.001	<0.001	0.108
Sodium (g/kg DM)	0.56	0.55	0.53	0.54	0.002	0.539	<0.001	0.054
Copper (mg/kg DM)	5.55	5.51	6.19	5.68	0.258	0.300	0.145	0.394

[1] Estimated from chemical composition and gas production at 24 h incubation as described by Menke and Steingass (1988); [2] UFL (Forage units for milk production) and UFV (Forage units for meat production) were calculated according to INRA Feeding System (2018). [3] SEM: standard error of the mean.

The two AH varieties analysed in the present study showed some differences in mineral content, with AH from Guara variety having more ($P < 0.001$) Ca, Mg and K but less P ($P = 0.010$) than Soleta-AH (Table 5.1). In contrast, no differences ($P \geq 0.300$) between varieties were detected in Na and Cu content. The content in minerals was similar to that reported previously by Feedipedia (2020) and NRC (2021) and by other studies analyzing different AH varieties (Jafari et al., 2011; Jafari et al., 2015; DePeters et al., 2020), confirming the high K

content of AH compared to forages and fibrous by-products such as cereal bran (INRA, 2018; Feedipedia, 2020; NRC, 2021). Harvesting year affected ($P \leq 0.025$) the content of all minerals analysed, except Mg and Cu, despite that the same fertilizer treatment was applied to each plot in both years.

Table 5.2 shows the gas production and fermentation parameters of the AH samples after *in vitro* incubation with ruminal fluid from sheep. The lower ($P < 0.001$) PGP and AGPR values of Soleta compared with Guara AH can be explained by the greater NDF and ADF content of Soleta, as fibre is less fermentable in the rumen than other chemical fractions (i.e. sugars, soluble carbohydrates, CP) and therefore generates less gas. However, in the analysis of relationships among gas production parameters and chemical composition (Table 5.3), no significant correlations ($P \geq 0.266$) between PGP and either fibre (NDF, ADF and CF fractions) or CP content were detected. In contrast, PGP was negatively correlated with lignin content ($r = - 0.489$; $P = 0.029$) and lignin/NDF ratio ($r = - 0.607$; $P = 0.005$), indicating that lignin limited the ruminal degradability of AH as generally observed for ruminant fibrous feeds (Van Soest, 1994).

Table 5.2 Influence of almond variety (VAR) and harvesting year (HY) on gas production parameters and fermentation parameters of Guara and Soleta almond hulls after 6 and 24 h of incubation with ruminal fluid from sheep.

Harvesting year	2020		2021			*P* =		
Almond variety	Guara	Soleta	Guara	Soleta	SEM[2]	VAR	HY	VAR*HY
Gas production parameters[1]								
PGP (mL/g dry matter (DM))	219	198	223	214	3.2	<0.001	0.301	0.051
Lag (h)	0.234	0.000	0.108	0.004	0.0337	<0.001	0.074	0.059
c (%/h)	5.54	5.13	4.39	4.38	0.020	0.306	<0.001	0.338
AGPR (mL/h)	8.42	7.27	6.98	6.73	0.241	0.005	<0.001	0.067
Fermentation parameters (6 h)								
Total volatile fatty acids (VFA; µmol/g DM)	3.21	2.80	3.54	3.14	0.087	<0.001	<0.001	0.964
Molar proportion of VFA (mol/100 mol)								
Acetate (Ac)	61.5	57.5	61.0	56.4	0.82	<0.001	0.338	0.710
Propionate (Pr)	30.9	32.3	31.2	34.5	0.83	0.007	0.138	0.254
Butyrate	6.38	8.56	6.25	7.27	0.306	<0.001	0.024	0.062
Isobutyrate	0.367	0.458	0.969	1.179	0.1963	0.445	0.001	0.762
Isovalerate	0.128	0.622	0.052	0.111	0.0340	<0.001	<0.001	<0.001
Valerate	0.673	0.589	0.469	0.554	0.0563	0.993	0.038	0.138
Ac/Pr (mol/mol)	2.01	1.80	2.01	1.68	0.075	<0.001	0.413	0.464
NH_3-N (mg/l)	68.4	55.7	73.3	71.3	7.36	0.324	0.166	0.472
Fermentation parameters (24 h)								
pH	6.81	6.86	6.74	6.75	0.008	<0.001	<0.001	0.025
Total VFA (µmol/ g DM)	6.40	5.20	7.47	6.97	0.150	<0.001	0.007	0.022
Molar proportion of VFA (mol/100 mol)								
Acetate (Ac)	62.7	61.1	62.2	59.6	0.45	<0.001	0.033	0.264
Propionate (Pr)	28.7	26.7	26.8	27.7	0.46	0.226	0.383	0.003
Butyrate	6.94	9.62	7.39	8.87	0.304	<0.001	0.624	0.051
Isobutyrate	0.331	0.720	2.565	2.618	0.1551	0.162	<0.001	0.285
Isovalerate	0.536	0.981	0.248	0.394	0.0612	<0.001	<0.001	0.018
Valerate	0.832	0.914	0.716	0.858	0.0875	0.207	0.331	0.733
Ac/Pr (mol/mol)	2.20	2.31	2.33	2.16	0.046	0.527	0.752	0.004
NH_3-N (mg/l)	124	114	105	109	8.0	0.693	0.155	0.398

[1] PGP: potential gas production; *c*: fractional rate of gas production; *Lag*: time before starting gas production; AGPR: average gas production rate; [2] SEM: standard error of the mean.

Almond hulls from Soleta variety presented lower ($P < 0.001$) *Lag* times than Guara-AH over both harvesting years, indicating that rumen degradation started more rapidly for Soleta samples (Table 5.2). *Lag* time is usually negatively related to the content of rapidly degradable substances such as sugars and easily fermented carbohydrates (Marcos et al., 2019). However, in the present study no correlation was detected between *Lag* time and sugars content (r = 0.185; $P = 0.426$; Table 5.3), suggesting that other chemical fractions, not analysed in the present study, were responsible for the reduced *Lag* time observed for Soleta AH. *Lag* was positively correlated with CP content and negatively with the content in NDF, ADF and lignin, which are fractions more slowly degraded (NDF and ADF) or undegradable (lignin). Similarly, the AGPR was negatively correlated with NDF, ADF and lignin content and the lignin/NDF ratio (Table 5.3).

In agreement with the observed differences in PGP, the fermentation of Guara samples generated more total VFA ($P < 0.001$) than Soleta samples at both 6 and 24 h of *in vitro* incubation (Table 5.2). The negative relationships observed between VFA production at 9 and 24 h and the lignin/NDF ratio (Table 5.3) are consistent with the negative effect of lignin on fibre degradation in the rumen (Van Soest, 1994). As observed previously for other feeds (Marcos et al., 2019), PGP was positively correlated with total VFA production at 6 and 24 h of incubation (r = 0.762 and 0.774, respectively; $P < 0.001$). The lower pH values ($P < 0.001$) observed for Guara than for Soleta also indicate greater fermentability of Guara samples, and pH values were negatively correlated with total VFA production (r = - 0.942; $P < 0.001$; n = 20) when both were measured after 24 h of incubation.

Table 5.3 Correlation matrix (Pearson coefficient and *P* values in brackets; n = 20) of the chemical composition of almond hulls samples with gas production parameters measured in 120-h *in vitro* incubations and fermentation parameters after 6 and 24 h of *in vitro* incubations (only $P < 0.05$ values are shown).

	Gas production parameters[1]				Fermentation parameters[2]				
Item	PGP	*Lag*	*c*	AGPR	VFA 6 h	VFA 24 h	NH_3-N 6 h	NH_3-N 24 h	pH 24 h
Dry matter		0.579 (0.008)	0.598 (0.005)	0.684 (<0.001)					
Ash		0.761 (<0.001)		0.498 (0.025)	0.508 (0.022)				
Crude protein		0.449 (0.047)	0.536 (0.015)	0.502 (0.024)				0.831 (<0.001)	
Neutral detergent fibre		-0.581 (0.007)	- 0.804 (<0.001)	- 0.854 (<0.001)					- 0.449 (0.047)
Acid detergent Fibre		- 0.670 (0.001)	- 0.807 (<0.001)	- 0.885 (<0.001)					
Lignin	- 0.487 (0.029)	-0.796 (<0.001)	- 0.582 (0.007)	- 0.812 (<0.001)					
Lignin/NDF	- 0.607 (0.005)	- 0.736 (<0.001)		- 0.529 (0.016)	- 0.578 (0.008)	- 0.523 (0.018)			
Crude fibre						- 0.542 (0.014)			- 0.521 (0.018
Total sugars								- 0.640 (0.002)	
Ether extract				-0.456 (0.043)					

[1] PGP: potential gas production; *c*: fractional rate of gas production; *Lag*: time before starting gas production; AGPR: average gas production rate; [2] Total volatile fatty acid (VFA) production and NH_3-N concentrations measured after 6 or 24 h of incubation.

The VFA profile differed between almond varieties, with Guara AH resulting in greater ($P < 0.001$) molar proportions of acetate and lower ($P \leq 0.007$) of propionate, butyrate and isovalerate than Soleta AH at 6 h of incubation. Although differences between AH varieties were reduced at 24 h of incubation, Guara AH still produced greater ($P < 0.001$) proportions of acetate and lower ($P < 0.001$) of butyrate and isovalerate compared with Soleta. The greater propionate proportions of Soleta at 6 h of incubation could be due to the presence of soluble and rapidly degraded fractions that contribute to propionate production, and this hypothesis is consistent with

the lower *Lag* time of Soleta AH. At 6 h of incubation, Guara samples showed greater ($P < 0.001$) acetate/propionate ratios than Soleta, but differences disappeared after 24 h of incubation ($P = 0.527$). We are not aware of studies reporting the fermentation profile of AH, but Durmic et al. (2014) observed that adding AH to a mixed diet (1:1 ratio) resulted in a significative increase in acetate/propionate ratio after 24 h of *in vitro* incubation, which agrees with the high acetate proportions observed in the present study for all AH samples. Similarly, Rad et al. (2016) observed no changes in acetate and propionate proportions and acetate/propionate ratio in the rumen of lambs when AH were included up to 40% of the diet replacing a fibrous feed (alfalfa hay).

Despite the greater CP content in Guara than in Soleta samples, there were no differences between AH varieties in NH_3-N concentrations ($P \geq 0.324$) at any incubation time. However, there was a positive correlation ($r = 0.830$; $P < 0.001$; Table 5.3) between NH_3-N concentrations at 24 h and CP content of AH samples. Ruminal CP degradability of AH has been reported to be low due to the high proportion of CP linked to the ADF (INRA, 2018; NRC, 2021). In the present study, NH_3-N concentrations were adequate for *in vitro* microbial growth (Satter & Slyter, 1974) due to the use of a N-enriched culture medium for the *in vitro* incubations. Moreover, it has to be noted that the measured NH_3-N concentrations are the balance between the NH_3-N generated in the degradation of CP and the NH_3-N captured by the microbiota for microbial protein synthesis.

5.1.2. Effects of including almond hulls on **in vitro** *ruminal fermentation of dairy sheep diets*

Several studies have investigated the effects of replacing conventional feed ingredients in ruminant diets with AH, but results have been inconsistent (Reed et al., 1988; Williams et al., 2014; Durmic et al., 2014; Rad et al., 2016). These controversial results can be attributed to variations in the nutritive value of AH and their inclusion level in the diet, but also to differences

in the quality of the feeds being replaced in the diets. Therefore, the second part of this study was designed to assess the effects of including increasing amounts of AH (0, 8, 16 and 24%) replacing different feeds in the diet. Almond hulls partly replaced concentrate ingredients (corn, wheat bran, and sugar beet pulp) in HP (high-producing) dairy diets, whereas in MP (medium-producing) dairy diets AH mostly replaced forage (alfalfa hay; 60.0% in MP0 to 40.3% in MP24 diet) and only a little proportion of corn (15% in MP0 to 10.2% in MP24 diet). Within HP and MP, all experimental diets were formulated to contain similar N and NDF levels. In addition, the culture medium of Goering and Van Soest (1970) used in the *in vitro* incubations was modified to exclude the N-containing compound (NH_4HCO_3) to avoid extra N supplementation.

As shown in Table 5.4, including AH in HP diets did not affect any gas production parameter except for the fractional gas production rate (*c*), which was linearly reduced ($P < 0.001$) as dietary AH level increased. Similarly, the DMED was linearly decreased ($P < 0.001$) by increasing the amount of AH in the diet. At 9 h of incubation, VFA production was unaffected ($P > 0.05$) by AH inclusion, but VFA profile was modified. Propionate proportion was linearly increased ($P = 0.027$) and valerate proportion ($P = 0.003$) and acetate/propionate ratio ($P = 0.028$) was linearly reduced by increasing AH levels in the diet, although only the HP24 diet showed significant differences ($P < 0.05$) with the control diet. The increase in propionate proportion observed in diets with AH is consistent with the presence of sugars and other rapidly-degradable substances in AH. Neither the amount of AFOM nor CH_4 production and CH_4/total VFA ratio were affected ($P \geq 0.303$) by including AH in the diet, indicating that diet fermentation was relatively unchanged at 9 h of incubation.

Table 5.4 Gas production and fermentation parameters of diets for high-producing dairy sheep without almond hulls (HP0) and with almond hulls at 8 (HP8), 16 (HP16) and 24% (HP24) of the fresh matter at 9 and 24 h of *in vitro* incubation with ruminal fluid from sheep.

Item	HP0	HP8	HP16	HP24	SEM[2]	*P* =	
						Lineal	Quadratic
Gas production parameters[1]							
PGP (mL/g dry matter (DM))	267	259	256	248	6.1	0.066	0.976
Lag (h)	0.060	0.057	0.056	0.056	0.0032	0.367	0.621
c (%/h)	2.07	1.62	1.28	0.86	0.120	<0.001	0.904
AGPR (mL/h)	9.71	9.34	9.44	9.43	0.420	0.702	0.679
DMED (%)	38.0^{c}	36.3^{b}	34.3^{a}	35.3^{a}	0.31	<0.001	0.002
Fermentation parameters (9 h)							
Total volatile fatty acids (VFA; mmol/g DM)	3.95	3.94	3.85	3.82	0.087	0.266	0.889
Molar proportion of VFA (mol/100 mol)							
Acetate (Ac)	63.0	62.8	62.2	62.5	0.27	0.141	0.608
Propionate (Pr)	22.2^{a}	22.2^{ab}	23.3^{ab}	23.5^{b}	0.42	0.027	0.867
Butyrate	10.8	10.6	10.5	10.4	0.15	0.055	0.921
Isobutyrate	0.605	0.697	0.733	0.465	0.0968	0.470	0.170
Isovalerate	2.04	2.35	2.04	1.93	0.046	0.151	0.318
Valerate	1.33^{b}	1.30^{ab}	1.21^{a}	1.22^{a}	0.021	0.003	0.311
Ac/Pr (mol/mol)	2.85^{b}	2.84^{ab}	2.68^{ab}	2.66^{a}	0.061	0.028	0.969
NH_3-N (mg/L)	143	139	141	143	2.5	0.720	0.219
CH_4 (mL)	18.3	18.6	18.4	18.0	0.39	0.662	0.401
CH_4/VFA (mL/mol)	4.65	4.75	4.81	4.74	0.111	0.528	0.449
AFOM (mg/g)[3]	342	328	332	330	7.0	0.303	0.701

[a,b,c] Within each row, the means with different superscripts are different ($P < 0.05$).

[1] PGP: potential gas production; *c*: fractional rate of gas production; *Lag*: time needed to start gas production; AGPR: average gas production rate; DMED: dry matter effective degradability; [2] SEM: standard error of the mean; [3] Apparently fermented organic matter was calculated from VFA production according to Demeyer (1991).

Table 5.4 (continued) Gas production and fermentation parameters of diets for high-producing dairy sheep without almond hulls (HP0) and with almond hulls at 8 (HP8), 16 (HP16) and 24% (HP24) of the fresh matter at 9 and 24 h of *in vitro* incubation with ruminal fluid from sheep.

Item	HP0	HP8	HP16	HP24	SEM[2]	*P* =	
						Lineal	Quadratic
Fermentation parameters (24 h)							
pH	6.84[a]	6.88[b]	6.89[bc]	6.91[c]	0.007	<0.001	0.171
Total VFA (mmol/g DM)	6.73[b]	6.30[ab]	6.35[ab]	5.98[a]	0.146	0.008	0.714
Molar proportion of VFA (mol/100 mol)							
Acetate (Ac)	62.3	62.7	63.0	62.5	0.45	0.699	0.411
Propionate (Pr)	20.1	19.9	20.1	20.8	0.24	0.087	0.116
Butyrate	13.2[b]	13.2[b]	12.8[ab]	12.5[a]	0.25	0.042	0.610
Isobutyrate	1.19	1.07	1.06	1.10	0.056	0.327	0.178
Isovalerate	1.75	1.77	1.72	1.80	0.027	0.289	0.627
Valerate	1.35	1.34	1.30	1.32	0.448	0.733	0.642
Ac/Pr (mol/mol)	3.10	3.15	3.13	3.01	0.052	0.264	0.144
NH_3-N (mg/L)	222[b]	222[b]	210[ab]	206[a]	4.8	0.026	0.707
CH_4 (mL)	53.0[b]	50.8[ab]	51.4[b]	47.7[a]	1.12	0.013	0.527
CH_4/VFA (mL/mol)	7.89	8.07	8.11	7.99	0.158	0.642	0.376
AFOM (mg/g)[3]	593[b]	557[ab]	559[ab]	523[a]	12.3	0.004	0.965

[a,b,c] Within each row, the means with different superscripts are different ($P < 0.05$).
[1] PGP: potential gas production; *c*: fractional rate of gas production; *Lag*: time needed to start gas production; AGPR: average gas production rate; DMED: dry matter effective degradability; [2] SEM: standard error of the mean; [3] Apparently fermented organic matter was calculated from VFA production according to Demeyer (1991).

In contrast, after 24 h of incubation more differences among diets were detected. Both total VFA production and the amount of AFOM were linearly reduced ($P = 0.008$ and 0.004, respectively) and pH was linearly increased ($P < 0.001$) by dietary AH inclusion, indicating a reduction of diet fermentability. In addition, butyrate proportion linearly decreased ($P = 0.042$) with increasing AH levels. Altogether, these results might indicate that some AH fractions were more rapidly fermented than other fractions in some of the replaced feeds (corn, wheat bran and sugar beet pulp), resulting in no differences in VFA production or AFOM at 9 h of incubation, but afterwards AH were less fermented resulting in reduced VFA production for diets containing AH.

There were no differences among diets in NH_3-N concentration at 9 h of incubation, but after 24 h NH_3-N concentration was linearly reduced (P = 0.026; Table 5.4) by including AH in the diet. The observed reduction in NH_3-N concentration is consistent with the reported low degradability of CP of AH (INRA, 2018; NRC, 2021). The potential effects of AH on ruminal CH_4 production have been investigated in a limited number of studies, and the results are contradictory. Whereas Durmic et al. (2014) observed a reduction in *in vitro* CH_4 production when AH were added to a mixed substrate, Williams et al. (2014) observed no change in ruminal CH_4 emissions of dairy cows when dietary alfalfa cubes were partly replaced by AH. In the present study, CH_4 production was unaffected at 9 h of incubation, but at 24 h of incubation was linearly decreased (P = 0.013) by increasing the amounts of AH in the diet (Table 5.4). Although these results might indicate an antimethanogenic effect of AH, the similar CH_4/total VFA ratios detected for all diets (P = 0.642 and 0.376 for linear and quadratic effects, respectively) indicate that the observed reduction in CH_4 production was due to decreased ruminal fermentability of the diets containing AH. The CH_4/total VFA ratio can be used as an indicator of ruminal fermentation efficiency (Vanegas et al., 2017), as CH_4 is an energy loss and VFA are the main energy source for the host animal and precursors for the synthesis of other compounds such as propionate for glucose synthesis and acetate and butyrate for fatty acids synthesis (Van Soest, 1994).

Table 5.5 shows the gas production and fermentation parameters of the diets formulated for medium-producing dairy sheep (MP diets), in which AH replaced partly alfalfa hay and corn. The inclusion of AH reduced linearly the time at which gas production started (*lag*; P = 0.008) and the fractional rate of gas production (*c*; P < 0.001) without affecting PGP and AGPR. The DMED was quadratically decreased (P = 0.030) by increasing the amount of AH in the diet.

Table 5.5 Gas production and fermentation parameters of diets for medium-producing dairy sheep without almond hulls (MP0) and with almond hulls at 8 (MP8), 16 (MP16) and 24% (MP24) of the fresh matter at 9 and 24 h of *in vitro* incubation with ruminal fluid from sheep.

Item	MP0	MP8	MP16	MP24	SEM[2]	*P* =	
						Lineal	Quadratic
Gas production parameters[1]							
PGP (mL/g dry matter (DM))	259	257	257	264	4.9	0.664	0.447
Lag (h)	0.058^{b}	0.058^{b}	0.057^{b}	0.052^{a}	0.001	0.008	0.060
c (%/h)	1.75^{b}	1.69^{b}	1.66^{b}	1.09^{a}	0.102	<0.001	0.028
AGPR (mL/h)	9.49	9.40	9.33	9.16	0.210	0.294	0.851
DMED (%)	34.6^{ab}	35.2^{b}	34.9^{b}	33.6^{a}	0.36	0.066	0.030
Fermentation parameters (9 h)							
Total volatile fatty acids (VFA; mmol/g DM)	3.93	3.91	3.70	3.87	0.089	0.379	0.316
Molar proportion of VFA (mol/100 mol)							
Acetate (Ac)	65.7^{c}	65.2^{bc}	64.3^{b}	62.4^{a}	0.36	<0.001	0.078
Propionate (Pr)	21.4^{a}	22.2^{ab}	22.8^{b}	24.5^{c}	0.39	<0.001	0.298
Butyrate	9.34	9.21	9.56	9.79	0.181	0.067	0.344
Isobutyrate	0.658	0.370	0.675	0.668	0.1276	0.572	0.301
Isovalerate	1.75	1.63	1.63	1.67	0.064	0.469	0.240
Valerate	1.18^{c}	1.36^{b}	1.04^{a}	0.96^{a}	0.028	<0.001	0.002
Ac/Pr (mol/mol)	3.08^{c}	2.94^{bc}	2.83^{b}	2.57^{a}	0.015	<0.001	0.285
NH_3-N (mg/L)	143	138	137	142	3.8	0.814	0.183
CH_4 (mL)	16.9	16.8	16.9	17.1	0.43	0.726	0.656
CH_4/VFA (mL/mol)	4.37	4.33	4.59	4.43	0.430	0.586	0.742
AFOM (mg/g)[3]	336	335	318	334	0.3	0.472	0.279

[a,b,c] Within each row, the means with different superscripts are different ($P < 0.05$).

[1] PGP: potential gas production; *c*: fractional rate of gas production; *Lag*: time before starting gas production; AGPR: average gas production rate; DMED: dry matter effective degradability; [2] SEM: standard error of the mean; [3] Apparently fermented organic matter was calculated from VFA production according to Demeyer (1991).

Table 5.5 (continued) Gas production and fermentation parameters of diets for medium-producing dairy sheep without almond hulls (MP0) and with almond hulls at 8 (MP8), 16 (MP16) and 24% (MP24) of the fresh matter at 9 and 24 h of *in vitro* incubation with ruminal fluid from sheep.

Item	MP0	MP8	MP16	MP24	SEM[2]	*P* =	
						Lineal	Quadratic
Fermentation parameters (24 h)							
pH	6.87[a]	6.86[a]	6.89[b]	6.90[b]	0.005	<0.001	0.244
Total VFA (mmol/ g DM)	6.69[c]	6.40[b]	6.09[a]	6.11[a]	0.078	<0.001	0.078
Molar proportion of VFA (mol/100 mol)							
Acetate (Ac)	64.6[c]	64.1[bc]	63.3[ab]	62.6[a]	0.39	0.003	0.863
Propionate (Pr)	19.2[a]	20.2[b]	20.6[b]	21.5[c]	0.18	<0.001	0.843
Butyrate	11.9	11.5	12.3	12.1	0.25	0.311	0.636
Isobutyrate	1.20	1.06	1.08	1.07	0.095	0.395	0.496
Isovalerate	1.78	1.65	1.67	1.65	0.041	0.076	0.204
Valerate	1.26[b]	1.54[c]	1.12[a]	1.12[a]	0.024	<0.001	<0.001
Ac/Pr (mol/mol)	3.36[c]	3.18[b]	3.07[ab]	2.91[a]	0.049	<0.001	0.786
NH_3-N (mg/L)	223[b]	188[a]	183[a]	191[a]	3.6	<0.001	<0.001
CH_4 (mL)	50.2	46.7	46.1	47.2	1.55	0.204	0.176
CH_4/VFA (mL/mol)	7.50	7.28	7.57	7.76	0.315	0.471	0.530
AFOM (mg/g)[3]	583[c]	556[b]	531[a]	536[a]	6.3	<0.001	0.033

[a,b,c] Within each row, the means with different superscripts are different ($P < 0.05$).
[1] PGP: potential gas production; *c*: fractional rate of gas production; *Lag*: time before starting gas production; AGPR: average gas production rate; DMED: dry matter effective degradability; [2] SEM: standard error of the mean; [3] Apparently fermented organic matter was calculated from VFA production according to Demeyer (1991).

Similarly to that observed for HP diets, the inclusion of AH in the MP diets did not affect either total VFA production or the amount of AFOM at 9 h of incubation, but after 24 h resulted in a linear decrease ($P < 0.001$) of both total VFA production and AFOM and in a linear increase of pH ($P < 0.001$), altogether indicating lower fermentation of AH-containing diets compared with the control diet (MP0). Changes in VFA profile were also observed at both measurement times, with VFA production being shifted to propionate at the expense of acetate when AH were included in the diet, with acetate/propionate ratios being lower for the diets with AH. Moreover, proportions of valerate were linearly decreased ($P < 0.001$) at both 9 and 24 h of incubation for

the diets with AH. Decreased proportions of valerate can be related to a reduction in CP degradation or amino acids deamination, as valerate is a breakdown product of proline (Dehority et al., 1958), and these results are consistent with the reduced NH_3-N concentrations observed at 24 h of incubation when AH were included in the diet. Finally, in accordance with that observed for HP diets, including AH in the diet did not affect either CH_4 production or CH_4/total VFA ratios at any measurement time, indicating that the used AH did not contain antimethanogenic compounds.

5.2. Experiment 2. Effects of including two levels of AH in the concentrate of light lambs on feed intake, growth performance, diet digestibility, post-mortem ruminal and caecal fermentation, blood parameters, and meat quality

The FA profile of the concentrates and cereal straw fed to the lambs and of the AH included in the concentrates used in Experiment 2 is given in Table 5.6. Although there is limited information on the FA profile of AH, the main FA are usually C18:1 *cis*-9 (oleic), C18:2 n-6 (linoleic) and C16:0 (palmitic) acids and they can account for 50, 25 and 13% of total FA (Williams et al., 2018). In accordance with these observations, the AH used in this experiment had 49.7% of C18:1 *cis*-9, 24.4% of C18:2 n-6 and 13.4% of C16:0 acids.

The inclusion of AH in the concentrate was accompanied by other modifications in feed ingredients to get isoenergetic concentrates, such as the increase in the amount of calcium soap in AH6 and AH12 concentrates. According to the manufacturer, the calcium soap used in the concentrates (Magnapac®; Norel SL) contains 50% of C16:0 and 35% of C18:1 *cis*-9, and this together with the high content of both FA in the AH can explain the greater C16:0 and C18:1 *cis*-9 content in the concentrates containing AH compared with the control concentrate. The substitution of barley grains and wheat bran by AH could have caused a decrease in the concentration of C18:2 n-6 in AH6 and AH12 concentrates. The C18:2 n-6 can account for

more than 50% of the FA present in both barley and wheat bran (FEDNA, 2019), being this content greater than that in the AH (24.4%).

Table 5.6 Fatty acids (FA) profile of the concentrates and barley straw fed to lambs and of the almond hulls (AH) included in AH6 and AH12 concentrates.

Item	Concentrate			Barley straw	AH
	Control	AH6	AH12		
FA (% total FA)[1]					
C16:0	24.5	27.9	30.0	31.0	13.4
C17:0	0.00	0.10	0.12	0.00	0.32
C18:0	3.07	3.37	3.86	3.87	4.45
C20:0	0.423	0.444	0.463	4.18	1.07
C22:0	0.263	0.242	0.224	5.99	1.06
C24:0	0.225	0.172	0.196	3.70	0.537
TOTAL SFA	**28.5**	**32.2**	**34.9**	**48.8**	**20.9**
C18:1 *cis* 9	26.9	29.5	31.5	16.8	49.7
C20:1	0.507	0.400	0.351	0.00	0.00
TOTAL MUFA	**27.4**	**29.8**	**31.9**	**16.8**	**49.7**
C18:2 n-6	41.3	35.5	31.1	25.3	24.4
C18:3 n-3	2.82	2.34	2.10	9.11	5.51
TOTAL PUFA	**44.1**	**37.9**	**33.2**	**34.4**	**29.5**

[1] SFA: saturated FA; MUFA: monounsaturated FA; PUFA: polyunsaturated FA.

5.2.1. Growth performance and diet digestibility

There were no effects ($P \geq 0.182$) of either the inclusion of AH in the concentrate or lamb sex on final BW, concentrate intake, straw intake, and total feed intake of lambs (Table 5.7), and no concentrate x sex interactions were detected for any of these parameters. The lack of differences between groups in final BW and feed intake agrees well with the results of Scerra et al. (2022), who reported no effects on these parameters when either 15 or 30% of AH were included in the concentrate fed to Sarda lambs replacing partially barley and corn grains. Similarly, Phillips et al. (2015) did not observe changes in feed intake and growth performance of Suffolk x Hampshire lambs when AH replaced alfalfa hay and were included at 5 or 10% of total diet. Likewise, Rad et al. (2016) reported no effects of substituting alfalfa hay with urea-treated AH up to 40% of the diet on feed intake and growth of Shall lambs. On the other hand,

Omar and Abdallah (2019) reported significantly better feed conversion ratio in Assaf lambs fed ensiled AH compared with feeding wheat straw, both fed at 20% of total diet (DM basis). All these studies indicate that AH had no negative effect on diet palatability and feed intake. In the present study, straw intake was low in all groups (< 7 g per 100 g of total DM intake), confirming previous observations in fattening light lambs under similar feeding conditions (Haro et al., 2019; de Evan et al., 2020).

The type of concentrate tended to affect ADG (P = 0.092) and lambs fed the AH12 concentrate gained 40 g/d less than control-fed lambs, whereas the difference for AH6-fed group was only 16 g/d. Although the differences did not reach the significance level, they could be of importance for the farmers.

The lack of sex effects on feed intake and growth performance can be explained by the low slaughter BW of lambs, as sex differences became more marked with advancing age (Burfening and Carpio, 1993). Moreover, lamb males usually have greater BW at birth than females (Bennett et al., 1991), but in the present study there was no difference between sexes in the initial BW of lambs (Table 5.7), which could also have contributed to the absence of differences between sexes in feed intake and FCR.

Table 5.7 Initial and final body weight, feed intake, average daily gain, feed conversion rate, and carcass weight of growing lambs fed barley straw and concentrate without almond hulls (AH; Control), with 60 (AH6) and with 120 (AH12) g of almond hulls per kg (fresh matter basis).

Item	Concentrate				Sex			*P* =		
	Control	AH6	AH12	SEMc[1]	Female	Male	SEMs[1]	Concentrate	Sex	Concentrate x Sex
Initial body weight (kg)	12.3	12.4	12.3	0.36	12.4	12.4	0.29	0.910	0.990	0.634
Final body weight (kg)	23.5	22.8	22.1	0.65	22.2	22.3	0.53	0.372	0.182	0.133
Feed intake (g/d)										
Concentrate	768	740	718	23.3	747	737	19.0	0.349	0.701	0.233
Straw	52.1	52.5	50.0	3.76	52.6	50.4	3.07	0.883	0.623	0.109
Total	820	793	768	23.7	799	787	19.4	0.331	0.673	0.288
Straw intake (% total intake)	6.51	6.42	6.47	0.449	6.47	6.46	0.366	0.991	0.981	0.110
Average daily gain (g/d)	238	222	198	11.8	208	231	9.6	0.092	0.111	0.131
Feed conversion rate (g/g)	3.55	3.59	3.96	0.184	3.89	3.51	0.150	0.274	0.099	0.069

[1] Standard error of the mean for concentrate (SEMc; n = 10) and sex (SEMs; n = 15) effects.

The inclusion of AH in the concentrate did not affect OM, NDF and ADF digestibility ($P \geq 0.205$; Table 5.8), but tended to decrease DM and CP digestibility ($P \leq 0.099$). The reduced DM and CP digestibility could be related to the high ash and lignin content of the AH used in the present study. In addition, CP of AH has been reported to have low ruminal degradability, as a large proportion is usually linked to ADF (Omar and Abdallah, 2017). Moreover, the trend towards reduced DM and CP digestibility is consistent with the reduced ADG of the lambs fed the concentrates with AH; especially those fed the AH12 concentrate. Other studies reported that dietary AH inclusion either had no effects on diet digestibility in lambs (Rad et al., 2016) or even increased linearly diet digestibility in dairy cows with increasing AH levels (Swanson et al., 2021). The effect of AH on diet digestibility would be influenced by the quality of AH, but also by the quality of the dietary ingredients that are replaced with AH. All digestibility values were within the range reported by others (Haro et al., 2019; De Evan et al., 2020) for light lambs fed high-cereal concentrate and cereal straw *ad libitum*.

Neither sex ($P \geq 0.585$) nor diet x sex interactions ($P \geq 0.431$) were detected on the digestibility of any nutrient. The absence of sex differences in diet digestibility is consistent with the similar feed intake and ADG values observed for both sexes, confirming the results of previous studies with lambs of different breeds (Zhao et al., 2015; Al-Khaza'leh and Obeidat, 2024). In agreement with our results, Zhao et al. (2015) did not detect differences between males and females in N utilization in either Highlander or Texel lambs.

Although there were no differences ($P \geq 0.234$) between groups, either due to concentrate type or to lamb sex, in N intake (g/d) and faecal N (g/d), lambs fed AH12 concentrate had greater ($P < 0.05$) urinary N excretion (expressed as both g/d or g/100 g N intake) than control lambs, resulting in lower ($P < 0.05$) amount of N retained (expressed as g/d and g/100 g N intake) in AH12 compared with control and AH6 lambs (Table 5.8). The decreased N retention observed in the lambs fed the AH12 concentrate contrasts with the lack

of changes in N retention of lambs when alfalfa hay was replaced with 20 or 40% of urea-treated AH in the diet reported by Rad et al. (2016).

As discussed by Galo et al. (2003), urinary N losses increase when NH_3-N accumulates in the rumen or great deamination occurs as consequence of feeding diets with either N excess or unbalanced amino acid profile. However, both N intake and plasma urea concentrations were similar in all lamb groups in our study, and ruminal NH_3-N concentrations were lower in AH12-fed than in control lambs, despite that both AH-concentrates contained urea-N that is rapidly degraded in the rumen. The increased urinary N excretion of AH12-fed lambs could be a consequence of lower DM fermentation in the rumen, as indicated by the *in vitro* results given in Table 5.18, which would reduce microbial growth and NH_3-N capture by ruminal microorganisms. Moreover, it should be considered that the measured ruminal NH_3-N concentrations are the balance between NH_3-N generation and the amount of NH_3-N absorbed and captured by ruminal microorganisms, and therefore they may not reflect actual NH_3-N production.

There were no effects of lamb sex ($P \geq 0.330$) or concentrate x sex interactions ($P \geq 0.185$) on N intake and urinary and faecal N losses.

Table 5.8 Diet digestibility and nitrogen balance in growing lambs fed barley straw and a concentrate without almond hulls (AH; Control), with 60 (AH6) and with 120 (AH12) g of almond hulls per kg (fresh matter basis).

Item	Concentrate				Sex			*P* =		
	Control	AH6	AH12	SEMc[1]	Female	Male	SEMs[1]	Concentrate	Sex	Concentrate x Sex
Digestibility (g/g)										
Dry matter	0.790	0.780	0.756	0.0097	0.773	0.778	0.0079	0.076	0.648	0.576
Organic matter	0.802	0.797	0.778	0.0093	0.789	0.795	0.0761	0.205	0.585	0.594
Crude protein	0.719	0.701	0.678	0.0123	0.701	0.698	0.0101	0.099	0.848	0.563
NDF (Neutral detergent fibre)	0.550	0.526	0.502	0.0317	0.528	0.525	0.0177	0.394	0.892	0.489
ADF (Acid detergent fibre)	0.451	0.459	0.449	0.0252	0.459	0.449	0.0206	0.839	0.753	0.431
Nitrogen balance (N)										
N intake (g/d)	20.5	21.3	19.2	0.79	20.4	20.2	0.65	0.234	0.808	0.185
N faeces (g/d)	5.72	6.35	6.24	0.378	6.18	6.03	0.309	0.481	0.729	0.566
N faeces (g/100 g N intake)	0.279	0.299	0.323	0.0128	0.300	0.301	0.0104	0.092	0.983	0.407
N urine (g/d)	2.30[ab]	2.02[b]	2.98[a]	0.242	2.59	2.28	0.197	0.046	0.293	0.084
N urine (g/100 g N intake)	0.115[b]	0.095[b]	0.154[a]	0.0112	0.128	0.115	0.0091	0.009	0.330	0.282
Retained N (g/d)[2]	12.5[a]	12.9[a]	9.94[b]	0.55	11.7	11.9	0.45	0.005	0.725	0.415
Retained N (g/100 g N intake)[2]	0.606[b]	0.606[b]	0.523[a]	0.0142	0.572	0.585	0.0116	0.001	0.456	0.910

[a, b:] Within values for each concentrate, means in the same row with different superscript differ (P < 0.05; Tukey test). Superscripts are only shown when a trend (*P* < 0.10) or a significant (*P* < 0.05) effect of concentrate was detected.

[1] Standard error of the mean for concentrate (SEMc; n = 8) and sex (SEMs; n = 12) effects.

[2] Calculated as N intake - N faeces - N urine.

5.2.2. Blood parameters

As shown in Table 5.9, AH inclusion in the concentrate did not affect ($P \geq 0.213$) any of the plasma metabolites and hepatic enzymes measured. The lack of differences between groups in plasma parameters is consistent with the similar nutrient intake in all lamb groups, and the values were within the normal range reported for light lambs fed high-cereal concentrates and straw (De Evan et al., 2020; Lobón et al., 2020). The similar levels of cholesterol, bilirubin, and enzymatic activities (ALP, GPT, ALA and GOT) observed in all groups indicate that feeding AH did not negatively affect liver function. The increased albumin, cholesterol, glucose and ALP concentrations with lamb age are in accordance with previous reports (De Evan et al., 2020; Lobón et al., 2020).

The lower plasma urea concentrations observed at the end of the trial compared with the start of the trial are consistent with the greater concentration of CP in the starter concentrate fed to lambs before starting the experiment (17.3 g/100 g; fresh matter basis) compared with the experimental concentrates (14.5 g/100 g). Similarly, there were no effects of sex ($P \geq 0.112$) on these parameters, although concentrate x sex interactions were detected for cholesterol ($P = 0.047$) and ALP ($P = 0.020$) concentrations. No concentrate x time interactions ($P \geq 0.184$) were detected for any plasma parameter, but there were sex x time interactions for the concentrations of total proteins ($P = 0.011$) and glucose ($P < 0.001$), as both concentrations increased over time in males and decreased in females.

Table 5.9 Plasma concentrations of metabolites and enzymes in growing lambs fed barley straw and a concentrate without almond hulls (AH; Control), with 60 (AH6) and with 120 (AH12) g of almond hulls per kg (fresh matter basis) at the beginning (day 0) and end (day 47) of the trial.

Item[1]	Day	Concentrate			SEMc[2]	Sex		SEMs[2]	*P* =				
		Control	AH6	AH12		Female	Male		Concentrate	Sex	Time	Concentrate x Sex	Sex x Time
Albumin	0	2.85	2.87	2.72	0.029	2.83	2.80	0.023	0.306	0.486	<0.001	0.155	0.089
(g/100 mL)	47	3.15	3.14	3.14		3.10	3.19						
Globulins g/100 ml	0	3.00	3.16	3.04	0.084	3.17	2.96	0.068	0.891	0.827	0.088	0.941	0.087
(g/100 mL)	47	2.95	2.88	2.84		2.82	2.97						
Total proteins	0	5.86	6.03	5.77	0.074	6.00	5.77	0.060	0.537	0.989	0.089	0.826	0.011
(g/100 mL)	47	6.10	6.03	5.99		5.92	6.16						
Urea	0	38.5	39.1	34.6	2.12	35.9	38.9	1.73	0.572	0.572	<0.001	0.073	0.089
(mg/100 mL)	47	25.5	21.4	22.7		26.1	20.2						
Cholesterol	0	42.7	41.6	38.2	1.25	40.0	41.7	1.02	0.412	0.112	<0.001	0.047	0.113
(mg/100 mL)	47	57.3	61.6	56.7		55.3	61.8						
Creatinine	0	0.510	0.521	0.530	0.0135	0.514	0.527	0.0110	0.861	0.630	<0.001	0.102	0.149
(mg/100 mL)	47	0.400	0.397	0.407		0.418	0.384						
Glucose	0	88.8	90.3	87.9	1.70	92.6	85.3	1.39	0.590	0.518	0.003	0.294	<0.001
(mg/100 mL)	47	93.9	97.2	95.2		90.4	100						
Bilirubin	0	0.341	0.358	0.339	0.0049	0.350	0.342	0.0040	0.213	0.808	0.136	0.635	0.123
(mg/100 mL)	47	0.336	0.341	0.335		0.332	0.342						

Table 5.9 (continued) Plasma concentrations of metabolites and enzymes in growing lambs fed barley straw and a concentrate without almond hulls (AH; Control), with 60 (AH6) and with 120 (AH12) g of almond hulls per kg (fresh matter basis) at the beginning (day 0) and end (day 47) of the trial.

Item [1]	Day	Concentrate			SEMc[2]	Sex		SEMs[2]	*P* =				
		Control	AH6	AH12		Female	Male		Concentrate	Sex	Time	Concentrate x Sex	Sex x Time
ALP	0	6.10	4.60	5.12	1.480	4.73	5.81	1.209	0.433	0.892	<0.001	0.020	0.636
(U/l)	47	12.4	15.3	9.02		12.5	11.9						
GPT	0	13.6	12.9	13.2	0.58	12.7	13.7	0.47	0.845	0.237	0.495	0.463	0.775
(U/l)	47	13.9	13.7	13.5		13.4	14.0						
ALA	0	29.7	24.9	26.6	2.34	25.2	28.9	1.91	0.645	0.468	0.042	0.231	0.751
(U/l)	47	23.7	21.8	18.1		20.2	22.2						
GOT	0	119	90.6	102	8.68	114	93.6	7.09	0.235	0.194	0.186	0.302	0.540
(U/l)	47	127	113	112		121	114						

[1] ALP: Alkaline phosphatase, GPT: Glutamic pyruvic transaminase, GOT: ALA: Alpha-amylase, GOT: Glutamic oxaloacetic transaminase.
[2] Standard error of the mean for concentrate (SEMc; n = 20) and sex (SEMs; n = 30) effects; No concentrate x time interactions were detected ($P \geq 0.184$).

5.2.3. Carcass measurements and organs weights

The inclusion of AH in the concentrate did not affect ($P \geq 0.175$) hot and cold carcass weight, carcass yield and carcass cooling losses (Table 5.10). These results are in accordance with those reported by Phillips et al. (2015), who found no effects from including 10% of AH in the diet of fattening lambs on these parameters. Hot and cold carcass weights were similar to those reported by de Evan et al. (2020), who analysed the influence of replacing cereals with agroindustrial by-products on the characteristics of the carcass of light lambs slaughtered at similar weights to those in the present study.

No significant differences ($P \geq 0.053$; Table 5.10) among treatments were found in any carcass measurement, but both carcass internal length and leg length tended to decrease ($P = 0.053$ and 0.090, respectively) in lambs fed the AH concentrates compared with control lambs. These results are in accordance with the lower, although not significant, final BW of the lambs fed the concentrates with AH, especially those fed the AH12 concentrate (Table 5.7). No differences among groups were detected in carcass fatness and pelvic-renal fat ($P = 0.218$ and 0.131, respectively), despite numerical differences in pelvic-renal fat between control and AH12 lambs were considerable (2.43 and 1.97, for control and AH12 groups, respectively).

No effects of lambs sex were observed on carcass measurements, excepting that females tended to have greater carcass yield ($P = 0.062$) and pelvic-renal fat ($P = 0.067$) than males (Table 5.10). Values of pelvic-renal fat were in the range reported by Sañudo et al. (1998) for Manchega lambs slaughtered at similar BW. Females usually deposit larger amounts of fat at an earlier age than males, and have even been reported to have greater expression of lipogenic genes compared to males (Dervishi et al. 2012), which could help to explain the trend to greater pelvic-renal fat observed for females in our study (2.37 *vs.* 2.03). Rodríguez et al. (2008) also found greater fat deposition in females than

in males in lambs under different feeding systems, and observed that sexual dimorphism was correlated with animal precocity and consequently with fat deposition. As shown in Table 5.11, lambs fed AH12 had smaller livers (both expressed as g and as % of BW; $P < 0.05$) than those fed control and AH6 concentrates, whereas spleen and kidneys weights were unaffected by the type of concentrate ($P \geq 0.127$). The lower liver weights of lambs fed the AH12 concentrate could be related to the reduced, although not significant, final BW of these lambs. In fact, Pérez et al. (2014) observed that the weight of the liver of lambs from Canary breeds expressed in relation to BW increased with increasing BW at slaughter.

The sex of lambs, significantly affected spleen and liver weights, with males having greater spleens (expressed as g) and livers (expressed as both g and % of BW) than in females ($P < 0.031$). As indicated by Expósito et al. (2003), males are larger and more compact than females, although differences between sexes are smaller when animals are slaughtered at low BW as in our study. In agreement with our results, Al-Khaza'leh and Obeidat (2024) reported that in Awassi lambs, liver spleen and kidney weights were higher in males than in females, and attributed these differences to the positive correlation with the BW at slaughter, which was also greater in males.

Table 5.10 Slaughter weight, carcass yield and carcass conformation in growing lambs fed barley straw and a concentrate without almond hulls (AH; Control), with 60 (AH6) and with 120 (AH12) g of almond hulls per kg (fresh matter basis).

Item	Concentrate				Sex			*P* =		
	Control	AH6	AH12	SEMc[1]	Female	Male	SEMs[1]	Concentrate	Sex	Concentrate x Sex
Hot carcass weight (kg)	10.9	10.7	10.6	0.34	10.6	10.8	0.28	0.735	0.588	0.113
Cold carcass weight (kg)	10.7	10.4	10.4	0.32	10.4	10.6	0.26	0.655	0.518	0.092
Carcass yield (%)	45.8	45.7	46.9	0.45	46.7	45.6	0.36	0.194	0.062	0.152
Chilling losses (%)	1.86	2.37	2.16	0.187	2.28	1.98	0.153	0.175	0.183	0.383
Carcass conformation										
Buttock width (cm)	17.5	17.1	17.3	0.23	17.2	17.4	0.19	0.564	0.379	0.131
Buttock perimeter (cm)	50.4	50.6	50.5	0.68	50.7	50.2	0.55	0.978	0.505	0.201
Thoracic width (cm)	1.3	17.4	17.4	0.45	17.3	17.4	0.37	0.986	0.816	0.260
Internal carcass length (cm)	55.7	55.1	54.3	0.35	54.7	55.2	0.28	0.053	0.188	0.086
Thoracic depth (cm)	23.0	22.2	22.5	0.58	22.4	22.7	0.47	0.621	0.620	0.529
Leg length (cm)	23.6	24.0	23.1	0.27	23.6	23.5	0.22	0.095	0.536	0.586
Carcass fatteness[2]	2.53	2.11	2.16	0.174	2.41	2.12	0.142	0.218	0.165	0.085
Pelvic-renal [2]	2.43	2.20	1.97	0.149	2.37	2.03	0.122	0.131	0.067	0.013

[1] Standard error of the mean for concentrate (SEMc; n = 10) and sex (SEMs; n = 15) effects.
[2] Measured according a 1 to 4 points scale (1: minimum score; 4: maximum score).

Table 5.11 Spleen, liver and kidneys weigh in growing lambs fed barley straw and a concentrate without almond hulls (AH; Control), with 60 (AH6) and with 120 (AH12) g of almond hulls per kg (fresh matter basis).

Item	Concentrate				Sex			*P* =		
	Control	AH6	AH12	SEMc[1]	Female	Male	SEMs[1]	Concentrate	Sex	Concentrate x Sex
Spleen weight (g)	47.2	49.1	43.9	2.38	43.6	49.9	1.94	0.327	0.031	0.362
Liver weight (g)	528[b]	506[b]	426[a]	16.9	455	518	13.8	0.001	0.004	0.046
Kidneys weight (g)	87.4	85.0	80.1	2.37	82.3	86.1	1.93	0.127	0.179	0.970
Spleen weight (% body weight (BW))	0.200	0.215	0.198	0.0072	0.196	0.213	0.0059	0.205	0.053	0.566
Liver weight (% BW)	2.25[b]	2.22[b]	1.93[a]	0.058	2.05	2.21	0.047	0.001	0.025	0.455
Kidneys weight (% BW)	0.373	0.376	0.364	0.0087	0.371	0.370	0.0071	0.618	0.904	0.072

[a,b] Within each effect (concentrate or sex), means in the same row with different superscript differ ($P < 0.05$).
[1] Standard error of the mean for concentrate (SEMc; n = 10) and sex (SEMs; n = 15) effects.

5.2.4. Colour parameters, lipid oxidation and pH of meat

The effects of the concentrate and sex lamb on colour, lipid oxidation measures (TBARS) and pH of meat are detailed in Table 5.12. No significant effect of either the concentrate type ($P \geq 0.375$) or sex ($P \geq 0.109$) was observed in any colour parameter evaluated, but a concentrate x sex interaction was detected for L* and h* parameters ($P = 0.003$ and 0.15, respectively). No sex x time or concentrate x time interactions were detected, excepting for a* that showed a concentrate x time interaction ($P = 0.015$). The lack of effects of lamb sex on meat colour is in agreement with previous results in two breeds of lambs (Horcada et al., 1998).

Storage time increased L* ($P < 0.001$) and decreased b* and C* values ($P = 0.015$ and 0.031, respectively). Meat colour is affected by numerous factors, such as breed, diet, age and weight of the animal at slaughter, exercise, stress during slaughter, and meat storage conditions, among others. The colour of lamb meat is crucial for consumer's purchase choice and contributes greatly to the value of the product, since a bright colour of the meat is generally associated with freshness and quality. Therefore, an increase in L* values lambs' meat could be favourable for consumer acceptance. The decrease in b* and C* after 6 days of storage is in accordance with the results of Inserra et al. (2014), who also reported a decrease in C* after 6 days of aerobic storing the meat of Comisana lambs. However, it should be noted that although the change in some colour parameters was significant, it was numerically small, indicating little relevance in the practice. Moreover, as discussed by Inserra et al. (2014) it is possible that using a modified atmosphere packaging would reduce time changes.

There was no effect of either the AH inclusion in the concentrate or sex on myoglobin, metmyoglobin and oxymyoglobin values ($P \geq 0.643$), but metmyoglobin values were increased over the storage period ($P < 0.001$). However, metmyoglobin values were below 40%, which is the limit established to qualify a meat as degraded according to trained consumer panels (de Evan et al., 2020).

Table 5.12 Colour parameters and concentration of thiobarbituric acid reactive substances (TBARS) of the meat (measured at slaughter (0) and after 6 days) and meat pH values (at slaughter (0) and after 24 h chilling) in growing lambs fed barley straw and a concentrate without almond hulls (AH; Control), with 60 (AH6) and with 120 (AH12) g of almond hulls per kg (fresh matter basis).

Item [1]	Time	Concentrate			SEMc[2]	Sex		SEMs[2]	*P* =					
		Control	AH6	AH12		Female	Male		Concentrate	Sex	Time	Concentrate x Sex	Sex x Time	Concentrate x Time
L*	0	47.9	47.2	47.5	1.02	48.3	46.9	0.83	0.649	0.109	<0.001	0.003	0.343	0.867
	6	51.1	50.5	51.2		51.3	50.6							
a*	0	5.94	5.27	6.91	0.210	6.20	5.87	0.171	0.385	0.981	0.417	0.169	0.209	0.015
	6	5.46	6.13	5.91		5.68	5.99							
b*	0	10.4	9.63	11.2	0.535	10.6	10.2	0.437	0.375	0.978	0.015	0.080	0.212	0.256
	6	9.40	9.54	9.85		9.39	9.80							
h*	0	60.2	61.2	58.3	0.34	59.6	60.2	0.28	0.586	0.650	0.459	0.015	0.945	0.327
	6	60.0	58.3	59.1		58.9	59.3							
C*	0	12.0	11.0	13.2	0.22	12.3	11.8	0.18	0.383	0.981	0.031	0.158	0.142	0.059
	6	10.9	11.4	11.5		11.0	11.5							
Myoglobin	0	32.5	35.5	28.0	0.22	31.2	32.8	0.18	0.713	0.945	0.309	0.082	0.807	0.936
(%)	6	25.6	28.6	25.3		27.0	26.0							
Metmyoglobin	0	20.9	21.6	22.7	0.22	21.4	22.1	0.18	0.643	0.687	<0.001	0.062	0.921	0.364
(%)	6	28.6	26.0	29.0		27.5	28.3							
Oxymyoglobin	0	46.6	42.9	49.3	0.22	47.4	45.1	0.16	0.806	0.792	0.882	0.164	0.771	0.837
(%)	6	45.8	45.4	45.7		45.6	45.7							

Table 5.12 (continued) Colour parameters and concentration of thiobarbituric acid reactive substances (TBARS) of the meat (measured at slaughter (0) and after 6 days) and meat pH values (at slaughter (0) and after 24 h chilling) in growing lambs fed barley straw and a concentrate without almond hulls (AH; Control), with 60 (AH6) and with 120 (AH12) g of almond hulls per kg (fresh matter basis).

Item [1]	Time	Concentrate				Sex			*P* =					
		Control	AH6	AH12	SEMc[2]	Female	Male	SEMs[2]	Concentrate	Sex	Time	Concentrate x Sex	Sex x Time	Concentrate x Time
TBARS	0	0.199	0.193	0.197	0.0052	0.194	0.198	0.0041	0.056	0.725	<0.001	0.792	0.726	0.111
	6	0.228	0.214	0.247		0.230	0.230							
pH														
Longissimus dorsi	0	6.73	6.73	6.88	0.034	6.73	6.83	0.028	0.369	0.022	<0.001	0.030	0.870	0.287
	24	5.54	5.73	5.64		5.63	5.71							
Semitendinosus	0	6.35	6.37	6.46	0.316	6.37	6.42	0.258	0.467	0.108	<0.001	0.116	0.402	0.432
	24	5.71	5.88	5.76		5.70	5.86							

No concentrate x sex x time interactions were detected for any treatment ($P > 0.05$).

[1] L*: Lightness; a*: Redness; b*: Yellowness; h*: Hue; C*: Chroma; TBARS concentrations are expressed as mg of malondialdehyde (MDA/kg)

[2] Standard error of the mean for concentrate (SEMc; n = 20) and sex (SEMs; n = 30) effects.

Concentrations of TBARS tended to be affected ($P = 0.056$) by AH inclusion in the concentrate but were unaffected by lamb sex ($P = 0.725$), and no interaction concentrate x sex was detected ($P = 0.792$). The trend to increased TBARS concentration in the meat of lambs fed the AH12 concentrate after 6 storage days (0.228 *vs.* 0.247 mg MDA/kg for control and AH12 groups, respectively) might be related to the greater PUFA content in the meat (see Table 5.14), since PUFA are the main substrates in the oxidative reactions of lipids. These results agree with previous studies (Morán et al., 2012; Holman et al., 2022) indicating that the enrichment of meat with PUFA increases its susceptibility to peroxidation, as a result of preferential free radical autoxidation of the carbon-hydrogen bonds of long chain PUFA.

In contrast to our results, Scerra et al. (2022) observed that the inclusion of AH in the diet of lambs actually decreased the TBARS concentrations in the meat, which was attributed to the antioxidant capacity of AH. Some authors report that a reduction of lipid oxidation could have positive effects on colour stability of meat, since FA oxidation could promote oxidation of myoglobin (Alderton et al., 2003; Faustman et al., 2010). The observed increase ($P < 0.001$) in TBARS concentrations with advancing storage time is in agreement with previous studies (Inserra et al., 2014; de Evan et al., 2020) reporting similar increases in the meat of lambs after storage. However, it should be noted that in our study TBARS concentrations in all groups were below 0.5 mg MDA/kg of meat, which is the threshold proposed by a trained sensory panel for the detection of off-flavours (rancidity) (Lanari et al. 1995). Moreover, the lack of interactions between time and both concentrate and sex ($P \geq 0.111$) on TBARS concentrations indicates that the changes observed over the storage period were not affected by either concentrate type or lamb sex.

No significant effects of the inclusion of AH in the concentrate were observed in the pH of the meat ($P \geq 0.369$; Table 5.12), and pH values agree well with those previously reported (de Evan et al., 2020; Haro et al., 2020) for lambs fed high-concentrate diets and slaughtered at a similar BW (25-26 kg). As expected, the pH decreased ($P < 0.001$) after 24 h of chilling in both *Longissimus dorsi* and *Semitendinosus* muscles, and values were below 6.0 within the optimal range of commercial quality (Beriain et al., 2000).

Sex differences in meat's pH are usually associated with a sudden drop in muscle pH, which produces protein denaturation and affects both the amount of free water that enters the extracellular spaces and the structure of the meat (Velasco et al., 2000). In fact, the pH of *Longissimus dorsi* muscle was greater ($P = 0.022$) in males than in females (6.83 *vs.* 6.73, and 5.71 *vs.* 5.63; respectively). Whereas the results of some studies (Forcada, 1985; Sañudo et al., 1986) indicate that the meat of male lambs can have greater pH values than that of females, other studies (Vergara et al., 1999; Al-Khaza'leh and Obeidat, 2024) detected no significant differences between sexes in the pH of meat from lambs slaughtered at similar BW than in our study. Finally, the lack of interactions ($P \geq 0.111$) between time and either concentrate type or sex indicate that these factors did not influence the drop in meat pH after chilling.

5.2.5. Chemical composition and FA profile of meat

The type of concentrate had no effect ($P \geq 0.187$) on chemical composition of lambs' meat (Table 5.13), and values for the analysed fractions were within the range reported by others authors (De la Fuente et al., 2014; Haro et al., 2020; Estrada et al., 2024) for lambs from different breeds slaughtered at similar BW. In agreement with our results, Scerra et al. (2022) reported no effects from including 15 or 30% of AH in the concentrate fed to Sarda lambs on chemical composition of lambs' meat.

Moisture content of the meat was greater ($P = 0.016$) in males than in females, but both protein and fat content tended to be greater ($P = 0.082$ and 0.057, respectively) in females compared with males. In agreement with our results, Pérez et al. (2012) observed a greater moisture content in the meat of males compared to females (70.6 *vs.* 69.3%) in Merino suckling lambs, although Tejada et al. (2008) reported no effect of sex on the pH of the meat from Merino lambs slaughtered at 24 kg of BW.

The effects of the type of concentrate and lamb sex on FA profile of meat are presented in Table 5.14. Although a greater number of FA were detected in the chromatographic analysis, only those with proportions greater than or equal to 0.3% are reported. No concentrate x sex interactions were observed ($P \geq 0.212$) for any of the FA analysed.

In agreement with previous studies (Velasco et al., 2004; Borys et al., 2011; Facciolongo et al., 2018), the most abundant FA was C18:9 *cis*-9, followed by C16:0 and C18:0 acids in all groups of lambs. Compared with control lambs, feeding the concentrates with AH decreased ($P < 0.05$) the proportion of C15:0 and C17:0, but did not affect ($P = 0.338$) the proportion of total SFA. In addition, the proportion of total MUFA was decreased ($P < 0.05$) while that of total PUFA was increased ($P < 0.05$) when the lambs were fed the concentrates including AH. The meat of the lambs fed AH6 and AH12 concentrates had lower ($P < 0.05$) proportions of C17:1 *cis*-9, C18:1 *cis*-9 and C18:1 *cis*-12 than that from control lambs, but had greater ($P < 0.05$) proportions of C18:2 n-6, C20:3 n-3, C20:4 n-6, C22:4 n-6 and C22:5 n-3.

Table 5.13 Chemical composition of the meat (24 h after slaughter) of growing lambs fed barley straw and a concentrate without almond hulls (AH; Control), with 60 (AH6) and with 120 (AH12) g of almond hulls per kg (fresh matter basis).

Item	Concentrate			SEMc[1]	Sex		SEMs[1]	*P* =		
	Control	AH6	AH12		Female	Male		Concentrate	Sex	Concentrate x Sex
Moisture (%)	73.7	73.9	74.8	0.59	73.3	75.1	0.49	0.393	0.016	0.910
Ash (%)	1.04	0.99	1.05	0.031	1.01	1.05	0.026	0.459	0.394	0.788
Protein (%)	21.2	22.2	21.7	0.42	22.2	21.3	0.34	0.235	0.082	0.753
Fat (%)	3.96	3.31	2.95	0.371	3.84	2.97	0.303	0.187	0.057	0.382

[1] Standard error of the mean for concentrate (SEMc; n = 10) and sex (SEMs; n = 15) effects.

In addition, including AH in the concentrate increased ($P < 0.05$) the proportions of both n-3 and n-6 FA in the meat, although it did not affect n-6/n-3 ratio (12.4, 13.0 and 13.4 for control, AH6 and AH12, respectively). These results contrast with those reported by Scerra et al. (2023), who unexpectedly did not detect any changes in meat FA profile when 15 or 30% of AH replaced partly the barley and corn grains in the concentrate of Sarda lambs that were slaughtered at 36 kg of BW.

Altogether, the results of our study indicate that including AH in the concentrate improved the FA profile of meat by increasing PUFA concentrations. A greater proportion of PUFA could indicate a potentially healthier fat profile for humans, as PUFA are reported to protect from osteoarthritis, cancer, and autoimmune disorders (Kapoor et al., 2021), whereas a high intake of SFA has been related to atherosclerotic cardiovascular disease risk and high cholesterol levels (Maki et al., 2021). Differences in meat FA profile can be due to differences in FA intake but also to other factors, such as the presence of plant secondary compounds that can influence the biohydrogenation of PUFA in the rumen (Vasta et al., 2008). Although the proportion of C18:1 *cis* 9 in the concentrates was increased by including AH (Table 5.6), the meat of the lambs fed AH-concentrates had lower proportions of this FA than that of control lambs. In contrast, including AH in the concentrate reduced the proportion of C18:2 n-6, but the meat of the lambs fed the AH-concentrates with AH had greater proportions of this FA than that of control lambs. These results indicate that variations in FA intake could not explain the observed differences between groups in FA profile of the meat and that other factors are possibly implied. In fact, AH contain bioactive compounds (such as phenolic acids, flavonoids, terpenoids; Prgomet et al., 2017) that, as suggested by Scerra et al. (2022), can modulate the rumen biohydrogenation process.

Table 5.14 Fatty acid profile (FA; g/100 g total FA) of meat in growing lambs fed barley straw and a concentrate without almond hulls (AH; Control), with 60 (AH6) and with 120 (AH12) g of almond hulls per kg (fresh matter basis).

Item[1]	Concentrate				Sex			*P* =		
	Control	AH6	AH12	SEMc[2]	Female	Male	SEMs[2]	Concentrate	Sex	Concentrate x Sex
C12:0	0.430	0.564	0.506	0.0464	0.452	0.548	0.0379	0.145	0.092	0.858
C14:0	1.84	1.77	1.66	0.106	1.82	1.69	0.086	0.507	0.320	0.399
C15:0	0.837 [b]	0.505 [a]	0.302 [a]	0.0763	0.558	0.538	0.0623	<0.001	0.826	0.934
C16:0	18.9	19.6	20.0	0.41	19.7	19.3	0.34	0.174	0.436	0.480
C17:0	4.30 [b]	2.90 [a]	2.12 [a]	0.272	3.24	2.97	0.222	<0.001	0.404	0.505
C18:0	15.7	15.8	15.5	0.61	15.5	15.9	0.50	0.948	0.584	0.425
Total SFA	**42.6**	**41.6**	**40.6**	**0.89**	**41.7**	**41.4**	**0.73**	**0.338**	**0.795**	**0.479**
C16:1 *cis*-9	1.06	0.858	0.991	0.131	1.06	0.873	0.107	0.568	0.232	0.788
C17:1 *cis*-9	1.90[b]	1.18 [a]	1.00 [a]	0.097	1.52	1.21	0.079	<0.001	0.015	0.436
C18:1 *cis*-10, *trans* 11	5.76	6.48	6.17	0.542	6.20	6.08	0.442	0.645	0.852	0.212
C18:1 *cis*-9	29.5 [b]	26.9 [a]	26.4 [a]	0.71	28.8	26.5	0.58	0.014	0.011	0.323
C18:1 *cis*-11	3.45	3.03	2.89	0.194	3.25	3.00	0.159	0.146	0.300	0.308
C18:1 *cis*-12	0.436 [b]	0.325 [a]	0.278 [a]	0.0367	0.397	0.296	0.0299	0.020	0.029	0.232
Total MUFA	**42.8 [b]**	**39.4 [a]**	**38.3 [a]**	**0.84**	**41.8**	**38.5**	**0.68**	**0.003**	**0.003**	**0.963**
C18:2 n-6	8.34 [b]	11.2 [a]	12.4 [a]	0.466	9.51	11.8	0.380	<0.001	<0.001	0.516
C18:3 n-3	0.363	0.408	0.434	0.0217	0.391	0.412	0.0177	0.093	0.423	0.812
C20:3 n-9	0.345	0.381	0.359	0.0468	0.327	0.397	0.0382	0.862	0.214	0.643
C20:3 n-3	0.238 [a]	0.322 [b]	0.365 [b]	0.0179	0.280	0.337	0.0146	<0.001	0.013	0.658
C20:4 n-6	2.96 [a]	4.06 [b]	4.69 [b]	0.271	3.45	4.36	0.222	<0.001	0.009	0.439
C22:4 n-6	0.365 [a]	0.500 [b]	0.566 [b]	0.0333	0.405	0.549	0.0272	0.001	0.001	0.589
C22:5 n-3	0.230 [a]	0.332 [b]	0.367 [b]	0.0183	0.275	0.344	0.0149	<0.001	0.004	0.818
Total PUFA	**14.6 [a]**	**18.9 [b]**	**21.1 [b]**	**0.73**	**16.4**	**20.0**	**0.59**	**<0.001**	**<0.001**	**0.455**
Σn-3	0.985 [a]	1.27 [b]	1.37 [b]	0.052	1.13	1.28	0.042	<0.001	0.022	0.853
Σn-6	12.1 [a]	16.4 [b]	18.3 [b]	0.73	13.9	17.3	0.60	<0.001	<0.001	0.447
Σn-6/Σn-3	12.4	13.0	13.4	0.54	12.3	13.5	0.44	0.447	0.068	0.727

[a,b] Within values for each concentrate, means in the same row with different superscript differ ($P < 0.05$; Tukey test). Superscripts are only shown when a significant ($P < 0.05$) effect of concentrate was detected.

[1] SFA: Saturated FA; MUFA: Monounsaturated FA; PUFA: Polyunsaturated FA.

[2]Standard error of the mean for concentrate (SEMc; n = 10) and sex (SEMs; n = 15) effects.

The greater proportions of C20:3 n-3, C20:4 n-6, C22:4 n-6 and C22:5 n-3 in the meat of the lambs fed the AH-concentrates could not be attributed to differences in the intake of these FA, as they were not detected in any concentrate. Both C20:3 n-3 and C22:5 n-3 can be synthetized from C18:3 n-3, whereas C18:2 n-6 is the precursor for the synthesis of C20:4 n-6 and C22:4 n-6 (Lauridsen, 2019). The reasons for the increased levels of these FA in the meat of the lambs fed the AH-concentrates are unknown, but they might be related with differences in ruminal FA biohydrogenation. The long-chain n-3 FA contribute to human health and well-being, although C20:3 n-3 and C22:5 n-3 are less studied than other n-3 FA such as 20:5 n-3 and 22:6 n-3 (Calder, 2018).

There were some differences between sexes in meat FA profile, but the proportion of total SFA was similar (P = 0.795) for both sexes. In accordance with a previous study (Facciolongo et al., 2018), the meat of females had greater proportions of total MUFA (P = 0.003) than that of males, which was due to the greater ($P \leq 0.029$) content of C17:1 *cis*-9, C18:1 cis-9 and C18:1 cis-12. In contrast, the proportion of all detected PUFA, excepting C18:3 n-3 and C20:3 n-9, was greater ($P \leq 0.013$) in males compared with females and consequently the meat of males had greater ($P < 0.001$) content in total PUFA. Similarly, the proportion of total n-3 and n-6 FA was also greater in males than in females ($P \leq 0.022$), leading to a trend (P = 0.068) towards greater n-6/n-3 ratio in the meat of males. Our results are consistent with previous studies indicating that sex has no influence on meat quality or chemical composition of light lambs, although the meat of males can have a FA profile more favourable to consumer health than females due to enhanced PUFA content (de Araújo et al., 2017). Contrary to these observations, Horcada et al. (1998) and Tsiplakou et al. (2018) did not detect differences between sexes in the FA profile of the meat of lambs slaughtered at 90 and 35 days of age, respectively. It seems that the effects of sex on FA profile of lamb meat can be influenced by diet, breed and slaughter age (Tsiplakou et al., 2018), but also by the analysed muscle. In fact,

Tejeda et al. (2018) observed that total PUFA content in *Longissimus lumborum* muscle was greater ($P < 0.01$) in females than in males when they were slaughtered at 24 kg BW, but differences between sexes disappeared when lambs were slaughtered at 29 kg BW. Moreover, in the same study no differences between sexes in FA profile were detected in *Semimembranosus* muscle at any slaughter BW.

5.2.6. Colour parameters and FA profile of fat

As shown in Table 5.15, there were no differences ($P \geq 0.312$) in colour parameters of the subcutaneous fat of the tail root due to either concentrate type or sex, and no concentrate x sex interactions were detected ($P \geq 0.201$). Díaz (2001) observed similar values of L* but greater b* values for lamb fat, and de Evan et al. (2020) reported comparable values of L*, a* and h* but greater values of b* and C* in the subcutaneous fat of the tail root of light lambs fed a high-cereal concentrate and slaughtered at 26 kg BW. Differences between studies in fat colour parameters may be due to variations in slaughter BW, diet and breed of the lambs. The lack of effect of sex on fat colour is in accordance with previous observations (Tejada et al., 2008; Prache et al., 2022).

The FA profile of the subcutaneous fat of the tail root is given in Table 5.16. Similarly to that observed in meat, C19:1 *cis*-9 was the most abundant FA in all groups of lambs, followed by C16:0. Including AH in the concentrate reduced the proportion of C15:0 and C17:0 and increased that of C16:0, although the proportion of total SFA was unaffected ($P = 0.623$). The increase in the proportion of C16:0 in the lambs fed the AH-concentrates might be due to the greater content in this FA in AH-concentrates as a result of both the increased amount of calcium soap that contained 50% of C16:0 and the high C16:0 content in the AH (13.4%; Table 5.6).

Total MUFA content was unaffected ($P = 0.224$) by the concentrate type, although the proportion of both C17:1 *cis*-9 and C19:1 *cis*-9 was reduced ($P < 0.001$) by feeding the

concentrates with AH. The proportion of total PUFA and total n-6 tended (P = 0.051 and 0.062, respectively) to increase when AH was included in the concentrates, but the n-6/n-3 ratio remained unchanged. In general, the effects of the concentrate type on FA profile of subcutaneous fat were less marked than those on meat FA profile.

Lamb sex had no effect (P = 0.701) on the proportion of total SFA but influenced significantly ($P \leq 0.040$) the total content of MUFA, PUFA, n-6 and n-3 FA, as well as the n-6/n-3 ratio (Table 5.16). Similarly to that observed in meat, the fat of females had greater (P = 0.040) MUFA and lower ($P < 0.001$) PUFA proportion compared with that of males. The lack of concentrate x sex interactions for all FA analysed (excepting for C17:0; P = 0.012) indicates that sex did not influence the observed response to feeding the different concentrates.

Table 5.15 Colour parameters of the subcutaneous fat of the tail root in growing lambs fed barley straw and a concentrate without almond hulls (AH; Control), with 60 (AH6) and with 120 (AH12) g of almond hulls per kg (fresh matter basis).

Item	Concentrate				Sex			*P* =		
	Control	AH6	AH12	SEMc[1]	Female	Male	SEMs[1]	Concentrate	Sex	Concentrate x Sex
L* (Lightness)	68.3	69.7	70.2	0.89	68.9	69.9	0.72	0.312	0.324	0.201
a* (Redness)	2.72	2.65	2.45	0.291	2.73	2.49	0.237	0.813	0.489	0.864
b* (Yellowness)	9.44	9.63	9.52	0.556	9.53	9.52	0.454	0.970	0.989	0.612
h* (Hue)	74.0	75.6	75.9	1.04	74.8	75.6	0.85	0.385	0.547	0.669
C* (Chroma)	9.83	10.02	9.83	0.607	9.93	9.85	0.496	0.968	0.905	0.674

[1] Standard error of the mean for concentrate (SEMc; n = 10) and sex (SEMs; n = 15) effects.

Table 5.16 Fatty acid profile (FA; g/100 g total FA) of subcutaneous fat of the tail root in growing lambs fed barley straw and a concentrate without almond hulls (AH; Control), with 60 (AH6) and with 120 (AH12) g of almond hulls per kg (fresh matter basis).

Item[1]	Concentrate			SEMc[2]	Sex		SEMs[2]	*P* =		
	Control	AH6	AH12		Female	Male		Concentrate	Sex	Concentrate x Sex
C12:0	0.287	0.321	0.299	0.0455	0.234	0.331	0.0371	0.866	0.296	0.813
C14:0	2.51	2.60	2.55	0.067	2.49	2.61	0.055	0.640	0.155	0.592
C15:0	1.85^{b}	1.77^{ab}	1.29^{a}	0.142	1.73	1.55	0.116	0.026	0.283	0.129
C16:0	18.2^{a}	20.8^{b}	22.2^{b}	0.57	19.8	20.9	0.46	<0.001	0.107	0.387
C17:0	7.38^{c}	6.03^{b}	4.68^{a}	0.300	6.61	5.45	0.246	<0.001	0.004	0.012
C18:0	15.3	15.4	14.7	0.85	14.8	15.5	0.69	0.837	0.503	0.184
Total SFA	**46.1**	**47.6**	**46.7**	**1.08**	**46.6**	**47.1**	**0.88**	**0.623**	**0.701**	**0.916**
C16:1 *trans*-13	0.286	0.331	0.349	0.0447	0.245	0.398	0.0365	0.611	0.009	0.983
C16:1 *cis*-9	0.728	0.695	0.902	0.0713	0.730	0.820	0.0583	0.127	0.295	0.334
C17:1 *cis*-9	2.05^{b}	1.25^{a}	1.06^{a}	0.098	1.64	1.27	0.079	<0.001	0.004	0.051
C18:1 *cis*-10, *trans*-11	9.53	10.9	10.7	0.79	10.4	10.3	0.65	0.419	0.890	0.572
C18:1 *cis*-9	27.9	25.3	26.9	0.83	27.9	25.5	0.68	0.097	0.022	0.849
C18:1 *cis*-11	3.30	3.29	2.63	0.220	3.11	3.05	0.180	0.084	0.829	0.923
C18:1 *cis*-12	0.337^{ab}	0.422^{b}	0.252^{a}	0.0441	0.310	0.364	0.0360	0.048	0.309	0.700
C19:1 *cis*-9	0.348^{b}	0.203^{a}	0.149^{a}	0.0244	0.292	0.175	0.0199	<0.001	<0.001	0.145
Total MUFA	**45.7**	**42.3**	**43.6**	**1.02**	**45.5**	**42.9**	**0.83**	**0.224**	**0.040**	**0.830**

[a,b] Within values for each concentrate, means in the same row with different superscript differ ($P < 0.05$; Tukey test). Superscripts are only shown when a significant ($P < 0.05$) effect of concentrate was detected.

[1] SFA: Saturated FA; MUFA: Monounsaturated FA; PUFA: Polyunsaturated FA.

[2] Standard error of the mean for concentrate (SEMc; n = 10) and sex (SEMs; n = 15) effects.

Table 5. 16 (continued) Fatty acid profile (FA; g/100 g total FA) of subcutaneous fat of the tail root in growing lambs fed barley straw and a concentrate without almond hulls (AH; Control), with 60 (AH6) and with 120 (AH12) g of almond hulls per kg (fresh matter basis).

	Concentrate				Sex			*P* =		
Item[1]	Control	AH6	AH12	SEMc[2]	Female	Male	SEMs[2]	Concentrate	Sex	Concentrate x Sex
C18:2 *trans*-11, *trans*- 15	0.312	0.370	0.375	0.0360	0.310	0.394	0.0294	0.416	0.059	0.424
C18:2 n-6	5.40	6.06	6.67	0.352	5.22	6.87	0.287	0.068	<0.001	0.427
C18:3 n-3	0.421	0.474	0.482	0.0265	0.415	0.503	0.0216	0.243	0.010	0.861
CLA *cis*-9, *trans*-11	0.396	0.328	0.402	0.0552	0.324	0.427	0.0451	0.591	0.128	0.107
Other CLA isomers	0.347	0.386	0.398	0.0283	0.329	0.425	0.0231	0.438	0.008	0.431
C20:4 n-6	0.243	0.326	0.318	0.0267	0.284	0.306	0.0219	0.078	0.489	0.192
Total PUFA	**8.23**	**9.14**	**9.71**	**0.395**	**7.96**	**10.1**	**0.323**	**0.051**	**<0.001**	**0.341**
Σn-3	0.474	0.534	0.536	0.0291	0.465	0.565	0.0237	0.258	0.008	0.727
Σn-6	5.99	6.76	7.35	0.373	5.82	7.59	0.304	0.062	<0.001	0.350
Σn-6/Σn-3	12.6	12.7	13.6	0.34	12.5	13.4	0.28	0.106	0.032	0.617

[a,b] Within values for each concentrate, means in the same row with different superscript differ ($P < 0.05$; Tukey test). Superscripts are only shown when a significant ($P < 0.05$) effect of concentrate was detected.

[1] SFA: Saturated FA; MUFA: Monounsaturated FA; PUFA: Polyunsaturated FA.

[2] Standard error of the mean for concentrate (SEMc; n = 10) and sex (SEMs; n = 15) effects.

5.2.7. Post-mortem ruminal and caecal fermentation

Table 5.17 shows the effects of both concentrate type and sex on *post-mortem* fermentative parameters in the rumen and caecum of lambs. No concentrate x sex interactions were observed for any parameter, excepting for ruminal NH_3-N concentration ($P = 0.036$) and caecal acetate proportion ($P = 0.042$).

The type of concentrate did not affect pH values and total VFA concentrations either in the rumen ($P = 0.355$ and 0.591, respectively) or in the caecum ($P = 0.764$ and 0.145), but VFA profile was modified. Changes in VFA concentrations agree well with that reported by Rad et al. (2016) when urea-treated AH replaced alfalfa hay in the diet of fattening lambs. However, these authors did not report changes in VFA profile, which contrasts with our results. Compared with control lambs, the AH12-fed lambs had greater ($P < 0.05$) proportions of butyrate and minor VFA (calculated as the sum of isobutyrate, isovalerate and valerate) and lower ($P < 0.05$) propionate proportions in the rumen, resulting in greater acetate/propionate ratios in AH12-fed lambs. The reduced proportions of propionate observed in the lambs fed AH in our study are in accordance with the lower proportions of non-structural carbohydrates in AH6 and AH12 concentrates (Van Soest, 1994), whereas all the diets used in the study of Rad et al. (2016) had similar levels of non-structural carbohydrates. In contrast, there were no differences ($P > 0.05$) between control and AH6-fed lambs in ruminal VFA profile.

Table 5.17 Fermentation parameters in the rumen and caecum measured *post-mortem* and rumen epithelium colour in growing lambs fed barley straw and a concentrate without almond hulls (AH; Control), with 60 (AH6) and with 120 (AH12) g of almond hulls per kg (fresh matter basis).

Item	Concentrate			SEMc[1]	Sex		SEMs[1]	*P* =		
	Control	AH6	AH12		Female	Male		Concentrate	Sex	Concentrate x Sex
Rumen										
pH	5.48	5.70	5.64	0.110	5.63	5.59	0.090	0.355	0.755	0.142
Total volatile fatty acids (VFA; mmol/L)	168	153	159	10.5	161	159	8.6	0.591	0.907	0.529
Individual VFA (mol/100 mol)										
Acetate	52.0[b]	53.0[b]	57.7[a]	1.76	51.0	57.4	1.44	0.088	0.005	0.530
Propionate	37.6[a]	36.5[a]	28.0[b]	2.41	38.3	29.9	1.96	0.024	0.007	0.365
Butyrate	6.70[b]	6.85[ab]	9.40[a]	0.757	6.60	8.69	0.618	0.039	0.028	0.177
Minor VFA[2]	3.64[b]	3.62[b]	4.91[a]	0.337	4.12	3.99	0.275	0.023	0.747	0.324
Acetate/propionate (mol/mol)	1.57[b]	1.62[b]	2.40[a]	0.231	1.40	2.33	0.189	0.039	0.002	0.380
NH_3-N (mg/L)	103[a]	48.4[b]	33.3[b]	14.72	61.7	61.2	12.01	0.009	0.977	0.036
Rumen epithelium colour[3]	0.65[c]	3.15[b]	3.93[a]	0.233	2.70	2.45	0.190	<0.001	0.372	0.925
Caecum										
pH	5.70	5.66	5.70	0.053	5.68	5.73	0.043	0.764	0.470	0.582
Total VFA (mmol/ g)	243	256	270	8.9	264	248	7.2	0.145	0.137	0.260
Individual VFA (mol/100 mol)										
Acetate	69.6	67.5	70.9	1.12	68.9	69.6	0.99	0.162	0.563	0.042
Propionate	17.3[b]	19.7[a]	16.2[b]	0.74	18.5	16.8	0.60	0.012	0.056	0.077
Butyrate	12.2	11.6	11.4	0.87	11.7	11.8	0.71	0.820	0.976	0.333
Minor VFA[2]	1.96[a]	1.33[b]	1.44[b]	0.136	1.47	1.68	0.111	0.008	0.209	0.156
Acetate/propionate (mol/mol)	4.12[a]	3.59[b]	4.47[a]	0.221	3.92	4.23	0.180	0.036	0.274	0.124
NH_3-N (mg/L)	226[a]	138[b]	108[b]	24.6	171	144	20.1	0.008	0.375	0.520

[a, b] Within values for each concentrate, means in the same row with different superscript differ ($P < 0.05$; Tukey test). Superscripts are only shown when a significant ($P < 0.05$) effect of concentrate Lwas detected.

[1] Standard error of the mean for concentrate (SEMc; n = 10) and sex (SEMs; n = 15) effects.

[2] Calculated as the sum or isobutyrate, isovalerate and valerate proportions.

[3] Scored from 0 (pale) to 5 (dark).

Concentrations of NH_3-N in the rumen and caecum were greater ($P = 0.009$) in the lambs fed the control concentrate than in those fed the concentrates with AH, which is in agreement with the low degradability of the AH protein. In addition, the colour of the ruminal epithelium was darker ($P < 0.05$) in the lambs fed AH compared with the control group, being darker ($P < 0.05$) for AH12 than for AH6-fed lambs. The darker colour of the ruminal epithelium in AH-fed lambs could be related to the presence of small pieces of hard shells or other hard debris in the AH that possibly caused an abrasion of the epithelium, as previously observed by Haro et al. (2019) when lambs were fed sunflower seeds treated with a malic acid solution.

There were also some effects of concentrate type on molar proportions of propionate and acetate/propionate ratio in the caecum, which were greater ($P < 0.05$) and lower ($P < 0.05$), respectively, in AH6 compared with both control and AH12 lambs. Caecal proportions of minor VFA were lower ($P < 0.05$) in AH6 and AH12 groups than in control-fed lambs.

Sex had a significant influence on ruminal VFA proportions, with acetate, butyrate proportions, and acetate:propionate ratio being greater ($P = 0.005$, 0.028 and 0.002 respectively) and propionate proportions being lower ($P = 0.007$) in males compared with females. The effect of lamb sex on ruminal parameters has been only investigated in a limited number of studies. Hanim et al. (2022) observed that female Garut lambs (25.0 kg BW) had lower concentrations of total VFA and proportions of acetate and butyrate in the rumen than the male lambs when both were fed a mixed diet (60:40 forage:concentrate ratio) and concluded that males had more efficient ruminal fermentation. Similarly, Ünlü et al. (2021) detected significantly lower concentrations of total VFA and proportions of acetate in female Menemen lambs than in males lambs fed a mixed diet (36:64 forage:concentrate) and slaughtered at 40.5 kg BW. These results partially agree with the lower acetate and butyrate proportions observed in the rumen of females in the present study, although no difference between sexes in total VFA concentrations was detected.

The greater ruminal propionate proportions detected in female lambs in our study might indicate sex-differences in microbiota in both digestive organs. In fact, the gut microbiota in mice has been reported to differ in males and females after puberty but this trend was reversed by males castration, which suggests an influence of sexual hormones (Markle et al., 2013). Further research is needed to fully understand the mechanisms underlying potential sex differences in ruminal fermentation in lambs.

5.2.8. **In vitro** *trial*

The results of the *in vitro* trial (Table 5.18) indicate a reduced fermentability of AH12 concentrate, as indicated by the lower ($P < 0.05$) values of PGP, AGPR, and total VFA production at both 9 and 24 h of incubation, compared with control and AH6 concentrates. These results agree well with the trend to lower ADG and digestibility of DM and CP observed *in vivo* for the lambs fed AH, especially for those fed the AH12 concentrate. In the *in vitro* trial there were no differences between control and AH6 concentrates in gas production parameters, and *Lag* values were similar ($P = 0.114$) for all concentrates.

After 9 h of *in vitro* incubation, AH12 concentrate had lower ($P < 0.05$) production of total VFA than control and AH6 concentrates, but VFA profile was similar for all concentrates excepting that of AH6 and AH12 concentrates had greater ($P < 0.05$) butyrate proportions than the control concentrate (Table 5.18). Most of these differences disappeared after 24 h of incubation, but control concentrate still showed lower ($P < 0.05$) pH values than both AH-concentrates and greater ($P < 0.05$) production of total VFA than AH12, indicating increased fermentation. These results contrast with the differences observed *postmortem* in the VFA profile in the rumen of lambs fed the different concentrates.

Although, *in vitro* NH_3-N concentrations were lowest ($P < 0.05$) for control and greatest ($P < 0.05$) for AH12 concentrate after 9 h of incubation, the differences disappeared at 24 h of incubation. In contrast, NH_3-N concentrations in the rumen of the lambs were decreased by AH

inclusion. As discussed by Mateos et al. (2015), both VFA and NH_3-N are removed from the rumen by absorption and flow to the lower gastrointestinal tract, whereas there is no absorption or digesta flow in the *in vitro* batch cultures used in this study, which can help to explain the observed differences in the *in vivo* and *in vitro* fermentation parameters.

Table 5.18 Gas production parameters and fermentation parameters of experimental concentrates without almond hulls (AH; Control), with 60 (AH6) and with 120 (AH12) g of almond hulls per kg (fresh matter basis) after *in vitro* incubation with sheep ruminal fluid.

Item	Concentrate			SEM[2]	*P* =
	Control	AH6	AH12		
Gas production parameters[1]					
PGP (mL/g dry matter)	287[a]	280[ab]	271[b]	2.1	0.006
c (% / h)	6.34[a]	6.40[a]	6.08[b]	0.059	0.019
Lag (h)	1.81	1.97	2.19	0.108	0.114
AGPR (mL/g dry matter)	11.4[a]	11.1[a]	10.1[b]	0.13	0.001
Ruminal parameters (9 h of incubation)					
Total volatile fatty acids (VFA; mmol/g incubated DM)	5.03[a]	4.97[a]	4.25[b]	0.156	0.022
Individual VFA (mol/100 mol)					
Acetate	59.1	58.3	58.0	0.51	0.339
Propionate	28.2	28.7	28.6	0.58	0.824
Butyrate	9.41[b]	10.1[a]	10.4[a]	0.18	0.020
Minor VFA[3]	3.28	2.94	3.02	0.336	0.757
Acetate/propionate (mol/mol)	2.13	2.06	2.05	0.054	0.585
NH_3-N (mg/L)	43.9[c]	52.0[b]	63.3[a]	2.52	0.005
Ruminal parameters (24 h of incubation)					
pH	6.54[c]	6.58[b]	6.61[a]	0.006	0.001
Total VFA (mmol/g incubated DM)	9.47[a]	9.36[a]	8.79[b]	0.109	0.009
Individual VFA (mol/100 mol)					
Acetate	59.4	59.3	60.1	0.61	0.628
Propionate	25.1	24.5	24.1	0.44	0.365
Butyrate	11.2	12.0	11.6	0.19	0.054
Minor VFA[3]	4.37	4.20	4.15	0.195	0.727
Acetate/propionate (mol/mol)	2.37	2.43	2.50	0.068	0.480
NH_3-N (mg/L)	109	115	120	3.7	0.170

[a, b, c] Means in the same row with different superscript differ ($P < 0.10$). Superscripts are only shown when a significant ($P < 0.05$) effect of concentrate was detected.
[1] PGP: potential gas production; *c:* fractional rate of gas production; *Lag*: time before the onset of gas production; AGPR: average gas production rate until half of PGP is reached.
[2] SEM: standard error of the mean (n = 4).
[3] Calculated as the sum or isobutyrate, isovalerate and valerate proportions.

5.3. Effect of different additives on characteristics and nutritive value of AH silage

The AH used in trial had 60.6% DM, and (all values on DM basis) 8.71% ash, 7.49% CP, 3.01% EE, 39.4% NDF, 29.4% ADF, 13.9% lignin and 27.6% total sugars. All chemical fractions were within the range previously reported for AH (Feedipedia, 2020). The silage bags were checked every 3-4 days over the storage period and no incident or vacuum failure was detected in any bag.

5.3.1. Characteristics and chemical composition of AH silages after 90 days of storage

At the time of opening, no visible signs of moulds or effluents were detected in any silage, and the organoleptic characteristics observed were considered typical of well-preserved silages. All silages had a suitable odour and colour characteristic of AH, although the FA silages seemed slightly darker than the other silages.

As shown in Table 5.19, the DM content of all silages was slightly above the expected value (50%). Although none of the additives changed the DM content compared to CON silage, the DM of FA silages was greater ($P < 0.05$) than that of the LAB-treated silages. In agreement with previous observations in silages of different crops (Derbyshire et al., 1976; Haigh and Chapple, 1998), the use of FA decreased ($P < 0.05$) DM losses. In contrast, the inoculation with LAB increased ($P < 0.05$) DM losses compared with CON silage, which could be attributed to the fermentation of soluble carbohydrates by the acid lactic bacteria added. The low DM losses (<4%) observed in all silages is consistent with the small production of gas and the lack of effluent observed in all bags.

Compared with CON silages, the inclusion of all additives significantly decreased ($P < 0.05$) the pH, with LAB-treated silages having the lowest ($P < 0.05$) values. The final pH of all silages treated with additives was below 4.6, being within the desirable pH range for legume

silages (4.3-5.0) proposed by Kung et al. (2018). The significant reduction in pH when LAB was added to the silages could be attributed to the heterolactic fermentation produced by the lactic acid bacteria, which can result in greater acidification and consequently better quality of the silage (Merlo, 2017). Moreover, it has been reported that as DM increases above 40-45%, silage pH increases because the metabolic water available for growth of lactic acid bacteria starts to become limiting as silage DM increases (Whiter and Kung, 2001). It is possible that ensiling AH with lower DM would result in lower pH values in the silage.

Table 5.19 Characteristics of almond hulls silages with no additives (CON) and treated with formic acid (FA), acetic and lactic acids (ALA) or a lactic acid bacteria inoculant (LAB) after 90 days of storage.

Item	Silage treatment[1]				SEM[2]	*P* =
	CON	FA	ALA	LAB		
Dry matter (DM; %)	53.9^{ab}	54.5^{b}	54.0^{ab}	53.7^{a}	0.19	0.020
DM losses (%)	2.31^{b}	1.46^{a}	2.41^{b}	3.65^{c}	0.065	<0.001
pH	5.04^{d}	4.56^{c}	4.43^{b}	4.40^{a}	0.007	<0.001
Volatile fatty acids (VFA; g/kg DM)						
Acetic	3.27^{b}	0.97^{a}	13.0^{c}	18.3^{d}	0.433	<0.001
Propionic	-	-	-	-	-	-
Butyric	0.00^{a}	0.00^{a}	0.00^{a}	2.22^{b}	0.127	<0.001
Isobutyric	4.10^{b}	3.97^{b}	2.63^{a}	2.16^{a}	0.253	<0.001
Isovaleric	0.82^{ab}	0.83^{ab}	0.75^{a}	0.90^{b}	0.032	0.039
Valeric	0.03^{a}	0.22^{b}	0.14^{b}	0.00^{a}	0.020	<0.001
Total VFA (g/kg DM)	8.22^{a}	5.99^{a}	16.6^{b}	23.6^{c}	0.604	<0.001
NH_3-N (g/kg DM)	1.26	1.24	1.30	1.24	0.031	0.518
Lactic acid (g/kg DM)	10.5^{b}	1.24^{a}	11.7^{b}	16.7^{c}	0.388	<0.001
Lactic/acetic (g/g)	3.20^{c}	1.29^{b}	0.91^{a}	0.92^{a}	0.068	<0.001

[a, b, c] Means in the same row with different superscript differ ($P < 0.05$).
[1] FA: formic acid at 4 mL/kg silage; ALA: acetic and lactic acids at 2 mL of each acid/kg silage; LAB: *Lactobacillus buchneri* at 5×10^9 CFU (colony forming units)/kg of silage.
[2] SEM: Standard error of the mean.

Significant differences ($P \leq 0.039$) between treatments were detected in all individual VFA analysed except for propionic acid, which was not detected in any silage. The LAB-silages had greater ($P < 0.05$) acetic acid content than the other silages. *Lactobacillus buchneri* is considered an obligatory heterofermenter, which can ferment hexoses and pentoses to equimolar proportions of lactic acid, CO_2, acetic acid and/or ethanol, thus explaining the greater

content of acetic acid in LAB-silages (Oude Elferink et al. 1999). An increase in the concentration of acetic acid could be beneficial since it can improve aerobic stability and decreases fungal growth in the silage (Kung et al. 2018). Likewise, acetic acid can be easily absorbed in the rumen and used as an energy source or as precursor for the synthesis of FA by the host ruminant (Van Soest, 1994). In agreement with our results, Kung et al. (2018) stated that a reduction in acetic acid concentration is usually observed when FA is added to silages, which could indicate reduced growth of *Enterobacteriaceae* spp. that can degrade proteins to NH_3-N and ferment sugars to acetic acid.

The LAB-treated were the only silages in which butyric acid was detected. However, it should be noted that butyric content in LAB-silages was less than 5 g/kg DM, which is the limit that could indicate the development of clostridia (Sirakaya, 2023). In fact, no *Clostridium perfringens* growth was detected in any silage in the microbiological analysis (Table 5.21).

Isobutyric content was greater ($P < 0.05$) in CON and FA silages than in ALA and LAB ones, whereas valeric concentrations were greater ($P < 0.05$) in the silages treated with acids (FA and ALA treatments) compared with both CON and LAB silages. Differences in the concentrations of isobutyric, isovaleric and valeric acids could suggest a lower proteolytic activity during the fermentation process in silages with lower concentrations of these VFA, since they are produced by the degradation of branched chain amino acids (Apajalahti et al., 2019). As consequence of the changes in the individual VFA, total VFA content was greater ($P < 0.05$) in ALA and LAB silages than in those without additives (CON) and treated with FA.

The NH_3-N concentration is considered as an indicator of proteolytic activity during silage fermentation (Apajalahti et al., 2019), but in our study it was unaffected ($P = 0.518$) by the silage treatments, ranging from 1.24 to 1.30 g/kg DM. The lack of differences in NH_3-N concentrations is consistent with the lack of clear differences between treatments in the concentrations of isobutyric, valeric and isovaleric acids, and altogether would indicate that the

treatments did not affect the proteolytic activity in the silages. In contrast, the concentration of lactic acid was lowest ($P < 0.05$) for FA silage and highest ($P < 0.05$) for LAB silage, with CON and ALA silages having intermediate values. The reduced lactic acid content detected when FA was added to the silages differs from the results of Ojeda and Cáceres (1984), who observed that using FA (3.5 mL/kg silage) as a preservative in King grass silages resulted in an increase in the concentration of lactic acid and a decrease in NH_3-N content. Similarly, de Evan et al. (2023) reported that FA (4 mL/kg silage) increased the concentration of lactic acid in *Typha* silages but had no effect on NH_3-N concentration. In contrast, Haigh (1999) observed that FA (4 mL/kg silage) did not influence the concentration of lactic acid in grass silages but decreased significantly their NH_3-N content. These contrasting results would indicate that the effects of FA could vary with the type of vegetal material ensiled.

In general, lactic acid concentrations in our study were below the range of 20-40 g/kg DM reported as optimal by Kung et al. (2018) for corn silages, but it should be noted that most probably the ensilability of AH is lower than that of corn. Kung et al. (2018) also indicate that the lactic/acetic ratio in good silages is usually 2.5 to 3.0, but in our study the silage without additives (CON) was the only silage that had values close to this range (3.20) and the ratios for the silages with additives were below 1.30. These low lactic/acetic ratios were due to the high acetic content in ALA and LAB silages, whereas in FA silages both acetic and lactic concentrations were low.

Although FA has been extensively used as a silage additive, it is gradually being substituted by other additives due to its corrosive nature, safety concerns and environmental problems. In contrast, both lactic and acetic acids are less corrosive and generally safer for human exposure compared to FA. Moreover, a mixture of lactic and acetic acids can have synergic effects, as lactic acid lowers the pH and promotes rapid fermentation, and acetic acid inhibits the growth of undesirable bacteria and fungi (Kung et al., 2018). Compared with FA, the ALA silages had

greater ($P < 0.05$) DM losses and concentrations of acetic acid, lactic acid and total VFA, but lower ($P < 0.05$) pH, isobutyric content and lactic/acetic ratio. The lower pH in ALA silages is consistent with the observation that lactic acid contributes the most to the pH drop during ensiling fermentation, because it is stronger than any of the other major acids (Kung et al., 2018). These results indicate that a mixture of lactic and acetic acids could be a good alternative to FA as preservative for AH silage.

As shown in Table 5.20, chemical composition was variable ($P \leq 0.039$) among silage treatments. Ash content was greater ($P < 0.05$) in ALA and LAB silages compared to CON and FA silages, but differences were low and of small practical relevance. The decrease in ash content observed in the silage treated with FA is in agreement with the results of de Evan et al. (2023), who reported a reduction in the amount of ash when FA was added to *Typha* silages. Haigh (1999) also reported a reduction in ash content by adding FA to grass silages. The ash content in all silages was relatively low compared with other values reported in the literature for AH samples (Carro, 2023), which could be attributed to the almond harvest system used in this study, as the almonds had no contact with the ground and therefore the AH was not contaminated with soil particles and dust.

Compared to CON silage, the use of FA as an additive reduced ($P < 0.05$) the CP, NDF, ADF and lignin content, but it did not affect EE and total sugars content. In agreement with our results, others have detected a reduction in fibre content by adding FA to herbage silages (Haigh and Chapple, 1998) and *Saccharina latissima* silages (Marcos et al., 2023), although no changes in fibre content after adding FA have been reported in other studies for grass silages (Haigh, 1999) and *Typha* silages (de Evan et al., 2023). As discussed by Leibeinsperger and Pitt (1988), the final effects of FA on chemical composition of silages depend on application rate, crop DM content, and other factors.

Table 5.20 Chemical composition (g/100 dry matter) of almond hulls silages with no additives (CON) and treated with formic acid (FA), acetic and lactic acids (ALA) or a lactic acid bacteria inoculant (LAB) after 90 days of storage.

Item	Silage treatment[1]				SEM[2]	*P* =
	CON	FA	ALA	LAB		
Ash	9.22^{ab}	9.02^{a}	9.35^{bc}	9.63^{c}	0.071	<0.001
Crude protein	7.71^{b}	7.38^{a}	7.45^{ab}	7.55^{ab}	0.059	0.039
Ether extract	3.33^{a}	3.68^{ab}	4.38^{b}	3.44^{a}	0.210	0.012
Total sugars	23.8^{c}	24.3^{c}	17.0^{b}	12.4^{a}	0.24	<0.001
Neutral detergent fibre	41.0^{bc}	39.3^{a}	40.6^{b}	41.9^{c}	0.24	<0.001
Acid detergent fibre	30.1^{b}	29.0^{a}	31.3^{c}	32.1^{c}	0.20	<0.001
Lignin	13.0^{a}	12.5^{a}	13.7^{b}	13.9^{b}	0.14	<0.001

[a, b, c] Means in the same row with different superscript differ ($P < 0.05$).
[1] FA: formic acid at 4 mL/kg silage; ALA: acetic and lactic acids at 2 mL of each acid/kg silage; LAB: *Lactobacillus buchneri* at 5×10^9 CFU (colony forming units)/kg of silage.
[2] SEM: Standard error of the mean.

Compared with CON silage, the ALA treatment resulted in greater ($P < 0.05$) content of EE, ADF and lignin and lower ($P < 0.05$) content of total sugars. The observed changes were in general low, excepting the reduction of total sugars (from 23.8 to 17.0%) that was attributed to sugars fermentation during ensiling. As expected, LAB-treated silage had lower ($P < 0.05$) content in total sugars than the other treatments. As already discussed, *L. buchneri* can ferment sugars to lactic acid, CO_2, acetic acid and/or ethanol, and the reduction of sugars content is in accordance with the greater content in acetic and lactic acids detected in LAB-silages (Table 5.19). The observed increase in the content of ash, NDF, ADF and lignin in ALA silages compared with CON ones was probably due to the shift in the proportion of the chemical fractions in ALA silages caused by the reduction of total sugars content.

In the microbiological analysis, no *Escherichia coli*, *Salmonella* spp., *Listeria* spp., *Clostridium perfringens* or *Staphylococcus coagulase+* were detected in any silage (Table 5.21). However, there were significant differences ($P < 0.001$) between treatments in the amount of yeasts and moulds and a trend ($P = 0.095$) was detected for *Enterobacteria* spp. counts. All additives reduced ($P < 0.05$) yeast and moulds, counts compared with CON silage,

with FA showing the lowest values ($P < 0.05$). These results are in accordance with previous studies reporting the ability of formic, lactic and acetic acids (Selwet, 2008) and of *L. buchneri* (Kung et al., 2001) to reduce the growth of both yeast and moulds. The numbers of yeast and moulds in all silages treated with additives were low (< 3.0 log CFU/g), and even in the CON silage the values were below the maximum value of 5 log CFU/g proposed by Kung et al. (2018) for all silages.

Table 5.21 Microbiological analysis (log CFU (colony forming units)/g silage) of almond hulls silages with no additives (CON) and treated with formic acid (FA), acetic and lactic acids (ALA) or a lactic acid bacteria inoculant (LAB) after 90 days of storage.

Item	Silage treatment[1]				SEM[2]	*P* =
	CON	FA	ALA	LAB		
Enterobacteria spp.	1.20	0.954	0.954	0.954	0.078	0.095
Yeasts	4.66^{d}	0.00^{a}	2.85^{c}	1.51^{b}	0.178	<0.001
Moulds	2.80^{c}	1.31^{a}	2.09^{b}	1.55^{ab}	0.190	<0.001

$^{a, b, c, d}$ Means in the same row with different superscript differ ($P < 0.05$).
[1] FA: formic acid at 4 mL/kg silage; ALA: acetic and lactic acids at 2 mL of each acid/kg silage; LAB: *Lactobacillus buchneri* at 5×10^{9} CFU (colony forming units)/kg of silage.
[2] SEM: Standard error of the mean.

5.3.2. **In vitro** *ruminal fermentation of silages: Gas production kinetics and fermentative parameters*

The gas production technique was used as an indicator of rumen fermentation kinetics of the silages, since the amount of gas produced is positively related to the amount of organic matter fermented (Menke et al., 1979). The gas production and fermentation parameters measured after *in vitro* incubation of the different silages are shown in Table 5.22.

Table 5.22 Gas production parameters and values of pH, NH_3-N concentration and production of volatile fatty acids (VFA) after 24 h of *in vitro* fermentation of almond hulls silages with no additives (CON) and treated with formic acid (FA), acetic and lactic acids (ALA) or a lactic acid bacteria inoculant (LAB) with ruminal fluid from sheep, and estimated metabolizable energy (ME) content of the silages.

Item	Silage treatment[1]				SEM[3]	*P* =
	CON	FA	ALA	LAB		
Gas production parameters[2]						
PGP (mL/g DM)	218^{c}	211^{bc}	203^{ab}	195^{a}	6.0	<0.001
c (%/h)	2.89^{a}	3.16^{a}	3.20^{a}	3.84^{b}	0.000	0.001
Lag (h)	0.00	0.00	0.00	0.02	0.016	0.375
AGPR (mL/h)	4.57^{a}	4.82^{ab}	4.67^{ab}	5.16^{b}	0.268	0.016
Fermentation parameters						
pH	6.71	6.71	6.70	6.71	0.009	0.549
NH_3-N (mg/L)	196^{ab}	194^{a}	202^{b}	202^{b}	4.1	0.006
Total volatile fatty acids (VFA; mmol/g DM)	6.60^{b}	6.21^{a}	6.66^{b}	6.45^{ab}	0.207	0.016
Individual VFA (mol/100 mol)						
Acetate (Ac)	58.1^{c}	57.8^{b}	57.4^{b}	56.7^{a}	0.18	<0.001
Propionate (Pr)	27.9^{a}	28.4^{b}	29.3^{c}	30.2^{d}	0.27	<0.001
Butyrate	12.0^{b}	11.8^{b}	11.3^{a}	11.0^{a}	0.18	<0.001
Isobutyrate	0.462	0.434	0.379	0.375	0.0690	0.213
Isovalerate	1.02^{a}	1.05^{ab}	1.04^{ab}	1.07^{b}	0.020	0.003
Valerate	0.523^{a}	0.503^{a}	0.538^{b}	0.560^{b}	0.0165	<0.001
Ac/Pr (mol/mol)	2.11^{d}	2.05^{c}	1.98^{b}	1.88^{a}	0.027	<0.001
ME (Kcal/kg DM)	1857	1883	1891	1854	12.7	0.131

$^{a, b, c, d}$ Means in the same row with different superscript differ ($P < 0.05$).
[1] FA: formic acid at 4 mL/kg silage; ALA: acetic and lactic acids at 2 mL of each acid/kg silage; LAB: *Lactobacillus buchneri* at 5×10^9 CFU (colony forming units)/kg of silage.
[2] PGP: potential gas production; *c:* fractional rate of gas production; *Lag:* time before starting gas production; AGPR: average gas production rate; ME: metabolizable energy.
[3] SEM: Standard error of the mean.

There were differences ($P \leq 0.016$) between treatments in all gas production parameters except for *Lag*. Compared with CON silage, the use of FA did not affect any gas parameter and ALA treatment only resulted in a reduction of the PGP ($P < 0.05$). In contrast, LAB-silages were fermented more rapidly than CON silages as indicated by their greater ($P < 0.05$) *c* and AGPR values. The lower PGP values in LAB-treated silages compared with CON silages could be related to the lower sugars content of LAB-silages, as sugars are extensively fermented in the rumen.

There were no differences ($P = 0.549$) between treatments in the final pH of vials at the end of the incubation period. The concentration of NH_3-N was greater ($P < 0.05$) in ALA and LAB silages than in FA silages, but the magnitude of the difference was small. Total VFA production was greater ($P < 0.05$) for CON and ALA silages than for FA silages, and there were differences between treatments ($P \leq 0.003$) in the proportions of the individual VFA excepting for isobutyrate. The effects of FA on silage digestibility reported in the literature are contrasting (Haigh and Chapple, 1998; Menezes et al., 2022; de Evan et al., 2023) but in general, no negative effects of FA on ruminal digestibility are reported, although it has been suggested that FA might inhibit the growth of ruminal microorganisms and decrease the fermentation end-products.

Compared with CON silage, all additives produced an increase ($P < 0.05$) in molar proportions of propionate at expense of acetate, with LAB silage having the greatest ($P < 0.05$) propionate and the lowest ($P < 0.05$) acetate and butyrate proportions. These shifts in VFA profile may be related to changes produced in the chemical fractions during the ensiling process that have affected the ruminal degradation of the silages. Despite the differences between treatments observed in both total VFA production and VFA profile, the estimated ME content was similar ($P = 0.131$) for all silages.

5.3.3. Evolution of silage characteristics over the storage period

A second objective of this experiment was to analyse the evolution of the characteristics of the silages and their chemical composition over the ensiling period. Figure 4.1 and Table 5.23 show the timely evolution of silages' pH. Values of pH were affected by both the silage treatment ($P < 0.001$) and the silages opening time ($P < 0.001$), and there was a treatment x time interaction ($P < 0.001$).

Compared with CON silages, the use of all additives produced a more sharply decrease in pH at day 21, reaching adequate pH values (≤ 4.38) in all treated silages. In accordance with previous observations in corn silage (Diepersloot et al., 2022), the pH increased from day 21 to 90 in all silages, but in all treated-silages remained below 4.5 by day 90.

The final pH of a silage is mainly related to its lactic acid concentration and the buffering capacity of the ensiled material (Kung et al. 2018). Therefore, it was expected that the treatments involving acids (FA and ALA treatments) and inoculating LAB would decrease the silage pH, as the acids directly acidify the silage and induces antibacterial activity (Zhang et al. 2017). Although CON silages had greater pH values at day 90 than other silages, the pH in all

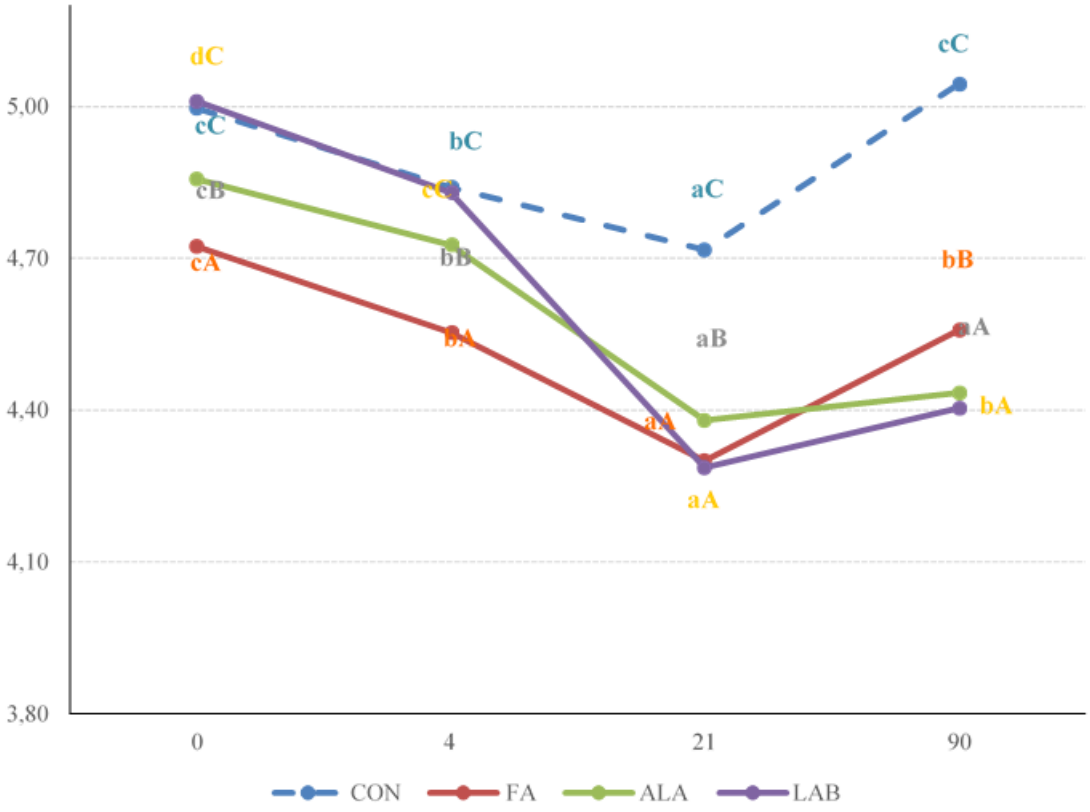

Figure 5.1 Evolution of pH values (0, 4, 21 and 90 days of ensiling) of almond hull silages with no additives (CON) formic acid (FA; 4 mL/kg silage); acetic and lactic acids (ALA; 2 mL/kg of each acid/kg silage) and with lactic acid bacteria (LAB; *Lactobacillus buchneri* at 5×10^9 CFU (colony forming units)/kg silage). Values with different lower case letters within each treatment indicate changes ($P < 0.05$) over time, and values with different upper case letters indicate differences ($P < 0.05$) between treatments within each sampling time.

silages were within the range, (4.3-5.0) established by Kung et al. (2018) as adequate for silages with DM ≤ 55%.

Vasta et al. (2008) stated that one of the most important factors determining the nutritional quality of the silage is its content in fermentation products, as these products can affect the voluntary intake of silage and the production of the animals. As shown in Table 5.23, a significant effect of time ($P < 0.001$) was observed on the concentrations of NH_3-N, total and individual VFA and lactic acid. Propionic acid was not detected in any silage, which is indicative of good silage quality (Kung et al. 2018). Although no effect ($P = 0.368$) of silage treatments was observed on NH_3-N content, the concentration of NH_3-N gradually increased ($P < 0.001$) over time. As previously discussed, NH_3-N concentration can be considered an indicator of proteolytic activity during silage fermentation and the greater values observed at day 90 compared to day 0 could be attributed to protein degradation and amino acids deamination. The NH_3-N content at day 90 was less than 15% of total N in all silages (10.2, 10.6, 10.9 and 10.3% for CON, FA, ALA and LAB silages, respectively), which is a value indicative of high proteolysis rate (Kung et al., 2018; Meneses et al., 2007). These results indicate that protein was not extensively degraded during ensiling.

Table 5.23 Temporal evolution of pH, NH_3-N concentrations, volatile fatty acids (VFA) and lactate content of almond hulls silages with no additives (CON) and treated with formic acid (FA), acetic and lactic acids (ALA) or a lactic acid bacteria inoculant (LAB).

Item	Treatment[1]	Opening time (days)				SEM[2]	*P* =		
		0	4	21	90		Treatment	Time	Treatment x Time
pH	CON	5.00^{cC}	4.84^{bC}	4.72^{aC}	5.04^{cC}	0.014	<0.001	<0.001	<0.001
	FA	4.72^{cA}	4.55^{bA}	4.30^{aA}	4.56^{bB}				
	ALA	4.86^{cB}	4.73^{bB}	4.38^{aB}	4.43^{aA}				
	LAB	5.01^{dC}	4.83^{cC}	4.29^{aA}	4.40^{bA}				
NH_3-N (g/kg DM)	CON	0.276^{a}	0.522^{b}	0.923^{c}	1.26^{d}	0.0341	0.368	<0.001	0.502
	FA	0.279^{a}	0.431^{a}	0.864^{b}	1.25^{c}				
	ALA	0.276^{a}	0.431^{a}	0.896^{b}	1.30^{c}				
	LAB	0.292^{a}	0.450^{a}	0.939^{b}	1.24^{c}				
Acetic (g/kg DM)	CON	0.586^{Aa}	0.644^{aA}	1.92^{abA}	3.27^{bB}	0.408	<0.001	<0.001	<0.001
	FA	0.553^{A}	0.364^{A}	0.921^{A}	0.972^{A}				
	ALA	3.81^{abB}	2.79^{aB}	5.03^{bB}	13.0^{cC}				
	LAB	0.695^{aA}	0.890^{bAB}	7.81^{bC}	18.3^{cC}				
Butyric (g/kg DM)	CON	0.153	0.283	0.000	0.000^{A}	0.1412	<0.001	<0.001	<0.001
	FA	0.178	0.102	0.118	0.000^{A}				
	ALA	0.139	0.077	0.118	0.000^{A}				
	LAB	0.212^{a}	0.239^{a}	0.040^{a}	2.22^{bB}				
Isobutyric (g/kg DM)	CON	0.000^{a}	0.000^{a}	0.000^{a}	4.10^{bB}	0.231	0.006	<0.001	<0.001
	FA	0.000^{a}	0.000^{a}	0.000^{a}	3.97^{bB}				
	ALA	0.000^{a}	0.000^{a}	0.000^{a}	2.63^{bA}				
	LAB	0.000^{a}	0.000^{a}	0.000^{a}	2.16^{bA}				
Valeric (g/kg DM)	CON	0.117^{b}	0.000^{Aa}	0.115^{b}	0.028^{abA}	0.0201	0.003	<0.001	<0.001
	FA	0.129^{bc}	0.000^{Aa}	0.121^{b}	0.216^{cB}				
	ALA	0.138^{ab}	0.033^{ABa}	0.130^{ab}	0.138^{Bb}				
	LAB	0.150^{b}	0.110^{bB}	0.114^{b}	0.000^{aA}				

Table 5.23 (continued) Temporal evolution of pH, NH_3-N concentrations, volatile fatty acids (VFA) and lactate content of almond hulls silages with no additives (CON) and treated with formic acid (FA), acetic and lactic acids (ALA) or a lactic acid bacteria inoculant (LAB).

Item	Treatment[1]	Opening time (days)				SEM[2]	*P* =		
		0	4	21	90		Treatment	Time	Treatment x Time
Isovaleric (g/kg DM)	CON	0.853	0.909	0.936	0.818	0.0361	0.179	<0.001	0.145
	FA	0.897	0.860	0.914	0.829				
	ALA	0.936	0.814	0.934	0.851				
	LAB	0.938	0.836	0.981	0.896				
Total VFA (g/kg DM)	CON	1.71^{Aa}	1.84^{a}	2.97^{Aa}	8.22^{bA}	0.570	<0.001	<0.001	<0.001
	FA	1.76^{Aa}	1.33^{a}	2.07^{Aa}	5.99^{bA}				
	ALA	5.02^{aB}	3.72^{a}	6.22^{aB}	16.6^{Bb}				
	LAB	1.99^{aA}	2.07^{a}	8.95^{bB}	23.6^{cC}				
Lactic (g/kg DM)	CON	2.88^{a}	5.51^{b}	7.23^{bB}	10.5^{cB}	0.380	<0.001	<0.001	<0.001
	FA	2.05^{a}	5.31^{b}	4.43^{bA}	1.24^{aA}				
	ALA	3.30^{a}	5.81^{b}	6.19^{bB}	11.7^{cB}				
	LAB	2.34^{a}	5.34^{b}	9.09^{cC}	16.8^{dC}				
Lactic/acetic (g/g)	CON	4.93^{bC}	8.57^{cC}	3.78^{aB}	3.20^{aB}	0.2021	<0.001	<0.001	<0.001
	FA	3.71^{Bb}	14.64^{dD}	4.81^{cB}	1.29^{aA}				
	ALA	0.866^{aA}	2.12^{bA}	1.23^{abA}	0.905^{Aa}				
	LAB	3.37^{Bb}	6.00^{cB}	1.17^{aA}	0.918^{aA}				

[a, b, c, d] Within each treatment, means in the same row with different superscript differ ($P < 0.05$).
[A, B, C, D] Within each time, means in the same column with different superscript differ ($P < 0.05$).
[1] FA: formic acid at 4 mL/kg silage; ALA: acetic and lactic acids at 2 mL of each acid/kg silage; LAB: *Lactobacillus buchneri* at 5×10^9 CFU (colony forming units)/kg of silage.
[2] SEM: Standard error of the mean of treatment x day interaction (n=3).

The use of additives significantly affected the content of all VFA ($P \leq 0.003$) except for isovaleric acid. The content of acetic acid increased gradually with time, reaching the greatest values at day 90 in all silages. Whereas the increase with time was relatively small in FA silages, the content of acetic acid increased markedly for both ALA (from 3.81 to 13.0 g/kg DM) and LAB (from 0.695 to 18.3 g/kg DM) silages. These high increases are probably due to the conversion of lactic acid (either directly added to silage in ALA treatment or produced by *L. buchneri* in LAB silages) to acetic acid, as previously reported (Kung et al., 2018).

Butyric acid content in CON, FA and ALA silages was very low over the first 21 days of ensiling and no butyric acid was detected in these silages by day 90. In contrast, butyric acid was detected at all opening times in LAB silages. Concentrations of butyric acid in LAB silages (2.22 g/kg DM) were below the 0.5% proposed by Kung and Shaver (2001) as the upper recommended limit for grass silages.

No isobutyric acid was detected in any silage over the first 21 days of silage storage but it was present in all silages by day 90, with greater ($P < 0.05$) values in CON and FA silages than in ALA and LAB ones. Concentrations of valeric acid remained low (< 0.25 g/kg DM) in all silages at all opening times, and it was not detected by day 90 in LAB silages. The small concentrations of isobutyric, valeric and isovaleric acids observed in all silages are in accordance with the low proteolysis rate suggested by the NH_3-N concentrations, as these VFA are generated in the degradation of branched-chain amino acids (Apajalahti et al., 2019).

Concentrations of total VFA remained relatively unchanged over the 21 first days of ensiling, except for LAB silages that showed an increase ($P < 0.05$) by day 21 compared with days 0 and 4. Total VFA concentrations on day 90 were greater ($P < 0.05$) than those measured previously for all silages. As expected, total VFA concentrations were greater

in ALA-treated than in the other silages due to the addition of acetic acid, but there were no differences between treatments on day 4. From day 21 onwards, ALA and LAB silages had greater concentrations of total VFA than CON and FA silages.

Lactic acid concentration increased over storage time in all silages, except in those treated with FA, which showed a decrease ($P < 0.05$) from day 21 onwards. As expected, LAB-treated silages had greater lactic acid concentrations than the other silages at both 21 and 90 days due to lactic acid production by *L. buchneri* (Arriola et al., 2021). The lactic/acetic ratio is considered an indicator of silage quality, and according to Monllor et al. (2020) the desired range is between 1 and 3. In our study, lactic/acetic ratio changed markedly over storage time for all silages, mainly due to variations in acetic acid concentrations. By the end of the storage period, both CON and FA silages had ratios within the recommended range, but ALA and LAB silages had smaller ratios (close to 1.0).

5.3.4. Evolution of chemical composition of silages over the storage period

The variability in chemical composition of the silages over the storage period is detailed in Table 5.24. The silage treatment affected ($P \leq 0.023$) all chemical fractions except the DM and CP content ($P \geq 0.108$). There were no treatment x time interactions for DM, ash, CP and EE, but interactions were detected for fibre fractions, lignin and total sugars content. In addition, a time effect ($P < 0.001$) was detected for all chemical fractions.

The main objective of ensiling is to preserve forage with a minimum loss of DM and nutrients. In our study, the DM content remained unchanged over the storage period for all treatments except for LAB silages that showed a small decline in DM content at day 90. The DM content of all silages was to the intended value (50%) and overall the values were within the range considered suitable for legume silages (35-55%) by Kung et al.

(2018). The changes in DM content of LAB silages could be due related to the DM fermentation by *L. buchneri*, which would also explain the notable decrease in total sugars content observed at day 90 (Table 5.24). McDonald et al. (1991) stated that the DM loss due to the activity of lactic acid bacteria usually account for 2 to 5% of the initial DM in the silage, and in our study it was less than 2% at day 90 for LAB silages.

The amount of ash increased over the storage time for ALA treatment, which had greater ($P < 0.05$) ash content at day 90 compared to that at day 0, although differences between opening times for other treatments were only numerical and did not reach statistical significance ($P > 0.05$). The CP content presented slight changes over the storage period, but values at day 90 did not differ from the initial values for any silage. This might indicate a reduced proteolytic activity, as it has been previously discussed. Similarly, the EE content of the silages had limited changes over the storage period, but values at day 90 were similar to the initial ones for all silages except for ALA treatment that showed an increase ($P < 0.05$) in the EE content as storage time advanced.

In general, there was an increase of NDF and ADF content through storage time, but differences between opening times only reached significance for ALA and LAB treatments. The lignin content of silages presented some small changes over the storage time, but values at day 90 were similar to those at day 0 except for FA, that showed lower ($P < 0.001$) values at day 90 compared with the initial content. Monllor et al. (2020) observed similar changes in NDF, ADF and lignin content of silages of horticultural by-products with advancing storage time.

In all silages, the content in total sugars decreased with advancing time ($P < 0.001$). The LAB silage showed the most marked decreased from day 0 to day 90, with a reduction of about 50% (26.9 *vs.* 12.4%). This marked decrease could be attributed to sugars fermentation by *L. buchneri* (Arriola et al., 2021).

In general, the observed changes in chemical composition agree well with the results from previous studies with corn silage (de Souza et al., 2022; Diepersloot et al., 2022), although de Souza et al. (2022) observed that the changes in chemical composition of corn silages over the storage time were influenced by the maturity stage of the forage at harvest before ensiling. The increases in NDF and ADF concentrations have been attributed by some authors (Jacovetti et al., 2018; de Souza et al., 2022) to the losses of soluble carbohydrates that cause a passive increase in fibre fractions.

Table 5.24 Temporal evolution of chemical composition (g/100 g dry matter) unless otherwise stated) of almond hulls silages with no additives (CON) and treated with formic acid (FA), acetic and lactic acids (ALA) or a lactic acid bacteria inoculant (LAB).

Item	Treatment[1]	Opening Time (days)				SEM[2]	*P* =		
		0	4	21	90		Treatment	Day	Treatment x Day
Dry matter (%)	CON	55.3	55.0	53.9	53.9	0.31	0.108	<0.001	0.584
	FA	55.5	55.2	54.5	54.5				
	ALA	54.8	55.3	54.3	54.0				
	LAB	54.6^{ab}	55.4^{b}	54.3^{ab}	53.7^{a}				
Ash	CON	8.71	8.96	9.02	9.22^{AB}	0.108	<0.001	<0.001	0.146
	FA	8.62	8.96	8.84	9.02^{A}				
	ALA	8.62^{a}	9.32^{b}	9.10^{ab}	9.35^{bB}				
	LAB	8.81	9.11	9.13	9.30^{B}				
Crude protein	CON	7.49^{bc}	6.97^{ab}	6.94^{a}	7.71^{c}	0.105	0.448	<0.001	0.134
	FA	7.55^{b}	6.88^{a}	6.89^{a}	7.38^{b}				
	ALA	7.49^{b}	7.15^{ab}	6.75^{a}	7.45^{b}				
	LAB	7.69^{b}	6.84^{a}	6.99^{a}	7.55^{b}				
Ether extract	CON	3.00	3.99	3.95	3.33^{A}	0.197	0.023	<0.001	0.134
	FA	3.18	3.69	4.05	3.68^{AB}				
	ALA	2.98^{a}	4.24^{b}	4.37^{b}	4.38^{bB}				
	LAB	2.92	3.94	3.85	3.78^{AB}				
Neutral detergent fibre	CON	39.3	39.5	40.6^{AB}	41.0^{B}	0.33	<0.001	<0.001	0.032
	FA	38.7	39.5	40.0^{A}	39.3^{A}				
	ALA	39.3^{ab}	38.9^{a}	40.1^{bAB}	40.6^{bAB}				
	LAB	39.5^{a}	40.1^{a}	41.8^{bB}	41.9^{bB}				

Table 5.24 (continued). Temporal evolution of chemical composition (g/100 g dry matter unless otherwise stated) of almond hulls silages with no additives (CON) and treated with formic acid (FA), acetic and lactic acids (ALA) or a lactic acid bacteria inoculant (LAB).

Item	Treatment[1]	Opening Time (days)				SEM[2]	*P* =		
		0	4	21	90		Treatment	Day	Treatment x Day
Acid detergent fibre	CON	29.3	29.5	30.5^{A}	30.1^{B}	0.26	<0.001	<0.001	<0.001
	FA	29.3^{ab}	29.7^{ab}	30.6^{bA}	29.0^{aA}				
	ALA	29.2^{a}	29.6^{ab}	30.9^{bcAB}	31.3^{cC}				
	LAB	29.8^{a}	30.6^{a}	31.9^{bB}	32.1^{bC}				
Lignin	CON	13.9^{ab}	13.6^{ab}	14.2^{bA}	13.0^{aB}	0.24	<0.001	<0.001	0.004
	FA	13.8^{b}	13.9^{b}	14.5^{bA}	12.5^{aA}				
	ALA	13.2	13.9	14.1^{A}	13.7^{B}				
	LAB	14.0^{ab}	14.6^{bc}	15.8^{cB}	13.9^{abB}				
Total sugars	CON	27.5^{cB}	25.2^{bc}	24.2^{bB}	23.8^{abC}	0.46	<0.001	<0.001	<0.001
	FA	26.4^{bcAB}	27.1^{c}	26.8^{bcC}	24.3^{aC}				
	ALA	25.2^{bA}	24.9^{b}	23.7^{bB}	17.0^{aB}				
	LAB	26.9^{cB}	26.4^{c}	19.0^{bA}	12.4^{aA}				

[a, b, c, d] Within each treatment, means in the same row with different superscript differ ($P < 0.05$).
[A, B, C, D] Within each time, means in the same column with different superscript differ ($P < 0.05$).
[1] FA: formic acid at 4 mL/kg silage; ALA: acetic and lactic acids at 2 mL of each acid/kg silage; LAB: *Lactobacillus buchneri* at 5×10^9 CFU (colony forming units)/kg of silage.
[2] SEM: Standard error of the mean of treatment x day interaction (n=3).

6. GENERAL DISCUSSION

In addition to the 20 samples of AH reported in Experiment 1, another 18 samples of AH were obtained from 2019, 2020 and 2023 campaigns. There were 4 samples from Marta and Soleta varieties, 3 samples from Lauranne, Penta and Guara varieties, and one sample from Belona variety. All samples were collected in Andalusia and were processed, analysed for chemical composition and incubated *in vitro* with ruminal fluid from sheep as described in Experiment 1. The ME content of AH was estimated from CP and EE content and the gas produced after 24 h of incubation with ruminal fluid as described in Experiment 3. The content in UFL (forage units for milk production) and UFV (forage units for meat production) was estimated according to INRA (2018) equations, and the content in NEm (net energy for maintenance), NEl (net energy for milk production) and NEf (net energy for meat production) was calculated following the equations proposed by the NRC (2021). Finally, the relationships among chemical fractions of AH samples (n = 38) and between chemical composition and the energy content were assessed by Pearson correlation using the PROC CORR of SAS (SAS Institute, 2017).

Table 6.1 shows the mean, maximum and minimum values of chemical composition fractions and the estimated energy content of the 38 samples of AH, as well as the coefficient of variation (CV) for each item. The CV was high (> 21%) for most chemical fractions, excepting the fibrous fractions (NDF, ADF and CF) that had CV lower than 17%. Some variability was also observed in the energy content, with values ranging from 0.60 to 0.82 UFL/kg DM and from 0.52 to 0.71 UFV/kg DM. Variations in NEm, NEl and Nef were similar, and for all estimations of energy content the CV was below 10%. The wide range in energy content reflects the high variability in chemical composition of AH samples. Furthermore, it has to be taken into account that all AH samples were obtained from the same region, and it is possible that this variability would increase if

samples from other almond cultivation areas and/or from other almond varieties were analysed.

Table 6.1 Mean, minimum, maximum and coefficient of variation (CV) of chemical composition fractions and energy content of almond hulls (n = 38).

Item[1]	Mean	Minimun	Maximun	CV
Dry matter (DM, %)	65.7	40.6	92.9	21.6
Ash	9.48	5.14	15.4	23.2
Crude protein	7.29	3.70	13.1	33.3
Ether extract	4.01	4.01	5.95	24.3
Crude fibre	15.4	11.7	20.7	14.7
Neutral detergent fibre (NDF)	33.9	23.8	43.6	14.8
Acid detergent fibre (ADF)	24.7	16.6	32.6	16.9
Lignin	10.8	5.71	15.8	26.0
(Lignin/NDF) * 100	13.9	20.7	42.6	17.1
Total sugars	26.8	10.4	44.3	24.7
UFL (/kg DM)	0.72	0.60	0.82	7.19
UFV (/kg DM)	0.62	0.52	0.71	7.19
NEm (Kcal/kg DM)	1390	1143	1591	7.60
NEl (Kcal/kg DM	1280	1046	1591	7.80
NEf (Kcal/kg DM)	852	852	1472	9.26

[1] Values are expressed as g/100 g dry matter, unless stated otherwise. UFL: forage units (net energy) for milk production; UFV: forage units (net energy) for meat production; NEm: net energy for maintenance; NEl: net energy for milk production; NEf: net energy for meat production.

Table 6.2 shows the chemical composition and energy content of AH reported in different Feed Tables. The average DM content of the AH analysed in our study (national samples) was 65.7%, while in American samples (NRC, 2001; Dairy One laboratory database, 2022), INRA Tables (2018) and Feedipedia (2000) the DM content ranged from 84.5 to 89.6%. This variation can be explained by the AH harvesting procedure, as the national samples were collected after different periods of drying in the field, while the samples reported in the Feed Tables were mostly commercial samples that had been dried to a lower percentage of moisture content. In California, the official regulations state that to be marketed as ‘almond hulls’ a sample should contain no more than 13% moisture, 15% crude fibre (CF) and 9% ash, the latter two fractions being expressed on a fresh

matter basis (DePeters et al., 2020). If the CF content is between 15 and 29%, the sample should be labelled as "almond hulls and shells". The Portuguese samples showed in Table 6.2 were also collected after drying of almonds in the field and had a slightly greater DM content than the national samples (72.4 *vs.* 65.7%), but the average value was also lower than that reported in Feed Tables.

Table 6.2 Chemical composition and energy content of almond hulls (AH) reported in different Feed Tables (adapted from Carro, 2023).

Item[1]	Dairy One, (2022)[2]	NRC (2001)	INRA (2018)	Feedipedia (2020)	Portuguese AH (Subpromais)[3]
Number of samples	159 - 333	23 - 32	-	8 - 70	10
Dry matter (DM; %)	89.6	86.9	84.5	89.1	72.4
Ash	8.41	6.12	11.5	7.21	11.4
Crude protein	5.89	6.51	6.0	5.70	6.23
Ether extract	3.16	2.91	-	3.02	1.28
Crude fibre	19.2	-	12.5	15.3	14.7
Neutral detergent fibre (NDF)	38.8	36.8	35.6	32.0	31.5
Acid detergent fibre (ADF)	32.2	28.7	14.9	26.0	24.3
Lignin	12.4	14.9	-	10.7	9.46
(Lignin/NDF) * 100	32.0	40.5	-	32.3	38.9
Total sugars	24.4	-	-	29.7	25.6
Starch	0.91	-	-	2.61	1.86
Energy content					
ME (Kcal/kg DM)	-	1890	2176	2078	1615
NEm (Kcal/kg DM)	1221	1220	-	-	-
NEl (Kcal/kg DM)	1334	1140	-	-	-
NEf (Kcal/kg DM)	657	650	-	-	-
UFL (kg DM)	-	-	0.75	-	0.53
UFV (kg DM)	-	-	0.66	-	0.44

[1]Chemical composition expressed in g/100 DM, unless stated otherwise; UFL: forage units (net energy) for milk production; UFV: forage units (net energy) for meat production; NEm: net energy for maintenance; NEl: net energy for milk production; NEf: net energy for meat production. [2]Average values from commercial samples analysed between 2004 and 2022 in the United States by Dairy One Laboratory (https://apps.dairyone.com/feedcomposition); [3]Values available at https://subpromais.pt/conteudo2.php?idm=15&filt_prod=89

The content in all chemical fractions were expressed on DM basis to allow the comparison of values from different databases. The average ash, NDF, ADF, lignin, CF and total sugars content of the national samples were within the range reported in the

different databases, but the national samples showed greater CP and EE contents. Differences in EE content could be related to variations in the methods of analysis used in the different laboratories, as diethyl ether was used as solvent in our laboratory and this reagent increases the extraction of fat-soluble matter compared to petroleum ether used in other laboratories (Hart and Fisher, 1971). However, it should also be noted that as mentioned above, the composition of AH is highly variable and these values may actually reflect actual differences between national samples and those from other countries, as many factors, including almond variety and growing conditions, can influence AH composition (Jafari et al., 2015; DePeters et al., 2020).

The available data indicate that AH are a by-product with low CP and starch content, but with high content of total sugars that are rapidly and extensively degraded in the rumen. The main sugars in AH are sucrose, glucose and fructose (Holtman et al., 2015). Glucose and fructose proportions can range between 35-44% and 30-40% of total sugars, respectively, although the values depend on the variety of almond (Offeman et al., 2014). These characteristics make the AH a good source of energy for ruminants, which can be used as a substitute for both forage (due to its fibre content) and concentrates (due to its energy content). The estimates of energy content of the national samples are in accordance to those in INRA Tables (2018), with an average supply of 0.72 UFL and 0.62 UFV per kg DM (Table 6.2). The average NEl content (1297 Kcal/kg DM) is also similar to that estimated in the American samples analysed by Dairy One (2022) and the NRC (2001), but the NEm and NEf content was greater than estimated for the American samples.

The low CP content, together with the high proportion of CP linked to ADF (average values of 31.4% in 34 American AH samples analysed by Dairy One (2022) and 38.3% in 6 samples analysed in our laboratory), makes the protein value of the AH very low.

There are few data on ruminal degradability of the CP of AH, but both the NRC (2001) and Dairy One (2022) data indicate that it is below 50% for a DM intake level of 2% of BW, with lower values (about 44%) in dairy cows with greater intake levels (4% BW), although INRA (2018) reported greater values (67%). The intestinal digestibility of the ruminally undegraded CP is low, with mean values of 50 and 58% according to NRC (2001) and INRA (2018), respectively.

The correlations among the chemical composition fractions of the 38 samples of AH analysed in our laboratory are shown in Table 6.3. No significant correlations between the moisture content and any of the chemical fractions analysed were observed. Ash content was positively correlated ($P = 0.017$) with CP content. The NDF content was positively correlated ($P < 0.001$) with, ADF, lignin and CF content. The amount of EE was positively correlated with NDF, ADF and CF ($P \leq 0.016$) and negatively with lignin and total sugars content ($P \leq 0.029$). Total sugars were negatively correlated ($P \leq 0.001$) with CP and NDF, ADF and CF, indicating that sugars are reduced as the CP and fibre content in AH increases.

Table 6.3 Pearson coefficients and *P* values (in brackets) of the correlations among the chemical composition fractions of almond hulls (n = 38). Only correlations with $P < 0.05$ are shown. [1]

Item	Ash	CP	NDF	ADF	Lignin	CF	EE	Total sugars
Ash		0.386 (0.017)			-0.335 (0.040)			
CP	0.386 (0.017)							-0.483 (0.002)
NDF				0.974 (<0.001)	0.814 (<0.001)	0.701 (<0.001)	0.398 (0.013)	-0.567 (<0.001)
ADF			0.974 (<0.001)		0.897 (<0.001)	0.586 (<0.001)	0.387 (0.016)	-0.498 (0.001)
Lignin	-0.335 (0.040)		0.814 (<0.001)	0.897 (<0.001)		0.323 (0.048)	-0.476 (0.003)	
CF			0.701 (<0.001)	0.586 (<0.001)	0.323 (0.048)		0.465 (0.003)	-0.538 (<0.001)
EE			0.398 (0.013)	0.387 (0.016)	-0.476 (0.003)	0.465 (0.003)		-0.354 (0.029)
Total sugars		-0.483 (0.002)	-0.567 (<0.001)	-0.498 (0.001)		-0.538 (<0.001)	-0.354 (0.029)	

[1] CP: crude protein; NDF: neutral detergent fibre; ADF: acid detergent fibre; EE: ether extract. CF: crude fibre. All chemical fractions are expressed as g/100 g dry matter.

Table 6.4 shows the correlations between chemical composition fractions and the gas production parameters. As expected, PGP was negatively correlated with lignin content (r = -0.474; $P = 0.004$) since there is a negative effect of lignin on fibre degradation in the rumen (Van Soest, 1994). In contrast, total sugars are highly-fermentable and contribute to gas production, which would explain the positive correlation between PGP and total sugars content ($P = 0.018$). The fractional rate of gas production (*c*) was negatively correlated with the fibrous fractions (NDF, ADF and CF) and positively correlated with total sugars content (r = 0.537; $P < 0.001$), demonstrating the more slowly fermentation rate of fibre components compared with the rapid fermentation of sugars.

Lag time is usually negatively related to the content in rapidly degradable substances such as sugars and other easily-fermented carbohydrates (Marcos et al., 2019). However, when the 38 samples of AH were analysed together, *Lag* was positively related to total

sugars content ($P = 0.041$). Although AH contain rapidly fermentable sugars (i.e. glucose, fructose, and sucrose) that should reduce the *Lag* time, this result could be due to the presence of other compounds that were not analysed in this study. Thus, AH are reported to contain a wide range of phytochemicals, mainly phenolic compounds (flavonoids and non-flavonoids), and triterpenoids (Silva et al., 2025) that can delay the fermentation start and produce an increase in *Lag*. *Lag* was negatively ($P < 0.001$) related to NDF, ADF and lignin content, which are fractions either more slowly degraded (NDF or ADF) or undegradable (lignin). The negative correlations of AGPR with NDF and ADF content were similar to those observed in Experiment 1 for 20 samples of AH from Soleta and Guara varieties (Table 5.3), although when including the 38 samples no correlation was observed between AGPR and lignin content. In both analysis (20 and 38 AH samples), AGPR was positively correlated with total sugars content.

Table 6.4 Pearson coefficients and *P* values (in brackets) of the correlations between chemical composition fractions and gas production parameters (n = 38). Only correlations with $P < 0.05$ are shown. [1]

Item	PGP	*c*	*Lag*	AGPR
Ash				
CP				
NDF		-0.455 (0.006)	-0.590 (<0.001)	-0.498 (0.002)
ADF		-0.352 (0.038)	-0.590 (<0.001)	-0.414 (0.013)
Lignin	-0.474 (0.004)		-0.592 (<0.001)	
CF		-0.377 (0.026)		
EE			-0.426 (0.011)	
Total sugars	0.398 (0.018)	0.537 (<0.001)	0.348 (0.041)	0.644 (<0.001)

[1]CP: crude protein; NDF: neutral detergent fibre; ADF: acid detergent fibre; CF: crude fibre; EE: ether extract; PGP: potential gas production; *c*: fractional rate of gas production; *Lag*: time before starting gas production; AGPR: average gas production rate. All chemical fractions were expressed as g/100 g dry matter.

A further objective of this Doctoral Thesis was to investigate the potential antimethanogenic effects of AH, and therefore the *in vitro* ruminal fermentation of mixed diets for either high-producing (HP) or medium-producing (MP) dairy sheep including different levels (0, 8, 16 and 24% of total diet) of AH was assessed. All experimental diets were formulated to have similar CP and NDF content. Because AH can be used as substitutes for both concentrates and forage, AH partly replaced concentrate ingredients (corn, wheat bran, and sugar beet pulp) in HP diets, whereas in MP diets, AH mostly replaced forage (alfalfa hay) and only a little proportion of corn.

Regarding the results of gas production and fermentative parameters, similar responses to AH inclusion were observed for both diets. Including AH tended to decrease the PGP of HP diets whereas no effect was observed for MP diets. This variable response could be attributed to differences in the fermentability of the feed ingredients replaced in each diet, as all feeds replaced in HP diet (corn, wheat bran, and sugar beet pulp) are extensively fermented in the rumen and therefore generate more gas than AH.

For both diets, the inclusion of AH decreased total VFA production after 24 h of incubation, indicating that AH were less fermentable than the feed ingredients that were replaced. Interestingly, the inclusion of AH in both diets (HP and MP) did not affect either VFA total production or AFOM after 9 h of incubation, which could be related to the rapid fermentation of AH during the first hours of incubation. The *in vitro* results also confirmed the low ruminal degradability of the AH protein, as NH_3-N concentrations decreased as the inclusion level of AH increased.

Although a previous study (Durmic et al., 2014) suggested the presence of antimethanogenic compounds in AH, the CH_4 production was not affected after 9 h of incubation of any diet in our study. After 24 h of incubation, the inclusion of AH in the HP diets decreased CH_4 production but the CH_4/total VFA ratio was unaffected. These

results indicate that the AH does not present antimethanogenic properties and that the decrease in CH_4 production could rather be due to the reduction in ruminal fermentability of the diets including AH.

Altogether, the results of the *in vitro* trial indicate that AH can be included in diets for dairy ruminants up to 16% without negative effects on ruminal fermentation at short incubation times (9 h), but at longer incubation times (24 h), including AH decreased diet fermentability. These results would indicate that AH may be more adequate for ruminants with rapid digesta passage rates through the rumen (i.e. low rumen digesta retention times), such as dairy ruminants.

Almond production is continuously growing worldwide, thus causing an increase in the AH produced. Although AH are a by-product widely used in ruminant feeding in some non-European countries (i.e. USA, Australia), its use in Spain and other Mediterranean countries is still quite limited and it can be considered that AH are unknown to many livestock producers. An *in vivo* experiment (Experiment 2) was designed to evaluate the effects of including 6 (AH6) or 12% (AH12) of AH in the concentrate of fattening light lambs (slaughtered at 23 kg BW) on productive parameters and the quality of carcass and meat. It is important to notice that the AH used in this trial was not cleaned and therefore contained soil particles and field debris (i.e. small twigs, leaves, …), which resulted in high ash and lignin content compared with other samples analysed in this Doctoral Thesis and data from the literature (Jafari et al., 2011; DePeters et al., 2020). No cleaning of AH was performed in order to use a representative sample of the AH currently available in the practice for livestock farmers in Spain. Based on the results of the *in vitro* trials conducted previously showing that up to 16% of AH can be included in the diet partially substituting corn, wheat bran and sugar beet pulp without detrimental effects on *in vitro*

VFA production at short incubation times, barley and wheat bran were partially replaced with 6 or 12% of AH in the experimental concentrates in the *in vivo* trial.

The inclusion of AH in the concentrates did not affect their palatability, as indicated by the lack of differences in feed intake between groups. The values of productive parameters were in the range reported by others (Manso et al., 1998; Carro et al., 2006; Carrasco et al., 2009; Haro et al., 2019) for light lambs fed high-cereal concentrates and slaughtered at similar BW. Nevertheless, it should be noticed that although no significant differences between groups were detected in final BW in this study (P = 0.372), the numerical differences among treatments are remarkable (23.5, 22.8 and 22.1 kg for control, AH6 and AH12, respectively) and can have important implications in the practice for the livestock farmers. These differences are also noticeable for ADG values (238, 222 and 198 g/d for control, AH6 and AH12, respectively).

The inclusion of AH tended to decrease DM and CP digestibility, possibly due to the high ash and lignin content of the AH used in this trial. Moreover, these results are in accordance with the reduced ADG of the lambs fed AH, especially those fed the AH12 concentrate. Including AH in the concentrate increased the excretion of N and decreased the amount of N retained, which can be related to the low digestibility of AH protein. The lack of differences among treatments in plasma metabolites and hepatic enzymes, and the physiological values obtained for light lambs with similar feeding system (De Evan et al. 2020; Lobón et al., 2020) indicate that the inclusion of AH did not negatively affect liver function or the metabolism of the lambs.

In general, no effects of the concentrate type were observed on carcass measurements, colour parameters, pH or chemical composition of the meat, and values were similar to those reported by other authors for light lambs (Pérez et al., 2012; Inserra et al., 2014; de Evan et al., 2020; Haro et al., 2020; Serra et al., 2022; Estrada et al., 2024). Lipid

oxidation is a complex phenomenon induced by oxygen in the presence of initiators such as free radicals, which affects mainly to PUFA. The trend to a greater TBARS concentration in the meat of AH12-fed lambs after 6 days storage period (P = 0.056) could be related to the greater PUFA content of the meat of these lambs, since PUFA increase meat's susceptibility to lipid peroxidation (Morán et al., 2012; Holman et al., 2022). However, it should be noted that despite this result, TBARS concentrations were below 0.5 mg MDA/kg of meat, which is considered the limit for detection of rancidity (Lanari et al. 1995).

The most noticeable differences between the experimental groups were observed in the FA profile of the meat. The proportion of FA in the diet is a key factor impacting consumer health. Whereas SFA are associated with increased cholesterol and LDL (low-density lipoproteins) levels linked to a greater risk of developing heart disease, the intake of PUFA can have the opposite effect (Tejeda et al. 2008). Therefore, one of the main challenges for ruminant nutritionists is to obtain animal products with a healthier FA profile. The observed differences in the FA profile of the three concentrates were reflected in the meat FA profile, and the meat from AH-fed lambs had significantly greater proportion of PUFA compared with control lambs. It should be taken into account that the variations in meat FA profile observed can be related to differences in FA intake, but also to the presence of secondary compounds in AH (i.e. phenolic acids, flavonoids, terpenoids, etc.) that can influence ruminal biohydrogenation of PUFA (Vasta et al., 2008; Prgomet et al., 2017). Altogether, the results of our study indicate that including AH in the concentrate improved the FA profile of meat towards a healthier profile.

The darker colour of the ruminal epithelium observed in the lambs fed the AH-concentrates supports the importance of using clean AH, without field debris (i.e., small

twigs, leaves, …) and hard shell pieces, as the darker colour was attributed to the abrasion of the epithelium caused by these contaminants.

In general, there were few differences due to the sex of the lambs on growth performance and meat characteristics, which confirms previous results in lambs slaughtered at low BW (Burfening and Carpio, 1993).The tendency to greater carcass yield and greater pelvic fat observed in females was expected, since there is a sexual dimorphism related to animal precocity leading to greater fat deposition in females compared to males that develop more muscle (Rodríguez et al. 2008; Dervishi et al. 2012).

Finally, a simple economic analysis was conducted to evaluate the feeding costs of the three groups of lambs based on actual market prices (March 2025). The estimated cost of AH was 60 €/t. The cost of all experimental concentrates was similar (Table 6.5) due to the high market prices of urea (550 €/t) and calcium soap (1200 €/t). In order to get isonitrogenous and isoenergetic concentrates, urea was included, and the amount of calcium soap was increased in the concentrates with AH. Using these values and the concentrate intake, the feeding cost during the experiment was 9.74, 9.38, and 9.10 € for Control, AH6, and AH12 groups, respectively (Table 6.5). The total BW gain of the lambs was 11.19, 10.43 and 9.31 kg for Control, AH6 and AH12 groups, respectively, and the concentrate cost per kg of BW gain was 0.870, 0.899 and 0.978 €/kg for Control, AH6 and AH12 treatment, respectively. Under the study conditions, the inclusion of AH did not result in a reduction of feeding costs. However, it should be considered that using clean AH with greater nutritive value would reduce the amount of calcium soap needed to get isoenergetic concentrates. Moreover, feed prices are highly volatile, and the results can differ under other market circumstances.

Table 6.5 Economic analysis to evaluate the feeding costs based on actual market prices (March 2025).

Trial duration (days)	Concentrate intake (kg/d)	Concentrate price (€/kg)	Total cost of concentrate (€)	Body weight gain (BWG) (kg)	Concentrate (€)/kg BWG (€)
47	0.768	0.2697	**9.74**	11.19	0.870
47	0.740	0.2697	**9.38**	10.43	0.899
47	0.718	0.2698	**9.10**	9.31	0.978

The fourth and last objective of this Doctoral Thesis was to evaluate the effects of different silage treatments to identify the optimal treatment for AH preservation during a long-time period. Although AH can be left in the field for drying before being stored, this process can take time and AH is exposed to moisture absorption, which can lead to mold growth and eventually loss of nutritional value (Jafari et al., 2011; Sirakaya, 2022). Another option, but more expensive, is the use of fossil energy to dry the AH, but this would increase their cost for livestock farmers. In the search for an affordable and easy method of preservation, silage emerged as a viable option. Although AH have high content in sugars, they also have high water content and high buffer capacity (Fadel, 1999) that are characteristics indicative of low ensilability. As the information on actual ensilability of AH was limited, the last trial of this Doctoral Thesis was designed to assess the ensiling capacity of AH and to analyse the effects of different silage additives, whose effects were evaluated at different times (0, 4, 21 and 90 days) over the silage storage period. Two types of additives were selected to be tested: acids and lactic acid bacteria. Formic acid was chosen because it has been extensively used as a silage additive, but it is gradually being substituted by other additives due to management and environmental problems. Therefore, it was decided to test also a mixture of lactic and acetic acids that can have synergic effects. The bacterial inoculant was a commercial product based on *Lactobacillus buchneri* and was used at a greater dose than that recommended by the manufacturer.

After 90 days of storage, there were some differences in the chemical composition of the silages due to the inclusion of additives, but some of them (i.e. ash, NDF, ADF and lignin content) were of small magnitude and would be unimportant in the practice. The most relevant difference was the reduced sugar content observed for ALA and LAB treatments compared with CON and FA silages, which was attributed to sugars fermentation to lactic acid and other acids during ensiling. In fact, both ALA and LAB silages showed greater concentrations of lactic and acetic acids than CON and FA silages. This could also explain the greater DM losses and lower pH values observed in ALA and BAL silages compared to CON and FA treatments. Despite these differences, all silages reached the pH values (4.3-5.0) recommended for silages with 30-55% of DM (Kung et al., 2018). In addition, all silages showed good microbial quality, as no *Escherichia coli*, *Salmonella* spp., *Listeria* spp., *Clostridium perfringens* or *Staphylococcus coagulase+* were detected. All tested additives were effective at reducing both yeast and moulds counts compared with CON silage, thus confirming the results from previous studies using the same additives (Selwet, 2008; Kung et al., 2001). The total counts of yeasts and moulds were below the maximum value of 100000 log CFU/g proposed by Kung et al. (2018) in all silages, even in CON silage (49900, 31, 2222 and 92 log CFU/g silage for CON, FA, ALA and LAB, respectively). Moreover, in all silages DM losses were low (< 4%) and the NH_3-N content was less than 15% of total N, indicating low rate of proteolysis.

It is noteworthy that the lactic/acetic ratio, which is commonly used as an indicator of silage fermentation quality, was lower than the 2.5 - 3.0 recommended value and only the CON silages were close to this value (3.2). The low ratios observed for the silages with additives (< 1.30) were due to the high acetic content in ALA and LAB silages, whereas in FA silages both acetic and lactic concentrations were low.

The effect of additives on gas production parameters measured in the *in vitro* trial were consistent with the results of others using similar additives (Zhang et al. 2017; Evan et al. 2023). The decreased PGP values and total VFA production in LAB silages compared with CON silages could be attributed to the consumption of sugars produced during ensiling. However, these changes did not affect the ME content of the silages, which was similar for all treatments.

Because the available information on AH ensilability was limited, the evolution of the characteristics and chemical composition of the silages were monitored over the storage period by opening silage bags at days 4, 21 and 90. A significant effect of storage time was observed in most parameters, and the observed changes agree well with those reported by others using the same additives (McDonald et al., 1991; Chaverra and Bernal, 2000; Meneses et al., 2007; Kung et al., 2018; Monllor et al., 2020; de Evan et al., 2023).

The pH values progressively decreased, reaching the minimum value at day 21 and afterwards increasing slightly by day 90. All additives reduced significantly the pH at 21 and 90 days compared with CON silages. Total VFA content increased with storage time in all silages, but the increase was especially marked in ALA and LAB silages. The lack of propionic acid in any silage would indicate good fermentation, and butyrate was only detected in LAB silages at 90 days, but concentrations were low (< 0.25%) and could be beneficial due to the stabilizing and antifungal action of this acid (Kung et al., 2018; Kung et al., 2020).

Chemical composition of silages changed with storage time, and changes were in accordance with those previously reported of silages of different crops (Monllor et al., 2020; de Souza et al., 2022; Diepersloot et al., 2022). As expected, total sugars content decreased with storage time in all silages, but the reduction was more pronounced in LAB silage due to sugars fermentation by *L. buchneri* (Arriola et al., 2021).

In summary, the results indicate that well fermented AH silages were obtained by adding ALA and LAB, compared to FA. Both ALA and LAB could be recommended as additives to enhance lactic acid production and thus generate greater acidification. The results of our study also indicate that AH could be ensiled without additives, although the use of additives resulted in lower pH and reduced counts of yeast and molds. Further studies are necessary to confirm these results in the practice.

7. CONCLUSIONS

1. Chemical composition and energy value of Spanish almond hulls were in the range of the values published in foreign Feed Tables, and both were highly variable, being affected by almond hull variety and harvest year. Therefore, analysis of chemical composition is recommended before using the almond hulls in practical feeding of ruminants.

2. Under *in vitro* conditions, the level of almond hulls that could be included in the diet without negatively affecting ruminal fermentation depended on the feed ingredients replaced by the almond hulls, but in some cases up to 16% of almond hulls could be included without negative effects on ruminal fermentation. No antimethanogenic effects of almond hulls were detected *in vitro*.

3. Almond hulls can be included up to 12% in the concentrate of light lambs without significatively affecting feed intake, lamb growth and meat characteristics (pH, colour and chemical composition), although slaughter weight of lambs was numerically lower. The use of almond hulls did not decrease feeding costs but in situations of higher prices of feed ingredients could be economically profitable.

4. Including either 6 or 12% of dried almond hulls in the concentrate of light lambs resulted in a healthier meat fatty acid profile for the consumer due to an increased proportion of polyunsaturated fatty acids, but it also augmented lipid oxidation during meat storage.

5. Almond hulls can be ensiled without additives, but using either acids (formic acid or acetic:lactic mixture) or a *Lactobacillus buchneri* inoculum helped to decrease the pH without affecting the energy content of silages.

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