



UNIVERSIDAD MIGUEL HERNÁNDEZ DE ELCHE

Escuela Politécnica Superior de Orihuela

Programa de Doctorado en Recursos y Tecnologías
Agrarias, Agroambientales y Alimentarias



TESIS DOCTORAL

Valorización del subproducto de brócoli (*Brassica oleracea*, var. Italica) y del subproducto y la planta de alcachofa (*Cynara scolymus*) para alimentación de cabras Murciano-Granadinas

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Co-director: JOSÉ RAMÓN DÍAZ SÁNCHEZ



2021



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TESIS DOCTORAL presentada por Paula Monllor Guerra

Directora: Gema Romero Moraleda

Co-director: José Ramón Díaz Sánchez

2021

Dña. Gema Romero Moraleda, Dra. en Ingeniería Agrónoma y Profesora Titular de la Universidad Miguel Hernández de Elche y

D. José Ramón Díaz Sánchez, Dr. en Ingeniería Agrónoma y Catedrático de Universidad de la Universidad Miguel Hernández de Elche,

CERTIFICAN:

Que la Tesis Doctoral titulada “**Valorización del subproducto de brócoli (*Brassica oleracea*, var. *Italica*) y del subproducto y planta de alcachofa (*Cynara scolymus*) para alimentación de cabras Murciano-Granadinas**” de la que es autora la graduada en Ingeniería Agrícola, Ingeniería Agroalimentaria y del Medio Rural, con Máster de Producción Animal, **Dña. Paula Monllor Guerra**, ha sido realizada bajo nuestra dirección y autorizamos a que sea presentada para optar a la obtención del grado de Doctor por la Universidad Miguel Hernández.

Y para que conste a los efectos oportunos se firma el presente certificado.

Firmado:

Dra. Dña. Juana Fernández López, Catedrática de Universidad y Coordinadora del Programa de Doctorado en Recursos y Tecnologías Agrarias, Agroambientales y Alimentarias (ReTos-AAA) de la Universidad Miguel Hernández de Elche (UMH),

CERTIFICA:

Que la Tesis Doctoral titulada “**Valorización del subproducto de brócoli (*Brassica oleracea*, var. *Italica*) y del subproducto y planta de alcachofa (*Cynara scolymus*) para alimentación de cabras Murciano-Granadinas**” de la que es autora la graduada en Ingeniería Agrícola, Ingeniería Agroalimentaria y del Medio Rural, con Máster de Producción Animal, **Dña. Paula Monllor Guerra**, ha sido realizada bajo la dirección de la **Dra. Gema Romero Moraleda** y la codirección del **Dr. José Ramón Díaz Sánchez**, actuando como tutora de la misma la **Dra. Esther Sendra Nadal**. Considero que la Tesis es conforme, en cuanto a forma y contenido, a los requerimientos del Programa de Doctorado ReTos-AAA, siendo por tanto apta para su exposición y defensa pública.

Y para que conste a los efectos oportunos firmo el presente certificado.

Dra. Dña. Juana Fernández López
Coordinadora del Programa Doctorado ReTos-AAA

Esta Tesis Doctoral, titulada “Valorización del subproducto de brócoli (*Brassica oleracea*, var. Italica) y del subproducto y planta de alcachofa (*Cynara scolymus*) para alimentación de cabras Murciano-Granadinas”, se presenta bajo la modalidad de **tesis por compendio** de las siguientes publicaciones:

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Ensiling Process in Commercial Bales of Horticultural By-Products from Artichoke and Broccoli

Autores: Paula Monllor, Gema Romero, Raquel Muelas, Carlos A. Sandoval-Castro, Esther Sendra y José Ramón Díaz

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Autores: Paula Monllor, Carlos A. Sandoval-Castro, Armín J. Ayala-Burgos, Amparo Roca, Gema Romero y José Ramón Díaz

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Autores: Paula Monllor, Raquel Muelas, Amparo Roca, Alberto S. Atzori, José Ramón Díaz, Esther Sendra y Gema Romero

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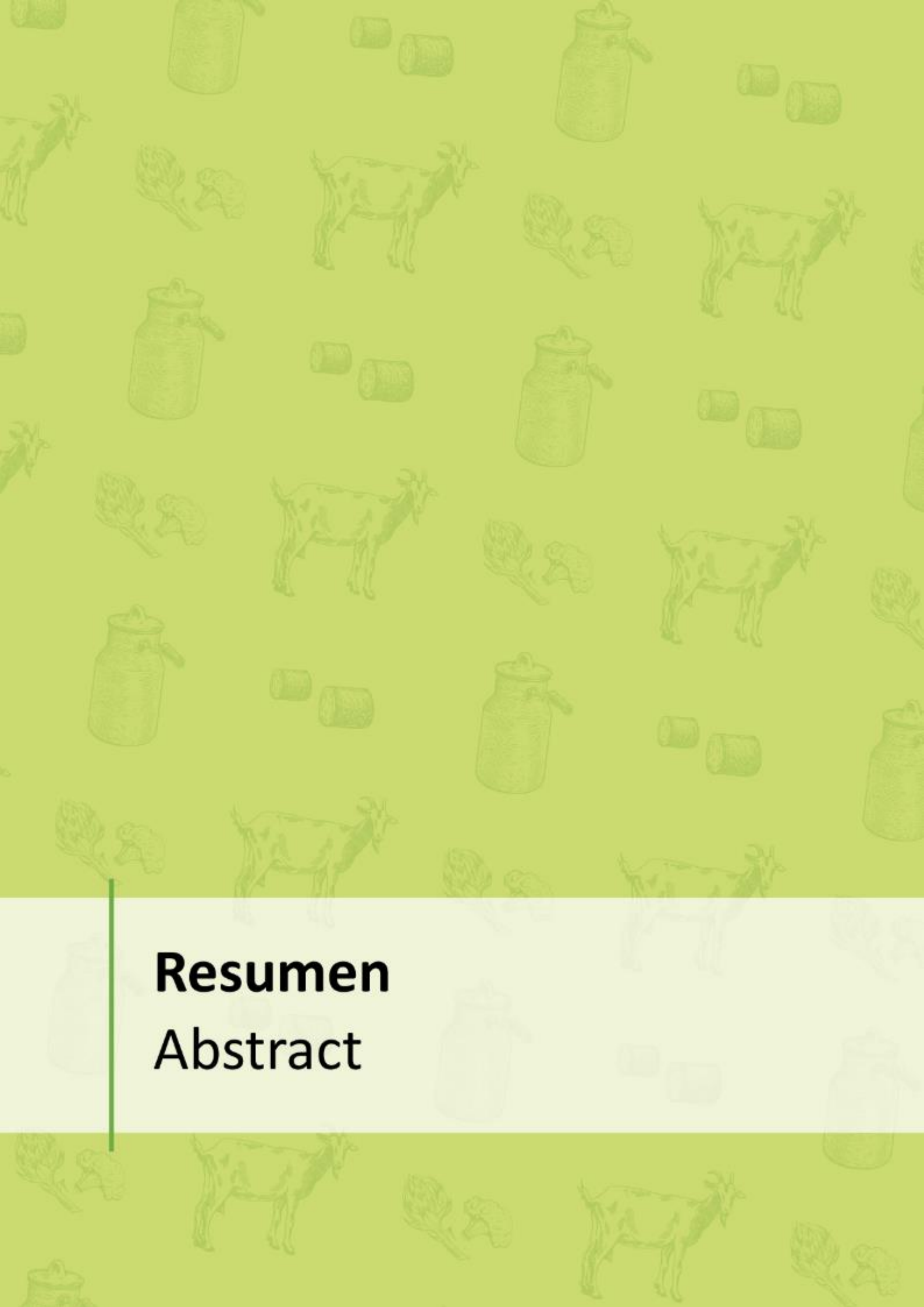
Listado de abreviaturas

| | | | |
|--------|--|-------|---|
| ADN | Ácido desoxirribonucleico | MO | Materia orgánica |
| AGV | Ácidos grasos volátiles | MS | Materia seca |
| BHB | β-hidroxibutirato | NEFA | Ácidos grasos no esterificados |
| C | Carrizo | OBCFA | Ácidos grasos de cadena ramificada |
| CLA | Ácido linoleico conjugado | ORS | Octapole Reaction System |
| CNE | Carbohidratos no estructurales | PA | Planta de alcachofa |
| DCC | DeLaval Cell Counter | PA25 | Dieta que incluye un 25% de PA ensilada |
| DI | Diámetro interno | PA40 | Dieta que incluye un 40% de PA ensilada |
| EE | Extracto etéreo | PA60 | Dieta que incluye un 60% de PA ensilada |
| EM | Energía metabolizable | PB | Proteína bruta |
| ESM | Extracto seco magro | PDA | Potato Dextrose Agar |
| ESU | Extracto seco útil | PT | Polifenoles totales |
| FB | Fibra bruta | PUFA | Ácidos grasos poliinsaturados |
| FAD | Fibra ácido detergente | PV | Peso vivo |
| FND | Fibra neutro detergente | RCS | Recuento de células somáticas |
| GLM | Análisis de modelos lineales en SAS | SA | Subproducto de alcachofa |
| HPLC | High-Performance Liquid Chromatography | SB | Subproducto de brócoli |
| IA | Índice de aterogenicidad | SB25 | Dieta que incluye un 25% de SB ensilado |
| ICP-MS | Inductively Coupled Plasma-Mass Spectrometry | SB40 | Dieta que incluye un 40% de SB ensilado |
| ID | Índice de desaturación | SB60 | Dieta que incluye un 60% de SB ensilado |
| IT | Índice de trombogenicidad | SFA | Ácidos grasos saturados |
| LAD | Lignina ácido detergente | ST | Sólidos totales |
| LCG | Leche corregida por grasa | T | Dieta testigo |
| LCGP | Leche corregida por grasa y proteína | UFA | Ácidos grasos insaturados |
| LRCS | Logaritmo en base 10 del RCS | UFC | Unidades formadoras de colonias |

Estructura de la tesis doctoral

El contenido de esta memoria se ha redactado de acuerdo con la normativa vigente de la Universidad Miguel Hernández de Elche para defender esta Tesis Doctoral bajo la modalidad de tesis por compendio de publicaciones. Por ello, esta memoria se ha estructurado de acuerdo con los siguientes puntos:

- **Resumen/Abstract:** Descripción de los resultados y conclusiones más relevantes (en castellano e inglés).
- **Introducción:** Descripción de la problemática actual de la ganadería caprina e introducción del uso de subproductos agrícolas como solución a dicha problemática, con especial énfasis en el subproducto de brócoli y el subproducto y la planta de alcachofa.
- **Objetivos:** Objetivo global y objetivos parciales de la investigación.
- **Materiales y métodos:** Breve descripción de la metodología empleada para la consecución de los objetivos.
- **Publicaciones científicas:** Se adjuntan los artículos originales publicados en las revistas *Animals* y *Foods*, y la transcripción literal del artículo publicado en *Small Ruminant Research*.
- **Resultados y discusión:** Breve resumen de los resultados obtenidos en cada uno de los capítulos.
- **Conclusiones generales:** conclusiones de la Tesis Doctoral.
- **Investigaciones futuras:** Enumeración de futuras investigaciones que pueden desarrollarse a partir de los resultados obtenidos.
- **Referencias bibliográficas:** Se indican las referencias empleadas en las secciones complementarias a las publicaciones.



Resumen
Abstract

Resumen

La alimentación de los animales representa más del 60% de los costes de una explotación ganadera. Además, la producción de alimentos para uso ganadero comporta un consumo elevado de recursos naturales limitados, como tierra, agua y combustibles fósiles. También es frecuente que los ingredientes de las raciones de los animales tengan que recorrer largas distancias antes de acabar en la granja, con el consiguiente gasto y contaminación por transporte. Todo ello repercute negativamente en el sector de la producción animal, tanto de forma económica como a través del impacto sobre el medioambiente, lo cual fomenta la creciente animadversión entre la sociedad actual, fundamentalmente en el entorno urbano. Una solución a estos problemas es el aprovechamiento de subproductos locales para alimentación animal, que conlleva varias ventajas para la sociedad actual y futura, como la reducción de los recursos necesarios para la producción de alimentos de origen animal y las emisiones de gases de efecto invernadero, transformando los residuos contaminantes para el medio ambiente procedentes de la agricultura en materias primas y produciendo alimentos de calidad y de alto valor biológico para el hombre.

España, junto con Italia, es uno de los principales países productores de brócoli y alcachofa de la Unión Europea. La producción nacional se concentra en el sur de la provincia de Alicante y Región de Murcia, donde 2018 se alcanzaron cifras superiores a 9.000 ha de superficie de cultivo de alcachofa, cifrándose la producción total de esta hortaliza en más de 120.000 t, y 260.000 t de brócoli. El resto de cultivo de alcachofa que queda en campo tras su recolección equivale a 11 t de forraje por hectárea. Por otra parte, solo el 50 y 70% de las alcachofas y brócolis que se transportan a las industrias envasadoras y congeladoras es utilizado, el resto se compone de las brácteas, tallos y partes no comestibles. Todo ello conlleva una gran disponibilidad de estos subproductos que pueden ser aprovechados mediante su utilización para alimentación animal.

La hipótesis principal de esta Tesis Doctoral es que el empleo del subproducto y planta de alcachofa y el subproducto de brócoli en raciones balanceadas para alimentación de ganado caprino lechero no interfiere en el rendimiento productivo, calidad de la leche ni en la salud de los animales. Sin embargo, dado el alto contenido en humedad y estacionalidad de estos subproductos agrícolas, es necesario someterlos a un proceso de conservación que asegure una óptima calidad y disponibilidad durante tiempos prolongados. Para ello se puso a punto la técnica de fabricación de silos de tipo bala de 300 kg de peso con subproducto y planta de alcachofa y subproducto de brócoli, determinando la estabilidad del proceso de fermentación en el día 30 y asegurando su viabilidad hasta 200 días desde el comienzo del ensilado.

Para conocer la aceptación y el grado de preferencia de las cabras por este tipo de silos, se realizó una prueba “en cafetería” en la que se correlacionó la ingestión de ensilado con su composición, dando como resultado que las cabras mostraban mayor preferencia por aquellos ensilados que habían pasado por un proceso homofermentativo, donde la producción de ácido láctico había sido mayoritaria, como en el caso del ensilado de subproducto y planta de alcachofa. Por otra parte, el ensilado de subproducto de brócoli dio lugar a un proceso heterofermentativo donde se produjo un nivel superior de ácido butírico, reduciendo con ello la ingestión de este alimento.

Debido al bajo coste de estos subproductos y a su contenido en nutrientes, resulta interesante incluir la máxima cantidad posible en las dietas de los animales, pero sin llegar a perjudicar su salud ni nivel de producción ni calidad de la leche. Para conocer la cantidad máxima óptima a incluir, se formularon dietas para cabras lecheras que incluían diferentes concentraciones de subproducto de brócoli y subproducto y planta de alcachofa ensilados. El subproducto de alcachofa ensilado solo se testó hasta un nivel de inclusión de 25%, consiguiendo resultados satisfactorios al no observar diferencias relevantes con la ración testigo. Respecto al subproducto de brócoli y planta de alcachofa ensilados, se vio que, a corto plazo, con hasta un 40% las cabras no mostraban diferencias relevantes con aquellas que habían sido alimentadas con una ración con ingredientes convencionales, como alfalfa y cereales (estos competidores de la alimentación humana). Incluso se observó una mejor calidad en el perfil lipídico de la leche de aquellas cabras alimentadas con planta de alcachofa ensilada, dado la menor concentración de algunos ácidos grasos saturados, como láurico, mirístico y palmitico, y mayor de poliinsaturados, especialmente los ácidos vacénico y ruménico, además de unos menores valores de los índices de aterogenicidad y trombogenicidad, obteniendo así un perfil más beneficioso desde el punto de vista de la prevención de enfermedades cardiovasculares en seres humanos. Finalmente, se realizó otra prueba para conocer cómo les afectaba a los animales el consumo de estos subproductos ensilados en un 40% de la ración durante una lactación completa, comprobando la ausencia de diferencias importantes en producción y calidad de la leche de la dieta que incluía planta de alcachofa en comparación a una ración convencional, y corroborando los resultados obtenidos anteriormente en cuanto a una grasa más beneficiosa de la leche, debido a mayor contenido en ácido α -linolénico y menor ratio n6/n3, así como menores Índices de Aterogenicidad y Trombogenicidad. Sin embargo, la inclusión de un 40% de subproducto de brócoli ensilado no fue del todo satisfactoria, ya que generó la selección de ingredientes dentro de la ración y se redujo la ingestión, afectando de forma negativa al rendimiento productivo y a la recuperación de peso vivo del animal tras el pico de lactación.

Abstract

Animals feeding represents more than 60% of the costs of a livestock farm. In addition, the food production for livestock involves high consumption of limited natural resources, such as land, water and fossil fuels. Often, the ingredients for animal rations come from far away, with the consequent expense and contamination due to transport. All this has a negative impact on the animal production sector, both economically and through the impact on the environment, which increases growing animosity among society, mainly in the urban areas. A solution to these problems is the use of local by-products for animal feeding, which entails several advantages for current and future society, such as reducing the resources necessary for the animal production and greenhouse gas emissions, transforming polluting waste for the environment from agriculture into animal feedstuffs and producing quality food with high biological value for human beings.

Spain, along with Italy, is one of the main broccoli and artichoke producing countries in the European Union. National production is concentrated in the south of the province of Alicante and the Region of Murcia, where in 2018 figures of over 9,000 ha of artichoke crop, which a total yield of 120,000 t of artichoke, and 260,000 t of broccoli have been reached. The rest of the artichoke crop that remains in the field after its harvest is equivalent to 11 t of forage per hectare. On the other hand, only 50 and 70% of the artichokes and broccolis that are transported to the packaging and freezing industries are used, the remaining is made up of bracts, stems and inedible parts. All this entails a great availability of these by-products that can be used for animal feeding.

The main hypothesis of this Thesis is that the use of the artichoke plant by-product and the broccoli by-product in well-balanced rations for dairy goat feeding does not interfere with productive performance, milk quality or the health of the animals. However, due to the high moisture content and seasonality of these agricultural by-products, it is necessary to submit them to a preservation process that ensures optimum quality and availability for long periods of time. For this reason, a manufacturing technique of 300 kg bale-type silos production with artichoke plant and by-product and broccoli by-product, as well as artichoke by-product, was developed, determining the stability of the fermentation process on day 30 and ensuring its viability up to 200 days from the beginning of silage process.

To know the acceptance and preference degree of goats for this type of silos, a study was carried out "in a cafeteria" design, in which the silage intake was correlated with its composition, resulting in the goats showed greater preference for those silages that had undergone a homofermentative process, where lactic acid production had been the majority, as in the case of artichoke plant and by-product silages. On the other hand, the broccoli by-product silage gave rise to a heterofermentative process where a higher level of butyric acid was produced, thereby reducing its intake.

Due to the low cost of these by-products and their nutrient content, it is interesting to include them as a maximum amount possible in the animals' diets, but without damaging their health, production level or milk quality. To know the optimal maximum amount to include, diets were formulated for dairy goats that included different concentrations of broccoli by-product and artichoke plant and by-product silages. Artichoke by-product silage only was tested to a level of

25%, reaching satisfactory results because there were no relevant differences with the control ration. Regarding broccoli by-product and artichoke plant silages, it was observed that, in a short term, with up to 40% the goats did not show relevant differences with those that had been fed a ration with conventional ingredients, such as alfalfa and cereals (these competitors for human food). It was observed a better lipid profile in the milk of those goats fed with artichoke plant silage, because it had lower concentration of some saturated fatty acids, such as lauric, myristic and palmitic, and higher of polyunsaturated acids, especially vaccenic and rumenic; in addition to lower values of the atherogenicity and thrombogenicity indices, thus obtaining a more beneficial profile from the point of view of the prevention of cardiovascular diseases in humans. Finally, another study was carried out to know how the consumption of these by-products silages affected the animals in 40% of the ration during a full lactation, verifying the absence of important differences in production and quality of milk in the diet that included artichoke plant silage compared to a conventional ration, and corroborating the results obtained previously in terms of a more beneficial fat in milk, due to a higher α -linolenic acid and a lower n6/n3 ratio and lower Atherogenicity and Thrombogenicity Indices. However, the inclusion of 40% broccoli by-product silage was not entirely satisfactory, since it generated the selection of ingredients within the ration and intake was reduced, affecting negatively productive performance and weight recovery after the peak of lactation.

1

Introducción

1. Introducción

1.1. Situación socioeconómica del sector caprino lechero

1.1.1. Situación actual

El ganado caprino se ha utilizado de forma tradicional para la obtención de leche, carne, pieles y estiércol. La cabra es la especie rumiante que se utiliza en ganadería que mejor se adapta a las zonas semiáridas y de montaña, en las que la producción agrícola resulta menos rentable y los pastos son escasos y de peor calidad (Molina-Alcaide y Romero-Huelva, 2017). Además, dado que en estas zonas se encuentra muy limitado el tipo de agricultura o sistema ganadero a implantar, las granjas de ganado caprino contribuyen a la fijación de población rural.

El censo caprino ha seguido una tendencia creciente a partir de la década de los noventa, aumentando como consecuencia la producción de leche de cabra en el mundo, como se observa en la Figura 1. En 2018, el número de cabezas ganaderas fue de 216.231.667, mientras que se obtuvo una producción total de 18.712.088 t de leche (FAOSTAT, 2019). En las Figuras 2 y 3 se muestra la distribución del ganado caprino a nivel mundial, siendo Asia el continente con mayor cabaña ganadera, estando China a la cabeza en cuanto a censo caprino (138.237.000 cabezas) e India en cuanto al país más productor de leche de esta especie (6.098.730 t).

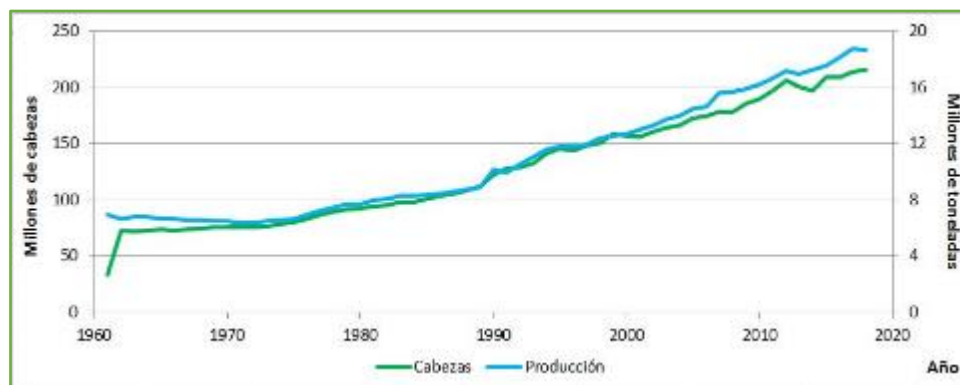


Figura 1. Evolución del censo y producción de leche de cabra en el mundo (FAOSTAT, 2019).

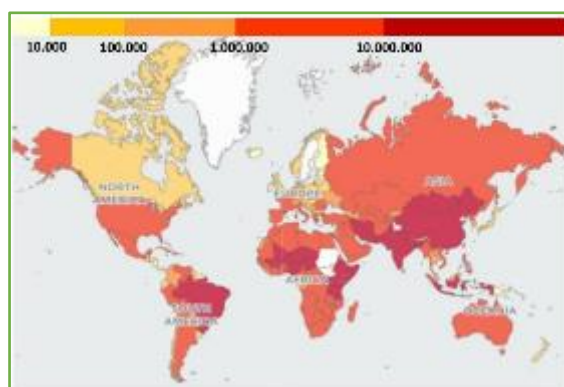


Figura 2. Distribución del ganado caprino en el mundo en 2018 (FAOSTAT, 2019).

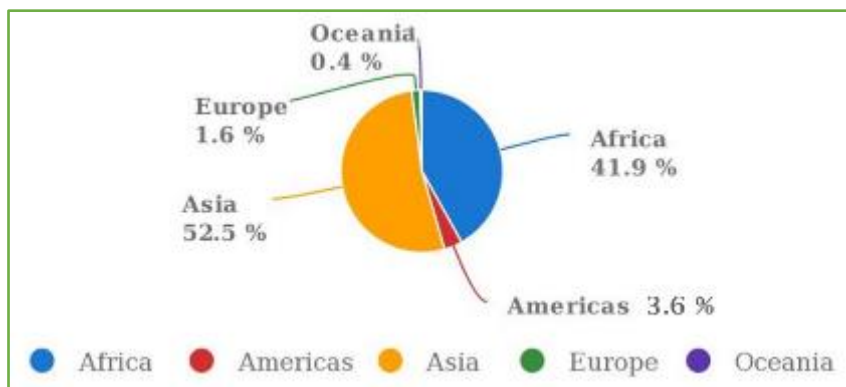


Figura 3. Distribución del ganado caprino por continentes en 2018 (FAOSTAT, 2019).

La producción lechera se ha incrementado a un ritmo más acelerado que el censo caprino total debido al desarrollo de esquemas de selección y mejora de la raza, especialmente en países europeos, con razas especializadas en la producción de leche, que ha dado como resultado una mejora de la productividad de los animales. Aunque el censo caprino a nivel de la Unión Europea no llega al 6% del censo mundial, la producción de leche de cabra representa un 16% de la producción mundial, debido a que las cabras de las granjas europeas triplican la media de productividad mundial (304,9 vs. 86,5 kg/animal al año), aunque esto depende en gran medida de varios factores, como el sistema de explotación, grado de tecnificación, raza, alimentación, etc. En la Figura 4 se muestra la distribución del censo caprino a nivel europeo, donde este predomina en los países de la cuenca del Mediterráneo. Debido al reducido formato corporal de la cabra, a su agilidad y habilidad para el pastoreo y a su reconocida rusticidad, el ganado caprino es susceptible de ser utilizado en sistemas extensivos y semiextensivos, siendo más idóneo que el ovino y el bovino para el aprovechamiento de zonas áridas y semiáridas caracterizadas por la escasez de lluvias y la baja disponibilidad de forraje, así como una topografía accidentada, que permite la utilización de rastrojos y subproductos derivados de cultivos agrícolas, logrando de esta forma una productividad aceptable en regiones ecológicas difíciles (Léiras *et al.*, 2014). No obstante, también se practica el sistema intensivo para sacar el máximo partido al potencial productivo de razas mejoradas y así aprovechar las características saludables y organolépticas de la leche de cabra y sus productos derivados.

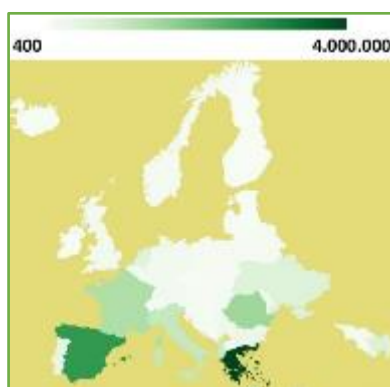


Figura 4. Distribución del ganado caprino en Europa en 2018 (FAOSTAT, 2019).

En la Figura 5 se indican datos relativos al conjunto de la Unión Europea y los cinco países miembros con mayor relevancia en el sector caprino, donde puede observarse que la producción total no depende únicamente del censo ganadero, sino que la productividad de los animales juega un papel muy importante en la producción anual de leche de cada país. Este es el caso de

Grecia y Francia, donde la producción total de este último casi duplica a la del otro (652.327 vs. 397.785 t en 2018) teniendo menos de la mitad de cabaña ganadera (1.298.656 vs. 3.997.519 cabezas) debido a una mayor productividad media (735,1 vs. 133,8 kg/animal y año).

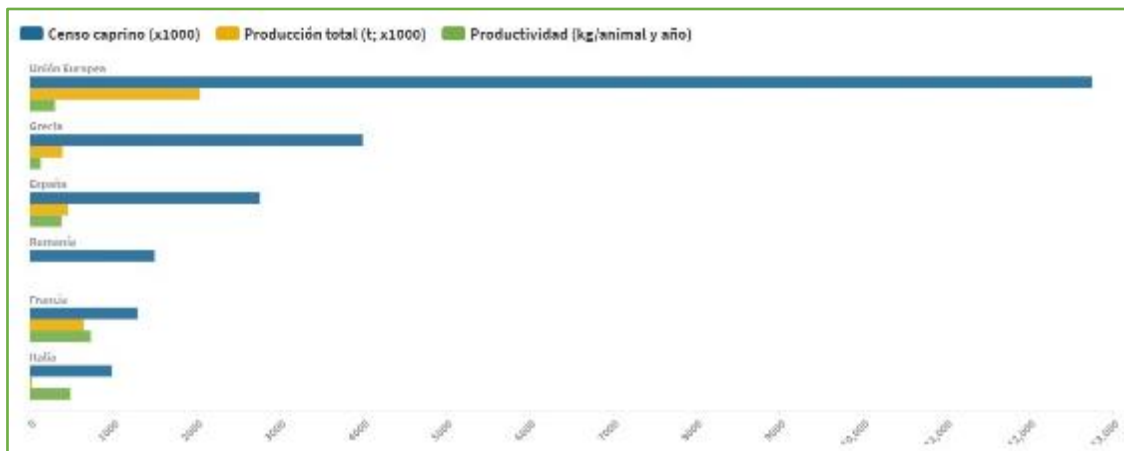


Figura 5. Censo caprino, producción total de leche y productividad media en la Unión Europea y cinco principales países miembros en 2018 (FAOSTAT, 2019).

Datos de producción y productividad de Rumanía no disponibles

A nivel de la Unión Europea y según datos de la Organización de las Naciones Unidas para la Alimentación y la Agricultura, FAO por sus siglas en inglés, España es el segundo país en importancia censal caprina (2.764.790 cabezas de ganado) y en producción de leche anual (461.375 t), detrás de Grecia y Francia, respectivamente (FAOSTAT, 2019). La producción media anual de leche por cabra lechera productiva puede estimarse en 380 kg. El sur de España es la región donde se localiza la mayor parte de esta cabaña ganadera, como se observa en la Figura 6, siendo Andalucía la Comunidad Autónoma con mayor población (1.029.654 cabezas; MAPA, 2019), seguida de Castilla-La Mancha y Extremadura.



Figura 6. Distribución del ganado caprino en España en 2018 (MAPA, 2019).

El conjunto censal de la Región de Murcia y la Comunitat Valenciana supone más del 10% de la cabaña nacional. Las explotaciones de caprino lechero de esta región suelen tener un tamaño medio (600 cabras aproximadamente), siendo la Murciano-Granadina la raza predominante, y carecen o disponen de poca superficie de tierra asociada. Existe un predominio del uso de mano de obra familiar y la alimentación está basada en el consumo de forrajes y concentrados

comprados. El número de partos al año por animal fija el ciclo productivo, que está en torno a 1 parto al año. Este tipo de explotaciones tienen una producción en torno a 480 litros leche por cabra y año y los cabritos se llevan a matadero con un peso vivo (PV) de 8 kg y 30 días de vida, aproximadamente (Informe RENGRATI, 2018).

El consumo nacional de leche de cabra y derivados aún es muy bajo (solo un 0,3% del total de leche y derivados lácteos), siendo de 0,63 kg consumidos per cápita en 2018 (MAPA, 2019). Sin embargo, es un mercado que durante ese año creció de forma muy notable (Figura 7), pudiendo ampliarse en el futuro su segmento de mercado.

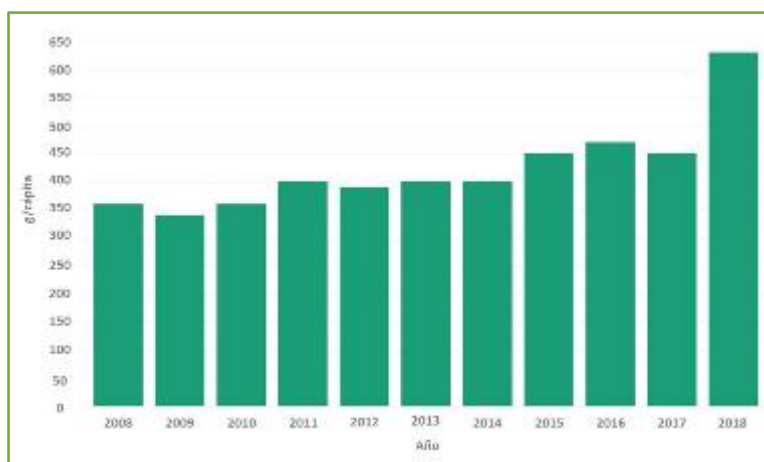


Figura 7. Evolución del consumo del conjunto de leche y queso de cabra en España (MAPA, 2019).

1.1.2. Problemática y perspectivas futuras de la ganadería caprina

Con el paso de los años, el margen de beneficios que se obtiene en las explotaciones ganaderas se ha reducido, como se muestra en la Figura 8, que representa la evolución del balance económico de una granja típica de caprino lechero de la Región de Murcia (Informes RENGRATI 2015-2019). En el caso del caprino lechero, esta reducción de beneficios se debe a un menor precio del hectogrado¹ de leche y al aumento de los costes de alimentación, que representan en torno a un 60% de los costes totales de una granja.

¹Hectogrado de leche: Según Real Decreto 115/2013, de 15 de febrero, sobre declaraciones a efectuar por los compradores y productores de leche y productos lácteos de oveja y cabra, el hectogrado es el valor porcentual de extracto seco quesero (ESQ; grasa + proteína) en 100 litros de leche.

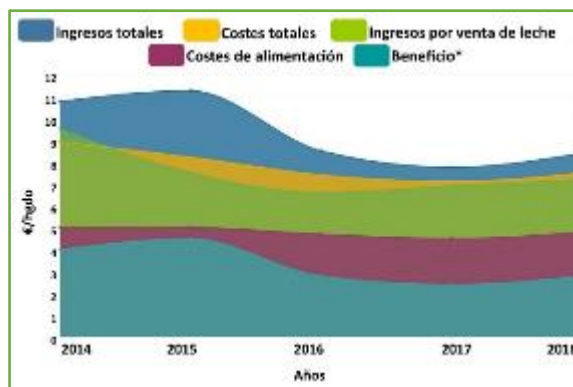


Figura 8. Evolución del balance económico en granjas de caprino lechero típicas de la Región de Murcia (informes RENGRAI de años 2015-2019).

*Beneficios según cuenta de explotación: beneficio efectivo (ingresos totales –costes efectivos) menos los costes no efectivos (costes de amortización, +/- cambios en inventario de animales y +/- ganancias y/o pérdidas de capital).

Por otra parte, toda actividad ganadera conlleva el gasto de recursos naturales finitos, como la tierra, el agua y combustibles fósiles que, además, liberan gases de efecto invernadero; por lo que es necesario establecer sistemas que optimicen el aprovechamiento de estos recursos. En la Figura 9 y 10 se observa como en la década pasada, en España descendió la superficie de tierra destinadas a cultivos (MAPA, 2019), con la consiguiente reducción en el gasto en combustible (EUROSTAT, 2019) y consumo de agua en actividades agrícolas (AQUASTAT, 2017). El aprovechamiento más eficiente de los recursos hídricos también favoreció el descenso del consumo de agua. Sin embargo, el consumo de agua destinada a actividades agrícolas (incluyendo la ganadería y la acuicultura) sigue siendo muy alto, más del doble del consumo combinado de agua para uso industrial y municipal (Figura 11).

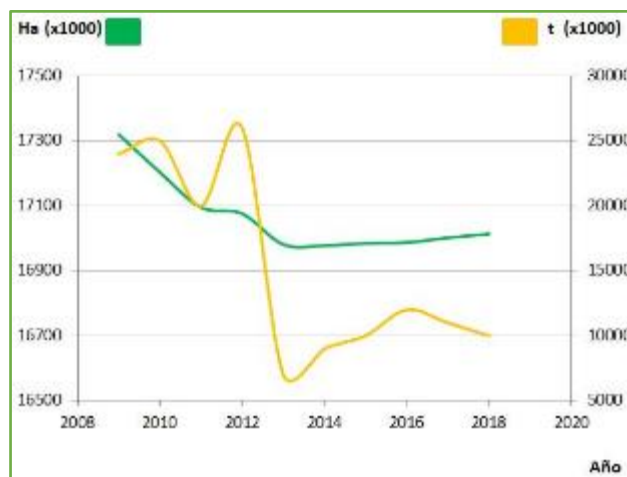


Figura 9. Variación de los insumos de tierra (miles de ha) y combustibles fósiles (miles de t) destinados a cultivos agrícolas en España (elaborado a partir de MAPA 2019 y EUROSTAT 2019).

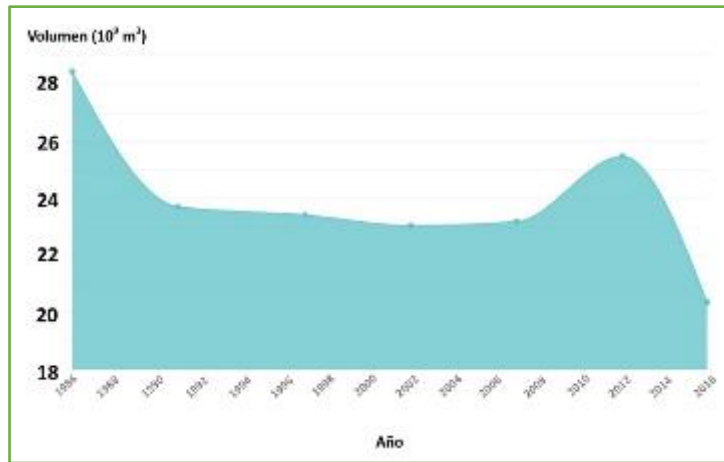


Figura 10. Evolución del consumo de agua en actividades agrícolas y ganaderas en España (AQUASTAT, 2017).

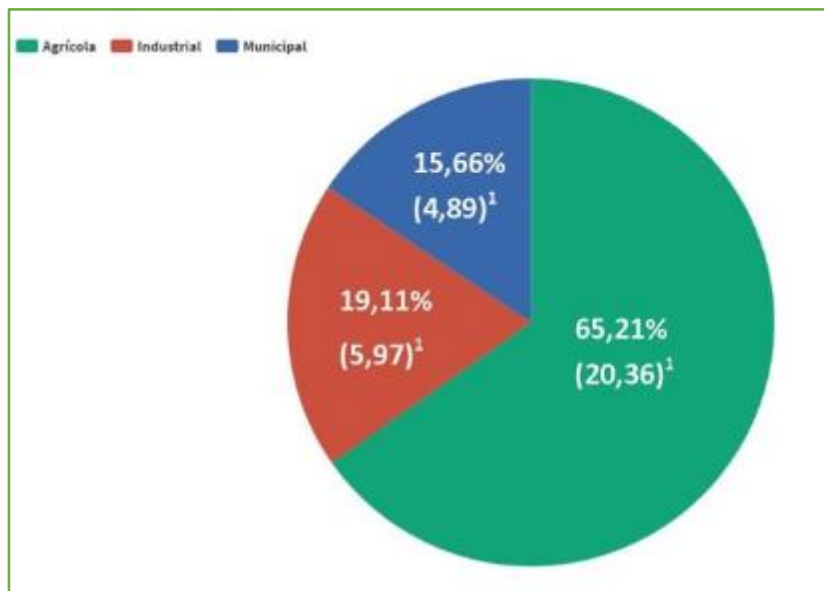


Figura 11. Consumo de agua para distintos usos en España en 2016 (AQUASTAT, 2017).

¹Volumen de agua en 10⁹ m³

El sector caprino, así como otros tipos de explotación ganadera y agrícola, y la red de servicios que se genera a su alrededor generan una actividad económica que tiene el potencial de fijar población en el medio rural, contribuyendo de este modo al desarrollo de zonas rurales y evitando el despoblamiento de las mismas, problema que se observa desde la segunda mitad del siglo XX (Figura 12).

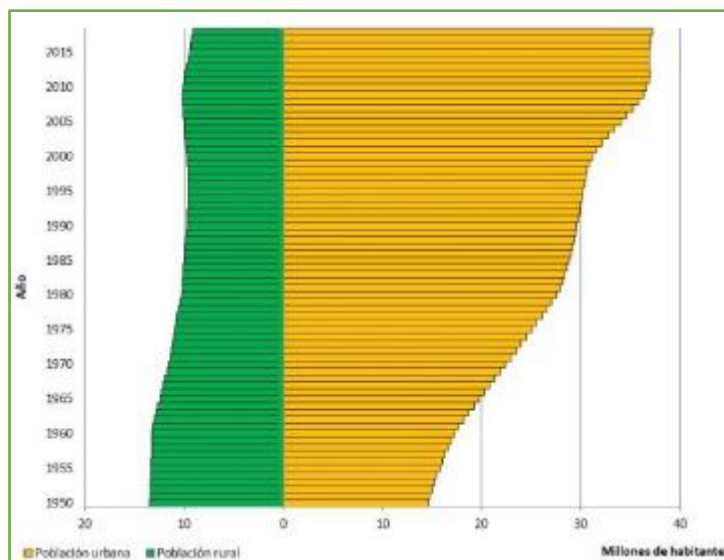


Figura 12. Evolución de la población rural y urbana en España (FAOSTAT, 2019).

1.2. Empleo de subproductos agroindustriales para alimentación animal

1.2.1. Generalidades

Desde mitad del siglo XX, la producción animal y vegetal se han ido intensificando para abastecer de alimentos a una población humana que está en constante ascenso. Alimentos aptos para consumo humano, como los cereales y la soja, son importantes fuentes de energía y de aminoácidos esenciales que son utilizados como ingredientes principales de las raciones de animales de alta producción, suponiendo con ello una competencia entre ganadería y población humana por una limitada superficie cultivable a nivel mundial. Con el crecimiento de la población mundial, la producción de alimentos crea cada vez más presión sobre el medio ambiente y acelera el cambio climático, al mismo tiempo que se agotan los recursos naturales. Se estima que para 2050, la población mundial será de 9 billones de personas, lo cual supondrá un incremento del 70% en la demanda de alimentos (CE, 2012). La intensificación en la producción global de alimentos ha dado lugar a grandes cantidades de subproductos y residuos (Waldron, 2007), que suponen graves problemas de contaminación y representan una pérdida de biomasa y nutrientes de gran valor (Laufenberg *et al.*, 2003). La Figura 12 representa la producción de residuos procedentes de la agricultura en la Unión Europea junto con las emisiones de gases de efecto invernadero asociados a estos residuos en caso de que no se les dé un tratamiento o aprovechamiento.

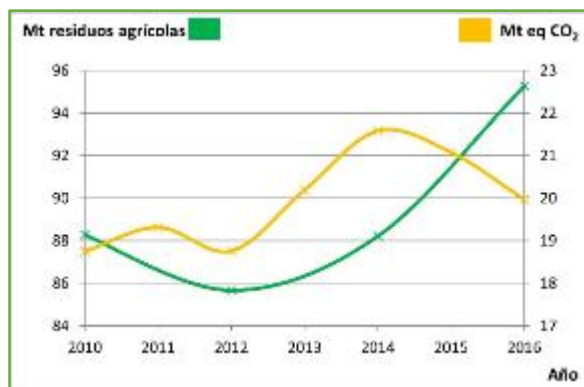


Figura 13. Evolución de la generación de residuos agrícolas (Mt) y de las emisiones de equivalentes de CO₂ (Mt) asociadas a esos residuos en la Unión Europea (elaborado a partir de EUROSTAT, 2019 y FAOSTAT, 2019).

Ante esta situación, la Comisión Europea incita a llevar a cabo cambios en el modelo productivo de alimentos, así como en el manejo que se hace de los recursos naturales, poniendo el enfoque en el reciclado de los mismos. Esta estrategia recibe el nombre de Bioeconomía y pretende desarrollar una sociedad más competitiva y eficiente en la utilización de recursos, aunando la seguridad alimentaria con una producción de alimentos sostenible y promoviendo cambios en el patrón de consumo de alimentos, que den lugar a dietas más saludables y respetables con el medio ambiente (CE, 2012).

La intensificación de la producción ganadera que permita satisfacer la mayor demanda de alimentos necesitará realizar un aprovechamiento racional de los recursos existentes, como los subproductos agroindustriales. Debido a su gran disponibilidad y al bajo precio, son productos muy atractivos para el sector ganadero, sobre todo en aquellas zonas con escasez de forrajes naturales (Gasa y Castrillo, 1992). La Ley 22/2011, de 28 de julio, de residuos y suelos contaminados, diferencia residuos de subproductos. Mientras que los primeros son cualquier sustancia u objeto que se deseché o se tenga la intención u obligación de desechar, los subproductos son elementos resultantes de un proceso de producción cuya finalidad primaria no era la producción de dichos elementos. La diferencia con el residuo viene de que en el subproducto se han de cumplir las siguientes condiciones:

- Va a ser utilizado posteriormente.
- No es necesario someterlo a ningún proceso de transformación.
- Su utilización cumple con todos los requisitos pertinentes para tal fin (en el caso de alimentación animal, que los niveles de fitosanitarios estén por debajo del límite máximo de residuos) y no supone un riesgo para la salud humana y medio ambiente.

Los subproductos agroalimentarios se pueden clasificar en diferentes categorías según el tipo de cultivo o tipo de industria del que proceden (Tabla 1). En el caso de frutas y verduras destinadas a industrias conserveras y de procesado, el porcentaje de desecho puede alcanzar el 30%.

Tabla 1. Porcentaje de subproducto generado de frutas y verduras utilizadas en diferentes tipos de procesos industriales

| Industria agroalimentaria | % de subproducto |
|---------------------------|------------------|
| Vinos | 20-30 |
| Zumos | 30-50 |
| Conservas | 5-30 |
| Aceite | 40-70 |
| Azucarera | 85 |

Kesapidou *et al.* (2015).

1.2.2. Aplicación de subproductos en alimentación animal

Como se observa en la Figura 14, el precio de las raciones ganaderas para caprino lechero se ha mantenido alto y variable en los últimos años (FAO, 2011), lo que ha despertado el interés del sector ganadero hacia la utilización de recursos locales alternativos (Romero-Huelva *et al.*, 2012; Magersa *et al.*, 2013), como los subproductos de la industria agroalimentaria. Algunos de estos subproductos son suministrados de forma gratuita por la industria a los ganaderos, mientras que otros tienen un pequeño coste, como el bagazo de cerveza (0,042 €/kg), aunque mucho inferior al de la alfalfa (0,192 €/kg), según datos del catálogo de productos de Poballe SA (campaña 2019-2020; www.poballe.com/alimentacionanimal/).

La utilización de subproductos agroalimentarios en nutrición animal permite reducir el impacto medioambiental de la industria agroalimentaria, además de aumentar la rentabilidad de la agricultura, industrias agroalimentarias y ganadería por producir alimentos de origen animal de alta calidad y valor biológico para el ser humano a partir de materiales de media-baja calidad, algunos de ellos con elevado contenido en humedad, que no tienen ningún valor alimenticio para el ser humano (Elferink *et al.*, 2008). De este modo, se cumple con las actuales políticas de “residuo-cero” que piden a la industria agroalimentaria la búsqueda de nuevas formas de aprovechamiento de subproductos (Panouillé *et al.*, 2007; CE, 2012), potenciando así la ecología industrial y la economía circular (Mirabella *et al.*, 2014). Otro beneficio añadido al empleo de subproductos en alimentación animal es que no se establece una competencia por el alimento o el uso de la tierra entre ganado y seres humanos, reduciéndose la demanda de cereales y semillas oleaginosas, alimentos también consumidos por las personas (Schader *et al.*, 2015). Con el empleo de subproductos agrícolas locales en la alimentación ganadera también se consigue proporcionar forrajes de alta calidad nutritiva en zonas en las que no se producen, reduciendo la importación de esos forrajes y la consiguiente emisión de gases de efecto invernadero. Además, en ganadería ecológica se pueden suministrar materias primas locales a un precio muy competitivo.

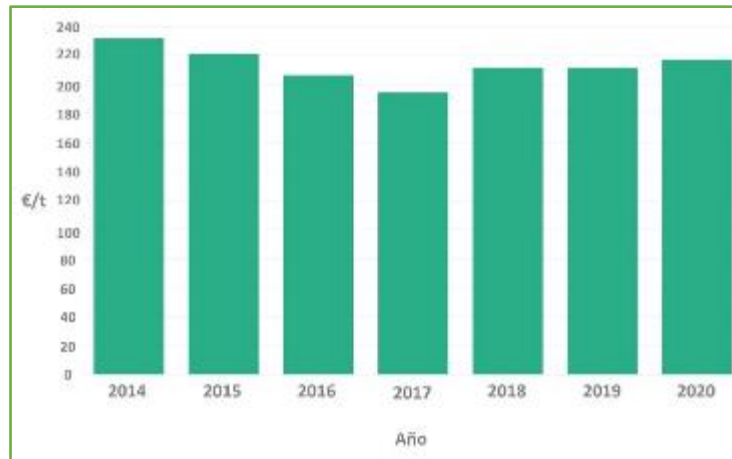


Figura 14. Evolución del precio medio de la ración para caprino lechero en España (MAPA, 2020).

Los subproductos agrícolas son aquellos materiales vegetales sólidos o semisólidos de la actividad agraria y agroalimentaria, que pueden proceder tanto de la producción primaria o recolección del producto principal, como las hojas de olivo, o de alguno de los eslabones de la industria donde se procesa, como las pulpas de las industrias de zumos. Estos subproductos pueden ser utilizados tal cual (en fresco) o deberán pasar por algún tipo de proceso de conservación, como la deshidratación en el caso de aquellos que tengan un alto contenido en humedad, o refinado para la obtención de compuestos específicos como ácidos grasos, vitaminas, fenoles o carotenoides. Los subproductos pueden ser incorporados en las raciones de los animales como ingredientes principales que aporten proteína o energía, o como suplementos con propiedades funcionales. El proceso se completa con la obtención de productos de origen animal a partir de esos subproductos vegetales, como se muestra en la Figura 15.



Figura 15. Proceso simplificado de obtención de subproductos vegetales y su utilización en alimentación animal (Kesapidou *et al.*, 2015).

La gran variabilidad de composición que hay entre los subproductos utilizados en alimentación animal da lugar a un amplio abanico de ingredientes potenciales que puedan sustituir total o parcialmente algunos de las materias primas convencionales que se incluyen en las raciones. Por ejemplo, los subproductos ricos en fibra, como la hoja de olivo (Molina-Alcaide y Yáñez, 2008) o el subproducto de alcachofa (Salman *et al.*, 2014), pueden reemplazar a forrajes, mientras que los subproductos con una concentración de energía, como la pulpa de naranja (Lashkari y Taghizadeg, 2013; Oltramari *et al.*, 2016) o de remolacha azucarera (Abo-Zeid *et al.*, 2017), pueden sustituir a los cereales de la dieta.

Los subproductos agrícolas pueden ser utilizados de dos formas en alimentación animal. El primer procedimiento es utilizarlo directamente en granja tal y como llega del campo o la industria agroalimentaria. El segundo procedimiento consiste en someter al subproducto a un tratamiento industrial (deshidratación, ensilado, molienda, homogenización, mezcla con otros ingredientes, etc.) y comercializarlo de forma individual o como parte integrante de un pienso compuesto. Esta ha sido la forma en que en los últimos años se han utilizado grandes cantidades de subproductos de olivo (orujos de aceituna, hoja de olivo) y pulpa de cítricos y remolacha (Gasa y Castrillo, 1992). Este proceso entraña pocos riesgos para la producción ganadera y no presenta inconvenientes en el manejo a nivel de granja. Sin embargo, el método de utilización de subproductos directamente en granja conlleva problemas de abastecimiento, transporte, conservación y administración a los animales.

La inclusión de estos alimentos alternativos en la dieta de los animales debe atender a criterios técnico-económicos. El primero de ellos es la relación precio/valor nutritivo, es decir, la utilización del subproducto será recomendable cuando el coste por unidad de energía y/o proteína sea menor que el de ingredientes convencionales. El contenido en materia seca (MS) es un parámetro muy importante a tener en cuenta porque, cuanto mayor sea su valor, más rentable será la utilización del subproducto porque conllevará menores costes de transporte y de conservación en la explotación (Williams, 2014). Además, el valor nutritivo también se puede ver afectado por la presencia de contaminantes del suelo. Otro criterio que influye en el uso de subproductos tiene que ver con el nivel de mecanización y mano de obra de la explotación, ya que es probable que se produzcan cambios en las tareas de reparto de alimentos para asegurar un óptimo aprovechamiento y evitar problemas digestivos; por lo que a veces resulta necesario dedicar más mano de obra en las labores de alimentación o que la explotación cuente con cierto grado de mecanización, como el remolque *uni-feed*. Finalmente, es muy importante la salubridad del subproducto, es decir, que no esté contaminado con elementos peligrosos de la cadena de producción o que contenga residuos químicos, tanto de origen natural como antrópico, que resulten tóxicos para los animales (Gasa y Castrillo, 1992). Además, es de especial relevancia que no se vea afectado el rendimiento normal de los animales, tanto a nivel de crecimiento, producción o la calidad del producto final.

En la Tabla 2 se muestra una lista de aspectos por los que se caracterizan los subproductos agroalimentarios utilizados en alimentación animal y los requisitos mínimos que han de cumplir para que resulte rentable y segura su utilización. Como se puede observar, resulta necesaria la investigación científica para asegurar la idoneidad de un subproducto como ingrediente de una ración ganadera.

Tabla 2. Aspectos característicos de los subproductos vegetales y requisitos que han de cumplir para poder ser utilizados en alimentación animal.

| Aspectos característicos | Requisitos |
|--|---|
| Estacionales y de ámbito local | Cercanía y disponibilidad durante tiempos prolongados (en fresco o tratados con algún método de conservación) |
| Disponibilidad en grandes cantidades | |
| Bajo coste de procesado, transporte y almacenamiento | Bajo coste |
| Costes de producción difíciles de estimar | Almacenamiento y manejo seguro |
| Conocimiento limitado de condiciones de procesado, almacenamiento y manejo | |
| Conocimiento limitado de efectos sobre digestibilidad | |
| Conocimiento limitado de niveles de inclusión en las dietas | -Conocimiento del modo de acción -Compuestos activos biodisponibles -Conocimiento de la presencia de factores antinutricionales |
| Bioseguridad | Producto que cumpla con legislación |
| Composición variable | Producto estandarizado con una descripción precisa y evaluación nutricional |

Kasapidou *et al.*, 2015.

1.3. Estudios con subproductos agroindustriales en alimentación de rumiantes

El empleo de subproductos agrícolas en alimentación animal es una práctica sostenible de los sistemas productivos que se viene utilizando desde que coexisten agricultura y ganadería, mucho antes de las políticas que promocionan los principios de las 3 Rs (Reducir, Reutilizar y Reciclar). Sin embargo, ha sido durante las últimas décadas cuando la investigación científica se ha volcado con la valorización de subproductos agroindustriales como sustitutos de los alimentos convencionales (Kasapidou *et al.*, 2015).

1.3.1. Composición de diferentes subproductos

Los residuos de las cosechas y las industrias envasadoras de frutas y verduras representan una fuente de nutrientes muy importante para los rumiantes. El análisis de la composición química es una herramienta fundamental para la evaluación de estos alimentos, en especial aquellos que han sido poco estudiados. La gran variabilidad de composición química y valor nutricional que presentan los subproductos agroindustriales representa una dificultad a la hora de hacer recomendaciones generales para su uso en alimentación animal. En la Tabla 3 se muestra el contenido nutricional de algunos subproductos agroindustriales, donde se observa la amplia variabilidad antes mencionada.

Tabla 3. 1. Composición química (g/kg MS), energía metabolizable (Mcal/kg MS) y digestibilidad de MS in vitro (g/kg MS) de algunos subproductos agroindustriales.

| Subproducto | MS | MO | PB | EE | FND | FAD | LAD | CNE | EM | DMS | DMO | PB/EM | LAD/FND (%) |
|--|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-------|-------------|
| ¹ Pulpa de remolacha azucarera (deshidratada) | 899 | 869 | 116 | 3 | 460 | 237 | 40 | 291 | 2,4 | 929 | 806 | 48,3 | 8,7 |
| ¹ Pulpa de remolacha azucarera ensilada | 750 | 937 | 110 | 3 | 430 | 246 | 25 | 394 | 2,5 | 940 | 815 | 44,0 | 5,8 |
| ¹ Hojas de remolacha azucarera | 223 | 874 | 157 | 15 | 290 | 113 | 27 | 412 | 2,3 | 957 | 830 | 68,3 | 9,3 |
| ¹ Semillas de uva | 916 | 960 | 116 | 52 | 682 | 584 | 437 | 110 | 1,1 | 362 | 321 | 106 | 64,1 |
| ² Orujo de uva | 422 | 947 | 85 | 60 | 510 | 530 | 10 | 192 | 1,2 | 326 | 290 | 70,8 | 2,0 |
| ³ Bagazo de cerveza | 931 | 961 | 282 | 71 | 545 | 201 | 46 | 110 | 2,8 | 892 | 877 | 30,6 | 8,4 |
| ¹ Hojas de olivo | 493 | 842 | 98 | 47 | 529 | 345 | 231 | 168 | 1,5 | 745 | 648 | 65,3 | 43,7 |
| ² Orujo de aceituna | 895 | 956 | 68 | 115 | - | - | - | - | 1,1 | 329 | 293 | 61,8 | - |
| ¹ Peladuras de almendra | 866 | 914 | 53 | 18 | 322 | 224 | 110 | 520 | 1,9 | 840 | 730 | 27,9 | 34,2 |
| ¹ Hojas de lechuga | - | 823 | 184 | 23 | 224 | 158 | 34 | 392 | 2,1 | 967 | 838 | 87,6 | 15,2 |
| ¹ Corteza de espárragos | - | 912 | 206 | 16 | 455 | 293 | 67 | 235 | 1,9 | 836 | 726 | 108 | 14,7 |
| ¹ Tallos de judías | 871 | 769 | 169 | 34 | 415 | 323 | 136 | 152 | 2,1 | 859 | 746 | 80,5 | 32,8 |
| ⁴ Subproducto de alcachofa fresca | 166 | 950 | 134 | 14 | 574 | 399 | 82 | 228 | 2,4 | 798 | 660 | 55,7 | 18,6 |
| ⁴ Subproducto de alcachofa escaldada | 205 | 943 | 119 | - | 475 | 368 | 72 | - | 2,3 | 771 | 710 | 51,5 | 11,2 |
| ¹ Subproducto de alcachofa ensilado | 118 | 921 | 126 | 19 | 591 | 404 | 110 | 185 | 1,8 | 759 | 775 | 70,0 | 5,0 |
| ¹ Subproducto de alcachofa henificado | 166 | 948 | 177 | 41 | 678 | 467 | 76 | 51 | 2,0 | 817 | 804 | 88,5 | 5,4 |
| ¹ Tallos de guisantes | 322 | 902 | 199 | 32 | 282 | 146 | 14 | 389 | 2,4 | 893 | 595 | 82,9 | 46,7 |
| ¹ Tallos de habas | 182 | 902 | 173 | 21 | 335 | 185 | 18 | 373 | 2,4 | 927 | 740 | 72,1 | 17,7 |
| ¹ Pulpa de tomate | 51 | 965 | 190 | 51 | 557 | 427 | 260 | 167 | 1,9 | 683 | 381 | 100 | 50,9 |
| ¹ Corazones de pimiento deshidratados | 973 | 893 | 192 | 67 | 311 | 222 | 55 | 323 | 2,1 | 852 | 740 | 91,4 | 17,7 |

MS: Materia seca (g/kg), MO: Materia orgánica (g/kg MS), PB: Proteína bruta (g/kg MS), EE: Extracto etéreo (g/kg MS), FND: Fibra neutro-detergente (g/kg MS), FAD: Fibra ácido-detergente (g/kg MS), LAD: Lignina ácido-detergente (g/kg MS), CNE: Carbohidratos no estructurales (g/kg MS), EM: Energía metabolizable (Mcal/kg MS), DMS: Digestibilidad de MS (g/kg), DMO: Digestibilidad de MO (g/kg).

¹García-Rodríguez *et al.* (2019); ²Gasa y Castrillo (1992); ³Mirzaei-Aghsaghali y Maheri-Sis (2008); ⁴Megías *et al.* (2002); ⁵Molina-Alcaide *et al.* (2017); ⁶Wadhwa y Bakshi (2005); ⁷Bakshi y Wadhwa (2013); ⁸Lagos-Burbano y Castro-Rincón (2019).

Tabla 3.2. (continuación) Composición química (g/kg MS), energía metabolizable (Mcal/kg MS) y digestibilidad de MS in vitro (g/kg MS) de algunos subproductos agroindustriales.

| Subproducto | MS | MO | PB | EE | FND | FAD | LAD | CNE | EM | DMS | DMO | PB/EM | LAD/FND (%) |
|--|-----|-----|-----|----|-----|-----|-----|-----|-----|-----|-----|-------|-------------|
| ¹ Piel de pimienta | 106 | 949 | 99 | 33 | 753 | 641 | 383 | 64 | 1,1 | 432 | 495 | 90,0 | 22,3 |
| ² Coliflor | 149 | 811 | 120 | 58 | 275 | 194 | 42 | 358 | 2,6 | 900 | 883 | 46,2 | 15,3 |
| ¹ Pulpa de manzana | 205 | 984 | 51 | 60 | 672 | 460 | 150 | 201 | 1,8 | 566 | 845 | 28,3 | 1,2 |
| ¹ Pulpa de clementina | 177 | 972 | 73 | 20 | 139 | 96 | 20 | 740 | 2,7 | 991 | 857 | 27,0 | 0,9 |
| ¹ Pulpa de limón | 49 | 957 | 76 | 77 | 247 | 171 | 30 | 558 | 3,0 | 975 | 694 | 25,3 | 14,3 |
| ¹ Pulpa de naranja | 155 | 969 | 80 | 26 | 222 | 126 | 20 | 640 | 2,8 | 989 | 671 | 28,6 | 15,2 |
| ⁴ Subproducto de calabaza | 164 | 937 | 132 | - | 636 | 415 | 132 | - | 1,8 | 616 | 538 | 71,7 | 20,8 |
| ⁴ Subproducto de melón | 79 | 909 | 99 | 18 | 274 | 239 | 23 | 518 | 2,4 | 846 | 735 | 40,6 | 8,4 |
| ⁴ Tallos de brócoli | 68 | 882 | 182 | - | 242 | 212 | 23 | - | 2,4 | 850 | 738 | 76,6 | 9,5 |
| ⁵ Destrío de berenjena | 74 | 823 | 175 | 7 | 383 | 214 | 148 | 258 | 2,6 | 997 | 864 | 67,4 | 38,6 |
| ⁵ Destrío de calabacín | 153 | 724 | 281 | 12 | 263 | 130 | 48 | 168 | 2,5 | 972 | 945 | 113 | 18,3 |
| ⁵ Destrío de berza | 91 | 779 | 225 | 14 | 223 | 119 | 2,4 | 317 | 2,7 | 984 | 956 | 82,8 | 1,1 |
| ⁵ Destrío de pepino | 54 | 760 | 198 | 13 | 168 | 131 | 2,5 | 381 | 2,6 | 970 | 944 | 75,6 | 1,5 |
| ⁵ Destrío de zanahoria | 152 | 826 | 108 | 16 | 305 | 140 | 109 | 397 | 2,8 | 960 | 935 | 38,3 | 35,7 |
| ⁶ Hojas de repollo | 100 | 842 | 199 | 26 | 337 | 226 | 89 | 280 | 2,2 | 938 | 813 | 90,5 | 26,4 |
| ⁶ Hojas de rábano | 88 | 779 | 194 | 45 | 279 | 219 | 70 | 261 | 2,1 | 980 | 849 | 92,4 | 25,1 |
| ⁷ Vainas de guisantes | 141 | 920 | 198 | 10 | 481 | 354 | 114 | 231 | 1,7 | 865 | 751 | 117 | 23,7 |
| ⁸ Cogollo de caña de azúcar | 250 | 936 | 49 | 7 | 682 | 445 | 42 | 198 | 2,2 | 737 | 642 | 22,4 | 6,2 |
| ⁸ Bagazo de caña de azúcar | 446 | 977 | 22 | 4 | 718 | 407 | 66 | 233 | 2,4 | 780 | 678 | 9,1 | 9,2 |

MS: Materia seca (g/kg), MO: Materia orgánica (g/kg MS), PB: Proteína bruta (g/kg MS), EE: Extracto etéreo (g/kg MS), FND: Fibra neutro-detergente (g/kg MS), FAD: Fibra ácido-detergente (g/kg MS), LAD: Lignina ácido-detergente (g/kg MS), CNE: Carbohidratos no estructurales (g/kg MS), EM: Energía metabolizable (Mcal/kg MS), DMS: Digestibilidad de MS (g/kg), DMO: Digestibilidad de MO (g/kg).

¹García-Rodríguez *et al.* (2019); ²Gasa y Castrillo (1992); ³Mirzaei-Aghsaghali y Maheri-Sis (2008); ⁴Megías *et al.* (2002); ⁵Molina-Alcaide *et al.* (2017); ⁶Wadhwa y Bakshi (2005); ⁷Bakshi y Wadhwa (2013); ⁸Lagos-Burbano y Castro-Rincón (2019).

Al valor nutritivo de los subproductos le afectan varios factores, como son el origen botánico o la variedad de la especie vegetal de la que proceden, el estado fisiológico en que ese vegetal es recolectado y el método de recolección (García-Rodríguez *et al.*, 2019). En la Tabla 4 se muestra la variabilidad existente en los diferentes componentes nutricionales de dos de los principales subproductos utilizados en alimentación ganadera debida a esos factores. Además, el proceso de industrialización al que se somete el vegetal, condicionado por variables como presión, temperatura, humedad, tiempo de procesado o grado de molturación, también influirá en el valor nutritivo del subproducto originado. Un ejemplo de ello es que la digestibilidad total de un subproducto procedente de un vegetal que ha sido sometido a condiciones de temperatura y humedad elevadas (cocción) es mayor que la del vegetal fresco (Gasa y Castrillo, 1992); pudiendo incluso desactivarse los principios tóxicos o antinutritivos. Por otro lado, estas mismas condiciones afectan negativamente al contenido proteico del subproducto, como comprobaron Megías *et al.* (2002) entre el subproducto de alcachofa fresca y escaldada.

Tabla 4. Variabilidad en la composición química y digestibilidad de los subproductos de oliva y tomate en función de la variedad vegetal, estado fisiológico y método de recolección.

| | Torta de oliva | Pulpa de tomate |
|---|----------------|-----------------|
| Materia orgánica | 90-97 | 92-97 |
| Proteína bruta | 5-14 | 19-25 |
| Fibra bruta | 15-50 | 15-38 |
| Extracto etéreo | 4-30 | 6-20 |
| Digestibilidad de materia orgánica | 32-54 | 52-68 |

Gasa y Castrillo (1992)

Los subproductos agroindustriales se pueden agrupar en diferentes categorías atendiendo al valor nutricional que presentan. Existe un grupo de subproductos que presentan una digestibilidad muy baja debido a un alto nivel de lignificación de la pared celular, lo cual reduce su valor nutritivo. En este grupo entrarían las hojas de olivo, la piel de pimiento, las semillas de uva o la pulpa de tomate deshidratada, cuyo contenido en lignina ácido detergente (LAD) representa más de un 40% de la fracción de fibra neutro detergente (FND). Estos subproductos servirían como sustitutos de forraje, tanto para aumentar el llenado del rumen (práctica poco usual en los sistemas ganaderos modernos), como para proporcionar fibra insoluble en aquellas dietas con alto contenido en almidón y, de esta forma, reducir el riesgo de acidosis ruminal.

Por otro lado, se encuentra el grupo de subproductos contrario al anteriormente comentado, el de aquellos muy digestibles y rápidamente degradables en el rumen debido a un contenido rico en carbohidratos no estructurales (CNE), como la pulpa de cítrico, la peladura de almendras o el destrío de hortalizas. Otra particularidad común a este grupo de subproductos es que tiene un contenido bajo de fibra y de proteína bruta (PB), lo cual los convierte en fuentes altas de energía que pueden incorporarse en dietas de rumiantes como reemplazo de los granos de cereal.

El resto de subproductos se encuentran en un grupo intermedio entre los dos anteriores, con una digestibilidad y contenido energéticos aceptables y, en algunos casos, con alto valor proteico, como la corteza de espárragos, las hojas de lechuga, los corazones de pimientos, los tallos de leguminosas y de brócoli o el destrío y hojas de hortalizas como la berza y el calabacín

o la lechuga y el repollo, superior al contenido proteico de una alfalfa (174 g/kg de MS; FEDNA, 2016). Su utilización en dietas de rumiantes depende tanto del coste como de su disponibilidad, así como de la composición del resto de la ración.

Otro factor importante de la composición de los subproductos que permite discernir entre cuáles de ellos pueden ser utilizados como ingrediente único de las raciones ganaderas, o tienen que complementarse con otros ingredientes que sirvan de fuente de energía o proteína es la relación entre estos dos componentes. Según Gasa y Castrillo (1992), las necesidades medias de proteína degradable en el rumen para la síntesis de proteína microbiana son de 35 g/Mcal de energía metabolizable (EM) y, teniendo en cuenta que la degradabilidad proteica media de un forraje convencional oscila en torno al 80%, habría que administrar 44 g de PB/Mcal de EM para satisfacer las necesidades en nitrógeno de los microorganismos del rumen. De los subproductos recogidos en la Tabla 3, aquellos con una alta relación PB/EM (más de 80 g PB/Mcal de EM), como la pulpa de tomate, los destríos de algunas hortalizas, como el de calabacín, o las vainas de guisantes pueden servir como suplemento proteico en piensos compuestos. También puede ocurrir el caso contrario, que el subproducto tenga una relación PB/EM baja por un exceso de energía, como las pulpas de frutas que, con un elevado contenido en carbohidratos rápidamente fermentables en el rumen, necesitan ser suplementadas con una fuente de nitrógeno. Por otro lado, algunos de los subproductos, pese a contar con una relación idónea PB/EM, su concentración es demasiado baja como para que sean administrados al ganado como ingrediente único de la ración. Tal es el caso de los subproductos de olivo, las peladuras de almendras y la pulpa de manzana. Los subproductos procedentes de la recolección e industrialización de hortalizas presentan una relación PB/EM equilibrada, que permite su empleo como ración única en dietas de mantenimiento o de baja producción o como sustitutos parciales o totales de forrajes convencionales en dietas mixtas para producciones medias y altas.

Otro factor limitante a tener en cuenta en el empleo de subproductos es su contenido en extracto etéreo (EE). Contenidos de EE superiores al 5-10% (sobre MS) perjudican el normal funcionamiento de la flora celulolítica del rumen (Gasa y Castrillo, 1992), afectando de esta forma a la digestión de la fibra, componente principal de las dietas de rumiantes. Es por ello que subproductos como el orujo de aceituna debe ser administrado en dosis bajas.

1.3.1.1. Propiedades funcionales de los subproductos

Los subproductos de frutas y verduras constituyen un reservorio de fitoquímicos (carotenoides, fenoles y flavonoides), antioxidantes, antimicrobianos, vitaminas o ácidos grasos con propiedades nutricionales y actividades tecnológicas favorables (Schieber *et al.*, 2001; Fernández-López *et al.*, 2008). Es por ello que pueden utilizarse como aditivos en alimentación ganadera para mejorar la calidad de los productos animales.

Según la Autoridad Europea de Seguridad Alimentaria, EFSA por sus siglas en inglés (2008), para demostrar que un aditivo favorece la calidad de un alimento de origen animal, debe mejorar las características sensoriales y la aceptación de ese producto, como ocurre con los antioxidantes y colorantes. También debe incrementar el valor nutricional de ese producto, tal y como sucede con los ácidos grasos poliinsaturados (PUFA) de cadena larga y el ácido linoleico conjugado (CLA). Otra opción es que asegure una calidad microbiana correcta del alimento. En la Tabla 5 se presentan algunos de los subproductos de frutas y vegetales junto con algunos de sus componentes funcionales más importantes.

Tabla 5. Componentes funcionales de subproductos de frutas y vegetales

| Subproducto | Componente funcional |
|---|---|
| ¹ Torta de oliva | Polifenoles |
| ¹ Piel de zanahoria | Polifenoles y carotenoides |
| ^{1, 2} Pulpa/piel/semillas de manzana | Polifenoles, capacidad antioxidante |
| ^{1, 3, 4, 5} Pulpa/Semillas/Piel de uva | Polifenoles, PUFA, tocoferoles, actividad antioxidante |
| ^{1, 6} Piel de patata | Polifenoles y actividad antioxidante |
| ^{1, 7, 8} Piel pulpa y semillas de naranja | Hesperidina, carotenoides y polifenoles |
| ⁶ Vainas de guisantes | Actividad antioxidante |
| ⁷ Semillas y piel de tomate | Ácidos grasos insaturados y carotenoides (licopeno) |
| ⁹ Piel y semillas de melón | Polifenoles, carotenoides, actividad antioxidante |
| ¹⁰ Piel de banana | Polifenoles y actividad antioxidante |
| ¹¹ Semillas de dátil | Polifenoles, flavonoides, actividad antioxidante, antibacteriana, antifúngica y anti-inflamatoria |

¹Galanakis (2012); ²Xu *et al.* (2016); ³Ovcharonova *et al.* (2016); ⁴Tangolar *et al.* (2011); ⁵Harbeoui *et al.* (2017); ⁶Shehata *et al.* (2020); ⁷Oreopoulou y Tzia (2007); ⁸Jorge *et al.* (2016); ⁹Vella *et al.* (2019), ¹⁰Silva *et al.* (2020); ¹¹Barakat *et al.* (2020).

1.3.2. Métodos de conservación

La mayoría de los cultivos de los que pueden utilizarse sus subproductos en alimentación animal son estacionales, como se refleja en la Tabla 6. Durante la época de recolección y procesado de los cultivos, los subproductos de estos son generados en grandes cantidades y a un ritmo superior al de su consumo por el ganado, provocando que se acumulen grandes cantidades que quedan inservibles, dado el reducido contenido en MS (menos de 300 g/kg) de la gran mayoría de ellos, que los hace altamente perecederos; convirtiéndose de este modo en un riesgo de contaminación medioambiental. Por lo tanto, resulta imprescindible adoptar métodos de conservación apropiados para estos subproductos que mantengan una calidad adecuada durante un largo periodo de tiempo y que así, puedan ser incorporados en la dieta de los animales. Los métodos de conservación de subproductos agrícolas más utilizados son la deshidratación y el ensilado.

Tabla 6. Calendario de recolección de varias hortalizas.

| | Enero | Febrero | Marzo | Abril | Mayo | Junio | Julio | Agosto | Septiembre | Octubre | Noviembre | Diciembre |
|-----------------|-------|---------|-------|-------|------|-------|-------|--------|------------|---------|-----------|-----------|
| Lechuga | | | | | | | | | | | | |
| Espinaca | | | | | | | | | | | | |
| Berenjena | | | | | | | | | | | | |
| Tomate | | | | | | | | | | | | |
| Pimiento | | | | | | | | | | | | |
| Habas | | | | | | | | | | | | |
| Berza | | | | | | | | | | | | |
| Alcachofa | | | | | | | | | | | | |
| Repollo | | | | | | | | | | | | |
| Brócoli | | | | | | | | | | | | |
| Coliflor | | | | | | | | | | | | |
| Col de Bruselas | | | | | | | | | | | | |
| Apio | | | | | | | | | | | | |
| Cardo | | | | | | | | | | | | |
| Pepino | | | | | | | | | | | | |
| Lombarda | | | | | | | | | | | | |
| Zanahoria | | | | | | | | | | | | |
| Espárrago | | | | | | | | | | | | |
| Fresa | | | | | | | | | | | | |
| Cebolla | | | | | | | | | | | | |
| Guisantes | | | | | | | | | | | | |
| Sandía | | | | | | | | | | | | |
| Melón | | | | | | | | | | | | |
| Calabaza | | | | | | | | | | | | |
| Judías verdes | | | | | | | | | | | | |

En amarillo los meses de cosecha (MAPA, 2019).

1.3.2.1. Deshidratación

La deshidratación consiste en la eliminación total o parcial del agua que contiene un alimento a través de un proceso térmico. Normalmente se emplea en aquellos subproductos con más de un 90% de humedad, como las pulpas de frutas y verduras. El proceso de deshidratación comienza con la acumulación del material sobre una superficie inclinada, permitiendo el drenaje del exceso de agua, que se puede facilitar con una prensa mecánica. El tratamiento térmico puede hacerse mediante la inyección de aire caliente en un desecador industrial o aprovechando el calor solar en aquellas regiones donde el clima lo permita. En este caso, basta con expandir el material sobre una superficie lisa de hormigón, formando una capa que no supere los 5-7 cm de grosor. Es necesario voltear el alimento 2-3 veces al día para que se deshidrate de forma homogénea hasta alcanzar un contenido de materia seca con el que la actividad biológica sea básicamente nula, como ocurre con la paja o el salvado de cereal (Muck, 1988).

1.3.2.2. Ensilado

Definición

El ensilado es un método de conservación de forraje húmedo que está ampliamente extendido por todo el mundo, alcanzándose a principios de la década de los 90 una cifra de 200 millones de toneladas de MS de vegetales almacenados de esta forma entre Europa Occidental y Estados Unidos (Wilkinson, 1988; USDA, 1991) y, en 2019 en España, una cantidad de más de 7 millones de t de forrajes ensilados (MAPA, 2019). El ensilado consiste en una fermentación natural bajo condiciones de anaerobiosis que llevan a cabo bacterias ácido-lácticas, que transforman carbohidratos solubles o azúcares en ácidos orgánicos, principalmente ácido láctico. Como consecuencia, el pH del material vegetal se reduce y se inhibe el crecimiento de microorganismos perjudiciales, obteniendo un alimento que conserva la humedad y las características nutritivas (Weinberg y Muck, 1996).

Condiciones para un buen ensilado

Una correcta fermentación láctica necesita de tres factores: anaerobiosis, sustrato rápidamente fermentable por bacterias ácido-lácticas (principalmente mono- y disacáridos) y una población suficiente de este tipo de bacterias. La ausencia de aire en el silo viene marcada tanto por factores que dependen del material vegetal, como el tamaño de corte o el nivel de humedad (si es escasa, el material vegetal es difícil de apisonar), o la técnica de ensilado, como el nivel de prensado y estanqueidad del silo (Noguer y Valles, 1977). La cantidad necesaria de sustrato fermentable depende de la capacidad tampón y MS del material vegetal. A mayor capacidad tampón, mayor resistencia se opone a la bajada de pH, por lo que es necesario una cantidad mayor cantidad de azúcares que sean fermentados para producir más ácido (Melvin, 1965). Por otro lado, la MS afecta negativamente a la actividad microbiana, por lo que altas cantidades de azúcares en ensilados con alto contenido en MS resulta ineficaz debido a que la actividad de bacterias ácido-lácticas está limitada (Muck y Speckhard, 1984). Una población microbiana aproximada de $8 \log_{10}$ unidades formadoras de colonias (ufc) de bacterias lácticas/g de materia fresca es suficiente para asegurar un rápido descenso del pH y una buena calidad del ensilado (Muck, 1988). Los géneros más comunes de bacterias ácido-lácticas que producen ácido láctico como metabolito principal de la fermentación de azúcares son *Lactobacillus*, *Pediococcus*, *Leuconostoc* y *Streptococcus* (McDonald, 1981). Para favorecer una correcta calidad del ensilado y reducir al máximo las pérdidas de MS, las bacterias homofermentativas son las idóneas para tal propósito, pues solo producen ácido láctico como resultado de la fermentación de los azúcares, el cual tiene un potencial mayor para reducir el pH que el resto de ácidos (McDonald,

1981). Sin embargo, de forma natural en el material vegetal se encuentra una mezcla de bacterias ácido-lácticas homo- y heterofermentativas. Mientras que con la homofermentación de la glucosa en ácido láctico se obtienen unas pérdidas de MS y energía de 0 y 0,7%, respectivamente, con la heterofermentación de glucosa en ácido láctico, etanol y CO₂, las pérdidas son del 24% de MS y del 1,7% de energía (McDonald, 1981), por lo que la homofermentación resulta más eficiente y produce menos gases de efecto invernadero.

La actividad proteolítica se reduce considerablemente cuando el pH alcanza un valor cercano a 4, aunque nunca llega a desaparecer (Muck, 1988). En estudios con ensilados de maíz y alfalfa (Bergen *et al.*, 1974; Muck, 1987) se comprobó que en el primer día de ensilado era cuando se daba mayor actividad proteolítica, disminuyendo esta tras cinco días de ensilado. Por lo que las medidas encaminadas a reducir este proceso metabólico deben ser efectivas en el momento del ensilado o poco tiempo después. Para aquellos ensilados considerados como húmedos (MS<50%), como tal es el caso de los subproductos agroindustriales, el descenso agudo del pH suele darse en los primeros 5 días.

Otro factor que influye en la calidad del ensilado es la temperatura que se alcanza en el interior del silo. Estudios con alfalfa ensilada a diferentes temperaturas indican que cada 10 °C de incremento de temperatura, la actividad proteolítica era significativamente mayor y aumentaba el contenido en sustancias nitrogenadas no proteicas (Muck *et al.*, 1987). Una forma de prevenirlo es asegurar un llenado rápido de los silos y un buen sellado.

Un nivel óptimo de humedad es necesario para un deseable crecimiento de la población de bacterias lácticas y un nivel de viabilidad de estas que lleve a cabo el metabolismo de azúcares y la producción de ácido láctico, de forma que se reduzca el pH y se evite la proliferación de microorganismos de putrefacción. En función de las ecuaciones de regresión de Leibensperger y Pitt (1987) a partir de los datos de Wieringa (1957) y Greenhill (1964), en los ensilados en torno a 300 g/kg de MS haría falta que el pH descendiera a 4,5 para detener la actividad clostridia. El correcto nivel de humedad también minimiza la producción de efluentes y, de esta forma, la pérdida de nutrientes (McDonald *et al.*, 1991; Gebrehanna *et al.*, 2014). Respecto a esto, Muck (1990) estableció un contenido en MS óptimo entre 40-55% para una correcta fermentación en silos torre de alfalfa, sin que se dieran problemas de sobrecalentamiento del material vegetal. En el caso de los silos trinchera, se permite que el contenido en MS sea inferior, del orden de un 25% (Noguer y Valles, 1977).

A través de la utilización de ingredientes que sirven como coadyuvantes, se puede mejorar la calidad del ensilado. En cuanto a reducir la porosidad del material vegetal, se han utilizado como coadyuvantes harina de maíz o pulpa de cítricos, que ocupan los poros de aire que quedan tras la compactación al inicio del ensilado, evitando así la acumulación de O₂. Esto provoca la reducción de la respiración celular, disminuyendo la generación de calor, y acelera la fermentación anaeróbica y la caída del pH del silo (Lee *et al.*, 2020; Sibanda *et al.*, 1997). Un rápido descenso del pH y una reducción en el incremento de la temperatura del silo minimiza la proteólisis, evitando de esta forma la pérdida de nutrientes (Muck, 1988).

Fases del ensilado

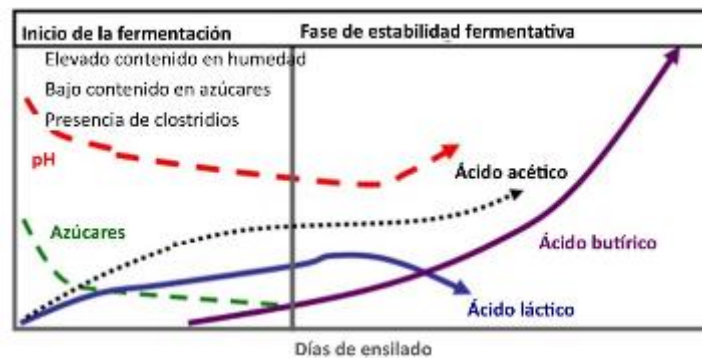
- A) Fase aeróbica en campo: Tras la cosecha, en las plantas se mantiene la respiración celular que metaboliza los azúcares y carbohidratos de reserva en CO₂ y agua (Muck *et al.*, 2003). De la misma forma, la actividad proteolítica de la planta continúa, hidrolizando proteínas en aminoácidos y péptidos. Dependiendo de las condiciones

climatológicas y del tiempo de exposición en el campo, la pérdida de estos nutrientes podrá ser más acentuada. Es por ello que el ensilado inmediato tras el corte de la planta minimiza las pérdidas de nutrientes. Sin embargo, cuando el contenido en humedad es muy alto, en ocasiones conviene alargar un poco la fase aeróbica en campo con el objetivo de deshidratar parcialmente el material a ensilar, inhibiendo de esta forma el crecimiento de clostridios y enterobacterias, que provocarían una pérdida de nutrientes durante las fases siguientes (Muck *et al.*, 2003).

- B) Fase aeróbica *in silo*: Al comienzo del ensilado, algo de O₂ permanece retenido junto con el alimento. Esta cantidad dependerá del diseño del silo, estructura, tamaño de corte del material vegetal y del nivel de compactación del silo. Un buen sellado del silo favorecerá un rápido consumo del O₂ durante la respiración celular (Muck *et al.*, 2003). Si el nivel de compactación o el sellado del silo es inadecuado, la fermentación aeróbica se prolongará en el tiempo, produciendo efectos adversos en contra de la calidad del ensilado: pérdida de MS por la fermentación de carbohidratos (McDonald, 1981), que provocará una reducción del contenido energético y limitará la fermentación anaeróbica, desacelerando la caída de pH (Ohyama *et al.*, 1975). Además, incrementará la temperatura del silo, lo cual conlleva una mayor proteólisis, debido a que se dan unas condiciones más favorables para el crecimiento de los clostridios (fermentación secundaria o clostridia) y las enterobacterias, y la formación de productos de Maillard cuando la temperatura supera los 60 °C, produciendo nitrógeno ligado a la fracción insoluble (Van Soest, 1982; Rooke y Hatfield, 2003). En esta fase también se da una fermentación acética llevada a cabo por bacterias coliformes aerobias, que producen ácido acético en el intervalo de temperaturas entre 18 y 25 °C cuando el pH aun no ha bajado de 5. Una fermentación acética de baja intensidad es recomendable para asentar las bases del siguiente paso en el ensilado, la fermentación láctica (Noguer y Valles, 1977).
- C) Fase de fermentación anaeróbica: Con la desaparición del O₂ y cuando el pH desciende por debajo de 4,2, se produce un rápido incremento de microorganismos con capacidad para liberar productos neutros y ácidos al medio, tal es el caso de las enterobacterias y las bacterias ácido-lácticas, las cuales son las más eficientes para reducir el pH del silo, tanto por el potencial acidificante del ácido láctico como por el menor gasto energético en la fermentación de los azúcares (Tabla 7). Los ácidos liberados por estos microorganismos reducen el pH del ensilado hasta valores comprendidos entre 3,2 y 3,8 (Weinberg y Muck, 1996), permitiendo la proliferación de otras bacterias ácido-lácticas más tolerantes, siempre que el sustrato (existencia de carbohidratos fermentables) no sea un factor limitante (Figura 16a). Cuando el sustrato es un factor limitante y el ritmo de fermentación es demasiado lento, el pH no disminuirá por debajo de 4,5, haciendo que la población de enterobacterias y clostridios reanude su crecimiento, con la consecuente actividad sacarolítica y proteolítica asociada a estos microorganismos (Rooke y Hatfield, 2003), produciendo ácido butírico, amoníaco y aminos, que reducen la ingestión *ad libitum* en rumiantes (Wilkins *et al.*, 1971; Figura 16b).



(a)



(b)

Figura 16. Evolución de varios indicadores de la calidad del ensilado a lo largo del tiempo en ensilados con buena calidad (a) y mala calidad (b) (adaptado de Kung, 2018).

- D) Fase de almacenamiento anaeróbico: Un silo se considera estable una vez que queda inhibido el crecimiento de bacterias ácido-lácticas, lo cual puede tardar entre 17 y 21 días en ensilados de forrajes convencionales (Noguer y Valles, 1972). Sin embargo, tras alcanzar este punto, pueden seguir dándose algunas fermentaciones llevadas a cabo por levaduras, que transforman el exceso de azúcar en etanol o, en el caso contrario que el contenido en azúcares sea escaso, las bacterias ácido-lácticas pueden transformar el ácido láctico en otros metabolitos (Rooke y Hatfield, 2003). Durante esta fase, es necesario asegurar que el silo permanezca estanco para evitar la entrada de O_2 , lo cual permitiría el crecimiento de microorganismos potencialmente patógenos.
- E) Fase aeróbica de apertura del silo: La apertura del silo conlleva la proliferación de microorganismos aerobios, principalmente levaduras y bacterias ácido-acéticas, seguidas de bacilos, mohos y enterococos. Estos microorganismos fermentan los azúcares, ácido láctico y demás ácidos del silo en CO_2 , agua y calor, provocando una pérdida de MS y energía (Wilkinson, 1986). Un incremento elevado de temperatura indicará un fuerte deterioro del ensilado. Por otra parte, al reducirse los ácidos presentes, el pH se incrementará, favoreciéndose la proteólisis y la degradación de carbohidratos estructurales, reduciéndose la calidad del silo por cambios en la textura y decoloración, y aumentando la concentración de sustancias tóxicas, como micotoxinas (McDonald, 1981). Todo ello reduce la palatabilidad del alimento y, consecuentemente,

su ingestión (McDonald *et al.*, 1991). Es por ello que, una vez abierto el silo, se recomienda su uso en la mayor brevedad posible.

Tabla 7. Condiciones de pH y temperatura (°C) y gasto energético (cal/g de azúcar) de los procesos metabólicos del ensilado.

| Proceso metabólico | pH | Temperatura | Gasto energético |
|-----------------------|-----------|-------------|------------------|
| Respiración | > 3,5 | - | 4,0 |
| Fermentación acética | > 5 | 18 – 25 | 1,32 |
| Fermentación láctica | 4,2 – 3,2 | 16 – 65 | 0,16 |
| Fermentación butírica | 4,2 | 20 - 50 | 0,90 |

Noguer y Valles, 1977.

Calidad del ensilado

Para valorar la calidad final de un ensilado, se puede recurrir tanto a medidas organolépticas como de composición. Respecto a las primeras, el color del ensilado debe tener un tono más amarillento que el del material vegetal de partida, con olor a fruta o ligeramente agrio. Si el ensilado se ha pardeado y huele a tabaco o caramelo, esto será indicativo de que ha habido una aireación excesiva que ha provocado un aumento de la temperatura y pérdidas de nutrientes, pero sin peligro de toxicidad para los animales. Cuando ha ocurrido una fermentación acética intensa, los ensilados desprenden un olor a vinagre muy fuerte que puede reducir la palatabilidad e ingestión en los animales, llegando a niveles tóxicos si se administra en grandes cantidades. Por último, los ensilados que han sufrido una fuerte fermentación butírica por clostridios suelen ser de color oscuro y, en principio, inodoros o con ligero olor a amoníaco. Sin embargo, al frotar durante unos instantes una porción del ensilado, se desprenderá un olor repugnante.

Las medidas de los metabolitos de fermentación también sirven para valorar la calidad final de un ensilado (Tabla 8). Entre ellas también se incluyen el contenido en MS y pH. Un índice relativo a estos dos parámetros es la escala de puntos Flieg (Kilic, 1986), que otorga una puntuación entre 0-100 a los ensilados dependiendo de su calidad (Tabla 8):

$$\text{Puntos Flieg} = 220 + (2 \times \text{MS} (\%) - 15) - 40 \times \text{pH}$$

Una buena técnica de ensilado puede conservar el alimento perfectamente durante 3 años.

Tabla 8. Clasificación de la calidad de los ensilados en función de los niveles de metabolitos de fermentación.

| Calidad | Muy buena | Buena | Regular | Baja | Muy baja |
|-----------------------------------|-----------|-----------|---------|---------|----------|
| pH | 3,8 - 4,2 | 4,2 - 4,5 | 4,5 - 5 | > 5 | > 6,5 |
| Puntos Flieg | 80 - 100 | 60 - 80 | 40 - 60 | 20 - 40 | 0 - 20 |
| Ácido láctico | > 5% | > 3% | < 3% | < 3% | < 3% |
| Ácido acético | < 0,3% | < 0,5% | > 0,5% | > 0,5% | > 0,5% |
| Ácido butírico | < 0,1% | < 0,3% | > 0,5% | > 0,5% | > 0,5% |
| N-NH ₃ /N _T | < 5% | < 10% | < 12% | > 12% | > 12% |

Adaptado de Noguer y Valles, 1977.

1.3.3. Inclusión en dietas de rumiantes

1.3.3.1. Efectos sobre rendimiento productivo y calidad de la leche

Las referencias a los efectos de la inclusión de subproductos ensilados sobre la ingestión y producción lechera encontrados en la literatura son escasas y se deben fundamentalmente al nivel de inclusión utilizado, así como a la composición nutritiva del ingrediente de la ración que ha sido reemplazado (Arco-Pérez *et al.*, 2017) como, por ejemplo, la presencia de un alto nivel de polifenoles totales (PT) y taninos, los cuales pueden reducir la palatabilidad de la dieta o provocar alteraciones en la flora ruminal. Además, la formulación de las raciones que incluyen el subproducto en estudio de manera iso-energética e iso-proteica en comparación a la ración testigo también influye en el rendimiento productivo de los animales. A continuación, se exponen los resultados obtenidos del uso de algunos subproductos agroindustriales en la alimentación de rumiantes, destacando su efecto sobre la producción y calidad de la leche.

El primer grupo de estos subproductos corresponde a aquel formado por destríos en campo debido a que el producto cosechado no da la talla comercial o presenta anomalías que deprecian su valor en mercado. Tal es el caso de los destríos de zanahorias y patatas. En experimentos llevados a cabo en vacas lecheras donde se les suministraban más de 25 kg/día de zanahorias frescas, no se observaron efectos adversos sobre el rendimiento lechero (Morel d'Arleux, 1990; Car, 1985). Morel d'Arleux (1990) destaca la combinación de zanahorias, ricas en azúcares rápidamente fermentables, con fuentes fibrosas para prevenir acidosis y trastornos de diarrea, no superando la mezcla de zanahorias y concentrado más del 50% (sobre MS) de la ración. Además, sugiere hacer una introducción progresiva de este alimento en la dieta durante unos 8-10 días. En el caso de los pequeños rumiantes, este mismo estudio comprobó que cantidades de 5 kg/día (27% de la ración) y 2-4 kg/día eran bien aceptadas por ovejas y cabras lecheras, respectivamente, durante una lactación completa. En cuanto a la calidad de la leche, solo Fuller (2004) menciona el amarillamiento de la leche de vaca por el alto contenido de carotenos de las zanahorias cuando estas se utilizaron en la alimentación durante un uso prolongado. En cuanto al uso de los destríos de patatas, estos necesitan ser sometidos a un proceso térmico para mejorar la digestibilidad del almidón y aumentar su valor nutritivo. Ese calor necesario puede ser el generado por los procesos de fermentación durante el ensilado. De Boever *et al.* (1983) no observaron efectos adversos en vacas alimentadas con una cantidad comprendida entre 15-20 kg de patatas al día.

Otro grupo de subproductos lo constituyen aquellos compuestos por semillas o partes de estas. En un estudio realizado con ovejas, se llegó a incluir hasta un 50% de semillas de mango en la dieta (Göhl, 1982). Debido al alto contenido en taninos de las semillas, la ingestión de la dieta fue baja (1,2% del PV); sin embargo, se alcanzó una digestibilidad del 70% de la ración. La inclusión de un 18% de piel de almendras en sustitución del heno de alfalfa de la dieta de vacas Holstein redujo la producción lechera y contenido en proteína, debido al déficit de proteína que presentaba la dieta con subproducto (Williams *et al.*, 2018), poniendo de manifiesto la importancia de formular de forma equilibrada las dietas que incluyen subproductos agrícolas. La sustitución de un 36% de pulpa de remolacha por piel de avellanas no conllevó diferencias en el rendimiento lechero de ovejas Comisanas (Campione *et al.*, 2020). Además, disminuyó el recuento de células somáticas (RCS) debido a la mayor concentración de PT que tiene este alimento, los cuales tienen actividad anti-inflamatoria (Shabtay *et al.*, 2012). La utilización de bayas de mirto en la alimentación de ovejas Sardas ha reportado resultados diversos, desde la no observación de efectos sobre la producción de leche (Nudda *et al.*, 2017) hasta una reducción del rendimiento lechero (Nudda *et al.*, 2019). La discrepancia de resultados no reside en el

contenido en polifenoles de las bayas de mirto, pues fue el mismo para los dos estudios (28 vs. 22,6 y 44,3 g/kg MS), sino al mayor nivel de FND y taninos hidrolizables de la dieta del segundo experimento (Nudda *et al.* 2019), que redujo el consumo de alimento.

El siguiente grupo de subproductos lo forman los residuos forrajeros (hojas y tallos) de cultivos industriales. En un experimento realizado con vacas alimentadas con hojas de mandioca henificada (Wanapat, 2009), la ingestión y digestibilidad de este alimento fue alta, dado su moderado contenido en taninos condensados (3,9% MS). Según Reed (1995), un contenido de taninos condensados superior al 6%, reduciría la ingestión y la digestibilidad de la dieta, mientras que contenidos comprendidos entre 2 y 4% blindaría las proteínas de la dieta, protegiéndola de la degradación en el rumen y, por tanto, aumentando la cantidad total de proteína by-pass. Wanapat *et al.* (1997) indican que las hojas de mandioca en estadíos tempranos de madurez son las que presentan un contenido en taninos bajos y un nivel de PB más alto (25% MS). El empleo de hojas de mandioca henificadas entre 0,56 y 1,7 kg/animal y día en vacas lecheras redujo entre 0,1 y 1,6 kg (27-42%) la cantidad de concentrado diaria administrada a cada animal (Wanapat *et al.*, 2000a; Wanapat *et al.*, 2000b), suponiendo con ello un ahorro económico en los costes de alimentación. Este aumento de la inclusión de hojas de mandioca en la dieta no afectó a la producción de leche y mejoró la producción de leche corregida por grasa al 3,5% (LCG), así como el contenido en grasa y proteína. En cuanto a las hojas y tallos de plátano, estos tienen baja digestibilidad debido a que su contenido en taninos es del 8% (Marie-Magdeleine *et al.*, 2010). Su valor nutricional es comparable al de la paja de arroz o los cogollos de la caña de azúcar (Viswanathan *et al.*, 1989), dado su reducida concentración de PB (7,2%) y elevado contenido en FND (67,2%). En un estudio llevado a cabo por El-Ghani (1999), se consiguió reemplazar hasta un 50% del ensilado de maíz utilizado para alimentar búfalas por un ensilado compuesto por hojas de plátano y paja (75:25) tratado con melaza y urea para incrementar su valor nutricional, sin reducir la producción de leche. Respecto a la piel del plátano, su valor nutricional es similar al de la piel de mandioca, con un 10% de PB y un 63% de FND. Hernan Botero *et al.* (2000) reportaron una buena aceptación y alta palatabilidad de este subproducto en cebúes cuando fue incluido en niveles de 15 a 30% en la dieta. Además, se observó un incremento de la producción lechera en vacas lecheras cuando se introdujo en la dieta entre 14 y 21 kg/día (Dormond *et al.*, 1998).

El cultivo industrial de la caña de azúcar genera gran variedad de subproductos, desde los cogollos y hojas de la parte superior y las vainas y hojas del tronco, que respectivamente representan un 8% y 20% de la biomasa total de la planta, hasta los subproductos resultantes del proceso de extracción del azúcar (bagazo y melaza) (Lagos-Burbano y Castro-Rincón, 2019). Respecto a los residuos forrajeros (cogollos y hojas), la mayoría de los estudios que incluyen su utilización en alimentación de rumiantes destacan la suplementación con urea como fuente de N no proteico para mejorar el funcionamiento de la flora ruminal y aumentar la digestibilidad (Garriz y López, 2002; Sousa *et al.*, 2015). La digestibilidad de la fibra también puede mejorar añadiendo productos alcalinizantes (NaOH, KOH, Ca(OH)₂, NH₃, CaO), pero teniendo en cuenta los límites máximos recomendados para evitar toxicidades. Hill y Leaver (2002) observaron pérdidas de hasta el 30% de MS en el ensilado de caña de azúcar si no se añadían los aditivos antes mencionados, debido a una fuerte fermentación alcohólica. Los estudios realizados con caña de azúcar como alimento para ganado en diferentes países muestran que, si esta es suplementada convenientemente, la ganancia media diaria de peso y la producción de leche no se ven afectadas (Sierra, 1981; Gutiérrez *et al.*, 2014). Vacas Holstein alimentadas con una ración compuesta por ensilado de caña y ensilado de maíz (70:30) mejoraron la condición corporal y el índice de conversión (Reyes *et al.*, 2014). La misma raza de vacas, alimentadas con una dieta que

incluía un 63% de cogollos de caña de azúcar picados y tratados con una solución deslignificante y urea, disminuyeron la producción lechera en comparación con aquellas que recibieron una ración convencional (de 25 a 18,2 l/día), pero a cambio de menores costes de alimentación, los cuales se redujeron en un 59,8% (Timana *et al.*, 2017).

El bagazo de cerveza es otro subproducto comúnmente utilizado en las explotaciones de vacuno lechero debido a su alto contenido en proteína (253 g/kg; Rooke *et al.*, 2015). En varios estudios llevados a cabo en vacas Holstein con dietas que incluían bagazo de cerveza en niveles de inclusión entre 11 y 29% se observó una mayor ingestión de las dietas que incluían este subproducto, consiguiendo con ello una mayor producción lechera (hasta un 13% superior a la dieta testigo en Cozzi y Polan, 1994) y un contenido superior de proteína en leche, además de mayor nivel de urea en sangre y hematocrito (Polan *et al.*, 1985). En otros estudios, la inclusión de bagazo de cerveza no incrementó la ingestión de la ración, pero sí el PV (Rooke *et al.*, 2015) y la producción lechera (Imaizumi *et al.*, 2015) debido al mayor contenido proteico e interesante perfil de aminoácidos que presenta este subproducto, especialmente en relación al contenido de metionina, asociada a una proteína menos degradable a nivel ruminal.

El último grupo de subproductos es aquel formado por la pulpa de hortalizas y frutas generados en las industrias conserveras y de zumos. Estos subproductos son muy abundantes en el sur de la provincia de Alicante y Región de Murcia, por lo que han sido ampliamente utilizados por ganaderos de la zona. La pulpa de tomate, en sus formas fresca, ensilada o deshidratada, puede reemplazar el 50% del forraje (Caluva *et al.*, 2003). Belibasakis (1990) comprobó como la ingestión de alimento, salud y producción de vacas lecheras, con rendimientos de 26 kg/día, no se veían afectadas al sustituir un 36,5% del concentrado de la dieta por pulpa de tomate deshidratada. La combinación con ensilado de maíz no provocó diferencias de ingestión, producción y composición de la leche con vacas alimentadas solo con silo de maíz (Weiss *et al.*, 1997). Arco-Pérez *et al.* (2017) evaluaron la inclusión de 200 g/kg de ensilado de subproducto de tomate y de oliva, en sustitución del heno de avena, en cabras lecheras, no observando diferencias de ingestión, producción y RCS a corto plazo (3 días). A medio plazo (un mes), las cabras alimentadas con la dieta que incluía subproducto de tomate ensilado aumentaron su consumo y PV, lo cual contrasta con Molina-Alcaide *et al.* (2010) y Romero-Huelva *et al.* (2012), quienes no observaron diferencias en la ingestión en cabras lecheras alimentadas con *feed-blocks* que incluían un 12% de torta de oliva y de subproducto de tomate, respectivamente. Sin embargo, la alimentación con el *feed-block* de torta de oliva redujo la producción lechera (Molina-Alcaide *et al.*, 2010). Abdollahzadeh *et al.* (2010) sustituyeron un 15% del heno de alfalfa de la ración de vacas lecheras por una mezcla de subproducto de tomate y pulpa de manzana (ambos ensilados), observando un aumento de la producción lechera, pero sin cambios en la composición. Sin embargo, otros autores observaron como la inclusión de un 4,5% (Nudda *et al.*, 2019) y un 15 y 30% (Abbeddou *et al.*, 2015) de pulpa de tomate en dietas de ovejas redujo el contenido de proteína de la leche, debido a una reducción del aporte energético de la dieta y a la baja degradabilidad en rumen del subproducto de tomate. Por el contrario, la inclusión a largo plazo de los subproductos de tomate y oliva en el estudio de Arco-Pérez *et al.* (2017) causó una mayor ingestión de alimento y una deposición de reservas corporales,

Respecto a la pulpa cítrica, debido a su alta digestibilidad de materia orgánica (MO; 85-90%) y alto valor energético (2,76 – 2,90 Mcal EM/kg MS), se ha utilizado como sustituto de los cereales en la dieta de vacas lecheras de alta producción (dietas ricas en concentrados y bajas en forrajes), (Crawshaw, 2004) y de cabras Murciano-Granadinas (López *et al.*, 2014; Ibáñez *et al.*, 2016), donde se sustituyó un 60% del maíz y la cebada de la dieta, respectivamente, por pulpa

cítrica deshidratada, sin afectar a la ingestión de alimento, PV y rendimiento lechero. El alto valor energético de la pulpa cítrica no procede del almidón, como ocurre con los cereales, sino de los carbohidratos solubles, fibra digestible y pectinas. Debido a la presencia de limonina en las semillas y la piel, la cual confiere un sabor amargo a la pulpa cítrica, este subproducto ha de ser incorporado de forma gradual a las dietas, sin superar el 40% de inclusión, pues la palatabilidad se reduce en exceso (Bhattacharya y Harb, 1973). En este sentido, Williams *et al.* (2018), tras incluir un 13% de pulpa cítrica ensilada en la dieta de vacas Holstein, observaron que la ingestión disminuyó debido a que el subproducto tenía un alto nivel de humedad y un exceso de piel y albedo grueso que redujo su palatabilidad. Piquer *et al.* (2009) y Jaramillo *et al.* (2009) observaron lo mismo en ovejas Manchegas y cabras Murciano-Granadinas alimentadas con un 39% y 30%, respectivamente, de mandarinas de destrío (siendo las dietas iso-energéticas e iso-proteicas en comparación a la dieta testigo utilizada en cada estudio). En ambos estudios, el elevado volumen de la dieta que contenía las mandarinas redujo la ingestión de los animales, provocando un menor contenido en grasa y MS de la leche de las cabras (Jaramillo *et al.*, 2009). Fegeros *et al.* (1995) y Assis *et al.* (2004) utilizaron niveles de inclusión del 30% en ovejas y del 20% en vacas, respectivamente, sin observar cambios en el consumo de alimento, digestibilidad, producción y composición de la leche. Sin embargo, otros autores observaron cambios en la producción y composición de leche de pequeños rumiantes cuando se utilizaba pulpa cítrica, como Salvador *et al.* (2014) en cabras lecheras, donde la inclusión de pulpa de naranja afectó a la calidad de la leche y la composición del queso, mejorando sus características sensoriales e incrementando la aceptación del consumidor; o Liotta *et al.* (2019) con ovejas Comisanas alimentadas con un 10% de pulpa cítrica pelletizada junto con melaza, donde se produjo un incremento de la producción y contenido en grasa. Otro ejemplo es el de Volanis *et al.* (2006), donde ovejas alimentadas con un 70% de ensilado de pulpa cítrica al final de la lactación incrementaron el contenido en grasa de la leche. Finalmente, cabe decir que un nivel de inclusión elevado de pulpa cítrica en la dieta conlleva el riesgo de acidosis y paraqueratosis de las paredes ruminales, reduciendo con ello también el rendimiento de los animales (Martínez-Pascual y Fernández-Carmona, 1980).

Según el estudio de Nudda *et al.* (2019), la utilización de un 4,5% de orujo de uva en la alimentación de ovejas incrementó la producción de leche debido a que la dieta que incluía orujo de uva tenía un elevado contenido de PT (1,48%), lo cual habría favorecido que la proteína sea degradada en menor medida en el rumen por la formación de complejos insolubles con éstos (Patra y Saxena, 2011) y, por tanto, se digiera una mayor cantidad de proteína en el intestino delgado. Sin embargo, en otros estudios no se han observado efectos sobre el rendimiento lechero de ovejas al incluir este subproducto en la dieta de los animales (Nudda *et al.*, 2015; Manso *et al.*, 2016; Alba *et al.*, 2019). La pulpa de manzana se ha utilizado de forma ensilada en alimentación de vacas Holstein con un nivel de inclusión del 30%, sin cambios sobre la producción y composición de la leche, aunque mejorando el índice de conversión cuando la dieta solo incluía un 15% de este subproducto (Ghoreishi *et al.*, 2007). El subproducto de piña procedente de la industria conservera ha sido utilizado como reemplazo parcial o total del forraje en dietas de vacas, ovejas y cabras, dada su alta palatabilidad y digestibilidad de la MO (73-75%) (Müller, 1978). Sruamsiri (2007) no observó efectos sobre la producción lechera de vacas cuando se incorporó una mezcla del subproducto de piña con paja de arroz en la ración de los animales. Respecto al efecto de la inclusión de subproductos en la dieta de rumiantes sobre la concentración de urea en leche, no se encuentra demasiada información en la bibliografía. Solo Nudda *et al.* (2017), Wang *et al.* (1996) y Toral *et al.* (2011) en ganado ovino

evidencian un nivel inferior de urea en leche debido a la reducción de la degradabilidad de las proteínas dietarias en el rumen a causa de los polifenoles.

1.3.3.2. Efectos sobre el perfil lipídico de la leche

En los últimos años, la investigación en la mejora de la calidad nutricional de la leche se ha enfocado hacia el incremento del contenido de los ácidos grasos con propiedades beneficiosas en la salud humana, tales como los ácidos grasos de cadena ramificada e impar (OBCFA), que son biomarcadores potenciales de la actividad ruminal, ya que van adheridos a los lípidos sintetizados por las bacterias ruminales y cuentan con actividad potencial contra enfermedades metabólicas (Pfeuffer y Jaudszus, 2016). Otros ácidos grasos de relevancia son el ácido ruménico (también conocido como CLA C18:2, cis9, trans 11), con efectos beneficiosos contra enteropatías (Bergamo *et al.*, 2016), aterosclerosis (Bachmair *et al.*, 2012), cáncer (Lu *et al.*, 2015) e inflamación (Mollica *et al.*, 2014), el ácido vaccénico (C18:1, trans 11) y la mejora del ratio de ácidos n6/n3 (Nudda *et al.*, 2020).

La cantidad y calidad de la grasa de la leche de los pequeños rumiantes es afectada principalmente por la dieta (Ferlay *et al.*, 2017). Los PUFA ingeridos en la dieta son biohidrogenados por las bacterias ruminales y transformados en los ácidos ruménico y vaccénico (Kepler *et al.*, 1966). Parte de ese ácido vaccénico no es metabolizado en ácido esteárico (C18:0) y es reducido por la acción de la enzima Δ^9 -desaturasa en CLA en la glándula mamaria (Figura 17). Además, los PUFA n3, como el ácido α -linolénico (C18:3n3), son importantes desde el punto de vista de la prevención de enfermedades cardiovasculares (Ganguly *et al.*, 2018) y la mejora de desórdenes metabólicos (Yamagishi *et al.*, 2017). A este respecto, Ulbricht y Southgate (1991) definieron dos índices acerca de la calidad de la grasa dietaria sobre la salud cardiovascular en humanos. El Índice de aterogenicidad (IA) se relaciona con la habilidad de adhesión de los lípidos a las células del sistema circulatorio e inmunitario, mientras que el Índice de trombogenicidad (IT) indica la tendencia a formar trombos en los vasos sanguíneos. Es por ello que la investigación en materia de calidad de leche va enfocada a reducir esos índices, mediante la reducción de ácidos grasos saturados (SFA) e incremento de monoinsaturados (MUFA) y PUFA, especialmente CLA y ácidos n3.

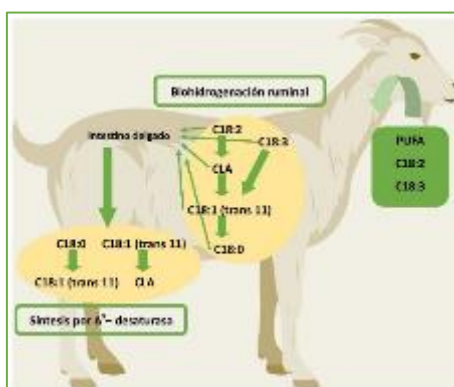


Figura 17. Metabolismo de los ácidos grasos poliinsaturados en los rumiantes (adaptado de Ferlay *et al.*, 2017).

A continuación se detalla el modo de acción en que la inclusión en la dieta de algunos subproductos agrícolas ha modificado el perfil lipídico de la leche de los rumiantes. La inclusión de 200 g/kg, tanto del ensilado de subproducto de tomate como del de oliva, en sustitución del

heno de avena de la ración, provocó un incremento del 188% en el ácido vaccénico, del 32% del ácido ruménico y del 38% del CLA total en la leche de cabras Murciano-Granadinas (Arco-Pérez *et al.*, 2017). De igual forma ocurrió con el subproducto de tomate en ovejas, donde se observó un incremento del 43,3% (Abbedou *et al.*, 2015) y del 19,8% (Romano *et al.*, 2010) en el CLA total de la leche, o con los *feed-blocks* de torta de oliva (Molina-Alcaide *et al.*, 2010) y de subproducto de tomate (Romero-Huelva *et al.*, 2012) en un nivel de inclusión de 12% en cabras lecheras, encontrando que la leche presentó mayor nivel de ácidos vaccénico y ruménico que las alimentadas con la dieta convencional. A largo plazo, las cabras del estudio de Arco-Pérez *et al.* (2017) aumentaron su peso corporal por una elevada deposición de reservas, lo cual hizo que también se incrementaran los niveles de SFA y el IA (Nogalski *et al.*, 2012) y se redujeran los de oleico, ruménico, MUFA y CLA total.

Estudios con pulpa cítrica arrojan diferentes resultados, como un mayor contenido en CLA en quesos de ovejas Comisanas alimentadas con un 10% de pulpa cítrica pelletizada (Liotta *et al.*, 2019), o un mayor contenido en anteiso C15:0 (de la familia de los OBCFA) en leche de vacas alimentadas con un 13% de pulpa cítrica ensilada (Williams *et al.*, 2018). Ibáñez *et al.* (2016) observaron una reducción del C15:0 (lo cual es indicador de un efecto negativo sobre el metabolismo de las bacterias ruminales y de la actividad fermentativa), ácido linoleico y PUFAs totales en cabras Murciano-Granadinas alimentadas con un 60% de pulpa cítrica deshidratada. Sin embargo, los contenidos de C16:0, C17:0, ácidos linolénico y vaccénico aumentaron por una mayor movilización de reservas debido a que la dieta no cubrió las necesidades en energía de los animales (Jorjong *et al.*, 2015). De esta forma, queda patente de nuevo la importancia de que las dietas que incorporan subproductos estén bien balanceadas y cubran todas las necesidades de los animales.

La piel de algunos frutos secos también se ha utilizado para modificar el perfil lipídico de la leche. Williams *et al.* (2018) como la inclusión de un 18% de piel de almendras en sustitución de la alfalfa de la ración de vacas lecheras dio lugar a un mayor nivel de OBCFAs, como C15:0 y anteiso C15:0, mientras que los contenidos de otros OBCFA, como C17:0, anteiso C17:0 e iso C17:0, además de los ácidos linoleico, linolénicos y PUFAs se redujeron. Campione *et al.* (2020) utilizaron la piel de avellanas para sustituir la pulpa de remolacha de la dieta de ovejas Comisanas en un 36%, observando con ello un incremento de MUFAs y ácidos vaccénico y ruménico y una reducción de los SFA debido a al mayor contenido en PT de la dieta que incluía la piel de avellanas (48,2 g/kg).

Un alto contenido de polifenoles en la dieta puede tener efectos anti-nutritivos, como una menor digestibilidad de la proteína y los carbohidratos y una reducción de la palatabilidad del alimento, lo que provocaría una menor ingestión y producción de leche (Jöbstl *et al.*, 2004). Por otro lado, cantidades moderadas de PT (entre 3 y 40 g/kg de MS) son capaces de modular el metabolismo ruminal de los lípidos, mediante la reducción o inhibición de la biohidrogenación de PUFAs llevada a cabo por la microbiota ruminal, y con ello, modificar el perfil lipídico de la leche (Correddu *et al.*, 2019). En términos generales, la acción de los polifenoles sobre los PUFAs a nivel ruminal puede incrementar los contenidos en leche de los ácidos linoleico y α -linolénico, especialmente este último, mediante la inhibición del último paso de la biohidrogenación ruminal de los PUFA, es decir, la reacción enzimática de reducción del ácido vaccénico a esteárico, aumentando con ello el contenido total de PUFAs en leche, con el consiguiente efecto beneficioso sobre el valor nutricional. También, se observa una tendencia general de que la inclusión de cantidades moderadas de PT incrementa el contenido de MUFAs en detrimento de los SFAs (Nudda *et al.*, 2020). Abbedou *et al.* (2011) y Razzaghi *et al.* (2015) observaron como

la inclusión de hojas de olivo en la dieta de ovejas y semillas de granada, piel de pistachos y pulpa de tomate en dietas de cabras, respectivamente, con un contenido de PT comprendido entre 3,2 y 22,5 g/kg de MS, provocaron un incremento del nivel de PUFAs en la leche, concretamente de los ácidos ruménico (365%), vaccénico (93%), linoleico (6%) y α -linolénico (197%) y una reducción moderada de los SFA (-2%), en comparación a la leche de los otros animales que no habían recibido la suplementación con estos subproductos.

Por otra parte, como se indica en la Figura 17, la concentración de los PUFAs en la leche no solo depende de los procesos de biohidrogenación llevados a cabo en el rumen, sino que también de la actividad de la enzima estearil-CoA 9-desaturasa, la cual interviene en la glándula mamaria para convertir el ácido esteárico (C18:0) en oleico (C18:1 cis9) y el ácido vaccénico (C18:1 trans 11) en ácido ruménico (CLA C18:2 cis9, trans 11), siendo la concentración de PUFAs y de taninos de la dieta factores importantes que afectan al modo de acción de esta enzima (Vasta *et al.*, 2009).

1.3.3.3. Efectos sobre niveles plasmáticos

Los datos hematológicos sirven para evaluar el estado sanitario de los animales.

Dietas con altos contenidos en grasa incrementan los niveles de colesterol en sangre debido a que se da una mayor demanda de este componente plasmático para metabolizar la grasa, es decir, para su digestión, absorción y transporte (Douglas *et al.*, 2004). Esto fue lo que observaron Faccenda *et al.* (2020) y Razzaghi *et al.* (2015) al emplear, respectivamente, bagazo de cerveza deshidratado en vacas Holstein y pulpa de semillas de granada y pulpa de tomate en cabras Saanen. Con el bagazo de cerveza se incrementó el contenido de urea plasmática debido a que los carbohidratos del bagazo de cerveza son más difíciles de fermentar que los del maíz, lo que causó un déficit de energía con la dieta que incluía este subproducto (241 g/kg MS; Faccenda *et al.*, 2020). Ante un balance energético negativo, la flora ruminal no es capaz de sintetizar toda la proteína microbiana y el excedente de N amoniacal es convertido en urea en el hígado (Santos y Pedroso, 2011). Lo contrario sucedió en Ibáñez *et al.* (2016) al alimentar cabras Murciano-Granadinas con pulpa cítrica, debido a que la alta tasa de fermentación de los carbohidratos de la naranja incrementaron la actividad ruminal para la síntesis de proteína microbiana (Spek *et al.*, 2013).

Sin embargo, es común que los niveles de urea desciendan al emplear subproductos de frutas y verduras en la dieta, ya que la mayoría están constituidos por la piel, pulpa o semillas, que contienen elevados contenidos en PT, en especial taninos, los cuales reducen la digestibilidad de la proteína en el rumen al formar complejos indegradables (Patra y Saxena, 2011; Ghasemi *et al.*, 2012). Tales fueron los casos de Razzaghi *et al.* (2015) y Sedighi-Vesagh *et al.* (2015), donde observaron una reducción de urea en sangre al incluir cáscara de pistacho en la dieta de cabras Saanen. Previamente, Ghaffari *et al.* (2013) y Shakeri *et al.* (2012) comprobaron lo mismo con subproducto de pistacho en moruecos Baluchi y terneros, respectivamente; Romero *et al.* (2000) con moruecos Manchegos alimentados con hojas de vid, Fernández *et al.* (2012) con ovejas alimentadas con quebracho y Ferreira *et al.* (2017) con vacas alimentadas con diferentes dosis (200, 400 y 600 g/kg) de torta de licuri (*Syagrus coronate*), semilla utilizada para la producción de biodiesel.

Respecto a la glucosa, las referencias encontradas en la bibliografía apuntan a un descenso generalizado cuando se emplean subproductos agrícolas en la dieta, aunque por causas distintas. En el experimento realizado por Sedighi-Vesagh *et al.* (2015) con cabras Saanen alimentadas con un 32% de cáscara de pistacho en la dieta, la glucosa se redujo debido al alto

contenido en taninos, que causó tanto una menor digestibilidad de los carbohidratos en el rumen (Barry y Manley 1986) como una menor producción de ácidos grasos volátiles (AGV), precursores de la glucogénesis (Makkar, 2003). Por otro lado, Flores-Fernández señala la mejora de metabolismo de la glucosa debido a una mayor sensibilidad de la insulina en aquellas dietas que incluyen *Citrus spp.* Tal fue el caso de Guzmán *et al.* (2020) y Alnaimy *et al.* (2017), quienes observaron una reducción de los niveles de glucosa en cabras Payoyas y terneros, respectivamente, al incluir en la dieta de los animales pulpa cítrica deshidratada. Sin embargo, en el caso de Ibáñez *et al.* (2016), la dieta que incluía pulpa de naranja causó un balance energético negativo, por lo que se redujo la glucosa y se movilizaron reservas lipídicas corporales, incrementando los niveles de β -hidroxibutirato (BHB) y ácidos grasos no esterificados (NEFA) en sangre, estos últimos necesarios para ser metabolizados en el hígado y producir energía.

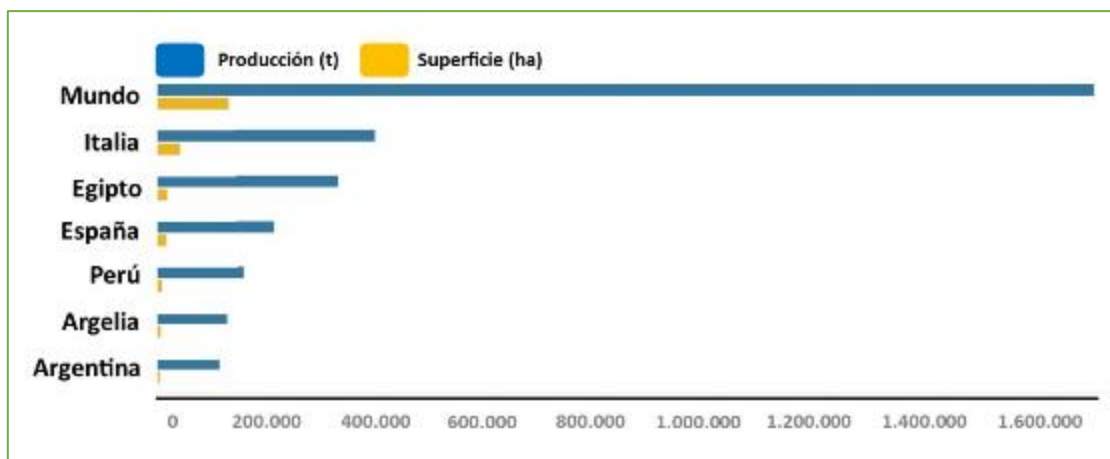
1.4. Planta y subproducto de alcachofa, subproducto de brócoli y otros subproductos de la familia de las Brassicáceas

1.4.1. Generalidades y datos de producción

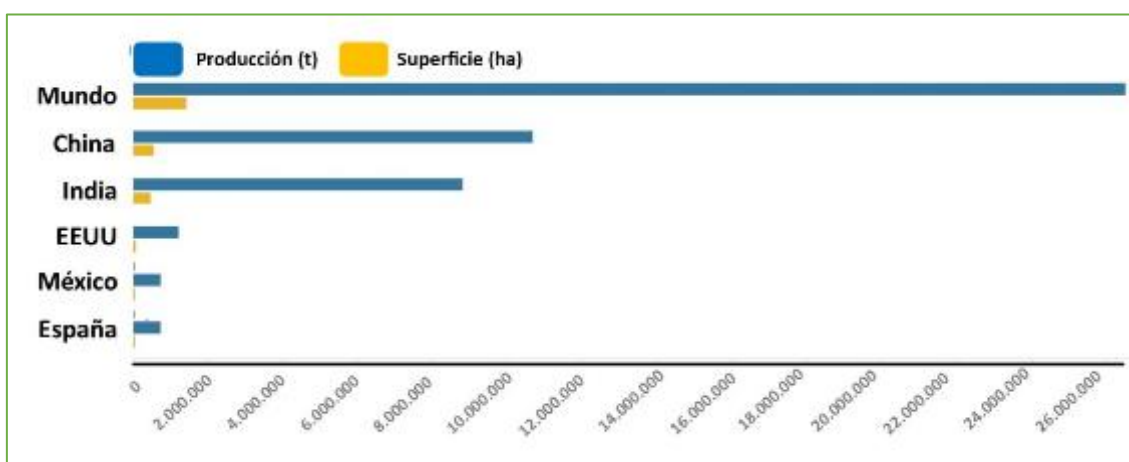
La alcachofa (*Cynara scolymus*) recibe otros nombres, como alcaucil o cardo. Es una hortaliza perteneciente a la familia de las Asteráceas, formada por un tallo perenne de 1 a 2 m de altura, con inflorescencias en capítulos apicales formando pseudo-corimbos, los cuales son consumidos en un estadio temprano de desarrollo. Es un cultivo de inviernos templados (10-25°C) que precisa suelos profundos y fértiles bien drenados. La siembra es directa y en líneas separadas entre 60 y 90 cm, siendo el tiempo de cosecha entre los meses de otoño y primavera (Baixauli, 2017; Hurrell *et al.*, 2009).

El brócoli (*Brassica oleracea* L. var. *Italica*), también conocido como albenga, brécol o brócoli, es una hortaliza de la familia de las Brassicáceas. Está formado por hojas grandes, carnosas y glaucas. La floración tiene lugar entre los meses de abril y septiembre, con una inflorescencia hipertrofiada, muy ramificada y pluriflora, con flores amarillas agrupadas en racimos, que forman una cabeza (pella) que, en un estado aun inmaduro, constituye la parte comestible por el ser humano. Es una planta de clima templado que presenta elevada resistencia al frío y al calor y se adapta a suelos muy diversos. El sistema de cultivo más utilizado es el de regadío, aunque también se suele cultivar en secano en aquellos climas con abundantes precipitaciones. En condiciones óptimas, se realiza siembra directa, aunque también puede hacerse siembra en plántulas y posteriormente el trasplante en campo. La densidad de plantación ronda las 30.000-50.000 plantas/ha y el ciclo vegetativo es de 100-130 días (Maroto y Baixauli, 2017; Hurrell *et al.*, 2009), siendo el tiempo de cosecha desde octubre a abril-mayo.

A nivel mundial, en 2018 el cultivo de alcachofa alcanzó una producción de 1.678.872 t en una superficie cultivada de 127.368 ha, siendo Italia el principal país productor (389.813 t), seguido de Egipto y España (323.866 y 208.463 t, respectivamente) (FAOSTAT, 2019). Como se observa en la Figura 18a, los países con mayor producción de alcachofa se encuentran en la cuenca mediterránea (Italia, Egipto, España, Argelia). Sin embargo, el cultivo de brócoli se encuentra especialmente asentado en el continente asiático, siendo primero China y después India los dos mayores productores de esta hortaliza, abarcando entre ellos dos más del 70% de la producción mundial, que en 2018 fue de 26.504.006 t con una superficie cultivada de 1.417.806 ha (FAOSTAT, 2019). Con 725.261 t, España se sitúa en el quinto puesto, como se observa en la Figura 18b.



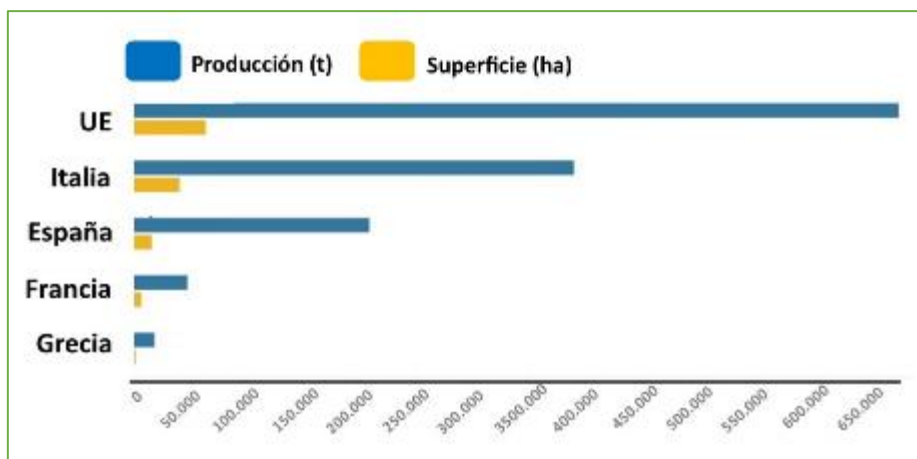
(a)



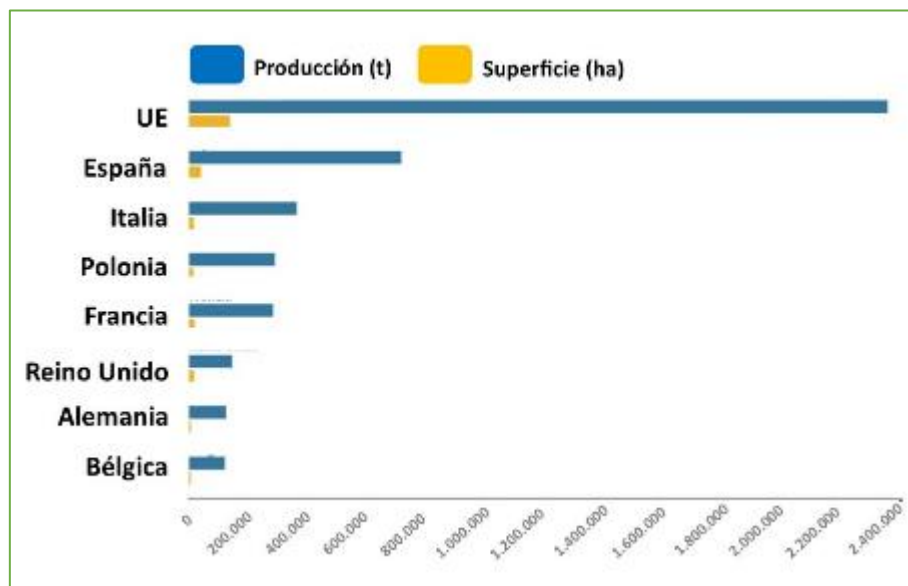
(b)

Figura 18. Producción y superficie cultivada a nivel mundial de alcachofa (a) y brócoli (b). (FAOSTAT, 2019. Datos de 2018).

A nivel europeo, la producción de alcachofa y brócoli en 2018 fue de 677.703 y 2.387.488 t, respectivamente, ocupando unas superficies de 63.462 y 139.479 ha (FAOSTAT, 2019). Dentro de la Unión Europea, en relación al cultivo de la alcachofa, después de Italia y España se sitúan Francia y Grecia (Figura 19a), incrementando así la importancia de la alcachofa como cultivo típicamente mediterráneo. Sin embargo, el brócoli presenta una mayor distribución, observándose mayoritariamente su producción en países mediterráneos (España, Italia y Francia), aunque también se encuentra presentes en latitudes más al norte, como se muestra en la Figura 19b.



(a)



(b)

Figura 19. Producción y superficie cultivada en la Unión Europea de alcachofa (a) y brócoli (b). (FAOSTAT, 2019. Datos de 2018).

La Figura 20 muestra la evolución de ambos cultivos de hortalizas en España en las últimas décadas. Mientras que la alcachofa alcanzó su pico de producción a principios de los 90, la producción y la superficie cultivada de brócoli no han dejado de incrementarse (FAOSTAT, 2019). Esto se debe a los nuevos hábitos de los consumidores, los cuales cada vez más están introduciendo el brócoli en la dieta. Además, en los últimos años se han desarrollado variedades de brócoli con una vida útil más larga, lo cual unido a una mejor conservación del brócoli congelado en comparación con la alcachofa, fomenta el incremento de las exportaciones (Fernández, 2019). La Región de Murcia concentra la producción de ambas hortalizas (Figura 21), alcanzando un 43% (90.450 t) y un 37% (209.122 t) de la producción nacional de alcachofa y brócoli, respectivamente, en la campaña 2018-2019 (MAPA, 2020). Respecto a la alcachofa, a la Región de Murcia se le une la Comunidad Valenciana, con un 28% de la producción total

(59.189 t). Por otro lado, dejando al margen la Región de Murcia, la producción de brócoli se encuentra más repartida por el territorio nacional. Destacan comunidades autónomas como la navarra, extremeña y valenciana, donde en esta última, todo el peso se lo lleva la provincia de Alicante (52.360 t).

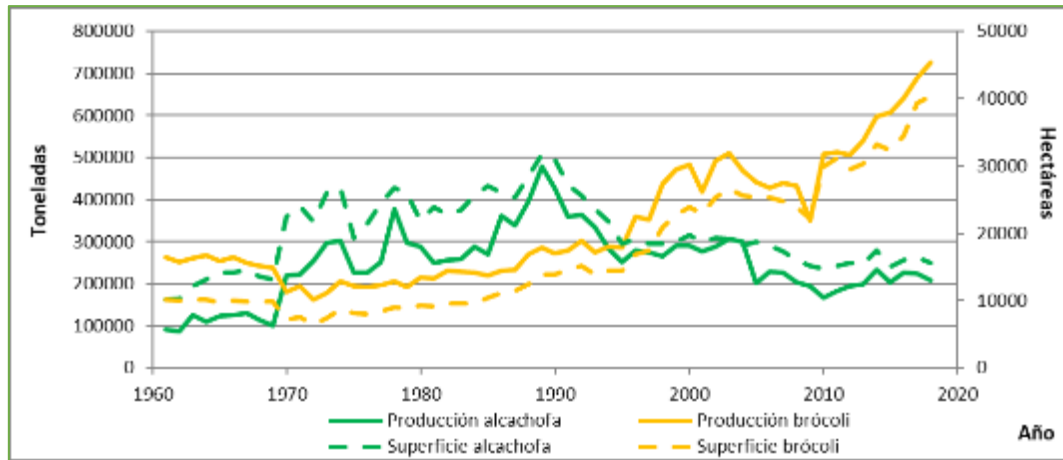


Figura 20. Evolución de la producción y superficie cultivada de alcachofa y brócoli en España (FAOSTAT, 2019).

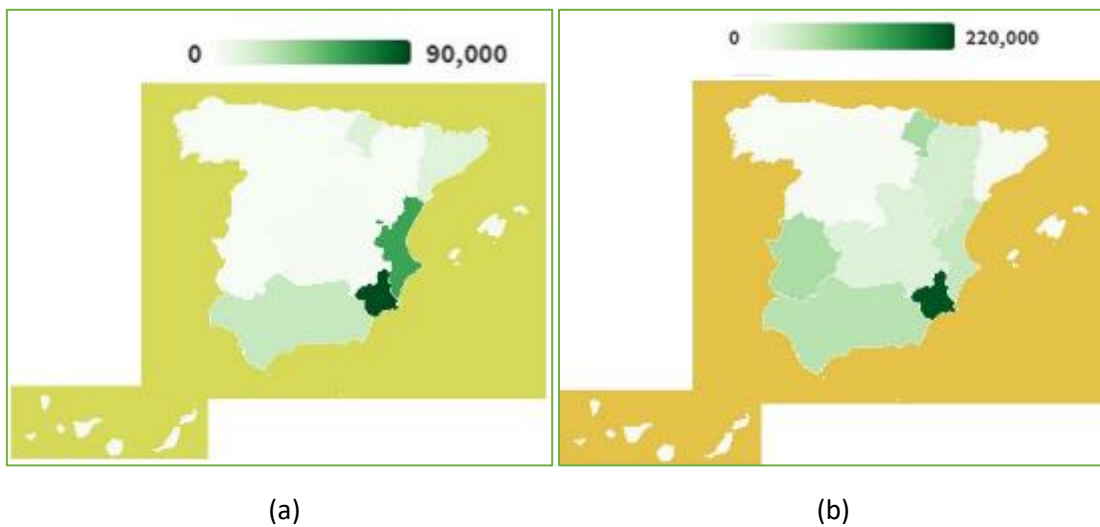


Figura 21. Distribución de la producción de alcachofa (a) y brócoli (b) en España durante la campaña 2018-2019 (MAPA, 2020).

La cosecha en campo y procesado en industrias hortofrutícolas de estos cultivos da lugar a grandes cantidades de subproductos. Según Lattanzio *et al.* (2009), solo un 20% del cultivo de la alcachofa es aprovechable para el consumo humano, estando el 80% restante constituido por el tallo, hojas, pedúnculos y brácteas externas del capítulo floral. Wernli y Tames (1989) estimaron en 11,1 t/ha de forraje verde en el cultivo de alcachofa, es decir, 449.053 t de planta de alcachofa disponibles teniendo en cuenta el dato de superficie cultivada en España en 2018 (40.437 ha; FAOSTAT, 2019). Por otro lado, en relación a los subproductos de alcachofa y de brócoli, Ros *et al.* (2012) contabilizaron que un 50% del corimbo de la alcachofa y 29.5% de la pella cosechadas

lo formaban brácteas y tallos y troncos, hojas e inflorescencias que no superan el control de calidad, por lo que de acuerdo a la cifra aportada por FAOSTAT (2019) sobre las toneladas de alcachofa y brócoli cosechados en 2018 en España (208.463 y 725.261 t, respectivamente), las cantidades de subproducto de alcachofa y de brócoli alcanzarían unas cifras de 104.232 y 213.952 t. Es por ello que, de acuerdo a la elevada disponibilidad de subproducto de estas dos hortalizas, tanto a nivel nacional como a nivel local, resulta interesante valorar su utilización en alimentación animal.

1.4.2. Antecedentes

La planta y el subproducto de alcachofa y el subproducto de brócoli se han utilizado en alimentación de animales desde siempre como una forma de abaratar costes, bien sacando a pastar a los animales a los campos para que comieran el rastrojo donde las alcachofas ya habían sido cosechadas, bien llevando los subproductos de alcachofa y brócoli de las centrales hortícolas a las granjas, como es el caso de las granjas de vacuno de la Región de Murcia (Martínez-Teruel *et al.*, 1998). Sin embargo, no ha sido hasta recientemente cuando se ha puesto el foco en el estudio científico de los efectos del consumo de estos subproductos en el rendimiento ganadero.

1.4.2.1. Composición de los subproductos de alcachofa y de brassicáceas

Las Tablas 9 y 10 recogen la composición nutricional de la planta y subproducto de alcachofa y el subproducto de brócoli, según diversos autores. En el caso de Cajarville *et al.* (1999), el subproducto utilizado fue el rastrojo del cardo (*Cynara cardunculus*), especie muy parecida a la alcachofa, en un estado fisiológico muy temprano en comparación al que alcanza la planta de alcachofa en el momento de cosechado de los capítulos florales. Es debido a ello a lo que se deben las diferencias en MS, PB, CNE y fibra con la planta de alcachofa utilizada por Wernli y Tames (1989), la cual presentó un estado vegetativo bastante avanzado y con un contenido en MS adecuado para ser ensilada ya que se limita la actividad negativa de las bacterias proteolíticas y la lixiviación de efluentes. Además, con un nivel de MS cercano al 30%, los ácidos resultantes del proceso de fermentación se encuentran más concentrados, por lo que el descenso de pH es más rápido y se alcanza antes la estabilización del ensilado (Ede y Blood, 1972). Por otro lado, la presencia de un 14% de CNE favorece la fermentación láctica (Kearney y Kennedy, 1962). El contenido en PB resulta inferior en comparación al de otros forrajes comúnmente utilizados en alimentación animal, como la alfalfa. El contenido en sílice es bastante alto, lo cual puede reducir el aprovechamiento de la fibra y la digestibilidad del forraje. Por último, mientras que la cantidad de Ca no es limitante, la planta de alcachofa es deficitaria en P.

Respecto al subproducto de alcachofa, la mayoría de la información recogida en la bibliografía se refiere al conjunto de brácteas externas y tallo de la alcachofa, tanto en fresco como tras haber pasado por un proceso de escaldado en la industria (Megías *et al.*, 2002; Meneses *et al.*, 2007 y Meneses *et al.*, 2020). Sin embargo, Molina-Alcaide *et al.* (2017) presentan la composición de alcachofas completas procedentes del destrío realizado en la industria. En general, la composición de este subproducto es similar a las vainas de judías o la corteza de espárragos, caracterizados por un contenido moderado de FND (400-600 g/kg) y una concentración de PB media-alta (160-200 g/kg; García-Rodríguez *et al.*, 2019), por lo que podría sustituir de forma parcial los forrajes y concentrados proteicos de la dieta, como la alfalfa o la torta de soja.

Tabla 9. Composición de la planta y subproducto de alcachofa (% MS), según varios autores.

| Ref. | PLANTA DE ALCACHOFA | | SUBPRODUCTO DE ALCACHOFA | | | | | |
|---------------|---------------------------------|-----------------------|-------------------------------------|------------------------------|------------------------------|------------------------------|------|---------------------------------|
| | Cajarville <i>et al.</i> (1999) | Wernli y Tames (1989) | Molina-Alcaide <i>et al.</i> (2017) | Meneses <i>et al.</i> (2020) | Meneses <i>et al.</i> (2007) | Megías <i>et al.</i> (2002) | | Salman <i>et al.</i> (2014) |
| Parte | Cardo | Planta de alcachofa | Destrío de alcachofa | Brácteas externas y tallos | | Brácteas y tallos escaldados | | Brácteas externas deshidratadas |
| MS | 13,05 | 31,5 | 17,8 | 16,6 | 29,7 | 16,6 | 20,5 | 92,3 |
| MO | 83,1 | | 77,1 | 94,3 | 94,2 | 95,0 | 94,3 | 87,3 |
| PB | 14,4 | 6,8 | 13,8 | 11,5 | 10,1 | 13,4 | 11,9 | 16,6 |
| EE | 2,105 | 2,1 | 1,38 | 2,07 | | | | 5,46 |
| FB | 12,9 | 27,3 | | | | 34,0 | 15,0 | 24,2 |
| CNE | 74,9 | 14 | | 11,6 | 5,61 | | | |
| FND | 25,15 | 51,2 | 51,4 | 43,1 | 42,9 | 57,4 | 47,5 | 42,9 |
| FAD | 16,35 | 38,5 | 34,0 | 31,1 | 30,0 | 39,9 | 36,8 | 30,0 |
| LAD | 3,025 | 11,3 | 15,4 | 10,5 | 10,1 | 8,2 | 7,2 | 10,1 |
| Cutina | 1,74 | | | | | | | |
| NFND* | 6,83 | | | | | 8,40 | 5,20 | |
| NFAD* | 2,28 | | | | | | | |
| Sílice | | 3,6 | | | | | | |
| Ca | | 1,8 | | | | | | |
| P | | 0,08 | | | | | | |

*Expresado en % nitrógeno total

MS: materia seca, MO: materia orgánica, PB: proteína bruta, EE: extracto etéreo, FB: fibra bruta, CNE: carbohidratos no estructurales, FND: fibra neutro detergente, FAD: fibra ácido detergente, LAD: lignina ácido detergente, NFND: nitrógeno ligado a FND, NFAD: nitrógeno ligado a FAD.

Tabla 10. Composición del subproducto de brócoli (% MS), según varios autores

| Ref. | SUBPRODUCTO DE BRÓCOLI | | | | | |
|------------|-----------------------------|---------------|------------------------------|-----------------------------|------------------------------------|------------------------------------|
| | Megías <i>et al.</i> (2014) | | Meneses <i>et al.</i> (2020) | Panwar <i>et al.</i> (2017) | Yi <i>et al.</i> (2015) | Muhammed Aziz <i>et al.</i> (2018) |
| Parte | Inflorescencias hervidas | Tallos crudos | Subproducto hervido | | Subproducto de brócoli pelletizado | Planta de brócoli |
| MS | 10,4 | 7,31 | 11,2 | 12,1 | 85 | 34,5 |
| MO | 94 | 89,2 | 99,4 | 94,2 | | 83,1 |
| PB | 31,4 | 14,5 | 31,1 | 27,2 | 21,6 | 22,4 |
| EE | | | 3,08 | 5,12 | | 3,6 |
| FB | | | | 7,99 | | 14,3 |
| CNE | 12,9 | 62,0 | 15,0 | | | |
| FND | 14,8 | 17,4 | 20,4 | 24,3 | 47,1 | |
| FAD | 12,4 | 12,9 | 13,1 | 21,9 | 38,3 | |
| LAD | 1,6 | 1,53 | 1,81 | 3,3 | | |
| Ca | | | | 4,45 | | |
| P | | | | 0,47 | | |

MS: materia seca, MO: materia orgánica, PB: proteína bruta, EE: extracto etéreo, FB: fibra bruta, CNE: carbohidratos no estructurales, FND: fibra neutro detergente, FAD: fibra ácido detergente, LAD: lignina ácido detergente.

Respecto al brócoli, el contenido en nutrientes varía dependiendo de la parte de la planta. Tal es el caso de los subproductos de brócoli del estudio de Megías *et al.* (2014) en cuanto al contenido de PB, el cual fue superior en las inflorescencias hervidas respecto a los tallos crudos. Otra causa que puede afectar al valor nutritivo es el proceso industrial al cual se hayan sometido los vegetales (Megías *et al.*, 2002), la época en que se ha dado la cosecha (Mirzaei-Aghsaghali y Maheri-Sis, 2008) o la variedad del cultivo (Panwar *et al.*, 2017). El alto contenido en PB, unido al bajo nivel de FB del subproducto de brócoli, aumentan el valor nutritivo de este alimento. Además, presenta un contenido en Ca superior al de los alimentos tradicionales (Panwar *et al.*, 2017). Wright y Lackey (2003) estimaron con ecuaciones de Petersen que el subproducto de brócoli podría reemplazar un 7,01 y 3,25% del contenido de soja y maíz, respectivamente, de la dieta de los rumiantes.

Otros subproductos de la familia de las Brassicáceas son los relativos a la coliflor y el romanesco. Según datos extraídos del estudio de Evan *et al.* (2020), del total de la cosecha de estas hortalizas, un 29,9% y un 21,8% corresponden, respectivamente, a las hojas y tallos de la coliflor, mientras que en el romanesco, las hojas representan un 45,8% y los tallos un 23,1%. Los subproductos de ambas brasicas tienen un alto contenido de proteína (superior al 20%) y un bajo nivel de FND (inferior al 30%), que a su vez presenta un bajo nivel de lignificación, lo que hace que estos subproductos sean altamente digestibles para los rumiantes.

1.4.2.2. Conservación de los subproductos de alcachofa y brassicáceas

El ensilado de planta de alcachofa presenta un contenido en carbohidratos superior al de los ensilados convencionales, el cual se sitúa entre el 1 y 3% Zhang *et al.*, 2018; Cai *et al.*, 1999). La MS de la planta de alcachofa en el momento de cosecha de los capítulos florales se sitúa en torno al 25-30%, lo cual beneficia el proceso de ensilado porque limita la actividad clostridial (Henderson, 1993). Wernli y Thames observaron en planta de alcachofa ensilada tras 240 días que la producción de AGV era menor respecto a los niveles usuales del resto de ensilados (5-10 %MS), debido a una escasa transformación de los carbohidratos. En estos mismos silos de 4,3 m³, también se observó unas pérdidas de MS entre el 9 y 22%, por descomposición de la capa superficial del silo, sin encontrarse pérdidas por lixiviación. En el estudio de conservación por ensilado de la planta de cardo (*Cynara cardunculus* L.) de Cajarville *et al.* (1999), se observó que la planta de cardo, de composición y características muy similares a las de la planta de alcachofa, presentó una fuerte capacidad tampón, es decir, una alta resistencia a la bajada de pH, superior a la que tienen la alfalfa y el trébol (McDonald *et al.*, 1988), lo cual supone una dificultad para el ensilado.

En aquellos casos en que el material a ensilar no tuviera un contenido de MS adecuado (25-30 %MS), un desecado previo es beneficioso para el proceso de ensilado (Cajarville *et al.*, 1999), el cual debe ser lo más rápido posible para reducir las pérdidas de carbohidratos. La planta de cardo fue fácil de compactar dada su alta densidad, lo cual favoreció el desarrollo de las condiciones adecuadas de anaerobiosis para la conservación del alimento. El pH se redujo hasta 4,5 y el proceso de ensilado fue homofermentativo debido a la alta producción de ácido láctico que tuvo lugar, respecto a la del resto de AGV, siendo la producción de láctico 5 veces superior a la de acético). Además, la desecación previa consiguió reducir las pérdidas de nutrientes, tanto por efluentes como por fermentación, siendo estas pérdidas de un 19,8% en el material sin desecar y de un 4,6% en el material vegetal sometido a 94 h de desecación.

El tamaño de partícula de la planta de alcachofa también afecta a su conservación mediante ensilado. Wernli y Thames (1989) comprobaron que un picado mayor de la planta de alcachofa

(tamaño de partícula entre 6,3 y 6,9 cm) frente a un picado inferior (14,0-14,4 cm) mejoraba el contenido de PB al final del ensilado y reducía el nivel de nitrógeno amoniacal, debido a que se producía más ácido láctico y el pH bajaba más rápidamente en el ensilado con menor tamaño de corte, inhibiendo de esta forma la acción de bacterias proteolíticas. Además, con menor tamaño de partícula se conseguía una mejor compactación del silo, dándose antes la anaerobiosis y limitando así la respiración y fermentación de nutrientes.

Estos mismos autores también evaluaron la aplicación de aditivos en el ensilado de planta de alcachofa. La adición de melaza limitó la proteólisis y redujo las pérdidas por fermentación y putrefacción, aunque tuvo poco efecto en la producción de ácido láctico, ya que el contenido en carbohidratos de la planta de alcachofa es suficiente para alcanzar el pico de producción de este ácido. Tanto el uso de melaza como de ácido fórmico tuvieron un efecto inhibitorio sobre la producción de ácido butírico.

El ensilado de brácteas de alcachofa es de índole heterofermentativa, debido a la alta producción de AGV que se genera durante el proceso de fermentación en el silo, tal y como observaron Meneses *et al.* (2020), indicando un valor de AGV total de 34,6 g/kg y un ratio AGV:láctico superior a 0,7 en las brácteas de alcachofa tras 24 días de ensilado a escala de laboratorio. Cabe decir que el tipo de formato de ensilado afecta a la composición del material vegetal, pues en varios estudios se encuentran diferencias notables en el pH, ácido láctico y AGV según el tipo de ensilado que hayan utilizado: microsilos a escala de laboratorio o silos trinchera (Meneses *et al.*, 2007; Muhammed Aziz *et al.*, 2018; Meneses *et al.*, 2020).

El subproducto de brócoli ensilado en condiciones de laboratorio (contenedores de polietileno de 12,5 L; Megías *et al.*, 2014) mostró un olor agradable y buenas características visuales. Sin embargo, la producción de efluentes fue alta hasta el día 24 del ensilado, siendo mayor en aquellos silos compuestos de tallos crudos de brócoli (286 mL/kg) que los que contenían inflorescencias y tallos pequeños hervidos, debido a un mayor contenido en humedad de los primeros. Esta alta producción de efluentes supone una pérdida de nutrientes y una contaminación medioambiental, y se da en los primeros días del ensilado como resultado de la alta intensidad de fermentación que caracteriza esa fase del proceso. McDonald *et al.* (1991) recomiendan un nivel de MS superior al 25% para controlar la producción de efluentes, tal y como comprobaron Muhammed Aziz *et al.* (2018) en ensilados de planta de brócoli que había sido desecada previamente hasta alcanzar un contenido en MS del 70%. En estos silos, las pérdidas de MS fueron entre un 4 y un 6%. Es por ello que los tallos de brócoli crudos necesitan de un proceso de desecado previo a ser ensilados. Muhammed Aziz *et al.* (2018) observó como en la planta entera de brócoli, pese a haber pasado por un proceso de desecado hasta alcanzar una MS del 70%, se reducía el contenido en proteína. La causa de ello fue que la desecación afectó en menor grado al interior de los tallos que al resto de la planta, siendo el contenido proteico de los tallos fermentado por la acción de enzimas y microorganismos. Los ensilados del estudio de Megías *et al.* (2014) mostraron una baja proteólisis y un reducido contenido en N amoniacal, al igual que en Meneses *et al.* (2020), pese a tener un reducido contenido en MS, aunque se dieron diferencias según el subproducto utilizado, siendo mayor el nivel de NH₃ en los silos con tallos de brócoli crudos que en los de inflorescencias hervidas. Tanto los valores de N amoniacal como de ácido láctico se situaron en el rango normal de valores para ensilados de buena calidad (Kung *et al.*, 2018). El pH final de ambos tipos de silos fue adecuado, similar a los de ensilados de forrajes con MS superior al 30% (Keady y Murphy, 1998).

En la calidad del ensilado también afecta el origen de la materia prima, ya que, dependiendo de su procedencia, pueden haberse dado condiciones de crecimiento y clima que afecten al

posterior valor nutricional del ensilado. Muhammed Aziz *et al.* (2018) observaron diferente puntuación en la escala Flieg con plantas de brócoli de diferentes orígenes.

1.4.2.3. Estudios con subproductos de alcachofa y de brasicáceas en rumiantes

La planta de alcachofa presenta una composición y un valor nutricional que la hacen apta para el consumo de la especie caprina. La planta de alcachofa ensilada puede manejarse fácilmente en microsilos (Hernández *et al.*, 1992) y se puede incorporar para dar volumen a la ración siempre y cuando vaya acompañada de una correcta suplementación, especialmente de proteína. Hernández *et al.* (1992) observaron un consumo diario de este tipo de ensilado en cabras de 50,2 g MS/kg PV^{0,75}, lo cual no fue suficiente para cubrir las necesidades de mantenimiento. Este nivel de ingestión fue ligeramente superior en Cajarville *et al.* (1998) con la planta de cardo ensilada (64 g MS/kg PV^{0,75}), ya que los animales en estudio fueron ovejas Manchegas. Sin embargo, los animales no mostraron rechazo por el alimento, pese a caracterizarse por su sabor amargo (Maymone y Battaglini, 1962), debido a que las cabras toleran mejor que otras especies de rumiantes este tipo de sabores (Goacher y Church, 1970). La digestibilidad *in vivo* del ensilado de planta de alcachofa (47,8%, según Hernández *et al.*, 1992) es similar a la del subproducto de tomate (48,2%, Fonollá *et al.*, 1988), lo cual es un valor considerablemente inferior si comparamos con otros subproductos agrícolas, como el subproducto de coliflor, con un 95% de digestibilidad *in sacco* de MS (Gasa *et al.*, 1986). Esta menor digestibilidad del ensilado de planta de alcachofa contrasta con la del cardo, la cual fue bastante superior en ovejas (78,3%, Cajarville *et al.*, 1998) debido a su mayor contenido en CNE y menor lignificación de las paredes celulares. La digestibilidad del alimento es la causante del contenido energético de este. Debido a ello, la concentración de energía digestible y EM del ensilado de planta de alcachofa fue considerablemente menor (1,72 y 1,40 Mcal/kg MS, respectivamente) que la del cardo (3,15 y 2,62 Mcal/kg MS), siendo necesario la incorporación de alguna fuente energética, como cereales o melazas, en el caso de la alimentación con planta de alcachofa. Respecto al efecto de este alimento sobre la calidad de la leche, en la bibliografía solo se encuentra el estudio de Muelas *et al.* (2017), en el que se observó que la inclusión de hasta un 25% de ensilado de planta de alcachofa no modificaba la composición y propiedades tecnológicas de la leche de cabras Murciano-Granadinas, ni afectaba a las cualidades sensoriales de la leche y yogur.

El ensilado de brácteas de alcachofa posee las condiciones necesarias para ser utilizado como ingrediente en las raciones de rumiantes. Meneses *et al.* (2020) observaron que la ingestión de una ración compuesta por 40% de alfalfa y 60% de brácteas de alcachofa ensiladas en machos de Murciano-Granadina fue de 30,2 g/kg PV^{0,75}, inferior a la observada con la ración de alfalfa como único ingrediente. Sin embargo, es importante señalar que estas raciones no estaban balanceadas. En otros estudios donde se midió el efecto de la inclusión de este subproducto en las raciones de pequeños rumiantes, Jaramillo *et al.* (2010) y Muelas *et al.* (2017) no observaron diferencias significativas en el rendimiento lechero, composición, valoración sensorial y aptitud tecnológica de la leche de ovejas y cabras alimentadas con raciones que incluían un 30 y 25%, respectivamente, de brácteas de alcachofa ensiladas.

El subproducto de brócoli presenta un alto valor nutritivo para rumiantes, comparable al de los subproductos fibrosos utilizados comúnmente en las raciones de estas especies, como la pulpa de remolacha y de cítricos (INRA, 2007). Esta característica le viene tanto por su alto contenido en proteína, vitaminas y polifenoles (Martínez-Villaluenga *et al.*, 2008), como por su alto valor de digestibilidad de MO, como observaron Meneses *et al.* (2020) en el ensilado de este subproducto (85,3%) y Panwar *et al.* (2017) en subproducto de brócoli fresco. Dado el elevado

contenido en PB y el reducido nivel de FND, el ensilado de subproducto de brócoli presenta mayor digestibilidad que la alfalfa (80,1 y 58,0%, respectivamente), aunque la ingestión en machos de Murciano-Granadina de una dieta compuesta por 55:45 de subproducto de brócoli ensilado y alfalfa fue menor que la de una ración compuesta por alfalfa como ingrediente único (29,1 y 56,0 g/kg PV^{0,75}; Meneses *et al.*, 2020). Este dato de ingestión contrasta con el de Panwar *et al.* (2017) en cabras Beetal en mantenimiento alimentadas con subproducto de brócoli fresco (57,5 g/kg PV^{0,75}) como ingrediente único de la ración, lo cual indica que este alimento es más palatable en fresco que ensilado, dado los metabolitos producidos durante la fermentación.

De Ruitter *et al.* (2009) establecen unas pautas en el manejo de esta especie vegetal, así como del resto de Brassicas, a la hora de alimentar el ganado. Fijan un nivel máximo de inclusión del 33 y 70% para vacas en lactación y secas, respectivamente, debido a que el bajo contenido en fibra y alta concentración de azúcares de estos vegetales podría conllevar problemas de acidosis y, por ello, es recomendable que los animales tengan acceso a una fuente fibrosa. Respecto a esto, Yi *et al.* (2015) fijaron un porcentaje máximo de inclusión de subproducto de brócoli pelletizado en dietas de vacas lecheras en un 20%, pues con este nivel de inclusión no se observaron diferencias con la dieta testigo en la producción y composición de la leche, salvo en la concentración de grasa, la cual fue 0,5 puntos porcentuales superior a la del testigo debido a un ligero mayor contenido de acetato en el subproducto de brócoli, el cual es un precursor de la síntesis de grasa (Sutton *et al.*, 1993). Por otro lado, en el caso del ovino en pastoreo, la dieta de estos animales puede estar formada por hasta un 90-100 % de Brassicas, según de Ruitter *et al.* (2009). Wadhwa *et al.* (2006) evaluaron la aceptación de las hojas de col y coliflor en caprino, observando una ingestión similar de estos subproductos con la del testigo (1,4 kg/día), formado por avena verde. Además, la digestibilidad de los subproductos de Brasicáceas fue mayor a la del testigo, debido a la rápida tasa de fermentación. Por último, cabe destacar dos factores anti-nutricionales por los que el consumo de Brassicas ha de ser controlado. Uno de ellos son los sulfóxidos de S-metil cisteína, los cuales se encuentran en alta concentración en los ramilletes florecidos. Estos compuestos dañan los glóbulos rojos, provocando anemia y reduciendo el consumo de alimento. Los glucosinolatos son otro de los factores anti-nutricionales que afectan al metabolismo del tiroides (de Ruitter *et al.*, 2009).

2

Objetivos

2. Objetivos

El objetivo global de la Tesis Doctoral fue la valorización del subproducto de brócoli y del subproducto y planta de alcachofa como alimento para el ganado caprino lechero como forma de reducir los residuos generados por la industria agroalimentaria, aprovechar el material vegetal que se queda en campo tras la cosecha, abaratar los costes de la ración ganadera y contribuir a la economía circular.

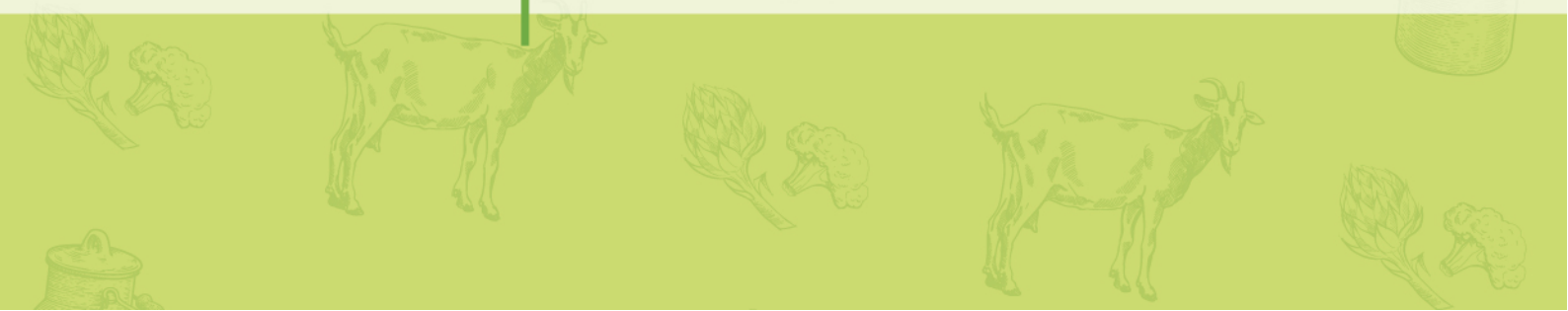
Para la consecución de tal fin, se establecieron los siguientes objetivos específicos:

1. Caracterizar la evolución del proceso de ensilado del subproducto de brócoli y subproducto y planta de alcachofa en microsilos comerciales.
2. Conocer el grado de aceptación de estos subproductos ensilados por parte de los animales y el porcentaje óptimo de su inclusión en la ración
3. Estudiar el efecto de la inclusión de los subproductos en la ración sobre el estado sanitario de los animales y en la producción y calidad nutritiva y funcional de la leche



3

Materiales y métodos



3. Materiales y métodos

3.1. Instalaciones

3.1.1. Instalaciones de Aprovertia S.L.

APROVERTIA S.L. es una empresa cuyo objetivo es el de producir y comercializar productos alimenticios de alto valor añadido para la alimentación animal, obtenidos a partir de la valorización de subproductos agrícolas, forestales, marinos, de la industria agroalimentaria y/o de las cadenas de distribución de alimentos. Realiza su actividad en la planta del Centro de Gestión de Residuos de San Javier, Murcia (37.8475, -0.8859), y es ahí donde tuvo lugar la fabricación de los silos que luego serían utilizados en los diferentes estudios que componen esta Tesis Doctoral.

Tanto la planta de alcachofa como los subproductos de alcachofa y brócoli procedían de las fincas agrícolas y centrales hortícolas de la comarca del Campo de Cartagena. La planta de alcachofa estaba formada por el rastrojo forrajero que queda en campo tras la cosecha de los capítulos florales, constituido por tallos hojas y alguna inflorescencia no recogida. Para favorecer la compactación de los silos y mejorar las condiciones de anaerobiosis, la planta de alcachofa se troceó con una picadora John Deere 8100 (Deere & Company, Moline, IL, EEUU) hasta alcanzar un tamaño de partícula de 2 cm. El subproducto de alcachofa estaba compuesto por las brácteas externas y los tallos de las alcachofas, además de pequeñas alcachofas completas procedentes del destrío. El subproducto de brócoli estaba formado principalmente por los troncos de la pella, aunque también había pequeñas inflorescencias descartadas en el triaje.

La fabricación de estos silos se encuentra recogida en la patente de Díaz *et al.* (2015). Brevemente, los silos fueron fabricados con una empacadora Agronic MR 820 (AGRONIC OY, Haapavesi, Finlandia. Figura 22), con un tamaño de 0,64 m³ y un peso de 300 kg. Para compactar correctamente los silos, se utilizaron 5 capas de red, mientras que para embalarlos e impedir la entrada de oxígeno, se usaron 13 capas de film plástico (Karatzis, Heraklion, Grecia).



Figura 22. Empacadora Agronic MR 820 (www.agriexpo.online.html).

3.1.2. Escuela Politécnica Superior de Orihuela

Los estudios llevados a cabo con animales, así como los análisis de muestras de ensilados, raciones y leche se realizaron en las instalaciones de la Escuela Politécnica Superior de Orihuela (EPSO), Alicante (38.0677, -0.9823), de la Universidad Miguel Hernández.

Granja de pequeños rumiantes

La granja cuenta con cabras de la raza Murciano-Granadina y ovejas de raza Manchega. La granja se compone de una nave principal dividida en dos por un pasillo de alimentación, ubicándose en cada lado una zona de alojamiento para cabras. También cuenta con dos patios de ejercicio a los que los animales tienen acceso durante el día y una sala de lactancia artificial con control ambiental y suelo enrejillado de PVC elevado sobre solera, la cual tiene una ligera pendiente para facilitar la limpieza y evacuación de residuos orgánicos. Dos salas separadas de los corrales de las hembras adultas sirven para alojar a los machos y la reposición. La lechería está provista de dos tanques de refrigeración de 600 y 800 L de capacidad, y está separada de la sala de máquinas y de la sala de ordeño tipo Casse con dos plataformas de 12 plazas cada una. En esta sala se encuentra instalada una máquina de ordeño en línea alta (LA) (GEA, Bönen, Alemania) con 12 juegos de ordeño Top Flow (GEA, Bönen, Alemania) con la que se pueden ordeñar las 12 plazas de las dos plataformas (1x12x12). Las dos plataformas disponen de un amarre de salida rápida frontal. La sala cuenta con todos los automatismos necesarios para realizar una gestión integral de la explotación: medidores de flujo Lactoflow controlados por el programa informático DairyPlan (GEA, Bönen, Alemania) y sistemas de lectura automática de bolos ruminales/crotales electrónicos montados en mangas de manejo situadas en la entrada de la plataforma. En la Figura 23 se muestran dos imágenes de la sala de ordeño a pleno funcionamiento.



Figura 23. Sala de ordeño de la granja de pequeños rumiantes de la EPSO.

El ritmo reproductivo de las cabras es de 1parto/año, agrupándose los animales en varios lotes productivos en función de su estado de lactación. Tras el parto, los cabritos se separan de las madres y van a la sala de lactancia, donde reciben el calostro durante los dos primeros días de vida y, después, son alimentados con leche artificial reconstituida a través de un sistema de tetinas conectadas a máquinas nodrizas. Las cabras se ordeñan mecánicamente una vez al día.

La rutina de ordeño es de “puesta y retirada automática de pezoneras” y sellado de pezones tras el ordeño mediante inmersión en solución yodada. La dieta convencional que reciben las cabras consiste en 2,5 kg/animal y día de una ración *unifeed* de alfalfa en rama y mix de granos y pellets y paja *ad libitum*.

La granja cumple la normativa sobre protección de animales utilizados en experimentación y otros fines científicos (RD 53/2013). Además, los estudios incluidos en esta Tesis Doctoral forman parte dos proyectos de investigación avalados por la Oficina de Investigación Responsable de la UMH (códigos UMH.DTA.GRM.01.14 y UMH.DTA.GRM.01.15). Además, se realiza un seguimiento periódico en colaboración con la Unidad de Análisis de Sanidad Animal de la Conserjería de Agricultura de la Generalitat Valenciana del estado sanitario de los animales (Tuberculosis, Brucelosis, AEC y Lengua Azul). De igual forma, se hace un seguimiento de micoplasmas en leche de tanque. La granja cumple con los criterios de Ecocondicionalidad y Bienestar Animal establecidos por la Generalitat Valenciana.

3.1. Diseño experimental

La presente Tesis Doctoral consta de cinco estudios consecutivos donde las conclusiones de los estudios previos sirvieron como premisas de los estudios posteriores. A continuación se detalla el diseño experimental de cada estudio.

Estudio 1: Proceso de ensilado de balas comerciales de subproductos hortícolas de la alcachofa y el brócoli (*Ensiling process in commercial bales of horticultural by-products from artichoke and broccoli*).

Se planteó un diseño experimental en el que la recogida de muestras tendría lugar durante diferentes días del proceso de ensilado, siendo mayor la frecuencia de recogida al principio, dado la mayor actividad de fermentación en las primeras etapas del ensilado, y menor al final. Se estableció que los días de recogida de muestras fueran el 0, 4, 7, 10, 15, 30, 60 y 200. En el día 0, o día de fabricación de los silos, se cogieron tres muestras de tres partes diferentes del montón de subproducto a ensilar: planta de alcachofa (PA), subproducto de brócoli (SB) y subproducto de alcachofa (SA). El resto de días de muestreo, se muestrearon tres silos de cada subproducto. La muestra se obtenía de tres partes diferentes del silo (parte superior, centro y parte inferior) con una barrena manual (Figura 24), para obtener mayor representatividad de la muestra.



Figura 24. Toma de muestras de los silos.

De las muestras obtenidas se determinaron poblaciones microbianas, bromatología, PT, digestibilidad *in vitro* de MS, productos de fermentación y residuos fitosanitarios.

Estudio 2: Estudio de preferencia de cuatro forrajes alternativos ensilados de la región del Mediterráneo en cabras Murciano-Granadinas (*Preference study of four alternative silage fodders from the Mediterranean region in Murciano-Granadina goats*).

En este estudio se ideó un diseño en cuadrado múltiple o “en cafetería”, muy utilizado en pruebas de preferencia en rumiantes (Borman *et al.*, 1991; Meier *et al.*, 2012). Se utilizaron cuatro cabras nulíparas alojadas en corrales individuales de 3 x 3 m. Los ensilados utilizados en el estudio de preferencia fueron: planta de alcachofa (PA), subproducto de alcachofa (SA), subproducto de brócoli con subproducto de alcachofa en una relación 50:50 (SBSA) y carrizo (C). La fase de adaptación a los nuevos alimentos duró 14 días, durante los cuales se administró a cada animal 150 g de cada ensilado por la mañana y la ración convencional por la tarde. La fase experimental duró 4 días. Tras un periodo de ayuno de 12 h, a cada animal se le ofrecían en cubos separados 2 kg de ensilado durante 4 horas, durante las cuales se registraba el tiempo que dedicaba cada cabra a comer de cada alimento. El peso del alimento remanente se registraba cada hora. Con estos datos, se calcularon la curva de ingestión en esas 4 horas (g de MS) y la tasa de ingestión (g MS/min). Tras las 4 horas de toma de datos, los cubos con ensilado se retiraban y a los animales se les ofrecía 2/3 de la ración convencional.

Para determinar la correlación y la curva de regresión de la composición de los alimentos con la ingestión, se determinó la composición bromatológica, PT, digestibilidad *in vitro* de MS y productos de fermentación de los cuatro ensilados.

Estudio 3: Efecto a corto plazo de la inclusión de subproductos de alcachofa ensilados en las dietas de cabras lecheras sobre la calidad de la leche. (*Short-term effect of the inclusion of silage artichoke by-products in diets of dairy goats on milk quality*).

Para este estudio se utilizaron 57 cabras Murciano-Granadinas en el cuarto mes de lactación. Los animales se dividieron en tres lotes de características homogéneas en cuanto a PV, producción lechera y RCS. A cada grupo se le asignó una dieta al azar: dieta testigo (T), que representa la ración convencional para este tipo de ganado, y dieta que incluía ensilados de

subproducto de alcachofa (SA) o planta de alcachofa (PA). Las dietas se formularon para que fueran isoenergéticas e isoproteicas.

El experimento se dividió en dos fases. La Fase 1 tuvo una duración de 8 semanas (las dos primeras sirvieron como periodo de adaptación) y el nivel de inclusión de subproductos ensilados fue de 12,5 % (en base seca). En esta fase se realizaron 4 controles bisemanales. Tras un mes de adaptación a las dietas de la Fase 2, esta duró 4 semanas y el nivel de inclusión utilizado fue del 25%, realizando en esta fase 3 controles bisemanales.

En cada control se registraba la ingestión de alimento y el PV de los animales. El día del control lechero, se extraía una muestra de sangre de los animales en ayunas para el análisis del perfil de metabolitos plasmáticos. Posteriormente, las cabras se ordeñaban y se recogía el dato de producción individual, además de muestras de leche de los animales y del tanque de refrigeración para los análisis de RCS, macrocomposición y perfiles mineral y lipídico.

Estudio 4: Composición y perfiles mineral y lipídico de la leche de cabras alimentadas con diferentes proporciones de subproductos de brócoli y planta de alcachofa (*Composition, mineral and fatty acid profiles of milk from goats fed with different proportions of broccoli and artichoke plant by-products*).

Se utilizaron siete lotes de 9 cabras Murciano-Granadinas en el cuarto mes de lactación. Los lotes eran homogéneos en PV, nivel de producción y RCS de los animales. A cada lote se le asignó una ración al azar, siendo T el grupo testigo, alimentado con una dieta convencional, y PA y SB los lotes alimentados con dietas que incluían ensilado de planta de alcachofa o subproducto de brócoli, respectivamente. Se utilizaron tres niveles de inclusión para cada subproducto: 25, 40 y 60%, en base seca. De esta forma, los grupos quedaron definidos como PA25, PA40, PA60, SB25, SB40 y SB60, en función del tipo y nivel de inclusión de ensilado que llevaba la dieta. Las siete dietas fueron formuladas para que fueran isoenergéticas e isoproteicas.

La parte experimental tuvo una duración de 4 semanas. Las 2 primeras semanas sirvieron de fase de adaptación a las dietas y en las dos semanas siguientes se llevaron a cabo 3 controles donde se registró la ingestión de alimento, PV y producción de leche, además de la recogida de muestras de leche (individuales y de tanque) y de sangre para su posterior análisis.

Estudio 5: Alimentación a largo plazo de cabras lecheras con ensilados de subproducto de brócoli y alcachofa: producción, calidad y composición de la leche (*Long-term feeding of dairy goats with broccoli by-product and artichoke silages: milk yield, quality and composition*).

En este último estudio se utilizaron 72 cabras Murciano-Granadinas al principio de la lactación (4ª semana). Los animales se dividieron en tres lotes homogéneos en PV, producción lechera y RCS. A cada lote se le asignó una dieta al azar: uno de ellos recibió una dieta testigo (T) compuesta por alfalfa, cereales y leguminosas, mientras que los otros dos fueron alimentados con dietas que incluían un 40% (en base seca) de ensilado de planta de alcachofa (PA) o de subproducto de brócoli (SB). Las raciones se calcularon para que fueran isoenergéticas e isoproteicas.

En comparación a los Estudios 3 y 4, el Estudio 5 fue de larga duración (23 semanas) para observar los efectos de la alimentación con los subproductos ensilados en estudio durante una lactación completa. Las primeras tres semanas sirvieron como periodo de adaptación a las

dietas. A partir de entonces, se realizaron 5 controles cada 5 semanas donde se tomaron datos de ingestión de alimento, PV, producción de leche y se recogieron muestras de sangre y leche de cada animal y del conjunto de todas las cabras de cada tratamiento (en tanque de refrigeración).

3.2. Variables analizadas

3.2.1. Ensilados y raciones

En el Estudio 1, las muestras de ensilados se tomaron por triplicado en cada día de muestreo estipulado en el diseño experimental (0, 4, 7, 10, 15, 30, 60 y 200). En el resto de estudios, las muestras de ensilados y de raciones ofrecidas a los animales se tomaron al inicio de los experimentos. De cada muestra, una parte se deshidrató en estufa a 60°C durante 48 h para los análisis de bromatología y digestibilidad *in vitro*, otra parte se congeló en fresco a -80 °C para las determinaciones de PT, AGV y perfil mineral, mientras que para la caracterización del perfil lipídico se utilizó muestra liofilizada.

Bromatología

Las determinaciones de MS, MO, EE, PB, fibra bruta (FB) y azúcares totales se hicieron sobre muestra deshidratada a 60°C, de acuerdo a los procedimientos recogidos en AOAC (1999). Las fracciones fibrosas FND, fibra ácido detergente (FAD) y LAD se analizaron según el método de Van Soest *et al.* (1991).

Polifenoles totales

La determinación de la cantidad de PT presentes en los ensilados y las dietas se realizó mediante el método de Folin-Ciocalteu recogido en Kim *et al.* (2003) sobre muestras en fresco.

Digestibilidad *in vitro* de MS

Se utilizó líquido ruminal extraído por vía esofágica de cinco cabras adultas de raza Murciano-Granadina, alimentadas con una dieta a base paja y granos de cereales y semillas oleaginosas. Doce horas antes de la recogida del líquido ruminal, se procedió a la retirada del alimento a los animales para así facilitar el proceso de extracción. Una vez obtenido el líquido ruminal, este se trasladó en un termo al laboratorio, donde se filtró y se mezcló con un medio de cultivo rico en nitrógeno, manteniendo una temperatura de 37°C y gaseando CO₂, tal y como se describe en Menke y Steingass (1988). Este caldo de cultivo se adicionó en dosis de 50 mL a viales de 120 mL con 0,5 g de muestra deshidratada a 60°C y molida. Los viales se incubaron en un baño orbital a 37°C durante 48 horas. Transcurrido este tiempo, el contenido de los viales se filtró en crisoles de placa porosa nº2 y se guardaron en estufa a 100°C durante 24 horas. La pérdida de MS se calculó como diferencia entre el peso en MS de la muestra al inicio de la incubación y el peso en MS al final de la misma, corrigiendo esa diferencia con los blancos.

Ácidos grasos volátiles y resto de metabolitos de fermentación

La determinación de AGV como acético, propiónico y butírico, además de otros metabolitos de la fermentación del ensilado, como el ácido láctico y el etanol, se llevó a cabo de acuerdo al método propuesto por Feng-Xia *et al.* (2013), utilizando cromatografía líquida de HPLC (Agilent

1200, Santa Clara, CA, EEUU), con una columna C610H de 30 cm y 7,8 mm DI (Supelcogel, Saint Louis, MO, EEUU) sobre muestras frescas de ensilados y raciones.

Perfil mineral

Para el análisis de minerales en las muestras de ensilados y raciones, se realizó una digestión previa de la muestra en microondas (Ethos Easy, Milestone SRL, Sorisole, Italia), según González-Arrojo *et al.* (2016). La digestión consistió en cuatro fases: 5 minutos a 1000 vatios a temperaturas entre 60 y 100 °C, 10 minutos a 1000 vatios a temperaturas entre 80 y 165 °C, 5 minutos a 1000 vatios a temperaturas entre 120 y 180 °C y 5 minutos a 700 vatios a temperaturas entre 120 y 180 °C. El equipo ICP-MS (Inductively Coupled Plasma-Mass Spectrometry) utilizado fue un Agilent 7700x (Santa Clara, CA, EEUU) ORS (*Octapole Reaction System*). Las condiciones de operación fueron optimizadas para determinar simultáneamente todos los elementos. Las soluciones estándar se prepararon diariamente mediante una dilución de 1000 mg/L de cada elemento en HNO₃ ultrapuro (2% v/v). Se utilizó un patrón interno para corregir las interferencias físicas y/o de matriz del equipo ICP-MS.

Perfil lipídico

Los ácidos grasos de las dietas se analizaron por metilación directa sobre la muestra liofilizada, sin extracción previa de la grasa, de acuerdo a Kramer *et al.* (1997). La identificación de los ácidos grasos se realizó con un detector de ionización de llama (FID) acoplado a un cromatógrafo de gases GC-17A (Shimadzu, Kioto, Japón), equipado con una columna CP Sil 88 de 100 m, 0,25 mm de DI y 0,20 mm de cobertura interna (Agilent, Santa Clara, CA, EEUU). Como patrones, se utilizó un mix de ésteres de ácidos grasos metilados (18912-1AMP, Sigma-Aldrich, Saint Louis, MO, EEUU).

Análisis microbiológicos

Las muestras de ensilados del Estudio 1 se transportaron al laboratorio en bolsas de plástico asépticas para las determinaciones microbiológicas el mismo día de su recolección. Se tomaron 10 g de cada muestra y se homogenizaron junto con 90 mL de agua de peptona en bolsas de Stomacher (BagMixer 400, Interscience, St. Nom la Bretèche, Francia). Los cultivos microbiológicos para el recuento de enterobacterias y aerobios mesófilos se incubaron directamente sobre placas Petrifilm (3M Microbiology, St. Paul, MN, EEUU) a 37 °C durante 24 h. Los cultivos para el recuento de bacterias ácido-lácticas se incubaron en placas Petri con caldo MRS (Liofilchem, Roseto degli Abruzzi, Italia) a 37 °C durante 48 h. Los cultivos para hongos y levaduras se incubaron en placas Petri con PDA (Potato Dextrose Agar) a 37 °C durante 72 h. Los resultados se expresaron como Log₁₀ ufc/g de muestra fresca, de acuerdo a los procedimientos de AENOR (2015).

El conteo de esporas del género *Clostridium* se llevó a cabo usando la técnica del Número Más Probable en caldo Bryant & Burkey (Merck), siguiendo la metodología de Arias *et al.* (2016). Los resultados fueron transformados a Log₁₀ para el análisis estadístico.

Análisis de residuos fitosanitarios

Un análisis multirresidual de los principales residuos fitosanitarios se llevó a cabo sobre las muestras de ensilado SB, SA y PA del día 200 del Estudio 1, utilizando para ello el Método QuEChERS, de acuerdo a la regulación de la Comisión Europea 901/2009.

3.2.2. Leche

Producción

La producción individual de las cabras se medía con un dispositivo Lactocorder (WMB, Balgach, Suiza. Figura 23) acoplado al tubo largo de leche del equipo de ordeño. Este dispositivo estima el flujo y cantidad de leche que pasa mediante infrarrojos. El mismo Lactocorder también recogía 100 mL de muestra de leche representativa de todo el ordeño de cada animal, para el posterior RCS y macrocomposición.



Figura 25. Equipo de medición de la producción de leche (Lactocorder).

Recuento de células somáticas

El RCS de las muestras de leche recogidas por los Lactocorder se determinaba mediante un equipo DCC (Delaval Cell Counter, DeLaval, Tumba, Suecia. Figura 24), utilizando un método electrónico fluoro-óptico en el que una cámara digital hace una fotografía de los núcleos de las células somáticas en el cassette, que está teñido con una tinción fluorescente específica para ácido desoxirribonucleico (ADN). Los resultados se transformaron a Log_{10} (LRCS) para el análisis estadístico.



Figura 26. Contador de células somáticas DCC de DeLaval (www.delaval.com)

Macrocomposición y urea

La muestra de leche tomada por el Lactocorder en granja fue utilizada en laboratorio para el análisis de la macrocomposición y contenido en urea de la muestra mediante un equipo de espectroscopía de infrarrojo medio (MilkoScan FT2, FOSS, Hillerød, Denmark. Figura 25), calibrado para leche de cabra. Las variables analizadas fueron: grasa, proteína, proteína verdadera, caseína, proteína del suero, lactosa, sólidos totales (ST), extracto seco magro (ESM), extracto seco útil (grasa + proteína, ESU), cenizas y urea.

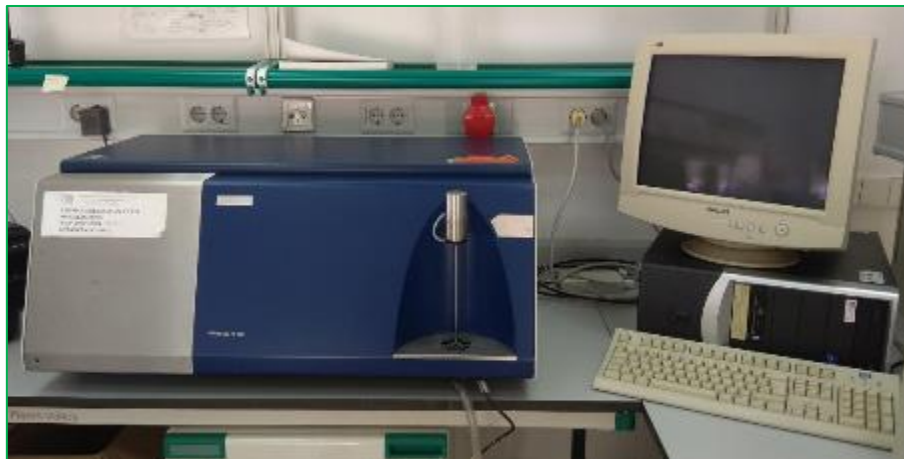


Figura 27. MilkoScan FT2 de FOSS.

Perfil mineral

El método de análisis de minerales de las muestras de leche fue el mismo que el utilizado en muestras de ensilados y raciones.

Perfil lipídico

A diferencia de las muestras de raciones y ensilados, en las muestras de leche se realizó una extracción de la grasa mediante el procedimiento de Folch con algunas variaciones, tal y como

se detalla en Romeu-Nadal *et al.* (2004). Los ácidos grasos fueron metilados según el método de Nudda *et al.* (2005). El equipo, columna y mix de patrones de ácidos grasos fueron los descritos anteriormente en el análisis del perfil lipídico de las muestras de alimento. Se calcularon los IA e IT, de acuerdo con Ulbricht y Southgate (1991), y los índices de desaturación (ID) para el C14:0, C16:0 y C18:0, según Lock y Garnsworthy (2003).

3.2.3. Sangre

Las muestras de sangre recogidas durante el control lechero de los Estudios 3, 4 y 5 se utilizaron para el análisis de glucosa, colesterol, urea, BHB, NEFA y hematocrito, utilizando espectrofotometría enzimática. La glucosa y el colesterol se determinaron mediante un kit de oxidasa/peroxidasa (Refs. 11503 y 11505, Biosystems, Barcelona, España). La urea se analizó con el método cinético GN 10125 (Gernon, España), BHB con el kit RB 1007 (Randox, Crumlin, GB) y los NEFA con el método FA 115 (Randox, Crumlin, GB). El porcentaje de hematocrito se determinó con un microhematocrito (ZIP Combo, CVM, Madrid, España).

3.3. Análisis estadístico

Todos los datos fueron registrados en hojas Excel (Microsoft, Redmond, WA, EEUU) y posteriormente procesados estadísticamente mediante el paquete informático SAS 9.2 (SAS, Cary, NC, EEUU). A continuación se detalla un resumen de los procedimientos utilizados en los diferentes estudios, los cuales están correctamente descritos en los artículos publicados.

Las variables relativas a la composición de los silos en el Estudio 1 y a los parámetros tomados directamente de las cabras, como ingestión y tasa de ingestión en el Estudio 2 y PV, producción y valores de macrocomposición y metabolitos plasmáticos en los Estudios 3, 4 y 5, fueron analizadas según un modelo lineal mixto con medidas repetidas (PROC GLIMMIX, SAS v9.2, 2012).

En el Estudio 2, los análisis de correlación y regresión de la composición de los ensilados con la ingestión de alimento se realizaron con los procedimientos Corr. y Reg del programa SAS (v9.2., 2012).

Para las variables analizadas a partir de la leche recogida en tanque en los Estudios 3, 4 y 5 se utilizó el procedimiento GLM, también del programa SAS (v9.2., 2012).

Las diferencias se consideraron estadísticamente significativas en $P < 0,05$.



4

Publicaciones

Publicación 1 (Artículo original)

**Ensiling Process in Commercial Bales of Horticultural By-Products from
Artichoke and Broccoli**





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Article

Ensiling Process in Commercial Bales of Horticultural By-Products from Artichoke and Broccoli

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Simple Summary: Artichoke and broccoli crops are widespread throughout the world, mainly in the Mediterranean region. After artichoke harvests and industrial processing of artichoke and broccoli, large amounts of by-products are generated. The use of these alternative and cheaper feedstuffs in ruminants' diets would reduce waste caused by the agri-food industry, disposal costs, and the land and natural resources used in animal feed production, contributing to the circular economy. Because of the high water content and the seasonality of these feedstuffs, ensiling might be a technology to preserve its nutritional quality for a long time, and this must be considered and studied at commercial scale. This paper looks into the viability of ensiling broccoli and artichoke by-products as commercial round bale silos (300 kg), their shelf life, and their suitability for ruminant feeding. The three silage by-products are stabilised on day 30. The high microbial quality and the appropriate nutritional composition at final stage (day 200) make them suitable for inclusion in ruminant diet, in combination with other energy and protein sources over a long period after the crop season.

Abstract: Wastes from artichoke and broccoli crops and cannery industries represent an environmental problem. A viable option to this problem is ensiling them for use as ruminants feed. The aim of this study was to characterise the ensiling process of broccoli and artichoke by-products and assess their suitability to be part of the ruminant diet, as well their minimum shelf life. Twenty-one commercial round bale silos (300 kg and 0.64 m³) of each by-product were made. Samples were analysed at days 0, 7, 15, 30, 60, and 200 to determine microbial populations, fermentation metabolites, nutritional components, and phytosanitary residues. Feedstuffs showed good suitability for ensiling, and stabilisation was achieved on day 30. The variables with the greatest significant differences among sampling times were microbial populations and fermentative components. There were no important dry matter losses, and some significant differences were observed in the nutritional composition, especially in crude protein and fibrous fractions, but they were not relevant for the loss of nutritional quality of silages. The phytosanitary residues determined on day 200 were below the maximum residue limits set by European legislation. So, ensiling these by-products in commercial round bale silos is a suitable and profitable technique that allows their preservation for a long time (200 days).

Keywords: Silage; feedstuff; nutritive value; ruminant feeding; alternative feeds

1. Introduction

In 2017, 1,505,328 tons of artichoke (*Cynara scolymus* L.) and 25,984,758 tons of broccoli (*Brassica oleracea* var. *Italica*) were harvested worldwide [1]. Artichoke crops contribute significantly

to the Mediterranean agricultural economy, the source of more than 60% of the world yield of this vegetable [2]. In the case of broccoli, more than 40% is produced in the Mediterranean and Southeast Asian regions [3].

The artichoke plant is a by-product consisting of leaves, stems and some inflorescences left in the field after harvest for human consumption. This stubble represents 80% of the total biomass of the plant [4] and has traditionally been used for feeding by grazing small ruminants or harvested and taken to farms [5]. Wernli and Thames [6] indicated that the yield was 11.105 t/ha of green forage in this crop, which, taking into account the FAO data on cultivated area worldwide (124,941 ha in 2016), would result in production figures of 1,387,470 t/year of available artichoke plant stubble. According to Ros et al. [7], 30% and 50% of the weight of processed broccoli and artichoke consist of by-products that could be destined for animal consumption and, considering the annual yield worldwide of these two vegetables, there will be 7,665,504 and 752,664 t of broccoli and artichoke by-products available, respectively.

Semi-intensive and intensive ruminant farms are characterised by high demand and dependence on feedstuffs, such as cereals, legume seeds, and alfalfa, which entail a high cost and require large crop areas. The use of local and alternative agri-food by-products and fodder in ruminant feeding reduces the external reliance on the aforementioned foods, thus minimising the use of land, water and supplies associated with livestock feeding and the feeding costs, which commonly exceed 60% of the total costs of a farm. Moreover, agricultural by-products, like artichoke and broccoli residues, are not competitive between human and livestock. Studies using other agricultural by-products have shown that if diets supplemented with this type of feedstuffs are formulated carefully, so that the nutritional needs of the animals are covered, their use may not compromise the milk yield and quality, or the technological properties and quality of the derived products [8]. Moreover, some recent publications showed that incorporation of by-products in diets would be profitable economically and nutritionally for dairy cattle production [9]. The use of broccoli and artichoke by-products can also be a way to minimise the waste originated by the canning industry and thus reduce disposal costs. However, the marked seasonality and high water content of these foods limit their direct use in animal feeding.

Ensiling is a way to preserve perishable products rich in lignocellulose and the main form of preservation of forage in many parts of the world, mainly due to its low dependence on climate, unlike hay [10]. Additionally, during ensiling, the bioactive components, such as polyphenols, undergo changes that might vary the antioxidant potential associated with them [11], which can positively affect animal health and performance, while improving the nutritional value of animal-derived products [12].

Only laboratory scale studies are published about artichoke and broccoli by-products silages [5,13–15], although no studies have been done in commercial size silos. The novelty of this experiment is that the evolution of these silos on a commercial scale are studied, so that the changes that occur during the silage process are better adjusted to reality, as they take place in a less controlled environment than laboratory scale studies. On the other hand, this study includes the variation of the microbiological populations of the three by-products, which better explains the changes in the fermentative and nutritional composition contents that occur in the three silages. Finally, no silage study with these by-products has reached the 200th day, so this study provides additional information about silage quality over a longer period of time. For this reason, the results derived from this study provide a more realistic approach to the suitability of these by-products for ensiling. Several studies have shown that these by-product silages, in total or partial replacement of conventional forage sources, were a viable option for ruminant feeding [16–24].

The aim of this study is to characterise the silage process of broccoli and artichoke by-products and artichoke plant stubble in commercial size silos (300 kg round bale) over 200 days to determine the quality and suitability of these types of silages as a ruminant feed and its shelf life. Changes in variables related to the fermentation process, microbial population dynamics, nutritional composition, fermentative components, and in vitro digestibility were studied. The hypothesis of this study is that the commercial-scale silage technique of these by-products in 300 kg round-bale silos will allow food

preservation for a long period of at least 200 days. The advantages of these types of silos over others are that they are easy to transport, can be sold as a package, have high compaction, good storage life, and no construction costs.

2. Materials and Methods

The materials studied were broccoli (*Brassica oleracea* var. *Italica*) and artichoke (*Cynara scolymus* L.) by-products (BB and AB, respectively) and artichoke plant stubble (APS). BB and AB were collected from agri-food industries from the Campo de Cartagena area, at the time they were leaving the horticultural facilities. APS was collected from a field of the same area after the artichoke harvest. All the materials were transported separately in refrigerated trucks to Aprovertia S.L. facilities, where the silo manufacturing took place. This process was in April, when the average temperature is 15 °C and relative humidity is 68% (values from San Javier, Murcia, Spain, weather station). To achieve suitable anaerobic conditions and correct compaction, the APS was cut and chopped to a size of 2 cm with a forage chopper (John Deere 8100, Moline, Illinois, USA) at silo manufacturing facilities. The same day, 21 round bale silos of each material of approximately 300 kg and 0.64 m³ each were made with a baler-wrapper (Agronic MR 820, Haapavesi, Finland), using five layers of inner conventional netwrap (Karatzis S.A., Heraklion, Greece) and 13 layers of conventional plastic film (Karatzis S.A., Heraklion, Greece) for each silo (Figure 1), following the description of Díaz et al. [25](2018) patent. No additives were used.

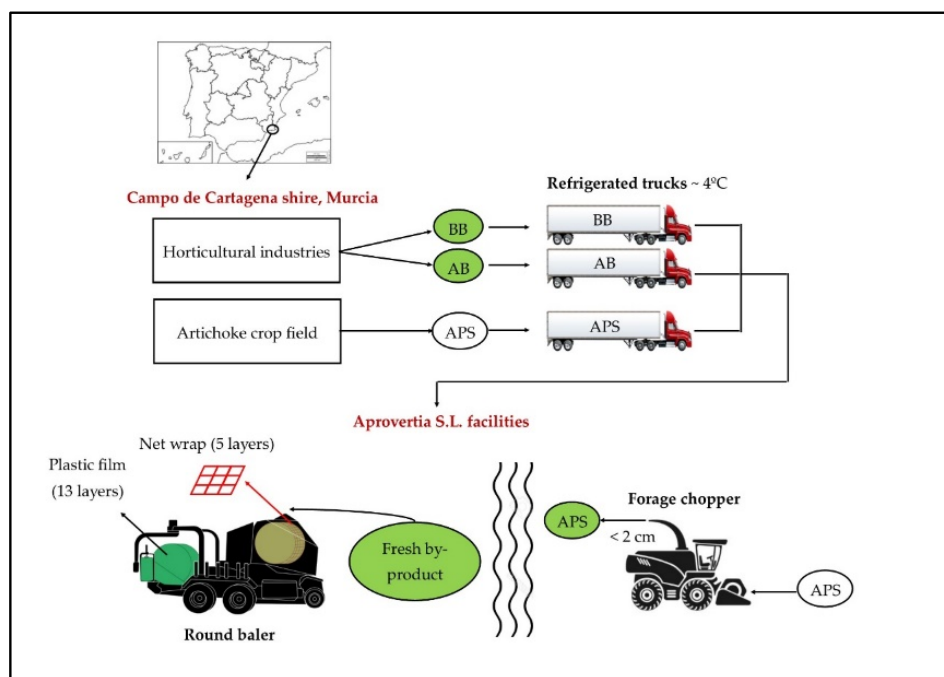


Figure 1. Ensiling process of the three by-products studied.

An experimental design was planned in which the sample collection days were 0 (silos manufacture day), 4, 7, 10, 15, 30, 60, and 200. For the sampling on day 0, three samples were collected from three different parts of the whole batch of the material to be ensiled. For the rest of the sampling days, each day a sample of 1 kg of three different bales was collected with a manual auger from three different zones of the bale: in the middle, and up, and down, 20 cm from the base. Then, the three sub-samples of a bale were mixed. Thus, three different representative samples of three different bales were obtained at every sampling day, which were taken to the laboratory, where they were separated into three aliquots: one for the analyses on the same day (dry matter, pH and microbiological cultures); another one was frozen at −20 °C; and another was dehydrated in an oven at 60 °C for 48 h.

On the same day of sampling, some variables were determined, such as pH (GLP 21, Crison, L'Hospitalet de Llobregat, Spain) and dry matter (DM, g/kg, AOAC, 1990, 948.12). With the samples from day 0, buffer capacity (meq NaOH 0.1N/100 g DM) was determined following the procedure in McDonald and Henderson [26]. Flieg scores were calculated to determine the quality of the silage, according to the equation given by Kilic [27]:

$$\text{Flieg scores} = 220 + (2 \times \text{DM} (\%) - 15) - 40 \times \text{pH} \quad (1)$$

According to this index, a score obtained below 20 would correspond to a very low quality silage; a low quality silage would have a score between 21 and 40; between 41 and 60 for medium quality silage; 61–80 in silages of high quality; and more than 81 for very high quality silages.

Samples for microbiological determinations were transferred to a laboratory in aseptic plastic bags. Then, 10 g of the sample was weighed into aseptic plastic bags with a lateral strainer and homogenised with 90 mL of peptone water in a stomacher (BagMixer[®] 400, Interscience, St Nom la Bretèche, France). Microbiological cultures for counts of enterobacteria and aerobic mesophilic bacteria were directly incubated on 3M[™] Petrifilm plates (3M Microbiology, St. Paul, Minnesota, USA) at 37 °C for 24 h. Samples for lactic acid bacteria counts were incubated in MRS broth (Liofilchem, Roseto degli Abruzzi, Italy) at 37 °C for 48 h and moulds and yeasts were incubated on PDA (potato dextrose agar) culture, expressing the results as Log₁₀ cfu/g of fresh matter and following AENOR procedures [28] in the fresh samples of days 0, 7, 15, 30, 60, and 200.

Spore count of Clostridium genus was performed on days 0, 15 and 200, using the most probable number technique (MPN) and Bryant & Burkey broth (BBB, Merck, Darmstadt, Germany) following the methodology indicated in Arias et al. [29]. The microbiology cfu counts were transformed to log₁₀ for further statistical analysis.

From samples dehydrated at 60 °C and further milled (1 mm), variables were analysed following AOAC procedures [30]. These variables were ash (g/kg DM, 934.01), crude protein (CP, g/kg DM, 988.05), ether extract (EE, g/kg DM, 920.39), crude fibre (CF, g/kg DM, 978.10), and total sugars (g/kg DM, 974.06). The contents of neutral detergent fibre (NDF, g/kg DM), acid detergent fibre (ADF, g/kg DM) and acid detergent lignin (ADL, g/kg DM) were also analysed according to Van Soest et al. [31]. In vitro dry matter digestibility (IVDMD, g/kg DM) was determined on days 0, 15, 30, 60, and 200, following the procedure of Menke and Steingass [32], using ruminal liquid extracted with oesophageal cannula from five goats from the farm of the Polytechnic School of Orihuela. A sample of 0.5 g was incubated in a 120 mL glass vial prewarmed at 39 °C before infusing with CO₂ and the injection of 60 mL of buffer rumen liquor. The incubation was in an orbital bath at 39 °C. After 48 h, vial contents were filtered using Whatman paper no.2 and the residue was oven dried at 105 °C for 48 h. This study was approved by the Responsible Research Office of the Miguel Hernández University (code UMH.DTA.GRM.01.15).

The frozen sample was used for analysis of the total polyphenols content (TP, g/kg DM) by the Folin-Ciocalteu method described in Kim et al. [33]. To study the fermentative changes during the ensiling process, some metabolites were measured, such as ammonia nitrogen (NH₃-N, g/kg DM, 941.04) according to AOAC [30]; and short-chain volatile fatty acids (VFA, g/kg DM: acetic, butyric and propionic acids, although the latter was not found in the samples), lactic acid (g/kg DM) and ethanol (g/kg DM) by HPLC liquid chromatography (Agilent 1200, Santa Clara, California, USA and Supelcogel C-610H column: 30 cm × 7.8 mm ID, Saint Louis, Missouri, USA), as described by Feng-Xia et al. [34]. A multi-residual analysis of the main phytosanitary products used in artichoke and broccoli crops was carried out on the samples of day 200 using QuEChERS Method, according to Commission Regulation (EC) No 901/2009.

All the determined variables were analysed following a mixed linear model (Proc. GLIMMIX, SAS V 9.2, 2012) due to the heterogeneous nature of by-products, according to the following equation:

$$Y = \mu + D_i + B_k + e \quad (2)$$

Where Y is the dependent variable, μ is the intercept, D_i is the fixed effect of the ensiling day, B_k is the random effect of the bale, and e is the residual error. The covariance model with a lower value of the Akaike criterion (lower AIC and BIC) was used for each variable.

A calculation of the approximate costs of tested silos (€/t) was made, including raw material, inner netwrap and plastic film, workforce, and other production and marketing costs (the company's commercial margin was not included).

3. Results

3.1. Microbiology

Figure 2 shows an increase in lactic populations ($p < 0.001$) in the early days (0–7) in the three silages, reaching the maximum on day 7 and becoming reduced and stabilised on day 30 in BB silage (6.88 log cfu/g FM) and on day 60 in AB and APS (7.13 and 7.08 log cfu/g FM, respectively). The enterobacteria population decreased markedly ($p < 0.001$) in the three silages from day 0 (7.26, 6.43 and 6.35 log cfu/g FM, respectively for BB, AB and APS) and disappeared completely on day 15 in BB and on day 60 in AB and APS, although in the latter there was a slight increase ($p < 0.001$) on day 200 (2.44 log cfu/g FM). The evolution of the mesophilic aerobes population varied according to the by-product. In BB, it decreased gradually ($p < 0.001$) from day 0 (8.84 log cfu/g FM) and stabilised on day 60 (4.56 log cfu/g FM), while in AB the reduction ($p < 0.01$) started on day 15 (7.77 log cfu/g FM) until day 200, reaching a value of 1.57 log cfu/g FM. In APS, a significant increase in mesophilic aerobes population was observed on day 7 (8.75 log cfu/g FM; $p < 0.05$), followed by a reduction, reaching stabilisation on day 30 (4.52 log cfu/g FM). As for yeasts, it was not observed at any time that they stabilised, but their number in the three silages was reduced ($p < 0.001$) throughout the experiment, reaching 3.17, 1.65 and 3.42 log cfu/g FM on day 200, respectively for BB, AB and APS. Regarding moulds, in BB and AB they remained stable throughout the experiment, where the average value was 1.07 and 2.11 log cfu/g FM, respectively. However, in APS, a reduction ($p < 0.001$) was observed from day 0 (5.15) until moulds disappeared on day 30. The spore count of the genus *Clostridium* spp. reached its lowest ($p < 0.05$) value on day 15 in the three by-products and, subsequently, increased to levels similar to the initial ones, but without significant differences in AB and APS.

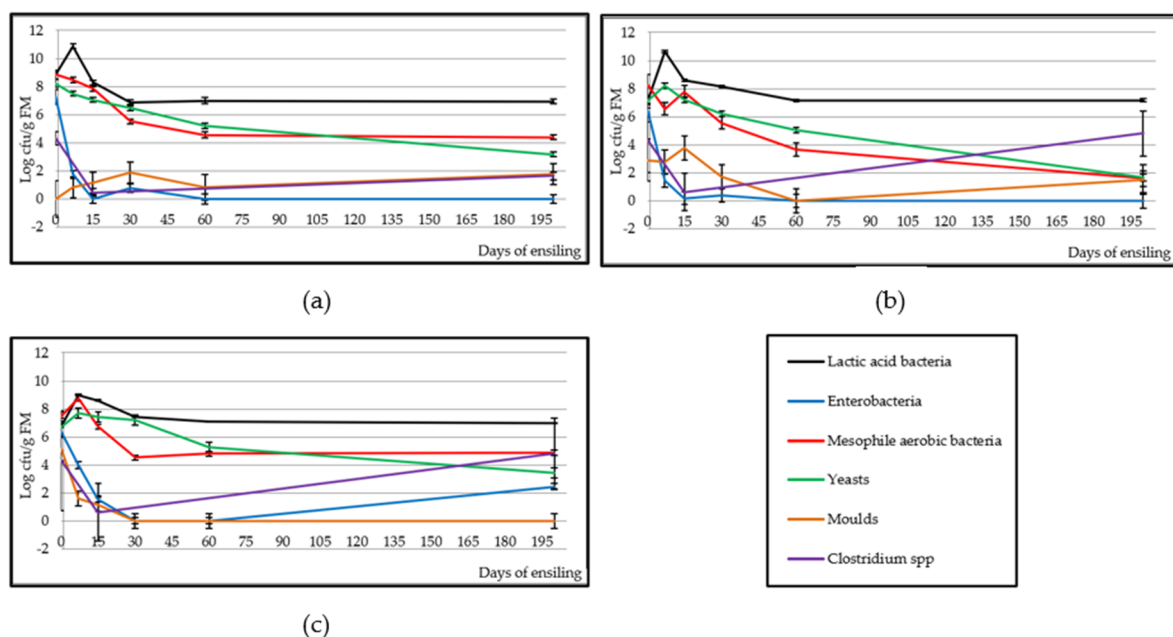


Figure 2. Effect of ensiling on microbial populations in broccoli by-product (a), artichoke by-product (b) and artichoke plant stubble (c) silages.

3.2. Physico-Chemical Parameters and Nutritional Composition

Figure 3 shows that pH decreased ($p < 0.001$) in the three silages, reaching its stabilisation on day 7 (4.30) in AB and on day 15 (4.47) in APS, while the lowest pH value of BB was observed on day 15 (4.09); thereafter, it increased slightly ($p < 0.001$) to reach a value of 4.71 on day 200. The buffer capacity of the raw materials before ensiling was 40.8, 21.8 and 19.6 meq/100 g DM in BB, AB and APS, respectively. Flieg scores increased to more than 80 from day 7 in all three silages, showing a very high quality. However, whereas AB and APS remained in this quality level, Flieg scores of BB decreased ($p < 0.01$) to the next lower level (60–80) from day 30, although silage quality was high quality.

Table 1 shows the changes in nutritional composition during the 200 days of silage. DM decreased ($p < 0.001$) in the three silages. While in AB and BB this decrease took place from the beginning until its stabilisation on day 30 (183 g/kg), in APS the value remained stable, decreasing slightly and gradually until reaching a value of 258 g/kg on day 200. While organic matter remained unchanged in AB during the whole experiment and its average value was 916 g/kg DM, it was slightly reduced ($p < 0.001$) in BB and APS, and reached values of 821 and 828 g/kg DM on day 200, respectively. Ether extract content increased slightly ($p < 0.05$) in BB and APS from day 30 and remained stable in AB. Crude protein level was very stable, except for a fluctuation ($p < 0.001$) observed in BB on day 60, in AB on day 200 and in APS on day 30. A decrease in NDF content was observed in the three silages from day 0 until day 30, then NDF increased on day 200 in BB and APS. Regarding ADF, AB did not show significant differences, BB and APS reduced their content until day 30 and 60, respectively, and then increased on day 200. The ADL content reduced from day 15 in BB and AB, and an increase ($p < 0.05$) on day 200 to levels similar to those on day 15 was observed in BB. In APS, the ADL level fluctuated slightly throughout the period, increasing on day 200. In vitro DM digestibility was reduced slightly ($p < 0.01$) in BB, while it increased in AB; in APS, it fluctuated with a few differences throughout the period. As for the content in TP, it rose ($p < 0.001$) in BB on day 15, remaining unchanged for the rest of the experiment; in AB it increased until day 60 and then decreased to a level similar to that presented on day 15, and in APS the value fluctuated slightly without major differences ($p < 0.05$).

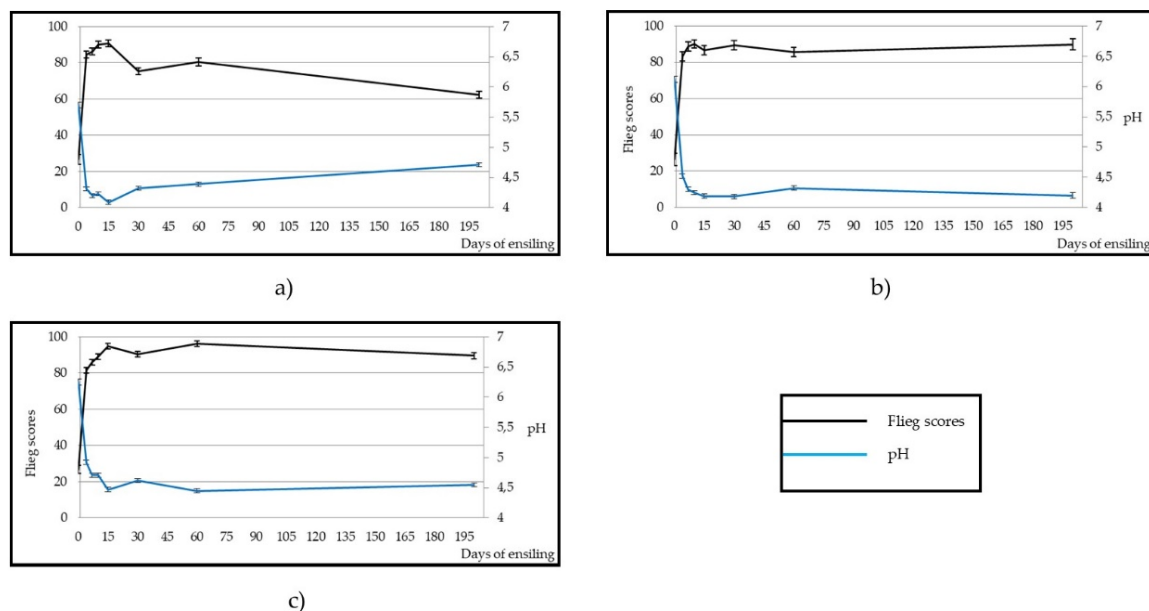


Figure 3. Effect of ensiling on Flieg scores and pH in broccoli by-product (a), artichoke by-product (b) and artichoke plant stubble (c) silages.

Table 1. Effect of ensiling on nutritional composition, in vitro dry matter digestibility and total polyphenols content in broccoli by-product (BB), artichoke by-product (AB) and artichoke plant stubble (APS) silages.

| Silage | Days of Ensiling | | | | | | | SEM | p-Value | |
|--------|--|--------|--------|--------|--------|--------|--------|--------|---------|------|
| | 0 | 4 | 7 | 10 | 15 | 30 | 60 | | | 200 |
| | Dry matter (g/kg) | | | | | | | | | |
| BB | 174bc | 184ab | 172bc | 197ab | 173bc | 142d | 181ab | 154cd | 6.79 | *** |
| AB | 256a | 220b | 203bcd | 201bcd | 207bc | 183d | 192cd | 190cd | 8.66 | *** |
| APS | 283abc | 291abc | 273cd | 286abc | 268d | 277bcd | 271cd | 258e | 4.21 | *** |
| | Organic matter (g/kg DM) | | | | | | | | | |
| BB | 849a | | | 840a | 826b | 828b | 821b | 821b | 3.5 | *** |
| AB | 912 | | | 927 | 912 | 912 | 916 | 916 | 9.4 | n.s. |
| APS | 839b | | | 849a | 837b | 833bc | 828c | 828c | 2.3 | *** |
| | Ether extract (g/kg DM) | | | | | | | | | |
| BB | 20.2b | | | 23.4b | 29.1a | 23.2b | 32.1a | 32.1a | 1.97 | ** |
| AB | 19.6 | | | 29.3 | 25.8 | 28.0 | 29.6 | 29.6 | 3.41 | n.s. |
| APS | 26.3b | | | 31.4ab | 35.7a | 34.7a | 34.6a | 34.6a | 2.22 | * |
| | Crude protein (g/kg DM) | | | | | | | | | |
| BB | 195a | | | 199a | 204a | 153c | 174b | 174b | 4.6 | *** |
| AB | 117b | | | 126b | 125b | 122b | 145a | 145a | 3.7 | *** |
| APS | 78.7a | | | 78.3a | 67.1b | 76.9a | 78.1a | 78.1a | 1.2 | *** |
| | Neutral detergent fibre (g/kg DM) | | | | | | | | | |
| BB | 395ab | | | 356bc | 311d | 342cd | 430a | 430a | 11.7 | *** |
| AB | 589a | | | 530ab | 510b | 541ab | 528ab | 528ab | 17.1 | * |
| APS | 547ab | | | 555ab | 540b | 532b | 571a | 571a | 9.9 | ** |
| | Acid detergent fibre (g/kg DM) | | | | | | | | | |
| BB | 272b | | | 266b | 233c | 259b | 326a | 326a | 8.2 | *** |
| AB | 398 | | | 359 | 353 | 379 | 354 | 354 | 13.3 | n.s. |
| APS | 358ab | | | 365a | 361a | 336b | 374a | 374a | 7.3 | *** |
| | Acid detergent lignin (g/kg DM) | | | | | | | | | |
| BB | 77.9a | | | 65.0b | 35.1c | 35.1c | 63.4b | 63.4b | 3.63 | *** |
| AB | 131a | | | 81b | 80b | 84b | 89b | 89b | 6.8 | *** |
| APS | 84.5bc | | | 87.1b | 91.9b | 77.1c | 107.9a | 107.9a | 2.67 | *** |
| | <i>In vitro</i> dry matter digestibility (g/kg DM) | | | | | | | | | |
| BB | 888a | | | 829b | 857ab | 800b | 822b | 822b | 14.7 | ** |
| AB | 670b | | | 724ab | 723ab | 737ab | 769a | 769a | 23.2 | * |
| APS | 606b | | | 579c | 632a | 629ab | 615ab | 615ab | 7.1 | *** |
| | Total polyphenols (g/kg DM) | | | | | | | | | |
| BB | 5.86c | | | 8.85a | 9.59a | 7.48b | 6.73ab | 6.73ab | 0.308 | *** |
| AB | 1.96d | | | 7.60c | 10.08b | 15.29a | 7.56c | 7.56c | 0.734 | *** |
| APS | 5.02b | | | 5.47ab | 5.95a | 5.55ab | 4.96b | 4.96b | 0.240 | ** |

a–e Different letters in the same row indicate significant difference between days. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; n.s.: non significant.

3.3. Fermentation

Figure 4 shows a rapid reduction ($p < 0.001$) in sugar content in the three silages from the outset, which ceased on day 15 in BB (29.2 g/kg DM) and AB (37.3 g/kg DM) and on day 7 in APS (29.0 g/kg DM). Lactic acid content increased ($p < 0.001$) in the three silages: while in BB and APS it reached its maximum value on day 60 (98.4 and 29.2 g/kg DM) and then was reduced ($p < 0.001$); in AB the maximum was observed on day 30 (51.9 g/kg DM) and remained at this level until the end of the experiment. Acetic acid concentration increased ($p < 0.001$) in the three silages gradually until the end of the experiment. Regarding butyric acid level, an increase ($p < 0.001$) was observed in the three silages: from day 30 in BB, where the maximum value was reached on day 60 (56.7 g/kg DM); the highest values in AB were given on days 7 and 15 (12.9 g/kg DM) and thereafter it was reduced ($p < 0.001$); in APS it increased until day 15 (12.6 g/kg DM), remaining stable until day 60. The ethanol concentration increased ($p < 0.001$) in the three silages from the beginning of the experiment, although it stabilised on different days: on day 30 for BB (17.8 g/kg DM), on day 60 for AB (9.19 g/kg DM) and on

day 7 for APS (3.59 g/kg DM). As with the rest of the fermentation products, ammoniacal N increased in the three silages: It remained at a constant increase throughout the experiment in BB ($p < 0.01$) until day 200 (1.65 g/kg DM), while it reached its maximum value on day 60 (1.15 and 0.242 g/kg DM) in AB and APS, respectively.

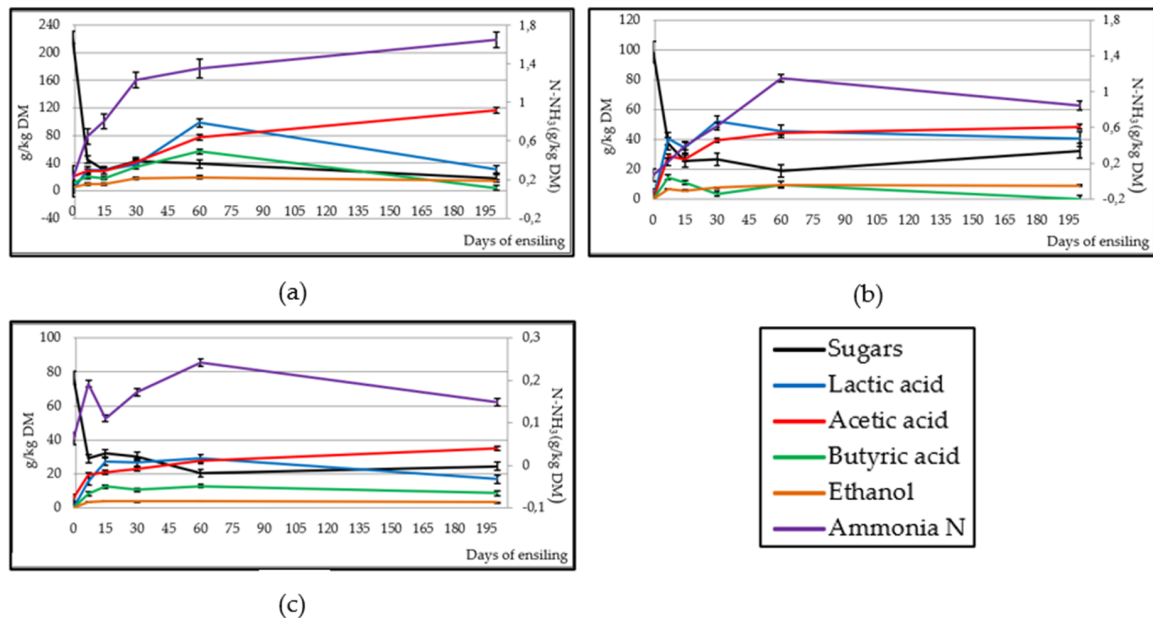


Figure 4. Effect of ensiling on sugar content and fermentative components in broccoli by-product (a), artichoke by-product (b) and artichoke plant stubble (c) silages.

3.4. Phytosanitary Residues Evaluation

Table 2 shows the phytosanitary residues determined on day 200 in the three silages. None of them exceeded the MRLs set by European legislation, although APS was the by-product in which a greater number of phytosanitary residues was detected.

Table 2. Phytosanitary residues (mg/kg) in silages after 200 days of ensiling in round bales broccoli by-product (BB), artichoke by-product (AB) and artichoke plant stubble (APS) silages.

| Phytosanitary | Type | BB | AB | APS | MRL | Legislation |
|---------------|-------------|-------|-------|-------|------|-------------|
| Cypermethrin | Insecticide | n.d. | n.d. | 0.240 | 2.00 | UE 520/2011 |
| Chlorpyrifos | Insecticide | n.d. | n.d. | 0.085 | 1.00 | CE 839/2008 |
| Imidacloprid | Insecticide | 0.023 | n.d. | 0.034 | 0.50 | UE 491/2014 |
| Miclobutanil | Fungicide | n.d. | 0.044 | 0.220 | 0.50 | UE 2016/567 |
| Spirotetramat | Insecticide | 0.053 | n.d. | n.d. | 1.00 | UE 2015/845 |
| Triadimefon | Fungicide | n.d. | n.d. | 0.700 | 1.00 | CE 459/2010 |

MRL: Maximum Residue Limit; n.d.: Not Detected.

3.5. Manufacturing Costs

Table 3 shows the different costs of the manufacturing process for the three types of silos. The main difference was observed in the cost of the raw material, since AB presented a higher cost (10 €/t of DM) than the other two by-products (4 and 5 €/tn of DM for BB and APS, respectively). However, final cost in €/kg of CP was higher in APS (1,01 €/kg CP) due to the lower content of CP of that silage.

Table 3. Approximate manufacturing costs (€/t) of broccoli by-product (BB), artichoke by-product (AB) and artichoke plant stubble (APS) silages in 300 kg round bale silos on commercial scale.

| Costs | BB | AB | APS |
|---|-------|-------|------|
| Raw material | 4 | 10 | 5 |
| Inner netwrap and plastic film | 7.9 | 7.6 | 8.1 |
| Workforce, other production and marketing costs | 7.3 | 7.3 | 7.3 |
| Total (fresh matter) | 19.2 | 24.9 | 20.4 |
| Total (dry matter) | 125 | 131 | 79 |
| Total (€/kg CP) | 0.718 | 0.903 | 1.01 |

CP: crude protein.

4. Discussion

4.1. Microbiology

The lactic acid bacteria naturally present in silage are responsible for carrying out the fermentation and influence its final quality [35]. The same variation shown by the lactic acid bacteria population in this study was observed in Khota et al. and Wen et al. [36,37] in tropical grasses and alfalfa silages. The population of lactic acid bacteria remained above the other microorganism populations throughout the experiment. The number of colonies of the other microorganisms decreased as a result of the anaerobiosis conditions and the high level of lactic acid bacteria. According to Muller et al. [38], they inhibit the growth of harmful microorganisms due to the production of antimicrobial substances (bacteriocins). The enterobacteria population decreased more rapidly in BB and AB than in APS, because in the latter silage pH dropped more slowly due to a lower sugar content on day 0. Woolford [39] indicates that a pH lower than 4.5 reduces enzymatic activity and prevents the proliferation of enterobacteria. Enterobacteria count values are lower than those found in other studies [36,40,41], which are close to 4 Log₁₀ cfu/g of FM from day 30 of ensiling. The yeast population was reduced due to the reduced availability of sugar and the increase in the concentration of acetic acid in the silages (Figure 4), which has antifungal and bactericide activity [42]. The low number of yeast colonies present after day 60 suggests that the silages will have high stability once the silo is opened, as the content was below the 6 Log₁₀ cfu/g recommended by Kung et al. [43] and will have good acceptance by animals [44]. The mould population observed in the three silages is lower than that found by Junges et al. [45] in corn silages on day 90. The *Clostridium* spp. spores value was lower than those found by other authors in alfalfa silages (0.84 Log₁₀ cfu/g on day 15, [37]) and corn silages (2–2.9 Log₁₀ cfu/g on day 90, [45]).

Considering all the microbiological variables studied, it was shown that the concentrations of microorganisms potentially harmful to the silage quality were kept below levels taken as a reference from the works of other researchers, in the absence of a regulation that sets the maximum permitted limits for these microorganisms.

4.2. Physico-Chemical Parameters and Nutritional Composition

The main purpose of ensiling is to preserve the nutrients in fresh food with minimum losses of DM and energy. During ensiling, DM is lost as effluents and gases [46]. McDonald et al. [47] stated that the loss of DM due to the activity of lactic acid bacteria is between 2% and 5%, which is transformed into CO₂ when the fermentation is carried out by heterofermentative bacteria. In this study, the loss of DM was between those values until day 30 in BB and day 60 in APS, while in AB a significant reduction in DM was observed from the beginning, although it subsequently stabilised. McDonald et al. [47] proposed 250 and 300 g/kg as appropriate initial DM contents for a correct silage process. Only in BB would it be below that range (174 g/kg), which would explain the slightest changes in the DM content in this silage, as happened in Megías et al. [14] with laboratory scale broccoli by-product silos. Wen et al. and Cai et al. [37,48] also found similar changes in DM content to those determined in this

study in the final stage of silage. DM contents were higher in BB and AB silages of Megías et al. and Meneses et al. [13,14] (71.6 and 266 g/kg, respectively as mean values throughout the ensiling process), but DM of APS of this study was higher than Hernández et al. [5] (203 g/kg).

The buffer capacity of silages was lower than often found in other foods, such as tropical forages (67.11 meq/100 g DM) or corn (50.8 meq/100 g DM) [36]. This allowed the pH to drop more quickly in these silages than in those foods, decreasing the risk of nutrient loss and proliferation of undesirable microorganisms. The greatest pH drops occurred at the beginning of the experiment, coinciding with the increase in the lactic acid bacteria population, which resulted in a significant increase in the lactic acid content, which is the most effective acid to reduce the pH [14], and which serves as a good indicator of silage [49] quality. Although pH of AB started from a higher value compared to Meneses et al. [13] (6.12 vs. 5.84), the final pH value of both silages reached similar levels. According to Wen et al. and Kung et al. [37,42], the pH of alfalfa and grass silages with an initial DM of 250–380 g/kg should decrease to 4.2–4.7 to ensure silage quality. In this study, the starting points of DM in AB and APS were 256 and 283 g/kg, respectively, and the pH reached on day 200 was 4.20 and 4.55 for AB and APS. Additionally, the rate of lowering of the pH is as important as the final pH that is reached, as fast acidification lowers the risk of growth of undesirable microorganisms during the first stage of silage [50]. The greatest drop in pH happened during the first 7 days, concurrent with a peak of lactic acid bacteria populations and lactic acid increase on day 7 (Figures 2 and 4), similarly to other studies [37,51,52].

Regarding Flieg scores, the values achieved were higher (indicating a higher quality) than those observed in other experiments with the same by-products, but with another ensiling technique, such as laboratory scale silos [14,18,21,53]. Only Meneses et al. [13] found a similar quality level for artichoke bracts laboratory scale silo on day 50 (95.6 Flieg points). However, in another experiment of the same researcher group with also laboratory size silos [54], the quality of artichoke bracts silage on day 100 was reduced to 64.2, whereas AB remained in the range 80–100 (very high quality) until day 200.

BB composition values did not match those of broccoli by-products studied in Megías et al. [14] because those were different from the one used in this experiment, as they were boiled inflorescences and raw stems, showing the great variability in the composition of by-products of the same class, as indicated by García and Castrillo [55]. However, the composition of AB and APS were similar to that of Meneses et al. and Hernández et al. [5,13], as they were the same raw materials, although ensiled in laboratory conditions and with a shorter silage process. The reduction in protein content in BB and AB was not excessive, as it did not exceed the 15% established by Meneses et al. [13] as an indicator of excessive proteolysis due to slow fermentation [50]. However, this reduction was not observed in other studies with the same by-products, because in BB it increased and in AB it remained stable [13,14]. The phenolic compounds have strong antioxidant activity that affects the antioxidant potential of a feed [11]. The increase in TP agreed with the lactic acid bacteria-producing β -glucosidase that catalyses the release of the phenolics during ensiled fermentation, making them more accessible to the solvent during the extraction and, consequently, enhancing the silage antioxidative potential [56]. Jung [57] observed the same in alfalfa and corn silages: the increase in TP concentration reduced fermentation of structural carbohydrates. As shown in Table 1, higher values of TP were achieved from day 30, which coincides with the end of the reduction of the NDF content in the three silages studied. The greatest changes in NDF and ADF contents occurred in BB because this silage had a higher concentration of sugars on day 0; however, in this study, the fibrous fractions of BB were reduced, but in Megías et al. [14] they increased. Ashbell and Donahay [58] indicated the relationship between the increase in ADF level as the sugar content is reduced, which causes an increase in the cell wall proportion in silage. Umana et al. [59] indicated that the highest digestibility of silage is achieved when populations of lactic acid bacteria stabilise, which also occurred on day 30 of this study. APS showed a higher IVDMD than Hernández et al. [5] in the same artichoke by-product (615 vs. 575 g/kg DM).

According to the silages composition on day 200, the high protein content and high DM digestibility of BB and AB increased CP/EM over 44 g of CP/Mcal EM (102 and 81.5 for BB and AB, respectively),

which was proposed by García and Castrillo [55] as a good ratio for well-balanced feedstuffs. For this reason, these two silages (BB and AB) should be incorporated into the ration in combination with an energy source to make the diets meet the nutritional requirements for ruminants. Regarding APS, it showed a balanced CP/EM ratio (50.4 g of CP/Mcal EM), so the need for an energy source is less than with the other two silages.

4.3. Fermentation

The fermentation products strongly determine the hygienic and nutritional quality of the silage, affecting the voluntary intake and production of the animals and the composition and quality of the milk and its derivatives [8]. Consequently, the quantification of organic acids and alcohols resulting from fermentation is essential for evaluation of the silage quality [42]. Several factors influence the synthesis of these fermentation products, such as the predominant microorganisms in the silage, the fermentable substrates present, and the types of fermentation that take place during the whole silage process [47].

Lactic acid production rate depends on the initial lactic acid bacteria populations and the availability of an easily fermentable substrate, such as sugars [60]. The sugar decrease and lactic acid increase observed in this experiment also occurred in other silages [37,52]. The lactic acid concentration values obtained were lower than those often found in grass silage (60–100 g/kg DM), although similar to values in ensiled legumes (20–40 g/kg DM, [42]). Lactic acid content was lower in BB than in Megías et al. [14] (103 g/kg DM on day 50) because BB started from a lower sugar content (223 vs. 620 g/kg DM). Until day 60, the three silages kept the lactic acid level above the rest of the VFA and fermentation products, which indicates a good concentration of nutrients and good palatability and pleasant smell, which would lead to good acceptance and high consumption by animals [14]. However, on day 200, it was observed that the acetic acid content was higher than that of lactic acid in BB and APS, which is a consequence of prolonged fermentation [47] and would cause the loss of nutrients, as can be observed in the reduction in DM between day 60 and 200 for these two silages. AB showed a higher level of acetic acid than Meneses et al. [13] (48.3 vs. 12.5 g/kg DM), as well as in the rest of fermentation metabolites, as AB had a higher content of rapidly fermentable carbohydrates (sugars; 98.3 vs. 56.1 g/kg DM), which favoured a more intense fermentation. Although moderate concentrations of acetic acid in the ration can be beneficial, as it is absorbed in the rumen and used as energy or incorporated as fat into milk or body reserves [61], the lactic:acetic ratio is considered a good indicator of the type of fermentation that has occurred in the silage and its quality, the best levels being between 3 and 1 [42]. In this study, that ratio was achieved from day 7 until day 60, but the final acetic acid increase observed at day 200 caused a slight decrease in the ratio. The final value of acetic acid in BB was higher than that recommended by Kung et al. [42] in grass silage (10–30 g/kg DM). Butyric acid and ethanol levels remained low throughout the experiment because enterobacteria, clostridia and yeasts populations were reduced as a result of a high lactic acid bacteria population [47]. Ammonia N yield was higher in BB because of the extended fermentation process, which caused a slight proteolysis, but without great effects on the reduction of the nutritional content of the silage [13]. The fermentation product concentrations were similar to those found by Driheuis and van Wikselaar [62] in grass silage in which a lactic fermentation has taken place.

The Flieg scale indicates that both AB and AP exhibited excellent silage quality. However, although BB quality remained high, it was on a lower level than the other two silages. Furthermore, its high CP content and high DM digestibility make it an interesting feedstuff to include in ruminant diets. Thus, the three silages can be used as part of livestock rations, although the high ammonia N concentration of BB may reduce its palatability and food intake in animals, so its inclusion in the diet should be done in moderation.

4.4. Phytosanitary Residues Evaluation

The phytosanitary residues found in the samples of the silages studied on day 200 do not exceed the MRLs imposed for each one by European legislation, so the three silages would not entail a risk to the health of the animals or for people who ingested the products obtained from these animals.

4.5. Manufacturing Costs

The cost of the three silages is much lower than that of other ingredients that are part of a conventional diet for ruminants. According to data prices from Lonja de Albacete [63] and feedstuff composition [64], alfalfa's price would be 1.36 €/kg of CP and the price of cereals ranges from 1.63 €/kg of CP for barley to 1.87 €/kg of CP for wheat, so the minimum difference would be between alfalfa and APS (0.350 €/kg of CP) and the maximum between wheat and BB (1.16 €/kg of CP). For this reason, despite the silos manufacturing costs, the economic profitability of these by-product silages is very great.

5. Conclusions

According to the values obtained for microbiology, physico-chemical parameters and fermentative and nutritional components, stabilisation of studied by-products was achieved on day 30. Thereafter, most variables remained stable or were modified very slightly, as occurred with the count of microorganism populations. The silage's quality remained high until day 200, as was the hypothesis of this experiment. We may state that ensiling broccoli and artichoke by-products and artichoke plant stubbles in commercial round bale silos is a practical and profitable technique that seems promising because it allows for their conservation over time, especially with artichoke by-products, not affecting their nutritional composition. Further studies should be carried out using them as feed for animals to explore voluntary intake and its effect on production and animal health.

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Publicación 2 (Transcripción literal)

Preference study of four alternative silage fodders from the Mediterranean region in Murciano-Granadina goats

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PREFERENCE STUDY OF FOUR ALTERNATIVE SILAGE FODDERS FROM THE MEDITERRANEAN REGION IN MURCIANO-GRANADINA GOATS

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Abstract: In Mediterranean countries, large quantities of by-products are generated from artichoke and broccoli crops, as well as large masses of common reed due to the cleaning of riverbeds. They have traditionally been applied directly in animal feed, especially for small ruminants, without undergoing any preservation process. Their inclusion in diets would allow three benefits: feeding animals with materials containing high nutrient contents all year round (not only in the crop season) in a well-balanced ration, lowering the feeding costs and reducing the amount of residues and their elimination cost for producers. Through the silage process, these feedstuffs can be preserved to ensure year-round availability. The aim of this short-term preference trial was to evaluate the goats' preference among four different ensiled feedstuffs and to study the relationship between animal preference and the silage composition. The four alternative silage-feedstuffs were offered to four nulliparous Murciano-Granadina goats (34.9 ± 1.9 kg) in a multiple square or cafeteria design. The feedstuffs tested were artichoke bracts (*Cynara scolymus*), artichoke plant, a 50:50 mixture of broccoli by-product (*Brassica oleracea*, var. *Italica*) and artichoke bracts, and common reed (*Phragmites australis*). To determine preference, data were collected on feed intake and the time each animal spent eating each feedstuff for four hours each day. Macrocomposition and some functional and chemical characteristic silages were analysed. The relation between DM intake and chemical composition of the tested feedstuffs was determined using correlation and regression analyses. The artichoke bracts silage presented the highest cumulative intake at 4 h (3.26 g DM/kg BW); being different from artichoke plant silage (2.17 g DM/kg BW), the second most consumed. Broccoli+artichoke and common reed silage intake was significantly lower than those of the other two feeds (0.36 and 0.67 g DM/kg BW, respectively). The strongest correlations between DM intake after 4 h (g of DM) and the nutritional and chemical characteristics of the silages were found with digestible energy ($r=0.60$, $P < 0.001$), lactic acid ($r=0.56$, $P < 0.001$) and lignin ($r= -0.54$, $P < 0.001$). The relation was described by a best fit equation of the formula $\text{DM intake} = -207.45 + 31.94 * \text{lactic acid} + 11.76 * \text{CP}$ ($R^2=0.48$, $P < 0.001$). Goats showed a clear preference for ensiled artichoke bracts, followed by artichoke plant silage, with the broccoli+artichoke and common reed silages being less accepted. The feedstuff composition, especially lactic acid and crude protein contents, affects preference. Since no feedstuff was rejected in its entirety by animals and previous results ensured a good quality of these silages, the four feedstuffs had the potential for incorporation into goat diet, due to their nutritional characteristics.

Keywords: Ruminant; By-Product; Artichoke; Broccoli; Common reed.

1. Introduction

Semi-intensive and intensive dairy goat farms are characterised by high demand and dependence on cereal-based energy concentrates and a rich source of protein and fibre such as alfalfa, which entail a high cost and require large crop surfaces. The use of alternative agrifood by-products, residues and fodder for feeding ruminants can minimise the external dependence on the aforementioned feeds and the feeding costs, while at the same time reducing the waste originated by the canning industry and the disposal costs. However, the marked seasonality and high-water content of these feedstuffs limit their use in animal feeding all year round.

World production of broccoli and artichoke is important and generates large amounts of by-products. In 2014, 1 575 700 tons of artichokes were harvested worldwide and Italy, Egypt and Spain were the largest artichoke-producing countries (more than 900 000 t; Food and Agriculture Organization, 2014). Artichoke by-products represent a high amount of waste material, roughly 80% of the total biomass of the plant (Lattanzio *et al.*, 2009). The artichoke plant is a by-product composed of leaves, stems and some unharvested inflorescences, while artichoke bracts are the outer leaves of the artichoke globe, along with some stems or peduncles. This material has traditionally been used for grazing small ruminants, or harvested and taken to farms (Hernández *et al.*, 1992) and incorporated into sheep (Jaramillo *et al.*, 2010) and goat (Monllor *et al.*, 2020ab) rations as silage. Wernli and Thames (1989) state that the yield is 11.105 t/ha of fresh forage in this crop, which, considering the Food and Agriculture Organization (2014) data on cultivated area worldwide (129 001 ha), would result in output of 1 432 556 tons/year of available artichoke plant. Regarding broccoli cultivation, 24 378 193 t of this vegetable were harvested worldwide, with China in first place with a production of 9 372 770 tons, followed by Spain (596 969 t) and Italy (405 053 t) (Food and Agriculture Organization, 2014). According to Ros *et al.* (2012), 29.5 % of broccoli inflorescence consists of stems and waste inflorescences that could be destined for animal consumption. Its potential value as silage has been partially evaluated (Megías *et al.*, 2014). Several studies have been carried out where broccoli by-product was included in the animals' diet, both as a single ingredient (Panwar *et al.*, 2017), where the daily intake was 624 ± 39.9 g/day or 6.27 % of body weight in Beetal goats, and integrated into TMR, reaching maximum values of 20 % in dairy cows (Yi *et al.*, 2015) and 40 % in dairy goats (Monllor *et al.*, 2020b). The assessment of the quality of the silages of both the artichoke and broccoli by-products as potential ingredients of a ration of ruminants is shown in Monllor *et al.* (2020c). The common reed (*Phragmites australis*) is a perennial rhizomatous grass that can be used as an alternative fodder. It grows close to aquatic ecosystems, such as rivers or lakes, invading cultivation areas and, in many cases, constitutes a residue from riverbed cleaning activities that can be used as ruminant feed, contributing to the circular economy. Recent studies revealed the potential use of common reed silage (Ogasawara *et al.*, 2004) or roughage (Baran *et al.*, 2002) as a component of livestock diets. Nevertheless, published studies have been carried out in laboratory conditions or mainly in sheep or cattle, with few studies being carried out in goats.

Silage is a way to preserve perishable products, such as the aforementioned by-products. Ideally, through lactic fermentation, the pH drops to a value of 4 or less, cellular respiration is suppressed, the degradation of proteins and vitamins is prevented and fermentation by clostridia is avoided. Studies using other agricultural by-products have shown that if diets supplemented with these types of feedstuffs are formulated carefully, so that the nutritional requirements of the animals are covered, their use must not compromise the milk production and quality, or the technological properties and quality of the derived products (Vasta *et al.*,

2008). On the other hand, due to the differences in composition between feeds and the presence of secondary fermentation metabolites, there is an inherent variability associated with their fermentation during the silage-making process. Once the silage is ready to be used, the short-term preference of animals towards these feeds can be a quick and useful tool to assess their suitability to be included in the diet.

The main objective of this experiment was to assess the goats' preference among four alternative ensiled feedstuffs and the relation among preference and silage composition, using a short-term trial to measure feedstuffs intake. The working hypothesis is that the short-term preference trial will serve to identify the preference scale of the four feedstuffs under study by measuring the intake of each one when they are offered at the same time.

2. Material and methods

2.1. Feed and experimental animals

This study was carried out in July, at the small ruminant farm of the Polytechnic School of Orihuela, Miguel Hernandez University (UMH), Spain. The feedstuffs studied were three agroindustrial by-products: artichoke bracts (*Cynara scolymus*) and broccoli by-products (*Brassica oleracea*, var. *Italica*), both from the canning industry, and artichoke plant and a residue from river cleaning, common reed (*Phragmites australis*). The three by-products are characteristic of the crops of this area and the common reed residue is from one of the predominant plants in the wild vegetation of the region. Three months before the start of the experiment, coinciding with the vegetable harvest, several microsilos of 300 kg and 0.64 m³ each of these four feeds were made with a baler-wrapper (Agronic MR 820, Netherlands), using five layers of inner netwrap (Karatzis S.A., Greece) and 13 layers of plastic film (Karatzis S.A., Greece). No additives were added. Aprovertia SL, a technology-based company of the Miguel Hernandez University, provided the facilities. For each silage, a sample was collected on the same day the silo was opened, for further analyses in the laboratory. Four non-pregnant nulliparous Murciano-Granadina goats at 10 months of age were used (34.9 ± 1.9 kg of BW). The usual diet of these goats consisted of alfalfa hay (290 g/kg), barley straw (140 g/kg) and concentrate based on cereal grains and oilseeds (570 g/kg), calculated for an intake of 1.4 kg of DM/animal and with 0.81 UFL/kg DM and 106.70 g of PDI/kg DM of digestible protein content.

2.2. Experimental design

A cafeteria or multiple square design was used because this methodology has been used successfully in ruminants for forage preference studies (Borman *et al.*, 1991; Meier *et al.*, 2012). The goats were housed in individual pens of 3×3 m. The pre-experimental phase lasted 14 days, during which each goat was given 150 g of each product in the morning: silage of artichoke bracts (AB), artichoke plant (AP), a mixture of broccoli with artichoke bracts with a 50:50 ratio (BRO) and common reed (C), and the usual ration in the afternoon. The experimental period lasted 4 days. After a 12-h fast, each goat was offered at the same time 2 kg as fed in individual plastic buckets housed in the feeder for periods of 4 h. The sequence of the buckets with each product was different for every goat and varied every day to avoid conditioned learning (association) between the positions of the buckets and the type of silage they contain. At all times, the buckets were not empty.

The orts were measured every hour to obtain a pattern of cumulative intake. During the 4 h that data collection took place each day in farm, the minutes that each goat spent eating each feed were recorded and, in this way, the intake rate could be obtained (g DM/min in which the animal

eats from each silage) in order to determine the intake pattern of each feed tested. After 4 h, the buckets were removed. Then goats were given two thirds of total amount of the usual diet. During this period, the same bale of each silage was used, thus avoiding differences in consumption. To ensure that the feed quality did not vary between the start and end of the experimental period, plastic bags with 2 kg of silage were vacuum packed and stored at 4 °C until use at the beginning of the trial.

2.3. Variables analysed

Prior to analysis of the macrocomposition, sugar content and *in vitro* DM digestibility (IVDMD), the feed samples were dehydrated at 60 °C in an oven for 48 h and subsequently ground (1 mm) and set aside in airtight containers for subsequent analysis. For the rest of the analysis, the sample was separated into individual plastic bags with an approximate content of 100 g of fresh sample in each and frozen at -20 °C until further analyses. The silage samples were analysed in duplicate using the official methods of the AOAC (1990) for DM (934.01), ash (942.05), CP (988.05), ammonia (NH₃) (941.04), ether extract (EE) (920.39), crude fibre (CF) (978.10) and total sugars (974.06). The pH was measured (GLP21 Crison, Spain) and Flieg Points were calculated to determine the quality of the silage, according to the equation given by Kilic (1986):

$$\text{Flieg points} = 220 + (2 \times \text{DM} (\%) - 15) - 40 \times \text{pH}$$

According to this index, a score obtained below 20, between 21 and 40, between 41 and 60, between 61 and 80 and over 81 would correspond to very low, low, medium, high and very high quality silages. NDF, ADF and ADL were also determined according to Van Soest *et al.* (1991). All fibrous fractions were corrected for the residual ash content. The total polyphenols (TP) content was analysed by the Folin-Ciocalteu method (Kim *et al.*, 2003). In addition, the proportion of short chain volatile fatty acids (VFA) present were determined (Feng-Xia *et al.*, 2013): acetic, propionic and butyric acid, also including lactic acid and ethanol by liquid chromatography by HPLC (Agilent 6890 with flame ionisation detector and column Supelcogel C-610H: 30 cm x 7.8mm ID). Finally, the energy fractions of gross energy (GE), digestible energy (DE), metabolisable energy (ME) and net energy of lactation (NEI) were determined, according to the equations proposed by Institut National de la Recherche Agronomique (1981). IVDMD was analysed in duplicate using the method of Menke and Steingass (1988), using rumen liquid extracted with the aid of an oesophageal probe from five adult Murciano-Granadina breed goats fed with a conventional ration based on barley straw and grain, which had been fasting for twelve hours prior to extraction.

2.4. Statistical analysis

The cumulative dry matter intake from hours 0–4, dry matter intake per kg of live weight of the animal accumulated at hour 4 and the dry matter intake rate (g DM/min) at hours 1 and 4 were analysed using a mixed linear model (Proc. Glimmix, SAS v9.2., 2012), which considered the day and hour of sampling (4 levels: 1–4) and the treatment (4 silages) as fixed effects, while animal was considered as random effect. A compound symmetry covariance structure was used, as it was the best fit of the model presented, according to the AIC and BIC criteria. Means and SD of dry matter intake (g DM), minutes of intake (min) in hour 1 and hour 4 of each feed were also calculated (Proc. Means, SAS v9.2, 2012).

The evolution of cumulative dry matter intake (g DM) was analysed throughout the sampling (hours 1–4), using a mixed linear model (Proc. Glimmix, SAS v 9.2., 2012), which considered the day of sampling (4 levels: 1–4), sampling time (4 levels: 1–4), and alternative fodder (4 levels) as

fixed effects, while animal was considered as random effect. A composite symmetry-type covariance structure was used, as it was the best fit of the model presented, according to the AIC and BIC criteria. Correlation (Proc. Corr, SAS v 9.2., 2012) of the cumulative DM intake in hour 4 with the variables of nutritional components of the feed: DM, OM, pH, EE, CP, CF, NDF, ADF, ADL, lignin, sugars, IVDMD, ash, ammonia, PT, volatile fatty acids (acetic, propionic and butyric), lactic acid, ethanol and energy fractions (GE, DE, ME and NEI) was analysed. Regression (Proc. Reg, SAS v 9.2., 2012) of DM cumulative intake in hour 4 on nutritional components of the feed: DM, pH, EE, CP, CF, NDF, ADF, ADL, sugars, IVDMD, ash, ammonia, PT, volatile fatty acids (acetic, propionic and butyric), lactic acid and ethanol was also analysed.

3. Results

3.1. Silage composition

The chemical composition, energy content and IVDMD of the four silages are shown in Table 1. AP was the silage where most lactic acid was detected (53.99 g/kg DM). However, in the case of BRO, no lactic acid could be detected. Other fermentation products of sugars such as short chain VFAs (acetic, propionic and butyric), as well as ethanol and ammonia, were also found in the silages. Some fermentation products (propionic and butyric acid) could not be detected in AB, AP and C silages. BRO was the only feed where the presence of butyric and propionic acid was detected (14.59 and 33.55 g/kg DM, respectively), coupled with a high amount of acetic acid (69.22 g/kg DM), and a higher NH₃ content (3.31 g/kg DM) than the other three silages. The four silages in this study had different pH, with AP showing the lowest value and BRO the highest (3.99 and 5.15, respectively). It can also be observed that three of the four feeds studied had a similar NDF content, with the exception of the common reed. Regarding the energy value, the three by-products reached similar values of both NEI (0.969-0.981 kcal/kg DM), and C presented the lowest (0.941 kcal/kg DM of NEI). In reference to the total polyphenols content, AB was the richest (17.01 g/kg DM), followed by BRO and C, with similar amounts and, finally, AP.

3.2. Intake and preference

The cumulative intake after 4 h of each feed is shown in Table 2. The intake was higher in AB, followed by AP, with C and BRO being significantly lower (Fig. 1; $P < 0.0001$). This figure shows how eating rate was reduced over time, being higher at the beginning. As can be seen in Table 2, at hour 1, the eating rate of AB was significantly higher than for the rest of the feed (4.8 g DM/min; $P < 0.01$). The lowest eating rate at hour 1 was recorded with BRO and C, reaching a value of 1.5 g DM/min. On the other hand, at hour 4 the intake rates were reduced in the four feedstuffs and AB was higher than BRO (3.0 vs. 1.4 g of DM/min, $P < 0.0495$), with no differences between AP and C. The intake of the four feedstuffs was greater in hour 1 than in hour 4 (Table 3), with AB presenting the highest intake, followed by AP. In the same way, the effective time the animals spent eating was also reduced in hour 4 compared to hour 1. No significant differences were observed among the days of the experiment ($P > 0.05$). The correlation coefficients between the nutritional components of the silages used in the experiment and the cumulative intake of DM in 4 h are shown in Table 4. The correlation with DM, EE, CP, ammonia, acetic acid and ethanol was not significant ($P > 0.05$). With a significance level of $P=0.05$, the intake correlated positively with the pH of the feed. The energetic fractions and the concentration of lactic acid present in the feed correlated positively with intake ($P < 0.001$), with an r higher than 0.5. Positive correlations were also found with concentration of OM and sugars. On the other hand, the lignin content had a significant negative influence on intake, being the one with the highest r (-0.54). The rest of the fibrous fractions also correlate negatively with

feed intake. Table 5 shows the result of the regression analysis of cumulative DM intake in 4 h and the composition variables. Only lactic acid and CP were significant predictors of intake ($R^2=0.48$; $P < 0.001$).

4. Discussion

4.1. Feedstuff composition

The four silages in this study had different pH, with AP showing the lowest value and highest lactic acid concentration. The pH, sugar and lactic acid contents of AB were similar to those reported in previous studies in artichoke silages (Meneses *et al.*, 2007), although other secondary VFAs (propionic and butyric), not reported by these authors, were detected. In the case of BRO, the acid lactic content was lower (almost null) and pH and butyric were especially higher than the results of Wang *et al.* (2017) in a mixed silage with rice straw, broccoli by-product and alfalfa (31.5 g/kg, 4.78 and 2.13 g/kg for lactic acid, pH and butyric acid), and over the limit set by Castle and Watson (1985) for a good quality silage (2 g/kg). Regarding the ammonia levels, all the silages studied had desirable levels according to McDonald *et al.* (1991), lower than 100 g of N-NH₃/kg TN, with BRO being the closest (94.4 g of N-NH₃/kg TN). Therefore, the score obtained in the Flieg scale showed that AP and C are silages of excellent quality, AB would be considered medium-quality silage and BRO would be a poor-quality silage.

The values of the fibrous fractions of C and AB were similar to those found in Tanaka *et al.* (2016) and Meneses *et al.* (2007), respectively; AP showed lower values of NDF, ADF and ADL and higher IVDMD than those reported by (Hernández *et al.*, 1992), probably due to a greater maturity of the artichoke plant used in that study. Values of NDF, ADF and ADL in BRO were higher than those found by Megías *et al.* (2014) in laboratory scale silos of broccoli raw stems on day 50 of silage.

All four silages have adequate PDI and ME contents to cover the nutritional needs of growing goats of 56.0 g PDI/kg DM and 1.74 Mcal/kg DM (Aguilera *et al.*, 1990; Bach *et al.*, 2010). Regarding the NDF content, Bach *et al.* (2010) recommend a level of 280–440 g/kg DM, which coincided with that of the three agroindustrial by-product silages (AB, AP and BRO). However, due to the high NDF content of C, it would be necessary to add it to the ration together with a starch source, such as cereals. In this way, it would also be possible to increase its energy content, which is slightly lower than that of the other three silages.

4.2. Intake and preference

The main objective of this experiment was to evaluate the relation between the composition of ensiled alternative fodders and short-term intake by goats, to validate the use of short-term preference as an index of silage quality. The moderate and high correlation of intake with pH, sugars, total protein, lignin, energy and lactic acid suggests that intake is a good indicator of the nutritional quality and type of fermentation that has occurred in the silage, and thus its final quality. Lactic acid plays an important role in intake, as has already been observed in its high correlation coefficient, which is a predictor of the regression equation to predict intake (kg DM/day). This acid is the most effective in reducing the pH of the silage and, therefore, the most effective in preserving the quality of the feed. Together with the pH and the concentration of VFAs, they are the best quality indicators for silage (Madrid *et al.*, 1999). Silage forages with high concentrations of lactic acid accumulate a higher concentration of nutrients, are more palatable and give off a more pleasant odour, resulting in greater intake (Stefanie *et al.*, 1999). Regarding the positive relation between intake and protein content, Rogosic *et al.* (2006) states that, in the

ruminal environment, there must be a sufficient amount of ammonium to allow the digestion of structural carbohydrates, so a CP content lower than 7 g/kg will reduce intake (Milford and Minson, 1965), but if the CP is not limited (higher than 120 g/kg), this variable will not limit the intake (Sandoval-Castro *et al.*, 2005; Alonso-Diaz *et al.*, 2008). Those concepts are also valid for single feeds, considering that a complete diet was offered on daily average and contributed to protein supply for ruminal fermentation. The negative and high correlation between intake and lignin agrees with Hadjigeorgiou *et al.* (2003) and Sandoval-Castro *et al.* (2005), which explained that lignin can act as an indicator of hardness and difficulty when chewing and reduces the preference by goats. That is why, despite the fact that C has more Flieg points than AB, the higher lignin content of this residue reduced the preference of the animals. These results, together with the lower correlation between IVDMD and intake compared to that obtained by Sandoval-Castro *et al.* (2005) in heifers in an experiment in cafeteria with five tree fodders ($r=0.92$), agree with the hypothesis that goats are able not only to discriminate the most digestible among several feeds to optimise the rate of nutrient intake and reduce exposure to predators (Duncan and Gordon, 1999), but also to select those feeds with better degree of preservation, higher palatability and more pleasant odour, whose main quality indicator in ensiled by-products seems to be the lactic acid and protein contents, especially if these feeds meet the nutritional requirements of the animals.

The positive correlation between total polyphenol content and feed intake does not agree with the numerous authors that affirm the opposite, either due to a pre-ingestive effect due to feed astringency (Ben Salem *et al.*, 2005) or a post-ingestive effect whereby DM digestibility is reduced (Rogosic *et al.*, 2006). Causes of this discrepancy may be the following: the amounts of total polyphenols in the present study were quite low (17.01 g/kg of DM in AB maximum), while in those studies, the feeds had a high level of condensed tannins, which are the cause of pre- and post-ingestive effects and, in the tested ensiled feeds in this study, a large part of the polyphenols probably corresponded to phenolic compounds from the solubilisation of lignin during the fermentation process, such as hydrolysable tannins or phenolic acids, which are the main phenolic constituents in artichoke heads and leaves (Azzini *et al.*, 2007; Pandino *et al.*, 2013). In addition, as it is a short-term study, there was not enough time for animals to develop effects of this kind, so they were not able to associate them with a particular feed (Sandoval-Castro *et al.*, 2005).

BRO had significantly lower intake, despite being the feed with the highest protein content, as its lactic acid content was zero added to the high level of butyric acid, which is usually less palatable (Watson and Smith, 1951). Additionally, Ruiters *et al.* (2009) pointed out that the vegetables of the Brassicaceae family, to which broccoli belongs, present some anti-nutritional factors, such as S-methylcysteine sulfoxide, which are found mainly in the inflorescence and can cause a depression in intake. The limited intake of C could be due to the fact that it was the silage with the highest level of NDF, which is related to the time spent on feed digestion and, consequently, reduces the intake rate (Verlinden and Wiley, 1989). The intake rates of this fodder (0.055 g/min kg BW) were similar to that reported by Vulink and Drost (1991) in cows, also for common reed (0.059 g/min kg BW). The intake of AP was lower than that obtained by (Hernández *et al.*, 1992) in castrated Murciano-Granadina males, where artichoke plant silage presented an intake of 50.2 g of DM/kg BW during 24 h. The reason for this difference may be the different sex and different time the animals had access to feed.

However, the total intake amounts of these four feedstuffs are lower than those found in the bibliography due to two fundamental aspects. The first is that those feedstuffs were not

provided as unique ingredient of the ration to animals, since after the four-hour period of daily data collection, the animals were fed with two thirds of the conventional diet. The second reason is the higher content of fermentation metabolites (especially BRO) that those silages presented compared to alfalfa hay or cereal grains. Despite this, this study did not show a complete rejection of those four alternative silage-feedstuffs by goats, which indicates that they can be incorporated into a complete ration. This is confirmed by the results of the experiments of Muelas *et al.* (2017) and Monllor *et al.* (2020ab), where the incorporation of AB, AP and BRO in a complete diet for dairy goats at different inclusion levels (12.5, 25 and 40 %, on a dry matter basis) did not affect the milk yield, quality and technological properties or their health status.

5. Conclusions

Goats showed a clear preference for ensiled artichoke bracts, followed by artichoke plant silage, with the broccoli+artichoke and common reed silages being less accepted. The feedstuff composition, especially lactic acid and crude protein contents, affects preference. As no feedstuff was rejected in its entirety by animals and previous results ensured a good quality of these silages, the four feedstuffs can be incorporated into ruminant diets due to their nutritional characteristics.

6. Ethics statement

The project was assessed by the Research Evaluation Office of Universidad Miguel Hernandez and qualified as suitable (Registration number: 2015.186.E.OEP, Reference: UMH.DTA.GRM.01.15).

7. Software and data repository resources

Not relevant.

Declaration of Competing Interest

None.

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Table 1. Chemical composition, energy content and in vitro dry matter digestibility of the four feeds used in the experiment.

| Composition | By-products | | | |
|--|-------------|-------|-------|-------|
| | AB | AP | BRO | C |
| Dry matter (g/kg) | 149 | 252 | 137 | 373 |
| Organic matter (g/kg DM) | 918 | 854 | 850 | 897 |
| pH | 4.79 | 3.99 | 5.15 | 4.59 |
| Flieg score | 43.2 | 95.9 | 26.4 | 96.1 |
| Ether extract (g/kg DM) | 26.3 | 52.6 | 25.3 | 25.2 |
| Crude protein (g/kg DM) | 159 | 89.5 | 184 | 137 |
| Digestible protein in intestine (g/kgDM) | 99.7 | 56.2 | 113.5 | 69.1 |
| Crude fibre (g/kg DM) | 376 | 355 | 338 | 375 |
| Neutral detergent fibre (g/kg DM) | 482 | 429 | 494 | 664 |
| Acid detergent fibre (g/kg DM) | 333 | 285 | 362 | 368 |
| Lignin (g/kg DM) | 47.7 | 48.6 | 67.1 | 52.6 |
| Sugars (g/kg DM) | 39.8 | 25.1 | 17.7 | 33.2 |
| <i>In vitro</i> dry matter digestibility (g/kg DM) | 745 | 644 | 775 | 412 |
| Ash (g/kg DM) | 82.4 | 146 | 150 | 103 |
| Ammonia (g/kg DM) | 1.83 | 0.270 | 3.31 | 0.691 |
| Total polyphenols (g/kg DM) | 17.0 | 4.61 | 8.30 | 7.41 |
| Acetic acid (g/kg DM) | 44.4 | nd | 69.2 | 17.1 |
| Propionic acid (g/kg DM) | nd | nd | 14.6 | nd |
| Butyric acid (g/kg DM) | nd | nd | 33.6 | nd |
| Lactic acid (g/kg DM) | 42.2 | 54.0 | nd | 24.1 |
| Ethanol (g/kg DM) | 12.0 | 5.82 | 13.6 | 3.91 |
| Gross energy (Mcal/kg DM) | 4.14 | 3.92 | 3.90 | 4.02 |
| Digestible energy (Mcal/kg DM) | 2.13 | 2.09 | 2.14 | 2.08 |
| Metabolisable energy (Mcal/kg DM) | 1.72 | 1.72 | 1.72 | 1.68 |
| Net energy of lactation (Mcal/kg DM) | 0.969 | 0.975 | 0.981 | 0.949 |

AB: Ensiled artichoke bracts; AP: Ensiled artichoke plant; BRO: Broccoli byproduct+artichoke bracts (50:50) silage; C: Ensiled common reed; DM: Dry matter; nd: Not detectable.

Table 2. Results of the comparison of means of cumulative intake by goats after 4 h of the four ensiled feedstuffs tested and eating rate at 1 and 4 h.

| Cumulative intake | Ensiled feedstuffs | | | | SEM | P-value |
|---|--------------------|-------|-------|-------|------|---------|
| | AB | AP | BRO | C | | |
| g DM in 4 h | 113.2a | 73.9b | 13.0c | 23.8c | 9.12 | <0.0001 |
| g DM in 4 h/kg BW | 3.2a | 2.2b | 0.4c | 0.7c | 0.28 | <0.0001 |
| Eating rate 1 st hour (g DM/min) | 4.8a | 3.1b | 1.5bc | 1.5c | 0.48 | 0.0030 |
| Eating rate 4 th hour (g DM/min) | 3.0a | 2.4ab | 1.4b | 2.4ab | 0.48 | 0.1495 |

abc: Different superscripts within the same variable indicate significant differences between experimental feeds.

AB: Artichoke bracts silage; AP: Artichoke plant silage; BRO: Broccoli by-product+artichoke bracts (50:50) silage; C: Common reed silage, DM: Dry matter; BW: Body weight.

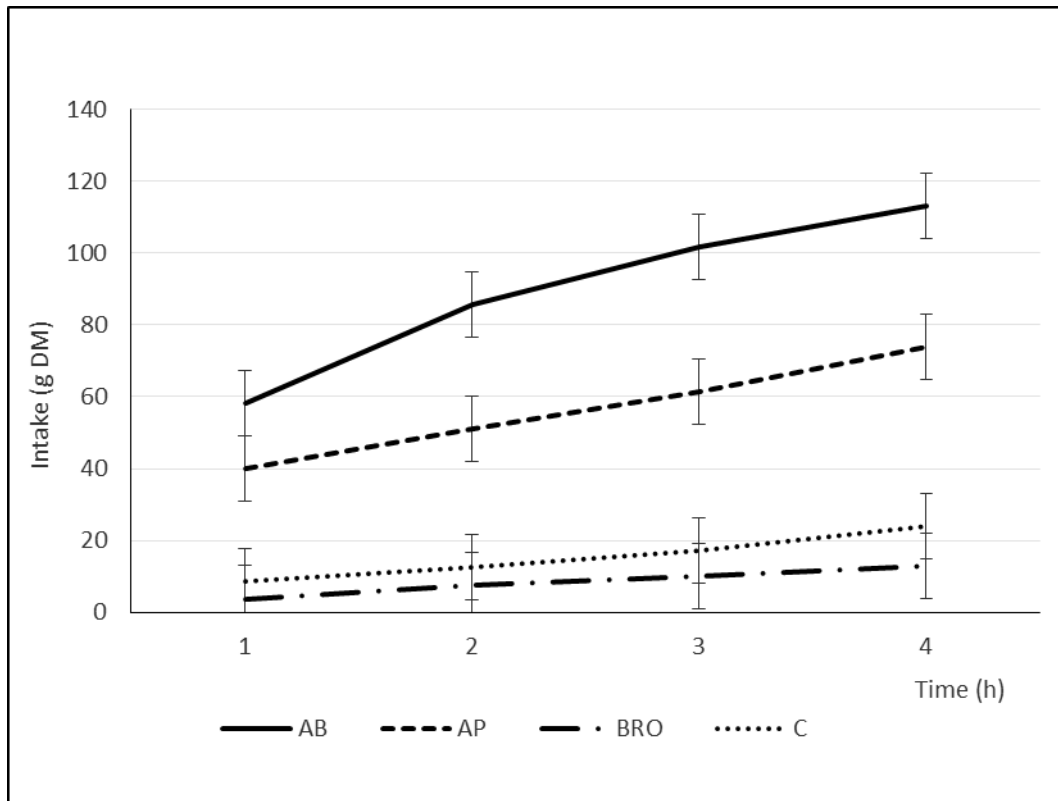


Figure 1. Goat's cumulative intake during 4 h of the four feeds used in the preference study (mean \pm standard error).

AB: Artichoke bracts silage; AP: Artichoke plant silage; BRO: Broccoli by-product+artichoke bracts (50:50) silage; C: Common reed silage.

Table 3. Feedstuff preference (intake g DM) and eating time (effective minutes of intake) by goats (mean \pm SD).

| | | Ensiled feedstuffs | | | |
|-------------|-------------|--------------------|------------------|----------------|-----------------|
| | | AB | AP | BRO | C |
| First hour | Intake | 58.2 \pm 33.95 | 40.1 \pm 25.99 | 3.8 \pm 6.07 | 8.6 \pm 13.31 |
| | Eating time | 11.6 \pm 4.17 | 12.9 \pm 7.02 | 1.1 \pm 1.92 | 4.4 \pm 5.67 |
| Fourth hour | Intake | 28.3 \pm 28.38 | 18.5 \pm 20.67 | 3.3 \pm 5.70 | 6.0 \pm 8.09 |
| | Eating time | 6.8 \pm 5.00 | 7.7 \pm 6.85 | 1.1 \pm 1.84 | 3.2 \pm 3.81 |

AB: Artichoke bracts silage; AP: Artichoke plant silage; BRO: Broccoli by-product+artichoke bracts (50:50) silage; C: Common reed silage.

Table 4. Correlation coefficients between chemical composition and IVDMD and dry matter intake of goats in preference experiment after four hours.

| Composition | r | P-value | Composition | r | P-value |
|-------------|-------|---------|-------------|-------|---------|
| Dry matter | -0.22 | ns | Ammonia | -0.20 | ns |
| pH | 0.24 | ns | TP | 0.43 | *** |
| OM | 0.37 | ** | AA | -0.16 | ns |
| EE | 0.20 | ns | PA | -0.43 | *** |
| CP | -0.21 | ns | BA | -0.43 | *** |
| CF | 0.36 | ** | LA | 0.56 | *** |
| NDF | -0.36 | ** | Ethanol | 0.09 | ns |
| ADF | -0.43 | *** | GE | 0.50 | *** |
| ADL | -0.54 | *** | DE | 0.60 | *** |
| Sugars | 0.47 | *** | ME | 0.57 | *** |
| IVDMD | 0.23 | ns | NEI | 0.57 | *** |
| Ash | -0.37 | ** | | | |

ns: Non-significant ($P > 0,05$); * $P < 0,05$; ** $P < 0,01$; *** $P < 0001$.

OM: organic matter; EE: ether extract; CP: crude protein; CF: crude fibre; NDF: neutral detergent fibre; ADF: acid detergent fibre; ADL: acid detergent lignin; IVDMD: *in vitro* dry matter digestibility TP: total polyphenols; AA: acetic acid; PA: propionic acid; BA: butyric acid; LA: lactic acid; GE: gross energy; DE: digestible energy; ME: metabolisable energy; NEI: net energy of lactation.

Energy value estimations were calculated according to Institut National de la Recherche Agronomique (1981).

Table 5. Linear regression equation between the chemical composition of the feedstuffs and the cumulative intake of DM in 4 h.

| Variable | Coefficient | SEM | R ² partial | P-value | Model |
|---------------|-------------|------|------------------------|---------|-------------------------|
| Lactic acid | 31.9 | 4.43 | 0.3095 | <0.0001 | R ² = 0.4833 |
| Crude protein | 11.8 | 2.60 | 0.1738 | <0.0001 | F= 28.52 |
| Intercept | -207 | 48.6 | | | P< 0.0001 |

Publicación 3 (Artículo original)

Short-Term Effect of the Inclusion of Silage Artichoke By-Products in Diets of Dairy Goats on Milk Quality

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Article

Short-Term Effect of the Inclusion of Silage Artichoke By-Products in Diets of Dairy Goats on Milk Quality

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Simple Summary: The use of artichoke by-products, both from the canning industry or from the stubble that remains in the field, provides a cheaper source of nutrients, suitable for ruminant feeding due to their ability to digest fibre-rich foods. The use of these by-products for animal feed is also a way to reduce waste caused by the canning industry and disposal costs, as well as the area and resources allocated to the production of food for livestock, contributing to the circular economy. Evaluating the effect of the inclusion of silage artichoke by-products (bracts and rest of crop plant) in dairy goat rations on the milk yield and composition, animal health status, mineral and lipid profile is an effective way to explore the suitability of these alternative feedstuffs for goat cattle. The use of artichoke bracts and whole plant silage in dairy goat diets does not lead to marked differences in the milk yield and quality or the animals' health status. From a nutritional point of view for human health, slightly better mineral and lipid profiles are observed in milk from goats fed artichoke plant silage, due to its higher polyunsaturated fatty acids and conjugated linoleic acid contents.

Abstract: Artichoke by-products represent a high amount of waste whose removal entails several costs. Moreover, feed is the main cost in a farm. So, including these by-products in ruminant diets would lower feed costs. Two experiments were conducted to evaluate the effect of two levels of inclusion, 12.5% and 25.0%, of two silages of artichoke by-products (artichoke bracts, AB and artichoke plant, AP) in the diet of goats on the milk yield, composition and quality and on the metabolic profile of the animals. AB presented the lowest blood urea content and there were no differences in milk yield in the two experiments. However, with 25.0% of silage by-product in the diet, a higher fat content was observed in AB and of protein in AP, as well as this treatment showing a slightly higher Se content. Regarding the milk lipid profile, milk from 12.5% of AP treatment presented a higher PUFA content. In conclusion, the use of silage artichoke by-products in dairy goat diets does not jeopardise milk yield and quality and health status of animals and, from a nutritional point of view for human health, a slightly better mineral and lipid profile is observed in milk from AP treatments.

Keywords: silage; lipid profile; minerals; metabolic profile

1. Introduction

The use of agri-food by-products and alternative fodder provides local food for livestock and it helps to reduce dependence on food from abroad. The use of vegetable by-products, both from the canning industry or from the stubble that remains in the field, is a cheaper source of nutrients, suitable for ruminant feeding due to their ability to digest fibre-rich foods. The use of these by-products

for animal feed is also a way to reduce waste caused by the canning industry and disposal costs, as well as the area and resources allocated to the production of food for livestock, contributing to the circular economy.

The marked seasonality of vegetables reduces the availability of these foods for much of the year and their high water content makes them a food with a short shelf life. Previous studies have shown that the silages of these by-products meet the fermentative conditions that ensure the nutritional and safety quality necessary to be part of the ration of small ruminants [1–3], and it allows their conservation for long periods of time (up to 200 days, according to [3]). The references found in the literature on the effect of silage consumption of these by-products in sheep on the milk quality and composition and the health status of the animals are scarce, but indicate their suitability for this purpose [1,4–6]. However, there has only been one study conducted in dairy goats on the effect on the technological properties of milk [7].

The consumption of fresh goat's milk worldwide is in third place behind that of cow and buffalo [8], but demand for it is increasing as a source of animal protein, calcium and phosphorus [9], and it has been catalogued by medical professionals as a perfect substitute for cow's milk in cases of food allergies [10].

The artichoke (*Cynara scolymus* L.) yield worldwide is important and generates a large quantity of by-products. In 2017, 1,505,328 t of artichoke were harvested worldwide [11]. This crop contributes significantly to the agricultural economy of the Mediterranean, where more than 60% of the world production of this vegetable originates [12]. Overall, artichoke by-products from the canning industry (leaves, external bracts and stems) represent a high amount of waste material—about 80% of the total biomass of the plant [13]—which entails the generation of 1,204,262 t/year of by-product. The artichoke plant is a by-product composed of leaves, stems and some unharvested inflorescences, which has traditionally been used for small grazing ruminants or has been harvested and taken to dairy farms [1]. According to Wernli and Thames [14], the yield of green fodder in this crop is 11.1 t/ha; which, taking into account the area cultivated worldwide (122,390 ha; [1]), results in a production of 1,358,529 t/year of available artichoke plant.

This study aims to evaluate the effect of the inclusion of silage artichoke by-products (bracts and the rest of the crop plant) at two levels (12.5 and 25.0% on a dry basis) in the ration of dairy goats on the milk yield and composition, animal health status, milk mineral and lipid profile and indices related to the nutritional quality of milk fat. The hypothesis of this study is that the inclusion of these by-product silages in well-balanced total mixed rations does not jeopardise goat performance and health status.

2. Materials and Methods

2.1. Animals and Facilities

Lactating Murciano-Granadina goats were used, housed at the teaching and experimental farm of the Miguel Hernández University, with a straw bed, access to outdoor yards, free access to water and enough feeding space for all animals (at least 35 cm/animal). The animals were fed twice a day, at 8:00 a.m. and 2:00 p.m., and milked once a day (Casse milking parlour, 2 × 12 × 12, GEA, Germany), as usual in the region. This study was approved by Responsible Research Office from Miguel Hernández University (code UMH.DTA.GRM.01.14).

2.2. Experimental Design

From a group of 70 goats that were in the middle of lactation (fourth month) fed with a conventional diet (control, C), pre-experimental sampling was performed and 57 animals were selected, with an average body weight of 44.7 ± 6.84 kg, an average production of 2.68 ± 0.68 kg/day and a somatic cell count (SCC) of 5.97 ± 0.42 Log cells/mL. They were divided into three homogeneous groups according to the variables mentioned (without significant differences between treatments). Each group was randomly assigned a diet: C (control, no by-products), AB (with silage artichoke bracts), AP (with silage artichoke plant).

The C diet was a conventional ration that included alfalfa hay and a mixture of grains in both experiments and was similar to that of the pre-experimental period. In Experiment 1, the level of inclusion of by-products in AP and AB was 12.5% (on dry matter basis), while in Experiment 2 the level of inclusion of silage by-products was increased to 25.0% (on dry matter basis) of the total ration, and the rest of the ration was composed of alfalfa hay and grain mixture. The groups of both experiments were composed of the same animals. All rations were calculated according to the formulation recommendations of Fernández et al. [15] for goats with a milk yield of 2.5 kg/day in Experiment 1 and 2.0 kg/day in Experiment 2, so that they were isoenergetic and isoproteic and adjusted to the milk yield. The animals were fed twice a day with fixed amounts, not ad libitum. Table 1 shows the amounts of the ingredients in each diet, as well as their composition and the amount offered daily.

Table 1. Ingredients and chemical composition of the experimental diets.

| Item | Experiment 1 | | | Experiment 2 | | |
|---|---|-------|-------|--------------|-------|-------|
| | C | AB | AP | C | AB | AP |
| | Ingredients (g/100 g DM) | | | | | |
| Alfalfa hay | 37.6 | 24.3 | 24.2 | 37.6 | 15.0 | 12.0 |
| Barley straw | - | 0.959 | - | - | 0.632 | - |
| Grains mix | 59.2 | 61.2 | 59.8 | 59.2 | 59.5 | 56.2 |
| Oat | 3.16 | 1.24 | - | 3.18 | - | - |
| Soybean meal 44% | - | - | 2.63 | - | - | 5.68 |
| Silage | - | 12.4 | 13.4 | - | 24.6 | 25.8 |
| Premix vitamins/minerals | - | - | - | - | 0.316 | 0.352 |
| kg DM offered/day | 2.25 | 2.19 | 2.24 | 1.98 | 1.90 | 1.99 |
| | Chemical composition | | | | | |
| DM (g/kg FM) | 872 | 577 | 707 | 868 | 398 | 516 |
| | g/kg DM | | | | | |
| OM | 935 | 935 | 930 | 929 | 937 | 922 |
| EE | 57.3 | 58.3 | 58.1 | 56.7 | 60.6 | 53.8 |
| CP | 146 | 148 | 148 | 150 | 143 | 144 |
| NDF | 432 | 411 | 454 | 452 | 443 | 442 |
| ADF | 206 | 203 | 204 | 205 | 200 | 210 |
| ADL | 44.6 | 41.1 | 54.6 | 45.2 | 42.9 | 41.0 |
| PT | 1.59 | 2.84 | 2.04 | 2.88 | 4.27 | 3.55 |
| IVDMD | 717 | 728 | 717 | 699 | 650 | 687 |
| ¹ ME (Mcal/kg DM) | 2.57 | 2.53 | 2.55 | 2.46 | 2.55 | 2.48 |
| | VFA and fermentation products (g/kg DM) | | | | | |
| Lactate | n.d. | n.d. | 11.1 | n.d. | n.d. | 24.9 |
| Acetate | 18.6 | 24.7 | 28.7 | 19.3 | 24.0 | 25.1 |
| Propionate | n.d. | n.d. | 5.35 | n.d. | 12.2 | n.d. |
| Butyrate | n.d. | n.d. | n.d. | n.d. | 4.60 | n.d. |
| Ethanol | n.d. | 3.09 | n.d. | n.d. | 8.20 | n.d. |
| Ammonia N (g N-NH ₃ /kg N _T) | 1.40 | 9.38 | 2.65 | 2.41 | 20.9 | 3.48 |
| | Fatty acids profile (g/100 g total fatty acids) | | | | | |
| C4:0 | 0.053 | 2.30 | 0.072 | 0.052 | 7.31 | 0.246 |
| C6:0 | 0.067 | 0.547 | 0.068 | 0.047 | 1.966 | 0.151 |
| C12:0 | 0.244 | 0.108 | 0.095 | 0.123 | 0.087 | 0.089 |
| C14:0 | 0.429 | 0.359 | 0.349 | 0.410 | 0.358 | 0.351 |
| C16:0 | 17.7 | 16.5 | 16.8 | 17.4 | 16.1 | 17.9 |
| C16:1c9 | 0.266 | 0.389 | 0.290 | 0.268 | 0.297 | 0.291 |
| C18:0 | 3.72 | 3.44 | 3.38 | 3.48 | 3.03 | 3.59 |
| C18:1c9 | 25.5 | 25.7 | 26.1 | 24.9 | 21.3 | 24.5 |
| C18:1c11 | 1.03 | 1.04 | 1.11 | 0.99 | 0.97 | 1.06 |
| C18:2n6 | 44.3 | 44.3 | 46.4 | 45.5 | 41.2 | 45.5 |
| C18:3n3 | 3.86 | 2.80 | 2.79 | 3.97 | 3.45 | 3.47 |

Table 1. Cont.

| Item | Experiment 1 | | | Experiment 2 | | |
|---------------|-----------------|-------|-------|--------------|-------|-------|
| | C | AB | AP | C | AB | AP |
| C20:0 | 0.498 | 0.422 | 0.497 | 0.472 | 0.420 | 0.486 |
| C20:1n9 | 0.314 | 0.314 | 0.329 | 0.312 | 0.297 | 0.309 |
| C22:0 | 0.510 | 0.184 | 0.483 | 0.462 | 0.369 | 0.411 |
| C24:0 | 0.211 | 0.279 | 0.367 | 0.392 | 0.322 | 0.330 |
| SFA | 24.2 | 24.9 | 22.5 | 23.3 | 32.2 | 24.1 |
| MUFA | 27.3 | 27.6 | 28.0 | 26.6 | 23.0 | 26.3 |
| PUFA | 48.5 | 47.6 | 49.4 | 50.1 | 44.8 | 49.6 |
| | Mineral profile | | | | | |
| Na (g/kg DM) | 2.02 | 2.24 | 4.16 | 2.57 | 2.58 | 4.56 |
| Mg (g/kg DM) | 3.14 | 3.12 | 3.00 | 3.13 | 3.01 | 2.67 |
| K (g/kg DM) | 13.6 | 14.7 | 15.1 | 13.9 | 15.4 | 15.5 |
| Ca (g/kg DM) | 8.45 | 7.96 | 8.33 | 8.60 | 6.66 | 9.12 |
| P (g/kg DM) | 2.72 | 3.33 | 3.07 | 3.17 | 3.31 | 3.19 |
| S (g/kg DM) | 3.17 | 2.94 | 3.10 | 3.10 | 2.98 | 2.94 |
| Se (mg/kg DM) | 0.243 | 0.188 | 0.176 | 0.375 | 0.336 | 0.350 |
| Zn (mg/kg DM) | 53.3 | 54.2 | 46.3 | 59.5 | 73.0 | 61.4 |
| Cu (mg/kg DM) | 6.84 | 6.68 | 6.60 | 6.16 | 6.50 | 6.09 |
| Fe (mg/kg DM) | 274 | 351 | 217 | 373 | 277 | 257 |
| Mn (mg/kg DM) | 45.6 | 54.4 | 44.8 | 62.7 | 61.0 | 62.5 |

C: Control diet, AB: Diet with artichoke bracts silage, AP: Diet with artichoke plant silage, DM: Dry matter, FM: Fresh matter, OM: Organic matter, EE: Ether extract, CP: Crude protein, NDF: Neutral detergent fibre, ADF: Acid detergent fibre, ADL: Acid detergent lignin, TP: Total polyphenols, IVDMD: In vitro dry matter digestibility, EM: Metabolic energy, VFA: Volatile fatty acids, SFA: Saturated fatty acids, MUFA: Monounsaturated fatty acids, PUFA: Polyunsaturated fatty acids; 1 [16].

After the pre-experimental sampling was carried out, Experiment 1 began, which lasted 8 w. The first 2 w served for each group of animals to adapt to their treatment diet (with 12.5% silage by-product). In the next 6 w, 4 biweekly samplings were performed. Next, Experiment 2 began. After a 4-week adaptation period to the diets that included 25.0% by-product, three biweekly samplings were performed in the next 4 w.

2.3. Variables Analysed

Representative samples of each ration were taken at the beginning of each experiment for subsequent laboratory analysis. The composition of the rations (Table 1) was determined by the AOAC [17] methods for dry matter (DM, g/kg; method 930.5), organic matter (OM, g/kg DM; method 942.05), ether extract (EE, g/kg DM; method 920.39) and crude protein (CP, g/kg DM; method 984.13). The neutral detergent fibre (NDF, g/kg DM), acid detergent fibre (ADF, g/kg DM) and acid detergent lignin (ADL, g/kg DM) contents were analysed according to Van Soest et al. [18]. The total polyphenol content (TP, g/kg DM) was analysed by the Folin-Ciocalteu method described in Kim et al. [19]. The apparent in vitro dry matter digestibility (IVDMD, g/kg DM) was analysed by the method of Menke and Steingass [20]. The proportion of short chain volatile fatty acids (VFA, g/kg DM): acetic, propionic and butyric acid, including also lactic acid and ethanol were determined by liquid chromatography (HPLC 1200 Agilent, Santa Clara, CA, USA) and Supelcogel C-610H column: 30 cm × 7.8 mm ID, (Saint Louis, MO, USA); [21].

The analysis of the fatty acid profile in the diets was performed by direct methylation on the lyophilised samples, without prior extraction of the fat, according to Kramer et al. [22]. Fatty acid methylated esters (FAME) were identified by a gas chromatograph (GC-17A Shimadzu, Kyoto, Japan) coupled to a flame ionisation detector (FID) equipped with a capillary column (DB23 30 m × 0.25 mm ID × 0.25 µm film coating JW Scientific, Agilent, USA). A mixture of FAME (18912-1AMP, Sigma-Aldrich, Saint Louis, MO, USA) was used for the identification of the fatty acids present in the samples.

For the analysis of dietary and milk minerals, a previous digestion of the samples was carried out according to González-Arrojo et al. [23]. Na, Mg, K, Ca, P, S, Se, Zn, Cu, Fe and Mn were determined by a quadrupole ICP-MS chromatograph (Agilent, Santa Clara, CA, USA) using an internal calibration.

The milk yield of each animal (kg/day) was determined during the milking of each sampling by a Lactocorder[®] device (Lactocorder, Balgach, Switzerland). The macrocomposition of milk (fat, protein, useful dry matter content, UDM; true protein, casein, whey protein, lactose, dry matter, DM; non-fat dry matter content, NFDm; ash; %) was analysed by medium infrared spectroscopy (MilkoScan[™] FT2, Foss, Hillerød, Denmark) and somatic cell count (SCC, 10³ × cell/mL) using the electronic fluoro-optical method (DCC, DeLaval, Tumba, Sweden). The fat-corrected milk yield was calculated according to Gravert [24]: FCM (3.5%) = 0.433 × yield (kg/day) + 16.218 × fat yield (kg/day), and fat and protein corrected milk yield according to Schau and Fet [25]: FPCM = yield (kg/day) × (0.337 + 0.116 × Fat (%) + 0.06 × Protein (%)). At each sampling, the animals were weighed using a scale with a precision of 100 g (APC, Baxtran, Vilamilla, Spain) to study the evolution of body weight (BW, kg) during the experiment. Food intake was determined by the difference from the amount (in DM basis) that was offered and refused, on two consecutive days in the week of each sampling and determining the DM of a representative sample of ration offered and refused, dried in an oven at 105 °C for 48 h. For the analysis of the fatty acid profile in the milk samples, an extraction was carried out using the Folch method with some variations described in Romeu-Nadal et al. [26] and a subsequent methylation according to the method of Trigueros and Sendra [27]. The chromatograph, column and FAME mix for the identification of milk fatty acids were the same as those used for diets. Indices related to the nutritional quality of milk fat were calculated: Atherogenicity Index (AI) and Thrombogenicity Index (TI) according to Batista et al. [28] and the Desaturase Index (DI) for C14:0, C16:0 and C18:0 according to Lock and Garnsworthy [29].

Milk samplings were carried out during the milking in the weeks in lactation 16 (pre-experimental sampling), 20 and 24 (in Experiment 1) and 28 and 32 (in Experiment 2), blood samples were taken from jugular vein of the fasting animals using an Eclipse[™] needle (BD Vacutainer, Franklin Lakes, NJ, USA) and collected in three test tubes with 4 mL capacity (BD Vacutainer, Franklin Lakes, NJ, USA): one of them contained potassium oxalate and NaF for the analysis of glucose, and another contained lithium heparin and was reserved for the analysis of urea, β-hydroxybutyrate (BHB) and haematocrit. Finally, the tube containing EDTAK2 was used to collect blood for the analysis of cholesterol and non-esterified fatty acids (NEFA). Blood samples were analysed by enzymatic spectrophotometry. A glucose oxidase/peroxidase kit (Ref. 11503 and 11505, Biosystems, Barcelona, Spain) was used for glucose and cholesterol (mg/dL), the kinetic method GN 10125 developed by Gernon (Spain) was used for urea (mg/dL), the Ranbut D-3-Hydroxybutyrate kit (RB 1007, Randox, Crumlin, UK) was used for BHB (mmol/L) and an enzymatic-spectrophotometric method (FA 115, Randox, Crumlin, UK) was used for NEFA (mmol/L). The percentage of haematocrit was determined with a microhaematocrit.

2.4. Statistical Analyses

The SCC values were transformed into base ten logarithm to carry out the statistical analysis. The data from each experiment (Exp. 1 and Exp. 2) were analysed separately following a mixed linear model with repeated measures PROC GLIMMIX (SAS v9.2, 2012), introducing in the model the covariate of the data obtained in the pre-experimental sampling, for the statistical analysis of Experiment 1, or of the last sampling of Experiment 1 for the analysis of Experiment 2, according to the following equation:

$$Y = \mu + D_i + S_j + D_i \times S_j + \text{cov}Y_0 + A_k + e \quad (1)$$

where Y is the dependent variable, μ is the intercept, D_i is the fixed effect of the diet ($i = C, AB, AP$), S_j is the fixed effect of sampling ($j = 1, 2, \dots$), $D_i \times S_j$ is the interaction of the diet with the sampling, $\text{cov}Y_0$ is the effect of the value of Y in the pre-experimental sampling, A_k is the random effect of the animal and e the residual error. The analysis of the data of the milk mineral and lipid profile did not take into account the effect of sampling or its interaction with the diet effect. A covariance model of composite

symmetry was used, as it presented the best modelling of the data (according to the AIC and BIC statistics).

3. Results

3.1. Body Weight, Milk Yield and Composition and Plasmatic Metabolism

In Exp. 1, the inclusion of by-products did not cause significant differences in almost any variable (Table 2). Although there were no differences in initial BW (homogeneous groups), and average BW increased by 3.90 kg ($p < 0.001$) during the experiment in the three treatments, the diet had a significant effect on BW, as it increased more in C and AP than in AB (49.8, 48.1 and 47.8 kg for C, AP and AB, respectively). On a daily average, the values of dry matter intake (DMI) for the different treatments were 2.14 ± 0.059 , 2.08 ± 0.073 and 2.12 ± 0.076 kg DM/day in C, AB and AP, respectively. Regarding the milk yield and composition, no differences due to diet were observed, but the effect of sampling and interaction diet \times sampling were observed. Milk yield, as well as FCM and FPCM yields and feed efficiency, were reduced at the end of the experiment, although FCM yield of AB and feed efficiency of AP remained stable. The ash content also decreased (-0.188% ; $p < 0.001$). The concentration of the rest of the variables related to the composition and LSCC increased throughout the experiment with slight differences between treatments, so the interaction was significant. Although the interaction diet \times sampling was significant in some of the studied variables, this was due to small fluctuations observed throughout the experiment and they did not cause important differences, as can be seen in Figure 1.

Table 2. Results of the comparison of means of the variables related to body weight, food intake, milk yield and composition, somatic cell count and basal metabolism, according to the effects considered in Experiment 1.

| Variable | Diet | | | | Signification | Sampling Signification | Interaction Signification |
|----------------------------------|--------|--------|----------|-------|---------------|------------------------|---------------------------|
| | C | AB | AP | SEM | | | |
| Initial BW (kg) | 43.7 | 44.6 | 45.7 | 1.44 | n.s. | — | — |
| Average BW (kg) | 49.8 a | 47.8 b | 48.1 a,b | 0.62 | * | *** | *** |
| Milk yield (kg/day) | 2.26 | 2.24 | 2.26 | 0.076 | n.s. | *** | n.s. |
| FCM (3.5%; kg/day) | 2.83 | 2.78 | 2.92 | 0.103 | n.s. | *** | * |
| FPCM (kg/day) | 2.66 | 2.61 | 2.72 | 0.087 | n.s. | *** | n.s. |
| Feed efficiency (Milk yield/DMI) | 1.06 | 1.07 | 1.07 | 0.060 | n.s. | *** | *** |
| Feed efficiency (FPCM/DMI) | 1.23 | 1.27 | 1.28 | 0.071 | n.s. | *** | ** |
| Fat (%) | 5.10 | 5.16 | 5.48 | 0.212 | n.s. | *** | *** |
| UDM (%) | 9.41 | 9.28 | 9.78 | 0.255 | n.s. | *** | *** |
| DM (%) | 14.1 | 14.0 | 14.4 | 0.26 | n.s. | *** | *** |
| NFDM (%) | 9.60 | 9.59 | 9.56 | 0.070 | n.s. | *** | *** |
| Protein (%) | 4.23 | 4.20 | 4.30 | 0.064 | n.s. | *** | *** |
| True protein (%) | 3.90 | 3.90 | 3.95 | 0.056 | n.s. | *** | *** |
| Casein (%) | 3.37 | 3.35 | 3.40 | 0.048 | n.s. | *** | *** |
| Whey protein (%) | 0.532 | 0.518 | 0.550 | 0.016 | n.s. | *** | *** |
| Lactose (%) | 4.21 | 4.23 | 4.16 | 0.029 | n.s. | *** | * |
| Ash (%) | 0.469 | 0.453 | 0.478 | 0.018 | n.s. | *** | * |
| LSCC (cell/mL) | 5.77 | 5.81 | 5.75 | 0.061 | n.s. | ** | * |
| Glucose (mg/dL) | 59.2 | 59.9 | 60.7 | 1.16 | n.s. | n.s. | n.s. |
| Cholesterol (mg/dL) | 113 | 117 | 116 | 2.4 | n.s. | * | * |
| Urea (mg/dL) | 47.7 a | 43.2 b | 44.6 a,b | 1.21 | * | *** | n.s. |
| BHB (mmol/L) | 0.442 | 0.400 | 0.382 | 0.022 | n.s. | n.s. | ** |
| NEFA (mmol/L) | 0.463 | 0.370 | 0.444 | 0.047 | n.s. | * | * |
| Haematocrit (%) | 29.6 | 28.9 | 29.3 | 0.43 | n.s. | *** | ** |

C: Control diet, AB: Diet with artichoke bracts silage, AP: Diet with artichoke plant silage, SEM: Standard error mean; BW: Body weight, DMI: Dry matter intake, FCM: Fat corrected milk, FPCM: Fat and protein corrected milk, UDM: Useful dry matter (% fat + % protein), DM: Dry matter, NFDM: Non-fat dry matter, LSCC: Log₁₀ somatic cell count, BHB: β -hydroxybutyrate, NEFA: Non-esterified fatty acids; a, b, c: different letters in the same row indicate significant difference between diets. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

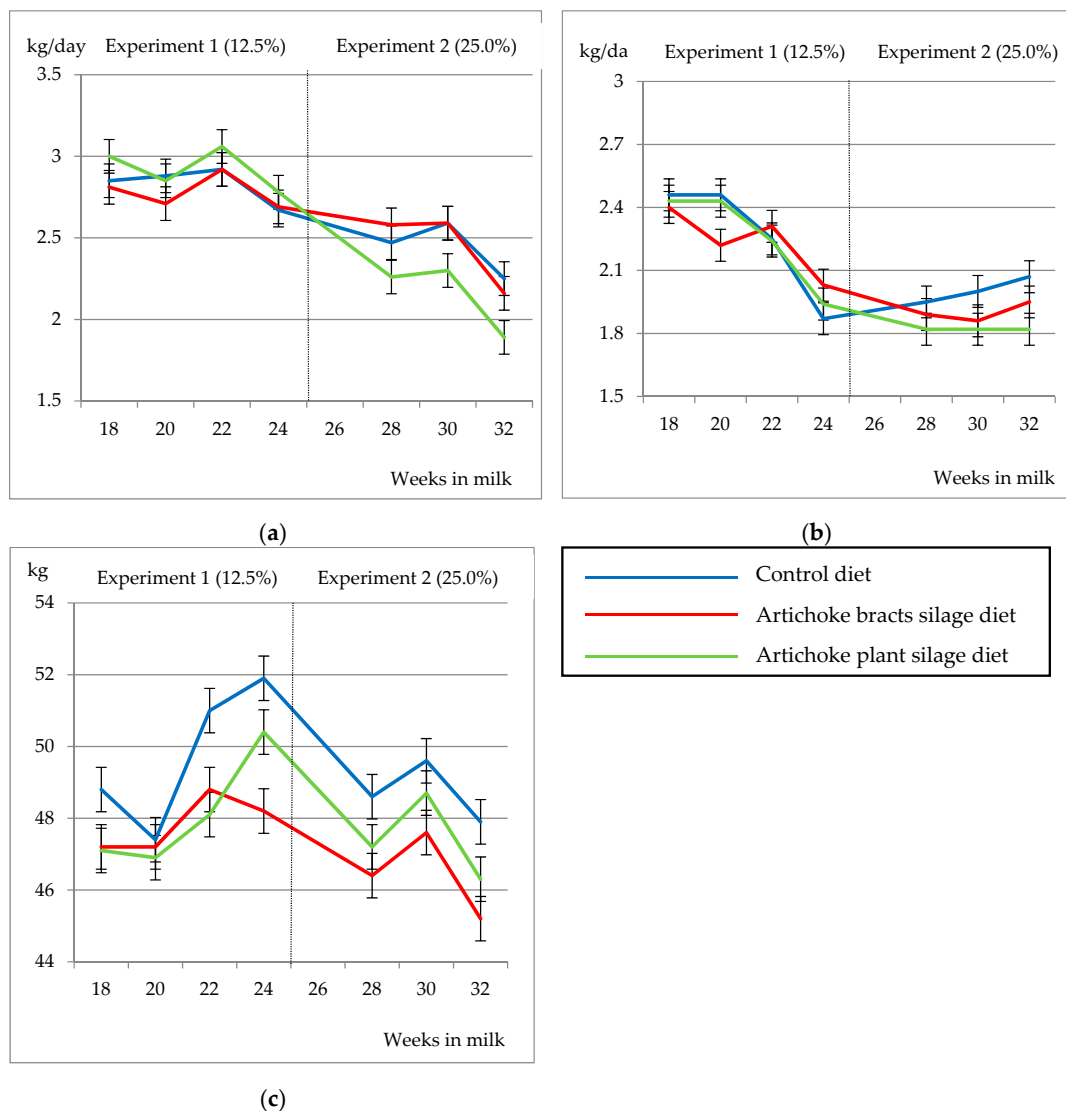


Figure 1. Changes in milk yield (a), fat corrected milk (b) and average body weight (c) due to diet throughout the lactation in experiments 1 and 2.

Regarding blood metabolites, the inclusion of 12.5% in the dry matter basis of AB and AP did not change the levels, except in the case of plasma urea, where AB had a lower content than C (43.2 vs. 47.7 mg/dL; $p < 0.05$) and AP obtained an intermediate content (44.6 mg/dL). During the experiment, urea levels increased equally in the three treatments (+4.84 mg/dL; $p < 0.001$) and haematocrit levels in AP (+1.71%; $p < 0.01$), while cholesterol levels were reduced in C (−7.40%; $p < 0.05$) and those of NEFA in AP (−0.201 mmol/L; $p < 0.05$).

In Exp. 2, the effect of diet was significant in many of the variables analysed (Table 3). There were no statistical differences in initial BW, but a reduction in average BW was observed in the three treatments, resulting in a lower BW in AB (46.4 kg; $p < 0.001$). Irrelevant differences in DMI were observed, resulting for AB, C and AP equal to 1.86 ± 0.051 , 1.92 ± 0.038 and 1.92 ± 0.052 kg DM/day, respectively. The treatments did not show differences in milk yield and feed efficiency, but in FCM and FPCM yields, which were higher ($p < 0.05$) in C and AB in contrast to AP, without differences between AB and AP in FPCM. The effect of sampling was significant in FCM and FPCM, which were reduced by 0.337 and 0.225 kg/day ($p < 0.001$), respectively, between the beginning and the end of the experiment, and also in feed efficiency related to FPCM. AB was the treatment with the highest fat content in milk (5.35%; $p < 0.01$), as well as in the parameters related to fat, such as UDM (9.62%) and DM (14.4%).

These three variables were reduced by 1.39%, 1.40% and 1.31% ($p < 0.001$) during the experiment without any diet \times sampling interaction. On the contrary, AP presented a higher concentration in the protein fractions—crude protein (4.36%), true protein (4.01%) and whey protein (0.556%)—as well as in NFDM (9.82%) and in LSCC (5.90 Log₁₀ cell/mL). During the experiment, the casein content was reduced 0.061% ($p < 0.001$) and those of whey protein and lactose increased by 0.048 and 0.100% similarly in all three treatments. However, NFDM and the crude and true protein levels increased only in AB and AP, while the concentration of these two protein fractions was reduced in C. As for LSCC, it only increased in AP (+0.192 Log₁₀ cell/mL; $p < 0.001$).

Table 3. Results of the comparison of means of the variables related to body weight, food intake, milk yield and composition, somatic cell count and basal metabolism, according to the effects considered, in Experiment 2.

| Variable | Diet | | | | Signification | Sampling Signification | Interaction Signification |
|----------------------------------|-----------|----------|---------|-------|---------------|------------------------|---------------------------|
| | C | AB | AP | SEM | | | |
| Initial BW (kg) | 50.1 | 48.6 | 50.9 | 0.92 | n.s. | — | — |
| Average BW (kg) | 48.7 a | 46.4 c | 47.4 b | 0.36 | *** | *** | n.s. |
| Milk yield (kg/day) | 2.01 | 1.90 | 1.82 | 0.072 | n.s. | n.s. | n.s. |
| FCM (3.5%; kg/day) | 2.44 a | 2.44 a | 2.15 b | 0.086 | * | *** | n.s. |
| FPCM (kg/day) | 2.31 a | 2.28 a,b | 2.05 b | 0.078 | * | *** | n.s. |
| Feed efficiency (Milk yield/DMI) | 1.02 | 1.05 | 0.95 | 0.058 | n.s. | n.s. | n.s. |
| Feed efficiency (FPCM/DMI) | 1.18 | 1.23 | 1.08 | 0.066 | n.s. | *** | n.s. |
| Fat (%) | 4.88 b | 5.35 a | 4.73 b | 0.149 | ** | *** | n.s. |
| UDM (%) | 9.14 a,b | 9.62 a | 9.06 b | 0.178 | * | *** | n.s. |
| DM (%) | 13.9 a,b | 14.4 a | 13.8 b | 0.17 | * | *** | n.s. |
| NFDM (%) | 9.69 b | 9.80 a,b | 9.82 a | 0.046 | * | *** | ** |
| Protein (%) | 4.22 b | 4.28 a,b | 4.36 a | 0.046 | * | ** | * |
| True protein (%) | 3.89 b | 3.94 a,b | 4.01 a | 0.041 | * | ** | * |
| Casein (%) | 3.37 | 3.43 | 3.45 | 0.033 | n.s. | *** | * |
| Whey protein (%) | 0.523 a,b | 0.506 b | 0.556 a | 0.013 | ** | *** | n.s. |
| Lactose (%) | 4.34 | 4.31 | 4.30 | 0.027 | n.s. | ** | n.s. |
| Ash (%) | 0.441 | 0.476 | 0.436 | 0.015 | n.s. | n.s. | n.s. |
| LSCC (cell/mL) | 5.77 a,b | 5.72 b | 5.90 a | 0.061 | * | ** | *** |
| Glucose (mg/dL) | 59.5 | 57.2 | 59.8 | 1.03 | n.s. | *** | n.s. |
| Cholesterol (mg/dL) | 109 a,b | 111 a | 102 b | 2.5 | * | *** | *** |
| Urea (mg/dL) | 43.2 a | 38.9 b | 42.2 a | 1.00 | ** | n.s. | n.s. |
| BHB (mmol/L) | 0.410 | 0.417 | 0.405 | 0.019 | n.s. | ** | ** |
| NEFA (mmol/L) | 0.631 | 0.717 | 0.625 | 0.054 | n.s. | * | *** |
| Haematocrit (%) | 31.6 | 32.1 | 32.1 | 0.55 | n.s. | *** | ** |

C: Control diet, AB: Diet with artichoke bracts silage, AP: Diet with artichoke plant silage, SEM: Standard error mean; BW: Body weight, DMI: Dry matter intake, FCM: Fat corrected milk, FPCM: Fat and protein corrected milk, UDM: Useful dry matter (% fat + % protein), DM: Dry matter, NFDM: Non-fat dry matter, LSCC: Log₁₀ somatic cell count, BHB: β -hydroxybutyrate, NEFA: Non-esterified fatty acids; a, b, c: different letters in the same row indicate significant difference between diets. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Regarding the blood metabolite profile, the differences found were small. A slightly higher level of cholesterol was observed in AB (111 mg/dL; $p < 0.05$) and urea in C and AP (43.2 and 42.2 mg/dL, respectively; $p < 0.01$). During the experiment, the glucose and haematocrit levels were reduced ($p < 0.001$), the concentration of cholesterol decreased in AB and NEFA in AP, while BHB increased slightly ($p < 0.01$) in this treatment.

3.2. Milk Mineral Profile

Regarding the mineral content of milk (Table 4) in Experiment 1, only Cu was affected by the diets. AP obtained a lower content than C (94 vs. 111 $\mu\text{g}/\text{kg}$; $p < 0.05$), without differences with AB (105 $\mu\text{g}/\text{kg}$). Including 25.0% of the by-products in the diet (Experiment 2), only S, Cu and Se were

affected by the diet. It was observed that the Cu level of the two experimental treatments was slightly lower than C (80.6 vs. 95.1 µg/kg; $p < 0.05$). Regarding the content of S, AB was lower than C (365 vs. 405 mg/kg; $p < 0.05$), without differences with AP (402 mg/kg). Similarly, the amount of Se present in AB milk (33.3 µg/kg) was slightly lower than that C and AP (36.6 and 40.7 µg/kg, respectively; $p < 0.05$), without differences between these two treatments.

Table 4. Effect of the diet on milk mineral profile in Experiments 1 and 2.

| Variable | Experiment 1 | | | | | Experiment 2 | | | | |
|------------|--------------|---------|------|-------|---------------|--------------|--------|---------|-------|---------------|
| | C | AB | AP | SEM | Signification | C | AB | AP | SEM | Signification |
| Na (mg/kg) | 354 | 373 | 369 | 11.7 | n.s. | 343 | 350 | 362 | 11.6 | n.s. |
| Mg (mg/kg) | 160 | 162 | 161 | 7.6 | n.s. | 167 | 156 | 166 | 3.9 | n.s. |
| P (mg/kg) | 1152 | 1245 | 1200 | 62.6 | n.s. | 1171 | 1142 | 1181 | 41.4 | n.s. |
| S (mg/kg) | 408 | 401 | 403 | 16.6 | n.s. | 405 a | 365 b | 402 a,b | 10.7 | * |
| K (mg/kg) | 1492 | 1513 | 1560 | 61.6 | n.s. | 1405 | 1415 | 1514 | 39.8 | n.s. |
| Ca (mg/kg) | 1394 | 1536 | 1449 | 58.9 | n.s. | 1437 | 1447 | 1451 | 36.8 | n.s. |
| Mn (µg/kg) | 64.2 | 77.2 | 89.8 | 13.36 | n.s. | 70.1 | 68.9 | 62.7 | 5.60 | n.s. |
| Fe (µg/kg) | 457 | 678 | 499 | 154.2 | n.s. | 483 | 403 | 468 | 26.7 | n.s. |
| Cu (µg/kg) | 111 a | 105 a,b | 94 b | 4.1 | * | 95.1 a | 80.6 b | 80.6 b | 3.74 | * |
| Se (µg/kg) | 25.8 | 26.5 | 26.3 | 2.93 | n.s. | 36.6 a,b | 33.3 b | 40.7 a | 1.67 | * |
| Zn (µg/kg) | 4811 | 4734 | 4968 | 225.3 | n.s. | 5387 | 4925 | 4987 | 286.1 | n.s. |

C: Control diet, AB: Diet with artichoke bracts silage, AP: Diet with artichoke plant silage; SEM: Standard error mean; a, b, c: different letters in the same row indicate significant difference between diets. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

3.3. Milk Lipid Profile

In Experiment 1, the significant differences obtained between treatments were small and can be considered irrelevant from a biological point of view (Table 5). In comparison to C, the inclusion of 12.5% of AB in the ration resulted in a small reduction ($p < 0.01$) of the milk PUFA (polyunsaturated fatty acids) content (−0.58%). AP slightly increased the level of MCFA (medium chain fatty acids) (+0.7%; $p < 0.05$), as the milk from this treatment also had the highest concentrations of myristic (C14:0) and palmitic (C16:0) acid and also a higher DI of C16:0 (+0.006; $p < 0.05$). A slightly lower content of n3 fatty acids and higher n6/n3 ratio ($p < 0.05$) were observed in treatments that included artichoke by-products in Experiment 1. The milk of the animals fed with a 25.0% of AB had a lower level of MUFA (monounsaturated fatty acids) and LCFA (long chain fatty acids) than C (−1.0 and −1.1%, respectively; $p < 0.05$), mainly because they also obtained lower oleic (C18:1 cis9, −1.3%) and arachidonic acid contents (C20:0, −0.130%).

Table 5. Effect of the diet on milk fatty acid profile (g/100 AGT) in Experiments 1 and 2.

| Variable | Experiment 1 | | | | | Experiment 2 | | | | |
|------------------|--------------|-----------|---------|-------|---------------|--------------|---------|-----------|-------|---------------|
| | C | AB | AP | SEM | Signification | C | AB | AP | SEM | Signification |
| C6:0 | 1.17 | 0.94 | 0.61 | 0.228 | n.s. | 0.420 | 0.945 | 0.881 | 0.250 | n.s. |
| C8:0 | 3.21 | 3.31 | 3.33 | 0.124 | n.s. | 3.29 | 3.18 | 3.36 | 0.081 | n.s. |
| C10:0 | 9.08 | 9.36 | 9.58 | 0.228 | n.s. | 9.07 | 9.16 | 9.36 | 0.177 | n.s. |
| C12:0 | 5.33 | 5.50 | 5.58 | 0.114 | n.s. | 5.17 | 5.27 | 5.40 | 0.086 | n.s. |
| C13:0 | 0.117 | 0.105 | 0.104 | 0.012 | n.s. | 0.108 | 0.077 | 0.104 | 0.020 | n.s. |
| C14:0 | 10.1 b | 10.3 a,b | 10.5 a | 0.11 | * | 10.1 | 10.1 | 10.5 | 0.13 | n.s. |
| C14:1c9 | 0.070 | 0.124 | 0.077 | 0.018 | n.s. | 0.153 | 0.104 | 0.115 | 0.036 | n.s. |
| C15:0 | 1.16 | 1.03 | 1.04 | 0.045 | n.s. | 1.12 | 1.24 | 1.16 | 0.076 | n.s. |
| C15:1 | 0.168 | 0.153 | 0.146 | 0.013 | n.s. | 0.168 | 0.150 | 0.159 | 0.013 | n.s. |
| C16:0 | 23.0 b | 23.5 a,b | 23.8 a | 0.20 | * | 24.0 | 24.3 | 24.2 | 0.45 | n.s. |
| C16:1 | 1.17 b | 1.24 a,b | 1.35 a | 0.052 | * | 1.22 | 1.19 | 1.29 | 0.034 | n.s. |
| C16:2 | 1.17 | 1.17 | 1.14 | 0.078 | n.s. | 0.968 | 1.133 | 1.182 | 0.092 | n.s. |
| C17:1 | 0.307 | 0.339 | 0.333 | 0.013 | n.s. | 0.345 | 0.390 | 0.363 | 0.023 | n.s. |
| C18:0 | 12.3 | 11.8 | 11.4 | 0.32 | n.s. | 13.2 | 12.7 | 12.2 | 0.34 | n.s. |
| C18:1t11 | 0.300 | 1.458 | 0.067 | 0.477 | n.s. | 0.193 | 1.866 | 0.106 | 0.814 | n.s. |
| C18:1c9 | 22.5 | 21.7 | 23.2 | 0.62 | n.s. | 22.5 a | 19.8 b | 22.5 a | 0.77 | * |
| C18:2t9,12 | 1.31 a | 0.72 b | 1.44 a | 0.107 | *** | 1.23 | 1.69 | 1.43 | 0.421 | n.s. |
| C18:2n6 | 3.40 | 3.47 | 3.56 | 0.063 | n.s. | 3.28 | 3.24 | 3.31 | 0.062 | n.s. |
| C18:3n6 | 0.156 a,b | 0.176 a | 0.142 b | 0.010 | * | 0.161 | 0.164 | 0.146 | 0.042 | n.s. |
| C19:0 | 0.121 | 0.118 | 0.089 | 0.014 | n.s. | 0.124 | 0.099 | 0.103 | 0.024 | n.s. |
| C18:3n3 | 0.302 | 0.269 | 0.275 | 0.013 | n.s. | 0.309 | 0.329 | 0.252 | 0.032 | n.s. |
| CLA c9t11 | 1.06 | 1.07 | 1.19 | 0.057 | n.s. | 0.866 | 0.674 | 0.876 | 0.079 | n.s. |
| CLA t10c12 | 0.208 | 0.232 | 0.145 | 0.056 | n.s. | 0.095 | 0.298 | 0.151 | 0.064 | n.s. |
| ∑CLA | 1.27 | 1.31 | 1.33 | 0.069 | n.s. | 0.961 | 0.971 | 1.027 | 0.112 | n.s. |
| C20:0 | 0.382 | 0.307 | 0.315 | 0.030 | n.s. | 0.374 a | 0.244 b | 0.307 a,b | 0.035 | * |
| C20:1n9 | 0.160 | 0.136 | 0.112 | 0.037 | n.s. | 0.100 | 0.064 | 0.090 | 0.022 | n.s. |
| C21:0 | 0.082 a | 0.048 a,b | 0.030 b | 0.017 | * | 0.046 | 0.027 | 0.047 | 0.011 | n.s. |
| C20:4n6 | 0.203 | 0.190 | 0.203 | 0.009 | n.s. | 0.225 | 0.211 | 0.225 | 0.017 | n.s. |
| C20:5n3 | 0.131 a | 0.093 a,b | 0.086 b | 0.014 | * | 0.124 | 0.116 | 0.102 | 0.020 | n.s. |
| C24:1 | 0.064 a | 0.027 b | 0.026 b | 0.011 | * | 0.052 | 0.072 | 0.029 | 0.030 | n.s. |
| C22:6n6 | 0.034 a,b | 0.015 b | 0.048 a | 0.008 | * | 0.039 | 0.031 | 0.033 | 0.005 | n.s. |
| SFA ¹ | 66.1 | 66.3 | 66.4 | 0.34 | n.s. | 66.9 | 67.4 | 67.7 | 0.41 | n.s. |

Table 5. Cont.

| Variable | Experiment 1 | | | | | Experiment 2 | | | | |
|-------------------|--------------|-----------|---------|-------|---------------|--------------|---------|----------|-------|---------------|
| | C | AB | AP | SEM | Signification | C | AB | AP | SEM | Signification |
| MUFA ² | 24.7 | 25.2 | 25.4 | 0.33 | n.s. | 24.6 a,b | 23.6 b | 24.6 a | 0.30 | * |
| PUFA ³ | 7.97 a | 7.41 b | 8.22 a | 0.154 | ** | 7.30 | 7.88 | 7.71 | 0.56 | n.s. |
| UFA ⁴ | 32.7 | 32.6 | 33.6 | 0.40 | n.s. | 31.9 | 31.5 | 32.3 | 0.44 | n.s. |
| SFA/UFA | 2.02 | 2.04 | 1.98 | 0.034 | n.s. | 2.10 | 2.14 | 2.09 | 0.042 | n.s. |
| SCFA ⁵ | 13.5 | 13.6 | 13.5 | 0.25 | n.s. | 12.8 | 13.3 | 13.6 | 0.37 | n.s. |
| MCFA ⁶ | 42.6 b | 43.4 a,b | 44.1 a | 0.41 | * | 43.3 | 43.9 | 44.5 | 0.53 | n.s. |
| LCFA ⁷ | 42.7 | 41.9 | 42.4 | 0.45 | n.s. | 42.8 a | 41.7 b | 41.9 a,b | 0.29 | * |
| n3 | 0.433 a | 0.362 b | 0.361 b | 0.022 | * | 0.433 | 0.445 | 0.353 | 0.047 | n.s. |
| n6 | 3.79 | 3.85 | 3.95 | 0.060 | n.s. | 3.71 | 3.64 | 3.72 | 0.095 | n.s. |
| n6/n3 | 8.94 b | 10.68 a | 11.03 a | 0.512 | * | 8.58 | 8.80 | 10.74 | 0.870 | n.s. |
| AI ⁸ | 2.38 | 2.39 | 2.41 | 0.039 | n.s. | 2.42 | 2.52 | 2.49 | 0.038 | n.s. |
| TI ⁹ | 3.14 | 3.10 | 3.08 | 0.042 | n.s. | 3.28 | 3.39 | 3.27 | 0.063 | n.s. |
| DI C14:0 | 0.007 | 0.012 | 0.007 | 0.002 | n.s. | 0.015 | 0.010 | 0.011 | 0.004 | n.s. |
| DI C16:0 | 0.051 b | 0.053 a,b | 0.057 a | 0.002 | * | 0.051 a,b | 0.049 b | 0.053 a | 0.001 | ** |
| DI C18:0 | 1.86 | 1.96 | 2.05 | 0.067 | n.s. | 1.71 | 1.70 | 1.85 | 0.062 | n.s. |

C: Control diet, AB: Diet with artichoke bracts silage, AP: Diet with artichoke plant silage; SEM: Standard error mean, DI: Desaturation index; ¹ SFA (saturated fatty acids) = C6:0 + C8:0 + C10:0 + C12:0 + C13:0 + C14:0 + C15:0 + C16:0 + C18:0 + C19:0 + C20:0 + C21:0; ² MUFA (monounsaturated fatty acids) = C14:1c9 + C15:1 + C16:1 + C17:1 + C18:1t11 + C18:1c9 + C20:1n9 + C24:1; ³ PUFA (polyunsaturated fatty acids) = C16:2 + C18:2t9,12 + C18:2n6 + C18:3n6 + C18:3n3 + CLAc9t11 + CLA t10c12 + C20:4n6 + C20:5 + C22:6n6; ⁴ UFA (unsaturated fatty acids) = MUFA + PUFA; ⁵ SCFA (short chain fatty acids) = C6:0 – C10:0; ⁶ MCFA (medium chain fatty acids) = C11:0 – C17:0; ⁷ LCFA (long chain fatty acids) = C18:0 – C24:0; ⁸ AI (atherogenic index) = $\frac{C12:0+4 \times C14:0+C16:0}{MUFA+n6+n3}$; ⁹ TI (thrombogenic index) = $\frac{C14:0+C16:0+C18:0}{0.5 \times MUFA+0.5 \times n6+0.3 \times n3+n3/n6}$; a, b, c: different letters in the same row indicate significant difference between diets. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

4. Discussion

4.1. Body Weight, Milk Yield and Composition and Plasmatic Metabolism

The BW values of the three treatments are within the normal range for the Murciano-Granadina breed [30]. The lower BW of AB can be explained by the lower initial BW of this treatment in both experiments, although there were no significant differences in the initial stage. In addition, the AB diet had higher values of ammonia (N), which also has a satiating effect due to the gamma-aminobutyric acid produced in the liver [31] and could have reduce DMI, Huhtanen et al. and Krizsan and Randby [32,33] observed in calves and dairy cows fed with grass silages. FCM and FPCM yields were higher in C and AB in Experiment 2, as C was the treatment with the highest milk yield (although without differences from the other two treatments) and AB had the highest fat content, so the milk of AB had the highest content in UDM and DM too. However, this did not happen in Jaramillo et al. [5], where the inclusion of up to 30% of artichoke silage in sheep diets did not lead to differences in milk composition. All the treatments showed a good level of feed efficiency, which were higher than those observed with other diets in goats of different breeds [34–36]. The absence of significant differences in milk yield and feed efficiency ensure the good feed quality of these by-products. The slightly higher protein content in the milk of the experimental treatments (especially AP) observed in Experiment 2 could be due to a higher level of TP in this diet, which would form complexes with the dietary protein, making it less soluble and, therefore, less digestible by ruminal microflora, so that it would increase the digested protein in the small intestine [37]. The decrease in milk yield and the increase in macrocomposition values observed in the three treatments during the experiment are related to the normal progress of lactation. The results for daily milk yield, fat, protein and lactose are slightly higher than those observed by Vacca et al. [38] in Murciano-Granadina goats, while the LSCC values are similar to those found in this study. Therefore, the use of artichoke silage at the levels of inclusion tested does not appear to have detrimental effects on the milk yield or composition, similar to that observed in cow's milk [39] and sheep [5]. In general, the inclusion of silages in well-balanced diets does not jeopardise the yield and composition of milk, as various authors observed by including different types of by-product silages in small ruminants' diets. While the inclusion of olive cake silage in sheep and goats' rations had no effects on milk yield [40,41], a higher fat content was observed in the milk of sheep and goats fed sliced oranges silage [42] and tomato and olive by-product silages [43], as occurred with AB in Experiment 2. The blood metabolite profile is one of the main indicators when evaluating the physiological state of animals [44]. The absence of relevant differences between treatments indicates the viability of the inclusion of these by-products in the ration for Murciano-Granadina dairy goats at the tested doses. The urea values measured in plasma were slightly higher than those of Ibáñez et al. [45] in Murciano-Granadina goats in mid-lactation (30.6 mg/dL), as the protein content of the diets was lower (132 g/kg DM), while the values of BHB and NEFA (1.74 and 1.06 mmol/L, respectively) were lower in our experiment due to lower mobilisation of body reserves, as also shown by the higher plasma glucose content compared to that observed by Ibáñez et al. [45] (43.9 mg/dL). The slight lower urea content of AB can be explained by two causes. On the one hand, the AB diet had a TP content slightly higher than the other two (Table 1). Frutos et al. [46] related the tannins (which are part of the TP) with a reduction in the digestibility of the protein in the rumen, thus releasing less ammonia, and the subsequent urea synthesis in the liver is reduced [47]. Decreases in milk urea were also observed in sheep fed diets with low levels of TP [48,49]. On the other hand, the slightly lower DMI of AB could cause a lower protein metabolism. However, the urea reduction was small and did not translate into lower protein content in milk or in FPCM yields.

4.2. Milk Mineral Profile

The milk mineral profile of the three treatments is similar to that reported by Guo [8] in goat milk. While in Experiment 1, the inclusion of 12.5% of AP and AB barely caused differences (only in Cu content), increasing the level to 25.0% caused a slight decrease in Se, as was observed in AB, although such small magnitudes that can be considered irrelevant.

4.3. Milk Lipid Profile

The milk lipid profile was not significantly modified by the inclusion of by-products in the diets at the doses tested. The milk lipid profile is strongly influenced by diet [48,49], in addition to the breed of animals. Some differences were observed between the results of this study with those of Ibáñez et al. [45], where they used the same breed: while the palmitic and α -linolenic (C18:3n3) contents were higher (42.6 and 0.400%, respectively), the oleic, linoleic (C18:2n6), conjugated linoleic (CLA) and vaccenic acid (C18:1t11) levels were lower (14.2, 2.46, 0.41 and 0.39%, respectively) than those observed in our experiment, with the exception of AB, which presented a higher level of vaccenic acid. In Mancilla-Leytón et al. [50], these differences were also found in grazing goats in the Mediterranean region, in addition to a higher ratio of SFA/UFA and AI index (3.03 and 3.00, respectively) and lower values in PUFA content (4.47%) and in the C16:0 and C18:0 DI (0.02 and 0.56). All this indicates that the inclusion of artichoke by-products in goat diets improves the milk lipid profile due to the higher content of oleic acid, PUFA and CLA, which have anti-atherogenic properties and reduce obesity [51]. In comparison to the rest of the treatments, the inclusion of 12.5% of AP in the diet showed a slightly higher content of PUFA, so it would have a more cardio-healthy lipid profile. On the other hand, the SFA/UFA ratio is a health indicator of the nutritional value of animal fat in human nutrition [52]. Simopoulos [53] recommends a value below 0.45 for the PUFA/SFA ratio for foods designed to slow cardiovascular diseases and cancer, which has been achieved by all treatments (0.118 in Experiment 1 and 0.113 in Experiment 2) and this means that the consumption of this milk can counteract the effects of diets high in saturated fatty acids, which are common in most western countries.

5. Conclusions

The use of silage artichoke bracts and whole plant in dairy goat diets at the doses studied (12.5 and 25.0%) in substitution for other forage sources does not lead to marked differences in the milk yield and quality or in the health status of the animals. From the point of view of nutritional quality of milk for human health, there is a slightly better lipid and mineral profile in AP. Consequently, the inclusion of artichoke bracts and plant silages in well balanced diets will allow us to take advantage of the high by-product availability for ruminant diets without negative effects on animal performance.

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SHORT-TERM EFFECT OF THE INCLUSION OF SILAGE ARTICHOKE BY-PRODUCTS IN DIETS OF

Publicación 4 (Artículo original)

Composition, Mineral and Fatty Acid Profiles of Milk from Goats Fed with Different Proportions of Broccoli and Artichoke Plant By-Products

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Article

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Abstract: In the Mediterranean region, artichoke and broccoli are major crops with a high amount of by-products that can be used as alternative feedstuffs for ruminants, lowering feed costs and enhancing milk sustainability while reducing the environmental impact of dairy production. However, nutritional quality of milk needs to be assured under these production conditions and an optimal inclusion ratio of silages should be determined. This work aimed to evaluate the effect of three inclusion levels (25%, 40%, and 60%) of these silages (artichoke plant, AP, and broccoli by-product, BB) in goat diets on milk yield, composition, and mineral and fatty profiles. Treatments with 60% inclusion of AP and BB presented the lowest milk yield. No differences were found on the milk mineral profile. Inclusion of AP in the animals' diet improved the milk lipid profile from the point of view of human health (AI, TI) compared to BB due to a lower saturated fatty acid content (C12:0, C14:0, and C16:0) and a higher concentration of polyunsaturated fatty acids (PUFA), especially vaccenic acid (C18:1 trans11) and rumenic acid (CLA cis9, trans11), without any differences with the control treatment.

Keywords: fatty acid profile; mineral profile; CLA; milk yield; circular economy

1. Introduction

Regarding milk consumption worldwide, cow's milk occupies first place, followed by buffalo and thirdly, that of goat [1], which continues to increase [2] due to its high level of calcium, phosphorus, and animal protein. In addition, goat milk has been classified as a substitute for cow's milk in those people who suffer from some type of allergy to this food [3]. Goat's milk is a source of nutrients in the human diet due to its content of Se and polyunsaturated fatty acids (PUFA), such as vaccenic and rumenic acid or CLA [4,5], which can influence the prevention of certain types of cancers and cardiovascular diseases [6,7]. The literature contains many studies of how diet affects the performance and quality of ruminant milk. Hilali et al. [8] and Cappucci et al. [9] found that the inclusion of agro-industrial and olive by-products in ewes' diets enhanced milk fatty acid profile, with no effects on performance and milk macro-composition. On the other hand, Schulz et al. [10] observed changes in milk fatty acid profile in cows fed with red clover silage in comparison with maize silage. Finally, Monllor et al. [11] showed slight differences in fat and protein levels of milk from goats fed with artichoke by-products and an increase of Selenium and polyunsaturated fatty acid contents.

The inclusion of agricultural by-products in ruminant diets does not have to affect the sensory quality of dairy products. Such is the case in Caputo et al. Ref. [12], who did not observe differences in the aromatic profile of milk and dairy products from cows fed with destoned olive cake.

It is necessary to enhance the sustainability of milk production and reduce the impact of animal feeding. The use of local resources, especially if recovered from by-products, may significantly enhance milk sustainability. Artichoke (*Cynara scolymus* L.) and broccoli (*Brassica oleracea* var. *Italica*) crops generate large quantities of by-products. According to Food and Agriculture Organization of the United Nations (FAO) [13], 1,505,328 t of artichoke and 25,984,758 t of broccoli were harvested worldwide in 2017. The artichoke plant is a waste, mainly formed of stems and leaves, and some unharvested inflorescences are left in the field after harvest of inflorescences for human consumption. This by-product has traditionally been used by grazing small ruminants or collected and brought to dairy farms [14]. The yield of green fodder in this crop is 11.1 t/ha [15], which, taking into account FAO's cultivated area data [13] (2017) worldwide (122,390 ha), would result in an annual production of more than 1,300,000 t of available artichoke plant. According to Ros et al. [16], 29.5% of harvested broccoli is composed of stems and inflorescences that are not suitable for human consumption. Broccoli by-product is considered, from the point of view of animal feed, more as a concentrate than as a forage, due to its low fibre content and high protein level [17].

Agri-food by-products, whether coming from stubbles left in the field or the canning industry, constitute a supply of alternative forage for livestock, allowing the use of local resources and reducing feed costs without damaging animal performance and productivity, as long as the rations that include these feeds are balanced. The use of these by-products can also be a solution to minimise residues produced by the agro-food industry and thus reduce removal costs and emissions of polluting gases caused by uncontrolled fermentation of these agricultural wastes. In addition, the use of agro-food by-products reduces the land and supplies dedicated to the development of livestock feed, thus aiding the circular economy. However, the strong seasonality and high water content of these feeds limits their systematic use in animal feeding. Through lactic fermentation, the silage is able to conserve perishable products so that cellular respiration is suppressed, protein and vitamin degradation is prevented, and clostridial fermentation is avoided [18], reaching levels of safety that do not endanger the health of animals and do not compromise the hygienic-sanitary quality of milk or derived products.

Previous studies have shown that these by-product silages have the proper fermentative and nutritional conditions to become part of sheep and goat diets [14,19,20]. The references found in the literature about the effect of consuming these silage by-products on milk quality and composition, as well as on the health status of animals, are scarce [21–23]. None of these studies have been conducted in dairy goats, except Muelas et al. and Monllor et al. [11,24], where the effect of up to 25% inclusion of silage artichoke plant on the technological aptitude of milk was studied.

With the previous background, it is hypothesised that these by-products may be incorporated into the diet of lactating goats without detriment to their milk yield and quality. Therefore, the objective of this experiment is to study the effect of the inclusion of by-product silages (artichoke plant and broccoli by-product) in the ration of goats on milk production, macro-composition, and quality and determine the optimum level of inclusion in the ration among the three levels tested (25%, 40%, and 60%), with the aim of assuring milk nutritional quality within an integrative approach of enhanced sustainability of milk production.

2. Materials and Methods

2.1. Animals and Facilities

The animals used in this experiment were Murciano-Granadina lactating goats housed in the experimental and teaching farm of the Miguel Hernández University, Spain, with access to outdoor yards (2.30 m²/animal), free access to water, and enough feeding space for all animals (at least 35 cm/animal and 1.50 m²/animal as total indoor space) with a straw bed. As usual in the region,

the animals were milked once a day (Casse milking parlour, 2 × 12 × 12, GEA, Germany) and fed twice a day, at 8:00 a.m. and 2:00 p.m. This study was approved by the Ethical Committee of Experimentation of the Miguel Hernández University (code UMH.DTA.GRM.01.15).

2.2. Experimental Design

On the fourth month of lactation, 63 lactating goats were selected (41.2 ± 7.15 kg, 2.25 ± 0.80 kg/day, 5.39 ± 0.48 Log cell/mL). The animals were divided into seven homogeneous groups regarding body weight (BW), daily milk yield, and somatic cell count (SCC).

A short-term experiment was conducted to study the effect of inclusion in the diet of two by-product silages (artichoke plant, AP, and broccoli by-product, BB), of which their composition and fermentation quality are shown in Table 1. They were included at three levels each (25%, 40%, and 60%, expressed on a dry matter basis of the total ration); thus, seven rations were tested: 25%, 40%, and 60% of artichoke plant silage (AP25, AP40, and PAP60, respectively), the same percentages of broccoli by-product silage (BB25, BB40 and BB60), and a control diet (C), which represents the conventional ration used to feed dairy goats (alfalfa hay and a mixture of grains). Diets were formulated according to the recommendations of Fernandez et al. Ref. [25], an average amount of 2.23 kg DM/day was offered, and the seven rations were isoenergetic and isoproteic. Table 2 shows the ingredient proportion and the chemical composition of each diet. Once the pre-experimental sampling was performed, the experiment lasted 4 weeks. In the first two weeks, each group of animals adapted to their diet. In the next two weeks, data on feed consumption, milk yield, and body weight were recorded and blood and milk samples from animals were collected weekly for subsequent laboratory analyses. Bulk milk samples were collected weekly and used to determine mineral and fatty acid profile concentrations.

Table 1. Chemical composition (g/kg DM) and fermentation quality (g/kg DM) of silages included in experimental diets.

| Item | BB | AP |
|---|------|-------|
| Chemical Composition | | |
| DM (g/kg of FM, as fed) | 154 | 258 |
| OM | 821 | 828 |
| CP | 174 | 78.1 |
| CF | 214 | 296 |
| NDF | 430 | 571 |
| ADF | 326 | 374 |
| ADL | 63.4 | 108 |
| EE | 32.1 | 34.6 |
| TP | 6.73 | 4.96 |
| VFA and Fermentative Metabolites | | |
| Lactate | 30.8 | 17.0 |
| Acetate | 117 | 35.2 |
| Propionate | 14.6 | n.d. |
| Butyrate | 3.80 | 8.56 |
| Ethanol | 14.6 | 3.25 |
| Ammonia N | 1.65 | 0.149 |

BB: Broccoli by-product silage; AP: Artichoke plant silage; DM: Dry matter; FM: Fresh matter; OM: Organic matter; CP: Crude protein; CF: Crude fibre; NDF: Neutral detergent fibre; ADF: Acid detergent fibre; ADL: Acid detergent lignin; EE: Ether extract; TP: Total polyphenols; VFA: Volatile fatty acids; n.d.: Not detected.

Table 2. Ingredients of experimental diets and their nutritional value.

| Item | Diets | | | | | | |
|--|---------|-------|-------|-------|-------|-------|-------|
| | C | AP25 | AP40 | AP60 | BB25 | BB40 | BB60 |
| Ingredients (g/100 g DM) | | | | | | | |
| Alfalfa hay | 38.0 | 14.7 | - | - | 13.5 | 8.50 | 4.60 |
| Oat | 16.0 | 15.0 | 13.0 | 8.0 | 35.0 | 26.5 | 26.6 |
| Barley | 9.50 | 9.00 | 8.00 | 4.51 | 5.50 | 3.72 | 1.23 |
| Corn | 9.08 | 8.43 | 8.00 | 4.35 | 5.16 | 3.60 | 1.19 |
| Dried sugar beet pulp | 7.36 | 7.00 | 6.50 | 3.53 | 4.18 | 3.00 | 0.960 |
| Sunflower meal | 3.36 | 3.12 | 3.00 | 1.61 | 2.00 | 1.33 | 0.440 |
| Peas | 2.50 | 2.32 | 2.09 | 1.20 | 1.42 | 0.990 | 0.330 |
| Cottonseed | 2.50 | 2.32 | 2.09 | 1.20 | 1.42 | 0.990 | 0.330 |
| Soybean meal 44% | 4.00 | 6.00 | 10.0 | 12.0 | 2.00 | 2.00 | 1.00 |
| Corn DDGS | 3.00 | 3.00 | 2.50 | 1.38 | 2.00 | 1.14 | 0.380 |
| Sunflower seeds | 2.00 | 1.74 | 2.40 | 1.00 | 1.07 | 0.740 | 0.250 |
| Beans | 1.25 | 1.16 | 1.05 | 0.600 | 1.00 | 0.500 | 0.160 |
| Wheat | 1.00 | 0.770 | 1.00 | 0.400 | 0.470 | 0.330 | 0.110 |
| Soy hulls | 0.420 | 0.390 | 0.350 | 0.200 | 0.240 | 0.160 | 0.050 |
| Silage | - | 25.0 | 40.0 | 60.0 | 25.0 | 40.0 | 60.0 |
| kg DM offered/day/animal | 2.24 | 2.26 | 2.20 | 2.30 | 2.22 | 2.21 | 2.20 |
| Chemical Composition | | | | | | | |
| DM (g/kg FM) | 893 | 554 | 448 | 361 | 438 | 334 | 254 |
| | g/kg DM | | | | | | |
| OM | 935 | 915 | 901 | 884 | 916 | 904 | 885 |
| CP | 162 | 160 | 163 | 157 | 162 | 165 | 169 |
| CF | 195 | 202 | 196 | 237 | 180 | 180 | 183 |
| NDF | 376 | 391 | 382 | 432 | 359 | 355 | 353 |
| ADF | 243 | 248 | 239 | 281 | 225 | 226 | 231 |
| ADL | 56.5 | 55.1 | 49.5 | 55.2 | 48.0 | 47.0 | 46.7 |
| EE | 41.9 | 36.5 | 35.1 | 30.5 | 41.3 | 38.5 | 34.7 |
| TP | 3.87 | 4.18 | 5.42 | 5.34 | 4.60 | 5.42 | 6.68 |
| IVDMD | 715 | 715 | 710 | 665 | 780 | 747 | 757 |
| ¹ ME (Mcal/kg DM) | 2.37 | 2.30 | 2.29 | 2.19 | 2.39 | 2.36 | 2.29 |
| VFA and Fermentative Metabolites (g/kg DM) | | | | | | | |
| Lactate | n.d. | 14.2 | 23.2 | 24.5 | 33.1 | 41.2 | 56.0 |
| Acetate | n.d. | 4.91 | 6.04 | 11.9 | 15.1 | 11.0 | 37.8 |
| Propionate | n.d. | n.d. | n.d. | n.d. | 2.63 | n.d. | 4.79 |
| Butyrate | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| Ethanol | n.d. | 1.50 | 1.80 | 1.69 | 9.64 | 12.5 | 23.2 |
| Ammonia N | 0.166 | 0.628 | 0.741 | 1.01 | 3.99 | 4.26 | 7.73 |
| Fatty Acids Profile (g/100 g Total Fatty Acids) | | | | | | | |
| C6:0 | 0.061 | 0.109 | 0.485 | 0.352 | 0.059 | 0.025 | 0.498 |
| C12:0 | 0.183 | 0.286 | 0.151 | 0.050 | 0.242 | 0.328 | 0.146 |
| C14:0 | 0.440 | 0.502 | 0.413 | 0.357 | 0.542 | 0.539 | 0.465 |
| C16:0 | 17.2 | 18.1 | 18.3 | 17.3 | 19.8 | 17.7 | 21.2 |
| C16:1 c9 | 0.300 | 0.348 | 0.369 | 0.364 | 0.374 | 0.312 | 0.592 |
| C18:0 | 3.25 | 3.08 | 2.93 | 3.63 | 2.96 | 3.34 | 2.76 |
| C18:1 c9 | 26.4 | 25.1 | 22.8 | 31.3 | 30.1 | 34.3 | 21.9 |
| C18:1 c11 | 1.06 | 1.11 | 1.33 | 1.12 | 2.00 | 2.23 | 3.74 |
| C18:2n6 | 44.0 | 42.0 | 40.5 | 32.3 | 35.5 | 29.4 | 29.4 |
| C18:3n3 | 4.07 | 4.79 | 6.75 | 6.43 | 5.79 | 8.18 | 13.0 |
| C20:0 | 0.463 | 0.757 | 0.884 | 1.19 | 0.493 | 0.679 | 0.838 |
| C20:1n9 | 0.323 | 0.408 | 0.300 | 0.336 | 0.464 | 0.386 | 0.423 |
| C22:0 | 0.457 | 0.546 | 0.519 | 0.960 | 0.393 | 0.784 | 0.640 |
| C24:0 | 0.336 | 0.493 | 0.392 | 0.411 | 0.365 | 0.600 | 0.652 |
| SFA | 23.3 | 24.7 | 26.4 | 26.8 | 25.5 | 24.6 | 29.5 |

Table 2. Cont.

| Item | Diets | | | | | | |
|-----------------|-------|-------|-------|-------|-------|-------|-------|
| | C | AP25 | AP40 | AP60 | BB25 | BB40 | BB60 |
| MUFA | 28.2 | 27.6 | 26.1 | 33.7 | 33.0 | 37.5 | 27.5 |
| PUFA | 48.7 | 48.3 | 47.7 | 40.0 | 41.5 | 38.1 | 43.2 |
| Mineral Profile | | | | | | | |
| Na (g/kg DM) | 2.89 | 5.83 | 7.34 | 12.1 | 2.37 | 5.28 | 5.09 |
| Mg (g/kg DM) | 2.66 | 3.24 | 3.05 | 3.63 | 2.06 | 2.52 | 2.43 |
| K (g/kg DM) | 13.5 | 14.3 | 14.1 | 17.8 | 17.8 | 19.4 | 30.1 |
| Ca (g/kg DM) | 5.90 | 10.8 | 11.2 | 17.0 | 5.62 | 8.91 | 7.49 |
| P (g/kg DM) | 2.76 | 4.09 | 3.69 | 3.56 | 3.40 | 3.61 | 4.18 |
| S (g/kg DM) | 2.89 | 3.45 | 3.06 | 3.78 | 3.40 | 4.27 | 6.58 |
| Se (mg/kg DM) | 0.198 | 0.190 | 0.150 | 0.243 | 0.183 | 0.135 | 0.167 |
| Zn (mg/kg DM) | 49.4 | 44.2 | 41.3 | 34.1 | 43.6 | 42.5 | 36.9 |
| Cu (mg/kg DM) | 6.15 | 6.42 | 5.83 | 6.76 | 5.68 | 4.67 | 5.41 |
| Fe (mg/kg DM) | 129 | 414 | 287 | 460 | 175 | 161 | 235 |
| Mn (mg/kg DM) | 42.1 | 47.7 | 44.2 | 54.0 | 38.5 | 34.6 | 35.7 |

C: Control diet; AP: Diet that includes artichoke plant silage; BB: Diet that includes broccoli by-product silage; DM: Dry matter; FM: Fresh matter; OM: Organic matter; CP: Crude protein; CF: Crude fibre; NDF: Neutral detergent fibre; ADF: Acid detergent fibre; ADL: Acid detergent lignin; EE: Ether extract; TP: Total polyphenols; IVDMD: In vitro dry matter digestibility; ME: Metabolisable energy; VFA: volatile fatty acids; SFA: Saturated fatty acids; MUFA: Monounsaturated fatty acids; PUFA: Polyunsaturated fatty acids, n.d.: Not detected ¹[26].

2.3. Analysed Variables

The body weight of the animals (BW, kg) was recorded by weighing them on a scale (± 100 g, APC, Baxtran, Vilamalla, Spain). The feed consumption was measured twice a week and calculated by the average of the difference of the feed offered and refused on dry matter basis. The chemical composition of the silages and diets was analysed as previously described by Monllor et al. [11]. Dry matter (DM, g/kg; method 930.5), organic matter (OM, g/kg DM; method 942.05), ether extract (EE, g/kg DM; method 920.39), crude protein (CP, g/kg DM; method 984.13), and crude fibre (CF; g/kg DM; method 962.09) were determined following AOAC [27] procedures. Neutral detergent fibre (NDF, g/kg DM), acid detergent fibre (ADF, g/kg DM), and acid detergent lignin (ADL, g/kg DM) were analysed according to Van Soest et al. [28]. Total polyphenol content (TP, g/kg DM) was measured by the Folin-Ciocalteu method [29]. Volatile fatty acids (VFA, g/kg DM) (acetic, propionic, and butyric acid, also including lactic acid and ethanol) were determined by HPLC liquid chromatography (Agilent 1200, Santa Clara, CA, USA and Supelcogel C-610H column: 30 cm \times 7.8 mm ID, Saint Louis, MO, USA), by Feng-Xia et al. [30] methodology. Apparent in vitro dry matter digestibility (IVDMD, g/kg DM) was measured according to Menke and Steingass [31]. Fatty acid profile analysis in diets was performed by direct methylation on the lyophilised samples, without prior extraction of the fat, according to Kramer et al. [32] and were identified by a gas chromatograph (GC-17A Shimadzu, Kyoto, Japan) coupled with a flame ionisation detector (FID) equipped with a capillary column (CP Sil 88 100 m \times 0.25 mm internal diameter and 0.20 μ m internal coverage, Agilent, Santa Clara, CA, USA). A mixture of fatty acid methylated esters (FAME;18912-1AMP, Sigma-Aldrich, Saint Louis, MO, USA) was used for identification of the fatty acids of the samples.

Dietary and milk minerals (Na, Mg, K, Ca, P, S, Se, Zn, Cu, Fe, and Mn) were determined by carrying out a previous digestion of the samples, according to González Arrojo et al. [6]. Microwave (MW) digestion unit Ethos Easy, Milestone (Milestone, Srl, Sorisole, Italy) equipped with a rotor for 10 TFM (chemically modified PTFE) vessels was used for sample mineralisation. The microwave program consisted of four phases (i) 5 min at 1000 W at temperatures from 100 to 60 °C; (ii) 10 min at 1000 W from 165 to 80 °C; (iii) 5 min at 1000 W from 180 to 120 °C; and, (iv) 5 min at 700 W from 180 to 120 °C. The ICP-MS (inductively coupled plasma mass spectrometry) instrument used in this study was an Agilent 7700 \times Octopole Reaction System (ORS) (Agilent Technologies, Tokyo, Japan). The ICP-MS operating conditions were optimised for the simultaneous determinations of all elements. ICP-MS

standard solutions were prepared daily by appropriate dilution of stock standard 1000 mg/L for each element in 2% *v/v* Suprapur HNO₃. An appropriate internal standard was also required for each analyte to correct physical and/or matrix interferences in ICP-MS.

The milk yield (kg/day) of every goat was determined during milking using a Lactocorder[®] device (Lactocorder, Balgach, Switzerland). This device collected a representative sample of 100 mL of milk at every milking of each animal for subsequent analysis. The macro-composition of milk (fat, protein, true protein, casein, whey protein, lactose, total solids, TS; non-fat total solids, NFTS; useful dry matter content, UDM, and ash; %) and urea content (mg/L) was determined by medium infrared spectroscopy equipment (MilkoScan[™] FT2, Foss, Hillerød, Denmark). The SCC (10³ cell/mL) was analysed by an electronic fluoro-optical method (DCC, DeLaval, Tumba, Sweden). Fat corrected milk yield (FCM) was calculated according to Gravert equation [33]: FCM (3.5%) = 0.433 × milk yield (kg/day) + 16.218 × fat milk yield (kg/day). Milk fatty acids were extracted by the Folch procedure, with some variations collected in Romeu-Nadal et al. [34] and were methylated following the Nudda et al. [35] method. The equipment, column, and FAME mix used for the identification of peaks of milk fatty acid profile were the same as for the diets. Atherogenicity index (AI) and thrombogenicity index (TI) were calculated according to Ulbricht and Southgate [36]. These indices provide important information because AI is related with the ability of lipids' adhesion to immunological and circulatory system cells and TI indicates the tendency to form clots in blood vessels [8]. Desaturase indices (DI) for C14:0, C16:0, and C18:0 were calculated according to Lock and Garnsworthy [37].

In order to assess the effect of the diets on goats' metabolism, blood samples were analysed. The same day as the milk sampling was performed, the fasting animals were bled and samples were collected for glucose, urea, and β-hydroxybutyrate (BHB) analysis. Blood samples were analysed with a glucose oxidase/peroxidase kit (Ref. 11503, Biosystems, Barcelona, Spain) for glucose (mg/dL), with a kinetic method (GN 10125, Gernon, Sant Joan Despi, Spain) for urea (mg/dL), and for the BHB (mmol/L), the Ranbut D-3-Hydroxybutyrate kit (RB 1007, Randox, Crumlin, UK) was used.

2.4. Calculations and Statistical Analysis

The SCC data were transformed into log₁₀ scores before statistical analysis (LSCC).

BW, milk yield and macro-composition, SCC, and plasmatic profile data were performed using SAS GLIMMIX (SAS Institute Inc., Cary, NC, USA) with repeated measures, introducing the covariate of the data obtained in the pre-experimental sampling into the model and considering DIET, SAMPLING, and interaction DIET × SAMPLING as fixed effects, according to the following equation:

$$Y = \mu + Di + Sj + DixSj + covY0 + Ak + e,$$

where Y is the dependent variable, μ is the intercept, Di is the fixed effect of the diet ($i = C, AP25, AP40, AP60, BB25, BB40, BB60$), Sj is the fixed effect of sampling ($j = 1, 2, 3$), $DixSj$ is the interaction of diet with sampling, $covY0$ is the effect of the value of Y in sampling 0, Ak is the random effect of the animal, and e is the residual error. The covariance model with a lower value of the Akaike criterion (lower AIC and BIC) was used for each variable.

Milk mineral and fatty acid profile data were analysed using SAS GLM (SAS Institute Inc., Cary, NC, USA), introducing the covariate of the data obtained in the pre-experimental sampling into the model and considering DIET as a fixed effect. The level of acceptance for significance was 0.05.

3. Results

3.1. Diet Effects on Body Weight and Feed Consumption

Body weight is an indicator of the health status of the animal and optimising the inclusion of by-products involves assuring the proper health status of the goats. The treatments with the highest by-product inclusion showed a lower BW (40.2 and 38.7 kg in AP60 and BB60, respectively), while with the inclusion of 25% and 40%, no differences were observed compared to C (42.9 kg, Table 3).

Sampling and interaction Treatment \times Sampling also had a significant effect on BW as an increase ($p < 0.001$) was observed in sampling 2 in treatments with 40% of by-product (+1.9 and +2.4 kg in BB40 and AP40, respectively) and then in sampling 3, they descended again. Diets were offered in a similar amount but the goats in the different treatments showed different consumptions, with group C showing the highest (2.21 kg DM/day), whereas the lowest consumption was observed in groups BB40 (1.38 kg DM/day) and BB60 (1.27 kg DM/day) compared to the other treatments, which showed intermediate consumption (AP25: 1.52, AP40: 1.54, AP60: 1.57, and BB25: 1.65 kg DM/day).

Table 3. Body weight, milk yield, and composition and SCC, according to the effects considered.

| Variable | Diets | | | | | | | SEM | Significance | | |
|----------------------------------|---------|---------|----------|----------|----------|---------|--------|-------|--------------|----------|------------------------|
| | C | AP25 | AP40 | AP60 | BB25 | BB40 | BB60 | | Diet | Sampling | Diet \times Sampling |
| BW (kg) | 42.9 a | 41.6 ab | 42.2 a | 40.2 bc | 41.9 ab | 41.9 ab | 38.7 c | 0.69 | *** | *** | *** |
| Milk yield (kg/day) | 2.24 a | 2.15 ab | 2.14 abc | 1.92 bcd | 1.90 cde | 1.76 de | 1.66 e | 0.090 | *** | ** | *** |
| LSCC (Log ₁₀ cell/mL) | 5.53 | 5.67 | 5.58 | 5.68 | 5.54 | 5.82 | 5.68 | 0.109 | n.s. | ** | ** |
| FCM (3.5%; kg/day) | 2.31 ab | 2.42 a | 2.26 ab | 2.17 abc | 2.03 bc | 2.00 bc | 1.88 c | 0.120 | ** | ** | * |
| Fat (%) | 3.76 b | 4.25 ab | 4.06 ab | 4.29 ab | 4.02 ab | 4.25 ab | 4.58 a | 0.218 | ** | n.s. | * |
| Protein (%) | 3.39 | 3.42 | 3.52 | 3.39 | 3.34 | 3.34 | 3.42 | 0.088 | n.s. | n.s. | n.s. |
| UDM (%) | 7.15 b | 7.68 ab | 7.59 ab | 7.68 ab | 7.36 ab | 7.61 ab | 8.01 a | 0.275 | * | n.s. | * |
| True protein (%) | 3.16 | 3.18 | 3.27 | 3.15 | 3.11 | 3.11 | 3.18 | 0.078 | n.s. | n.s. | n.s. |
| Casein (%) | 2.68 | 2.69 | 2.76 | 2.66 | 2.65 | 2.65 | 2.72 | 0.061 | n.s. | *** | n.s. |
| Whey protein (%) | 0.470 | 0.484 | 0.507 | 0.491 | 0.456 | 0.465 | 0.474 | 0.024 | n.s. | *** | ** |
| Lactose (%) | 4.25 | 4.16 | 4.20 | 4.16 | 4.23 | 4.20 | 4.18 | 0.045 | n.s. | *** | ** |
| TS (%) | 12.0 b | 12.5 ab | 12.4 ab | 12.4 ab | 12.2 ab | 12.4 ab | 12.9 a | 0.28 | * | * | * |
| NFTS (%) | 8.75 | 8.67 | 8.81 | 8.63 | 8.70 | 8.67 | 8.75 | 0.084 | n.s. | *** | n.s. |
| Ash (%) | 0.639 | 0.615 | 0.648 | 0.625 | 0.638 | 0.627 | 0.652 | 0.024 | n.s. | n.s. | * |
| Milk urea (mg/L) | 617 ab | 587 abc | 591 abc | 641 a | 558 bc | 588 abc | 542 c | 23.0 | ** | n.s. | n.s. |

C: Control diet; AP: Diet that includes artichoke plant silage; BB: Diet that includes broccoli by-product silage; 25, 40, and 60 inclusion level of by-product silage on dry matter basis %; SEM: Standard error mean; BW: Body weight; Milk yield (kg/day); LSCC: Log₁₀ somatic cell count; FCM: Fat corrected milk (3.5%); UDM: Useful dry matter content (fat + protein); TS: Total solids; NFTS: Non-fat total solids; abc: Least square means within a column having different superscripts differ significantly. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

3.2. Milk Yield, Macro-Composition, and SCC

A decrease in milk yield was observed as the percentage of inclusion of by-products increased (Table 3). C, AP25, and AP40 were the treatments with the highest milk daily yield (2.24, 2.15, and 2.14 kg/day, respectively; $p < 0.001$); BB60 was associated with the lowest yield (1.66 kg/day). A tendency to decrease FCM was also observed as the percentage of inclusion of the by-product in the diet increased. The highest yield was obtained in AP25, even without significant differences compared to C or other AP treatments; BB25 and BB40 did not show significant differences compared to C, AP40, and AP60, whereas BB60 showed the lowest value. The interaction among sampling and treatments was significant as the milk yield and FCM were only significantly reduced in AP25 and AP60 during the experiment, but remained stable in the rest of the treatments.

The diet had no significant effect on LSCC. An increase of + 0.28 Log cells/mL ($p < 0.01$) was observed in AP25 between samplings 2 and 3, so that sampling and interaction with treatment were significant.

As for the macro-composition of the milk shown in Table 3, the diet only had significant effects on fat (but also affected UDM and TS) and urea (Table 3). BB60 was the one with the highest fat value and T was the lowest. The significant interaction of the treatment with the sampling in fat, UDM, TS, whey protein, and lactose was due to specific increases or decreases in sampling 2 in AP40, which returned to similar values to the previous ones at sampling 3. Both the casein content of milk and NFTS were reduced in all treatments during the experiment ($p < 0.001$). The ash content increased 0.134 percentage units in AP25 at the end of the experiment, remaining stable in the rest of the treatments. Regarding the milk urea content, AP60 was the treatment that presented the highest level (641 mg/dL; $p < 0.01$) and BB60 the lowest (542 mg/dL).

3.3. Milk Mineral Content

Milk mineral profile is shown in Table 4. Only significant differences in the Mn concentration due to dietary treatment were observed, although of small magnitude. AP40 was the treatment that presented the highest level of Mn (0.233 mg/kg DM; $p < 0.05$), followed by BB25 (0.222 mg/kg DM), whereas BB40 was the treatment showing the lowest value (0.185 mg/kg DM). These differences between treatments are not considered biologically relevant because the greatest of them, which was between AP40 and BB40, was only 0.048 mg/kg DM.

Table 4. Milk mineral profile according to the effects considered.

| Mineral | Diets | | | | | | | SEM | Significance |
|---------------|---------|---------|---------|---------|----------|---------|---------|-------|--------------|
| | C | AP25 | AP40 | AP60 | BB25 | BB40 | BB60 | | |
| Na (g/kg DM) | 2.59 | 2.40 | 2.23 | 2.36 | 2.53 | 2.41 | 2.68 | 0.113 | n.s. |
| Mg (g/kg DM) | 0.888 | 0.837 | 0.835 | 0.932 | 0.884 | 0.813 | 0.853 | 0.047 | n.s. |
| K (g/kg DM) | 12.0 | 11.5 | 11.2 | 11.8 | 12.1 | 10.9 | 11.5 | 0.51 | n.s. |
| Ca (g/kg DM) | 8.85 | 7.56 | 8.64 | 8.81 | 8.07 | 7.85 | 7.81 | 0.495 | n.s. |
| P (g/kg DM) | 6.00 | 5.16 | 6.37 | 6.08 | 5.43 | 6.05 | 6.11 | 0.412 | n.s. |
| S (g/kg DM) | 2.45 | 2.29 | 2.44 | 2.45 | 2.35 | 2.40 | 2.37 | 0.107 | n.s. |
| Se (mg/kg DM) | 0.102 | 0.095 | 0.127 | 0.117 | 0.091 | 0.105 | 0.093 | 0.010 | n.s. |
| Zn (mg/kg DM) | 18.6 | 21.3 | 17.1 | 28.3 | 25.9 | 20.4 | 23.5 | 2.60 | n.s. |
| Cu (mg/kg DM) | 0.697 | 0.538 | 1.11 | 0.397 | 0.357 | 0.382 | 0.420 | 0.367 | n.s. |
| Fe (mg/kg DM) | 2.95 | 2.16 | 2.26 | 2.72 | 2.11 | 2.22 | 2.34 | 0.557 | n.s. |
| Mn (mg/kg DM) | 0.203 b | 0.198 b | 0.233 a | 0.201 b | 0.222 ab | 0.185 b | 0.192 b | 0.010 | * |

C: Control diet; AP: Diet that includes artichoke plant silage; BB: Diet that includes broccoli by-product silage; 25, 40, and 60 inclusion level of by-product silage on dry matter basis %; SEM: Standard error mean. abc: Least square means within a column with different superscripts differ significantly. * $p < 0.05$.

3.4. Milk Fatty Acid Profile

Regarding the milk fatty acid profile (Table 5), some significant variations were observed, although they were quantitatively limited. Regarding the content of vaccenic acid (C18:1t11), it was observed that this was higher ($p < 0.001$) in the AP treatments, without differences compared to C. There was a higher concentration of linoleic acid (C18:2n6) in AP60 (2.53%; $p < 0.001$); however, it was at C where a higher level of other C18: 2 isomers was observed. An increase ($p < 0.001$) of α -linolenic acid (C18:3n3) was observed as the level of AP inclusion in the ration was higher and AP60 presented a higher level (0.242%). AP treatments were also those with the highest content ($p < 0.01$) in rumenic acid (CLA c9, t11), although subsequently no significant differences were found in the sum of isomers of CLA (conjugated linoleic acid) between treatments, except of BB60, of which their content was the smallest of all. Table 6 shows that as the percentage of AP inclusion increased, so did the PUFA content, and AP60 was the treatment with the highest content ($p < 0.001$) compared to all the BB treatments, without differences from C or the rest of the AP treatments. AP60 presented the highest levels ($p < 0.001$) of n3 (0.275%) and n6 (2.79%) fatty acids, the latter without differences compared to C or the other AP treatments. It also achieved the lowest ($p < 0.001$) ratio n6/n3 obtained together with BB60 (10.3 and 12.3, respectively). Regarding the lipid quality indices related to human health (AI and TI), AP40 and AP60 were the ones with the lowest value ($p < 0.001$) and therefore, were healthier. Regarding the desaturation indices of the myristic (DI14), palmitic (DI16), and stearic (DI18) fatty acids, the differences found between treatments were of small magnitude. BB60 was the one with the highest value in DI14 and DI18 (0.012% and 2.08%, respectively; $p < 0.001$) and AP60 presented a higher value of DI16 (0.061%; $p < 0.01$).

Table 5. Fatty acid composition (g/100 g total fatty acids) measured in milk according to the effects considered.

| Fatty Acid | Diets | | | | | | | SEM | Significance |
|---------------|------------|-----------|-----------|-----------|------------|------------|-----------|-------|--------------|
| | C | AP25 | AP40 | AP60 | BB25 | BB40 | BB60 | | |
| C4:0 | 2.21 | 2.66 | 2.53 | 2.57 | 2.53 | 2.62 | 2.67 | 0.586 | n.s. |
| C6:0 | 3.05 | 3.59 | 3.41 | 3.51 | 3.54 | 3.55 | 3.61 | 0.795 | n.s. |
| C7:0 | 0.052 ab | 0.060 ab | 0.070 ab | 0.046 b | 0.073 ab | 0.071 ab | 0.077 a | 0.024 | * |
| C8:0 | 4.11 | 4.57 | 4.67 | 4.32 | 4.64 | 4.77 | 4.28 | 0.981 | n.s. |
| C9:0 | 0.065 b | 0.077 ab | 0.095 a | 0.088 ab | 0.102 a | 0.102 a | 0.102 a | 0.023 | * |
| C10:0 | 13.2 | 15.0 | 14.7 | 14.5 | 15.6 | 15.6 | 15.3 | 3.03 | n.s. |
| C10:1 c9 | 0.037 | 0.040 | 0.033 | 0.036 | 0.047 | 0.036 | 0.034 | 0.017 | n.s. |
| C11:0 | 0.197 ab | 0.171 bc | 0.186 abc | 0.157 c | 0.190 ab | 0.201 a | 0.193 ab | 0.022 | ** |
| C12:0 | 3.23 a | 2.81 bc | 3.10 abc | 2.66 c | 3.11 abc | 3.31 ab | 2.93 abc | 0.274 | *** |
| C12:1 c9 | 0.032 | 0.024 | 0.035 | 0.030 | 0.039 | 0.037 | 0.024 | 0.012 | n.s. |
| iso C13:0 | 0.017 b | 0.016 b | 0.026 ab | 0.028 a | 0.027 a | 0.016 b | 0.019 ab | 0.008 | * |
| anteiso C13:0 | 0.025 | 0.025 | 0.030 | 0.030 | 0.030 | 0.031 | 0.026 | 0.008 | n.s. |
| iso C14:0 | 0.055 b | 0.045 b | 0.060 b | 0.067 ab | 0.063 ab | 0.058 b | 0.084 a | 0.019 | ** |
| C14:0 | 7.62 ab | 7.08 ab | 6.92 ab | 6.74 b | 7.59 ab | 7.56 ab | 7.76 a | 0.568 | * |
| iso C15:0 | 0.174 abcd | 0.130 b | 0.178 abc | 0.184 a | 0.163 abcd | 0.154 bc | 0.152 bcd | 0.021 | *** |
| anteiso C15:0 | 0.226 a | 0.170 c | 0.208 ab | 0.223 a | 0.189 bc | 0.181 c | 0.181 c | 0.021 | *** |
| C14:1 c9 | 0.073 bc | 0.062 c | 0.067 bc | 0.076 abc | 0.071 bc | 0.080 ab | 0.090 a | 0.011 | *** |
| C15:0 | 0.652 bc | 0.524 d | 0.617 c | 0.753 ab | 0.675 bc | 0.717 b | 0.818 a | 0.066 | *** |
| C15:1 | 0.070 a | 0.042 d | 0.048 cd | 0.064 ab | 0.055 bc | 0.061 ab | 0.055 bcd | 0.011 | *** |
| iso C16:0 | 0.176 c | 0.147 d | 0.188 bc | 0.225 a | 0.178 c | 0.204 ab | 0.218 a | 0.022 | *** |
| C16:0 | 21.5 ab | 22.3 ab | 20.4 ab | 20.5 b | 22.1 ab | 22.0 ab | 23.9 a | 1.67 | ** |
| C16:1 t4 | 0.039 ab | 0.003 b | 0.040 ab | 0.070 a | 0.003 b | 0.024 ab | 0.048 ab | 0.049 | * |
| C16:1 t5 | 0.023 ab | 0.005 ab | 0.029 ab | 0.043 a | 0.000 b | 0.007 ab | 0.042 ab | 0.036 | * |
| C16:1 t6-7 | 0.105 | 0.089 | 0.112 | 0.139 | 0.097 | 0.060 | 0.085 | 0.148 | n.s. |
| C16:1 t9 | 0.193 | 0.168 | 0.187 | 0.166 | 0.188 | 0.175 | 0.137 | 0.114 | n.s. |
| C16:1 t10 | 0.028 | 0.002 | 0.020 | 0.013 | 0.030 | 0.007 | 0.012 | 0.034 | n.s. |
| C16:1 t11-12 | 0.012 | 0.041 | 0.023 | 0.048 | 0.019 | 0.063 | 0.041 | 0.037 | n.s. |
| C16:1 c7 | 0.203 | 0.182 | 0.205 | 0.204 | 0.191 | 0.178 | 0.176 | 0.043 | n.s. |
| C16:1 c9 | 0.436 c | 0.449 bc | 0.491 bc | 0.542 ab | 0.482 bc | 0.475 bc | 0.617 a | 0.080 | ** |
| C16:1 c10 | 0.029 ab | 0.000 b | 0.031 ab | 0.047 a | 0.000 b | 0.012 ab | 0.033 ab | 0.040 | * |
| C16:1 c11 | 0.000 | 0.002 | 0.004 | 0.006 | 0.000 | 0.003 | 0.011 | 0.009 | n.s. |
| iso C17:0 | 0.249 ab | 0.234 ab | 0.275 a | 0.223 ab | 0.207 ab | 0.184 b | 0.165 b | 0.060 | ** |
| anteiso C17:0 | 0.287 a | 0.218 bc | 0.263 ab | 0.293 a | 0.257 ab | 0.180 c | 0.282 a | 0.049 | *** |
| C17:0 | 0.555 b | 0.485 b | 0.516 b | 0.703 a | 0.536 b | 0.541 b | 0.636 a | 0.058 | *** |
| C17:1 c6-7 | 0.040 | 0.046 | 0.050 | 0.049 | 0.041 | 0.056 | 0.034 | 0.018 | n.s. |
| C17:1 c8 | 0.000 b | 0.002 b | 0.000 b | 0.003 b | 0.002 b | 0.014 b | 0.035 a | 0.012 | *** |
| C17:1 c9 | 0.104 b | 0.114 b | 0.121 b | 0.195 a | 0.119 b | 0.159 a | 0.215 a | 0.023 | *** |
| iso C18:0 | 0.034 ab | 0.041 ab | 0.047 b | 0.047 ab | 0.034 b | 0.057 a | 0.053 ab | 0.013 | * |
| C18:0 | 14.1 a | 12.5 ab | 13.2 ab | 12.2 ab | 12.7 a | 11.8 ab | 9.9 b | 0.85 | *** |
| C18:1 t4 | 0.068 ab | 0.085 a | 0.067 ab | 0.049 bc | 0.082 a | 0.043 c | 0.045 c | 0.016 | *** |
| C18:1 t5 | 0.030 ab | 0.024 b | 0.031 ab | 0.033 ab | 0.038 a | 0.017 b | 0.026 ab | 0.011 | ** |
| C18:1 t6-8 | 0.196 a | 0.166 abc | 0.180 ab | 0.134 d | 0.146 bcd | 0.171 abc | 0.123 cd | 0.027 | ** |
| C18:1 t9 | 0.269 a | 0.271 ab | 0.245 abc | 0.234 bcd | 0.233 bcd | 0.213 abcd | 0.193 d | 0.028 | ** |
| C18:1 t10 | 0.276 a | 0.235 ab | 0.230 ab | 0.205 b | 0.220 ab | 0.235 ab | 0.219 b | 0.047 | * |
| C18:1 t11 | 1.30 a | 1.33 a | 1.35 a | 1.25 ab | 0.98 bc | 0.95 c | 0.81 c | 0.169 | *** |
| C18:1 t12 | 0.492 a | 0.471 a | 0.460 abc | 0.396 b | 0.383 bcd | 0.377 bcd | 0.317 d | 0.049 | *** |
| C18:1 t13-14 | 0.059 | 0.000 | 0.058 | 0.000 | 0.062 | 0.114 | 0.037 | 0.117 | n.s. |
| C18:1 c9 | 18.0 ab | 17.6 ab | 18.2 ab | 19.0 a | 16.3 b | 16.9 ab | 17.5 ab | 1.45 | * |
| C18:1 c11 | 0.043 ab | 0.055 ab | 0.038 ab | 0.005 b | 0.045 ab | 0.155 a | 0.052 ab | 0.121 | * |
| C18:1 c12 | 0.587 a | 0.565 abc | 0.581 a | 0.536 abc | 0.511 bc | 0.569 ab | 0.511 c | 0.047 | ** |
| C18:1 c13 | 0.124 | 0.116 | 0.112 | 0.115 | 0.115 | 0.119 | 0.112 | 0.019 | n.s. |
| C18:1 c14 | 0.424 a | 0.395 ab | 0.375 ab | 0.326 b | 0.371 b | 0.365 b | 0.329 b | 0.040 | ** |
| C18:1 c15 | 0.206 | 0.192 | 0.195 | 0.213 | 0.198 | 0.208 | 0.209 | 0.028 | n.s. |
| C18:2 c9,t13 | 0.294 a | 0.229 abc | 0.246 ab | 0.188 c | 0.220 bc | 0.220 abc | 0.174 abc | 0.044 | ** |
| C18:2 t8,c13 | 0.098 a | 0.084 ab | 0.083 ab | 0.089 ab | 0.074 b | 0.089 ab | 0.092 ab | 0.019 | * |
| C18:2 t9,t12 | 0.000 | 0.000 | 0.007 | 0.057 | 0.000 | 0.000 | 0.008 | 0.034 | n.s. |
| C18:2 c9,t12 | 0.154 a | 0.117 ab | 0.112 b | 0.104 b | 0.106 b | 0.107 b | 0.101 b | 0.031 | ** |
| C18:2 t11,c15 | 0.011 ab | 0.004 b | 0.014 a | 0.017 a | 0.013 ab | 0.010 b | 0.017 a | 0.008 | ** |
| C18:2n6 | 2.59 abcd | 2.40 ab | 2.42 ab | 2.53 a | 2.10 c | 2.26 bc | 1.98 bcd | 0.193 | *** |
| C20:0 | 0.233 d | 0.267 bc | 0.280 b | 0.350 a | 0.237 cd | 0.241 cd | 0.225 d | 0.029 | *** |
| C18:3n6 | 0.025 | 0.022 | 0.027 | 0.023 | 0.015 | 0.010 | 0.019 | 0.014 | n.s. |
| C20:1 c9 | 0.012 ab | 0.010 b | 0.017 ab | 0.029 a | 0.000 b | 0.007 b | 0.008 b | 0.015 | ** |
| C20:1 c11 | 0.038 | 0.050 | 0.053 | 0.049 | 0.052 | 0.053 | 0.040 | 0.018 | n.s. |
| C18:3n3 | 0.183 b | 0.145 c | 0.152 bc | 0.242 a | 0.156 bc | 0.179 bc | 0.173 bc | 0.025 | *** |
| CLA c9,t11 | 0.486 bc | 0.510 abc | 0.527 ab | 0.538 ab | 0.370 bc | 0.377 c | 0.344 bc | 0.064 | ** |
| CLA t9,c11 | 0.044 b | 0.032 c | 0.038 bc | 0.058 a | 0.030 c | 0.032 c | 0.035 bc | 0.009 | *** |
| CLA t10,c12 | 0.024 | 0.026 | 0.029 | 0.039 | 0.013 | 0.010 | 0.024 | 0.024 | n.s. |
| CLA t12,t14 | 0.017 | 0.012 | 0.023 | 0.025 | 0.009 | 0.006 | 0.022 | 0.017 | n.s. |
| ΣCLA | 0.528 a | 0.550 a | 0.549 a | 0.532 a | 0.529 a | 0.531 a | 0.482 b | 0.019 | *** |
| C20:2n6 | 0.033 | 0.027 | 0.038 | 0.040 | 0.044 | 0.036 | 0.034 | 0.015 | n.s. |
| C20:3n9 | 0.070 b | 0.061 b | 0.075 b | 0.116 a | 0.080 b | 0.060 b | 0.069 b | 0.017 | *** |
| C22:0 | 0.023 | 0.027 | 0.019 | 0.025 | 0.018 | 0.021 | 0.027 | 0.015 | n.s. |
| C20:3n3 | 0.000 b | 0.004 b | 0.013 b | 0.031 a | 0.000 b | 0.000 b | 0.000 b | 0.012 | *** |
| C20:4n6 | 0.152 a | 0.126 b | 0.151 a | 0.165 a | 0.158 a | 0.146 ab | 0.153 a | 0.018 | *** |
| C23:0 | 0.021 bc | 0.019 c | 0.030 abc | 0.047 a | 0.045 a | 0.029 abc | 0.038 ab | 0.015 | ** |
| C20:4n3 | 0.001 | 0.001 | 0.001 | 0.001 | 0.010 | 0.001 | 0.001 | 0.009 | n.s. |
| C22:2n6 | 0.000 c | 0.026 b | 0.001 c | 0.009 bc | 0.051 a | 0.023 b | 0.057 a | 0.015 | *** |
| C24:0 | 0.049 | 0.031 | 0.047 | 0.073 | 0.126 | 0.036 | 0.042 | 0.092 | n.s. |

C: Control diet; AP: Diet that includes artichoke plant silage; BB: Diet that includes broccoli by-product silage; 25, 40, and 60 inclusion level of by-product silage on dry matter basis %; SEM: Standard error mean; abc: Least square means within a column having different superscripts differ significantly. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Table 6. Grouped fatty acids (g/100 g total fatty acids) and indices related to cardiovascular health and desaturation activity in milk according to the effects considered.

| Variable | Diets | | | | | | | SEM | Significance |
|----------|-----------|-----------|-----------|---------|-----------|----------|----------|-------|--------------|
| | C | AP25 | AP40 | AP60 | BB25 | BB40 | BB60 | | |
| SFA | 72.2 | 73.0 | 72.2 | 70.9 | 75.1 | 74.2 | 73.6 | 2.19 | n.s. |
| MUFA | 23.3 | 22.7 | 23.5 | 24.5 | 21.1 | 21.8 | 22.6 | 1.90 | n.s. |
| PUFA | 4.11 ab | 3.86 abc | 3.87 abc | 4.24 a | 3.40 d | 3.56 cd | 3.50 bcd | 0.335 | *** |
| UFA | 27.4 | 26.6 | 27.4 | 28.7 | 24.5 | 25.4 | 26.1 | 2.21 | n.s. |
| SFA/UFA | 2.64 | 2.77 | 2.64 | 2.50 | 3.10 | 2.95 | 2.85 | 0.326 | n.s. |
| SCFA | 22.9 | 26.1 | 25.7 | 24.7 | 26.6 | 26.9 | 25.7 | 5.38 | n.s. |
| MCFA | 36.2 b | 35.6 b | 34.3 b | 34.8 b | 36.5 b | 36.6 b | 39.4 a | 2.79 | * |
| LCFA | 39.8 abc | 37.4 abc | 38.7 abc | 41.6 ab | 36.4 abc | 35.4 bc | 36.0 c | 2.88 | ** |
| n3 | 0.182 b | 0.151 b | 0.164 b | 0.275 a | 0.157 b | 0.178 b | 0.174 b | 0.034 | *** |
| n6 | 2.78 a | 2.55 abc | 2.60 ab | 2.79 a | 2.30 c | 2.44 bc | 2.18 bc | 0.218 | *** |
| n6/n3 | 15.4 abc | 17.3 ab | 17.4 a | 10.3 d | 14.8 abc | 13.8 bc | 12.3 cd | 2.33 | *** |
| AI | 2.11 b | 2.11 bc | 1.95 cd | 1.83 d | 2.37 a | 2.28 ab | 2.31 ab | 0.127 | *** |
| TI | 3.32 b | 3.30 b | 3.09 cd | 2.94 d | 3.65 a | 3.39 b | 3.36 abc | 0.141 | *** |
| DI C14:0 | 0.010 abc | 0.009 abc | 0.010 abc | 0.011 c | 0.009 abc | 0.011 bc | 0.012 a | 0.001 | *** |
| DI C16:0 | 0.050 b | 0.044 b | 0.055 ab | 0.061 a | 0.044 b | 0.048 b | 0.050 ab | 0.009 | ** |
| DI C18:0 | 1.55 bc | 1.72 bc | 1.67 b | 1.80 ab | 1.54 d | 1.75 bc | 2.08 a | 0.049 | *** |

C: Control diet; AP: Diet that includes artichoke plant silage; BB: Diet that includes broccoli by-product silage; 25, 40, and 60 inclusion level of by-product silage on dry matter basis %; SEM: Standard error mean; SFA: Saturated fatty acids; MUFA: Monounsaturated fatty acids; PUFA: Polyunsaturated fatty acids; UFA: Unsaturated fatty acids (MUFA + PUFA); SCFA: Short chain fatty acids (C6:0 a C10:0); MCFA: Medium chain fatty acids (C11:0 a C17:0); LCFA: Long chain fatty acids (C18:0 a C24:0); AI: Atherogenic index; TI: Thrombogenic index; DI: Desaturation index; abc: Least square means within a column having different superscripts differ significantly. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

3.5. Plasma Metabolic Profile

Regarding the plasma metabolic profile (Table 7), it was observed that the greater the inclusion of BB in the diet, the higher the glucose level (49.5 and 50.0 mg/dL in BB40 and BB60; $p < 0.001$), although the differences were of small magnitude (42.5 mg/dL in BB25). Regarding urea, C and AP had a higher content ($p < 0.001$), while the BB treatments obtained lower levels and BB60 showed the lowest (33.2 mg/dL). The level of BHB was higher in treatments that included less by-product, such as AP25, AP40, and BB25, while it was lower in treatments that included more BB (0.299 and 0.304 mmol/L in BB40 and BB60, respectively; $p < 0.001$). There was significant interaction of treatment with sampling in the three variables due to the different behaviour throughout the experiment between treatments: Glucose increased ($p < 0.001$) with the progress of the experiment in all treatments except BB60; blood urea was reduced ($p < 0.001$) at sampling 2 in BB25 and BB40 and increased at sampling 3 in BB25, BB40, and BB60; BHB increased ($p < 0.01$) at the end of the experiment in BB25, BB60, and AP60, while in C, BB40, AP25, and AP40 remained stable.

Table 7. Plasmatic profile according to the effects considered.

| Variable | Diets | | | | | | | SEM | Significance | | |
|---------------------|----------|---------|----------|----------|----------|---------|---------|-------|--------------|----------|-----------------|
| | C | AP25 | AP40 | AP60 | BB25 | BB40 | BB60 | | Diet | Sampling | Diet x Sampling |
| Glucose (mg/dL) | 44.6 bc | 47.7 ab | 45.0 bc | 48.3 ab | 42.5 c | 49.5 a | 50.0 a | 1.52 | *** | *** | *** |
| Plasma urea (mg/dL) | 52.0 a | 50.7 a | 50.9 a | 49.2 a | 38.8 bc | 39.8 b | 33.2 c | 2.14 | *** | ** | *** |
| BHB (mmol/L) | 0.336 bc | 0.522 a | 0.424 ab | 0.376 bc | 0.421 ab | 0.299 c | 0.304 c | 0.040 | *** | n.s. | ** |

C: Control diet; AP: Diet that includes artichoke plant silage; BB: Diet that includes broccoli by-product silage; 25, 40, and 60 inclusion level of by-product silage on dry matter basis %; SEM: Standard error mean; BHB: β -hydroxybutyrate; abc: Least square means within a column having different superscripts differ significantly. ** $p < 0.01$; *** $p < 0.001$.

4. Discussion

4.1. Diet Effects on Body Weight and Feed Consumption

One of the factors that affects the total volume of the diet and its consumption by livestock is the moisture content, as Jackson and Forbes [38] pointed out. This effect is especially important in the

short term as herbivores are able to progressively modify the volume of the rumen to increase the speed of transit of the digesta [39], so in the long term, this effect would have less influence. In this experiment, carried out in the short term, diet C was the one presenting the highest DM content and feed consumption (2.21 kg DM/day). On the contrary, diets BB40 and BB60 contained a greater amount of water and were bulkier and presented less consumption. In addition, diets with silage showed higher concentrations of VFA and other substances resulting from fermentation. The presence of propionic acid in BB60 (4.79 g/kg DM), as well as a higher concentration of ammonia N in both BB40 and BB60, also occurred in treatments with lower consumption due to the depressing effect on feed consumption demonstrated by Baumont [40]. The feed consumption of the BB treatments was superior to those found by Meneses [41] (0.508 kg DM/day) in Murciano-Granadina castrated males, whose ration incorporated 55% of BB silage. All BW values were normal for the Murciano-Granadina breed [42,43]. The greatest reduction in BW was in BB60, as well as the greatest reduction in feed consumption (1.27 kg DM/day and 38.7 kg).

4.2. Milk Yield, Macro-Composition, and SCC

The treatments that presented a higher feed consumption were those that had a higher milk yield. The values obtained are similar to the yield obtained with the equation proposed by León et al. Ref. [44] for the modelling of the Murciano-Granadina lactation curve, which stands at 1.93 kg/day between the fourth and fifth lactation months, which is where the animals used in this experiment were located. The highest percentage of fat in BB60 (4.59%) was probably due to a concentration effect (being the treatment with the lowest yield) and its highest content in acetic acid (37.8 g/kg DM, triple the rest) in the diet, which is an extra-lipogenic nutrient precursor of fat synthesis. Van Kneysel et al. [45] observed similar effects in dairy cows when part of the corn in the diet was replaced by beet pulp. Due to a higher fat concentration in BB60, UDM and TS also reached the highest values in this treatment (8.03% and 12.9%, respectively), exceeding C by almost a percentage point. The urea level of all treatments was found to be within the optimal range for goats recommended by the Interprofessional Dairy Laboratory of Castilla-La Mancha (LILCAM), which is between 500 and 700 mg/L. The differences found in the milk urea content can be explained by the different levels of feed consumption of the treatments. BB60 presented less feed consumption, in particular refusing part of the offered BB, which probably induced lower total protein intake and lower levels of milk urea, as Jimeno et al. [46] noticed.

4.3. Milk Mineral Content

The macromineral values correspond to those found by Mellado and García [47] in goat crossings. The composition of the diet of animals largely determines the concentrations of minerals in milk [48]. As there were no large differences in the content of the different minerals in the diets, no significant differences were subsequently observed in the milk of the different treatments, which is important for the technological aptitude of the milk, given the relevance of Ca and P in the setting and development of the microstructure of cheese [49], the main destination of goat's milk. Only the Mn had a higher concentration in AP40 (0.233 mg/kg DM), although with such tight differences that they are not biologically relevant.

4.4. Milk Fatty Acid Profile

The milk of animals fed with AP60 had a higher content of n3 fatty acids, which caused a lower n6/n3 ratio, which is positive for the prevention of coronary and cardiovascular diseases [50]. On the other hand, C, AP25, AP40, and AP60, of which their diets had the highest levels of PUFA, were the treatments with milk richest in vaccenic, rumenic, and PUFA, as reported by Collomb et al. [51], who observed differences in the PUFA and vaccenic content in the milk of cows fed with high mountain pastures and in lowland plains because the plants that made up the mountain meadows had a higher concentration of PUFA.

Another factor that could influence the increase of PUFA in AP treatments was the slightly higher content of total polyphenols (TP) in the diet, although lower than that of BB60. However, the lower feed consumption of BB60 could mean that the total TP intake does not reach those of the AP treatments. Several studies have demonstrated the inhibitory action of dietary polyphenols on ruminal biohydrogenation of PUFA, without detrimental effects on milk yield and composition, due to interference with microbial flora [52–55]. These effects have also been observed in sheep with small amounts in the diet of by-products rich in TP [56,57]. Cappucci et al. Ref. [9] also observed how after increasing the TP content of the diet of Comisana sheep by including different levels of olive by-product, the concentration of linoleic (C18:2n6) and α -linolenic (C18:3n3) in milk was increased.

As a result of a lower milk content of C12:0, C14:0, C16:0, and C18:0, AP40 and AP60 had the lowest levels of AI and TI, so the milk of these animals would be of higher quality in terms of human health [42]. The values obtained from AI in all the treatments of this study are below those found by Molina-Alcaide et al. Ref. [42] in Murciano goats fed with conventional ration supplemented with feed blocks of olive by-products. The desaturation indices obtained in this experiment are similar to those provided by Baldin et al. Ref. [58] in a study in goats that received a dietary CLA supplement.

4.5. Plasma Metabolic Profile

Despite the differences found in the metabolic profile of the different treatments, glucose, urea, and BHB levels remained within the ranges considered optimal for goats [59], except for the urea value in BB60, which was slightly lower. As Friggens et al. [60] observed in goats' performance, the level of BHB was generally low and particularly in those treatments showing lower feed consumption (BB40 and BB60) because goats, as lactating animals, adapt their milk yield to the level of feed intake, as seen in Table 3. This reduces the metabolic load and allows them to maintain adequate body reserves turnover. Due to the strong relationship between plasma and milk urea content [61], the lower levels of blood urea were found in the same treatments with the lowest values of milk urea.

5. Conclusions

The findings of this study highlighted that a threshold level of AP or BB inclusion in dairy goat diets, without negative effects on milk yield, composition, mineral and fatty acid profile, as well as metabolic status of the animals, would be 40% of the dietary dry matter.

The inclusion of artichoke plant and broccoli by-product silages in high doses (60%) caused lower feed consumption and lower milk yield. Inclusion at 60% of AP and BB increased the milk TS, although not enough to compensate for the reduced yield, resulting in lower FCM in the case of BB60. No differences were found regarding the milk mineral profile. Inclusion of the artichoke plant silage in the animals' diet improved the milk lipid profile from the point of view of human health (AI, TI) compared to broccoli silage, due to a lower SFA content (C12:0, C14:0, and C16:0) and a higher concentration of PUFA, especially vaccenic acid (C18:1 trans11) and rumenic acid (CLA cis9, trans11), without any differences compared to the control treatment. Regarding sanitary status, the plasma metabolic profile in broccoli treatments reflects that goats ate grains and alfalfa, whereas broccoli was the last ingredient, impairing its consumption, especially at the high concentration (60%). In addition, the diets that included 60% of by-product silages caused a reduction in BW.

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Publicación 5 (Artículo original)

Long-Term Feeding of Dairy Goats with Broccoli By-Product and Artichoke Plant Silages: Milk Yield, Quality and Composition

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Article

Long-Term Feeding of Dairy Goats with Broccoli By-Product and Artichoke Silages: Milk Yield, Quality and Composition

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Simple Summary: In the Mediterranean region, artichoke and broccoli are major crops with a high number of by-products that can be used as alternative feedstuffs for ruminants, lowering feed costs and enhancing milk sustainability while reducing the environmental impact of dairy production. However, the nutritional quality of milk needs to be assured under these production conditions. A 40% inclusion of these silage by-products has proven to be a good short-term option (1 month). Therefore, it is interesting to study the effect of feeding animals with these by-products for a longer time, such as full lactation (6 months). With this type of feeding, the performance of the animals fed with the artichoke plant silage was similar to that of those fed with a conventional diet (alfalfa and cereals), even improving the milk quality from the point of view of bioactive compounds. However, the performance of goats fed with broccoli by-product silage in high amounts worsened due to a low intake of the diet containing that by-product. Therefore, it is desirable to reduce its level of inclusion.

Abstract: The aim of this experiment was to study the effects of a 40% inclusion of broccoli by-product (BB) and artichoke plant (AP) silages in dairy goat diets on the milk yield, composition and animal health status during a full lactation. Feed consumption was lower in AP and BB animals due to their composition and higher moisture content, and BB animals showed a significant reduction in body weight. Milk from the BB treatment had the highest fat content, total solids and useful dry matter content (5.02, 13.9 and 8.39%, respectively). The Se level was slightly lower in AP and BB animals; however, the milk of these treatments was the lowest in Na and, in the case of BB animals, the richest in Ca (1267 mg/kg). Control and AP milk showed a similar fatty acid profile, although AP had a more beneficial aptitude for human health (lower ratio of n6/n3, 12.5). Plasma components, as metabolic parameters, were adequate for goats. It was concluded that a 40% inclusion of AP is an adequate solution to reduce the cost of feeding without harming the animals' health or performance and to improve the nutritional milk quality. It is necessary to lower the BB level of inclusion to increase feed consumption.

Keywords: circular economy; alternative feedstuffs; milk yield; fatty acid profile

1. Introduction

According to the Food and Agriculture Organization (FAO) [1], the production of goat milk is in third place (behind cow and buffalo), with a world production of over 18,600,000 t. The main

goat milk-producing country worldwide is India, while in Europe the countries that top the list of the highest producers are France, Greece and Spain. Murciano-Granadina is one of the most cosmopolitan Spanish goat breeds [2] and is used on farms in various countries in Europe, Africa and South America. This breed is generally exploited in semiextensive systems under different climatic conditions, feeding on natural pastures or different crop stubble throughout the year, receiving supplementation in critical periods based on agricultural by-products or conventional feeds [3]. Goat's milk is a rich source of nutrients in the human diet that can influence the prevention of certain types of cancers and cardiovascular diseases due to its Se and polyunsaturated fatty acids (PUFAs) contents, such as vaccenic and rumenic acids or conjugated linoleic acid (CLA) [4,5].

Worldwide artichoke (*Cynara scolymus* L.) and broccoli (*Brassica oleracea* var. *Itálica*) productions are important, as they reached values of 1,505,328 and 25,984,758 t of artichoke and broccoli, respectively [1]. These crops generate large amounts of waste that represent an environmental problem due to their rapid decomposition [6]. Wernli and Thames [7] indicate that artichoke plant can generate a yield of 11.1 t/ha of forage, so 1,300,000 t/year of this feedstuff could be available for ruminant feeding. Regarding broccoli, Ros et al. [8] calculated that 29.5% of the total broccoli harvested consists of stems and inflorescences not suitable for human consumption and they could be considered a concentrate rather than a forage, due to their low fibre content and high protein level [9].

The use of by-products for ruminant feeding allows their transformation into high quality food for humans, such as meat and milk [10], while promoting the development of the circular economy and reducing the use of land and water for food production and decreasing competition between animals and humans [11]. However, the marked seasonality and high water content of these feeds limit their systematic use as fresh components in animal feeding.

The silage of these by-products manages to achieve the appropriate fermentation conditions to ensure the nutritional quality and safety necessary to be part of the ration of small ruminants [12–14] while allowing their conservation for prolonged periods of time (up to 200 days according to Monllor et al. [14]). The references found in the literature on the effect of silage consumption of these by-products on milk quality and composition, as well as on the health status of the animals, are scarce and tested in the short term [12,15–17]. Only three studies have been published about its use in dairy goats. Muelas et al. [18] observed that the inclusion of artichoke plant silage of up to 25% in the diet had no effect on the sensory properties of yogurt made from the milk of these animals, while Monllor et al. and Monllor et al. [19,20] observed that the inclusion of up to 40% of artichoke plant silage and broccoli by-product in the middle stage of lactation (fourth to fifth month) had no relevant effects on the milk yield, composition and technological properties, whereas the inclusion of 60% of the by-product reduced performance.

Broccoli by-product and artichoke plant silages were tested in a previous short-term study for 5 weeks [20], showing favourable results. With the present experiment, we aimed to study during a full lactation the effect of the inclusion of 40% (on a dry matter basis) of the same by-product silages in the ration of dairy goats on the milk yield, composition, mineral and lipid profile and health status of the animals.

2. Materials and Methods

2.1. Animals and Facilities

Murciano-Granadina goats from the experimental farm of the Miguel Hernández University were allocated with a straw bed, access to outdoor yards, free access to water and enough feeder space for all animals (at least 35 cm/animal). Animals were fed twice a day, at 8:00 and 14:00, and milked once a day (Casse milking parlour, 2 × 12 × 12, GEA, Germany), as usual in the region. The experiment was carried out between the months of May and October. This study was approved and authorized by the Ethical Committee of Experimentation of the Miguel Hernández University (code UMH.DTA.GRM.01.15),

authorized by the competent public body (Conselleria de Presidència i Agricultura, Pesca, Alimentació i Agua of Generalitat Valenciana, Spain).

2.2. Experimental Design

From a group of 80 goats at the onset of lactation (4th week) fed with a conventional diet (control, C), a pre-experimental individual sampling was performed and 72 animals were selected, with an average body weight (BW) of 44.6 ± 7.81 kg, an average milk yield of 2.43 ± 0.21 kg/day and a somatic cell count (SCC) of 5.14 ± 0.55 Log cells/mL. The animals were divided into three groups with homogeneous characteristics regarding the commented variables. Each group was assigned to one treatment: control (C), which represents the conventional diet of the region (alfalfa hay and a mixture of grains) and two treatment rations that included 40% (on dry matter basis) of broccoli by-product silage (BB) or artichoke plant silage (AP) in a total mixed ration (TMR). Silages were manufactured with a baler-wrapper (Agronic MR 820, Netherlands), with a weight of 300 kg and without additives, according to the work published by Monllor et al. [14]. All rations were calculated according to the formulation recommendations of Fernández et al. [21]. They were isoenergetic and isoproteic and the daily amount offered was 2.22 kg DM/day. BB and AP silages had a lower cost than other feedstuffs used in animal feeding (45 and 42 EUR/t, respectively). Tables 1 and 2 show the amounts of the proportions of ingredients in each diet, as well as costs and the composition and mineral and lipid profiles. Feeding was performed in a group to avoid the stress caused by the individual confinement of the animals during a long-term study (6 months), as was done by Nudda et al. [22], due to the gregarious behaviour of goats [23]. This experimental design allows the average food consumption of the animals to be determined, although not the exact amount of individual feed intake nor ingested nutrients. However, the facilities and devices present on the farm allowed the collection of individual milk samples.

Table 1. Ingredients and chemical composition of the experimental diets provided to goats of the present study.

| Item | Diets | | |
|---------------------------------|-------|-------|-------|
| | C | BB | AP |
| Ingredients (g/100 g DM) | | | |
| Alfalfa hay | 37.4 | 12.0 | - |
| Oat | 16.0 | 32.0 | 13.5 |
| Barley | 8.00 | 3.00 | 6.40 |
| Corn | 9.0 | 3.00 | 8.00 |
| Dried sugar beet pulp | 7.50 | 3.00 | 6.50 |
| Sunflower meal | 3.40 | 1.00 | 3.00 |
| Peas | 3.00 | 1.00 | 2.30 |
| Cottonseed | 3.00 | 1.00 | 2.30 |
| Soybean meal 44% | 4.50 | 1.00 | 11.0 |
| Corn DDGS | 3.00 | 1.00 | 2.50 |
| Sunflower seeds | 2.00 | 1.00 | 2.00 |
| Beans | 1.40 | 0.500 | 1.10 |
| Wheat | 1.00 | 0.300 | 1.00 |
| Soy hulls | 0.500 | 0.200 | 0.400 |
| Silage | - | 40.0 | 40.0 |
| Cost (EUR/kg DM) | 0.28 | 0.26 | 0.26 |

Table 1. Cont.

| Item | Diets | | |
|---|---------|-------|------|
| | C | BB | AP |
| Chemical Composition | | | |
| DM (g/kg FM) | 893 | 336 | 450 |
| | g/kg DM | | |
| OM | 935 | 904 | 901 |
| EE | 41.9 | 39.1 | 35.2 |
| CP | 162 | 164 | 164 |
| NDF | 376 | 354 | 381 |
| ADF | 243 | 227 | 238 |
| ADL | 56,5 | 48.0 | 49,3 |
| TP | 3,65 | 4,93 | 3,28 |
| IVDMD | 744 | 737 | 668 |
| ¹ ME (Mcal/kg DM) | 2.37 | 2.36 | 2.29 |
| VFA and Fermentative Metabolites (g/kg DM) | | | |
| Lactate | 0.00 | 40.8 | 49.4 |
| Acetate | 0.00 | 9.50 | 1.39 |
| Ethanol | 0.00 | 9.22 | 1.58 |
| Ammonia N | 0.03 | 0.410 | 0.09 |

C: Control diet; BB: Diet that includes broccoli by-product silage; AP: Diet that includes artichoke plant silage; DM: Dry matter; FM: Fresh matter; OM: Organic matter; CP: Crude protein; CF: Crude fibre; NDF: Neutral detergent fibre; ADF: Acid detergent fibre; ADL: Acid detergent lignin; EE: Ether extract; TPs: Total polyphenols; IVDMD: In vitro dry matter digestibility; ME: Metabolisable energy; VFAs: Volatile fatty acids. ¹ [24].

Table 2. Mineral and fatty acid profile of experimental diets provided to goats of the present study.

| Mineral Profile | Diets | | |
|--|-------|-------|-------|
| | C | BB | AP |
| Na (g/kg DM) | 1.41 | 5.65 | 7.33 |
| Mg (g/kg DM) | 2.56 | 2.61 | 2.58 |
| K (g/kg DM) | 16.2 | 20.9 | 14.2 |
| Ca (g/kg DM) | 8.66 | 10.30 | 10.00 |
| P (g/kg DM) | 3.36 | 3.88 | 4.18 |
| S (g/kg DM) | 3.36 | 4.42 | 2.85 |
| Se (mg/kg DM) | 0.11 | 0.20 | 0.13 |
| Zn (mg/kg DM) | 40.6 | 49.8 | 38.7 |
| Cu (mg/kg DM) | 7.61 | 5.58 | 7.44 |
| Fe (mg/kg DM) | 175 | 266 | 272 |
| Mn (mg/kg DM) | 36.4 | 44.1 | 40.5 |
| Fatty acid profile (g/100g total fatty acids) | | | |
| C6:0 | 0.048 | 0.121 | 0.108 |
| C12:0 | 0.150 | 0.086 | 0.100 |
| C14:0 | 0.349 | 0.374 | 0.441 |
| C16:0 | 15.9 | 18.1 | 18.4 |
| C16:1 c9 | 0.31 | 0.328 | 0.341 |
| C18:0 | 3.17 | 3.16 | 3.10 |
| C18:1 c9 | 24.4 | 36.1 | 22.7 |
| C18:1 c11 | 1.04 | 2.38 | 1.04 |
| C18:2n6 | 46.9 | 28.9 | 43.7 |
| C18:3n3 | 5.03 | 6.09 | 5.40 |
| C20:0 | 0.461 | 0.717 | 0.914 |

Table 2. Cont.

| Mineral Profile | Diets | | |
|--|-------|-------|-------|
| | C | BB | AP |
| Fatty acid profile (g/100g total fatty acids) | | | |
| C20:1n9 | 0.310 | 0.447 | 0.346 |
| C22:0 | 0.526 | 0.804 | 1.17 |
| C23:0 | 0.123 | 0.663 | 0.139 |
| C24:0 | 0.383 | 0.580 | 0.662 |
| SFA | 21.8 | 25.5 | 25.6 |
| MUFA | 26.1 | 39.3 | 24.6 |
| PUFA | 52.0 | 35.2 | 49.8 |

C: Control diet; BB: Diet that includes broccoli by-product silage; AP: Diet that includes artichoke plant silage; DM: Dry matter; SFAs: Saturated fatty acids; MUFAs: Monounsaturated fatty acids; PUFAs: Polyunsaturated fatty acids.

Once the pre-experimental sampling was done, the experiment lasted 23 weeks. The first three weeks for each group served as time to adapt to their diet. In the following 20 weeks, data on BW and milk yield were taken, and blood and individual milk samples were collected for a subsequent laboratory analysis every 5 weeks, with 5 samplings in total during the whole experiment (Figure 1). In addition, the same week of individual sampling, after the milking of each experimental group, milk samples from the refrigerating tank were collected on three consecutive days for an analysis of the mineral and lipid profile. Then, the leftover milk was transferred to another refrigerated tank to get an empty and clean tank to receive milk from the next experimental group.

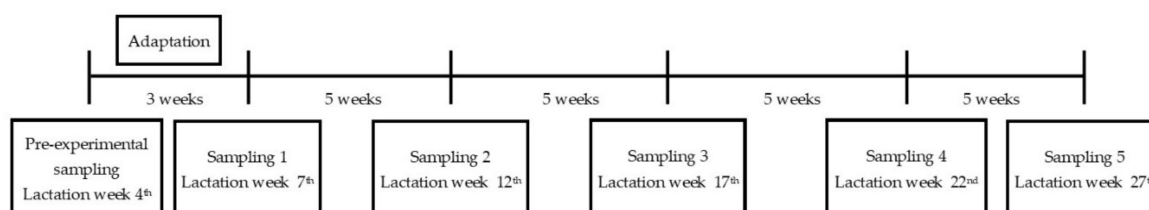


Figure 1. Chronogram of the experiment.

2.3. Variables Analysed

BW (kg) was determined by weighing animals with a precision scale of 100 g (APC, Baxtran, Spain). Feed consumption data were taken from two consecutive days in each sampling week and calculated as the average of the difference between the feed amount offered and refused, determining the dry matter by dehydration in an oven at 105°C for 48 h of a representative sample of the feed amount refused by the animals for each treatment. Representative samples were taken from each silage and ration at the start of the experiment for a subsequent laboratory analysis. To avoid providing the animals with spoiled silage, each bale was removed after two days of being open. In this way, it was possible to feed the animals with silage in optimal conditions that would not have been affected by aerobic degradation. The composition of the rations was determined in a similar way to Monllor et al. and Monllor et al. [19,20], using Association of Official Agricultural Chemists (AOAC) methods [25] for dry matter (DM, g/kg; method 930.5), organic matter (OM, g/kg DM; method 942.05), ether extract (EE, g/kg DM; method 920.39) and crude protein (CP, g/kg DM; method 984.13). The contents of neutral detergent fibre (NDF, g/kg DM), acid detergent fibre (ADF, g/kg DM) and acid detergent lignin (ADL, g/kg DM) were analysed according to Van Soest et al. [26]. The total polyphenol content (TP, g/kg DM) was determined by the Folin–Ciocalteu method reported in Kim et al. [27]. The proportion of short chain volatile fatty acids (VFAs, g/kg DM)—acetic, propionic and butyric acid, also including lactic acid and ethanol—were determined by high performance liquid chromatography (HPLC) (Agilent 1200 and Supelcogel C-610H column: 30 cm × 7.8 mm ID; [28]). The apparent in vitro dry matter

digestibility (IVDMD, g/kg DM) was determined in duplicate by the method of Menke and Steingass [29]. An analysis of the fatty acid profile in the diets was carried out by direct methylation on the lyophilised samples, without prior extraction of the fat, according to Kramer et al. [30]. Methylated fatty acid esters (FAMES) from diets were identified and quantified by a gas chromatograph (GC-17A Shimadzu, Japan) coupled with a flame ionisation detector (FID) equipped with a capillary column (CP Sil 88 100 m × 0.25 mm internal diameter and 0.20 µm internal coverage, Agilent, USA). A FAME standard mix (18912-1AMP, Sigma-Aldrich, USA) was used to identify the fatty acids present in the samples.

For the analysis of dietary and milk minerals, the same procedures were followed as in Monllor et al. [20], with a previous digestion of the samples performed according to González Arrojo et al. [4]. Na, Mg, K, Ca, P, S (g/kg DM) and Se, Zn, Cu, Fe and Mn (mg/kg DM) were determined by prior microwave digestion (Ethos Easy, Milestone, Italy) and identified with an ICP-MS octupole chromatograph (Agilent 7500 Reaction System, USA), using an internal standard.

The dairy yield and macrocomposition were determined similarly to Monllor et al. [20]. The milk production of each animal (kg/day) was recorded during milking using a Lactocorder[®] device (Lactocorder, Switzerland), which collected a 100 mL individual sample for subsequent analyses. The milk macrocomposition (fat, protein, useful dry extract, UDM; true protein, casein, whey protein, lactose, total solids, TSs; nonfat total solids, NFTSs; ash; %) and urea content (mg/L) was analysed by medium infrared spectroscopy (MilkoScan[™] FT2, Foss, Denmark) calibrated for goat milk. The somatic cell count (SCC, Log cell/mL) was determined by an electronic fluoro-optical method (DCC, DeLaval, Sweden). The fat corrected milk yield was calculated according to the Gravert equation [31]: fat corrected milk (FCM) (3.5%) = 0.433 × yield (kg/day) + 16,218 × fat yield (kg/day), and fat and protein corrected milk yield according to the equation of Schau and Fet [32]: FPCM = yield (kg/day) × (0.337 + 0.116 × fat (%) + 0.060 × protein (%)).

Analysis of the milk fatty acid profile was carried out in duplicate, with an extraction using the Folch method with some variations reported in Romeu-Nadal et al. [33] and a subsequent methylation according to the method of Nudda et al. [34], similar to what was done by Monllor et al. [21]. The chromatograph, the column and the FAME mix for the identification of the milk fatty acids were the same as those used in the diets. The indices related to the nutritional quality of milk fat were calculated—the Atherogenicity Index (AI) and Thrombogenicity Index (TI)—according to Ulbricht and Southgate [35], and the Desaturase Index (DI) for C14:0, C16:0 and C18:0, according to Lock and Garnsworthy [36].

On the same day that the milk sampling was performed, blood samples were taken from fasting animals for an analysis of glucose, urea, β-hydroxybutyrate (BHB), cholesterol, nonesterified fatty acids (NEFAs) and haematocrit, similarly as published by Monllor et al. [20]. Blood samples were analysed by enzymatic spectrophotometry. For glucose and cholesterol (mg/dL), a glucose oxidase/peroxidase kit was used (Refs. 11,503 and 11,505, Biosystems, Spain); for urea (mg/dL), the kinetic method GN 10,125 developed by Gernon was used (Spain); for the BHB (mmol/L), the Ranbut D-3-Hydroxybutyrate kit (RB 1007, Randox, UK) was used, and for the NEFAs (mmol/L), an enzymatic-spectrophotometric method FA 115 (Randox, UK) was used. The haematocrit (%) was determined with a microhaematocrit.

2.4. Statistical Analysis

SCC values were transformed into logarithm base 10 to carry out the statistical analysis. A normality test was performed on data (PROC UNIVARIATE. SAS v9.2, 2012) and the result was that all variables complied with normality and variance homogeneity. The variables obtained from individual animals were analysed according to a mixed linear model with repeated measures (PROC GLIMMIX. SAS v9.2, 2012), introducing into the model the covariate of the data obtained in the pre-experimental sampling, according to the following equation:

$$Y = \mu + Di + Wj + DixWj + covY0 + Ak + e, \quad (1)$$

where Y is the dependent variable, μ is the intercept, Di is the fixed effect of the diet ($i = C, BB, AP$), Wj is the fixed effect of the lactating week ($j = 7, 12, 17, 22, 27$), $Di \times Wj$ is the interaction of the diet with the lactating week, $covY0$ is the effect of the value of Y in control 0, Ak is the random effect of the animal and e is the residual error. For each variable, the covariance model that presented lower AIC and BIC statistics was used.

In the case of the milk lipid and mineral profile variables, an ANOVA (PROC. GLM, SAS v9.2, 2012) was performed, similar to the previous one, except that the random effect of the animal was not considered.

3. Results

No significant differences ($p > 0.05$) between groups were observed in the pre-experimental sampling (fourth week of lactation) for any of the variables analysed (body weight, milk yield and composition, SCC and milk mineral and fatty acid profile).

3.1. Body Weight and Milk Performance

BW remained constant in animals fed with AP throughout the experiment, and a significant increase ($p < 0.01$) was observed in C animals, while BW of animals fed with BB fluctuated throughout the experiment, with a decrease in the first half (weeks 7th to 17th) before reaching similar levels at the end of the experiment to those at the outset (Figure 2a). The C group had the highest average BW in the treatment (43.5 kg; Table 3), with significant differences ($p < 0.01$) with the BB group (41.5 kg), while the AP group did not show differences compared to any of the other treatments (42.1 kg). The average feed consumption was higher in the C group (1.98 ± 0.112 kg DM/day), followed by AP animals (1.82 ± 0.151 kg DM/day), while a remarkable lower consumption in BB animals (1.59 ± 0.124 kg DM/day) was observed. All three treatments reduced feed consumption mid-lactation: 160 and 180 g DM/day less between weeks 12th and 17th in C and AP animals, respectively, and 100 g DM/day less between weeks 17 and 22 in BB animals.

Table 3. Comparison of body weight, milk yield and composition and somatic cell count (SCC) in goat milk, according to the effects considered from 7th to 27th lactation week.

| Variable | n | Diets | | | | Significance | | |
|---------------------------------|----|---------|---------|---------|--------|--------------|------|--------------------|
| | | C | BB | AP | SEM | Diet | Week | Diet \times Week |
| BW (kg) | 24 | 43.5 a | 41.5 b | 42.1 ab | 0.50 | ** | ** | ** |
| Milk yield (kg/day) | 24 | 2.11 ab | 1.91 b | 2.21 a | 0.098 | * | *** | *** |
| FCM (kg/day) | 24 | 2.40 | 2.29 | 2.47 | 0.098 | n.s. | *** | *** |
| FPCM (kg/day) | 24 | 2.21 | 2.07 | 2.27 | 0.087 | n.s. | *** | *** |
| Fat (%) | 24 | 4.49 b | 5.02 a | 4.40 b | 0.157 | ** | *** | *** |
| UDM (%) | 24 | 8.05 ab | 8.39 a | 7.79 b | 0.199 | * | *** | ** |
| TS (%) | 24 | 13.5 ab | 13.9 a | 13.2 b | 0.21 | * | *** | ** |
| NFTS (%) | 24 | 8.82 | 8.73 | 8.68 | 0.071 | n.s. | *** | *** |
| Protein (%) | 24 | 3.55 a | 3.35 b | 3.42 ab | 0.059 | * | *** | * |
| True protein (%) | 24 | 3.29 a | 3.12 b | 3.18 ab | 0.052 | * | *** | * |
| Casein (%) | 24 | 2.86 | 2.76 | 2.77 | 0.048 | n.s. | *** | n.s. |
| Whey protein (%) | 24 | 0.431 a | 0.356 b | 0.404 a | 0.0119 | *** | *** | ** |
| Lactose (%) | 24 | 4.42 | 4.47 | 4.41 | 0.028 | n.s. | *** | *** |
| Ash (%) | 24 | 1.00 | 1.03 | 0.99 | 0.018 | n.s. | *** | * |
| Milk urea (mg/L) | 24 | 797 a | 745 b | 793 a | 15.5 | * | *** | *** |
| SCC (Log ₁₀ cell/mL) | 24 | 5.73 a | 5.55 b | 5.53 b | 0.062 | * | *** | ** |

C: Control diet; BB: Diet that includes broccoli by-product silage; AP: Diet that includes artichoke plant silage; SEM: Standard error of mean; BW: Body weight; FCM: Fat corrected milk (3.5%); FPCM: Fat and protein corrected milk; UDM: Useful dry matter content (fat + protein); TSs: Total solids; NFTSs: Nonfat total solids; SCC: Log₁₀ somatic cell count; abc: Least square means within a column having different superscripts differ significantly. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

The milk yield was slightly lower in the BB group (1.91 kg/day, Table 3) compared to the AP group (2.21 kg/day, $p < 0.05$), with no differences in the C group (2.11 kg/day) and with a significant

and gradual decrease ($p < 0.001$) throughout the experiment in the three treatments (Figure 2b), although in a different way, which meant the interaction of the treatment with the sampling was significant. The milk yield of animals fed with BB decreased markedly in the first sampling (week 7), increased in week 12 to levels close to the rest of the treatments and then decreased again significantly at week 17, with the three treatments reaching similar values at the end of lactation (Figure 2b); the AP group's milk yield decreased gradually throughout the experiment, and some oscillations were observed in the C group at the end of the experiment (week 22). Regarding the FCM and FPCM, no differences were observed between treatments in terms of mean values (Table 3); the value of both parameters decreased in the C and AP treatments as lactation progressed ($p < 0.001$), while in the BB treatment, the FCM remained (data not shown).

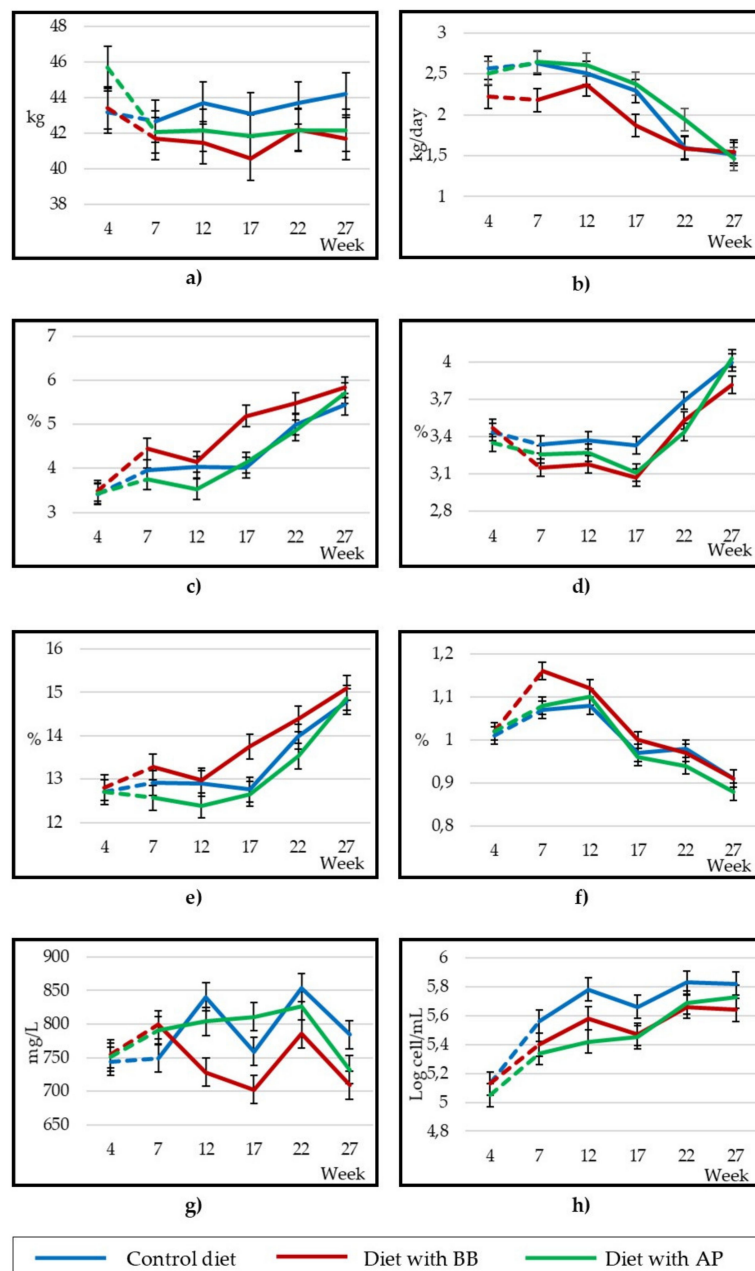


Figure 2. Comparison of changes in body weight (a), milk yield (b), milk fat (c), milk protein (d), total solids (e), ash (f), milk urea (g) and somatic cell count (h) in goat milk (24 goats per group) from 7th to 27th lactation week.

Regarding the milk macrocomposition, BB presented a higher average fat content ($p < 0.01$) (0.5% more, in absolute terms) than the other treatments (Table 3). A gradual increase ($p < 0.001$) in the fat content was observed in the three treatments throughout the experiment, generally with an inverse evolution to that observed in the milk yield, and the fat of the BB group was significantly greater than the other treatments at week 17 (Figure 2c), while in the rest of the samplings no significant differences ($p > 0.05$) were observed between treatments. The C treatment presented the highest content of crude and true protein (3.55 and 3.29%, respectively; $p < 0.05$, Table 3), while the BB treatment presented significantly lower values (3.35 and 3.12%) without significant differences between the two treatments with AP (3.42 and 3.18%). As observed with fat, the crude protein content increased ($p < 0.001$) as lactation progressed, and C presented a significantly higher content ($p < 0.001$) than the other treatments in weeks 17 and 22. The milk protein contents of the C and AP animals were equal at the end of the experiment (Figure 2d), but higher ($p < 0.05$) than in BB animals. In relation to whey protein, the C and AP groups were significantly higher than the BB group (0.431 and 0.404 vs. 0.356%, respectively; $p < 0.001$). In relation to TSs and UDM, BB animals presented significantly and slightly higher contents (13.9 and 8.39%, respectively; $p < 0.05$) than AP animals (13.2 and 7.79%), with no differences of both treatments in C animals (13.5 and 8.05%). These variables evolved in a similar way as fat and protein did throughout lactation (Figure 2e). There were no significant differences in the average content of ash between treatments and it decreased ($p < 0.001$) over the weeks (Figure 2f). C and AP animals presented higher ($p < 0.05$) milk urea contents (797 and 793 mg/L, respectively) than BB animals (745 mg/L) and the evolution throughout the experiment was different between treatments—more constant in AP and with 90 mg/L maximum oscillations in C and BB animals, as can be seen in lactation weeks 12 and 17 in Figure 2g. The SCC increased ($p < 0.001$) in the three treatments as lactation progressed (Figure 2h), with a higher content in C animals (5.73 Log cells/ML; $p < 0.05$) than BB and AP animals (5.55 and 5.53 Log cell/mL, respectively).

3.2. Milk Mineral Profile

The only significant differences were observed between treatments regarding the contents of Na, Ca, Mn and Se (Table 4). The C group obtained higher ($p < 0.001$) Na and Se contents (378 mg/kg and 17.2 $\mu\text{g/kg}$, respectively), although with small differences with the other treatments (19 and 47 mg/kg of Na in BB and AP animals, respectively, and 2.8 and 3 $\mu\text{g/kg}$ of Se in BB and AP animals, respectively). BB animals had the highest Ca level (1267 mg/kg; $p < 0.001$) and AP animals showed the lowest concentration of Mn (31.2 $\mu\text{g/kg}$; $p < 0.001$), with little difference from the other treatments (Table 4). In relation to the evolution of each mineral throughout the experiment, a reduction in the concentration of Cu was observed ($-27.5 \mu\text{g/kg}$), the levels of K and Zn fluctuated during lactation without significant differences between the beginning and the end and, in the rest of the minerals, an increase was observed over lactation in the three treatments—68 mg/kg Na, 40 mg/kg Mg, 204 mg/kg P, 72 mg/kg S, 89 mg/kg Ca, 16.1 $\mu\text{g/kg}$ Mn, 69 $\mu\text{g/kg}$ Fe and 4.1 $\mu\text{g/kg}$ Se—as the average increases from the beginning to the end of the experiment.

Table 4. Comparison of mineral profile from refrigerated goat milk tank according to the effects considered from 7th to 27th lactation week.

| Variable | n | Diets | | | | Significance | | |
|------------|----|-------|-------|-------|------|--------------|------|--------------------|
| | | C | BB | AP | SEM | Diet | Week | Diet \times Week |
| Na (mg/kg) | 15 | 378 a | 359 b | 331 c | 5.1 | *** | *** | *** |
| Mg (mg/kg) | 15 | 135 | 138 | 132 | 3.6 | n.s. | *** | *** |
| P (mg/kg) | 15 | 1025 | 992 | 988 | 63.0 | n.s. | *** | ** |
| S (mg/kg) | 15 | 394 | 381 | 380 | 17.8 | n.s. | *** | ** |
| K (mg/kg) | 15 | 1601 | 1557 | 1627 | 35.2 | n.s. | *** | *** |

Table 4. Cont.

| Variable | n | Diets | | | | Significance | | |
|------------|----|--------|--------|--------|-------|--------------|------|-------------|
| | | C | BB | AP | SEM | Diet | Week | Diet × Week |
| Ca (mg/kg) | 15 | 1208 b | 1267 a | 1200 b | 26.1 | *** | *** | * |
| Mn (µg/kg) | 15 | 46.5 a | 43.3 a | 31.2 b | 2.18 | *** | *** | n.s. |
| Fe (µg/kg) | 15 | 301 a | 301 a | 277 b | 15.7 | n.s. | *** | *** |
| Cu (µg/kg) | 15 | 72.4 | 55.7 | 63.0 | 5.0 | n.s. | *** | ** |
| Se (µg/kg) | 15 | 17.2 a | 14.4 b | 14.2 b | 0.540 | *** | *** | *** |
| Zn (µg/kg) | 15 | 3246 | 3017 | 3443 | 213 | n.s. | ** | ** |

C: Control diet; BB: Diet that includes broccoli by-product silage; AP: Diet that includes artichoke plant silage; SEM: Standard error mean; abc: Least square means within a column having different superscripts differ significantly. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

3.3. Milk Fatty Acid Profile

The greatest differences in the fatty acid contents were found in C15:0, C16:1cis9 and C17:0, where BB and AP animals presented the highest concentrations during the experiment (Figure 3a,b), and in some isomers of oleic acid, such as vaccenic (C18:1trans11) and rumenic acids (C18:2cis9,trans11), in which the highest concentration corresponded to the C animals (Table 5), although all differences were of small magnitude. As lactation progressed, the differences between treatments in linoleic acid (C18:2n6) and PUFA contents (Figure 3c,e respectively) were reduced. However, from week 17, α -linolenic acid (C18:3n3) and the ratio n6/n3 (Figure 3d,i) increased and the AP group obtained better results (higher level of α -linolenic acid and lower ratio n6/n3).

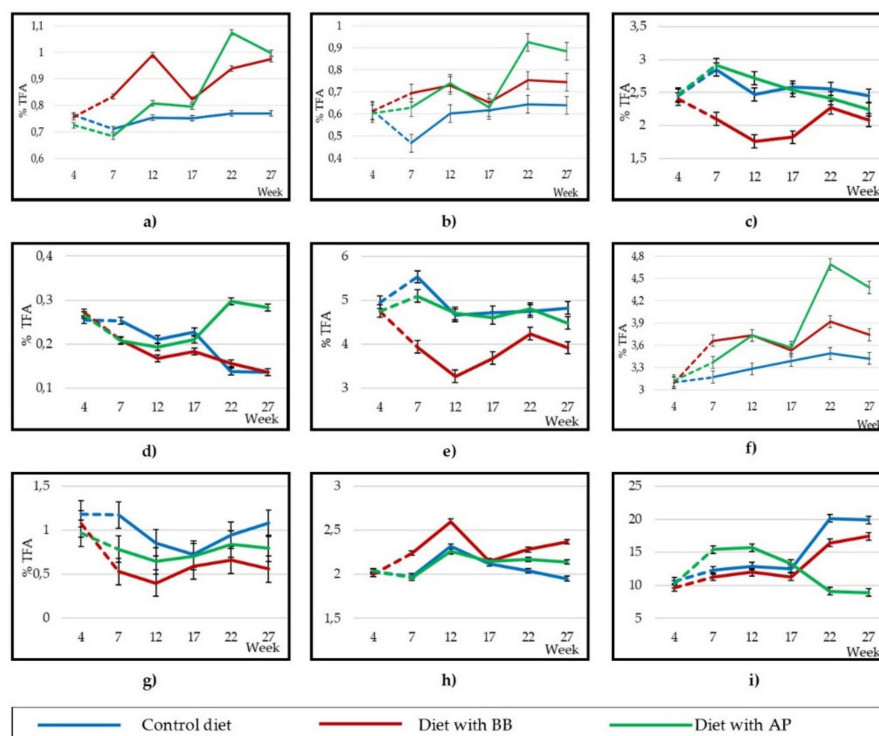


Figure 3. Changes in goat milk lipid profile from refrigerated tank (3 samples/sampling and treatment) during the experiment: C15:0 (a), C17:0 (b), C18:2n6 (c), C18:3n3 (d), polyunsaturated fatty acids (PUFA) (e), odd and branched chain fatty acids (OBCFA) (f), \sum CLA(g), SFA/unsaturated fatty acid (UFA) (h) y n6/n3 (i).

Table 5. Comparison of fatty acid composition (g/100 g total fatty acids) measured in goat milk from refrigerated tank according to the effects considered from 7th to 27th lactation week.

| Fatty Acids | n | Diets | | | | Significance | | |
|----------------------------|----|----------|----------|----------|-------|--------------|------|-------------|
| | | C | BB | AP | SEM | Diet | Week | Diet × Week |
| C4:0 | 15 | 1.34 a | 1.31 b | 1.38 a | 0.019 | * | *** | *** |
| C6:0 | 15 | 1.86 | 1.76 | 1.88 | 0.047 | n.s. | ** | * |
| C7:0 | 15 | 0.028 | 0.030 | 0.028 | 0.002 | n.s. | *** | *** |
| C8:0 | 15 | 2.41 b | 2.30 c | 2.49 a | 0.024 | *** | *** | *** |
| 4-methyloctanoic acid | 15 | 0.019 | 0.017 | 0.020 | 0.001 | n.s. | * | * |
| 4-ethyloctanoic acid | 15 | 0.015 | 0.017 | 0.018 | 0.002 | n.s. | * | n.s. |
| C9:0 | 15 | 0.042 b | 0.048 a | 0.040 b | 0.002 | ** | ** | *** |
| C10:0 | 15 | 8.10 b | 8.14 ab | 8.35 a | 0.075 | * | * | *** |
| C10:1 c9 | 15 | 0.027 b | 0.033 a | 0.027 ab | 0.002 | * | *** | n.s. |
| C11:0 | 15 | 0.236 c | 0.259 b | 0.278 a | 0.004 | *** | *** | *** |
| C12:0 | 15 | 3.74 | 3.83 | 3.81 | 0.033 | n.s. | *** | *** |
| C12:1 c9 | 15 | 0.013 | 0.015 | 0.015 | 0.002 | n.s. | n.s. | n.s. |
| iso C13:0 | 15 | 0.014 b | 0.015 b | 0.020 a | 0.001 | *** | n.s. | n.s. |
| anteiso C13:0 | 15 | 0.040 b | 0.041 ab | 0.045 a | 0.001 | * | *** | *** |
| iso C14:0 | 15 | 0.052 c | 0.066 b | 0.087 a | 0.003 | *** | *** | ** |
| C14:0 | 15 | 8.49 b | 8.73 a | 8.82 a | 0.044 | *** | *** | n.s. |
| iso C15:0 | 15 | 0.156 b | 0.158 b | 0.191 a | 0.003 | *** | *** | *** |
| anteiso C15:0 | 15 | 0.247 b | 0.220 c | 0.265 a | 0.003 | *** | ** | ** |
| C14:1 c9 | 15 | 0.124 c | 0.142 b | 0.153 a | 0.002 | *** | *** | *** |
| C15:0 | 15 | 0.751 c | 0.912 a | 0.872 b | 0.006 | *** | *** | *** |
| C15:1 | 15 | 0.083 ab | 0.075 b | 0.090 a | 0.003 | ** | *** | *** |
| iso C16:0 | 15 | 0.202 b | 0.240 a | 0.233 a | 0.004 | *** | *** | ** |
| C16:0 | 15 | 23.9 c | 26.5 a | 24.8 b | 0.10 | *** | *** | *** |
| C16:1 t4 | 15 | 0.022 b | 0.035 a | 0.029 ab | 0.004 | * | * | *** |
| C16:1 t5 | 15 | 0.026 | 0.036 | 0.031 | 0.004 | n.s. | *** | *** |
| C16:1 t6-7 | 15 | 0.061 | 0.036 | 0.042 | 0.010 | n.s. | n.s. | n.s. |
| C16:1 t9 | 15 | 0.182 a | 0.087 c | 0.126 b | 0.009 | *** | * | * |
| C16:1 t10 | 15 | 0.020 | 0.025 | 0.025 | 0.003 | n.s. | *** | *** |
| C16:1 t11-12 | 15 | 0.066 | 0.058 | 0.059 | 0.004 | n.s. | ** | * |
| C16:1 c7 | 15 | 0.228 | 0.240 | 0.238 | 0.006 | n.s. | * | *** |
| C16:1 c9 | 15 | 0.497 b | 0.666 a | 0.667 a | 0.015 | *** | *** | n.s. |
| C16:1 c10 | 15 | 0.032 ab | 0.026 b | 0.034 a | 0.003 | * | *** | ** |
| C16:1 c11 | 15 | 0.023 ab | 0.020 b | 0.028 a | 0.002 | * | n.s. | * |
| 3,7,11,15-Tetramethyl-16:0 | 15 | 0.056 | 0.025 | 0.029 | 0.018 | n.s. | n.s. | * |
| Cyclo C17:0 | 15 | 0.050 b | 0.081 a | 0.077 a | 0.003 | *** | *** | ** |
| iso C17:0 | 15 | 0.341 b | 0.305 c | 0.363 a | 0.005 | *** | *** | ** |
| anteiso C17:0 | 15 | 0.325 b | 0.347 a | 0.328 b | 0.004 | *** | *** | ** |
| C17:0 | 15 | 0.594 b | 0.714 a | 0.762 a | 0.020 | *** | *** | *** |
| C17:1 c6-7 | 15 | 0.045 a | 0.037 b | 0.043 a | 0.002 | * | ** | *** |
| C17:1 c8 | 15 | 0.019 b | 0.035 a | 0.017 b | 0.004 | ** | n.s. | n.s. |
| C17:1 c9 | 15 | 0.143 c | 0.212 b | 0.234 a | 0.006 | *** | *** | *** |
| Delta C17:2 | 15 | 0.021 | 0.023 | 0.024 | 0.002 | n.s. | n.s. | n.s. |
| isoC18:0 | 15 | 0.042 | 0.044 | 0.046 | 0.003 | n.s. | n.s. | n.s. |
| C18:0 | 15 | 14.1 a | 13.4 b | 12.4 c | 0.10 | *** | *** | *** |
| C18:1 t4 | 15 | 0.035 | 0.029 | 0.029 | 0.002 | n.s. | *** | ** |
| C18:1 t5 | 15 | 0.034 a | 0.027 b | 0.027 b | 0.002 | * | *** | ** |
| C18:1 t6-8 | 15 | 0.417 a | 0.286 b | 0.296 b | 0.008 | *** | *** | *** |
| C18:1 t9 | 15 | 0.408 a | 0.288 c | 0.324 b | 0.008 | *** | *** | *** |
| C18:1 t10 | 15 | 0.572 a | 0.318 c | 0.411 b | 0.013 | *** | *** | *** |
| C18:1 t11 (vaccenic) | 15 | 2.13 a | 0.83 c | 1.18 b | 0.050 | *** | *** | *** |
| C18:1 t12 | 15 | 0.550 a | 0.388 c | 0.427 b | 0.011 | *** | *** | *** |
| C18:1 t13-14 | 15 | 0.963 | 0.973 | 0.553 | 0.165 | n.s. | *** | *** |
| C18:1 c9 | 15 | 18.9 b | 19.5 b | 20.2 a | 0.21 | *** | *** | *** |
| C18:1 c11 | 15 | 0.451 b | 0.570 a | 0.555 ab | 0.038 | * | ** | ** |
| C18:1 c12 | 15 | 0.518 a | 0.429 c | 0.474 b | 0.015 | *** | *** | ** |
| C18:1 c13 | 15 | 0.120 | 0.101 | 0.102 | 0.007 | n.s. | n.s. | n.s. |
| C18:1 c14 | 15 | 0.493 a | 0.415 b | 0.388 c | 0.009 | *** | *** | *** |
| C18:1 c15 | 15 | 0.246 a | 0.227 b | 0.220 b | 0.004 | *** | *** | *** |
| C18:1 c16 | 15 | 0.023 a | 0.017 b | 0.019 ab | 0.002 | * | * | ** |
| C18:2 t8,c13 | 15 | 0.138 | 0.133 | 0.139 | 0.002 | n.s. | *** | ** |

Table 5. Cont.

| Fatty Acids | n | Diets | | | | Significance | | |
|------------------------|----|---------|----------|---------|-------|--------------|------|-------------|
| | | C | BB | AP | SEM | Diet | Week | Diet × Week |
| C18:2 t9,c12 | 15 | 0.027 | 0.029 | 0.032 | 0.003 | n.s. | *** | ** |
| C18:2 t9,12 | 15 | 0.021 | 0.018 | 0.021 | 0.002 | n.s. | n.s. | ** |
| C18:2 t10,14 | 15 | 0.070 a | 0.033 b | 0.039 b | 0.004 | *** | n.s. | * |
| C18:2 t11,c15 | 15 | 0.048 | 0.036 | 0.046 | 0.004 | n.s. | *** | * |
| C18:2 t11,15 | 15 | 0.012 | 0.008 | 0.010 | 0.003 | n.s. | n.s. | n.s. |
| C18:2 t12,c15 | 15 | 0.030 | 0.033 | 0.030 | 0.003 | n.s. | n.s. | n.s. |
| C18:2 c9,t12 | 15 | 0.121 a | 0.109 b | 0.117 a | 0.002 | *** | * | n.s. |
| C18:2 c9,t13 | 15 | 0.321 a | 0.299 c | 0.311 b | 0.003 | *** | *** | *** |
| C18:2 c12,15 | 15 | 0.026 | 0.026 | 0.024 | 0.002 | n.s. | ** | ** |
| C18:2n6 | 15 | 2.58 a | 2.01 b | 2.57 a | 0.047 | *** | *** | *** |
| C18:2 t9,c11 | 15 | 0.048 | 0.042 | 0.057 | 0.003 | *** | n.s. | * |
| C18:2 c9,t11 (rumenic) | 15 | 0.843 a | 0.443 c | 0.619 b | 0.018 | *** | *** | *** |
| C18:2 t10,c12 | 15 | 0.029 | 0.030 | 0.036 | 0.004 | n.s. | n.s. | n.s. |
| C18:2 t12,14 | 15 | 0.020 | 0.022 | 0.021 | 0.003 | n.s. | ** | n.s. |
| C18:3n3 | 15 | 0.193 b | 0.170 c | 0.238 a | 0.004 | *** | *** | ** |
| C18:3n6 | 15 | 0.029 b | 0.024 b | 0.036 a | 0.002 | *** | n.s. | *** |
| C20:0 | 15 | 0.241 c | 0.270 b | 0.314 a | 0.004 | *** | ** | *** |
| C20:1 c5 | 15 | 0.021 | 0.017 | 0.019 | 0.002 | n.s. | n.s. | n.s. |
| C20:1 c9 | 15 | 0.015 b | 0.016 b | 0.021 a | 0.002 | * | * | n.s. |
| C20:1 c11 | 15 | 0.052 a | 0.040 b | 0.048 a | 0.002 | *** | *** | ** |
| C20:1 c15 | 15 | 0.016 | 0.017 | 0.020 | 0.003 | n.s. | n.s. | n.s. |
| C20:2n6 | 15 | 0.038 | 0.037 | 0.038 | 0.003 | n.s. | ** | n.s. |
| C20:3n6 | 15 | 0.021 | 0.016 | 0.016 | 0.003 | n.s. | * | ** |
| C20:3n9 | 15 | 0.079 b | 0.075 b | 0.104 a | 0.004 | *** | n.s. | ** |
| C20:4n6 | 15 | 0.142 b | 0.139 b | 0.166 a | 0.004 | *** | * | * |
| C22:0 | 15 | 0.033 | 0.031 | 0.035 | 0.003 | n.s. | n.s. | n.s. |
| C22:2n6 | 15 | 0.021 b | 0.042 a | 0.023 b | 0.005 | ** | * | n.s. |
| C23:0 | 15 | 0.023 b | 0.034 ab | 0.039 a | 0.005 | * | * | n.s. |
| C24:0 | 15 | 0.031 b | 0.043 ab | 0.052 a | 0.005 | ** | *** | ** |

C: Control diet; BB: Diet that includes broccoli by-product silage; AP: Diet that includes artichoke plant silage; SEM: Standard error mean; abc: Least square means within a column having different superscripts differ significantly. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Regarding the fatty acid groups, BB animals presented the worst results from the point of view of functional compounds for human health—a lower content of CLAs, higher ratio of saturated fatty acids (SFAs)/unsaturated fatty acids (UFAs) and a higher AI and TI values (Table 6). The C group had the highest level ($p < 0.001$) of the sum of CLA isomers (0.954 g/100 g of total fatty acids (TFA)), followed by the AP group (0.752 g/100 g TFA) and BB group (0.547 g/100 g TFA). The MUFA content was significantly ($p < 0.0001$) higher in C (27.5 g/100 g TFA) than in AP (27.1 g/100 g TFA) and BB (26.2 g/100 g TFA). The PUFA content was similar in C and AP animals (4.89 and 4.73 g/100 g TFA, respectively), and both were significantly ($p < 0.0001$) higher than BB animals (3.81 g/100 g TFA). The SFA/UFA ratio was significantly ($p < 0.0001$) lower in C animals (2.08), followed by AP animals (2.14) and was significantly higher in BB animals (2.33). However, the n6/n3 ratio was lower ($p < 0.0001$) in AP animals (12.5), followed by BB animals (13.7) and higher in C animals (15.6). Related to odd and branched chain fatty acids (OBCFA), the silage treatments showed a higher content than the C group (3.35 g/100 TFA; $p < 0.001$), with higher contents in the AP compared to the BB group (3.95 and 3.71 g/100 TFA, respectively). The effect of lactation and diet × sampling interaction was also significant in OBCFA, because although in C animals it remained constant, in AP animals it increased in week 7 and later in week 17, and in BB animals it increased in week 22 ($p < 0.001$; Figure 3f). Regarding the evolution of the summations throughout the experiment, the three treatments showed similar changes, although at some points of the experiment small oscillations occurred (Figure 3e,g,h), except in the case of the ratio n6/n3 (Figure 3i), which decreased markedly and significantly in the AP group from week 17th, due to the increase in C18:3n3 (Figure 3d).

Table 6. Comparison of grouped fatty acids (g/100 g total fatty acids), indices related to cardiovascular health and desaturation activity in goat milk from refrigerated tank according to the effects considered from 7th to 27th lactation week.

| Variable | n | Diets | | | | Significance | | |
|----------|----|---------|---------|---------|-------|--------------|------|-------------|
| | | C | BB | AP | SEM | Diet | Week | Diet × Week |
| SFA | 15 | 67.4 c | 69.7 a | 67.9 b | 0.12 | *** | *** | *** |
| MUFA | 15 | 27.5 a | 26.2 c | 27.1 b | 0.14 | *** | *** | *** |
| PUFA | 15 | 4.89 a | 3.81 b | 4.73 a | 0.067 | *** | *** | *** |
| UFA | 15 | 32.4 a | 30.1 c | 31.8 b | 0.12 | *** | *** | *** |
| OBCFA | 15 | 3.35 c | 3.71 b | 3.95 a | 0.038 | *** | *** | *** |
| ∑CLA | 15 | 0.954 a | 0.547 c | 0.752 b | 0.019 | *** | *** | *** |
| SFA/UFA | 15 | 2.08 c | 2.33 a | 2.14 b | 0.013 | *** | *** | *** |
| SCFA | 15 | 13.9 b | 13.7 b | 14.3 a | 0.13 | ** | *** | * |
| MCFA | 15 | 40.8 c | 44.2 a | 42.8 b | 0.13 | *** | *** | n.s. |
| LCFA | 15 | 45.3 a | 42.1 c | 42.9 b | 0.20 | *** | *** | * |
| n3 | 15 | 0.193 b | 0.170 c | 0.238 a | 0.004 | *** | *** | ** |
| n6 | 15 | 2.83 a | 2.27 b | 2.84 a | 0.047 | *** | *** | *** |

C: Control diet; BB: Diet that includes broccoli by-product silage; AP: Diet that includes artichoke plant silage; SEM: Standard Error of Mean; SFAs: Saturated Fatty acids; MUFAs: Monounsaturated Fatty Acids; PUFAs: Polyunsaturated Fatty Acids; UFAs: Unsaturated Fatty Acids (MUFA + PUFA); OBCFA: Odd and Branched Chain Fatty Acids; CLA: Conjugated Linoleic Acid; SCFAs: Short Chain Fatty Acids (C6:0 a C10:0); MCFAs: Medium Chain Fatty Acids (C11:0 a C17:0); LCFAs: Long Chain Fatty Acids (C18:0 a C24:0); AI: Atherogenic Index; TI: Thrombogenic Index; DI: Desaturation Index; abc: Least square means within a column having different superscripts differ significantly. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

3.4. Plasmatic Metabolites Profile

Significant differences ($p < 0.001$) were observed between treatments (Table 7 and Figure 4). The glucose content decreased ($p < 0.001$) in C and AP animals during the first part of the experiment (Figure 4a) and stabilised later in C animals, while in BB animals it increased at the last sampling and oscillated in the last two samplings in AP animals (weeks 22 and 27). Regarding cholesterol, the C group reached levels significantly higher (100 mg/dL; $p < 0.0001$) than the BB and AP groups (91.9 and 86.1 mg/dL, respectively). The cholesterol levels of the C and BB groups decreased from lactation week 7 ($p < 0.001$) (−17.09 and −17.55 mg/dL, respectively; Figure 4b). C animals' cholesterol increased again later, while BB animals remained unchanged until the end; AP animals did not show significant differences between any sampling. The plasma urea content was slightly higher in C and AP animals (45.7 and 44.0 mg/dL, respectively) compared to BB animals (40.4 mg/dL), and the evolution throughout the experiment was similar in the three treatments (Figure 4c), except between weeks 17 and 22, where plasma urea content of animals fed with C and BB remained constant and decreased in AP animals. Finally, the urea content increased in the three treatments at the last sampling, although more markedly in C animals (+18.3, +8.1 and +7.4 mg/dL in C, BB and AP animals, respectively). Diet had a significant effect on the BHB concentration: the average value of the BB group was lower than the C and AP groups (0.356 vs. 0.456 and 0.450 mmol/L, respectively for BB, C and AP groups; $p < 0.01$). BHB levels decreased during the experiment, with slight fluctuations (Figure 4d). The NEFA level was higher in BB and AP animals at the start of the experimental phase (Figure 3e), and an increase in these treatments was observed since by-product silages were included in the diets, as the three treatments had similar levels in the pre-experimental period (0.424, 0.476 and 0.550 mmol/L, respectively for C, BB and AP animals). The NEFA levels of BB and AP animals decreased ($p < 0.001$) gradually throughout the experiment until they reached a similar level to C animals at week 22. Haematocrit decreased in all three treatments over the whole experiment (Figure 4f), and a slight increase was observed at the last sampling in C animals, which caused significant, although minor differences ($p < 0.01$) compared to BB animals, and without differences compared to AP animals (30.4, 28.2 and 29.3% for C, BB and AP animals, respectively).

Table 7. Comparison of plasmatic profile according to the effects considered from 7th to 27th lactation week.

| Variable | n | Diets | | | | Significance | | |
|---------------------|----|---------|---------|---------|-------|--------------|------|-------------|
| | | C | BB | AP | SEM | Diet | Week | Diet × Week |
| Glucose (mg/dL) | 24 | 50.8 a | 51.2 a | 47.6 b | 0.66 | *** | *** | *** |
| Cholesterol (mg/dL) | 24 | 100.8 a | 91.9 b | 86.1 b | 2.94 | *** | *** | *** |
| Urea (mg/dL) | 24 | 45.7 a | 40.4 b | 44.0 a | 1.14 | ** | *** | *** |
| BHB (mmol/L) | 24 | 0.456 a | 0.356 b | 0.450 a | 0.028 | * | *** | *** |
| NEFA (mmol/L) | 24 | 0.585 b | 0.856 a | 0.868 a | 0.048 | *** | *** | *** |
| Haematocrit (%) | 24 | 30.4 a | 28.2 b | 29.3 ab | 0.47 | ** | *** | *** |

C: Control diet; BB: Diet that includes broccoli by-product silage; AP: Diet that includes artichoke plant silage; SEM: Standard Error of Mean; BHB: β-Hydroxybutyrate; NEFAs: Nonesterified Fatty Acids; abc: Least square means within a column having different superscripts differ significantly. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

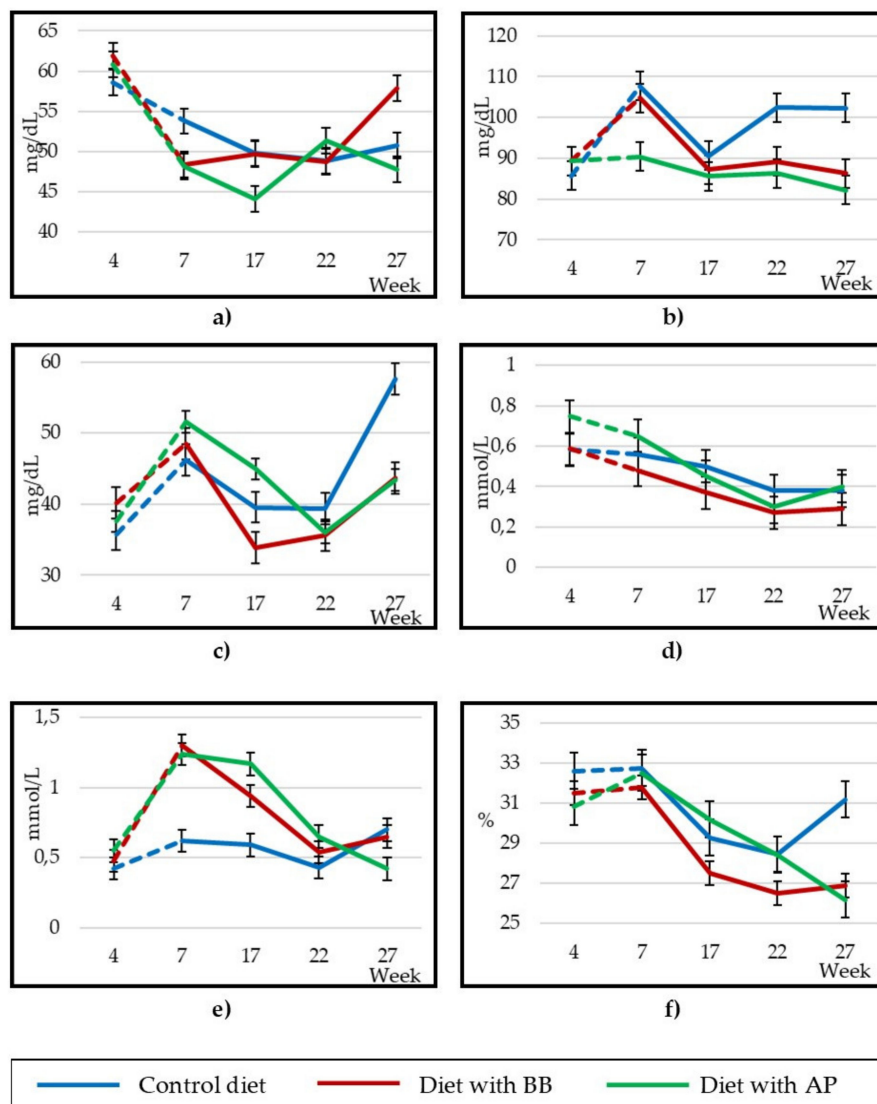


Figure 4. Changes in plasmatic metabolites (24 goats per group) throughout the experiment: glucose (a), cholesterol (b), urea (c), BHB (d), NEFA (e) and haematocrit (f).

4. Discussion

4.1. Body Weight and Milk Performance

The BW reduction observed in the first sampling (week 7 of lactation) was due to the fact that the animals had the greatest lactation needs at the same time, which did not coincide with the time of the maximum intake capacity, which encourages weight loss [37]. BW increased later, coinciding with the reduction in milk yield and the recovery of feed consumption typical of this lactation stage. BB animals showed a lower feed consumption than C and AP animals, because the diet that included BB contained a higher concentration of fermentation products, such as acetic acid, ethanol and ammonia N (Table 1), which had a depressive effect on feed consumption, as observed by Huhtanen et al. [38] through a meta-analysis of 240 studies. As in Monllor et al. [20], the higher moisture content of the BB diet and, therefore, the higher ration volume, decreased consumption. Other causes that affected feed consumption were the higher content of polyphenols in BB, which limits intake, as observed by Oliveira et al. [39] in growing calves and in grazing Sardinian goats, as shown by Decandia et al. [40]. The average feed consumption of C and AP animals (1.98 and 1.82 kg DM/day) was higher than that reported by Criscioni and Fernández [41] in Murciano-Granadina goats (1.7 kg DM/day) with an average milk yield of 2.2 kg/day and a BW of 46 kg, fed with alfalfa and concentrate. BB consumption was slightly lower than the values commented (1.59 kg DM/day).

The milk yield values observed in this experiment are similar, or even slightly higher in the case of the AP group, to those obtained from the equation proposed by León et al. [3] to model the lactation curve for Murciano-Granadina goats: $y = 1906 + 0.0229t - 0.000254t^2 + 0.000264(t - 57.28)^2$, where y is the daily production (kg/day) and t represents the days in lactation. The inclusion of BB silage at the level tested (40% DM in the diet) negatively affected the milk yield, as a marked decrease was observed in this treatment compared to C and AP groups in the first 17 weeks in lactation, although after the 22nd week, the differences between treatments disappeared. On the other hand, the AP group's milk yield was not significantly different from C group's, which indicates that the inclusion of 40% of artichoke plant silage during lactation allows for the maintenance of the productive level.

The higher fat content in BB animals' milk (5.02%) was due to its lower milk yield, also related to the higher NEFA content observed, so it indicated a greater tendency for a negative energy balance, probably due to a greater mobilisation of body reserves [42]. Even though the level of NEFA in AP animals was similar to that in BB animals (0.868 mmol/L), the higher milk yield (2.21 kg/day) of the AP group meant that fat concentration was higher in the BB group than the AP group. Another cause that explains the higher fat content of BB animals' milk is the higher concentration of acetic acid (9.50 g/kg DM) in the diet, which served as a precursor for the "de novo" synthesis of fat in the mammary gland [43]. Lough et al. [44], when they supplied a continuous intravenous infusion of acetate in dairy goats at different times of lactation, did not observe changes in milk yield or protein content. The higher fat content of BB animals' milk explains the higher level of TSs and UDM (13.9 and 8.39%, respectively) also observed in this treatment, even though C and AP animals had higher protein values.

The SCC increased as lactation progressed and the yield reduced, as occurred in Strzałkowska et al.'s study [45]. The SCC values are within normality for Murciano-Granadina goats with a correct health status of mammary gland [46].

4.2. Milk Mineral Profile

The mineral profile of this study coincides with that shown by Stergiadis et al. [47]. The Ca/P ratio of BB and AP animals is similar to that observed in local breeds in southern Europe [48,49]. The Ca/P ratio of C animals was lower due to its lower Ca content. The higher concentration of Ca in BB animals is due to the fact that the diet of this treatment also presented a higher level of Ca than the other two [50], which is beneficial for milk coagulation and curd firmness [51].

4.3. Milk Fatty Acid Profile

OBCFA levels were within the normal range for milk, between 2 and 3% of total fat, according to Patel et al. [52]. These same authors found a higher concentration of OBCFA in milk from cows fed grass silage, indicating that the more NDF content in the diet, the more OBCFA will be synthesised by ruminal microorganisms due to a higher production of VFAs. This could be the explanation for the higher total OBCFA content in AP animals, as well as C11:0, C17:1 cis9, iso C13:0, iso C14:0, iso C15:0 and iso C17:0. The higher levels of C15:0 and C17:0 in BB animals' milk come from a greater reserve mobilisation in the animals of this treatment, as these fatty acids are synthesised *de novo* from adipose tissue [53]. The initial increase (week 7) of OBCFA in AP animals could also be due to a mobilisation of reserves, coinciding with the peak of lactation; however, the OBCFA content stabilised in this group and the next increase that occurred at week 17 may already have been due to an adaptive response to the higher NDF content of the AP diet following the microbial origin of these fatty acids.

The lower concentration of linoleic, vaccenic and rumenic acids in BB was because the diet that included this by-product contained a lower proportion of linoleic and α -linolenic, precursors that are biohydrogenated in the rumen to form the aforementioned acids [54]. The Short Chain Fatty Acid (SCFA) concentrations in this study are similar to those found by Arco-Pérez et al. [55] in milk from Murciano-Granadina goats fed with a tomato by-product silage and to those commonly found in cow's milk [56]. This means that the inclusion of these by-product silages in the goat diet at the tested concentrations (40%) seems to have little impact on the flavour of milk, due to the role that SCFAs play in the organoleptic properties of milk [57]. From week 17 of lactation, the nutritional quality fatty acid profile of AP improved from the point of view of human health, because of a lower *n6/n3* ratio [58], which gives added value to the milk of goats fed with the silage, due to the current high demand for high quality healthy products for human consumption.

4.4. Plasmatic Metabolites Profile

Glucose levels coincided with those observed by Hamzaoui et al. [59] in goats of the same species. The glucose concentration was higher at the beginning of the experiment due to gluconeogenesis that takes place at the beginning of lactation [60], whereby the animals are able to reach the peak of production despite the limited intake capacity that occurs in this stage. The same could be the cause of the increase in glucose in BB animals at the last stage of the experiment, due to the lower feed consumption of the animals in this treatment, which caused the mobilisation of reserves and the reduction in BW. In addition, higher contents of TP in BB animals could cause an improvement in carbohydrate catabolism, as Makkar et al. and Zhong et al. [61,62] observed when glucose levels increased as the content of catechins and condensed tannins were higher in goat diets. The beneficial effects of TP on the lipid metabolism of small ruminants have been also demonstrated [63]. As a result of the lower feed consumption in AP and BB treatments in the stages with the highest energy demands, the NEFA content was higher in these treatments due to the mobilisation of adipose tissue to meet the animals' requirements [64,65]. These differences in NEFAs were not accompanied by differences in BHB, due to the availability of glucose (a result of the glycogenesis observed in BB), which is used to oxidise NEFA in the liver and produce energy [66]. As the level of feed consumption was adjusted to the milk yield, the concentration of NEFA was reduced, as occurred in Ríos et al. [67].

With the inclusion of BB in the diet, the cholesterol level increased between the 4th and 7th week of lactation, as in the C group, due to the synthesis of lipoproteins responsible for the transport of lipids to the mammary gland, habitual in this lactation stage [68]. This increase in cholesterol at the onset of lactation has been corroborated by other authors, such as Ruas et al. and Guedon et al. [69,70], who established that in beef cattle the minimum cholesterol level occurred at birth and the maximum was reached nine weeks later. The blood cholesterol content decreased as lactation progressed and milk production decreased, according to the Wood model [71]. However, it increased in the C group from week 17, probably due to a positive energy balance (excess of energy intake compared to the level of production at that time), as observed by Ríos et al. [67], coinciding with the increase observed

in BW. The cholesterol levels observed in this experiment are within the normal range of values (69.2–239 mg/dL) proposed by Merck et al. [72], in all treatments, indicating that the diets were adequate for the observed production levels.

The plasma urea level was higher at the onset of lactation due to the energy deficit typical of this stage [66]. The increase in urea in C animals at the end of the experiment is due to an excess of protein ingested in the ration [73], which, together with the excess of ingested energy discussed above, would indicate that the energy/protein balance was adequate, but the total amount of feed consumed was higher than the animals' requirements. This can be confirmed by the fact that the significant increase in the body weight of the goats fed the C diet (also compared to the by-products containing diets) at the end of the experiment. The average urea content of BB animals was lower than that of the other treatments due to the lower protein intake along with the higher TP content of this diet [74].

Taking into account the cost of the AP ration presented in Table 1, the feeding was reduced by 14.64% compared to diet C (76.19 EUR and 89.26 EUR/animal, respectively, during the 23 weeks that the study lasted), without major changes in the milk yield and composition, and with the benefit of a healthier lipid profile for human health, as already explained. On the other hand, in the BB diet, the feeding expenditure was even lower (66.56 EUR/animal), although with the consequent reduction in milk yield.

5. Conclusions

The use of artichoke plant silage in diets of dairy goats in the tested proportion (40% on a dry matter basis) over a full lactation did not lead to marked differences in milk yield and quality or compromise the animal health status compared to the diet commonly used in intensive farms (alfalfa hay and mix of cereals and legumes). Regarding the lipid profile, from a nutraceutical point of view, the milk of goats fed with AP, especially at the end of lactation, is more beneficial as it has a lower $n6/n3$ ratio. AP is therefore considered an efficient alternative for the use of residues derived from the agro-industrial sector, which allows the production of animal feed of high biological value for humans, with potential benefits for human health.

Regarding the inclusion of broccoli by-product silage in 40% DM in the diet during a full lactation, with a composition similar to that tested in this study, it could generate a dietary selection that is superior to what is desired, which would reduce the feed intake and lead to a lower milk yield, and at the same time promote a lower recovery of body reserves at the end of lactation, together with worse AI and TI indices in milk. Therefore, it is concluded that it is convenient to study the inclusion of a lower amount of this ingredient in the ration, especially during the peak of lactation, or the presentation of BB in other ways that stimulate its intake when it is part of a TMR, in order to maintain animal performance while benefits for the circular economy are achieved.

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5

Resultados y discusión

5. Resultados y discusión

5.1. Desarrollo del proceso de ensilado del subproducto de brócoli (SB) y subproducto y planta de alcachofa (SA y PA) en microsilos. Viabilidad de la técnica.

En este estudio se caracterizó el proceso de ensilado de los subproductos de brócoli y alcachofa y de la planta de alcachofa en microsilos comerciales de 300 kg de peso durante 200 días con el propósito de determinar la calidad e idoneidad de este tipo de ensilados como alimento para rumiantes, así como su vida útil.

Para ello se llevo a cabo el seguimiento de las dinámicas poblacionales de microorganismos y de los cambios en los perfiles de metabolitos de la fermentación, en el contenido en nutrientes y en la digestibilidad *in vitro* de la MS. También se analizó la presencia de residuos fitosanitarios en los ensilados transcurridos los 200 días del proceso. Finalmente, se presenta un análisis de costes de la fabricación para cada tipo de silo.

5.1.1. Microbiología

La población de bacterias lácticas de los tres ensilados alcanzó el pico de su crecimiento en el día 7. A partir de entonces se redujo, llegando a estabilizarse en el día 30 en SB (6,88 Log ufc/g) y en el día 60 en PA y SA (7,08 y 7,13 ufc/g, respectivamente). Las poblaciones del resto de microorganismos se redujeron a lo largo de proceso de ensilado como consecuencia de las condiciones de anaerobiosis y el alto nivel de bacterias ácido-lácticas, ya que son capaces de producir sustancias antimicrobianas que inhiben el crecimiento de microorganismos perjudiciales para la calidad del ensilado (Muller *et al.*, 1996). Las enterobacterias, al contrario que las ácido-lácticas, redujeron su población desde el inicio del ensilado hasta desaparecer el día 30 en SB y el día 60 en SA y PA, aunque posteriormente se observó un ligero repunte en el día 200 en PA (2,44 ufc/g). El lapso entre los ensilados de alcachofa y el de SB se debe a que la bajada de pH en PA y SA se dio de forma más lenta debido a una menor actividad enzimática por un menor contenido en azúcares que SB (Woolford, 1984). El recuento de aerobios mesófilos decreció en SB a partir del día 0 y se estabilizó en el día 60 (4,56 ufc/g), mientras que en PA se observó un pico de estos microorganismos el día 7 (8,75 ufc/g) seguido de una reducción, alcanzando un nivel estable en el día 30 (4,52 ufc/g). En SA, la reducción de aerobios mesófilos comenzó en el día 15 y mantuvo su ritmo decreciente hasta el día 200. La población de levaduras se mantuvo en recesión durante todo el ensilado debido a la reducción en el contenido de azúcares y al incremento del nivel de acético en el silo, debido a la actividad bactericida y antifúngica de este compuesto (Kung *et al.* 2018). Los niveles de levaduras alcanzaron unos valores en el día 200 de 3,17, 1,65 y 3,42 ufc/g en SB, SA y PA, respectivamente, lo cual asegura una buena estabilidad del silo una vez sea abierto y una buena palatabilidad para los animales (Kung *et al.*, 1998; Windle *et al.*, 2013). El nivel de mohos se mantuvo estable durante todo el proceso en SB (1,07 ufc/g) y SA (2,11 ufc/g), al contrario que en PA, donde la población de mohos se redujo desde el inicio del ensilado, desapareciendo el día 30. El conteo de esporas del género *Clostridium spp.* registró su valor más bajo el día 15 en los tres ensilados, siendo los valores de los días 0 y 200 similares, sin diferencias significativas en SA y PA.

5.1.2. Parámetros físico-químicos y composición nutricional

El pH descendió drásticamente en los tres ensilados al inicio del proceso de fermentación, cuando la población de bacterias ácido-lácticas había alcanzado su pico de población, y con el

ello, el máximo nivel de ácido láctico, el cual es el metabolito de la fermentación del ensilado con mayor capacidad acidificante (Megías *et al.*, 2014). Mientras que en el día 7 y 15 el pH se estabilizó en SA (4,30) y PA (4,47), en SB se incrementó ligeramente a partir de ese día, alcanzando un valor de 4,71 en el día 200. Este rápido descenso en los tres ensilados fue consecuencia de una baja capacidad tampón que presentó el material vegetal al inicio, siendo la de SB ligeramente superior al resto (40,8, 21,8 y 19,6 meq/100 g MS en SB, SA y PA, respectivamente), consiguiendo con ello un menor riesgo de pérdida de nutrientes y proliferación de microorganismos no deseables.

La escala Flieg mide la calidad de un ensilado en base a su contenido en MS y pH, otorgando unos valores entre 0 y 100, siendo la calidad mayor cuanto mayor es la valoración en esta escala. Mientras que SA y PA mantuvieron una puntuación superior a 80 durante todo el proceso de ensilado, la puntuación de SB permaneció en el rango de 80-100 hasta el día 15 y, a partir de entonces, descendió al rango de 60-80 debido a la mayor capacidad tampón comentada anteriormente, lo cual provocó un incremento de pH que explica las diferencias en los recuentos de poblaciones microbianas y perfil de AGV frente a PA y SA.

El contenido en MS se redujo en los tres ensilados como consecuencia de la actividad de las bacterias lácticas, pero mientras que en SB y SA se estabilizó en el día 30 (142 y 183 g/kg), en PA se mantuvo estable desde el inicio hasta el día 15, donde experimentó un ligero descenso. Estas pérdidas de MS no sobrepasaron el límite del 5% recomendado por McDonald *et al.* (1991) en las primeras fases del ensilado (hasta el día 30 en SB y el día 60 en PA) En cuanto al nivel de MO, este descendió en SB y PA, alcanzando unos valores de 821 y 828 g/kg de MS en el día 200, mientras que SA permaneció estable durante todo el experimento (916 g/kg MS).

La concentración de EE se incrementó ligeramente en SB y PA a partir del día 30, permaneciendo sin cambios en SA. En términos generales, el contenido en PB se mantuvo estable, salvo ligeras oscilaciones ocurridas en el día 60 en SB, en el día 200 en SA y en el día 30 en PA que no sobrepasaron el 15% de reducción del contenido en PB, valor límite fijado por Meneses *et al.* (2007) como indicador de una excesiva proteólisis. El contenido de FND descendió hasta el día 30 en los tres ensilados y se incrementó en el día 200 en PA y, principalmente, en SB por una reducción de la MS entre los días 60 y 200 debido a la fermentación de compuestos solubles, lo cual aumentó la proporción de la pared celular dentro del silo (Ashbell y Donahaye, 1984). Respecto a FAD, SA permaneció estable, mientras que en SB y PA siguió una tendencia similar a la de FND por la reducción de compuestos solubles. Por último, LAD descendió a partir del día 15 en SB y SA y se incrementó en en el día 200, mientras que en PA se mantuvo con oscilaciones durante todo el experimento. La digestibilidad *in vitro* de MS se redujo ligeramente en SB, se incrementó en SA y en PA fluctuó con pequeñas diferencias durante todo el estudio, aunque en términos generales, la estabilización de la digestibilidad se consiguió en el día 30 en los tres ensilados, lo cual coincide con la estabilización de la población de las bacterias lácticas, como apunta Umana *et al.* (1991).

El contenido en PT permaneció estable en PA durante todo el proceso, en SA se incrementó hasta el día 60, mientras que en SB creció hasta el día 15 (8.85 g/kg MS), nivel en el que se mantuvo estable durante el resto del ensilado. Este incremento de PT en SB se debe a la acción de la β -glucosidasa producida por las bacterias ácido-lácticas, la cual interviene en la liberación de compuestos fenólicos de las estructuras vegetales durante la fermentación (Sun *et al.*, 2009).

5.1.3. Fermentación

Los metabolitos resultantes de la fermentación de un ensilado afectan a la calidad nutricional de este, ya que juegan un papel relevante en el consumo de alimento y la producción y composición de la leche. A la síntesis de los productos de fermentación le afectan factores tales como las poblaciones de microorganismos y sustratos fermentables presentes en el silo. El contenido en azúcares se redujo en los tres ensilados al inicio, permaneciendo estable a partir del día 7 en PA (29,0 g/kg MS) y del día 15 en SB y SA (29,2 y 37,3 g/kg MS, respectivamente). La concentración de ácido láctico se incrementó en todos los ensilados, siendo esta subida más drástica en los primeros 7 días dado el mayor contenido en azúcares en ese momento. El pico de producción se alcanzó en el día 60 en SB (98,4 g/kg MS) y PA (29,2 g/kg MS) y en el día 30 en SA (51,9 g/kg MS), siendo el ácido láctico el producto de la fermentación predominante hasta este momento, lo cual indica una buena concentración de nutrientes y olor agradable que estimula el consumo de alimento (Megías *et al.*, 2014). El nivel de ácido acético subió de forma gradual en los tres tipos de silos, superando en el día 200 al contenido en ácido láctico en SB y PA, dado el prolongado tiempo de fermentación. En SB del día 200, el valor de ácido acético superó el límite recomendado por Kung *et al.* (2018) para silos de hierba con buena calidad (10-30 g/kg MS), lo cual afectaría posteriormente a la preferencia e ingestión de este ensilado en los experimentos con animales.

Respecto al ácido butírico, este se incrementó en los tres ensilados, alcanzando el nivel máximo en la primera mital del experimento (entre los días 7 y 60). La concentración de etanol aumentó desde el inicio en los tres ensilados y se estabilizó en los días 7, 30 y 60 en PA, SB y SA, respectivamente. Tanto el butírico como el etanol se mantuvieron dentro de los niveles aceptables para ensilados de buena calidad debido a los bajos recuentos en las poblaciones de enterobacterias, clostridios y levaduras (McDonald *et al.*, 1991). Por último, el nivel de N amoniacal ascendió de forma constante en SB durante todo el proceso de ensilado, siendo superior a SA y PA debido a una fermentación más intensa que ocasionó una ligera proteólisis, pero sin grandes consecuencias sobre el contenido de nutrientes del silo (Meneses *et al.*, 2007). El N amoniacal también se incrementó en PA y SA hasta el día 60, punto a partir del cual descendió.

5.1.4. Evaluación de residuos de fitosanitarios y análisis de costes de fabricación

Los compuestos fitosanitarios no excedieron los LMRs fijados en la legislación europea, por lo que estos ensilados no entrañan ningún tipo de riesgo para la salud de los animales o las personas que ingieren los productos de estos animales.

Por otra parte, el coste de los tres ensilados es menor que el de otros ingredientes típicos de las raciones de rumiantes, como por ejemplo la alfalfa o la cebada, cuyos precios en la semana 17 de 2020 de la Lonja de Albacete eran de 1,36 y 1,63 €/kg PB, mientras que los de los ensilados de este estudio fueron de 0,718, 0,903 y 1,01 €/kg PB para SB, SA y PA, respectivamente.

5.2. Grado de aceptación y preferencia de cabras Murciano-Granadinas por los ensilados de subproducto de brócoli y subproducto y planta de alcachofa.

Este experimento se basó en estudiar la preferencia de las cabras entre cuatro alimentos alternativos ensilados, estableciendo la relación entre la preferencia de cada alimento con su composición, mediante la toma de datos de la ingestión de alimento en una prueba a corto plazo.

Las cabras mostraron mayor preferencia por SA y PA que por SBSA, dado por los resultados de de ingestión acumulada (113, 73,9 y 23,8 g de MS en 4 h, respectivamente). Esta preferencia se determinó por los coeficientes de correlación obtenidos entre el nivel de ingestión de alimento y su composición. Variables como el pH ($r = 0,24$), el contenido en azúcares ($r = 0,47$), LAD ($r = -0,54$), EM ($r = 0,57$) y ácido láctico ($r = 0,56$) mostraron coeficientes de correlación moderados y elevados con la ingestión de alimento, demostrando así que la ingestión de alimento es un buen indicador de la calidad de los ensilados. Se observó también que tanto el ácido láctico como el contenido en PB mostraron una relación positiva con la ingestión de alimento, dado que fueron las variables que mejor definían la ecuación de regresión que mejor se adaptaba a la preferencia de los alimentos ($R^2=0,4833$). Esto se corresponde con que el ácido láctico sea el ácido más eficaz en reducir el pH del silo y, por tanto, el principal indicador de calidad del alimento ensilado (Madrid *et al.*, 1999), aportándole además un olor agradable; y a que el contenido en PB del alimento deba ser el adecuado (superior al 7%) para asegurar una correcta actividad microbiana en la fermentación ruminal de los carbohidratos y, de esta forma, la obtención de energía para el metabolismo de los rumiantes (Rogisic *et al.*, 2006).

Contenidos altos en FND, FAD y especialmente LAD redujeron la ingestión dado que el alimento es más duro y difícil de rumiar (Sandoval-Castro *et al.*, 2005), como ocurrió en el caso de SBSA y C. Algunos de los AGV de los ensilados, como el ácido propiónico y butírico, resultado de un proceso heterofermentativo como el que tuvo lugar en SBSA, también afectaron de forma negativa a la ingestión, debido a que reducen la palatabilidad del alimento (Watson y Smith, 1951). Este menor consumo del silo SBSA también pudo deberse a la presencia de sulfóxido de S-metilcisteína (no analizado), el cual es un factor anti-nutricional característico de la familia de las Brassicáceas (de Ruiter *et al.*, 2009).

Pese a la mayor preferencia mostrada por SA y PA que por SBSA, las cabras no mostraron un rechazo total por ninguno de los ensilados, indicando con ello que los tres alimentos pueden ser incorporados en la dieta de estos animales.

5.3. Efecto de varios niveles de inclusión de ensilados de SB, SA y PA en la dieta de cabras Murciano-Granadinas a corto plazo.

En estos estudios se evaluó el efecto de la inclusión de los ensilados de subproducto de brócoli y alcachofa y planta de alcachofa en distintos niveles (12,5 y 25% de SA y PA en el Estudio 3 y 25, 40 y 60% de PA y SB en el Estudio 4) en las raciones de cabras lecheras sobre la producción, composición y perfiles mineral y lipídico de la leche y estado sanitario de los animales a corto plazo (entre uno y dos meses). El objetivo último de los estudios fue determinar el nivel óptimo de inclusión de los ensilados de estos alimentos alternativos en la dieta de cabras lecheras.

5.3.1. Ingestión y peso vivo

La inclusión de un 12,5% y 25% de SA y PA no afectó al consumo de alimento, aunque con un 25% de este ensilado se redujo ligeramente el peso de los animales (47,8 y 48,1 kg) en comparación a aquellos alimentados con una dieta testigo (49,8 kg), pero sin salirse del rango normal para la raza Murciano-Granadina (MAPA, 2018). La reducción del PV fue mayor cuanto más cantidad de subproducto incluía la dieta, siendo con un 60% de SB donde más se notó este efecto, dado que fue con este tratamiento, además del que incluía un 40% de SB, donde la ingestión de alimento fue menor (1,27 y 1,38 kg MS/día para SB60 y SB40, respectivamente frente a los 2,21 kg MS/día del lote testigo). Las causas del menor consumo de alimento de las dietas que incluían mayor cantidad de SB fueron el mayor volumen de la ración, producido por un alto contenido en humedad del ensilado SB (15,4 %) (Jackson y Forbes, 1970) y la mayor proporción de metabolitos de fermentación del ensilado de las dietas SB40 y SB60, principalmente ácido propiónico y amoníaco, los cuales tienen un efecto depresivo sobre la ingestión de alimento (Baumont, 1996). En relación al amoníaco, este es metabolizado en el hígado y transformado en ácido γ -aminobutírico, que tiene un efecto saciante (Scherer *et al.*, 2015).

5.3.2. Producción, macrocomposición y recuento de células somáticas

En el Estudio 3 no se observaron diferencias entre el tratamiento testigo y el que incluía un 12,5 de PA y SA en relación a la producción de leche y macrocomposición, asegurando de este modo la buena calidad de los ensilados. Al incrementar el nivel de inclusión de SA en la dieta, se produjo un incremento del contenido graso de la leche (5,35%), así como el ESU y los ST (9,62 y 14,4%). PA25 del Estudio 3 mostró un contenido superior de proteína en la leche (4,36%) pudiendo estar explicado por un mayor contenido de PT en la dieta (3,55 g/kg MS), los cuales tienen la capacidad de formar complejos con la proteína dietaria, reduciendo su solubilidad en el medio ruminal y, por tanto, su digestibilidad por la microbiota del rumen, lo cual favorece una mayor tasa de pasaje de PB al intestino delgado (Patra y Saxena *et al.*, 2011).

Debido a la reducción del consumo de alimento en aquellos tratamientos que incluían más subproducto en el Estudio 4, se redujo la producción de leche y la de LCG. PA25 y PA40 fueron los que presentaron mayor producción de leche (2,15 y 2,14 kg/día, respectivamente), sin diferencias con T (2,24 kg/día), y SB60 el que menos (1,66 kg/día). Por el contrario, este último tratamiento presentó el mayor contenido de grasa en leche (4,58%), así como con un nivel mayor de MS y ESU (12,9 y 8,01%), debido a un efecto de concentración y a que la dieta SB60 contenía el triple de ácido acético (37,8 g/kg MS) que el resto de dietas, que es un precursor de la síntesis de grasa. Se encontraron diferencias en el contenido de urea en leche, aunque los valores de los siete tratamientos permanecieron dentro del rango normal para la especie caprina (500-700 mg/L; LILCAM). PA60 fue el tratamiento que presentó mayor nivel y SB60 el menor

(641 y 542 mg/L), debido al menor consumo de alimento que experimentaron los animales de este tratamiento. Ninguna de las dietas afectó al RCS, el cual se situó en valores normales.

5.3.3. Perfil mineral de la leche

Las diferencias halladas en el perfil mineral de la leche entre los distintos tratamientos son de tan escasa magnitud que se consideran biológicamente irrelevantes, lo cual es importante desde el punto de vista de la aptitud tecnológica de la leche, dada la relevancia del Ca y P en la formación y desarrollo de la microestructura del queso (Pastorino *et al.*, 2003). En el Estudio 3, los tratamientos que incluían 12,5 y 25% de PA y un 25% de SA mostraron un nivel significativamente inferior de Cu en leche, en comparación a T. También se redujo ligeramente el contenido en Se de la leche con la dieta que incluía un 25% de SA. El menor contenido en Cu obtenido con las dietas que incluían PA en el Estudio 3 se mantuvo en el Estudio 4 entre PA25 y T (0,538 y 0,697 mg/kg MS), aunque sin que fuera una diferencia significativa. Sin embargo, sí que lo fue el mayor contenido en Mn de la leche de PA40 (0,233 mg/kg MS). Respecto a los tratamientos con SB, no se observaron diferencias significativas con T.

5.3.4. Perfil lipídico de la leche

En el Estudio 3, SA mostró menor contenido en PUFA y ácidos n3 y mayor ratio n6/n3 que T, cuando el nivel de inclusión fue de 12,5%. Por otro lado, mientras que en el Estudio 3 la leche del tratamiento que incluía un 12,5% de PA presentó menor contenido en ácidos grasos n3 (0,361%) y mayor ratio n6/n3 (11,03) que T, en el Estudio 4 el tratamiento que incluía mayor cantidad de PA (PA60) mostró mejores valores que T, esto es más n3 (0,275%) y menor n6/n3 (10,3), lo que conlleva una mejor calidad del perfil graso de la leche en términos de prevención de enfermedades cardiovasculares (Hu, 2002). SB60 también presentó uno de los ratios de n6/n3 más bajo (12,3).

Por otra parte, los tratamientos con PA obtuvieron mayor concentración de ácido vaccénico en leche que los de SB. Además, se vio que cuanto mayor era el nivel de inclusión de PA en la dieta, mayor era también el contenido de ácido ruménico y PUFA en leche. Esto se debió a que las dietas con PA contenían mayor cantidad de PUFA y de PT, los cuales tienen un efecto inhibitorio en la biohidrogenación de los ácidos grasos en el rumen (Correddu *et al.*, 2019). Pese a que la dieta SB60 fue la que mayor contenido en PT presentó, no se observó un efecto en la leche debido al bajo consumo que se dio entre las cabras de este tratamiento.

Debido al mayor contenido en PUFA y de n3 y menores niveles de C12:0, C14:0, C16:0 y C18:0, PA40 y PA60 obtuvieron menores IA (1,95 y 1,83) e IT (3,09 y 2,94), lo cual es un punto a favor para este subproducto desde el punto de vista de la prevención de enfermedades cardiovasculares (Molina-Alcaide *et al.*, 2010).

5.3.5. Perfil de metabolitos plasmáticos

La inclusión de un 12,5 y 25% de SA en la dieta dio lugar a una reducción del contenido de urea plasmática en el Estudio 3 debido al ligeramente superior contenido de PT de estas dietas, lo cual redujo la degradación de la proteína dietaria en el rumen, liberando con ello menos amoníaco y reduciendo la síntesis de urea en el hígado (McMahon *et al.*, 2000), pero sin perjudicar al contenido proteico de la leche.

Pese a las diferencias halladas en el perfil de metabolitos plasmáticos del Estudio 4, los valores plasmáticos se encontraron dentro de un rango normal para cabras (Rivas *et al.*, 2014), excepto en el caso de la urea de SB60 (33,2 mg/dL), la cual se mostró en un nivel inferior al recomendado

debido a lo anteriormente expuesto con la urea en leche. T y los tratamientos que incluían PA obtuvieron los valores más altos. Se observó que los animales de los tratamientos que incluían mayor cantidad de SB, como SB40 y SB60, presentaron un nivel más bajo de BHB (0,299 y 0,304 mmol/L). Esto se debe a la respuesta adaptativa de las cabras lecheras, las cuales adaptaron su producción de leche al menor consumo de alimento que tuvo lugar con estos tratamientos, de forma que no se experimentó una movilización de reservas elevada (Friggens *et al.*, 2016).

5.4. Efecto de la inclusión de un 40% de ensilado de SB y PA en dietas de cabras Murciano-Granadinas durante una lactación completa.

Con este estudio se determinó el efecto de la inclusión de un 40% de ensilado de subproducto de brócoli y planta de alcachofa en la ración de cabras lecheras durante una lactación completa (largo plazo) sobre la producción, composición y calidad de la leche y el estado sanitario de los animales.

5.4.1. Ingestión y peso vivo

El consumo de alimento fue mayor en T (1,98 kg MS/día), seguido de cerca por PA (1,82 kg MS/día), mientras que el de SB fue considerablemente inferior (1,59 kg MS/día), debido a una mayor concentración de productos de fermentación, como ácido acético, etanol y N amoniacal, en esta dieta, los cuales tienen un efecto depresivo sobre la ingestión de alimento (Huhtanen *et al.*, 2004). Al igual que ocurrió en el Estudio 4, el elevado contenido en humedad de la dieta SB también influyó en que esta dieta fuera más voluminosa que T y PA, afectando negativamente al consumo de alimento. Otra de las causas por las que la ingestión de SB fue menor a la de los otros lotes fue el mayor contenido en PT de la dieta con SB, lo cuales reducen la palatabilidad del alimento (Decandia *et al.*, 2000). Los tres lotes redujeron la ingestión a partir de la mitad de la lactación debido al progreso de la lactación, por el cual la producción se reduce y, de ese modo, la ingestión de alimento también. En cuanto al PV medio, T y PA no presentaron diferencias. Solo SB mostró un PV medio inferior a T (41,5 vs. 43,5 kg) explicado por el menor consumo de alimento experimentado por las cabras. Mientras que el PV de PA se mantuvo estable durante toda la lactación, el de T se incrementó a medida que transcurría esta, conforme se reducían las necesidades energéticas para la producción de leche.

5.4.2. Producción, macrocomposición y recuento de células somáticas

La producción de leche fue superior en PA que en SB (2,21 y 1,91 kg/día), sin diferencias con T (2,11 kg/día). Con el transcurso de la lactación, la producción lechera fue decayendo en los tres tratamientos, reduciéndose a su vez las diferencias de producción entre lotes. LCG de T y PA también se disminuyó a medida que avanzaba la lactación, al contrario que la de SB, que se mantuvo estable.

En cuanto a la macrocomposición, SB mostró mayor contenido en grasa, en ST y ESU (5,02, 13,9 y 8,39%, respectivamente) debido a su menor producción lechera. Este hecho también se debió a un mayor contenido en ácido acético en la dieta SB (9,50 g/kg MS), el cual actúa como precursor en la síntesis *de novo* de grasa en la glándula mamaria (Safayi y Nielsen, 2013), y a un nivel superior de NEFA en sangre (0,856 mmol/L), resultado de una mayor movilización de reservas en este grupo (Criscioni y Fernández, 2016). Aunque PA también mostró un nivel similar de NEFA al de SB (0,868 mmol/L), la mayor producción de leche obtenida por el lote PA tuvo un efecto de dilución sobre el contenido graso. De forma inversa a la producción de leche, el

contenido en grasa y proteína fue incrementándose en los tres tratamientos a medida que avanzaba la lactación. T y PA presentaron mayores contenidos en proteína (3,55 y 3,42%), proteína verdadera (3,29 y 3,18%) y proteína del suero (0,431 y 0,4045). También fueron los tratamientos con mayor nivel de urea en leche (797 y 793 mg/L).

El RCS se incrementó en los tres lotes conforme la lactación progresaba, mostrando T un nivel superior a SB y PA (5,73 vs. 5,55 y 5,53 Log₁₀ células/mL, respectivamente) y estando los valores de los tres tratamientos dentro del rango normal para cabras Murciano-Granadinas con buen estado de salud de la glándula mamaria.

5.4.3. Perfil mineral de la leche

Las diferencias halladas entre tratamientos fueron de escasa magnitud. T presentó unos contenidos superiores de Na y Se en leche (378 mg/kg y 17,2 µg/kg), mientras que SB fue el tratamiento con mayor nivel de Ca (1267 mg/kg) debido a que la dieta SB también tenía mayor contenido en Ca que la de los otros dos tratamientos. En cuanto al Mn, T y SB mostraron una concentración mayor que PA (46,5 y 43,3 vs. 31,2 µg/kg). A medida que avanzaba la lactación se incrementaron los niveles de Na, Mg, P, S, Ca, Mn, Fe y Se en los tres tratamientos.

5.4.4. Perfil lipídico de la leche

Las principales diferencias en el perfil de ácidos grasos se dieron en los contenidos de C15:0, C16:1cis9 y C17:0, así como en el cómputo total de OBCFA, en los que SB y PA mostraron un contenido superior a T por una mayor movilización de reservas corporales al inicio de la lactación y un mayor contenido de FND en la dieta en el caso de PA (Patel *et al.*, 2013). A partir de la semana 17 de lactación, PA presentó un mejor perfil lipídico desde el punto de salud humana, ya que se incrementó la concentración de C18:3n3 y se redujo el ratio de ácidos grasos n6/n3, lo cual aporta un valor añadido a la leche de las cabras alimentadas con el silo PA debido a la alta demanda de productos saludables para el consumo humano.

SB fue el tratamiento que presentó peores resultados en el perfil lipídico, con menor sumatorio de CLAs (0,547%), mayor ratio SFA/UFA (2,33) y mayores IA e IT (2,29 y 3,39). También mostró menores niveles de ácido linoleico, vaccénico y ruménico porque la dieta SB contenía la menor concentración en precursores de estos ácidos, como linoleico y α-linolénico (Vlaemink *et al.*, 2006).

5.4.5. Perfil de metabolitos plasmáticos

Respecto a la glucosa, los niveles de este metabolito fueron superiores en el inicio de lactación debido a la glucogénesis intensa que tiene lugar en este momento, proceso que posibilita a los animales alcanzar el pico de lactación pese a la reducida capacidad de ingestión que caracteriza este estado de la curva de lactación (Grummer, 1995). El incremento de glucosa en SB al final del experimento también puede explicarse con la glucogénesis, dado el reducido consumo de alimento que los animales de este lote mostraron, así como al mayor contenido de PT de la dieta SB (4,93 g/kg MS), los cuales mejoran el catabolismo de carbohidratos (Zhong *et al.*, 2011).

Por otro lado, SB y PA fueron los tratamientos que presentaron mayor nivel de NEFA (0,856 y 0,868 mmol/L) debido a la movilización de reservas por el menor consumo de alimento en estos tratamientos (Correddu *et al.*, 2020). El nivel de NEFA de SB y PA se redujo hasta alcanzar niveles similares a T en la semana 22 de lactación, cuando la cantidad de alimento ingerida se ajustó mejor a la producción de leche de los animales, como ocurrió en Ríos *et al.* (2006). Respecto al nivel de BHB, SB mostró un valor inferior (0,356 mmol/L) al de los otros dos tratamientos (0,456

y 0,450 mmol/L respectivamente para T y PA), siendo la causa de esta discrepancia con el nivel de NEFA la mayor disponibilidad de glucosa de SB (51,2 mg/dL), la cual es utilizada en el catabolismo de NEFA para producir energía (Rivas *et al.*, 2014). T presentó un nivel de colesterol mayor que el de SB y PA (101 vs. 91,9 y 86,1 mg/dL, respectivamente). El nivel de colesterol se redujo a medida que descendía la producción de leche con el avance de la lactación, salvo en T a partir de la semana 17 debido a un exceso de energía ingerida en la dieta, al igual que ocurrió con el PV en esta etapa de la lactación. Sin embargo, pese a las diferencias halladas, los valores de colesterol de los tres tratamientos se encuentran dentro de los valores normales (70-240 mg/dL; Merck *et al.*, 1993).

En cuanto a la concentración de urea plasmática, T y PA obtuvieron valores superiores a SB (45,7 y 44,0 vs. 40,4 mg/dL) debido al déficit de ingestión proteica de la dieta SB y a su mayor contenido en PT. Al final de la lactación, el nivel de urea se incrementó especialmente en T debido, probablemente, a un consumo de alimento superior a las necesidades de los animales.

*FE DE ERRATAS

En el artículo “*Long-Term Feeding of Dairy Goats with Broccoli By-Product and Artichoke Silages: Milk Yield, Quality and Composition*” falta añadir la palabra Plant en el título (*Long-Term Feeding of Dairy Goats with Broccoli By-Product and Artichoke PLANT Silages: Milk Yield, Quality and Composition*). Además, en *Table 6*, faltan los datos referentes a los Índices de Aterogenicidad y Trombogenicidad y los Índices de Desaturación:

| Variable. | n | Diets | | | | Significance | | |
|-----------|----|--------|--------|--------|-------|--------------|------|-------------|
| | | C | BB | AP | SEM | Diet | Week | Diet × Week |
| AI | 15 | 2.02c | 2.29a | 2.12b | 0.015 | *** | *** | *** |
| TI | 15 | 3.05b | 3.39a | 3.04b | 0.016 | *** | *** | *** |
| DI C14:0 | 15 | 0.015c | 0.016b | 0.017a | 0.000 | *** | *** | *** |
| DI C16:0 | 15 | 0.048b | 0.046c | 0.051a | 0.001 | *** | *** | *** |
| DI C18:0 | 15 | 1.84b | 1.85b | 2.12a | 0.010 | *** | *** | ** |

Estos cambios han sido comunicados a la revista *Animals* (MDPI) y estamos a la espera de que publiquen la versión corregida del artículo.



6

Conclusiones Conclusions

6. Conclusiones

La estabilización de los ensilados de subproducto de brócoli y subproducto y planta de alcachofa tuvo lugar en el día 30 de ensilado y la calidad de los ensilados se mantuvo alta hasta el día 200. Por ello, el ensilado de estos subproductos en silos comerciales formato microsilo de 300 kg es una técnica viable que permite la conservación de los subproductos durante largos periodos de tiempo, permitiendo así la desestacionalización del subproducto.

Las cabras mostraron mayor preferencia por los ensilados de subproducto y planta de alcachofa que por el que incluía subproducto de brócoli y brácteas de alcachofa debido a que los primeros contenían más ácido láctico, el cual estimula la ingestión de alimento, y menor contenido en ácido butírico. Como ninguno de los tres ensilados fue rechazado por los animales, los tres pueden ser incluidos en la ración ganadera.

La utilización de los ensilados de subproducto de brócoli y subproducto y planta de alcachofa en niveles de inclusión de 12,5, 25 y 40% en la dieta de cabras lecheras no causó a corto plazo grandes diferencias en el rendimiento lechero y estado sanitario de los animales. Desde el punto de vista de la calidad nutricional para el ser humano, el uso del ensilado de planta de alcachofa mejoró el perfil lipídico de la leche. Sin embargo, la inclusión de un 60% de estos ensilados en la dieta de los animales redujo considerablemente la ingestión de alimento, así como el peso vivo y la producción de leche.

Finalmente, la utilización de un 40% de ensilado de estos subproductos a largo plazo (una lactación completa) causó diferentes resultados dependiendo del subproducto. Mientras que con la planta de alcachofa no se produjeron grandes diferencias en cuanto a la producción y calidad de la leche y estado sanitario de los animales en comparación a una dieta convencional, con el subproducto de brócoli se redujo el consumo de alimento de tal forma que afectó negativamente a la producción de leche y a la recuperación de reservas al final de la lactación. Por otra parte, mientras que con la planta de alcachofa se mejoró ligeramente el perfil lipídico de la leche con respecto a la ración convencional, con el subproducto de brócoli se empeoró.

Por todo ello se concluye que el ensilado de planta de alcachofa puede ser incluido hasta un 40% en dietas equilibradas para caprino lechero durante una lactación completa, sin riesgo de afectar negativamente la producción y salud de los animales y, además, mejorando la calidad de la leche. El ensilado de subproducto de brócoli también puede ser utilizado con ese nivel de inclusión, pero durante un periodo corto de tiempo y una vez transcurrido el pico de lactación. Durante los mayores momentos de necesidades energéticas (inicio de lactación) es conveniente emplear niveles de inclusión menores que favorezcan el consumo de alimento.

6. Conclusions

The stabilisation of the broccoli by-product and the artichoke by-product and plant silages took place on day 30 of silage process and the quality of the silage remained high until day 200. Therefore, the silage of these by-products in commercial microsilos of 300 kg is a viable technique that allows the preservation of by-products for long periods of time and their availability throughout the year.

Goats showed a greater preference for artichoke plant and by-product silages than for silage that included broccoli by-product and artichoke bracts because artichoke silages had higher lactic acid content, which stimulates feed intake, and less butyric acid content. As neither of the three silages were rejected by the animals, both can be included in the livestock ration.

The use of broccoli by-product and artichoke plant and by-product silages at inclusion levels of 12.5, 25 and 40% in the diet of dairy goats did not cause great differences in the milk yield and health status of the animals in a short-term. From the point of view of nutritional quality for humans, the use of artichoke plant silage improved the milk lipid profile. However, the inclusion of 60% of those silages in the diet of the animals considerably reduced feed intake as well as body weight and milk yield.

Finally, the use of 40% silage of those by-products in a long-term (a full lactation) caused different results depending on the by-product. While with the artichoke plant there were no great differences in terms of the milk production and quality and the health status of the animals compared to a conventional diet, with the broccoli by-product the feed consumption was reduced in such a way that it negatively affected milk production and the recovery of reserves at the end of lactation. On the other hand, while with the artichoke plant the milk lipid profile was slightly improved in comparison to control ration, with the broccoli by-product it worsened.

Therefore, it is concluded that artichoke plant silage can be included up to 40% in balanced diets for dairy goats during a full lactation, without the risk of negatively affecting the production and health of the animals and, in addition, improving milk quality. Broccoli by-product silage can also be used at this level of inclusion, but for a short period of time and after peak lactation. During the greatest moments of energy needs (beginning of lactation) it is convenient to use lower inclusion levels that favour the feed consumption.



7

**Investigaciones
futuras**

7. Investigaciones futuras

Como consecuencia de los resultados obtenidos en la presente Tesis Doctoral, surge la necesidad de seguir profundizando en el estudio de los efectos de estos subproductos sobre otros factores de la producción animal. A continuación se detallan algunos con mayor interés:

- Estudiar los efectos de la inclusión del ensilado de subproducto de alcachofa con niveles superiores al 25% a corto plazo sobre el nivel productivo, sanitario y calidad de la leche de los animales, determinando de este modo el nivel óptimo. Posteriormente, realizar una prueba a largo plazo con el nivel que haya dado mejores resultados a corto plazo.
- Estudio de nuevas formas de conservación/presentación de estos subproductos, como el pelletizado en el caso del subproducto de brócoli, que mejore la aceptación de los animales y estimule un mayor consumo de alimento.
- Determinación de los efectos de las dietas que incluyen estos subproductos a nivel ruminal mediante técnicas in vitro o in vivo.
- Caracterización de la dinámica de producción de gas mediante ensayos in vitro que permitan cuantificar la producción de gases de efecto invernadero.

8

Referencias

8. Referencias

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