Universitat Autònoma de Barcelona

Valoración de nuevas variedades de cebada en alimentación de rumiantes

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Assessment of new barley varieties in ruminant feeding

TESIS DOCTORAL

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Assessment of new barley varieties in ruminant feeding

Tesis presentada por **Santiago Alexander Guamán Rivera** y dirigida por los doctores Elena Albanell Trullás y Gerardo Caja López, del Departament de Ciència Animal i dels Aliments de la Universitat Autònoma de Barcelona

Bellaterra, 13 de Junio de 2022

Elena Albanell Trullás catedrática de universidad **y Gerardo Caja López** profesor emérito, del Departament de Ciència Animal i dels Aliments de la Universitat Autònoma de Barcelona.

Certifican:

Que la memoria titulada "**Valoración de nuevas variedades de cebada en alimentación de rumiantes**", presentada por **Santiago Alexander Guamán Rivera** para optar al grado de Doctor en Producción Animal, ha estado realizada bajo su dirección, y considerándola finalizada, autorizan su presentación para que sea juzgada por la comisión correspondiente.

Y para que conste a los efectos oportunos, firman la presente a Bellaterra (Cerdanyola del Vallès), a 13 de Junio de 2022.

Dra. Elena Albanell Trullás Dr. Gerardo Caja López

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A Dios por concederme salud y proteger mi hogar y a mi familia por el apoyo incondicional

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Santiago Alexander Guamán Rivera

LISTA DE ABREVIATURAS

ADF Acid detergent fiber

ADL Acid detergent lignin

BCS Body condition score

BG β-glucans

BW Body weight

CF Crude fiber

CON Control

CP Crude protein

CPd Crude protein digestibility

DE Digestible energy

DIM Days in milk

DM Dry matter

DMd Dry matter digestibility

DMI Dry matter intake

GE Gross energy

HBB High β-glucans barley

IL-1α Anti-inflammatory cytokine

IL-1β Pro-inflammatory cytokine

INP Intraperitoneally injected

LC Lacaune breed

LPS Lipopolysaccharide

ME Metabolizable energy

MN Manchega breed

NDF Neutral detergent fiber

NDFd Neutral detergent fiber digestibility

NDFI Neutral detergent fiber intake

NEL Net energy for lactation

NFE Nitrogen-free extract

OM Organic matter

OMd Organic matter digestibility

OMI Organic matter intake

PAMPs Pathogen associated molecular patterns

PDI Protein digestible in the intestine from dietary and microbial origin

PDIA Protein digestible in the intestine from dietary origin

PRR Pattern recognition receptors

RFV Relative feed value

RPB Rumen protein balance

RT Rectal temperatures

SCC Somatic cell count

SEM Standard error of the mean

sFV Sheep fill value

UFL Feed units for lactation

RESUMEN

Valoración de nuevas variedades de cebada en alimentación de rumiantes

Esta tesis evaluó el potencial nutricional y las propiedades funcionales de nuevas variedades de cebada. Para ello, se valoró una nueva variedad de cebada capuchona (Exp. 1) para forraje y una nueva variedad de cebada para grano con alto contenido en beta-glucanos (BG) (Exp. 2). En la primera experiencia, se compararon los forrajes de la cebada capuchona cv. Mochona y de un triticale comercial (cv. Titania) en dos años consecutivos (2016 y 2017), que se sembraron (0,75 ha de cada cultivo), cosecharon y procesaron para heno y ensilado. El consumo voluntario y digestibilidad se determinó con 32 ovejas secas alojadas en jaulas metabólicas durante un periodo de adaptación (15 d) y de medición y muestreo (5 d). Los resultados mostraron diferencias en la composición química entre años (P < 0,003 a 0,001), probablemente debido a condiciones meteorológicas, excepto en los contenidos de FAD de los henos (P = 0,20) y de PB (P = 0,18) de los ensilados. La especie vegetal condicionó la composición química de ambos forrajes (P < 0,001), excepto para la PB de henos y ensilados (P = 0,25 a 0,61), así como para la FAD en henos (P = 0,12). La ingestión de la MS de los forrajes fue similar entre años (P = 0,24 a 0,47) y especies (P = 0,10 a 0,70). Sin embargo, el heno de triticale presentó mayor digestibilidad de MO que la cebada, aunque sin diferencias entre ensilados (P = 0.36). Consecuentemente, los henos de cebada tuvieron menor valor energético que los de triticale (P < 0,001 a 0,014), pero los ensilados mostraron valores superiores de PDIA (P = 0,020) y RPB (P = 0,010). Para la segunda experiencia, en la que se valoraron cebadas grano de distintos contenidos de BG, se utilizaron 36 ovejas de dos razas lecheras (Manchega, n = 18; Lacaune, n = 18) al final de lactación. Las ovejas fueron sometidas a un periodo de adaptación (10 d), en el que recibieron heno de alfalfa y 350 g/d de grano de cebada cv. Meseta (3,8% BG), y se distribuyeron en 3 grupos homogéneos que recibieron los tratamientos: (i) CON, misma suplementación que en el periodo de adaptación (13,3 g BG/d); (ii) HBB, suplementadas con una nueva variedad de cebada (cv. Annapurna) con alto contenido en BG (10% BG, 35 g BG/d); y (iii) INP, misma suplementación que el grupo CON, pero a las ovejas se les inyectó por vía intraperitoneal una única dosis de una solución con 1,4% BG (2 g BG/oveja). A los 9 d de aplicados los tratamientos, se realizó un desafío LPS, para lo que a todas las ovejas se les infundió una dosis de 1 mL de solución de *E. coli* (O55:B5) (5 μg/mL) en media ubre, mientras que recibieron 1 mL de suero fisiológico en la otra media ubre. La temperatura rectal se midió durante toda la experiencia y a los 4 d del desafío LPS se tomaron muestras de leche y sangre para determinar la concentración de citoquinas. La producción de leche disminuyó un 38% en las ovejas INP (P = 0,006) pero no se observaron diferencias entre CON y HBB (P = 0,71). A pesar de ello, se observó una menor concentración de IL-1β pro-inflamatoria en las ovejas INP (P = 0,06 a 0,09), respecto a CON y HBB (P = 0,99). El desafío con LPS, desencadenó un mayor incremento de la temperatura rectal en CON y HBB (P = 0,043), siendo menor para INP (P = 0,27 a 0,32). La producción de leche disminuyó por efecto del LPS (P = 0,019), pero no hubo diferencias por efecto de los tratamientos de BG (P = 0,29). Por lo tanto, las ovejas de los tratamientos HBB y INP mostraron numéricamente menor concentración de citoquinas pro y anti-inflamatorias que las CON. Como conclusión, a partir de los resultados obtenidos se puede afirmar que la cebada capuchona (cv. Mochona), mostró una calidad nutritiva similar a la del triticale y superior a la establecida para cebadas convencionales. Además, los beta-glucanos de cebada, incluidos en los granos o extraídos como componentes funcionales, podrían reforzar la respuesta inmunitaria y contribuir a reducir el uso de aditivos antimicrobianos en la producción de rumiantes.

RESUM

Valoració de noves varietats d'ordi en alimentació de remugants

En aquesta tesi es va avaluar el potencial nutricional i les propietats funcionals de noves varietats d'ordi. Per a això, es va valorar una nova varietat d'ordi caputxona (Exp. 1) per a farratge i una nova varietat d'ordi per a gra amb alt contingut en beta-glucans (BG) (Exp. 2). En la primera experiència, es va comparar el farratge d'un ordi caputxona (cv. Mochona) amb un triticale comercial (cv. Titania), en dos anys consecutius (2016 i 2017), que es van sembrar (0,75 ha de cada cultiu), collir i processar per a fenc i ensitjat. El consum i digestibilitat es va determinar amb 32 ovelles seques allotjades en gàbies metabòliques durant un període d'adaptació (15 d) i de mesures i mostreig (5 d). Els resultats van mostrar diferències en la composició química entre collites (P < 0,003 a 0,001), probablement a causa de condicions meteorològiques, excepte en el contingut de FAD dels fencs (P = 0,20) i la PB (P = 0,18) dels ensitjats. L'espècie vegetal va condicionar la composició química de tots dos farratges (P < 0,001), excepte per a la PB de fencs i ensitjats (P = 0,25 a 0,61), així com per a la FAD en fencs (P = 0,12). La ingestió de la MS dels farratges va ser similar entre anys (P = 0,24 a 0,47) i espècies (P = 0,10 a 0,70). No obstant, el fenc de triticale va presentar major digestibilitat de la MO que l'ordi, encara que sense diferències entre ensitjats (P = 0,36). Conseqüentment, el fenc d'ordi va tenir menor valor energètic que el de triticale (P < 0,001 a 0,014), però els ensitjats van mostrar valors superiors de PDIA (P = 0,020) i RPB (P = 0,010). Per a la segona experiència, en la qual es va valorar ordi gra de diferents continguts de BG, es van utilitzar 36 ovelles de dues races lleteres (Manxega = 18; Lacaune = 18) al final de lactació. Les ovelles van ser sotmeses a un període d'adaptació (10 d), rebent fenc d'alfals i suplementades amb 350 g/d de gra d'ordi cv. Meseta (3,8% BG) i es van distribuir en 3 grups homogenis que van rebre els tractaments: (i) CON, mateixa suplementació que el període d'adaptació, 13,3 g BG/d; (ii) HBB, suplementades amb una nova varietat d'ordi (cv. Annapurna) amb alt contingut en BG (10% BG, 35 g BG/d); i (iii) INP, mateixa suplementació que el grup CON, però a les ovelles se'ls va injectar per via intraperitoneal una única dosi d'una solució amb 1,4% BG (2 g BG/ovella). Als 9 d d'aplicats els tractaments, es va realitzar un desafiament LPS, per al que a totes les ovelles se'ls va infondre, una dosi d'1 ml de solució d'*E. coli* (O55:B5) (5 μg/mL) en mig braguer, mentre que van rebre 1 mL de sèrum fisiològic en l'altre mig braguer. La temperatura rectal es va mesurar durant tota l'experiència i als 4 d del desafiament LPS es van prendre mostres de llet i sang per a determinar la concentració de citocines. La producció de llet va disminuir un 38% en les ovelles INP (P = 0,006) però no es van observar diferències entre CON i HBB (P = 0,71). Malgrat això, es va observar una menor concentració de IL-1β pro-inflamatòria en les ovelles INP (P = 0,06 a 0,09), respecte a CON i HBB (P = 0,99). El desafiament amb LPS, va desencadenar un major increment de la temperatura rectal en CON i HBB (P = 0,043), sent menor per a INP (P = 0,27 a 0,32). La producció de llet va disminuir per efecte del LPS (P = 0,019) però no va haver-hi diferències per efecte dels tractaments BG (P = 0,29). Per tant, les ovelles dels tractaments HBB i INP van mostrar numèricament menor concentració de citocines pro i anti-inflamatòries que les CON. Com a conclusió, a partir dels resultats obtinguts es pot afirmar que l'ordi caputxona (cv. Mochona), va mostrar una qualitat nutritiva similar a la del triticale i superior a l'establerta per a ordis convencionals. A més, els beta-glucans d'ordi, inclosos en els grans o extrets com a components funcionals, podrien reforçar la resposta immunitària i contribuir a reduir l'ús d'additius antimicrobians en la producció de remugants.

ABSTRACT

Assessment of new barley varieties in ruminant feeding

This thesis evaluated the nutritional potential and functional properties of new barley varieties. For that, a new variety of hooded barley (Exp. 1) and barley grain with high β-glucan (BG) contents (Exp. 2) were assessed. In the first experiment, hooded barley (cv. Mochona) and commercial triticale (cv. Titania) were compared during 2 consecutive years (2016 and 2017), which were sowed (0.75 ha each variety) and harvested and processed as hay and silage. Thirty-two dry ewes were allocated in metabolic cages during an adaptation period (15 d) and sampling (5 d) for dry matter intake (DMI) and apparent digestibility determination. There were differences in chemical composition between years ($P < 0.003$ to 0.001), probably due to the meteorological conditions, except for ADF ($P = 0.20$) in hays and CP ($P = 0.18$) in silages. The vegetal species conditioned the chemical composition of both forages ($P < 0.001$), except for CP of hay and silage ($P = 0.25$ to 0.61), as well as for ADF ($P = 0.12$) in hay. The DMI was similar between years ($P = 0.24$ to 0.47) and species ($P = 0.10$ to 0.70). However, triticale hay had a higher OM digestibility than barley, although no differences between silages ($P = 0.36$). Consequently, barley hays had lower energetic values than triticale ($P < 0.001$ to 0.014), but with greater PDIA ($P = 0.020$) and RPB ($P = 0.010$) as silages. For the second experiment, in which were evaluated grain barley with different BG contents. Thirty-six ewes of two breeds (Manchega, n = 18; Lacaune, n = 18) in late lactation were used. The ewes had an adaptation period (10 d) fed with alfalfa hay and supplemented 350 g/d of barley grain cv. Meseta (3.8% BG). Then, the ewes were distributed into 3 homogeneous groups and assigned the follow treatments: (i) CON, same supplementation as adaptation (13.3 BG/d); (ii) HBB, ewes supplemented with a new barley variety (cv. Annapurna) with 10% of BG content (35 g BG/d); and (iii) INP, fed as CON and intraperitoneally injected at d 1 with a unique dose of 1.4% BG solution (2g BG/ewe). After 9 d of treatment applications, a LPS challenge was done, all ewes in one udder half received 1 mL (5 μg/mL) from *E. coli* solution (O55:B5) meanwhile, 1 mL of saline solution was infused in the other. Rectal temperature was measured throughout the experiment, milk samples and blood for cytokine concentration were collected daily for 4-d after the challenge. The milk yield decreased by 38% ($P = 0.006$) in the INP ewes, but no differences were observed between CON and HBB ewes ($P = 0.71$). Nonetheless, the pro-inflammatory IL-1β was lower in INP ewes ($P = 0.06$ to 0.09) compared with CON and HBB ($P = 0.99$). The LPS challenge, triggered a higher rectal temperature in CON and HBB ($P = 0.043$), being mild in the INP ewes ($P = 0.27$ a 0.32). The milk yield decreased by LPS effect ($P = 0.019$), but there was no difference between BG treatments ($P = 0.29$). Therefore, the HBB and INP ewes showed lower pro- and anti-inflammatory cytokines than those from CON. In conclusion, based on the results obtained, it can be stated that the hooded barley (cv. Mochona) showed a similar nutritional quality to that of triticale and superior to that reported for conventional barley. In addition, barley β-glucans, included in the grains or extracted as functional components, could strengthen the immune response and help reduce the use of antimicrobial additives in ruminant production.

TABLA DE CONTENIDOS

CAPÍTULO 1

Revisión bibliográfica

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Revisión bibliográfica

1.1. Introducción general y motivación

La cebada *(Hordeum vulgare* L.*)* es un cereal utilizado, ya sea como forraje o grano, en muchas partes del mundo para la alimentación animal, así como para usos alimentarios humanos. En España, la cebada es el primer cultivo herbáceo extensivo que, debido a su mayor rusticidad y resistencia a la sequía, ocupa las superficies agrícolas de secano de menor pluviometría y muchos de los suelos calcáreos de peor calidad. Además, los sistemas agropastorales ovinos relacionados con el aprovechamiento de la cebada, tienen un peso importante en la España semi-árida y seca, así como en otros países de la cuenca Mediterránea (Correal y Sotomayor, 2011).

Bajo un escenario de lucha contra el cambio climático, el reto actual de los sistemas ganaderos es obtener productos de calidad, mediante el aprovechamiento sostenible y el manejo racional de los recursos naturales. En este sentido, el desarrollo experimentado por la agricultura mundial, y la puesta en práctica de técnicas modernas de mejora genética han permitido, de manera gradual, un importante aumento de la productividad con la obtención de nuevos cultivares de cebada. Dada la creciente preocupación de la población mundial por el cuidado de la salud, así como por la resistencia microbiana, el uso de compuestos bioactivos, entre los cuales destacan los derivados de la cebada, representa un interesante recurso alimenticio tanto para uso en humanos como animales. Estos aspectos han sido poco investigados en España, donde dada la importancia que tiene la cebada, podría darle un nuevo valor añadido.

En la República del Ecuador, la cebada fue introducida por los españoles (Jamieson y Sayre, 2010) y está asociada a comunidades indígenas locales, que la cultivan tradicionalmente entre 1.200-3.800 msnm como recurso alimentario humano y para pastoreo de pequeños rumiantes (Ponce-Molina et al., 2019). Así pues, nuevas variedades de cebada podrían también resultar de interés para el Ecuador y ser consideradas como alternativas a los cultivares locales de bajo rendimiento.

El doble interés para España y la Republica del Ecuador justifica la realización de esta tesis doctoral.

1.2. Cebada (*Hordeum vulgare* L.*)*

1.2.1. Historia y origen

Existen considerables pruebas históricas y arqueológicas que documentan el papel de la cebada como fuente de alimentación en la evolución de la humanidad. Newman y Newman (2008), revisaron retrospectivamente el desarrollo de la cebada como alimento junto con el desarrollo de las civilizaciones a través de los tiempos. En general, se acepta como un hecho que la transformación del cultivo de "cebada silvestre" en "cebada cultivada" se produjo a lo largo de muchos milenios. Aunque, el lugar o lugares exactos en los que se produjeron estos hechos ha sido un tema de controversia.

De acuerdo con Badr et al. (2000) y Grando y Macpherson (2005), la teoría más aceptada es que la cebada se originó hace unos 10.000 años en la zona de Oriente medio conocida como el creciente fértil (que abarca el actual Israel, el norte de Siria, el sur de Turquía, el este de Irak y el oeste de Irán). Además, evidencias arqueológicas indican que, desde el Oriente medio, la agricultura y el uso de la cebada se extendieron siguiendo las migraciones neolíticas y las rutas comerciales agrícolas, hasta el norte de África, el continente europeo y muchas partes de Asia (China, Japón, India) (Zapata et al., 2004; Newman y Newman, 2008). Aunque otras teorías proponen Etiopía, Tíbet-Nepal o la región mediterránea occidental como lugar de domesticación de la cebada. De todas maneras, evidencias científicas de los últimos años sugieren una hipótesis de origen multicéntrico para la cebada.

En España, la cebada junto con otras plantas domesticadas, aparecieron durante la expansión de la cultura neolítica hace unos 7.000 años (Blasco et al., 2006; Martínez-Moreno et al., 2017). Se presume que llegaron por tres posibles rutas: la primera por la costa valenciana desde el Mediterráneo (Araus et al., 2001), una segunda por los Pirineos y una última ruta por el norte de África por mar hasta el sur de España (Zapata et al., 2004; García-Martínez, 2015).

La cebada silvestre fue un alimento ampliamente utilizado por los seres humanos y clave en el desarrollo de muchas civilizaciones (por ejemplo, egipcia, griega, árabe y romana), incluso considerado como un alimento con virtudes medicinales. Es así que los gladiadores romanos la consumían habitualmente, ya sea en sopas o gachas porque pensaban que les proporciona fuerza, de ahí su nombre en latín de *"hordearii*" o "hombres cebada" (Grando y Macpherson, 2005; Stanca et al., 2016). Sin embargo, a medida que

otros cereales (por ejemplo, el trigo, centeno y la avena) fueron ganando importancia y se hicieron más abundantes, la cebada quedó relegada al estatus de "pan de pobres" (Newman y Newman, 2008).

1.2.2. Clasificación taxonómica

La cebada es una herbácea monocotiledónea de ciclo vegetativo anual, clasificada dentro de la familia de las Poaceae, tribu *Triticaeae* y al género *Hordeum*. A su vez, *Hordeum* consta de más de 40 especies, de las cuales casi tres cuartas partes son perennes, siendo la mayoría diploides (2n = 14), mientras que las restantes son tetraploides (2n = 28) y hexaploides (2n = 42) (Jacobsen y Bothmer, 1992). *H. vulgare ssp. vulgare* (cebada cultivada) es la especie más importante de este género.

1.2.3. Situación actual del cultivo

Según datos de la FAO (2022), la cebada se cultiva en más de 100 países, y representa el cuarto cereal en cuanto a superficie y producción mundial, además de acuerdo con la misma fuente, hoy en día, aproximadamente el 73% de la cosecha mundial de cebada se destina para la elaboración de piensos, un 21% la industria de la malta, cerveza y destilación, siendo solamente un 6% para consumo humano. Además, Tricase et al. (2018) remarcan un creciente interés de la cebada, como recurso utilizado en la generación de energías renovables como bio-combustible.

En la última década, Europa ha contribuido con aproximadamente el 62% de la producción mundial de cebada, mientras que Asia y América han representado el 15% y el 14%, respectivamente, como se ilustra en la Figura 1.1. A nivel de la Unión Europea y según datos de superficie de cebada cultivada durante la campaña de 2020, España ocupó el primer lugar, seguida de Francia y Alemania (Eurostat, 2021). Según el anuario de Estadística Agraria del Ministerio de Agricultura, Pesca y Alimentación (MAPA, 2022), a nivel español en el año 2020, la cebada fue el cultivo que ocupó más superficie agrícola ($2{,}7 \times 10^6$ ha). Además, de acuerdo con Martínez-Moreno et al. (2017) y De-Vega et al. (2018), aproximadamente en España el 90% de la superficie cultivada de cebada se destina para la producción de pienso, siendo el restante hacia la industria cervecera.

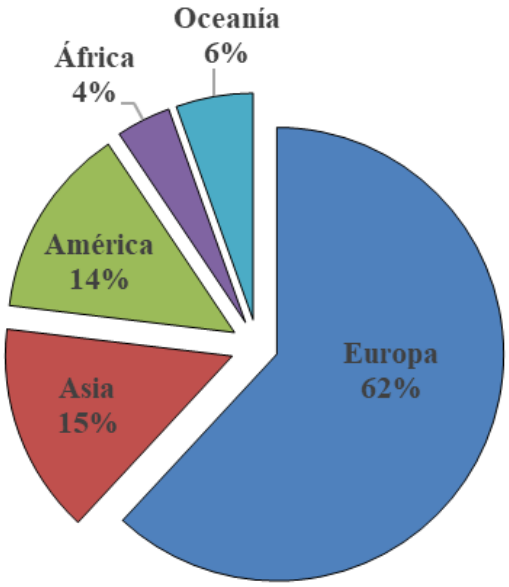

Figura. 1.1. Distribución porcentual de la producción mundial de cebada. Elaborado a partir de datos (FAOSTAT, 2022).

1.2.4. Tipos de cebadas

La cebada, debido a su alta variabilidad genética, se considera un cultivo modelo para la realización de estudios de mejora vegetal (Taner et al., 2004; Idehen et al., 2017). Además, de acuerdo con Schulte et al. (2009), su maduración temprana y su gran adaptabilidad a las condiciones de estrés (frío, sequía, suelos alcalinos y salinos) hacen que se pueda cultivar en todo el mundo, desde las regiones boreales hasta las ecuatoriales. Por lo tanto, dadas las peculiaridades que presenta el cultivo de cebada, los diferentes grupos de variedades pueden clasificarse según diversas características resumidas en la Figura 1.2.

En general, la cebada de 6 carreras (también denominada cebada caballar), se destina mayoritariamente para la alimentación animal, mientras que la de 2 carreras (cebada cervecera) para la producción de cerveza. De igual forma, las variedades de 6 carreras están asociadas a las siembras de otoño, mientras que las de 2 carreras mayoritariamente a finales de invierno, en siembras llamadas de primavera (Panizo-Casado, 2015). En la actualidad, se ha producido un claro incremento en la siembra de variedades de 2 carreras, independientemente de la época de siembra, que ha ido en detrimento de la superficie dedicada a la cebada de 6 carreras (López-Querol et al., 2016; MAPA, 2022).

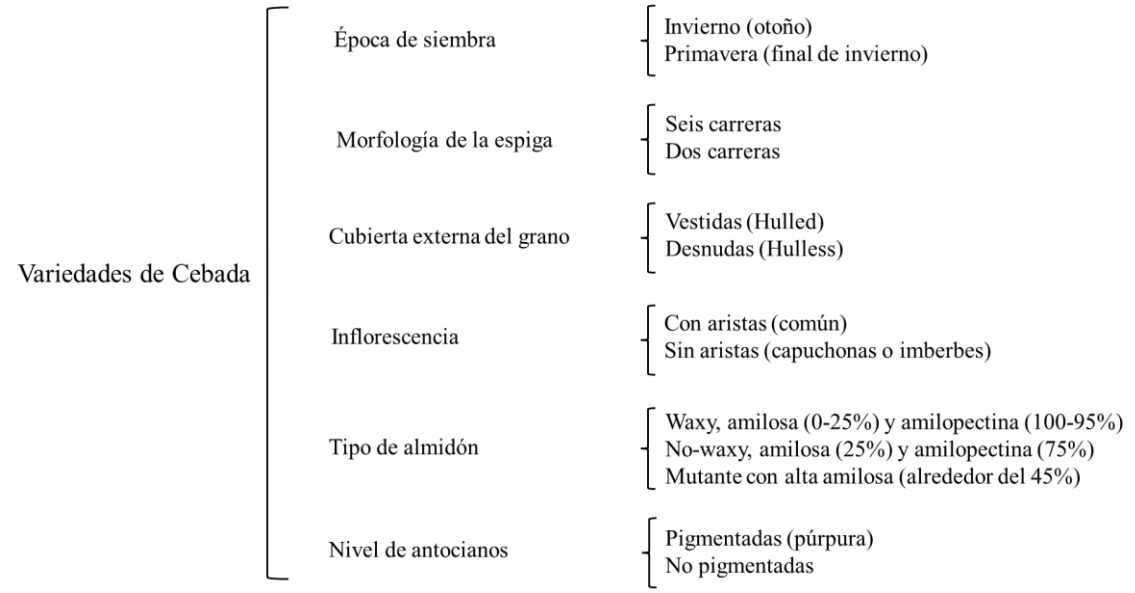

Figura 1.2. Clasificación de las variedades de acuerdo con sus características físicas y agronómicas (Elaboración propia).

En cuanto al aprovechamiento de la cebada, tiene gran interés tanto el grano como la planta entera (forraje). La cebada grano desempeña un importante rol en la industria ganadera y en la fabricación de piensos, por su valor energético y coste económico (Eskandari et al., 2009; Lenssen et al., 2015). En el caso de la cebada forrajera, por su mayor rusticidad y resistencia a la sequía, en los países del Mediterráneo ha ocupado tradicionalmente las superficies de secano de menor pluviometría y suelos de peor calidad (Francia et al., 2006; Stanca et al., 2016). En consecuencia, el alto potencial de producción y nutricional de nuevos cultivares de cebada podría proporcionar ventajas frente a otros cereales usualmente utilizados en la alimentación de rumiantes en condiciones similares a las mediterráneas españolas.

1.2.5. La nutrición animal clave en los sistemas de producción

En economías en desarrollo y debido al crecimiento de la población, el sector ganadero ha evolucionado como respuesta al rápido aumento de la demanda de productos de origen animal (Den-Hartog y Sijtsma, 2013). Estos productos ganaderos proporcionan

el 17% del consumo mundial de kcal y el 33% de proteínas (Rosegrant et al., 2009). Además, se estima que la demanda de productos ganaderos se duplicará en el año 2050 (Rojas-Downing et al., 2017).

La Agenda 2030 propuesta por las Naciones Unidas, propone 17 objetivos de desarrollo sostenible (ODS), para orientar y coordinar políticas en la búsqueda de la equidad y la sostenibilidad así como erradicar la pobreza (ONU, 2020). Aunque la ganadería se relaciona con varios de los ODS, seis de ellos son especialmente relevantes para el sector: ODS-1 (fin pobreza), ODS-2 (hambre cero), ODS-12 (producción y consumo responsable), ODS-13 (acción por el clima), ODS-15 (vida y ecosistemas terrestres) y ODS-17 (alianzas). En consecuencia, la estimación precisa del valor energético y proteico de los recursos alimentarios utilizados en la industria ganadera, es fundamental para obtener sistemas eficientes de producción (Kański et al., 2013).

Según Sauvant y Nozière, (2016, 2018) los sistemas de valoración de alimentos son un factor esencial de progreso para el sector ganadero y han favorecido los avances en investigación sobre la nutrición de rumiantes. Es por ello que la mayoría de los sistemas de valoración de alimentos actuales (NASEM, INRA, DVE/OEB, NorFor, AFRC) tienen un enfoque mecanicista que busca mejorar el rendimiento de los animales, la calidad de los productos, la eficiencia en el uso de los piensos y forrajes, al mismo tiempo tienen en cuenta el impacto medioambiental al reducir la excreción de nitrógeno y las emisiones de metano (Arias et al., 2020).

En este sentido, la gran demanda de energía por parte de los rumiantes de alta producción, requiere una determinación precisa de la energía disponible en las diferentes fuentes utilizadas para la formulación en los programas de alimentación (Weiss, 1993; McDonald et al., 2010). En el sistema INRA (2018), el contenido de energía se expresa en unidades forrajeras leche (UFL) que corresponden a 1,76 Mcal y, de acuerdo con diferentes autores, éste es el principal factor que afecta al rendimiento y composición de leche en los rumiantes (Caja y Bocquier, 2000; Coleman y Moore, 2003).

Como ya se ha comentado en el apartado anterior, hay muchas variedades de cebadas, y la mayoría de ellas pueden ser utilizadas en alimentación animal, ya sea en forma de grano como de forraje. Sin embargo, en esta tesis doctoral el estudio se ha centrado exclusivamente en una cebada forrajera, denominada “capuchona” y en una cebada grano con alto contenido en beta-glucanos.

1.3. Cebada forrajera: capuchona

1.3.1. Morfología y origen

La inflorescencia de la cebada es una espiga, con tres espiguillas en cada nudo del raquis. Las espiguillas contienen en su interior una flor que puede ser fértil o estéril. Las glumas (vaina estéril a modo de bráctea rodea la espiguilla) de las espiguillas acostumbran a tener aristas largas que se caracterizan por ser fibrosas. Estas aristas o barbas han sido descritas como elongaciones filamentosas ásperas de las lemas (glumillas) que pueden medir entre 3-15 cm de longitud. En las cebadas denominadas capuchonas (en inglés hooded), en lugar de la arista se desarrolla una flor extra estéril, dando lugar a una espiga aparentemente imberbe.

El origen de las primeras cebadas capuchonas se remonta a finales del siglo XVIII en las regiones del Himalaya (Harry, 1931; Stebbins y Yagil, 1966). Teoría fundamentada aparentemente por la obtención de un mutante de cebada sin las características aristas en la segunda generación (F2) de un cruce de dos variedades nativas Everest × Manchuria, confiriéndoles genéticamente el carácter dominante (Bonnett, 1938). El fenotipo mutante Hooded (Kap) dominante ha sido referido por conducir la sobreexpresión del gen Knox3 en la cebada, lo que da lugar al desarrollo de la flor extra estéril adicional sobre la lema (Figura 1.3) (Müller et al., 1995; Badr et al., 2000; Osnato et al., 2010).

Würschum et al. (2020), han indicado que esta característica contribuye al almacenamiento de carbohidratos, así como en la eficiencia del uso del agua y su fotosíntesis después de la senescencia de las hojas (Motzo y Giunta, 2002; Tambussi et al., 2007). Sin embargo, la presencia de aristas limita el consumo voluntario en animales, debido al efecto áspero de estos filamentos sobre la mucosa oral (Karren et al., 1994; Wallsten, 2008).

En los sistemas ganaderos, se necesita forraje en cualquiera de sus formas de aprovechamiento (verde, heno y ensilado), durante todo el año. En el contexto de las condiciones españolas, la siembra de cebadas capuchonas podría ser una opción para producir forraje con posibles ventajas en el consumo voluntario de rumiantes, debido a la ausencia de aristas en las espigas. Por lo tanto, la cebada capuchona cosechada antes de la madurez podría ser una buena alternativa para conseguir ensilados de alta calidad

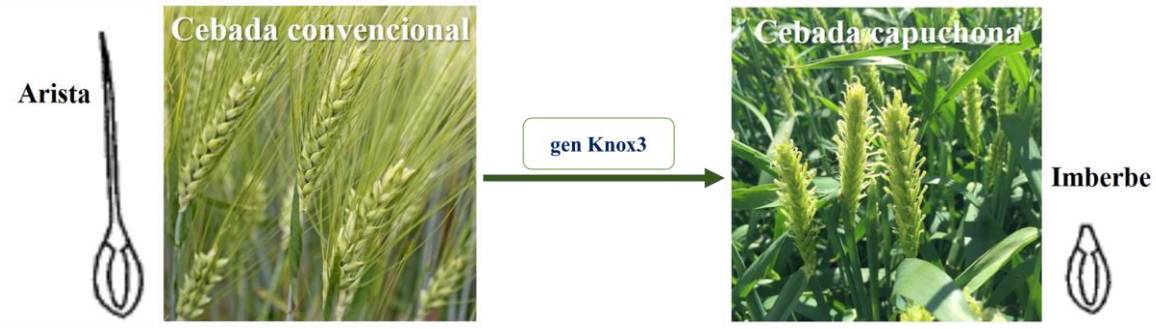

Figura 1.3. Expresión del gen *Knox3* en la cebada convencional dando origen a una flor extra estéril en lugar de aristas.

nutricional. Al mismo tiempo, en primaveras lluviosas con alta disponibilidad de otros forrajes alternativos, podrían dejarse madurar con baja penalización en el rendimiento en grano.

1.3.2. Composición química

Se ha realizado una extensa revisión bibliográfica sobre la composición química (proteína, PB; Fibra Neutro Detergente, FND y Fibra Ácido Detergente, FAD) del forraje de cebadas capuchonas. En la Tabla 1.1, se describen los estudios más representativos, indicándose el país y el estado de madurez del forraje en el momento del análisis.

Robinson et al. (2001) evaluaron el forraje de dos variedades comerciales de cebadas capuchonas (Haybet y Westford), obteniendo adecuados valores proteicos (>7% PB/MS) aptos para ser utilizados en nutrición de rumiantes. Hadjichristodoulou (1979) y Hadjipanayiotou et al. (1981) en Grecia, compararon el heno de variedades de cebadas capuchonas frente a variedades de cebadas convencionales, observando valores proteicos similares o superiores en las variedades capuchonas frente a las convencionales. Resultados similares obtuvieron Todd et al. (2003) y Carr et al. (2004) en EEUU, Park et al. (2008) en Corea del Sur, Nikkhah (2013) en Irán y Romero-Bernal et al. (2013) en México (Tabla 1.1). En cuanto a los valores de fibra, como era de esperar, variaron ostensiblemente según el estado de maduración. Los resultados son variables y no se observa una clara tendencia al comparar cebadas convencionales con capuchonas. Brummer y Pearson (2004), compararon dos cebadas capuchonas frente a otros cereales (cebada común, triticale, avena y trigo), obteniendo valores proteicos de las capuchonas

Tabla 1.1. Composición química de forraje de cebadas capuchonas. Se indica si se ha realizado comparación con un cultivo control

Referencia	País	Etapa de madurez	Capuchona				Control			
			Variedad	PB %	FND %	FAD %	Variedad	PB %	FND %	FAD %
Hadjichristodoulou (1979)	Grecia	Estado lechoso	116	10,0	N/D	N/D	Cebada cv. Athenais	10,2		N/D
			144	10,7			Cebada cv. 628	9,0	N/D	
			Sanokrithi 79	11,0			Cebada cv. 48 Alger	9,8		
Hadjipanayiotou et al. (1981)	Grecia	Estado lechoso	Sanokrithi 79	5,3	N/D	N/D	Cebada cv. 48 Alger	7,8	N/D	N/D
Robinson et al. (2001)	EEUU	No informado	Haybet	11,7	63,2	38,2	-	-	-	-
			Westford	10,7	61,1	31,8				
		No informado	Haybet	9,4	51,7	32,6	-	-	-	-
			Westford	10,1	62,8	41,0				
Todd et al. (2003)	EEUU	Estado pastoso	Haybet	9,7	53,9	30,1	Cebada cv. Valier	9,0	50,4	28,8
			MT981060	10,8	52,5	28,8				
			Westford	11,5	55,1	29,5				
Brummer y Pearson (2004)	EEUU	Estado pastoso	Washford	8,5	N/D	N/D	Cebada cv. Steptoe	8,3	N/D	N/D
			Westford	8,7			Triticale cv. 105, 301 y 2700	11,1		
							Avena cv. Ajay, Colo 37, Monida y Rusell	9,9		
							Trigo, cv. Sylvan	10,1		
		Estado pastoso	Washford	11,3	N/D	N/D	Cebada cv. Steptoe	10,4	N/D	N/D
			Westford	12,2			Triticale cv. 105, 301 y 2700	13,3		
							Avena cv. Ajay, Colo 37, Monida y Rusell	12,3		
							Trigo cv. Sylvan	13,9		

Tabla 1.1. Continuación

Referencia	País	Etapa de madurez	Capuchona				Control			
			Variedad	**PB %**	**FND %**	**FAD %**	**Variedad**	**PB %**	**FND %**	**FAD %**
Carr et al. (2004)	EEUU	Estado pastoso	Haybeat	8,3	61,0	35,0	Cebada cv. Foster	8,7	58,0	34,0
			Horsford	8,9	56,6	34,6	Cebada cv. Robust	8,8	58,4	35,3
			Westford	9,3	62,3	39,2	Cebada cv. Stander	9,5	56,2	32,0
Park et al. (2008)	Corea del Sur	Estado pastoso	Yuyeon	10,0	52,8	28,5	Cebada cv. Wooho	9,9	54,1	29,1
Nikkhah (2013)	Irán	No informado	Haybet	8,3	60,9	35,2	Cebada cv. Robust	8,8	58,4	35,3
			Westford	9,3	62,3	39,2	Cebada cv. Foster	8,7	57,9	34,4
Romero-Bernal et al. (2013	México	Estado pastoso	Emerald	11,0	51,8	24,3	Cebada cv. Cabuya	12,0	61,8	32,3
			Capuchona	12,8	60,0	31,6	Cebada cv. Petunia	13,2	62,1	30,0

Composición química expresada como % de materia seca; PB, proteína bruta; FND, Fibra neutro detergente; FAD, Fibra ácida detergente; N/D, no determinado;

superiores a la de la cebada común, similar a la avena, aunque inferiores a trigo y triticale (Tabla 1.1).

1.3.3. Valor nutricional: ingestión y digestibilidad

Los forrajes son un recurso esencial para la alimentación animal en el mundo debido a que proveen energía y proteína para el ganado (Aguilar-López et al., 2013). Normalmente son incluidos en la dieta de rumiantes alrededor del 20-100% en base a sus necesidades de materia seca. Aunque, su calidad está condicionada a parámetros de consumo y digestibilidad (Givens et al., 2000). Con este antecedente, podemos mencionar que el rendimiento de los animales depende de la ingesta de nutrientes digestibles y metabolizables. Es así que al comparar la variación entre animales y alimentos, diferencias entre el 60-90% estarían relacionadas con la ingesta, mientras que del 10-40% dependerían de la digestibilidad (Van Soest, 1994; Mertens, 2015). Además, la alimentación representa aproximadamente el 60% del coste de producción y, por tanto, tiene un gran impacto en la economía del sistema de producción.

A pesar de la existencia de diferentes ecuaciones desarrolladas para predecir el consumo voluntario de alimentos en rumiantes (Ingvartsen, 1994; Dijkstra et al., 2002), obtener predicciones satisfactorias es siempre complejo. Factores relacionados con el animal, alimento, manejo, alojamiento y/o condiciones medioambientales (Figura 1.4), pueden influir en la regulación del consumo y, por tanto en su predicción (Jarrige et al., 1986; Ingvartsen, 1994). En consecuencia, la medida del consumo voluntario es una función tanto del potencial de ingesta del alimento, como de la demanda de nutrientes por parte del animal (Givens et al., 2000; Baumont et al., 2000; Coleman y Moore, 2003).

De acuerdo con Van Soest (1994) y McDonald et al. (2010), la determinación de la digestibilidad en ovejas es el método estándar para determinar el valor nutritivo de los forrajes en la mayoría de los sistemas de alimentación de rumiantes. Aunque los valores expresados en las tablas de referencia de composición química y valor nutritivo de los alimentos para rumiantes, tanto del INRA (2018; sistema francés) como del NRC (2001; sistema estadounidense), dependen de las condiciones de alimentación en las que se midieron. En el caso del INRA, las mediciones de digestibilidad se realizan en condiciones *ad libitum* (Demarquilly et al., 1995), mientras que en el NRC (2001), son realizadas a un nivel de alimentación de mantenimiento, pero ambos en condiciones *in vivo*.

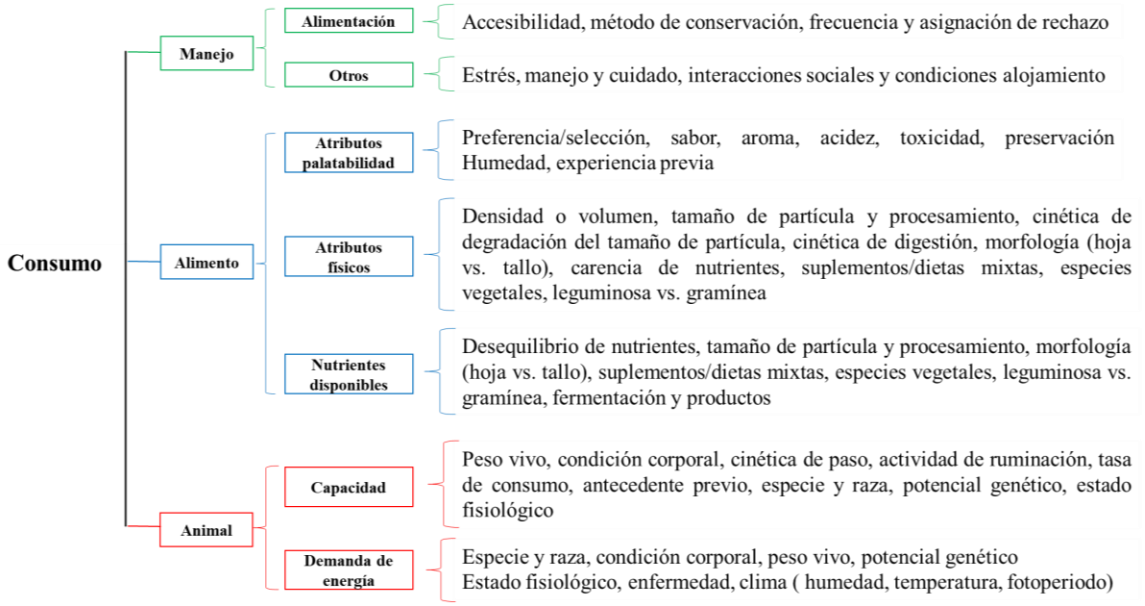

Figura 1.4. Factores relacionados con el consumo voluntario de alimentos en rumiantes (Baumont et al., 2000; Mertens y Grant 2020).

Aunque se han desarrollado ecuaciones para predecir los valores de digestibilidad, basadas en la composición química de los alimentos (Andueza et al., 2011), y sistemas basados en la simulación *in vitro* del proceso de digestión ruminal utilizando inóculos de líquido ruminal (Tilley y Terry, 1963), los resultados no han sido del todo satisfactorios. Los experimentos *in vivo* son la forma más adecuada para medir la ingesta voluntaria (McDonald et al., 2010) y, además, permiten determinar la digestibilidad aparente por diferencia entre lo consumido y excretado (Demarquilly et al., 1995). Lo excretado contiene, además del alimento no digerido, pérdidas fecales endógenas, procedentes de las células microbianas no digeridas y generadas en las fermentaciones del tracto gastrointestinal (Mertens y Grant, 2020).

En la Tabla 1.2 se hace referencia a tres principales trabajos que han medido en condiciones *in vivo* el potencial de consumo de MS de forraje de cebadas capuchonas, así como su digestibilidad aparente (d). Un primer estudio llevado a cabo por Hadjipanayiotou et al. (1981) en Grecia, no encontró diferencias en consumo voluntario de MS al comparar heno de cebada capuchona y cebada común en ovejas (43 vs. 41 g MS/kg $PV^{0.75}$). Además,

Tabla 1.2. Consumo voluntario de MS y digestibilidad aparente (d) de forraje de cebadas capuchonas en condiciones *in vivo* en rumiantes

Referencia	País	Especie y peso vivo	Forraje	Variedad	Capuchona				Variedad	Control			
					Consumo	Digestibilidad, %				Consumo	Digestibilidad, %		
					g MS/kg $PV^{0.75}$	MS	PB	FND		g MS/kg $PV^{0.75}$	MS	PB	FND
Hadjipanayiotou et al. (1981)	Grecia	Ovino 65 kg	heno	Sanokrithy69	43	57	28	N/D	cv. 48 Alger	41	56	42	N/D
Robinson et al. (2001)	EEUU	Bovino 268 kg	heno	Haybet	160	65	N/D	59					
				Westford	129	63		55					
Todd et al. (2003)	EEUU	Bovino 311 kg	heno	Haybet	130	61	N/D	45	cv. Valier	132	59	N/D	38
				MT981060	140	47		30					
				Westerford	109	68		56					

MS, materia seca; PB, proteína bruta; FND, fibra neutra detergente; N/D, no determinado; $PV^{0.75}$, peso metabólico

la dMS, no difirió al comparar ambos forrajes (57 vs. 56%, en promedio), aunque sí en dPB (28 vs. 42%), tal como se muestra en la Tabla 1.2.

Robinson et al. (2001), valoraron dos variedades de cebadas capuchonas Haybet y Westerford en bovino, obteniendo valores de consumo medio (129-160 g MS/kg $PV^{0.75}$), digestibilidad de MS (63-65%) y digestibilidad de FND (55-59%), respectivamente (Tabla 1.2). Finalmente, Todd et al. (2003) también en bovino, compararon tres cultivares de cebada capuchona (Westford, MT981060 y Haybet) frente a un cultivar de cebada común (Valier). Los resultados mostraron que el cultivar MT981060 fue más consumido que las otras dos variedades capuchonas y que la cebada común (140 vs. 132 g MS/kg $PV^{0.75}$; capuchona vs común). A pesar de ello, los cultivares Westerford y Haybet mostraron mayores porcentajes dMS (68-61%) y dFND (56-45%), tanto para MT981060 como para la cebada común (Tabla 1.2).

1.4. Cebada grano: beta-glucanos

1.4.1. Descripción y composición

El grano de cebada está constituido por una compleja estructura celular, en la que se distinguen tres componentes: el embrión o germen (3,5%), el pericarpio (18%) y el endospermo (78,5%) (Fox, 1996; Brouns et al., 2011). El embrión es vital para el proceso de germinación, y contiene el mayor contenido en lípidos y vitaminas liposolubles de todas las fracciones del grano (Fox, 1996). El pericarpio conocido técnicamente como el salvado del grano, es rico en polisacáridos altamente reticulados, como la celulosa, la lignina y el heteroxilano (Nirmala-Prasadi y Joye, 2020). Finalmente, el endospermo es de consistencia harinosa y está formado por una matriz proteica en donde se encuentran los gránulos de almidón, siendo el almidón el principal componente del grano de los cereales y el polisacárido de reserva más importante.

El almidón está formado por dos polisacáridos muy similares, la amilosa y la amilopectina. Ambos están formados por unidades de glucosa, en el caso de la amilosa unidas entre ellas por enlaces α-(1→4) dando lugar a una cadena lineal. En el caso de la amilopectina, forma cadenas ramificadas con uniones α-(1→4) y α-(1→6). En la mayoría de las variedades de cebada convencionales, el almidón se encuentra en una relación amilosa-amilopectina de 30:80, aunque trabajos de mejora genética han logrado obtener

cultivares con diferentes ratios en la relación amilosa-amilopectina (De-Paula et al., 2017). Respecto al contenido en grasa, en general el grano de cebada contiene una baja proporción (<2%, en base a MS), siendo más de la mitad de tipo poliinsaturadas (linoleico y linolénico). Además, el grano de cebada ha sido referido como un alimento rico en minerales, destacando su contenido en potasio, fosforo y magnesio (4,52; 2,64 y 1,33 %/MS, respectivamente). Es destacable también su contenido en calcio, hierro y cobre (3,3; 0,36 y 0,05 mg/kg MS, respectivamente).

Los contenidos en proteína bruta del grano de cebada varían entre 8-13% MS (Fox, 1996), aunque pueden variar de acuerdo a factores: genéticos (cultivar), medioambientales, la etapa de madurez y el nivel de fertilización (Acosta et al., 1991; Romero-Bernal et al., 2013). Respecto al tipo de proteínas (según su solubilidad), aproximadamente el 50% corresponde a prolamina (hordeína), mientras que el 50% restante corresponde a las otras tres fracciones proteicas: albúminas, globulinas y glutelinas (Fox, 1996; Newman y Newman, 2008).

Los polisacáridos no amiláceos (PNA), también denominados carbohidratos complejos, son componentes estructurales localizados en el pericarpio, la capa de aleurona y endospermo. De acuerdo con Newman y Newman (2008), los PNA, al igual que la fibra dietética total, no son digeridos por las enzimas digestivas de los mamíferos, por lo que apenas proporcionan energía. Sin embargo, debido a la capacidad de modular respuestas biológicas en organismos vivos, su uso como compuestos bioactivos tanto en alimentación humana como animal, han ganado interés (Ul-Ain et al., 2018).

1.4.2. Compuestos bioactivos del grano de cebada

Los compuestos bioactivos (CB) son sustancias obtenidas de una amplia gama de fuentes naturales, los cuales han mostrado en estudios *in vitro* e *in vivo* efectos beneficiosos sobre la salud tanto humana como animal (Patil et al., 2009). Los granos de cereales han sido referidos como excelentes fuentes de CB, dentro de los cuales se incluyen la fibra dietética (compuesta principalmente por arabinoxilanos, beta-glucanos, celulosa, lignina, lignanos) esteroles, tocoferoles, tocotrienoles, alquilresorcinoles, ácidos fenólicos, vitaminas y microelementos (Bartłomiej et al., 2012).

En el caso de la cebada, los arabinoxilanos están localizados mayoritariamente en la capa de aleurona y los beta-glucanos (BG) en el endospermo (Volman et al., 2008; Baik y

Ullrich, 2008), mientras que los compuestos fenólicos se localizan mayoritariamente en las capas externas del grano (Martínez-Subirá et al., 2018).

Ensayos clínicos en humanos, así como pruebas experimentales con animales, han demostrado que los CB entre los que destacan los BG y los compuestos fenólicos, debido a su alta actividad antioxidante, ejercen efectos beneficios sobre la salud (Wood et al., 2003; Reilly et al., 2010). Por esta razón, la Administración de Alimentos y Medicamentos de Estados Unidos (FDA, 2020), relacionó el consumo de fibra de cebada con la reducción de riesgo coronario y enfermedades del corazón. De igual forma, el Centro Conjunto de Investigación Europeo (JRC, 2021) ha hecho hincapié en el consumo de cereales integrales en la alimentación, reconociendo además la Autoridad Europea de Seguridad Alimentaria los beneficios sobre la salud humana del consumo de fibras solubles (EFSA, 2021).

El contenido en BG de la cebada (2-10%/MS) es superior al encontrado en avena (2,2-7,8%), centeno (1,2-2,0%) y trigo (0,4-1,4%) (Baik y Ullrich, 2008; Reilly et al., 2010). Se ha comprobado una correlación positiva entre el contenido de amilosa de los granos de cebada y sus niveles en BG (Izydorczyk y Dexter, 2008). Según Ellis et al. (1997), las cebada cultivadas en España suelen producir granos con mayor contenido de BG, posiblemente por efecto del clima cálido y seco.

Hoy en día, los BG son reconocidos como potenciales ingredientes funcionales que podrían ser utilizados en una amplia gama de productos farmacéuticos, con prometedoras propiedades fisicoquímicas y reológicas, para su aplicación tanto en alimentación humana como en animal. Con estos antecedentes, granos de cebada con altos contenidos en BG incluidos en la formulación de dietas para rumiantes como alimentos funcionales, podrían ser una alternativa para mejorar el status metabólico e inmunitario del animal.

1.4.3. Los beta-glucanos

Los BG son macromoléculas de polisacáridos formados por monómeros de D-glucosa unidos a través de enlaces β-glicosídicos β-(1→3) y β-(1→4) (Bai et al., 2019; Murphy et al., 2020). Los BG de diferentes especies de cereales comparten la misma estructura molecular (Izydorczyk y Dexter, 2008). Sin embargo, exhiben variaciones en características como: proporciones de enlaces β-glicosídicos, presencia y cantidad de fragmentos largos de oligosacáridos (1→4) y tamaño molecular (Reilly et al., 2010; Rieder y Samuelsen, 2012).

En la cebada, los BG corresponden a homopolisacáridos lineales compuestos por enlaces β-(1→3) y β-(1→4), en un proporción de 70:30%, respectivamente (Reilly et al., 2010). Su estructura está constituida por bloques de enlaces β-(1→4) consecutivos, separados por un único enlace β-(1→3), construyendo una estructura en forma de escalera (Figura 1.5) (Lazaridou y Biliaderis, 2007).

Los cereales no son la única fuente de BG, otras fuentes importantes son los hongos, levaduras, algas y bacterias (Hieke et al., 2014; Jin et al., 2018). Aunque en estas fuentes naturales los BG se encuentran como homopolisacáridos lineales de glucosa unidos a través de enlaces β-(1→3) y cadenas laterales con enlaces β-(1→6) y β-(1→9) (Brown y Gordon, 2005). Por lo tanto, su estructura macromolecular y en particular el tipo de enlaces de la cadena principal y de sus ramificaciones, permite diferenciarlos claramente de los BG de cereales (Murphy et al., 2020).

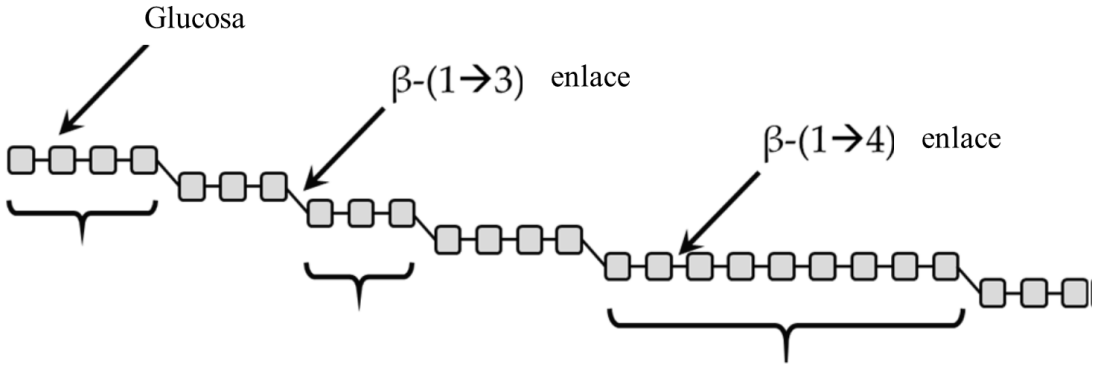

Figura 1.5. Estructura molecular de los beta-glucanos de cebada (Tosh et al., 2004).

1.4.4. Propiedades físicas de los beta-glucanos

De acuerdo con Bai et al. (2019), una mayor proporción de enlaces β-(1→3) en la estructura molecular aumenta la solubilidad de los BG, haciendo que la molécula sea más soluble y flexible (Lazaridou et al., 2008), aspecto de gran interés industrial (Schmidt, 2020). Otra característica importante, es la capacidad de formar soluciones altamente viscosas. En el caso de los BG de cereales, esta propiedad está condicionada por su estructura, peso molecular y concentración (Bai et al., 2019). En consecuencia, la cebada con altos contenidos de BG puede formar soluciones altamente viscosas, siendo un interesante ingrediente emulsificante para múltiples aplicaciones en la industria alimentaria aunque con implicaciones digestivas en monogástricos.

La viscosidad de los BG puede generar propiedades metabólicas beneficiosas atribuibles a su capacidad de formar soluciones viscosas en soluciones acuosas, como ocurre en el tubo digestivo (Storsley et al., 2003). La viscosidad retrasa el vaciamiento gástrico e interfiere en el contacto entre enzimas pancreáticas y sus substratos en el lumen intestinal (Henrion et al., 2019). Como consecuencia se ralentiza y reduce el ritmo de digestión del almidón (y la producción de mono y disacáridos asociada) y, por tanto, se provoca un retraso en la absorción de la glucosa (Nirmala-Prasadi y Joye, 2020). Estos efectos explicarían la reducción plasmática de colesterol y su bajo índice glicémico (Wang et al., 2017), lo que disminuye el riesgo de enfermedades coronarias, obesidad, diabetes y cáncer (Brouns et al., 2011).

1.4.5. Propiedades inmunológicas de los beta-glucanos

Los enlaces glucosídicos β-(1→3) son eficaces agentes inmunoestimulantes con capacidad de desencadenar respuestas inmunitarias (Legentil et al., 2015). El principal receptor para el reconocimiento de BG de cereales es la Dectina-1, que se expresa a altos niveles en la células inmunes innatas, tales como: monocitos sanguíneos y esplénicos, neutrófilos y macrófagos (Adams et al., 2008).

Estudios experimentales han revelado que las células del sistema inmune innato, tras ser estimuladas con BG extraídos de levaduras, se vuelven más activas para la fagocitosis de patógenos, producción de radicales libres (explosión oxidativa) y regulación de la producción de citoquinas (Meena et al., 2013; Guzman y Montoya, 2018). Por ello, actualmente, los BG están siendo incluidos como alimentos funcionales para alcanzar un estado de "inmunidad entrenada", caracterizada por una mayor capacidad de respuesta de las células inmunes innatas previamente activadas (Mourits et al., 2018), tal como se ilustra en la Figura 1.6.

En base a esta evidencia, granos de cebada con alto contenido en BG podrían ser un potencial agente biológico de entrenamiento del sistema inmune, pudiendo ser incluidos como alimentos funcionales en la formulación de raciones para rumiantes lecheros.

1.4.6. Uso de los beta-glucanos en alimentación animal

En la última década, garantizar la salud y el bienestar de los animales con la reducción

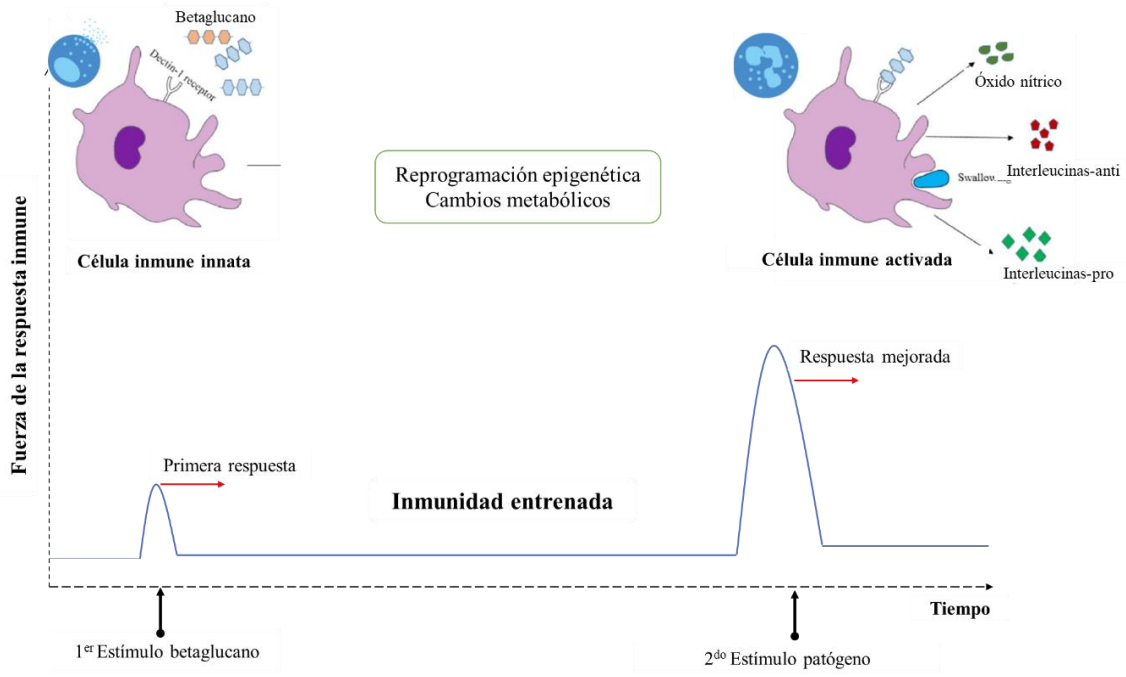

Figura 1.6. Proceso para alcanzar un estado de inmunidad entrenada de las células del sistema inmune innato con beta-glucanos extraídos de levaduras (Jin et al., 2018).

progresiva del uso de medicamentos, se ha convertido en una cuestión clave para los ganaderos y los consumidores de todo el mundo (Bronzo et al., 2020). En el marco de la política de lucha contra la resistencia antimicrobiana, la Unión Europea aprobó, con pleno consenso del Consejo de Ministros de Agricultura (UE: 22/7/2003), la normativa que prohíbe el uso de antibióticos como aditivo para piensos (Regulación CE 1831/2003, FDA-2010-N-0155). En consecuencia, a raíz de esta normativa, se evidencia un creciente interés por los CB por sus propiedades antimicrobianas, antiinflamatorias y antioxidantes, como alternativa a los aditivos químicos (Bartłomiej et al., 2012; Karásková et al., 2015).

Dentro de un amplio grupo de CB para uso en producción animal, la fibra dietética utilizada como aditivo zootécnico, ha sido objeto de muchos trabajos de investigación (Callaway et al., 2021). Las evidencias científicas de los últimos 20 años han documentado que suplementar la dieta de rumiantes con levaduras (*Saccharomyces cerevisiae*), conocidas por tener BG como componentes de su pared celular, mejoran los parámetros productivos. Por esta razón, en la actualidad se pueden encontrar productos comerciales a base de levaduras. La Tabla 1.3 presenta estudios en los que han suplementado rumiantes

lecheros con distintas dosis de *S. cerevisiae*. En general, los resultados muestran aumentos de la producción de leche (2-16%), consumo de MS (2-14%), ganancia media diaria de PV (1-4%), y eficiencia alimentaria (4-6%), además de mejorar las concentraciones de ácidos grasos volátiles. En el caso de la cebada, a pesar de que el grano es ampliamente incluido en la formulación de dietas para rumiantes, no hay trabajos que hayan incluido altos contenidos de BG como alimentos funcionales para evaluar sus efectos.

Tabla 1.3. Respuestas productivas de rumiantes suplementados con levadura (*Saccharomyces cerevisiae)* frente a un grupo no suplementado como control.

Especie	Suplementación	Respuestas productivas	País	Referencia
Bovina	60 g/día	+6% producción de leche +1% ganancia media diaria de PV +6% eficiencia alimentaria	Estados Unidos	Schingoethe et al. (2004)
	3 g/día	+4% producción de leche +2% ganancia media diaria de PV −3% acetato +16% propionato	Sudáfrica	Erasmus et al. (2005)
	56 g/día	+2% producción de leche +4% ganancia media diaria de PV +6% eficiencia alimentaria	Estados Unidos	Cooke et al. (2007)
	6 g/día	+4% producción de leche +2% consumo MS +4% eficiencia alimentaria	Israel	Moallem et al. (2009)
	3,3 g/día	+16% producción de leche +14% consumo MS	España	Bach et al. (2018)
Ovina	4 g/día	− 9% acetato +23% propionato	Turquía	Inal et al. (2010)

Por otro lado, de acuerdo con estudios de degradabilidad ruminal, los BG de cereales son degradados por la microflora bacteriana (Engstrom et al., 1992) y en consecuencia, no hay estudios que hayan incluido BG de cebada como ingredientes funcionales para modular respuestas inmunes.

Sin embargo, un interesante trabajo realizado en nuestro grupo de investigación por Torrent (2015), reveló que los BG de cebada abandonaron el rumen no completamente degradados, pudiendo ser absorbidos con posibles efectos metabólicos. Un segundo estudio, en el que se utilizaron ovejas lecheras suplementadas con BG de cebada, permitió detectar una mayor concentración de lactosa en su perfil metabolómico (Contreras-Jodar et al., 2017). En base a esta nueva evidencia, y considerando los posibles efectos inmunitarios de los BG, se propuso una nueva línea de trabajo dentro del proyecto Retos del Ministerio Economía y Competitividad español, respecto al papel de los BG de cebada como potenciales agentes biológicos de entrenamiento de las células del sistema inmune innato en rumiantes lecheros.

Hay muy pocas referencias bibliográficas con BG de cebada, por lo que se ha hecho un trabajo de revisión, encontrándose estudios con levaduras Tabla 1.4. En ella se puede observar, cómo distintos trabajos en donde se administró levaduras a dosis diarias entre 0,2-150 g/animal obtuvieron diferentes respuestas en marcadores específicos del sistema inmune en rumiantes, evidenciando los efectos inmunoestimulantes de su inclusión en programas de alimentación animal.

Por todo ello, el uso de cultivares con altos contenidos de BG como alternativa a los antibióticos en alimentación de rumiantes, podría ser un campo de investigación con resultados prometedores. Siendo los estudios en los que se incluya en la alimentación de rumiantes BG una opción no farmacológica interesante para mejorar el rendimiento productivo y modular el sistema inmune.

Tabla 1.4. Respuesta inmunitaria a la suplementación con levaduras (*Saccharomyces cerevisiae* o *Debaryomyces hansenii*) en rumiantes frente a un grupo control no suplementado.

Especie	Suplementación		Respuestas inmunes	País	Referencia
Bovina	*S. cerevisiae*	50 g/día	+76% inmunoglobulina IgG	República Checa	Fröhdeová et al. (2014)
		150 g/día	+47% inmunoglobulina IgG		
	S. cerevisiae	2,5 g/día	−40% haptoglobina	Estados Unidos	Word et al. (2019)
	S. cerevisiae	12 g/día	+70% TNF-α +42% proteína amiloide A	Estados Unidos	Burdick-Sanchez et al. (2020)
Ovina	*S. cerevisiae*	3 g/día	+22% inmunoglobulina IgG +31% lisozimas	Polonia	Wójcik (2010)
	S. cerevisiae	3 g/día	+23% gammaglobulinas +10% lisozimas −62% RCS	Polonia	Zabek et al. (2013)
Caprina	*D. hansenii*	2 g/día	−60% expresión TNF-α −40% expresión IL-1β	México	Angulo et al. (2019)
	S. cerevisiae	0,2 g/día	−43% TNF-α +15% IL-1β +18% lisozimas +35% óxido nítrico	México	Angulo et al. (2020)
	D. hansenii	0,2 g/día	+50% TNF-α +27% IL-1β +22% lisozimas +15% óxido nítrico		

RCS, recuento de células somáticas; marcadores de inmunidad humoral: gammaglobulinas, óxido nítrico y lisozimas; Citoquinas pro-inflamatorias, TNF-α, IL-6 y IL-1β; Proteínas de fase aguda, haptoglobina y proteína amiloide A sérica.

CAPÍTULO 2

Hipótesis y objetivos

CAPÍTULO 2

2.1. Hipótesis

La principal hipótesis de trabajo de esta tesis doctoral es la posibilidad de utilización de nuevas variedades de cebada, con innovadoras propiedades nutritivas y funcionales, en la alimentación de rumiantes. Esta tesis doctoral se centra en dos nuevas variedades de cebada una forrajera y otra de grano rica en beta-glucanos. Así, se planteó evaluar:

- Cebada forrajera capuchona, con una flor extra estéril en lugar de aristas en las espigas. Se espera que la ausencia de aristas favorezca la apetecibilidad del ganado y mejore su calidad nutritiva.
- Cebada grano con alto contenido en beta-glucanos (compuestos bioactivos), que se espera que estimulen el sistema inmune, confiriendo protección contra un amplio espectro de patógenos en rumiantes.

2.2. Objetivos

El objetivo general de la presente tesis doctoral fue:

1) Determinar el valor nutritivo de una cebada capuchona (cv. Mochona; Semillas Batlle) en ganado ovino en condiciones *in vivo* y *ad libitum*.

   Para ello, se evaluó la nueva variedad de cebada capuchona (cv. Mochona) y se comparó con un triticale comercial (cv. Titania) de elevado rendimiento en condiciones de secano. Ambos forrajes se cosecharon y procesaron como heno y ensilado en 2 años consecutivos, y se evaluó su ingestibilidad y digestibilidad en ganado ovino.

2) Explorar las propiedades inmunoestimuladoras de una cebada rica en beta-glucanos (cv. Annapurna; Semillas Batlle) en ovejas lecheras en lactación.

   Para ello, se evaluaron las respuestas productivas e inmunitarias de la suplementación oral de una nueva variedad de cebada grano de alto contenido en beta-glucanos (cv. Annapurna), comparada con una cebada convencional (cv. Meseta) y con la suplementación directa de los beta-glucanos por vía

intraperitoneal. La evaluación inmunitaria se completó con un desafió intramamario a corto plazo de endotoxina (LPS) de *Escherichia coli*.

CAPÍTULO 3

Comparison of the nutritional value of hooded barley (cv. Mochona) and triticale (cv. Titania) fed as hay or silage to sheep

Comparación del valor nutricional de cebada capuchona (cv. Mochona) y triticale (cv. Titania) como heno o ensilado para la alimentación de ovinos

CAPÍTULO 3

Comparison of the nutritional value of hooded barley (cv. Mochona) and triticale (cv. Titania) fed as hay or silage to sheep

Comparación del valor nutricional de cebada capuchona (cv. Mochona) y triticale (cv. Titania) como heno o ensilado para la alimentación de ovinos

3.1. ABSTRACT

The hooded barley phenotype produces the development of an extra sterile flower in spikelets instead of awns. Hence, it might improve the nutritive value of the whole plant. The aim of this work was to evaluate a new variety of hooded barley (cv. Mochona) compared to triticale (cv. Titania) harvested and processed as hay and silage during 2 consecutive years, in ruminants. Digestibility was determined using 32 dry and open dairy ewes, distributed in 2 balanced groups by treatment (barley or triticale). Both preservation methods (hay or silage) were sequentially compared under *ad libitum* feeding conditions. Chemical composition was determined by official methods and data were analyzed using the PROCMIXED of SAS v.9.4. The results showed differences on the chemical composition of hay and silage by harvesting year ($P < 0.001$ to 0.004), except for ADF ($P = 0.20$) and NFE ($P = 0.18$), in hays, and DM ($P = 0.33$) and CP ($P = 0.18$) in silages. Moreover, species conditioned most components of both forages ($P < 0.001$ to 0.066), except for CP, DM and ADF ($P = 0.10$ to 0.61) of hay, and CP, OM, Ash as well as ADF ($P = 0.13$ to 0.32) in silage. No differences were observed for voluntary intake (31 ± 3 g DM/kg $BW^{0.75}$, on average) by harvesting year ($P = 0.24$ to 0.47), species ($P = 0.10$ to 0.70) or their interaction ($P = 0.12$ to 0.43). Both forages showed high sheep fill values (sFV), either as hay or as silage (2.65 ± 0.030 and 3.09 ± 0.042, on average, respectively). Apparent digestibility (d) of nutrients varied according to harvesting year ($P < 0.001$ to 0.020), as well as DMd and CPd showed differences between species ($P < 0.001$) in case of silages. Respect to hays, hooded barley showed lower OMd ($P = 0.040$), NDFd ($P = 0.033$), and CPd ($P = 0.002$). Regarding nutritive values, hays did not differ in GE content (4.71 ± 0.010 Mcal/kg DM; $P = 0.37$), while that hooded barley silage showed greater GE than triticale (4.85 vs. 4.78 ± 0.010 Mcal/kg DM; $P = 0.002$). No differences were detected between species in digestible protein values for both preservation methods (68 ± 0.7 g PDI/kg DM, on average; $P = 0.12$ to 0.37), but RPB was greater in triticale than hooded barley (hay, −30 vs. −26 ± 1.5 g/kg DM; silage, −28 vs. −21 ± 1.4 g/kg DM, on average; $P = 0.010$ to 0.060, respectively). Harvesting year markedly affected PDIA and RPB in hay ($P < 0.001$ to 0.004) and silage ($P = 0.006$ to 0.020). In conclusion, marked compositional differences between years, but not between hooded barley and triticale species, were observed. However, despite of the similar intake and digestibility between species, hooded barley showed slightly lower nutritive values than triticale and were greater than those reported for conventional barleys in literature.

3.2. INTRODUCTION

Cereals are the most important source of carbohydrates and protein for humans and livestock, being a major source of food energy (Pascari et al., 2019). Although cereals are usually harvested for grain, they can also be used for forage (i.e. pasture, hay or silage) or harvested for double purpose (i.e. forage followed by grain).

Barley (*Hordeum vulgare* L.) has several advantages over other cereals, such as to be more vigorous, tolerant to drought and salinity, resistant to diseases and plagues, able to produce forage and grain in shorter time, and low production costs (Francia et al., 2006; Capettini et al., 2011; Nikkhah, 2013). Barley is cultivated in more than 100 countries of the world, being the fourth cereal in surface and production (FAO, 2022). Furthermore, barley is the typical cereal in Mediterranean arid lands, as in Spain, where it occupies more than 2.5×10^6 ha and is the first cereal commodity (MAPA, 2022).

As a consequence of its genetic diversity, there are barley cultivars able to be harvested in spring or in winter, with different row numbers in the spike (two to six rows), hulled or hulless grains, as well as with or without awns, among others (Baik and Ullrich, 2008; Martínez-Moreno et al., 2017). Awns are long distal appendages characteristic of most grass species (Poaceae) such as wheat, barley and rice (Roig et al., 2004; Würschum et al., 2020). As a plant protection strategy to avoid grazing, awns are an undesirable trait for forage crops (Wallsten, 2008; Würschum et al., 2020). The hooded barley mutant phenotype leads to the overexpression of the K gene, which produces the development of an extra sterile flower in the spikelet, instead of the characteristic awn, resulting in cultivars of seemingly imberbe spikes (Badr et al., 2000; Roig et al., 2004). Hooded barley cultivars may be used directly for feeding herbivores or preserved as hay or silage. Moreover, these cultivars without awns, may be more palatable in advanced stages of maturity by not injuring the mouth or the digestive mucosa (Karren et al., 1994).

The productive and nutritive use of hooded barley in ruminants has been compared to conventional barley in Cyprus (Hadjipanayiotou et al., 1981), the USA (Brummer and Pearson, 2004), South Korea (Park et al., 2008) and México (Romero-Bernal et al., 2013). Although chemical composition was indicative of greater nutritive values of hooded than those of conventional barleys, no differences were found on dry matter digestibility (DMd) and organic matter digestibility (OMd) in sheep (Hadjipanayiotou et al., 1981) and beef cattle (Todd et al., 2003). In Spain, with the exception of our preliminary work by Ajenjo-

Puigderrajols (2018), no further studies have assessed the nutritive value of hooded barley as forage for small ruminants.

With this aim, the hooded barley (cv. Mochona) was assessed as a possible more productive and palatable forage alternative for small ruminants in dry Mediterranean conditions. Most used preservation modes (hay and silage) were compared with a common commercial variety of triticale (cv. Titania) in two consecutive years, and their chemical composition and in vivo ingestibility and digestibility were evaluated.

3.3. MATERIALS AND METHODS

3.3.1. Cultural practices

In November of two consecutive years (2016 and 2017), two plots of 0.75 ha located at the experimental fields of the Servei de Granges i Camps Experimentals in Bellaterra (41°30’20’’N and 2°05’46’’E; elevation, 162 m) of the Universitat Autònoma de Barcelona, were sowed with hooded barley (*Hordeum vulgare* cv. Mochona; 150 kg/ha) or triticale (*Triticum* × *Secale* cv. Titania; 220 kg/ha) from Semillas Batlle (Bell-lloc, Lleida, ES) according to the common cultural practices done in the area. In May of the following years, both forages were harvested by a single cut using a tractor operated rotary disc mower (Mod. Manlleu, Compar, Sant Pere de Torelló, ES) and preserved as hay and silage. Hays were sun-cured (4 d), wilted, packed in rectangular bales (1 × 0.5 × 0.4 m), using a low-pressure baling machine (Mod. IH LBX 422, Case, Hamburg, DE) and stored indoors. For silage, the cut forages were immediately pick upped, chopped in a vertical chopper-mixer (Mod. Boy Mix 8 m3, Compar) and ensiled in 1 m^3 (1.1 × 0.9 × 1 m) rectangular plastic containers covered with a plastic film and with openable drainers. On the second year, a mix of heterofermentative inoculant (ABFs, Alter-entorn, Mora d´Ebre, ES) was used to enhance the aerobic stability of silages, minimizing DM losses. Both forages, hay and silage of barley and triticale were preserved for 10 months before use in the 2 harvesting years (H1, 2017; H2, 2018).

3.3.2. Animals, management and feeding conditions

Animal care conditions and management practices agreed with the Spanish Royal Decree 52/2013, on the protection of animals used for experimental purposes, and were

approved by the Ethical Committee of Animal and Human Experimentation of the Universitat Autònoma de Barcelona (CEEAH reference 3871).

A total of 32 adults, dry and open ewes, were divided in balanced groups of similar body weight (BW) and body condition score (BCS) and used for forage evaluation according to year (H1, n = 12; H2, n = 20). The ewes were of 2 dairy breeds (Manchega, n = 22, 78.0 ± 3.2 kg BW and 3.5 ± 0.1 BCS; Lacaune, n = 10, 75.6 ± 2.0 kg BW and 3.4 ± 0.1 BCS). Forages (barley or triticale) were offered in sequential periods, starting with hay and following with silage. Each ewe´s group was only fed with one forage species and, because the CEEAH requirements, each group only used for a year.

Ewes were adapted to diets and experimental conditions in free groups, on straw-bedded pens (3 m^2/ewe) for each forage type. Pens had free access to metabolic cages, which formed a front line and were used for feeding and watering, the forage and water being offered freely the whole day. The metabolic cages (Caja et al., unpublished) consisted of plastic containers (120 × 100 × 122 cm; Foldable large load carrier 1000 L, Auer Packaging, Amerang, DE) modified to allocate individuality 2 sheep in tied-stalls, each one with a plastic feeder (40 × 30 × 23 cm; Auer Packaging) and a water bowl (20 × 30 cm; Suevia 125, Kincheheimam Neckar, DE) connected to a water tank (20 L). A mineral block (Na, 36.74%; Ca, 0.32%; Mg, 1.09%; Zn, 5 g/kg; Mn, 1.5 g/kg; S, 912 mg/kg; Fe, 304 mg/kg; I, 75 mg/kg; Co, 50 mg/kg; Se, 25 mg/kg; Ovi bloc, Sal Cupido, Barcelona, ES) was also available individually. The metabolic cages were elevated 30 cm from the floor which were not an obstacle for the access of the ewes. To ensure the adaptation of the ewes and to avoid distress (Figure 3.1), they were first adapted to the pens as a group letting them to choose where to fed (d 1 to 10); secondly, they were trained to be tied in the metabolic cages (d 11 to 15) as tied-stalls, during the daytime (0800 to 2000 h) and released during the night (2000 to 0800 h). Finally, (d 16 to 20), the ewes were tied during day and night, as used conventionally in metabolic cages, for total feces collections and to measure daily intake during the measurement of ingestibility and digestibility (5 d). All ewes adapted to the experimental procedures.

3.3.3. Ingestibility y digestibility

All forages were offered once a day ad libitum (fixed at 115% of the previous day's consumption). Voluntary daily intake (g/kg $BW^{0.75}$) was calculated by measuring the

Facilities	Straw bedded pens with metabolic cages															Metabolic cages					Straw bedded pens						
Experimental period	Adaptation															Measurements					Washout						
Day (0800 to 2000)	Free										Tied stalls					Tied stalls					Free						
Night (2000 to 0800)	Free										Free					Tied stalls					Free						
Days	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27

Figure 3.1. Scheme of the experimental design used to assess the in vivo ingestibility and digestibility of the forages during both years.

difference between offers and orts and expressed as dry matter intake (DMI), neutral detergent fiber intake (NDFI) and organic matter intake (OMI), according to Demarquilly et al. (1995). Feces were collected during the tied-stalls period by using perforated plastic boxes (60 × 40 × 17 cm; latticed Auer Packaging) covered by plastic mesh to allow the drainage of urine to a solid plastic box (60 × 40 × 12 cm EG64-12; blind Auer Packaging) that were placed behind the ewes.

Offers, orts and feces were individually weighed using an electronic scale (Gram K3, Gram Precision, Barcelona, ES) and daily sampled taking aliquots (5 to 10%). Samples of offers orts and feces were kept at -20ºC until analyses.

The apparent digestibility of each forage was determined by using feed intake, fecal output, and chemical analyses according to Demarquilly et al. (1995) and McDonald et al. (2010). Digestibility coefficients were expressed as dry matter digestibility (DMd), organic matter digestibility (OMd), neutral detergent fiber digestibility (NDFd) and crude protein digestibility (CPd). At the end of each digestibility period, the ewes had a washout period (7 d) fed with the new forage under free pen conditions, to avoid digestive contamination with the feed used in the previous experimental period.

3.3.4. Chemical analyses

Previously to the analysis, the frozen hay and feces samples were conditioned at 60 °C for 24 h, whereas silage samples were ice-dried (LyoAlfa 15, Testar, Klosterneuburg, AT).

Thereafter, all samples were homogenized and grinded through a cyclone mill (Retsch SM2000, Retsch, Haan, DE) with a mesh of 1-mm.

The chemical analyses were carried out in duplicate according to AOAC (2000). Dry matter (DM) was determined at 103°C for 24 h and ashes burnt at 550°C for 5 h. Organic matter (OM) was calculated as the difference between DM and ashes content of each sample. Crude protein (CP) was calculated as percentage of N × 6.25 by the Dumas method using a Leco Analyser (Leco Corporation, St Joseph, MI, USA), corrected according to Müller (2017). Crude fiber (CF) was analyzed according to Weende method by acid hydrolysis with 1.25% H_2SO_4, followed by alkaline hydrolysis with 1.25% NaOH. Whereas, neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) were sequentially determined on an ash-free basis according to Van Soest et al. (1991) adding sodium sulphite and thermostable α-amylase. All fiber determinations above mentioned were performed using an Ankom200 Fiber Analyser (Ankom Technology, Fairport, NY, USA). Moreover, the nitrogen-free extract (NFE) was calculated as follows NFE = OM – CP – CF. Additionally, silage pH values were measured daily in each digestibility trial of both experimental years using a pH meter (senSION+pH31, Hach, CO, USA). Finally, the chemical composition and the measured digestibility values under ad libitum conditions, were used to calculate the feeding values of each forage for lactating ruminants, according to INRA (2018). Nutritional energy values, such as gross energy (GE), digestible energy (DE), metabolizable energy (ME) as well as net energy for lactation (NE_L), were expressed as Mcal/kg DM, and a conversion of 1 UFL = 1.76 Mcal/kg DM was used. With regard to protein partitioning, values were expressed in g/kg DM as protein digestible in the intestine (PDI), calculated as the total sum of protein digestible in the intestine from dietary origin (PDIA) and of microbial origin (PDIM). Moreover, the rumen protein balance (RPB, g/kg DM) was calculated using the equation RPB = -84.5 + 0.61 × CP according to INRA (2018).

3.3.5. Statistical analyses

All data were analyzed by the MIXED procedure of SAS v.9.4 (SAS Institute Inc., Cary, NC, USA). The statistical mixed model contained as fixed effects the forage species (hooded barley, triticale), experimental year (H1, H2) and their interaction. As random effects were considered the animal and the residual error. Furthermore, CORR procedure

of SAS, was used to identify Pearson correlation coefficients between chemical composition and voluntary intake and apparent digestibility values. Differences between least square means were determined by t-tests using the PDIFF option of SAS. Significance was declared at $P < 0.05$ and tendency at $P < 0.10$, unless otherwise indicated.

3.4. RESULTS Y DISCUSSION

3.4.1. Chemical composition of hays

The composition of hays varied according to the forage species and the harvesting year for all nutrients (Table 3.1; $P < 0.001$ to 0.003), except for DM (91.3 ± 0.10%, on average; $P = 0.10$), CP (10.6 ± 0.93%, on average; $P = 0.61$) and ADF (39.8 ± 0.10%, on average; $P = 0.12$) values between species, and between years as ADF ($P = 0.20$) and NFE ($P = 0.18$). The S×H interaction was significant in NDF ($P = 0.004$) and ADL ($P = 0.014$), as shown in Table 3.1.

Both forage species showed less CP content in H2 than in H1 (−30%, on average; $P = 0.003$; Table 3.1), mainly due to climatic conditions between years (i.e., drought in H1 and rain in H2 during sun-drying). Nevertheless, the hooded barley showed, on average, a numerically greater CP content (10%), than the triticale, but also with a higher mean ash content (15.3 vs. 11.9 ± 0.11%; respectively; $P < 0.001$). Consequently, the hooded barley showed a lower OM content than triticale (84.7 vs. 88.2 ± 0.11%, on average; $P < 0.001$) which conditioned the values of intake and digestibility, as later discussed.

Environmental conditions such as water availability, soil type and fertilization, mainly affect CP contents in forage cereals (Helsel and Thomas, 1987; Romero-Bernal et al., 2013). Aguilar-López et al. (2013) mentioned that conventional barley may reach 19% CP before ear emergence and that its content decreases dramatically with plant maturity (Juskiw et al., 2011; Nair et al., 2016). Value of CP is considered a key for assessing the quality of forages (Givens et al., 2000; McDonald et al., 2010) and it is also essential for rumen fermentation (Fahey et al., 1994). For ruminants, it is desirable that forages have CP values greater than 8% on DM basis (Waghorn and Clark, 2011; Gill et al., 2013). Reference values of conventional barley processed as hay show CP contents ranging from 7.8 to 13% (Carr et al., 2004; Greg and Marc, 2011), although Aboagye et al. (2021) did not find differences on CP contents between different cereals harvested as forages,

Table 3.1. Chemical composition, ingestibility (I) and apparent digestibility (d) of hay of cereal species (S) of hooded barley (cv. Mochona) and triticale (cv. Titania) in 2 consecutive harvesting years (H1, 2017; H2, 2018).

Hay	**Hooded barley**		**Triticale**		**±SEM**	***P* value**		
item	H1	H2	H1	H2		S	H	S×H[1]
DM**,** %	91.8^{a}	91.0^{b}	92.3^{a}	90.4^{b}	0.01	0.10	0.001	0.001
Composition, % DM								
CP (N × 6.25)	12.8^{a}	9.2^{b}	12.2^{a}	8.3^{b}	0.93	0.61	0.003	0.98
CF	32.0^{bj}	40.2^{aj}	32.2^{bk}	37.5^{ak}	0.15	0.001	0.001	0.001
NDF	65.0^{bj}	68.9^{aj}	64.1^{bk}	65.6^{ak}	0.20	0.001	0.001	0.004
ADF	40.0	39.8	39.8	39.7	0.10	0.12	0.20	0.86
ADL	5.5^{aj}	5.4^{bj}	4.4^{ak}	3.7^{bk}	0.10	0.001	0.002	0.014
NFE	39.0^{k}	36.4^{k}	43.0^{j}	43.0^{j}	0.56	0.001	0.18	0.14
OM	83.8^{bk}	85.6^{ak}	87.4^{bj}	88.9^{aj}	0.11	0.001	0.001	0.12
Ash	16.2^{aj}	14.4^{bj}	12.6^{ak}	11.1^{bk}	0.11	0.001	0.001	0.12
Ingestibility, g/kg BW$^{0.75}$								
DMI	27	29	36	31	2.6	0.10	0.47	0.43
OMI	23^{k}	27^{k}	33^{j}	31^{j}	1.9	0.001	0.52	0.10
NDFI	17^{k}	22^{k}	25^{j}	23^{j}	1.3	0.008	0.27	0.05
Digestibility, %								
DMd	64.4^{a}	52.4^{b}	70.5^{a}	50.8^{b}	3.07	0.40	0.001	0.12
OMd	55.6^{k}	58.9^{k}	64.4^{j}	60.3^{j}	1.99	0.040	0.88	0.13
NDFd	61.6^{ak}	33.8^{bk}	66.7^{aj}	34.0^{bj}	1.14	0.033	0.001	0.37
CPd	55.4^{bk}	59.7^{ak}	59.8^{bj}	61.4^{aj}	0.66	0.002	0.002	0.001

[1]S×H interaction; $^{a-b}$ Mean values with different letter in the same row differ for harvesting year ($P < 0.05$); $^{j-k}$ Mean values with different letter in the same row differ for forage species ($P < 0.05$); SEM, standard error of the mean.

including barley and triticale (9.2% CP, on average and on DM basis). The above indicated researchers also observed a strong effect of maturity stage on the CP content of cereals as forages.

The mean CP content of hooded barley cv. Mochona (11.0 ± 0.93%, on DM basis) was higher than other hays of hooded barley cultivars (cv. Haybet, Westford and Washford) reported by Robinson et al. (2001), Todd et al. (2003) and Brummer and Pearson (2004). Nevertheless, our CP value was lower than reported by Romero-Bernal et al. (2013) for hooded barley 12.4% CP. On the other hand, our hooded barley cv. Mochona preserved as hay, showed greater CP content than our triticale hay in both experimental years, and their values were greater than reported for hay of conventional barley (Feedipedia, 2021).

Regarding fiber composition (Table 3.1), hooded barley showed higher values than triticale (NDF, 67.0 vs. 64.8 ± 0.20%; ADL, 5.5 vs. 4.1 ± 0.10%, on average; $P < 0.001$). Additionally, both species showed lower ADL values in H2 (hooded barley, 5.4 ± 0.10%; triticale, 3.7 ± 0.10%; on average) than in H1 ($P = 0.002$; Table 3.1), although with higher NDF values in H2 (hooded barley, 68.9 ± 0.20%; triticale, 65.6 ± 0.20%; on average) than in H1 ($P < 0.001$; Table 3.1). Moreover, the fiber contents were inversely related to the CP content ($r^2 = -0.84$; $P = 0.002$) which agreed with Hoover (1986) and Harper and McNeill (2015).

3.4.2. Ingestibility of hays

No differences were observed on the voluntary intake of the ewes for both forage species (Table 3.1) expressed as DMI (31 ± 2.6 g/kgBW$^{0.75}$, on average; $P = 0.10$). Similarly, no differences were detected between values for both years (Table 3.1) as DMI ($P = 0.47$) and as OMI ($P = 0.52$) and NDFI ($P = 0.27$). However, the hooded barley showed lower digestibility data than triticale for OMI (25 vs. 32 ± 1.9 g/kgBW$^{0.75}$, on average; $P < 0.001$) and NDFI (20 vs. 34 ± 1.3 g/kgBW$^{0.75}$, on average; $P = 0.008$; Table 3.1). In addition, a S×H interaction was observed in NDFI ($P = 0.05$), as shown in Table 3.1.

According to Coleman and Moore (2003), CP content has a strong relationship with intake when dietary CP content is around 8%. Consequently, due to our greater CP contents obtained for both forages (hooded barley and triticale, 11.0 and 10.3%, respectively) did not observe correlation with DMI ($P = 0.47$) in our data, whereas NDF contents showed a slight negative correlation ($r^2 = -0.55$) but this value was not significant ($P = 0.31$). Moreover, Givens et al. (2000) and Harper and McNeill (2015) stated that the relationship between NDF contents and DMI is not always consistent.

Jeranyama and Garcia (2004) and Moore et al. (2020), proposed the use of relative feed value (RFV) as a useful method of ranking forages based on their fiber concentration. Forages with a RFV < 100 are considered of poor quality Amrita and Chairman (2012). The RFV of our hooded barley and triticale hays were 74 and 82, respectively. However, other values than the NDF and ADF used for RFV calculation (e.g., CP content, antinutritional factors) could interfere in its interpretation, so it should be only considered as a quality indicator (Gill et al., 2013).

Andueza et al. (2012) reported 17% greater DMI for triticale than the conventional barley (64 vs. 54 g/$BW^{0.75}$) in wethers. Instead, Hadjipanayiotou et al. (1981) observed that intake of hooded barley and conventional hays was not affected by the maturity stage in ewes although, as the authors stressed, both hays were not directly comparable because they were cultivated at different locations. Meanwhile, Todd et al. (2003) did not find differences for DMI (2.70 vs. 2.75 g/kg $BW^{0.75}$) between hooded barley and conventional barley, in ad libitum fattening steers.

According to Mertens and Grant (2020), DMI typically accounts for most of the variation of nutritive values among forages. In the same sense, Fahey et al. (1994) stated that 60 to 90% differences might be related to intake under ad libitum conditions. No marked effects were found in the DMI of our hooded barley cv. Mochona compared to triticale cv. Titania. Our hypothesis was that the absence of awns might give an advantage on the voluntary intake of barley in ewes, which was not supported by the obtained results. Both forages were similarly palatable for ewes under our conditions.

3.4.3. Digestibility of hays

Regarding apparent digestibility data, the results are summarized in Table 3.1. There was no difference between both forage species on DMd (59 ± 3.1%, on average; $P = 0.40$). By contrast, differences were detected by effect of harvesting year for DMd (H1 vs. H2, 67 vs. 52 ± 3.1%, on average; $P < 0.001$), the differences being a consequence of above indicated compositional values between years (Table 3.1). Therefore, a positive relationship was found between DMd and CP content ($r^2 = 0.70$; $P = 0.026$), but the relation was negative with NDF content ($r^2 = -0.70$; $P = 0.011$). On the other hand, the hooded barley showed lower digestibility of nutritional data than the triticale as OMd (57 vs. 62 ± 1.9%, on average; $P = 0.040$), NDFd (48 vs. 50 ± 1.1%, on average; $P = 0.033$), and CPd (58 vs. 61 ± 0.6%, on average; $P = 0.002$). Hence, OMd showed negative correlations but no significant with NDF content ($P = 0.65$) and ADL content ($P = 0.13$).

Regarding the effects of harvesting year, there were huge differences for NDFd (H1 vs. H2, 64 vs. 34 ± 1.1%, on average; $P < 0.001$), agreeing the greater CP content in H1 vs. H2 (Table 3.1). Congruently, the lower ADL values in the H2 may explain their greater CPd (H1 vs. H2, 58 vs. 61 ± 0.6%, on average; $P = 0.002$). The S×H interaction only was significant for CPd ($P < 0.001$; Table 3.1). Therefore, the CPd values were negatively

correlated with ADL contents ($r^2 = -0.75$; $P = 0.004$) and ADF ($r^2 = -0.66$; $P = 0.02$) contents. Consequently, differences of our DMd values may be a consequence of the above indicated variations in chemical composition due to the experimental year effect (Table 3.1).

Andueza et al. (2012) reported higher apparent digestibility data for conventional barley compared to triticale, expressed as DMd (69 vs. 65%), OMd (71 vs. 68%) and NDFd (65 vs. 56%), although values were lower for DMI (54 vs. 64 g/kg $BW^{0.75}$) and greater for NDF content (59 vs. 50%) in wethers. Todd et al. (2003) obtained greater digestibility values of DMd (65 vs. 59%) and NDFd (51 vs. 38%), when hooded vs. conventional barley were compared, under *ad libitum* conditions in steers, respectively. Nevertheless, Hadjipanayiotou et al. (1981) reported higher DMd in hooded barley than conventional barley (57 vs. 55%), although these results are not strictly comparable since both forages were grown at different locations.

Finally, despite the slight differences observed on the comparison of ingestibility and apparent digestibility of our hooded barley and triticale hays, both forages have greater CP contents (10.6 ± 0.93%, on average; $P = 0.61$) and lower OMd values (57 and 62 ± 1.9%, on average, respectively; $P = 0.040$) than those reported by Feedipedia (2021) for the reference conventional barley hay (8.7% CP and 66.7% OMd, on DM basis). Aguilar-López et al. (2013) reported greater CP and TDN (total digestible nutrients) values for conventional barley than for triticale hays, although the samples were processed at early maturation stages (145 d). No data on whole cereal crop hays are currently available in NRC (2001) and INRA (2007, 2018) feed tables.

3.4.4. Chemical composition of silages

Both forages showed adequate final pH values after ensiling (pH ≤ 4.1; Table 3.2), although they were greater for hooded barley than for triticale (3.9 vs. 3.6 ± 0.10; on average, respectively; $P < 0.001$) indicating differences between both forage species. The pH value is considered a silage quality indicator (Driehuis et al., 2001; Kung-Jr and Ranjit, 2001; Kung-Jr et al., 2018), and the high-quality silages must have pH ≤ 4.0 (Ryser et al., 1997; Driehuis et al., 2018).

The use of additives in H2, improved markedly the ensiling conditions and produced 10% lower pH than in H1 (Table 3.2). In this sense, the pH drops produced by adding

Table 3.2. Chemical composition, ingestibility (I) and apparent digestibility (d) of silage of cereal species (S) of hooded barley (cv. Mochona) and triticale (cv. Titania) in 2 consecutive harvesting years (H1, 2017; H2, 2018).

Silage	**Hooded barley**		**Triticale**		**±SEM**	***P* value**		
item	H1	H2	H1	H2		S	H	S×H[1]
pH	4.10^{aj}	3.72^{bj}	3.90^{ak}	3.34^{bk}	0.10	0.001	0.001	0.60
DM, %	28.0	32.2	28.2	29.7	3.60	0.10	0.33	0.33
Composition, % DM								
CP (N × 6.25)	12.5	10.5	10.7	10.0	0.63	0.25	0.18	0.47
CF	29.9^{by}	34.2^{ay}	31.4^{bx}	33.6^{ax}	0.77	0.066	0.001	0.002
NDF	52.2^{bk}	57.9^{ak}	52.5^{bj}	61.7^{aj}	0.13	0.008	0.001	0.001
ADF	31.6^{b}	35.4^{a}	33.2^{b}	34.9^{a}	0.44	0.13	0.001	0.001
ADL	4.0^{bj}	4.2^{aj}	3.2^{bk}	3.7^{ak}	0.04	0.001	0.001	0.060
NFE	40.3^{b}	47.3^{a}	42.8^{b}	46.4^{a}	0.91	0.15	0.004	0.20
OM	82.7^{b}	91.9^{a}	84.9^{b}	90.5^{a}	0.48	0.32	0.001	0.001
Ash	17.3^{a}	8.1^{b}	15.1^{a}	9.5^{b}	1.10	0.32	0.001	0.001
Ingestibility, g/kg $BW^{0.75}$								
DMI	27	38	32	30	2.7	0.70	0.24	0.12
OMI	23^{c}	37^{a}	30^{b}	30^{b}	1.7	0.85	0.012	0.013
NDFI	15^{b}	24^{a}	18^{b}	20^{a}	1.3	0.87	0.005	0.054
Digestibility, %								
DMd	52.6^{k}	52.5^{k}	61.5^{j}	61.0^{j}	0.77	0.001	0.74	0.82
OMd	56.6	60.6	64.9	57.0	1.78	0.36	0.44	0.027
NDFd	51.7^{b}	60.2^{a}	58.1^{b}	61.0^{a}	1.53	0.12	0.020	0.21
CPd	59.7^{k}	58.7^{k}	63.2^{j}	63.2^{j}	0.42	0.001	0.44	0.44

[1]S×H interaction; $^{a-c}$ Mean values with different letter in the same row differ for harvesting year ($p < 0.05$); $^{j-k}$ Mean values with different letter in the same row differ for forage species ($P < 0.05$); $^{x-y}$ Mean values with different letter tended to differ ($P < 0.10$); SEM, standard error of the mean.

inoculants in H2, were 0.4 and 0.5 units for hooded barley and triticale, respectively. According to Preston (2016), cereals as barley and triticale, have enough water-soluble carbohydrate concentration for lactic bacteria, as well as a low buffering capacity, so they ensiled adequately. In our study, the use of heterofermentative inoculant in H2 reduced the pH values (Table 3.2; $P < 0.001$), which numerically allowed more DM recovery after ensiling (Table 3.2; $P = 0.33$). On the other hand, a rapid decrease of pH would help to limit the protein breakdown in the silage by inactivating plant proteases, and to enhance its quality (Fahey et al., 1994; Muck et al., 2018). Nevertheless, no differences were observed in H1 data between CP values when hays and silages were compared (Table 3.1 vs. Table 3.2). No comparison was done for H2 because the hays of this year suffered rain during the

sun-curing process and, consequently, their CP contents were washed in the hays of both forage species and were lower than their corresponding silages (Table 3.1 vs. Table 3.2). No differences were detected between silages of both forage species (Table 3.2) for OM and ash contents ($P = 0.32$), as well as for CP ($P = 0.25$) and NFE ($P = 0.15$) contents.

By contrast, with the exception of CP content ($P = 0.18$), the harvesting year had marked influence (H1 vs. H2) for OM (83.8 vs. 91.2 ± 0.48%, on average; $P < 0.001$), ashes (16.2 vs. 8.8 ± 1.10%, on average; $P < 0.001$) and NFE (41.5 vs. 47.0 ± 0.91%, on average; $P = 0.004$), respectively (Table 3.2).

Regarding fiber contents, the hooded barley showed lower NDF than triticale (55.1 vs. 57.2 ± 0.13%; on average; $P = 0.008$), greater ADL content (4.1 vs. 3.5 ± 0.1, on average; $P < 0.001$) and no differences in ADF content (33.8 ± 0.44%, on average; $P = 0.13$), when both forage species were compared as silages. Respect to the effect of year (H1 vs. H2), we observed greater NDF (52.4 vs. 60.0 ± 0.13, on average; $P < 0.001$), ADF (32.4 vs. 35.2 ± 0.44, on average; $P < 0.001$), and ADL (3.6 vs. 4.0 ± 0.1, on average; $P < 0.001$) contents in H1 vs. H2. With regard to the S×H interaction, significant effects were observed in OM, NDF and ADF, as shown in Table 3.2 ($P < 0.001$).

Siefers et al. (1996) and González-García et al. (2016) reported greater CP content for conventional barley than triticale silages (12.5 vs. 9.2%, respectively). Instead, ZoBell et al. (1992) found lower CP content for conventional barley than triticale silages (8.5 vs. 10%, respectively). Nevertheless, several studies did not observe differences on CP content between conventional barley vs. triticale silages, as reported by McCartney and Vaage (1994; 12.0 vs. 11.6%), Jedel and Salmon (1995; 9.7 vs. 9.8%) and Nikkhah (2013; 12.4 vs. 12.7%), respectively.

3.4.5. Ingestibility of silages

No differences were observed in DMI (32 ± 2.7 g/kg $BW^{0.75}$, on average; Table 3.2) between forage species ($P = 0.70$) and harvesting year ($P = 0.24$), despite than in H2 the use of additives improved silage pH. Additionally, no differences in intake were detected between species when expressed as OMI (30 ± 1.7 g/kg $BW^{0.75}$, on average; $P = 0.85$) and NDFI (19 ± 1.3 g/kg $BW^{0.75}$, on average; $P = 0.87$). Nevertheless, harvesting year showed greater values in H2 than in H1 (Table 3.2) for OMI (37 vs. 23 ± 1.7 g/kg $BW^{0.75}$; $P = 0.012$) and NDFI (24 vs. 15 ± 1.3 g/kg $BW^{0.75}$; $P = 0.005$), respectively, accordingly with

the use of silage additives. The S×H interaction, was significant in the case of OMI (P = 0.013) and NDFI (P = 0.054), as shown in Table 3.2. No correlations were detected between DMI and CP content (P = 0.62), or NDF content (P = 0.79), nor pH (P = 0.63). In addition, silage quality calculated as RFV (Jeranyama and Garcia, 2004) gave approximately the same value in hooded barley (RFV, 106) and in triticale (RFV, 101) but, despite the use of silage additives, it was greater in H1 (RFV, 114) than in H2 (RVF, 96). Our results questioned the practical interest of RFV in winter cereal forages and when the fiber values are high.

On the contrary, McCartney and Vaage (1994) reported greater DMI in conventional barley than triticale silages (29 vs. 17 g/kg $BW^{0.75}$) in wethers, and ZoBell et al. (1992) in growing steers (89 vs. 83 g/kg $BW^{0.75}$). Nevertheless, our results agree with Nikkhah (2013) who did not observe DMI differences when comparing conventional barley and triticale in dairy cows.

With regard to the use of chemical and biological additives in the silage fermentation of conventional barley, the studies reported improved aerobic stability and small losses of DM during long preservation periods (Kung-Jr and Ranjit, 2001; Nishino et al., 2003; Gollop et al., 2005), although there are no convincing studies reporting improved voluntary intake, digestibility or feed efficiency in dairy cows (Driehuis et al., 2001; Oliveira et al., 2017; Muck et al., 2018). According to Buxton et al. (1996), the major regulators of DMI in herbivores include physical limitations, physiological control, and psychogenic factors. Consequently, in our study DMI could have been influenced by many other factors and among them: palatability, odour, proportion of $NH4^+$ and organic acids content (Campling, 1966; Chiofalo et al., 1992).

3.4.6. Digestibility of silages

Apparent digestibility data of the silages are summarized in Table 3.2. The DMd showed differences between species (Table 3.2), hooded barley values being lower than those of triticale (52 vs. 61 ± 0.7%, on average; P < 0.001). Similarly, differences were observed for CPd between hooded barley vs. triticale (59 vs. 63 ± 0.4%; P < 0.001) although no differences were observed between species for OMd (60 ± 1.7%, on average; P = 0.36) and NDFd (57 ± 1.5%, on average; P = 0.12).

Harvesting year affected NDFd (P = 0.020), which was lower in H1 vs. H2 (55 vs. 61± 1.5%, on average, respectively). No differences between years were detected in other nutrient digestibility coefficients (DMd, P = 0.74; OMd, P = 0.44; CPd, P = 0.44; Table 3.2). The S×H interaction showed differences only for OMd (P = 0.027). No correlations between CP content with DMd (P = 0.34), OMd (P = 0.98) or NDFd (P = 0.33) were found. Additionally, NDF content did not correlated with CPd (P = 0.80). McCartney and Vaage (1994) reported greater DMd (64 vs. 59%) in conventional barley vs. triticale silages, although no differences were found in OMd and NDFd in wethers, as in our results.

Our study did not show differences in DMd or OMd by harvesting year or by silage additive in both forages. The ensiled forages with additive might have improved their aerobic stability mainly due to the antifungal action (Oude-Elferink et al., 2001; Oliveira et al., 2017), preserving its nutritional quality once opened but without effects on intake and digestibility.

3.4.7. Nutritive values

Energy values of hays. Partitioning of energy values for our hays of hooded barley cv. Mochona and triticale cv. Titania, are shown in Table 3.3. Except for GE (4.71 ± 0.010 Mcal/kg DM, on average; P = 0.37) the hooded barley had lower energy values than the triticale when expressed as DE (2.54 vs. 2.77 ± 0.010 Mcal/kg DM, on average; P < 0.001), ME (2.23 vs. 2.34 ± 0.024 Mcal/kg DM, on average; P = 0.014) and NE_L (1.29 vs. 1.45 ± 0.042 Mcal/kg DM, on average; P = 0.010). Most likely this was a consequence of the lower value of OMd for hooded barley compared to triticale (57 vs. 62 ± 1.9%, on average; P = 0.004; Table 3.1), although, no correlation was detected between OMd and ME (P = 0.30). Consequently, the triticale hay had greater energy content for milk production, than hooded barley hay.

The harvesting year (H1 vs. H2) showed differences in GE (4.75 vs. 4.67 ± 0.010 Mcal/kg DM; P < 0.001), DE (2.69 vs. 2.62 ± 0.010 Mcal/kg DM; P < 0.001) and ME (2.35 vs. 2.22 ± 0.024 Mcal/kg DM; P = 0.010), although no differences were detected for NE_L (1.37 ± 0.042 Mcal/kg DM, on average; P = 0.54). Additionality, differences were detected in the S×H interaction of DE (P < 0.001) and NE_L (P = 0.020), as shown in Table 3.3.

The greater GE obtained in H1 when compared to H2, might be explained by the greater CP content of H1 (Table 3.1) and because the greater GE value of proteins with regard to carbohydrates. Accordingly, a positive correlation between GE and CP content was identified ($r^2 = 0.99$; $P < 0.001$), but not with NDF content ($P = 0.32$).

According to Feedipedia (2021), conventional barley hay had similar GE values as we obtained in the case of hooded barley hay (4.71 vs. 4.70 Mcal/kg DM, respectively). On the contrary, the DE value (2.72 vs. 2.54 Mcal/kg DM) was approximately 7% lower in hooded barley hay, because the greater OMd reported in Feedipedia (2021) with regard to the value measured in our hooded barley hay (66.7 vs. 57.3%, respectively), as previously reported in Table 3.1. This may be consequence of our greater NDF values compared to those of Feedipedia (2021), although no differences were detected on ME (2.22 vs. 2.23 Mcal/kg DM). No data are available for NE_L comparison in hooded barley vs. triticale hay, and consequently, our data are proposed.

Energy values of silages. With regard to silages, energy values for hooded barley cv. Mochona and triticale cv. Titania, are also shown in Table 3.3. Differences were detected between hooded barley vs. triticale only for GE (4.85 vs. 4.78 ± 0.010 Mcal/kg DM, on average; $P = 0.002$). A tendency was observed for ME (2.26 vs. 2.34 ± 0.025 Mcal/kg DM, on average; $P = 0.080$), without differences for DE (2.75 ± 0.050 Mcal/kg DM, on average; $P = 0.19$) and NE_L (1.40 ± 0.021 Mcal/kg DM, on average; $P = 0.12$).

The harvesting year effect (H1 vs. H2) showed differences in all energy values expressed as GE (4.88 vs. 4.75 ± 0.010 Mcal/kg DM; $P < 0.001$), DE (2.84 vs. 2.67 ± 0.050 Mcal/kg DM; $P = 0.023$), ME (2.36 vs. 2.23 ± 0.025 Mcal/kg DM; $P = 0.021$) and NE_L (1.45 vs. 1.35 ± 0.021 Mcal/kg DM; $P = 0.030$). Consequently, GE showed positive correlation with CP content ($r^2 = 0.93$; $P = 0.07$) and negative for NDF content ($r^2 = -0.94$; $P = 0.05$). The S×H interaction had significant effects for DE ($P = 0.003$), ME ($P = 0.002$) and NE_L ($P = 0.003$), as shown in Table 3.3.

When compared our hooded barley silage with the values reported in the feed tables of INRA (2018) for conventional barley silage (Code FE4790), our values were greater for GE (4.85 vs. 4.51 Mcal/kg DM) and ME (2.26 vs. 2.11 Mcal/kg DM), respectively. Additionally, the referential NE_L value informed by NRC (2001) for conventional barley silage is lower than our hooded barley silage (1.37 vs. 1.24 Mcal/kg DM, respectively).

Table 3.3. Nutritive values of hays and silages according to the cereal species (S) and harvesting year (H1, 2017; H2, 2018).

Item (DM basis)	Hay				±SEM	*P* value			Silage				±SEM	*P* value		
	Hooded barley		Triticale						Hooded barley		Triticale					
	H1	H2	H1	H2		S	H	S×H	H1	H2	H1	H2		S	H	S×H
Energy, Mcal/kg																
GE	4.75^{a}	4.67^{b}	4.74^{a}	4.66^{b}	0.010	0.37	0.001	0.95	4.92^{aj}	4.78^{bj}	4.84^{ak}	4.71^{bk}	0.010	0.002	0.001	0.64
DE	2.49^{ak}	2.59^{bk}	2.89^{aj}	2.65^{bj}	0.010	0.001	0.001	0.001	2.66^{a}	2.77^{b}	3.01^{a}	2.57^{b}	0.050	0.19	0.023	0.003
ME	2.27^{ak}	2.19^{bk}	2.43^{aj}	2.24^{bj}	0.024	0.014	0.010	0.10	2.19^{ay}	2.32^{by}	2.53^{ax}	2.14^{bx}	0.025	0.080	0.021	0.002
NE_L	1.24^{k}	1.34^{k}	1.52^{j}	1.38^{j}	0.042	0.010	0.54	0.020	1.32^{a}	1.42^{b}	1.58^{a}	1.28^{b}	0.021	0.12	0.030	0.003
Feeding values/kg[1]																
sFV[2]	2.62^{bj}	2.78^{aj}	2.47^{bk}	2.74^{ak}	0.030	0.052	0.003	0.20	3.07^{ak}	2.81^{bk}	3.56^{aj}	2.92^{bj}	0.042	0.010	0.002	0.034
UFL[3]	0.71^{k}	0.77^{k}	0.87^{j}	0.79^{j}	0.011	0.004	0.54	0.010	0.76^{a}	0.81^{b}	0.90^{a}	0.73^{b}	0.014	0.21	0.040	0.010
PDIA[4], g	22^{a}	16^{b}	21^{a}	15^{b}	0.7	0.37	0.004	0.97	22^{aj}	19^{bj}	19^{ak}	18^{bk}	0.4	0.020	0.020	0.12
PDI[5], g	68^{a}	65^{b}	72^{a}	65^{b}	0.7	0.12	0.008	0.12	68	69	69	67	0.4	0.37	0.37	0.040
RPB[6], g	−15^{by}	−36^{ay}	−18^{bx}	−41^{ax}	1.5	0.060	0.001	0.54	−15^{bk}	−27^{ak}	−26^{bj}	−30^{aj}	1.4	0.010	0.006	0.060

[1]Estimated according to INRA (2018); [2]Fill value for dairy sheep (sFV = 1 kg DM of reference grass); [3]Feed units for lactation (1.76 Mcal of NE_L); [4]Protein digestible in the intestine from dietary origin; [5]Protein digestible in the intestine from dietary and microbial origin; [6]Rumen protein balance; [a-b] Mean values with different letter in the same row differ for harvesting year ($P < 0.05$); [j-k] Mean values with different letter in the same row differ for forage species ($P < 0.05$); [x-y] Mean values with different letter tend to differ ($P < 0.10$); SEM, standard error of the mean.

According to Sauvant and Nozière (2016) and INRA (2018), the OMd has a key role for conversion efficiency from EM to NE_L, which explain our positive correlation found between OMd with EM ($r^2 = 0.99$; $P = 0.01$).

Feeding values of hays. According to INRA (2018), the ingestibility of forages is predicted from their intake capacity, conditioned by the physical bulk limitation and metabolic regulation in comparison to a forage of reference (i.e., standard grass hay); the value is expressed as sheep fill value (sFV). The sFV of our hays, varied according to the forage species (2.70 vs. 2.61 ± 0.030, on average; $P = 0.052$) and year (H1 vs. H2, 2.55 vs. 2.76 ± 0.030; $P = 0.003$), although these values corresponded to those of a poor forage of low intake and high bulk effect (Table 3.3).

Furthermore, in our data, the forage species determined the UFL values of hays, and the hooded barley was 11% lower than the triticale (0.74 vs. 0.83 ± 0.011 kg/DM; $P = 0.004$), although with no differences between years in their UFL values (0.79 ± 0.011 kg/DM, on average; $P = 0.54$). The S×H interaction had effect in UFL values ($P = 0.010$; Table 3.3). There are no referential data available for conventional barley hay in the feed tables of INRA (2018). Therefore, the values obtained in our study are proposed as referential data for hooded barley and triticale hays, respectively.

The protein nutritive values of hays are also summarized in Table 3.3. There were no differences between hooded barley vs. triticale in protein values expressed as PDIA (19 ± 0.7 g/kg DM, on average; $P = 0.37$) and PDI (68 ± 0.7 g/kg DM, on average; $P = 0.12$), but RPB showed a tendency to differ between both forages ($P = 0.060$), being lower in hooded barley than triticale (−26 vs.−30 ± 1.5 g/kg DM, on average). On the contrary, marked differences were observed by effect of the harvesting year (H1 vs. H2) on PDIA (22 vs. 16 ± 0.7 g/kg DM, on average; $P = 0.004$), PDI (70 vs. 65 ± 0.7 g/kg DM; $P = 0.008$) and RPB (-17 vs.−39 ± 1.5 g/kg DM; $P < 0.001$), respectively.

Rumen protein balance (RPB) integrates the quantitative effect of energy × nitrogen interactions on digestive processes and particularly on OMd and microbial growth (Sauvant and Nozière, 2016; Lapierre et al., 2018; INRA, 2018). The digestive microbiota dramatically decreases when RPB is under zero (Sauvant and Nozière, 2016). Therefore, when both forages were compared as hay, our hooded barley showed lower RPB value than triticale. Agreeing Sauvant and Nozière, 2016, the RPB of our hays was linearly

related to their CP content (r^2 = 0.99; *P* = 0.004). Unfortunately, there is no referential data available on RPB values for conventional barley hay in the INRA (2018) feed tables.

Feeding values of silages. Ensiled forages (Hooded barley vs. triticale) differed on sFV according to species (2.94 vs. 3.24 ± 0.042, on average; *P* = 0.010, respectively), years (H1 vs. H2, 3.32 vs. 2.87 ± 0.042; *P* = 0.002) with a significant S×H interaction (*P* = 0.034), as shown in Table 3.3. Both forages had higher values than the theoretical forage of reference (sFV = 1.36) of INRA (2018) as a consequence of their lower intake and high bulk effect.

No differences were observed for UFL values between silage species (0.80 ± 0.014 kg/DM, on average; *P* = 0.21). However, harvesting year (*P* = 0.040) and the S×H interaction (*P* = 0.010) had significant effects. According to the feed tables of INRA (2018; FE4790) and FEDNA (2016), the UFL values reported for conventional barley silage (0.73 and 0.72 UFL/kg DM, respectively) were lower than the value obtained in our hooded barley silage (0.79 UFL/kg DM; Table 3.3). No referential data are available for UFL of triticale in the FEDNA (2016) and INRA (2018) feed tables.

Table 3.3 shows the protein nutritive values of silages. With the exception of PDI, that did not differ between species (68 ± 0.4 g/kg DM, on average; *P* = 0.37), the hooded barley silage showed greater PDIA (21 vs. 19 ± 0.4 g/kg DM; *P* = 0.020) and RPB (−21 vs. −28 ± 1.4 g/kg DM; *P* = 0.010) contents than triticale. Differences were also observed between harvesting years (H1 vs. H2) for PDIA (21 vs. 19 ± 0.4 g/kg DM; *P* = 0.020) and RPB (−21 vs.−29 ± 1.4 g/kg DM; *P* = 0.006). Nevertheless, no differences were detected on PDI between years (*P* = 0.37; Table 3.3). Likely as previously reported for hays, a positive correlation was detected between CP content and RPB value (r^2 = 0.99; *P* < 0.001). Furthermore, S×H interaction, was significant in PDI content (*P* = 0.040) and a tendency was observed for RPB (*P* = 0.060), as shown in Table 3.3.

Therefore, our hooded barley silage had a lower RPB value than triticale, being also greater than the reported by INRA (2018) for conventional barley silage expressed as PDIA (21 vs. 14 g/kg DM), PDI (69 vs. 60 g/kg DM) and RPB (−21 vs.−27 g/kg DM).

3.5. CONCLUSIONS

Despite the marked differences between harvesting years, there were small differences in the chemical composition of the hooded barley (cv. Mochona) and the triticale (cv. Titania) species studied. However, comparing hooded barley and triticale, the first had greater CP content, when preserved as hay, but lower fiber contents when preserved as silage, than the second. These results seem to be a consequence of the absence of awns in barley. Nevertheless, DM intake in sheep did not differ for both preservation methods when forage species, harvesting years or their interaction were compared. Regarding apparent digestibility, the hooded barley hay had lower OMd values than triticale hay, apparently due to greater fiber contents, although no differences were detected for silages. Consequently, slightly lower energy values were found for hooded barley than triticale when preserved as hay, but values were opposite when preserved as silage. Moreover, greater PDI and RPB values were found in the hooded barley than triticale when preserved as silages.

Even though the high sFV values observed in all forages studied, hooded barley had lower bulk effect and lower deficit protein in the rumen than triticale when preserved as silage. Finally, as a consequence of the lack of awns, we conclude that the hooded barley cv. Mochona had greater nutritional value when compared to references of conventional barley in sheep.

CAPÍTULO 4

Effects of barley β-glucans on the performance and inflammatory responses of dairy ewes submitted to an endotoxin intramammary challenge in late lactating

Efectos de los beta-glucanos de cebada en el rendimiento y la respuesta inflamatoria de ovejas lecheras tratadas con endotoxina intramamaria al final de la lactación

CAPÍTULO 4

Effects of barley β-glucans on the performance and inflammatory responses of dairy ewes submitted to an endotoxin intramammary challenge in late lactating

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4.1. ABSTRACT

The objective of the current study was to explore the short-term immune responses of lactating dairy ewes supplemented with barley of different β-glucan (BG) content after a intramammary endotoxin challenge. Thirty-six ewes of two breeds (Lacaune and Manchega) in late lactation (210 ± 5 d in milk) were adapted to a diet of alfalfa hay ad libitum and 350 g/d of barley grain cv. Hispanic (3.8% BG, in DM basis), then they were allocated into 3 groups to which the experimental treatments were applied for 15-d. Treatments were: 1) Control (CON), fed the same diet as in adaptation (13.3 g BG/d); 2) High B-glucans barley (HBB), fed a new variety (cv. Annapurna) with 10% BG (35 g BG/d); and, 3) fed as CON and intraperitoneally injected (INP) at d 1 with a unique dose of 1.4% BG solution in distilled water (2 g BG/ewe). At d 9, all ewes in one udder half received 1 mL of an E. coli endotoxin solution (5 μg/mL), whereas 1 mL of saline solution was infused in the other. Rectal temperature (RT), milk yield and composition, somatic cell count (SCC) and plasma interleukins (IL-1α and IL-1β) were monitored daily for 4-d after the challenge (d 9 to 14) and analyzed by PROC MIXED for repeated measurements. The INP treatment induced a transitory increase (d 3 to 6) on RT (0.30 ± 0.05 ºC, on average; $P < 0.001$), peaked at d 4 (39.31 ± 0.05 ºC; $P < 0.001$), and decreased 38% milk yield ($P = 0.006$), on average for d 1 to 9, with regard to CON and HBB treatments. As a result of the challenge, RT increased from d 11 to 14 in all treatments ($P < 0.001$), whereas milk yield fell down 43% on d 13, without changes by effect of BG treatment ($P = 0.38$). Fat, protein and SCC contents increased in the udders LPS treated from (d 10 to 12; $P < 0.001$) but no for BG treatment. Plasma concentration of IL-1α and IL-1β, did not vary by BG treatment or challenge, although the INP ewes had lower IL-1β concentration than in the other treatments before the challenge ($P = 0.06$). This might support the greater trained immunity response in the INP ewes, that were BG injected, and the response to the amount of BG seemed to be more effective when used intraperitoneally, which needs further study.

4.2. INTRODUCTION

Nowadays, there is a growing concern with regard to animal health protection and welfare, which are key topics for farmers and consumers worldwide. Immunomodulation is a promising strategy to enhance the resistance to diseases, instead of using antibiotics (Volman et al., 2008; Byrne et al., 2020).

The β-glucans (BG) are natural cell wall polysaccharides found in yeast, mushrooms, some bacteria, seaweeds and cereals (Samuelsen et al., 2014; Jin et al., 2018). Their characteristics vary among groups according to BG linkage type, branching and molecular weight (Rieder and Samuelsen, 2012). Thus, in fungi and bacteria, BG consist of β-(1→3) and (1→6) linkages, whereas those of cereals have β-(1→3) and (1→4) linkages (Rieder and Samuelsen 2012; De-Arcangelis et al., 2019). Feeding BG from cereals has been related with improvements of the immune system stage and resistance against pathogens (Volman et al., 2008; Bartłomiej et al., 2012). The BG contents in barley range from 2 to 11%, which is higher than in other cereals (i.e., oat 2 to 7%, rye 1 to 3%, wheat 0.4 to 1.5%, as well as in sorghum and rice < 1%) (Rieder and Samuelsen, 2012; De-Arcangelis et al., 2019). Therefore, because of its generalized fodder use in animal nutrition and high content of soluble BG of low molecular weight, barley may have potential properties to reinforce the immune system of livestock.

According to Adams et al. (2008) and Jin et al. (2018), the innate immune system identifies pathogens (i.e. bacteria, viruses and parasites) through microbial structures (pathogen associated molecular patterns, PAMPs) by the so-called pattern recognition receptors (PRR). The BG molecules have the ability to bind to PRR through its main receptor (Dectin-1), which is present on the surface of macrophages, dendritic cells and neutrophils (Brown and Gordon, 2003). Therefore, supplementing with BG can initiate the complex signalling pathways of immunity. Consequently, barley with high BG contents might be a valuable source of biologically active compounds to include in ruminant feeding programs, as a possible method to train its immune system against a broad spectrum of pathogens.

Lipopolysaccharide (LPS) is a PAMPs of Gram-negative bacteria, such as *Escherichia coli* (Akarsu and Mamuk, 2007; Meneses et al., 2018), which acts as a potent inducer of inflammation by stimulating immune system cells to produce pro-inflammatory cytokines and acute-phase proteins (Sly et al., 2004; Hadfield et al., 2018). Consequently, LPS

challenge has been experimentally used as a common model to study the inflammatory response induced by pathogenic *E. coli*, or endotoxins in the mammary gland of lactating ruminants (Castro-Costa et al., 2014; Campos et al., 2018).

Grove et al. (2006) studied the disappearance and digestion of barley BG in situ (rumen) and in vivo (full digestive tract) in cows, concluding that they vary according to varieties and that is possible to expect enough BG concentration to produce effects in the gastrointestinal tract. In this sense, Torrent (2015) and Torrent et al. (2017) indicated that BG left the rumen not completely degraded in dairy ewes, being its metabolites detected in milk and urine (Contreras-Jodar et al., 2017). These evidences support that solubilized BG leave the rumen undigested and are detected in blood with potential metabolic and productive positive effects in ruminants. Moreover, although the mechanism is unknown, oral BG showed stimulation of the mammalian immune system in mice (Yun et al., 1992, 2003; Davis et al., 2004). No information is available in lactating ruminants.

Despite barley is a cereal widely used in the diet of ruminants, to our best knowledge, there are no studies on the effect of using barley BG as functional feed ingredient for modulating the immune system of ruminants. Therefore, the objective of this study was to evaluate the short-term immune responses of dairy ewes supplemented with barley BG and submitted to an intramammary LPS challenge.

4.3. MATERIALS AND METHODS

4.3.1. Ethical approval

This experiment fulfilled the Spanish requirements on the protection of animals for experimental purposes (RD 52/2013) and were approved by the Ethical Committee of Animal and Human Experimentation of the Universitat Autònoma de Barcelona (CEEAH reference #3871).

4.3.2. Animals and management

A total of 36 adult ewes of two dairy breeds (**MN**, Manchega, n = 18; **LC**, Lacaune, n = 18), with similar body weight (MN, 79.3 ± 2.1; LC, 77.4 ± 3.1 kg BW) and body condition score (MN, 3.50 ± 0.19; LC, 3.54 ± 0.26 units of BCS) from the herd of the Servei de Granges i Camps Experimentals of the UAB (Bellaterra, ES), were used. The

ewes were healthy, in late lactation (210 ± 5 DIM, on average) and differed in milk yield according to the breed (MN, 0.96 ± 0.12; LC, 1.68 ± 0.19 kg/d). Udder healthiness of the ewes was checked in each udder half using somatic cell count (SCC < 200×10^3 cells/mL) at milk recording at recruitment.

The ewes were kept in straw-bedded pens, ×1 feed indoors (0900 h) and ×2 machine milked (0730 and 1600 h) in a 2×12-parallel stalls milking parlor (DeLaval-España, Alcobendas, ES) equipped with silicone milking clusters (SG-TF100, DeLaval, Tumba, SE) and automatic milk-flow and milk-recording devices (MM25SG, DeLaval). Milking was conducted at a vacuum of 40 kPa, 120 pulses/min and 50% pulsation ratio. The milking routine included cluster attachment, machine milking, and automatic cluster detachment (milk flow rate <0.1 L/min or milking time >3 min). Teat dipping with a iodine solution (P3-io shield; Ecolab Hispano-Portuguesa, Barcelona, ES) was done at the end of milking. Data of ambient temperature and relative humidity during the study were recorded every 20 min by using an aerial probe suspended in the middle of the pens connected by Bluetooth to a weather station (Nexus 35.1075, TFA Dostmann, Reicholzheim, DE). Data were downloaded and processed using its own software (Nexus v.1.3, TFA Dostmann).

4.3.3. Experimental treatments

After an adaptation period of 10-d, the ewes were allocated into 3 experimental treatments for 15-d and submitted to an intramammary LPS challenge at d 9. During adaptation, the ewes were fed a diet consisting of alfalfa hay ad libitum (18% CP; in DM basis; 1.45 Mcal of NE_L/kg and RPB 25 g kg/DM) and individually supplemented at a.m. milking with 350 g/d barley whole grain cv. Meseta (Batlle, Bell-lloc, ES) containing 3.8% BG (13.3 g/d BG per ewe). The ewes had free access to water and commercial micromineral blocks (Na, 36.74%; Ca, 0.32%; Mg, 1.09%; Zn, 5 g/kg; Mn, 1.5 g/kg; S, 912 mg/kg; Fe, 304 mg/kg; I, 75 mg/kg; Co, 50 mg/kg; Se, 25 mg/kg; Ovi bloc, Sal Cupido, Barcelona, ES). After the adaptation period, the ewes were allocated into 3 balanced groups according to breed, milk yield, BW and BCS, and the groups randomly assigned to the experimental treatments.

Treatments were: i) Control (**CON,** n = 12) fed the same diet as during adaptation (13.3 g/d BG); ii) High B-glucans barley (**HBB,** n = 12) fed with 350 g/d of a new barley variety (cv. Annapurna, Batlle, Bell-lloc, ES) containing 10% BG (35 g/d BG); and, iii)

Intraperitoneally injected (**INP,** n = 12) at experimental period d-1 with a unique dose of a 1.4% BG solution (2 g BG/ewe).

The 1.4% BG solution was prepared the day before injection, using BG powder extracted from HBB barley (provided by Marian Moralejo; Agrotecnio Center, Universitat de Lleida, ES) dissolved in distilled water (37ºC for 1 h) with the help of a magnetic hot plate stirrer (MCG05E, Ovan, Barcelona, ES). The 1.4% BG solution was stored overnight in closed bottles at room temperature, and intraperitoneally injected (140 mL/ewe) in the right flank paralumbar fossa of the INP ewes, by using a 1.10×40-mm needle (19G×1½", Braun, Melsungen, DE) and plastic syringes (Ico plus3, Novico Médica, Barcelona, ES), the day after preparation.

4.3.4. Measurements, sampling, and analyses

4.3.4.1. Physiological and performance data

The rectal temperatures (RT) were measured daily at 0800 h using a digital clinical thermometer (AccuVet, Cei Technology, Taoyuan City, TW; range, 32 to 45°C; accuracy, ±0.1°C). Milk yield (kg/d) of individual ewes was recorded automatically at each milking (MM25SG, DeLaval). Furthermore, at the beginning and the end of the experimental period (d 1 and 15), BW was measured using an electronic scale (True-test, A6500, Auckland, NZ; accuracy, ± 0.2 kg), while BCS was assessed (0 to 5 points; accuracy, ± 0.25 points) according to Russel et al. (1969).

4.3.4.2. Udder health

Before milking on d 7, milk samples from each udder half of all ewes were collected for bacterial culture. With this aim, udder teats were dipped in a iodine solution (P3-io shield), dried with disposable paper towels, and whipped with ethanol 70%. Then, the initial milk squirts were hand-milked with sterile gloves and discarded, and 5 mL milk were collected in sterile tubes. The milk samples were preserved at 4ºC and 0.1 mL of milk streaked directly onto sheep blood agar plates (Agar Sangre 90 mm, Madrid, ES) on the same day and incubated at 37ºC (Heraeus B-5042,Hanau, DE) until examination. Plates were examined at 48 h for bacterial growth of major mastitis pathogens. All ewes enrolled in the experiment had culture-negative udder halves. In addition, milk samples (50 mL) for

SCC were collected in standard plastic containers and preserved with an antimicrobial tablet (Bronopol, Broad Spectrum Micro-tabs II, D&F Control Systems, San Ramon, CA) at 4°C according to the Dairy Herd Improvement Laboratory of Catalonia (ALLIC, Cabrils, ES) procedures. The SSC were determined by an automatic cell counter (Fossomatic 5000, Foss, Hillerød, DK).

4.3.4.3. Intramammary endotoxin challenge

The endotoxin solution of *E. coli* used to induce the intramammary challenge was prepared by diluting 5 mg of purified LPS (*E. coli*, serotype O55:B5:L2880; Sigma-Aldrich, St. Louis, MO) in 5 mL of physiological saline (0.9% NaCl; Braun, Barcelona, ES). The solution was homogenized using a vortex mixer (Heidolph instruments, Schwabach, DE) for 1 min, and aliquots of 5 µg/mL of LPS, as stock solution, were performed under aseptic conditions and stored at −20ºC in polypropylene Eppendorf tubes (Deltalab, Barcelona, ES) until use.

On the d 9 where the LPS challenge was performed, approximately 30 min after the a.m. milking, the teat tips were disinfected with the iodine solution (P3-io shield) and whipped with ethanol 70%. Thereafter, one udder half was infused via the teat canal with 1 mL LPS solution (5 µg/mL), at random, while the other half was infused with 1 mL of saline solution (0.9% NaCl), using aseptic polypropylene syringe cannulas (Distritip 1.5-mm o.d. and 12.5 mL, Gilson, Madrid, ES). A gentle massage in the cisternal direction was performed immediately after each injection.

Systemic and local signs of reactions to the LPS challenge were monitored every 24-h by measuring RT (AccuVet) pre- (d 9) and post-challenge (d 10 to 15). Moreover, milk samples (50 mL) before routine a.m. milking, from each udder half, were collected pre- (d 9) and post-challenge (d 10 to 12, and d 14) and preserved with antimicrobial tablets (Bronopol) at 4°C until analysis. Major milk components (fat, total protein, lactose, total solids, and urea) and SCC were determined using Milkoscan (MilkoScan FT2, Foss, Hillerød, DK) and Fossomatic 5000, respectively, in the ALLIC laboratory.

Blood samples pre- (d 9) and post-challenge (d 10 and 14) were taken by jugular venepuncture using Vacutainer tubes with lithium heparin 68 IU (BD, Belliver Industrial Estate, Plymouth, UK). Plasma was separated by centrifugation at 3,000 × *g* for 15 min at

4ºC using a swing-bucket rotor (Hettich, Tuttlingen, DE). Then, 1.5 mL of plasma were transferred into Eppendorf tubes and stored at −20ºC until analyses. Concentrations of IL-1α and IL-1β plasma interleukins, were determined using commercial ELISA kits (Cusabio High-Tech, Houston, TX) designed and validated for sheep. For IL-1α, the competitive inhibition enzyme immunoassay technique (detection range, 31.3 to 2000 pg/mL) was used, whereas for IL-1β the quantitative sandwich enzyme immunoassay technique (detection range, 15.6 to 1000 pg/mL) was used. The ELISA plates were read in an automatic reader (iEMS Reader MF V.2.9−0, Labsystems, Helsinki, FI) at 450 nm. Intra- and inter-assay precision coefficients were 8 and 10%, respectively, in both cases.

4.3.5. Statistical analyses

All statistical analyses were performed using SAS v.9.4 (SAS Institute Inc., Cary, NC, USA) based on a linear mixed model for repeated measures (PROC MIXED). Diagnostic tests were conducted using UNIVARIATE procedure to determine whether residual of the data had normal distribution. For all models, 3 covariance structures were tested (Compound Symmetry, Unstructured and Autoregressive order 1) and the one with the smallest Bayesian information criteria was selected. For SCC data, logarithmic transformation ($\log_{10}$) was done to ensure normal distribution. For performance data, the statistical models considered the treatments (CON, HBB and INP), the sampling day and their interactions as fixed effects, the ewe and residual error were taken as random effects. For the LPS challenge data, the statistical model included the treatments, the sampling day, and treatment × day interactions as fixed effects, considering the udder half nested within the animal and the residual error, as random effects. The measurements taken before the LPS infusions (d 9), were considered as covariates to obtain the specific effect of the challenge. Furthermore, when the main effects or interactions were significant, differences between least squares means were determined using the PDIFF option of SAS. Values were considered significant when $P < 0.05$ and tendencies declared at $P < 0.10$.

4.4. RESULTS AND DISCUSSION

Daily mean ambient temperatures slowly increased during the experiment (range from 22 to 28°C; 25°C, on average), which affected the RT values of the ewes in all treatments (Figure 4.1). Pearson correlations between ambient and RT were significant and positive

for all treatments ($r^2 = 0.99$; $P < 0.001$). The RT values of the ewes before the challenge (d 0 to 9), showed an increasing trend until d 4 and differed between treatments ($P < 0.001$; Figure 4.1). This was in part due to the increase of ambient temperatures, which decreased from d 5 to 9 pre-challenge. A quadratic response between ambient temperature and RT was observed for both pre- and post-challenge periods ($r^2 = 0.38$).

4.4.1. Pre-challenge period

Thermophysiological responses. On average, the RT values during the pre-challenge period were greater in INP ewes (39.12 ± 0.05°C), in comparison to CON (38.74 ± 0.05°C; $P < 0.001$) and HBB (38.90 ± 0.05°C; $P = 0.005$), the CON and HBB also differing between them ($P = 0.033$; Figure 4.1). Nevertheless, all values returned close to the initials (38.65 ± 0.03) at d 9 (38.52 ± 0.05ºC; $P = 0.35$), indicating that the response to the INP treatment faded, allowing us to initiate the LPS challenge without residual effects.

The variation of RT values in the CON ewes showed the impact of ambient temperatures and their adaptation to the experimental measurements (i.e., rectal thermometer), as the hyperthermia induced by the management stress (Piccione et al., 2002; Elhadi et al., 2019) . Additionality, the HBB showed differences with the CON ewes from d 4 to 8 ($P = 0.10$ to 0.03), which may be a consequence of the ingestion of the high BG barley. In our knowledge, no such effect was earlier reported in ruminants.

The pyretic reaction is a new evidence reported in sheep fed high BG and may be a result of the cascade immune response led by the synthesis and secretion of cytokines shown in humans (Appenheimer et al., 2005), as later discussed. The INP treatment induced its greatest RT increase during the pre-challenge period from d 3 to 6 ($P = 0.05$ to 0.001), when compared to CON and HBB. Regarding HBB, only RT differences to CON were observed after d 4 ($P = 0.030$ to 0.05), as shown in Figure 4.1. No interactions between BG treatment × day were detected ($P = 0.16$) during the whole pre-challenge period.

The pyretic reaction of the ewes to BG administration was greater in INP than HBB ewes, which indicated that the biological effects of BG depended on the amount supplied and on the administration way, agreeing Soltanian et al. (2009) and Brown and Williams (2009). Although Contreras-Jodar et al. (2017) reported that barley BG orally administrated escape partially from rumen degradation, the BG intraperitoneally injected

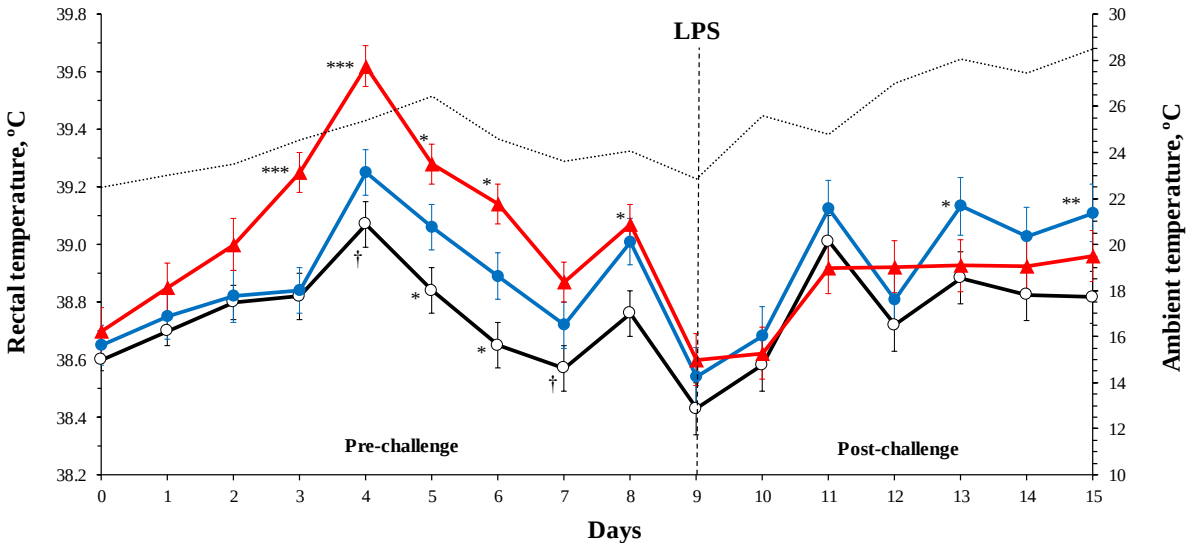

Figure 4.1. Variation of rectal temperatures according to B-glucans (BG) treatments (CON: ○, Control; HBB: ●, High BG barley; INP: ▲, Intraperitoneally) and by effect of the Lipopolysaccharide (LPS) challenge in dairy ewes. Values are LSM and vertical bars represent SEM (⋯, mean ambient temperature of the barn; significant differences between the closest measurements are indicated by symbols: †, $P < 0.10$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$).

(INP, 2 g BG/ewe), should have been more bioavailable than those orally administrated (35 g BG/ewe and d) or the rumen effective degradation lower than 94.2% (i.e., including rumen flow).

Even though the increase in body temperature represents an adaptative mechanism to facilitate host resistance and inhibition of pathogens spread (Ostberg et al., 2000), several reports documented that hyperthermia is associated with the enhancement of the innate and adaptive immune responses (Boltaña et al., 2013). Consequently, in our study, the RT increases could have been a signalling pathway to enhance animal immunity (Zhao et al., 2007).

Lactation performances. Milk yield responses to BG administration are shown in Figure 4.2. Although milk yield did not vary from d 0 to 9 in CON and HBB ewes (0.80 ± 0.07 kg/d, on average; $P = 0.71$), the INP treatment produced a 38% drop in milk yield,

when compared to them (0.50 ± 0.07 kg/d, on average; P = 0.004 and 0.009, respectively). Milk yield of INP ewes returned to their initial values at d 7, confirming the recovery observed for RT values. As a consequence, a treatment × day interaction was identified (P < 0.001) during the pre-challenge period in our ewes. According to Kvidera et al. (2017), a decrease in milk synthesis is one of the first observable signs of immunoactivation in dairy cows. Therefore, in our experiment, the dramatic decrease of milk yield in the INP ewes, could have been the result of addressing glucose to the activity of immune cells (Lochmiller and Deerenberg, 2000), instead of using the glucose for the synthesis of lactose in the mammary gland.

Regarding milk composition, no effects were detected by BG treatment at the end of the pre-challenge period (d 9; P = 0.27 to 0.94). Mean values of milk composition were characteristic of dairy ewes in late-lactation (fat, 7.67 ± 1.36%; protein, 6.56 ± 0.10%; lactose, 4.39 ± 0.29%; total solids, 19.46 ± 2.36%; urea, 85.9 ± 10.0 mg/dL), as also reported by Castro-Costa et al. (2014) in the same breeds and similar experimental conditions.

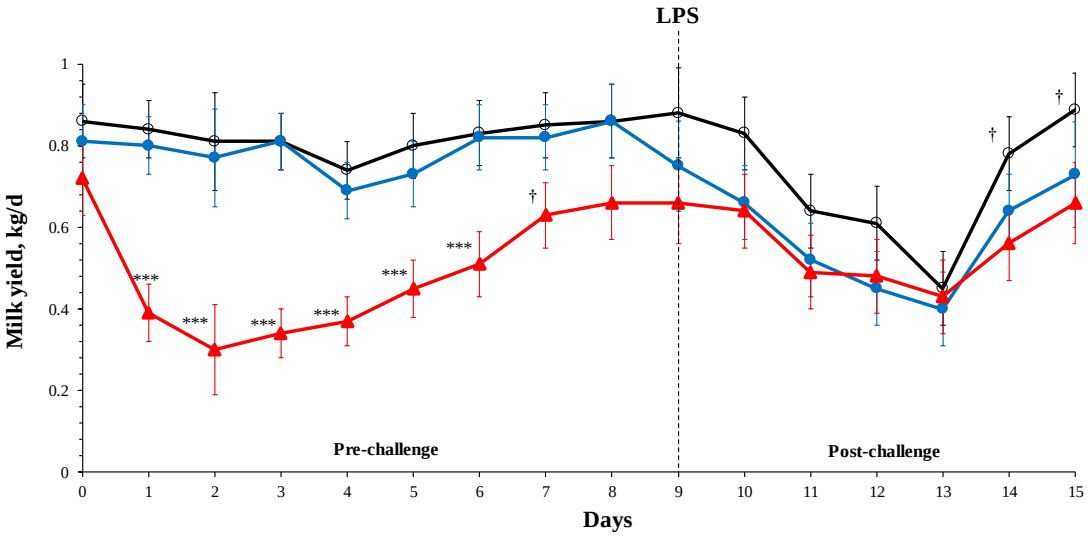

Figure 4.2. Milk yield of dairy ewes according to B-glucans (BG) treatments (CON: ○, Control; HBB: ●, High BG barley; INP: ▲, Intraperitoneally) and by effect of the Lipopolysaccharide (LPS) challenge in dairy ewes. Values are LSM and vertical bars represent SEM (significant differences between the closest measurements are indicated by symbols: †, P < 0.10; ***, P < 0.001).

***Immunity responses*.** Value of $\log_{10}$ SCC was 6% greater in the INP (5.15 ± 0.09; *P* = 0.04) than CON and HBB ewes (4.84 ± 0.09, on average; *P* = 0.55), during the pre-challenge period. The SCC provides an indication of the inflammatory response of the ewes' mammary gland, which agreed with the pyretic response of the INP ewes above described. This increase of SCC in the mammary gland of INP treated ewes (equivalent to 72,495 cells/mL), may be the response to the recruitment of sentinel cells against mastitis-causing pathogens, mainly neutrophils (Alhussien and Dang, 2018) in sheep, as well as fibroblasts (Chen et al., 2016), without developing a true mammary infection.

Regarding plasma interleukins, the value of the IL-1α (anti-inflammatory) in the blood of the ewes submitted to the INP treatment (249 ± 85 pg/mL), decreased by 36% than in CON (444 ± 85 pg/mL*; P* = 0.50) and HBB (333 ± 85 pg/mL; *P* = 0.12) ewes, on average, but the differences were only numerical. Moreover, the IL-1β (pro-inflammatory) blood concentration in the INP treated ewes, was 73% lower (47 ± 46 pg/mL), in comparison to the CON (*P* = 0.09) and HBB (*P* = 0.06) that were similar (174 ± 46 pg/mL, on average; *P* = 0.99). These results indicate both pre- and pro-inflammatory lower status of the INP ewes at the end of the pre-challenge period, which could be a signal of an enhanced immunity system. A similar decrease in pro-inflammatory status was also reported by Angulo et al. (2020) in newborn goats fed with BG extracted from *Debaryomyces hansenii*, a species of yeast from the family *Saccharomycetaceae.*

4.4.2. Post-challenge period

***Thermophysiological responses*.** Values of RT by BG treatment after the intramammary LPS challenge, are shown in Figure 4.1. The LPS infusion triggered a RT increase in all ewes until d 11 (range, 0.32 to 0.58°C; Figure 4.1), and stayed thereafter. On average, the RT values were greater in HBB than in CON ewes (38.92 ± 0.06 vs. 38.75 ± 0.05°C; *P* = 0.043), but no differences were detected to INP ewes, which were intermediate (38.84 ± 0.05°C; *P* = 0.32 and 0.27, respectively to HBB and CON). Moreover, RT values of the HBB ewes remained slightly higher from d 11 until the end of the experiment, but only the differences with the CON ewes were detected at d 13 (*P* = 0.05) and 15 (*P* = 0.02), as shown in Figure 4.1. No interaction treatment × sampling day was detected (*P* = 0.61).

Castro-Costa et al. (2014) and Shangraw et al. (2019), using a similar LPS concentration and dose per BW (5 μg/mL and approximately 0.06 μg/kg BW, in ewes and cows, respectively), observed a transitory RT peak immediately after LPS intramammary infusion (6 h), which disappeared the day after. Campos et al. (2018) reported a greater and longer RT increase (approximately, 1 to 1.5°C from 2 to 11-h post-challenge) when dairy cows were LPS treated (10 mL of 2.5 μg/mL LPS), as a response mechanism for thermoregulation and activation of the immune response. Although a RT increase of 2-d was also observed in our ewes, no decrease was later detected because the positive trend of ambient temperatures previously indicated. The mild and steady RT increase observed in our INP ewes, could be congruent with the hypothesis of the immunomodulatory effects of barley BG and its potentiality to alleviate the negative systemic impact of the local infusion of *E. coli* endotoxin.

Lactation performances. Effects of LPS on the milk yield of our ewes according to BG treatments, are shown in Figure 4.2. Overall, the LPS challenge decreased by 43% the whole udder milk yield for all BG treatments from d 9 to 13. This decrease disappeared after d 13 and only tendencies to differ were detected between the CON and INP ewes at d 14 and 15 post-challenge (P = 0.092 and 0.086, respectively; Figure 4.2). Moreover and on average, mean milk yield of the whole udder during the post-challenge period did not differ among treatments (0.57 ± 0.08 kg/d; P = 0.38). There was no interaction between treatment × sampling day (P = 0.32).

To assess the effects of BG treatments on individual mammary function, we compared milk yield between udder halves (i.e., LPS-infused vs. saline-infused), as proposed by Heyneman et al. (1990) and Hoeben et al. (2000) for assessing the systemic effects of *E. coli* infected quarters in dairy cows. According to the results shown in Table 4.1, values of milk yield by udder half varied by effect of LPS challenge (P = 0.019) and day (P < 0.001), but did not vary by effect of BG treatment (P = 0.29).

Similarly to our results, Mehrzad et al. (2001) and Silanikove et al. (2011), reported 30 to 80% milk yield decrease 24-h in the LPS challenged quarters of dairy cows. Moreover, Castro-Costa et al. (2014) reported 36% milk yield decrease 72-h post-challenge in the LPS treated udder halves, compared to those saline infused, in dairy ewes. A BG × Day (P < 0.001) interaction was also detected for milk yield, the INP ewes no showing differences

Table 4.1. Lactational effects of the intramammary LPS challenge or saline infusion by udder half according to B-glucans (BG) treatments in dairy ewes.

	Treatments[1]						Mean ± SEM	*P* value				
	CON		HBB		INP							
Milk	Saline	LPS	Saline	LPS	Saline	LPS		Challenge	BG	Day	BG × Chall[3]	BG × Day[4]
Yield, kg/d	0.36^{a}	0.33^{b}	0.28^{a}	0.26^{b}	0.28^{a}	0.23^{b}	0.29 ± 0.04	0.019	0.29	0.001	0.69	0.001
Composition												
Fat, %	8.26^{b}	8.63^{a}	8.85^{b}	9.46^{a}	8.62^{b}	8.91^{a}	8.79 ± 0.46	0.001	0.55	0.001	0.57	0.001
Protein, %	6.89^{b}	7.03^{a}	6.93^{b}	7.40^{a}	6.75^{b}	6.89^{a}	6.95 ± 0.29	0.003	0.65	0.001	0.27	0.73
Lactose, %	3.70^{x}	3.50^{xy}	3.47^{xy}	3.41^{xy}	3.85^{x}	3.64^{x}	3.59 ± 0.09	0.10	0.06	0.001	0.76	0.20
Total solids, %	19.54^{b}	20.13^{a}	20.17^{b}	21.26^{a}	20.17^{b}	20.39^{a}	20.28 ± 0.77	0.001	0.73	0.001	0.20	0.44
Urea, mg/dL	85	86	87	89	81	81	85 ± 3	0.22	0.30	0.001	0.82	0.23
Log_{10} SCC[2]	6.13	6.24	6.43	6.29	6.17	6.28	6.25 ± 0.13	0.77	0.42	0.001	0.44	0.001

[1]B-glucans (BG) treatments (CON: Control; HBB: High BG barley; INP: Intraperitoneally) and infusions by udder half: Saline (1 mL of 0.9% sterile saline solution) and LPS (5 μg/mL endotoxin solution from *E. coli*); [2]Somatic cell count were log_{10} transformed; [3]BG treatment × challenge interaction; [4]BG treatment × sampling day interaction; SEM, standard error of the mean; $^{a–c}$Means with different letter in the same row differ at $P < 0.05$; $^{x–y}$ Means with different letter in the same row differ at $P < 0.10$.

between LPS and saline infused udder halves at d 11 (P = 0.38), as observed in Figure 4.3a.

With regard to milk composition (Table 4.1), most milk components increased by effect of the LPS challenge (fat, $P < 0.001$; protein, $P = 0.003$; total solids, $P < 0.001$), on average, agreeing with the milk yield drop. On the contrary, lactose content tended to decrease (P = 0.10), on average, by effect of the impairment of mammary function produced by the LPS. The decrease in lactose milk content also tended to vary according to BG treatment (P = 0.06), as shown in Figure 4.3b.

One of the most marked local effects of LPS infusion in the mammary gland, is the increased permeability of the blood-milk barrier in the mammary epithelial tissue (i.e., leaking tight-junctions), which leads to the scape of milk components, specially lactose (Shangraw et al., 2019). This effect is mediated by the nitric oxide released by the milk SCC recruited during the LPS challenge (Bouchard et al., 1999).

In agreement with our results, Silanikove et al. (2011) and Castro-Costa et al. (2014), reported that lactose content dramatically decreased 24-h post-challenge in the LPS treated cow's quarters or ewe's half udders, respectively, indicating the opening of the tight junctions of the mammary epithelial cells. In our ewes, all milk components in the INP treatment were less affected than those of CON and HBB treatments (Table 4.1), which could indicate a lower affectation of the vascular and mammary epithelial cells permeability by effect of the LPS challenge.

***Immunity responses*.** The $\log_{10}$ SCC did not differ between half udders of the ewes of all BG treatments (P = 0.36 to 0.75) before infusions (4.94 ± 0.14, on average). Nevertheless, both saline and LPS infusions, provoked the migration of a large amount of blood leucocytes into the alveolar lumen and the milk (Figure 4.3c), which varied by day effect ($P < 0.001$), but not for challenge (P = 0.77) or BG treatments (P = 0.42; Table 4.1). A peak in the SCC from half udders was observed at d 10 which greater in the LPS infused than the saline infused udders for all BG treatments (P = 0.05 to 0.004; Figure 4.3c). On the contrary, SCC values of the LPS infused udders were lower than the saline infused udders for CON and INP treatments at d 14 (P = 0.02 to 0.001).

Only the BG × day interaction ($P < 0.001$) observed in the SCC values of our ewes seem to indicate that the evolution of the leucocyte recruitment varied on time according to

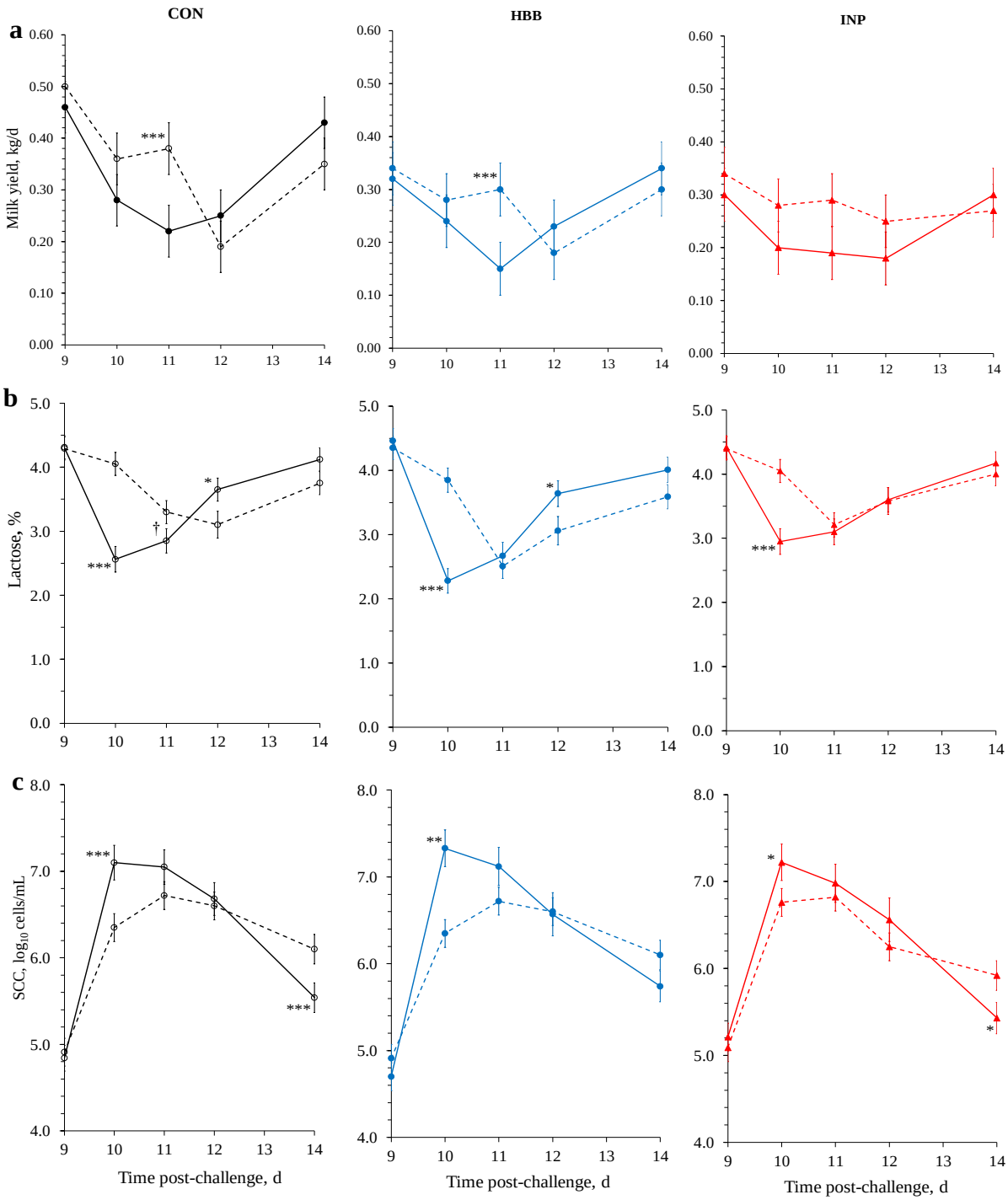

Figure 4.3. Milk yield (a), lactose (b) and somatic cell count (c) of dairy ewes in late lactation relative to B-glucans (BG) treatments (CON: ○, Control; HBB: ●, High BG barley; INP: ▲, Intraperitoneally) and according to infusion by udder half (CON-LPS: ○; CON-saline - -○- -; HBB-LPS: ●; HBB- Saline: - -●- -, and INP-LPS: ▲; INP- Saline - - ▲- -). Values are LSM, and vertical bars represent SEM. (significant differences are indicated by : †, $P < 0.10$; *** $P < 0.001$: ** < 0.01: * $P < 0.05$).

BG treatment, the HBB treated ewes returning earlier to the initial values. A faster SCC recruitment translates into a more effective capture and elimination of the endotoxin (Campos et al., 2018).

With regard to plasma interleukins, their evolution during the post-challenge period is shown in Figure 4.4. The IL-1α (anti-inflammatory) concentration did not differ by BG treatment (376 ± 92 pg/mL, on average; P = 0.69), time after challenge (P = 0.52) or their interaction (P = 0.85). Similarly, the IL-1β (pro-inflammatory) concentration did not vary among BG treatments (136 ± 51 pg/mL, on average; P = 0.66), time after challenge (P = 0.62) or their interaction (P = 0.33).

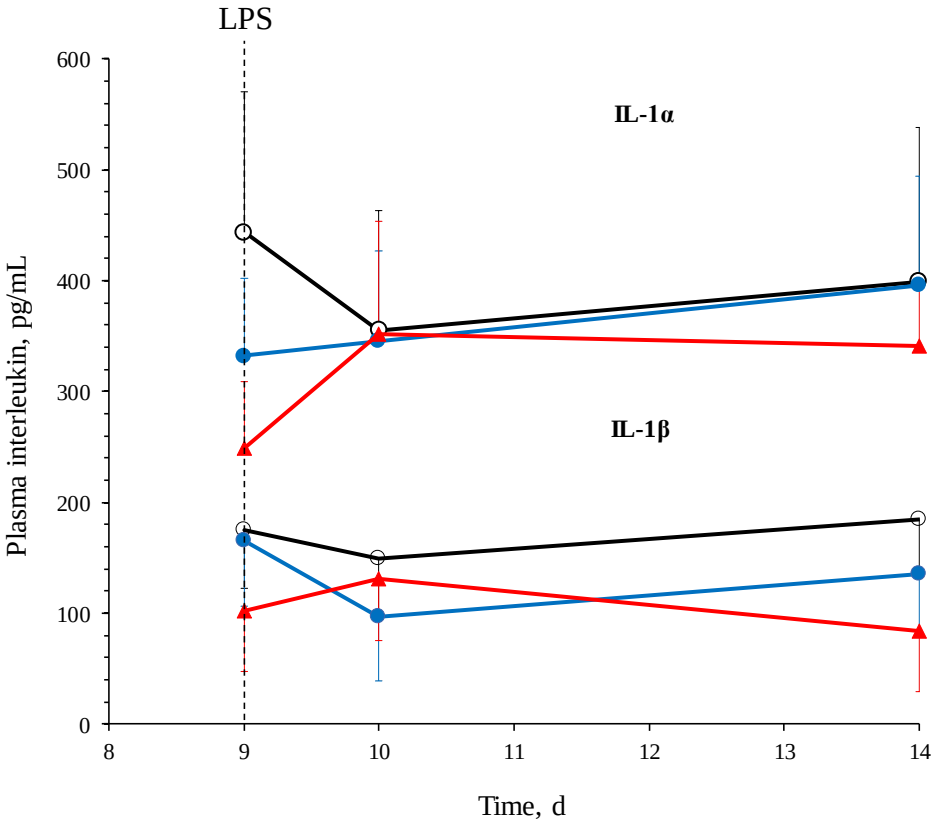

Figure 4.4. Plasma concentration of IL-α anti- and IL-1β pro-inflammatory interleukins of dairy ewes according to B-glucans (BG) treatments (CON: ○, Control; HBB: ●, High BG barley; INP: ▲, Intraperitoneally) after to the Lipopolysaccharide (LPS) challenge. Values are LSM, and vertical bars represent SEM.

Although no differences were detected in the IL-1α and IL-1β by effect of the BG treatment, in part due to the large analytical error of its determination, numerically lower values of HBB and INP ewes were observed for IL-1β (pro-inflammatory) during the experimental period. Both BG treatments seem to reduce the pro-inflammatory stage of the ewes with regard to the CON ewes.

Despite the IL-1β is one of the most potent and pleiotropic pro-inflammatory cytokine related to local and systemic inflammatory response (Herman et al., 2013). Bannerman (2009) and Shangraw et al. (2019) only reported marked IL-1β increases in milk, whereas blood values were very low, after a LPS challenge in cows. Unfortunately, interleukins were not analysed in our ewes. On the other hand, Mavrommatis et al. (2020) reported that dairy ewes fed with *Saccharomyces cerevisiae* live yeast, rich in BG, showed a better oxidative status (i.e., lower inflammation) during lactation and expressed less IL-1β transcripts in blood, which may be a proof of an enhanced immune system.

Suppression of IL-1β may be an evidence of trained immunity, as indicated by Bronzo et al. (2020) in dairy cows. According to these authors, mammary epithelial cells previously stimulated with LPS, develop endotoxin tolerance by means of epigenetic mechanisms, which include the down-regulation of the expression of proinflammatory cytokines (i.e., TNF-α, IL-1β). According to this, we considered that our results suggest the enhancement of the innate immune system of our dairy ewes by effect of the barley BG administered orally or intraperitoneally, in our HBB and INP treatments, respectively.

4.5. CONCLUSIONS

Our study provided new data on the potentiality of barley BG as training biological agents to induce immune activation of dairy ewes against *E. coli* endotoxin. Although further studies should be done to support our findings, this experiment showed that the BG treated ewes were able to regulate their innate responses at local level to maintain the integrity of the mammary epithelial barrier. As the metabolic availability of BG varied according to the way of administration, the use of low solubility or rumen bypass BG should be preferable.

CAPÍTULO 5

Conclusiones

CAPÍTULO 5

Conclusiones

Las conclusiones obtenidas de los experimentos realizados en la presente tesis doctoral son los siguientes:

5.1. Conclusiones específicas

5.1.1. Forraje de cebada capuchona

1. La cebada capuchona, conservada tanto como heno o ensilado, presentó mayores contenidos de PB que los valores obtenidos en el triticale.
2. Aunque los contenidos de FAD no difirieron entre forrajes, la FND varió de acuerdo con la forma de conservación, presentando menores contenidos en los ensilados que en los henos, y en especial en la cebada capuchona.
3. Independientemente de la forma de conservación del forraje, no se observaron diferencias entre cebada capuchona y triticale en el consumo voluntario de MS.
4. A pesar de ello, el heno de cebada capuchona mostró menor digestibilidad aparente de la MO que el triticale, aunque sin diferencias entre ensilados.
5. Respecto al contenido energético, los valores de los ensilados fueron superiores a lo de los henos, aunque en el caso de los henos de cebada capuchona sus valores fueron ligeramente inferiores a los del triticale.
6. El ensilado de la cebada capuchona mostró valores superiores de PDIA y RPB que los obtenidos en el ensilado de triticale.

5.1.2. Cebada grano rica en beta-glucanos

7. Los beta-glucanos de cebada indujeron una reacción pirética, independientemente de su vía de utilización, que fue más marcada en las ovejas en las que se aplicaron por vía intraperitoneal.

8. Se observó una moderada disminución de la producción de leche a corto plazo, por efecto de la administración intraperitoneal de beta-glucanos, que desapareció progresivamente.
9. Las concentraciones en plasma de citoquinas pro- y anti-inflamatorias fueron más bajas en las ovejas que recibieron beta-glucanos por vía intraperitoneal, lo que sugiere un mejor estado de su sistema inmunitario.
10. La producción de leche durante el desafío con LPS disminuyó en todas las ovejas, sin observar diferencias entre los tratamientos con beta-glucanos.
11. Los efectos negativos en la composición de leche por efecto del desafío con LPS, fueron menos pronunciados en el grupo de ovejas que recibieron beta-glucanos por vía intraperitoneal, lo que sugiere una menor afectación de su epitelio mamario.

Finalmente,

12. Las ovejas suplementadas con beta-glucanos, oral o intraperitonealmente mostraron una menor concentración de citoquinas en plasma, lo que indicó la activación previa de su sistema inmune (*trained immunity*).

5.2. Conclusiones generales

La cebada capuchona (cv. Mochona), tanto en heno como en ensilado, mostró tener calidad nutritiva similar a la del triticale y superior a las referencias bibliográficas de cebadas convencionales. Por ello, es un interesante recurso forrajero alternativo en la alimentación de rumiantes.

Por otro lado, la cebada grano de alto contenido en beta-glucanos (cv. Annapurna), demostró ser capaz de estimular el sistema inmune innato, y podría ser un interesante alimento funcional y una alternativa al uso de aditivos antimicrobianos en producción de rumiantes.

CAPÍTULO 6

Bibliografía

CAPÍTULO 6

Bibliografía

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