The impact of shearing and hormonal treatments (melatonin or cabergoline) in lactating dairy ewes

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El impacto del esquileo y de los tratamientos hormonales (melatonina o cabergolina) durante la lactación en ovejas lecheras

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Dr. Gerardo Caja López

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SHORT BIOGRAPHY

Abdelaali (Ali) El Hadi was born in Ksar El Kebir (Morocco, 1988) and he graduated in Agricultural Engineering "Animal Production" at the Agronomic and Veterinary Institute (IAV) Hassan II (Rabat, Morocco) in 2012. After obtaining a competitive grant from the Spanish Ministry of Foreign Affairs (2013-15), he earned the double official M. Sc. degree in "Animal Nutrition" (2015) from the International Centre for Advanced Agronomic Mediterranean Studies (CIHEAM-IAMZ) and the University of Zaragoza (Zaragoza, ES). Thereafter, he was contracted as Research Assistant at the Department of Animal and Food Sciences of the University Autonomous of Barcelona (UAB) in Bellaterra (ES), where he did applied research and performed his Ph.D. in Animal Production (2015-20). During this period, he updated his knowledge with several expertise courses on ruminant nutrition, analytical methodology, use of new technologies (Livestock Precision Farming), statistical analyses and languages. He also earned the official qualification of "Research staff user of animals for experimentation" (UAB-Generalitat de Catalunya). He attended national and international meetings (AIDA, ADSA, ISNH), received a grant from the EAAP for presenting in the 2019 Annual Meeting in Ghent (BEL) and made a short-stay of research at the Department of Animal Science of the Aarhus University in Tjele (DK) with an Erasmus+ grant. The current Ph.D. thesis is a final requisite for obtaining his PhD with a mention as International Doctorate.

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LIST OF ABBREVIATION

ACTH Adrenocorticotropic hormone
ADF Acid detergent fiber
ADG Average daily gain
BCS Body condition score
BW Body weight
CAB Cabergoline
CF Crude fiber
CLA Conjugated linoleic acid
CN Casein
CO Control
CP Crude protein
dDM Digestibility of dry matter
DM Dry matter
DMI Dry matter intake
ECM Energy corrected milk
EE Ether extract
Exp Experiment
FA Fatty acids
FAME Fatty acids methyl esters
FUs Fill units for sheep
FV Fill value
GGT γ-glutamyl transferase
GH Growth hormone
GnRH Gonadotropin-releasing hormone
IGF-I Insulin-like growth factor 1
IMI Intramammary infection
LC Lacaune breed
LCFA Long chain fatty acids
LD Long day
LH Luteinizing hormone
MEL Melatonin
MN Manchega breed
MUFA Monounsaturated fatty acids
NAS *N*-acetyl serotonin
NDF Neutral detergent fiber
NEFA Non esterified fatty acid
NIRS Near infrared reflectance spectroscopy
OM Organic matter
P4 Progesterone
PDO Protected denomination of origin
PEG Polyethylenglycol

PRL Prolactin
PRLR PRL receptors
PUFA Polyunsaturated fatty acids
RTD Recommended treatment dose
SCC Somatic cells count
SCFA Short chain fatty acids
SD Short day
SFA Saturated fatty acids
SH Shorn
T_A Ambient temperature
T_B Body temperature
TH Tyrosine hydroxylase
T_{LC} Lower critical temperature
T_{LL} Lower lethal temperature
TMR Total mixed ration
TNZ Thermoneutral zone
TP True protein
Trp Tryptophan
TS Total solids
TSH Thyrotropin
T_{UC} Upper critical temperature
T_{UL} Upper lethal temperature
US Unshorn

ABSTRACT

The impact of shearing and hormonal treatments (melatonin or cabergoline) in lactating dairy ewes

Three experiments were carried out in order to evaluate the lactational effects of shearing or treating with hormones (melatonin or cabergoline) in 2 breeds of dairy ewes (Lacaune, LC; Manchega, MN). In Exp. 1, a total of 48 ewes in mid-lactation were used under mild-winter conditions. Ewes were allocated in 8 balanced groups to which the experimental treatments (control unshorn, CO; shorn, SH) were applied. Responses to shearing varied according to breed, the rectal temperature after shearing only decreasing in MN ewes (–0.36ºC). Feed intake increased 5% in the LC-SH, when compared to LC-CO, but did not vary in MN ewes. Milk yield of LC-SH ewes was 10% greater than LC-CO, but no differences were detected in MN ewes. No effects in milk composition, nor on milk fatty acid profile, were observed in either breed, but LC-SH yielded 9% more milk protein than LC-CO ewes. Moreover, no effects were detected in on plasmatic glucose, NEFA, cortisol and insulin, as well as in body reserves of either breed.

In Exp. 2, a total of 72 dairy ewes in early-lactation under autumn conditions were penned indoors in 12 balanced groups. Treatments were: Control (CO), that did not receive any treatment, and Melatonin (MEL), that received 1 implant (18 mg/ewe) s.c. in the ear base. No MEL effects were detected on feed intake, milk yield and milk composition in either breed. MEL values in plasma showed a significant increase in the MEL-treated ewes of both breeds, but PRL in plasma only decreased in the MN ewes. No MEL effects were detected in plasmatic IGF-I and body reserves values of either breed throughout the experiment.

In Exp. 3, a total of 30 ewes in late-lactation and managed as a single flock under spring conditions, were used. Ewes were allocated in 3 groups to which the treatment were assigned. Treatments consisted of a single i.m. injection of cabergoline, a dopamine antagonist, at different doses per ewe: low (L, 0.56 mg), high (H, 1.12 mg) and control (CO, 1 mL saline). No local reaction in the injection site was detected but milk yield fell rapidly after treatment in both breeds (MN vs. LC, –54% vs. –27%) and milk fat and protein contents increased similarly in both breeds and at both cabergoline doses (23%, on average), when compared to CO ewes. PRL decreased dramatically in the cabergoline treated ewes, when compared to CO ewes. Nevertheless, on d 14 of treatment, PRL values rebounded transitorily, being 58% greater in the cabergoline treated than in CO ewes. Udder volume was similar for the cabergoline treated ewes, but both values were lower than those of CO ewes as a result of mammary involution.

In conclusion, shearing dairy ewes during lactation under mild-winter conditions, is a suitable management option that may increase feed intake and milk production, without deleterious effects on milk composition. The use of exogenous MEL implants in early-lactation and under autumn conditions has no effects on the lactational performances of dairy sheep in early lactation. Finally, cabergoline injection may be a useful tool to facilitate the decrease of milk production by inhibiting PRL secretion of dairy ewes at dry-off.

RESUMEN

El impacto del esquileo y de los tratamientos hormonales (melatonina o cabergolina) durante la lactación en ovejas lecheras

Se llevaron a cabo 3 experimentos para evaluar los efectos del esquileo o el tratamiento con hormonas (melatonina o cabergolina) sobre la lactación de 2 razas de ovejas lecheras (Lacaune, LC; Manchega, MN). En Exp. 1, se utilizó un total de 48 ovejas a mitad de lactación y en condiciones de invierno suave. Las ovejas se repartieron en 8 grupos equilibrados a los que se aplicaron los tratamientos (control sin esquileo, CO; esquileo, SH). Las respuestas al esquileo variaron según la raza, la temperatura rectal después del esquileo sólo disminuyó en las ovejas MN (–0.36ºC). La ingestión aumentó un 5% en las LC-SH, en comparación con las LC-CO, pero no en las MN. La producción de leche de las ovejas LC-SH fue un 10% mayor que las LC-CO, pero no se detectaron diferencias en las MN. No se observaron efectos en la composición de la leche ni en el perfil de ácidos grasos de la leche en ninguna de las razas, pero las ovejas LC-SH produjeron un 9% mas de proteína. Además, no se detectaron efectos en la glucosa, NEFA, cortisol e insulina en sangre, así como en las reservas corporales de ninguna de las razas.

En Exp. 2, se utilizaron 72 ovejas en el inicio de la lactación en condiciones de otoño que fueron estabuladas en 12 grupos equilibrados. Los tratamientos fueron: Control (CO), que no recibió ningún tratamiento, y Melatonina (MEL), que recibió 1 implante (18 mg/oveja) s.c. en la base de la oreja. No se detectaron efectos de MEL en la ingestión, producción o composición de la leche en ninguna de las razas. Los valores de MEL en plasma aumentaron en las ovejas tratadas con MEL de ambas razas, pero la PRL en plasma solo disminuyó en las MN. No se detectaron efectos de MEL en IGF-I en plasma ni en las reservas corporales de ninguna de las razas, durante el experimento.

En Exp. 3, se utilizaron un total de 30 ovejas en final de lactación en condiciones de primavera. Las ovejas se repartieron en 3 grupos equilibrados. Los tratamientos consistieron en 1 inyección i.m. de cabergolina, un antagonista de la dopamina, a diferentes dosis por oveja: baja (L, 0.56 mg), alta (H, 1.12 mg) y control (CO, 1 ml de solución salina). No se detectó reacción local en el lugar de la inyección, pero la producción de leche disminuyó rápidamente después del tratamiento en ambas razas (MN vs. LC, –54% vs. –27%) y el contenido de grasa y proteína de la leche aumentó de manera similar en ambas razas y en ambas dosis de cabergolina (23%, en promedio), en comparación con las ovejas CO. La PRL disminuyó drásticamente en las ovejas tratadas con cabergolina, en comparación con las ovejas CO. Sin embargo, en el día 14 de tratamiento, los valores de PRL rebotaron transitoriamente, siendo un 58% mayor en las ovejas tratadas con cabergolina que en las ovejas CO. El volumen de ubre fue similar para las ovejas tratadas con cabergolina, pero ambos valores fueron más bajos que los de las ovejas CO como resultado de la involución mamaria.

En conclusión, el esquileo de ovejas lecheras durante la lactación en condiciones de invierno suave, es una opción de manejo adecuada que puede aumentar la ingestión y la producción de leche, sin efectos perjudiciales sobre la composición de la leche. El uso de implantes de MEL al inicio de la lactación y en condiciones de otoño no tiene efectos sobre la producción de ovejas lecheras. Por último, la inyección de cabergolina puede ser una herramienta útil para facilitar la disminución de la producción de leche por la inhibición de la secreción de PRL de las ovejas lecheras en el periodo de secado.

RESUM

L’impacte de la xolla i dels tractaments hormonals (melatonina o cabergolina) durant la lactació en ovelles lleteres

Es van realitzar 3 experiments amb l'objectiu d'avaluar els efectes de la xolla o el tractament amb hormones (melatonina o cabergolina) a la lactació de 2 races d'ovelles lleteres (Lacaune, LC; Manchega, MN). En Exp. 1, es va utilitzar un total de 48 ovelles a meitat de lactació en condicions d'hivern suau. Les ovelles es van distribuir en 8 grups equilibrats als quals se'ls van aplicar els tractaments experimentals (control sense xolla, CO; xolla, SH). Les respostes a la xolla van variar segons la raça, la temperatura rectal després de la xolla va disminuir només en les ovelles MN (–0.36ºC). La ingestió va augmentar un 5% en les LC-SH, en comparació de les LC-CO, però no va variar en les MN. La producció de llet de les ovelles LC-SH va ser un 10% més gran que la de LC-CO, però no es van detectar diferències en les MN. No es van observar efectes en la composició de la llet ni en el perfil d'àcids grassos de la llet en cap de les races, però la producció de proteïna va augmentar un 9% en les ovelles LC-SH. A més a mes, no es van detectar efectes en la glucosa, NEFA, cortisol i insulina en sang, així com en les reserves corporals de cap de les races.

En Exp. 2, es van utilitzar 72 ovelles en l'inici de la lactació en condicions de tardor que van ser estabulades en 12 grups equilibrats. Els tractaments van ser: Control (CO), que no va rebre cap tractament, i Melatonina (MEL), que va rebre 1 implant (18 mg/ovella) s.c. a la base de l'orella. No es van detectar efectes de MEL sobre la ingestió, la producció de llet o la composició de la llet en cap de les races. Els valors de melatonina en plasma van mostrar un augment significatiu en les ovelles MEL de les dues races, però la PRL en plasma només va disminuir en les ovelles MN. No es van detectar efectes de MEL en l'IGF-I en plasma ni en els valors de les reserves corporals de cap de les races durant l'experiment.

En Exp. 3, es van utilitzar un total de 30 ovelles a final de lactació en condicions de primavera. Les ovelles es van distribuir en 3 grups equilibrats. Els tractaments van consistir en una sola injecció i.m. de cabergolina, un antagonista de la dopamina, a diferents dosis per ovella: baixa (L, 0.56 mg), alta (H, 1.12 mg) i control (CO, 1 ml de solució salina). No es va detectar cap reacció local en el lloc de la injecció, però la producció de llet va disminuir ràpidament després del tractament en ambdues races (MN vs. LC, –54% vs. –27%) i el contingut de greix i proteïna de la llet va augmentar de manera similar en ambdues races i en les dues dosis de cabergolina (23%, de mitjana), en comparació amb les ovelles CO. La PRL va disminuir dràsticament en les ovelles tractades amb cabergolina, en comparació amb les ovelles CO. No obstant això, en el dia 14 de tractament, els valors de PRL van rebotar transitòriament, sent un 58% més gran en les ovelles tractades amb cabergolina que en les ovelles CO. El volum del braguer va ser similar per a les ovelles tractades amb cabergolina, però tots dos valors van ser més baixos que els de les ovelles CO com a resultat de la involució mamària.

En conclusió, la xolla d'ovelles lleteres durant la lactació en condicions d'hivern suau, és una opció de maneig adequada que pot augmentar la ingestió i la producció de llet, sense efectes perjudicials sobre la composició de la llet. L'ús dels implants de MEL a l'inici de la lactació i en condicions de tardor no té efectes sobre la producció de ovelles lleteres. Finalment, la injecció de cabergolina pot ser una eina útil per facilitar la disminució de la producció de llet via la inhibició de la secreció de PRL de ovelles lleteres en el període de l'assecatge.

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Literature review

1.1. Introduction

Sheep farming is practiced in the majority of the world and has been fundamental to many civilizations (Haenlein, 2007). Sheep is widely considered as one of the most adapted species to different climates and is found in all livestock production systems. Sheep husbandry and industry provides employment to many millions of people all over the world in the undeveloped as much as developed countries (Ryder, 2007). Sheep sector also requires fewer capital than other agriculture sector, and sheep is the most appropriate of the ruminant species to utilize the poor vegetation in difficult and marginal areas.

There are relatively a small number of sheep breeds which are specialized only for dairy purpose (Haenlein, 2007). The most productive dairy sheep breeds in the world include Assaf, Awassi, East Friesian (Milchschaf), Chios, Lacaune, Manchega and Sarda. Most sheep milk is produced in the Mediterranean region (Pulina et al., 2018) and it is commonly used to make cultured dairy products.

In Spain, the dairy sheep sector grown quickly because of strong modernization of the sector, with considerable increases in milk yield per animal (224 kg/ewe; FAOSTAT, 2020) and total milk production (0.54 Mt; FAOSTAT, 2020), therefore, the intensive farms, the level of dairy specialization and size of sheep farms have increased (Pulina et al., 2018). Most sheep milk is sold to dairy industries to be processed into traditional sheep-cheeses types which many of them are sold as "Protected denomination of origin" quality highly appreciated by local and international markets (e.g., Manchego and Roquefort) as reviewed by Pulina et al. (2018).

The good management of dairy sheep is considered one of the key factors to improve the performance of dairy sheep farms through the increase of milk quantity as well as by improving its quality.

As sheep are a species characterized by its fleece of wool, shearing is a necessary management practice that allows sustaining the performance as well as the well-being of the ewes (i.e. it modify the limits of the thermoneutral zone, facilitate the movement of the ewes within the milking parlors, and improves milking hygiene). Many studies have touched the issue of shearing and its relationship with productive parameters, but most of

them were interested in the effects of shearing in late-pregnancy, usually at the spring-summer period, on the birth-weight of the lambs, but very few have studied the effects of shearing on lactation. In Chapter 3 of this thesis, we study the effects of shearing in early-lactation during winter (as cold stress factor) to evaluate the maximum response of shearing on milk yield and composition of 2 breeds of dairy ewes.

Sheep are also a species characterized by sexual seasonality, in which the reproductive activity is stimulated by short photoperiod, inducing the onset of ovulatory activity. In this context, melatonin implants are considered a useful tool to manage sheep production and seasonality by mimicking the stimulatory effect of autumn short days. Most of the bibliographic references on the use of melatonin in sheep talk about the effects of implants on the reproductive parameters (i.e. fertility, fecundity, prolificacy, birth-weight of the lamb...) under spring-summer photoperiod conditions. In the Chapter 4 of this thesis, we evaluate the effects of subcutaneous melatonin implants on the lactational performances in early-lactation of 2 breeds of dairy ewes under autumn photoperiod conditions.

In modern dairy sheep farms, it is very common to arrive to drying-off period with ewes that are still producing considerable volumes of milk. Usually, in these circumstances, farmers use techniques to reduce udder engorgement and mammary inflammation by nutrient restriction or antibiotic therapy, but sometimes, the use of these techniques has limitations (i.e. body condition, pregnancy toxaemia). Under this situation, the use of cabergoline (dopamine agonist) could be an interesting method to facilitate the dry-off by interfering with the transmission of hormonal signals from the pituitary gland to induce the cessation of milk production. Cabergoline, as dry-off facilitator, was used in dairy cow and also tested in dairy goat, but it was not valuated in dairy ewes. In the Chapter 5 of this thesis, we investigate the effects of two doses of cabergoline on prolactin suppression and milk secretion to determine the suitable dose and its effects in 2 breeds of dairy ewes in late-lactation.

1.2. Panorama of dairy sheep sector

1.2.1. Dairy sheep in the world

There are around 1,200 million sheep in the world, and approximately 20.7% are intended for dairy production (Table 1.1; FAOSTAT, 2020). Agreeing the review done by Pulina et al. (2018), the world's total sheep milk (10.6 Mt) is mainly produced in Asia (46.3%) with remarkable amounts in China and Turkey, followed by Europe (29.8%) and Africa (23.1%). Milk yield of dairy ewes in Europe (98 kg/ewe; Table 1.1) is more than the double of the world's average milk yield (42 kg/ewe). On average, milk yield is low in Asia, Africa, and America (27 to 39 kg/ewe).

Table 1.1. Dairy sheep in the world[1].

	Total sheep		Dairy sheep		Milk		Yield
Continent	$\times 10^6$ head	%	$\times 10^6$ head	%	Mt	%	kg/head
Asia	515	42.6	126	50.2	4.92	46.3	39.0
Africa	384	31.7	89	35.5	2.45	23.1	27.4
Europe	131	10.8	33	13.1	3.17	29.8	97.6
America	83	6.9	3	1.2	0.09	0.8	32.6
Oceania	97	8.0	-	-	-	-	-
Total	1 210	100	251	100	10.63	100	42.3

[1]Source: FAOSTAT (2020).

Zygoyiannis (2006), report that over 60% of sheep are found in temperate zones and less than 40% in tropical zones. Most world dairy sheep are mainly located in subtropical-temperate areas of Asia, Africa and Europe (Figure 1.1; FAO, 2010), in an area bounded by the 20°W and 50°E meridians and the 35°N and 45°N parallels (Pulina et al., 2018). The major sheep producing areas are in temperate zone areas, characterized by temperate pasture growing conditions and highest pasture production.

The world milk production in 2018 was 843 Mt (FAOSTAT, 2020). Despite the large number of sheep, sheep milk represented only 1.3 % of world's total milk production after cattle (81.0%), buffaloes (15.1%) and goat (2.2%). Camel milk represents less than 0.4%. Nevertheless, worldwide sheep milk production has more than doubled (+108%, Figure 1.2) during the last 60 years due to the improvement of feeding techniques, together with enhancement of the genetic merit, better control of reproduction and prevention of the principal pathologies associated with intensive breeding conditions (Boyazoglu and Morand-Fehr, 2001).

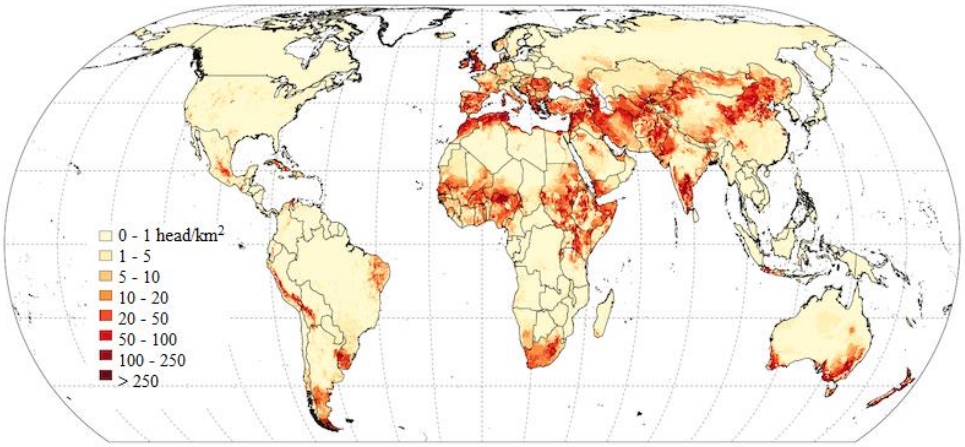


Figure 1.1. World sheep distribution in 2010 (FAO, 2010).

If this trend is sustained, it is expected to increase by approximately 2.3 Mt by 2030 (FAOSTAT, 2020). It should be noted that the increase of sheep population was not uniform in all continents and in all countries. With regard to sheep's milk, a linear growth was estimated by Pulina et al. (2018) from FAOSTAT data, as shown in Figure 1.2), expecting to reach 12 Mt in 2030.

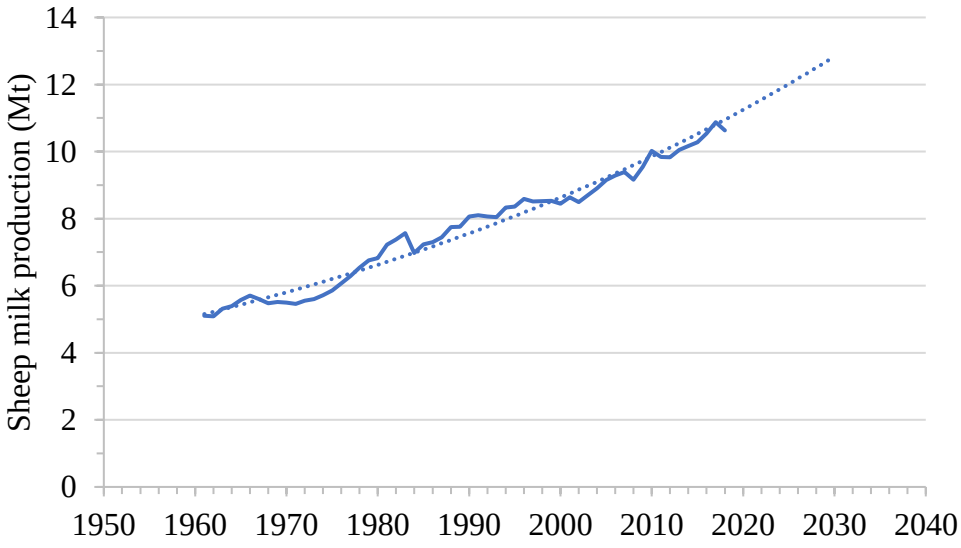


Figure 1.2. Sheep milk production trends in the world from 1961 to 2018 (solid line) and forecast to 2030 (dashed line) (FAOSTAT, 2020).

Sheep milk is usually not consumed directly as milk but it is especially suitable for cheese and yogurt production because of its high protein and solids content (Boyazoglu and Morand-Fehr, 2001; Pulina et al., 2018), but is sold as high-quality dairy products, representing one of the most important ingredients of the Mediterranean traditional diet.

According to Pulina et al. (2018), the global export of sheep-milk cheese accounted for approximately US$374 M in 2013. Italy is the leader in sheep-milk cheese exports, holding 36% of the market share, followed by France (20%).

1.2.2. Dairy sheep in the Mediterranean area

Dairy sheep farms are concentrated in the Mediterranean countries and the Black Sea regions, with relevant Greek or Roman cultural heritage, where their dairy products are typical ingredients of the human diet (Caja, 1990). The Mediterranean and the Black Sea regions, with only 27.1% of the world dairy sheep, produced 52.6% (5.59 Mt; FAOSTAT, 2020) of the world sheep milk in 2018, with Turkey as a first producer, followed by Greece, Syria, Romania, Spain, and Italy, each of them producing annually more than 0.5 Mt (near 10%) of sheep's milk (Table 1.2).

Table 1.2. Dairy sheep in the Mediterranean and the Black Sea regions[1].

	Milk		Yield
Country	Mt	%	kg/head
Turkey	1.45	25.9	76.9
Greece	0.75	13.5	107.4
Syria	0.65	11.6	56.2
Romania	0.63	11.2	81.9
Spain	0.54	9.7	223.7
Italy	0.52	9.4	104.3
France	0.32	5.8	251.6
Algeria	0.30	5.4	16.8
Egypt	0.10	1.7	48.2
Albania	0.08	1.5	62.1
Others[2]	0.24	4.3	91.3
Total	5.59	100.0	101.9

[1]Source: FAOSTAT (2020); [2]Mediterranean countries with less than 1% of milk production.

Systems for raising dairy sheep herds in different regions of the Mediterranean are characterized by the co-existence of modern exploitations with a very developed intensive systems and traditional farms with extensive systems which characterized by the use of marginal resources and rustic races milked by hand (Caja, 1990). The dairy sheep industry is based on intensive and semi-intensive and systems with local breeds and crossbreeds (Pulina et al., 2018). Average flock size varies from small to medium (140 to 333 ewes/farm), and individual milk yield from low to medium (16 to 251 kg/ewe), with France and Spain being the current leaders, followed by Greece and Italy (Table 1.2),

indicating the level of dairy specialization and modern dairy sheep systems in these countries, characterized by technically advanced farms and specialized dairy breeds.

Sheep's milk in the Mediterranean countries is mainly produced in specialized flocks of selected breeds, whereas the breeds subjected to milking in other countries are generally of mixed type and substantially less productive (Boyazoglu and Morand-Fehr, 2001). Most sheep milk is sold to industries and transformed into typical dairy products (e.g. cheese) that have a regional or local nomination of origin and high quality (e.g. Feta, Idiazabal, Manchego, Pecorino, Roquefort, etc.).

1.2.3. Dairy sheep in Spain

The dairy sheep sectors contributed 1.0% to the total agricultural output and 2.7% (€488 M) to the livestock output of Spain from 2016 to 2018 (MAPA, 2020).

There are approximately 15.9 million sheep in Spain, from which dairy sheep represent 21% (MAPA, 2020), with the remainder intended for lamb production. On average, size of Spanish dairy sheep farms is 140 ewes/farm. The large amount of sheep milk produced in Spain (0.54 Mt; FAOSTAT, 2020) is mainly a result of the high yield obtained per ewe (224 kg/ewe) by using specialized dairy breeds. Most Spanish sheep milk is produced in the Autonomous Communities of Castilla y León (54.9%), Castilla-La Mancha (32.0%), Navarra (2.9%), Extremadura (2.3%) and Madrid (2.3%) (MAPA, 2020).

During the last 50 years, Spanish sheep milk production has almost doubled (+97.4%, Figure 1.3), due to considerable improvements in production systems (i.e. genetics, feeding and management). However, it has slightly decreased (–9.3%) in the last 5 yr (FAOSTAT, 2020) because near 28.3% of dairy sheep farms left the dairy industry, which resulted in a noticeable depopulation of rural areas in Spain (MAPAMA, 2016).

One of the reasons for the increase of sheep milk production in Spain during the last 30 years was the introduction of high-yielding foreign breeds (Assaf, 400 L/ewe and Lacaune, 350 L/ewe), used as purebreds or crossed with local breeds (Ugarte et al., 2001). The most important local dairy breeds in Spain are Manchega (190 L/ewe), Latxa (168 L/ewe) and Churra (117 L/ewe), which are generally raised under intensive and semi-intensive systems (Pulina et al., 2018).

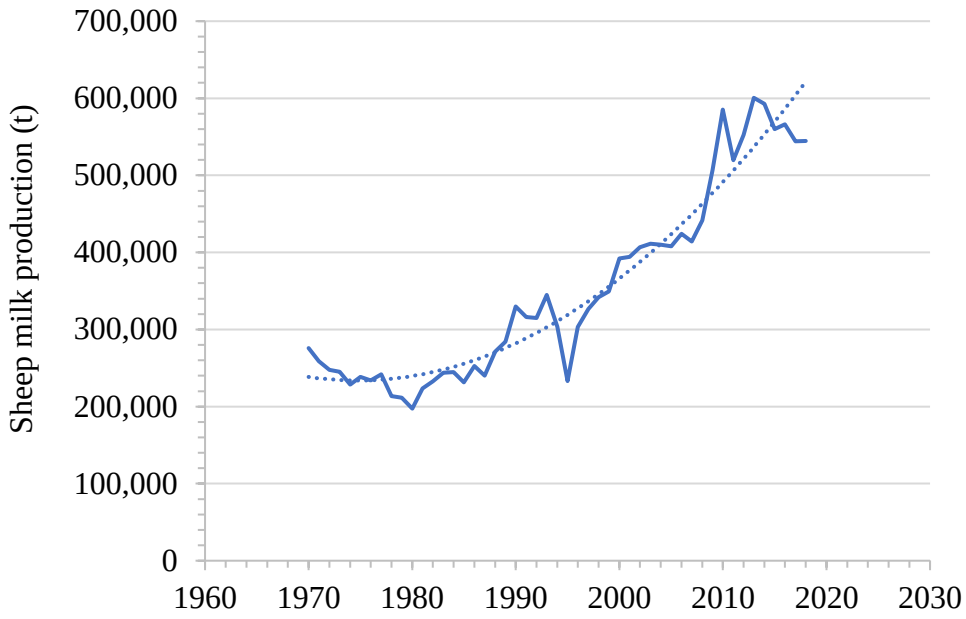


Figure 1.3. Sheep milk production trends in Spain (FAOSTAT, 2020).

In Spain, incomes of dairy sheep farms markedly vary with production system and Autonomous Community. In the case of intensive dairy sheep farms of Castilla y León, Milán et al. (2014) reported that milk sales were the main income (78.6%), followed by milk-fed lamb sales (13.2%), and EU subsidies (6.9%).

In general, most sheep milk (93.9%) in Spain is processed into cheeses in dairy industries for national and export markets (e.g. Manchego and Idiazabal). Approximately, 23.0% of Spanish production is sold as Protected Denomination of Origin (PDO) sheep-milk cheeses (Pulina et al., 2018). The most produced PDO Spanish sheep-milk cheese is Manchego (85.4%; Figure 1.4) from Castilla-La Mancha, followed by Idiazabal from the Navarre and Basque Country, Roncal from Navarra, Zamorano from Castilla y León, and Torta del Casar and De la Serena from Extremadura (MAPAMA, 2016).

More than 50% of Spanish PDO sheep-milk cheeses are exported. The volume of exports, since 2013, has been increased significantly to inside and outside the EU markets; this favorable evolution in foreign trade has resulted in constant increases in the economic value of exports in the last years (MAPAMA, 2016). The main destination of exports to outside the EU is the United States, where the Spanish PDO sheep-milk cheeses represent the highest percentage, both in volume and value, of the current market.

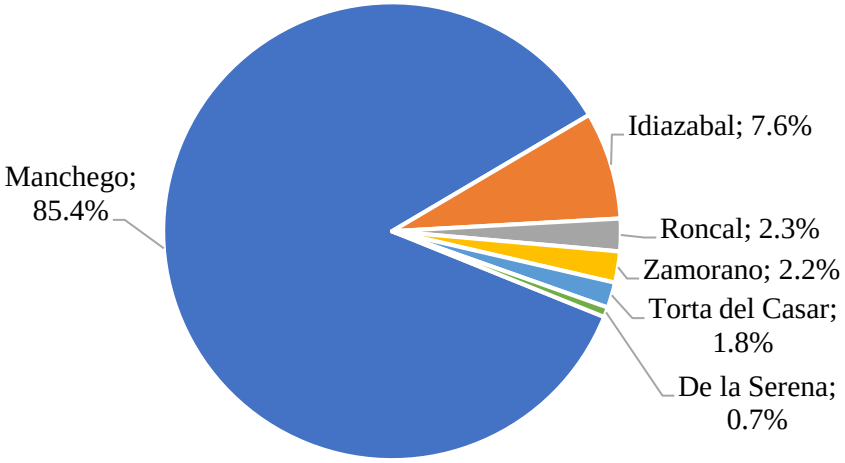


Figure 1.4. The Protected Denomination of Origin (PDO) Spanish sheep-milk cheeses (MAPAMA, 2016).

1.3. Shearing in dairy sheep

1.3.1. Thermoregulation in dairy sheep

Thermoregulation is the process by which animals maintain their body temperature (T_B) constant by the balance between heat gain and heat loss in order to optimize the energy used in physiological functions and cellular metabolic reactions (Randall et al., 1997). The degree of thermoregulatory activity that homeotherms require to maintain a constant T_B, increases with increasing the ambient temperature (T_A) extremes (Norris and Kunz, 2012).

According to Randall et al. (1997), the range of T_A within homeotherms can control its T_B by passive measures, without changing its metabolic rate to maintain thermal homeostasis, is known as the thermoneutral zone (TNZ). The TNZ is delimited by the lower critical temperature (T_{LC}), below which the basal metabolic rate becomes insufficient to balance heat loss, and the upper critical temperature (T_{UC}) which is the turning point at which evaporation of metabolic water is used to dissipate the excess of heat (Freer, 2007).

For sheep, The TNZ depend largely on amount of external insulation provided by the fleece (NRC, 1981). Shorn animals on a maintenance ration have a T_{LC} of about 25°C, but the value is estimated as –3°C with full fleece (Table 1.3).

Table 1.3. Estimates of lower critical temperatures for Sheep (NRC, 1981).

Fleece length, mm	Feeding	T_{LC}, °C
Shorn	Maintenance	25
Shorn	Fed ad libitum	13
5	Fasting	31
5	Maintenance	25
5	Fed ad libitum	18
10	Maintenance	22
50	Maintenance	9
100	Maintenance	–3

According to NRC (1981), the T_{LC} values vary considerably depending upon fleece, age, breed, lactational state, nutrition and housing conditions. The resistance to heat loss is provided basically by the fleece, and also by the insulation of the boundary layer of air surrounding the body which varies with wind speed and radiation (Freer, 2007).

When the T_B of the animal falls below its normal values, the animal enters a state of hypothermia. If this condition persists, the animal arrives to the lower lethal temperature (T_{LL}), which is the extreme cold temperature where an animal can no longer produce enough heat and dies by hypothermia (Randall et al., 1997). The range of temperatures between the T_{LC} and T_{LL} is known as the zone of metabolic regulation where heat production (through metabolic processes) is necessary to increase T_B (Norris and Kunz, 2012). On the other hand, the temperature range from the T_{UC} to the upper lethal temperature (T_{UL}) is known as the zone of heat dissipation where the most efficient method for dissipating the excess heat is by evaporation (Figure 1.5).

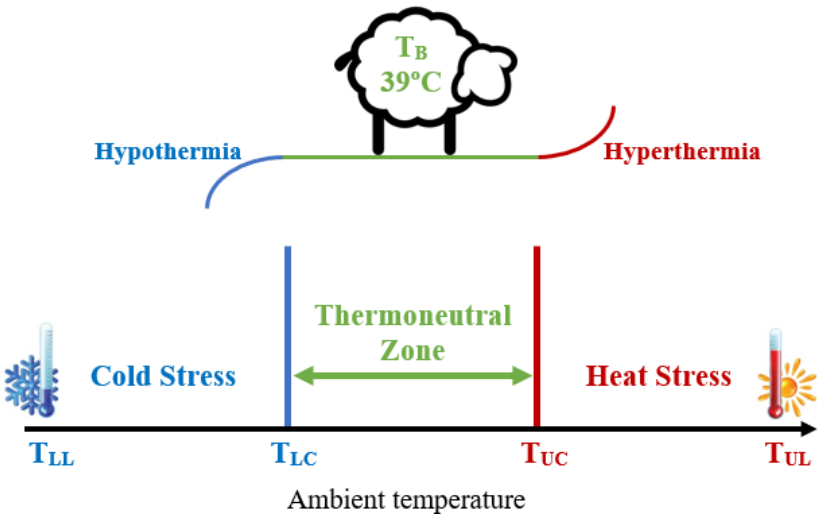


Figure 1.5. Environmental temperatures and sheep thermoregulation (T_B, body temperature; T_{LC}, lower critical temperature; T_{LL}, lower lethal temperature; T_{UC}, upper critical temperature; T_{UL}, upper lethal temperature).

1.3.2. Shearing effects on sheep thermoregulation

Shearing is usually considered to be a necessary practice for flock management in order to improve the sheep welfare and production (Swanson and McGlone, 2010). Shearing modifies the limits of the TNZ of the animals, increasing the T_{LC} and inducing different adaptive responses to maintain the body homeostasis (Aleksiev, 2008).

Sheep's fleece is considered as a thermoregulatory bio-structure, which its thermal insulation reduces the convective heat loss from the body under cold conditions (Piccione et al., 2010). Shearing boosts the heat transfer between the animal and its environment, especially in cold weather, resulting in a greater metabolic rate to match the increased energy demand for heat production.

The process of energy exchange between an animal and the environment is conditioned by its fleece, which play a remarkable role in the maintenance of thermal balance (Sleiman and Abi Saab, 1995). Maintenance of the homoeothermic conditions in sheep is influenced by the characteristics of the wool fleece, which is conditioned to intrinsic (i.e. breed, age, sex) and extrinsic (i.e. time from shearing, temperature, relative humidity and wind) factors (NRC, 1981).

When shearing is conducted during the end of winter or early spring, shorn sheep are exposed to cold stress as a consequence of losing their insulation. Different degrees of cold stress can be expected by breed according to their traits and adaptation to climate. A greater degree of cold stress would result in a greater metabolic rate, thus increasing the amount of feed necessary for the maintaining the T_B (Piccione et al., 2002).

Commonly, shearing is done once a year, traditionally in spring or early summer. However, in some countries and with high yielding wool breeds, shearing may take place twice or thrice annually. In the case of the Mediterranean countries, traditional sheep production systems involve shearing at the beginning of the summer to match with the onset of hot temperatures (Dyrmundsson, 1991). In Spain, sheep are usually shorn around mid of May, according to the traditional shearing time before mating.

1.3.3. Shearing effects on reproductive performances

Shearing out of the traditional season at start of the summer, implies in many cases to coincide with the mid- and late-pregnancy which makes hard the prediction of the metabolic and physiological consequences of shearing on the performances of the ewe

and the lambs. Shearing pregnant ewes has been associated with an increase in lamb birth weight in several studies (Dyrmundsson, 1991; Sphor et al., 2011; De Barbieri et al., 2012). In the case of heat stress, shearing may alleviate stress by reducing the negative effects of heat exposure on placental and fetal growth (Bell et al., 1989). However, in the case of cold stress, an increase in voluntary feed intake by the ewe following shearing (Parker et al., 1991) may be contribute to the birthweight increase.

Corner et al. (2006), reported that mid-pregnancy shearing resulted in lambs that were heavier and larger at birth than lambs of control ewes, and shearing at mid-pregnancy also produced long-term changes in lamb's growth, both before and after birth. Mid-pregnancy shearing also increased birth weight as reported by Kenyon et al. (2003), which is one of the major drivers to improve lamb survival (Morris et al., 1999). Moreover, it increased the birth weight of singleton- and twin-lambs (De Barbieri et al., 2014). However, Revell et al. (2000) reported that mid-pregnancy shearing was associated with a marked increase in the birth weight of twin-born lambs, without effects on singletons.

1.3.4. Shearing effects on lactational performances

Shearing practice has been studied in sheep especially during mid- and late pregnancy, because of its importance to increase lamb birth weight and to improve lamb survival. Other studies have evaluated the effects of winter shearing on milk yield and composition in mid lactation. Main lactational responses of shearing treatment are summarized in Table 1.4.

As shown in Table 1.4, most effects of shearing practices on dairy ewes, either in mid- or late- pregnancy (aiming increase lamb performances) or lactation (aiming to increase comfort), are highly variable and not consistent. The reasons for this variability are unknown. These main effects in sheep can be summarized as:

i) Increased feed intake (range, 0 to 20%),
ii) Reduced water consumption (range, –25 to 0%),
iii) Increased milk yield (range, 0 to 22%),
iv) Improved milk composition: fat (range, 0 to 24%), protein (range, 0 to 12%) and lactose (range, 0 to 3%),
v) Changes in the profile of milk FA, with increase of long chain FA (range, 0 to 9%) and tendencies to modify those of medium length (mainly, C8 to C12), and, finally

Table 1.4. Summary of the main effects of shearing or cold stress on the lactational performances of ewes (SH = shorn, CO = control).

Reference	Breed	Season	Ewe stage	Treatments	Main effects			
					Body	Intake	Water	Milk
McBride and Christopherson (1984)	Suffolk crosses (n = 16)	-	Early-lactation	CO vs. Cold[1]	n/d	n/d	n/d	No effect on yield. Composition: fat (+24%), protein (+12%), lactose (+3%), SCFA (–20%), LCFA (+9%)
Knight et al. (1993)	Dorset (n = 66)	Autumn & spring	Early-lactation	SH vs. CO	n/d	n/d	n/d	No effect on yield. Content: fat (+15%), protein (+10%)
Dabiri et al. (1996)	BL×Romney (n = 60)	Autumn & spring	Late pregnancy	SH vs. CO	No effect on BW	+14%	n/d	No effects
Avondo et al. (2000)	Comisana (n = 28)	Summer	Mid-pregnancy	SH vs. CO	No effect on BCS	+20%	n/d	No effects
Piccione et al. (2002)	Comisana, Pinzirita Siciliana (n = 60)	Spring	Dry	SH vs. CO	No effects, but body temperature increased (+1ºC			
Aleksiev (2008)	Tsigai (n = 50)	Spring	Mid-lactation	SH vs. CO	n/d	No effect	–25%	No effects
Ruiz et al. (2008)	Latxa (n = 60)	Winter	Late pregnancy	SH vs. CO	No effect on BW	+10%	n/d	No effects
Rassu et al. (2009)	Sarda (n = 12)	Spring	Mid-late lactation	Pre- vs. Post-[2]	n/d	n/d	n/d	No effect on yield. Composition: fat (+9%) and increased SCFA (C8, C10, C12 and C16). No effects on LCFA
Leibovich et al. (2011)	Assaf (n = 150)	Summer	Late pregnancy	SH vs. CO	No effect on BW	+8%	n/d	Milk yield (+7%), ECM (+10%)
Sphor et al. (2011)	Polwarth (n=10)	Winter	Early pregnancy	SH vs. CO	n/d	n/d	n/d	Milk (+22%). No effects on composition

[1]Housed (21ºC) vs. cold-exposed (0ºC); [2]Pre- vs. post-shearing; n/d = not determined; BW = body weight; FA = fatty acids; SCFA = short chain FA; LCFA = long chain FA.

vi) No changes in body reserves.

1.4. Melatonin and photoperiod in dairy sheep

1.4.1. Photoperiod effects on dairy sheep seasonality

Photoperiod is the main factor that determines the beginning and end of the reproductive season (Lehman et al., 2010). Sexual seasonality is a common factor in all sheep breeds and this is why it is considered that sheep has an endogenous reproduction rhythm, characterized by the photoperiod variations throughout the year, alternating the reproductive and anoestrus activity (Barrell et al., 2000).

Reproductive activity can be modulated by alternating periods of long days (16-h light, 8-h darkness) or short days (8-h light, 16-h darkness). So, exposure to short days induces the onset of ovulatory activity 40 to 50 d after the start of stimulation, while long days cause its inhibition, which ceases 20 to 30 d after the start of treatment (Lehman et al., 2010).

Sheep show 2 well-defined physiological periods annually (Henningsen et al., 2016):

- Seasonal anestrus period (long days), with the absence of regular estrous cycles, sexual receptivity, and ovulation, characterized by decreased libido.
- Seasonal reproductive period is the other physiological period of sheep during which ovarian and estrous cyclicity occurs. It is characterized by the succession at regular intervals of approximately 17 d duration and ovulation in the female; in the male, spermatogenesis and libido are restored. As a result, parturition occur at the most favorable time of the year (spring), with abundance of pasture and a comfortable ambient temperature (Barrell et al., 2000).

During the sexual season, the hypothalamic secretion of GnRH and the subsequent secretion of LH by the hipophysis determine the sexual cycle. LH secretion is inhibited during the luteal phase by high levels of progesterone (P4) produced by the corpus luteum (Lehman et al., 2010). After luteolysis, the fall in plasma P4 levels induces an increase in the GnRH and LH, stimulating estradiol (E2) secretion in the follicular phase that initiates the pre-ovulatory peak of GnRH and LH, thus activating the ovulation (Nestor et al., 2018). Stationary anestrus occurs as a consequence of a decrease in the activity of the hypothalamic-pituitary axis, by which the GnRH pulse frequency is considerably reduced and consequently the secretion of pituitary hormones (Lehman et al., 2010).

1.4.2. Melatonin biosynthesis

Biosynthesis of melatonin is done in 4 steps (Figure 1.6). First, the amino acid precursor tryptophan (Trp) is taken up by the pinealocyte from the blood and hydroxylated and converted into 5-hydroxytryptophan (5-HTP) by mean of the enzyme tryptophan hydroxylase in the mitochondria.

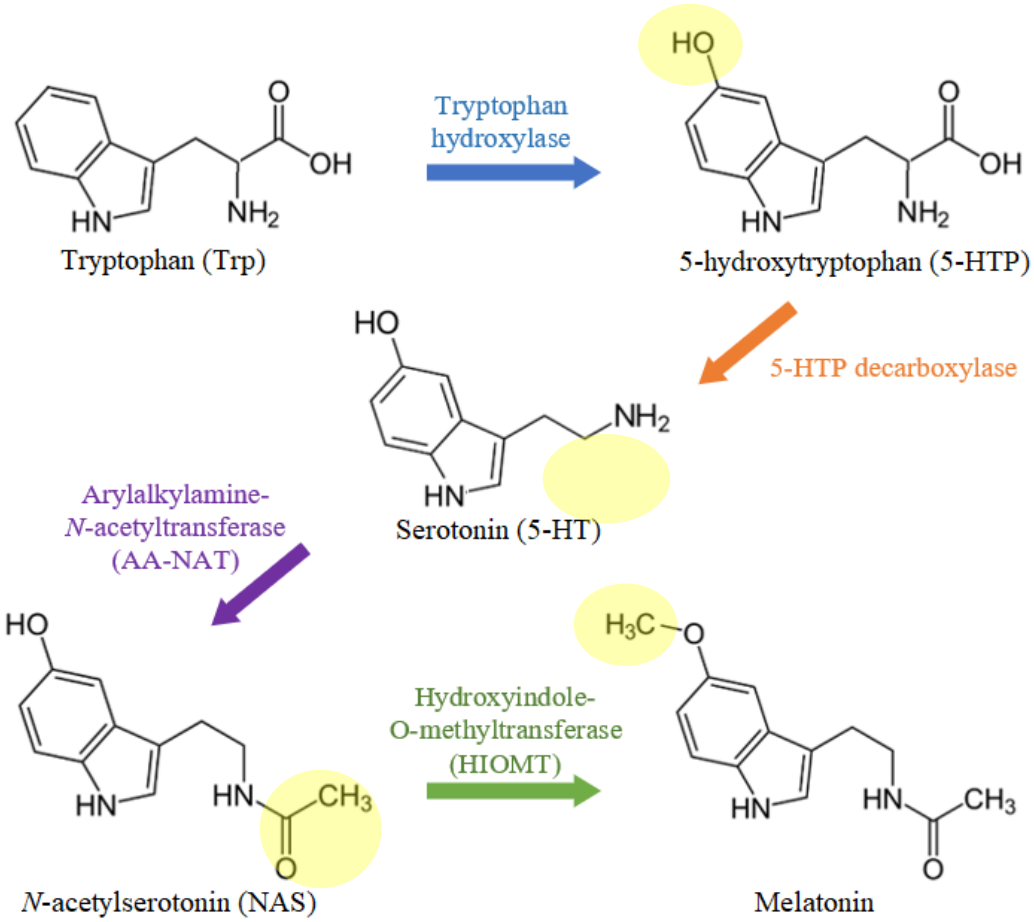


Figure 1.6. The pathway of melatonin biosynthesis.

Second, 5-HTP is decarboxylated by the Trp-5-monooxygenase hydroxylase (Reiter et al., 2014) and converted into serotonin (5-HT or 5-hydroxytryptamine) in the cytosol. Third, the 5-HT is first acetylated by the arylalkylamine-N-acetyltransferase (AA-NAT) into *N*-acetyl serotonin (NAS) which plays a key role in melatonin biosynthesis (Simonneaux and Ribeleyga, 2003). Finally, N-acetyl serotonin is O-methylated by hydroxyindole-O-methyltransferase (HIOMT) into melatonin in the pinealocyte of pineal gland (Reiter et al., 2014). According to Chowdhury and Maitra (2012), the AA-NAT switched melatonin synthesis on and off with photoperiodic variations in duration, whereas HIOMT tunes the amplitude of the nocturnal melatonin synthesis with photoperiodic variation in magnitude.

1.4.3. Melatonin secretion and metabolism

In mammalian species, melatonin is released from the pineal gland into the cerebrospinal fluid. It is secreted during the night under natural environment, for this reason it is recognized as the hormone of darkness. Melatonin secretion onset usually occurs around the sunset, and its offset is around sunrise, with a peak between 02:00 and 04:00 a.m. (Arendt, 1998). Melatonin profile in blood is low during the day (~10 pg/mL) and is significantly higher at night (>100 pg/mL).

Due to its amphiphilic nature, melatonin has a high lipid and water solubility, which simplifies its movement across the cell membrane and various body fluids. Melatonin is not stored within pineal cells but directly released by the pineal gland into the cerebrospinal fluid and the general circulation (Pevet et al., 2017). The pineal gland is not the only source of melatonin, which is also synthesized in several tissues and cells including the gastrointestinal tract, retina, skin, Harderian gland, kidney, liver, airway epithelium, lymphocytes, mononuclear cells, pancreas, platelets, red blood cells, thymus and thyroid glands (Acuña-Castroviejo et al., 2014).

Melatonin degradation can take one of three different metabolic pathways. First, it can be degraded through oxidative catabolism leading to the formation of unstable intermediary kynurenine derivative AFMK, which is further deformylated to be more stable, and metabolized to the primitive and primary active metabolite of melatonin N1-acetyl-5-methoxy-kynunerine (AMK) (Arendt, 2006). Second, melatonin can be metabolized via the hydroxylation pathway in the liver by microsomal enzymes to form 6-sulfatoxy-melatonin (aMT6s), which is eliminated in the urine and used to measure the plasma melatonin profile in urine (Arendt, 1998). Finally, melatonin can be hydroxylated and converted into cyclic 3-hydroxymelatonin (Arendt, 2006).

1.4.4. Melatonin effects on endocrine network

Melatonin affects the anterior pituitary activity and influences the synthesis and secretion of steroid and non-steroid hormones (Chowdhury and Maitra, 2012). Melatonin modifies the synthesis and secretion of different anterior pituitary hormones like adrenocorticotropin (ACTH), thyrotropin (TSH), and growth hormone (GH) by directly influencing the secretory activity of the pituitary cells or indirectly by influencing the hypothalamic neurons producing the neurohormones that inhibit or stimulate the release of these hormones (Pevet et al., 2017). Melatonin has a negative effect on the synthesis

of PRL and GH (Misztal et al., 2018) and it is involved in the regulation of Ca and P metabolism by stimulating the parathyroid gland or inhibiting calcitonin release and prostaglandin synthesis. Melatonin decreased PRL and IGF-I (Dahl et al., 2000) in dairy nevertheless, Lacasse et al. (2014) reported that melatonin decreased PRL but tended to increase IGF-I. Moreover, it affects the activity of pituitary–adrenal axis by modulating the peripheral action of corticoids (Chowdhury and Maitra, 2012).

1.4.5. Subcutaneous melatonin implants

In general, the form of melatonin application adopted for sheep has been subcutaneous implants (2 × 4 mm) applied to the base of the ear. Commercial subcutaneous implants of 18 mg melatonin (such as Melovine; CEVA Salud Animal, Barcelona), allow a slow and continuous release of melatonin without suppressing the endogenous nocturnal secretion of the hormone (Zarazaga et al., 1998). Administration of melatonin in this way provides photoperiodic information that the sheep interprets as short days (Malpaux et al., 1997). Haresign (1990) observed that a single implant was effective in in English sheep breed females, however, it also recommended the use of 3 implants for stimulating males.

Subcutaneous melatonin implants need at least 36-d to induce greater cyclicity in treated animals, obtaining better results with 93-d of implant exposure. According to Abecia et al. (2007), the usual duration of elevated plasma melatonin levels after treatment with subcutaneous implants is 70 d. Forcada et al. (2002) observed how implanted animals maintained elevated melatonin levels after 100 d of treatment, and those levels seemed to decline after 120 d of implant application.

1.4.6. Melatonin effects on reproductive performances

The exogenous melatonin implants are considered a useful tool to advance the onset of the breeding season and improve lamb production (i.e. fertility, prolificacy and litter size) in sheep (Abecia et al., 2011, 2012). Abecia et al. (2007) reported 15 to 30% increase of number of lambs produced in the Rasa Aragonesa, Assaf and Merino sheep breeds, but its efficacy vary according to the breed, season and farm. Melatonin seems to increase the number of cyclic ewes before introduction of rams (Abecia et al., 2012). Furthermore, melatonin treatment leads to increase the number of ewes exhibiting full length cycles in response to the contact with the rams (Abecia et al., 2006), as a consequence of its luteotrophic effect of melatonin. Likewise, it has been demonstrated that melatonin

administration is an effective method to induce cyclicity and to increase ewe's ovulation rate by increasing the number of ovulatory follicles (Forcada et al., 1995). Melatonin implants at lambing can also improve the viability of embryos of undernourished ewes during the reproductive season (Vázquez et al., 2013). Furthermore, Abecia et al. (2002) reported that melatonin favoured the viability of embryos in vitro, and it was capable to reduce the production of PGF2α in vitro during the ewe anestro.

1.4.7. Melatonin effects on lactational performances

Melatonin treatment has been studied in ruminants, with special interest in dairy sheep, because the impact of cyclicity in their reproduction and following lactation, to evaluate its secondary effects on milk yield and milk composition in different stages of lactation and seasons.

The use of exogenous MEL tries to improve cyclicity by emulating the effects of the short-day photoperiod of autumn. Nevertheless, as melatonin may decrease PRL, IGF-I and GH, negative effects are expected on milk yield whereas milk composition may be improved similarly to observed in late-lactation. On the other hand, when MEL and short-day photoperiod are used during late pregnancy and in the dry period, it may be expected increases in PRLR (PRL receptors) which will increase milk production in the following lactation (Dahl et al., 2000). So, the occurrence of short-days (winter) in the dry period will enhance the lactation performances in the next lactation (spring). Nevertheless, reported on the use of photoperiod and melatonin are in some cases contradictory, indicating that other factors may alter the hormonal responses.

As shown in Table 1.5, the most important effects of photoperiod (short- vs. long-days) on lactating dairy ruminants can be summarized as:

i) Inconsistent on feed intake (range, –16 to +12%),
ii) Decreased milk yield (range, –25 to –6%), during lactation, but increased milk yield in the following lactation when applied during pregnancy (+9 to +26%),
iii) Inconsistent on milk composition (fat, –5 to +10%; protein, 0 to +4%), tending to increase milk contents.
iv) Decreased plasmatic PRL (range, –44 to –7%) and IGF-I (–51 to –13%), and
v) No effects on body reserves.

In conclusion, short-day photoperiod (8-h light), as in autumn and winter, increases plasmatic melatonin and creates a negative hormonal environment for lactation which may reduce milk yield, although positively impacts on fat and protein milk contents.

Table 1.5. Summary of the effects of photoperiod treatments (SD = short day, 8-h light; LD = long day, 16-h light) in lactating ruminants.

Reference	Species (Breed)	Season	Stage	Treatments	Main effects (treatment vs. control)		
					Intake	Milk	Other
Photoperiod treatments:							
Bocquier et al. (1997)	Sheep (Sarda) (n = 38)	Controlled light	Early lactation	SD vs. LD	–16%	Yield (–25%). Composition: fat (+3%, NS) and protein (+4%)	No effects on BW
Dahl et al. (1997)	Cattle (Holstein) (n = 40)	Controlled light	Mid lactation	SD vs. LD	No effect	Yield (–6%), no effects on composition	IGF-I (–13%)
Miller et al. (2000)	Cattle (Holstein) (n = 34)	Controlled light	Dry, pregnant	SD vs. LD	+12%	Yield (+9%) in next lactation, no effects on composition	PRL (–44%), no effects on IGF-I
Auchtung et al. (2005)	Cattle (Holstein) (n = 39)	Controlled light	Dry, pregnant	SD vs. LD	No effect	Yield (+14%) in next lactation, no effects on composition	PRL (–7%), increased PRL receptors
García-Hernandez et al. (2007)	Goat (Alpine, Nubian & La Mancha) (n = 79)	Controlled light	Overall	SD vs. LD	+6%	Yield (–6%). Composition: fat (–5%)	BW (+5%), high frequency of pseudo-gestation in LD goats
Mabjeesh et al. (2007)	Goat (Saanen) (n = 8)	Controlled light	Dry, pregnant	SD vs. LD	No effect	Yield (+26%) in next lactation, no effects on composition	PRL (–38%), IGF-I (–51%)
Mikolayunas et al. (2008)	Sheep (East Friesian) (n = 22)	Controlled light	Dry, pregnant	SD vs. LD	n/d	Yield (+18%) in next lactation. Composition: fat (+10%) and no effect on protein	PRL (–7%)
Velasco et al. (2008)	Cattle (Holstein) (n = 40)	Controlled light	Dry, pregnant	SD vs. LD	+7%	Yield (+10%) in next lactation, no effects on composition	PRL (–24%)
Flores et al. (2011)	Goat (Creole) (n = 31)	Late autumn	Early lactation	SD vs. LD	n/d	Yield (–19%). No effects on composition that tended to increase	No effects on BW and BCS

On the other hand, exogenous MEL has been used to increase circulating melatonin to unleash the onset of reproductive season in sheep. The main effects in lactating ruminants, with special emphasis in small ruminants, are summarized in Table 1.6. Moreover, some data was included in control treatments of Table 1.7, and are:

i) Decreased milk yield during lactation (range, –23 to 0%) in cattle, but not in sheep,
ii) Increased milk yield in the following lactation, when applied during pregnancy (+11%) in goats,
iii) Improved milk composition: fat (range, 0 to +14%), protein (range, 0 to +6%) and casein (range, 0 to +7%),
iv) No effects on BW, and
v) Inconsistent effects on plasmatic PRL (range, –58 to +16%) and GH (range, –43 to +85%), depending on photoperiod.

The joint effect of MEL supplements and photoperiod were studied by several authors and their results are summarized in Table 1.7. and are:

i) Small increase in feed intake or feed efficiency (range, 0 to +4%),
ii) Inconsistent changes in milk yield (range, –35 to +26%),
iii) Inconsistent effects on milk composition: fat (range, –4 to 0%), protein (0 to +15%),
iv) No effects on BW, and
v) Decreased plasmatic PRL (range, –71 to 0%), but inconsistent GH (–43 to +85%), depending on photoperiod.

As final conclusions, the administration of exogenous MEL generally induces a decrease in milk yield with changes in milk composition similar to those observed in the end of lactation. MEL effects are mainly driven by the decrease in PRL secretion, which also decrease the circulating levels of IGF-1 and GH, although many results are inconsistent. This may be, in part, explained by an important interaction with photoperiod, the effects of MEL being greater under short-day conditions (autumn and winter). Although most results indicate that MEL cannot be used to mimic a short-day photoperiod during the dry period of cattle, positive effects in the following lactation have been reported in goats. The effects may be similar in dairy sheep, but they are currently unknown.

Table 1.6. Summary of the effects of melatonin implants (MEL = melatonin; CO = control) in lactating ruminants.

Reference	Species (Breed)	Season	Stage	Treatments	Main effects (treatment vs. control)		
					Intake	Milk	Other
Melatonin implants:							
Asher et al. (1994)	Red deer (n = 23)	Spring	Dry, pregnant	MEL vs. CO (1×18-mg)	n/d	Retardation of mammary gland development	Decreased PRL. No effects on BW
Misztal et al. (1997)	Sheep (Polish Lowland) (n = 8)	Spring	Dry	MEL vs. CO (1×18-mg)	n/d	n/d	PRL (+16%)
Abecia et al. (2005)	Sheep (Assaf & Lacaune) (n = 312)	Winter	Early-mid lactation	MEL vs. CO (1×18-mg)	n/d	No effects	Fecundity (+32%), no effects on litter size. Fertility (+276%) in Assaf
Auldist et al. (2007)	Cattle (NZ Friesian) (n = 12)	Summer[1] (solstice)	Mid-lactation	MEL vs. CO (3×6×18-mg)	n/d	Yield (–23%). Composition: fat (+14%), protein (+6%), casein (+7%) and lactose (–3%)	PRL (–58%) without changes in IGF-1
Morini et al. (2018)	Cattle (Holstein?) (n = 60)	Winter (21 Dec)	Dry, pregnant	MEL vs. CO (12×18-mg)	n/d	Yield tended to decrease (–8%)	n/d
		Summer (21 Jun)				No effects on yield	n/d
Aviles et al. (2019)	Goat (Creole) (n = 25)	Summer	Dry, pregnant	MEL vs. CO (1×18-mg)	n/d	Yield (+11%) in next lactation, no effects on composition	Increased kid's ADG

[1]South hemisphere.

Table 1.7. Effects of photoperiod (SD = short day, 8-h light; LD = long day, 16-h light) and melatonin treatments (MEL = melatonin; CO = control) in lactating ruminants.

Reference	Species (Breed)	Season	Lactation	Treatments	Main effects (treatment vs. control)		
					Intake	Milk	Other
Photoperiod and melatonin implants:							
Molik et al. (2010)	Sheep (Polish Longwool) (n = 20)	Autumn	Early suckling	SD-MEL vs. SD (1×18-mg)	n/d	n/d	No effects on PRL, increased GH (+85%)
		Spring		LD-MEL vs. LD (1×18-mg)	n/d	n/d	Decreased PRL (–71%) and GH (–43%)
Molik et al. (2012)	Sheep (Polish Longwool) (n = 60)	Controlled light	Early	SD vs. LD	n/d	No effects on yield. Composition: Protein (+6%)	No effects
				LD-MEL vs. LD (1×18-mg)	n/d	No effects on yield. Composition: Protein (+15%)	No effects
Molik et al. (2013)	Sheep (Polish Longwool) (n = 60)	Controlled light	Early	SD vs. LD .	n/d	Yield (–35%). No effects on composition	Decreased PRL (–34%) and GH (–28%)
				LD-MEL vs. SD (1×18-mg)	n/d	Yield (–16%). No effects on composition	Decreased PRL (–33%)
Lacasse et al. (2014)	Cattle (Holstein) (heifers, n = 29; cows, n = 32)	Controlled light	Dry-pregnant. LD after calving	SD vs. LD	No effect heifers Cows (+4%)	No effect in heifers. Yield (+11%) and fat content (+4%) in cows	Decreased PRL (–50%)
				LD-MEL vs. SD (orally 25 mg/d)	No effect	No effects on yield. Composition: Fat (+4%)	Decreased PRL (–51%)
Ponchon et al. (2017)	Cattle (Holstein) (n = 30)	Controlled light	Dry, pregnant	SD vs. LD	n/d	Yield tended to decrease	PRL tended to decrease
				LD-MEL vs. SD (orally, 4 mg/100 kgBW and d)	n/d	No effects on yield and composition	No changes in serum albumin, SCC and udder involution
Misztal et al. (2018)	Sheep (Polish Longwool) (n = 36)	Winter & summer	Early	SD vs. LD	n/d	Yield (+26%). No effects on composition	No effects
				LD-MEL vs. SD (1×18-mg)	n/d	No effects on yield and composition	Increased PRL. No effects on BW

Moreover, the reported studies in dairy ruminants imply that part of the seasonal variation in milk yield and composition cannot be mitigated by strategies involving only nutrition and it seems to be recommendable to add the effect of light supplementation to allow long-day photoperiod. Further studies are required to confirm this conclusion.

1.5. Cabergoline and dry off period of dairy sheep

1.5.1. The importance of the dry off period

Drying-off is a challenging period for dairy sheep because usually it coincides with late pregnancy period were pregnant ewes are susceptible to ketone bodies toxemia and new intramammary infections because of the increase of glucose demand and the decrease of immunocompetence (Zhao et al., 2019). The risks are greater in high-yielding and twin-bearing ewes (Silva-del-Río et al., 2010), especially when using low energy diets or feed restriction methods at dry-off period (Caldeira et al., 2007).

Usually, dry-off practices can be abrupt or gradual, including various milk cessation methods: restricted feeding, reduction milking frequency, application of internal teat sealants and administration of antibiotic dry therapy (Vilar and Rajala-Schultz, 2020), in order to have animals starting a new lactation with a healthy and uninfected mammary gland. In dairy sheep, where abrupt drying-off is commonly done, selective (i.e., IMI) or generalized antibiotic therapy is recommended at drying-off to improve udder health and milk yield in the following lactation (Gonzalo et al., 2004). Cessation of milking results in udder engorgement, which leads mammary gland epithelium to apoptosis and, if excessive, induces mammary inflammation and cell necrosis (Zobel et al., 2015).

To avoid inappropriate drying-off and to alleviate the nutritional stress or animal discomfort caused by milking cessation, the use of dry-off facilitator (e.g., cabergoline) could be an interesting method to provoking the cessation of milk production by interfering with the transmission of hormonal signals from the pituitary gland (Lacasse et al., 2019).

1.5.2. Cabergoline characteristics

Cabergoline, a dopamine agonist and ergot (*Claviceps spp.*) derivative (Schardl et al., 2006) is a potent and long-acting inhibitor of PRL secretion, with an elimination half-life ranging between 60 and 109 h (Del Dotto and Bonuccelli, 2003). The long duration of action stems from its slow elimination from pituitary tissue, high-affinity binding to

pituitary dopamine receptors, and extensive enterohepatic recycling (Andreotti et al., 1995). Although cabergoline is commonly described as a very specific and high affinity dopamine D2 receptor agonist (Odaka et al., 2014), it also possesses significant affinity for the D3, D4, $5HT_{1A}$, $5HT_{2A}$, $5HT_{2B}$, $5HT_{2C}$ receptors, as well as low affinity for the D1 receptors (Sharif et al., 2009).

Cabergoline had similar efficacy as bromocriptine in inhibiting lactation with the advantages of easier dosing, better tolerability and fewer drug interactions due to his own pharmacokinetic properties that differentiated it from all other ergotic (e.g., bromocriptine) and non-ergotic (e.g., quinagolide) dopamine agonists (Ferrari et al., 1995).

1.5.3. Cabergoline mode of action and main uses

Cabergoline, as a long-acting D2 receptor dopamine agonist, is used to suppress lactation by inhibiting PRL secretion, which is considered a key factor for mammary development and lactation (Webster, 1996). Cabergoline has a direct inhibitory effect on the lactotroph cells of the anterior pituitary gland by binding to dopamine D_2 receptors and suppressing PRL secretion (Figure 1.7). By this mode of action, cabergoline decreases milk yield in dairy animals, reducing the risk of milk leakage, new intramammary infections and discomfort at dry-off.

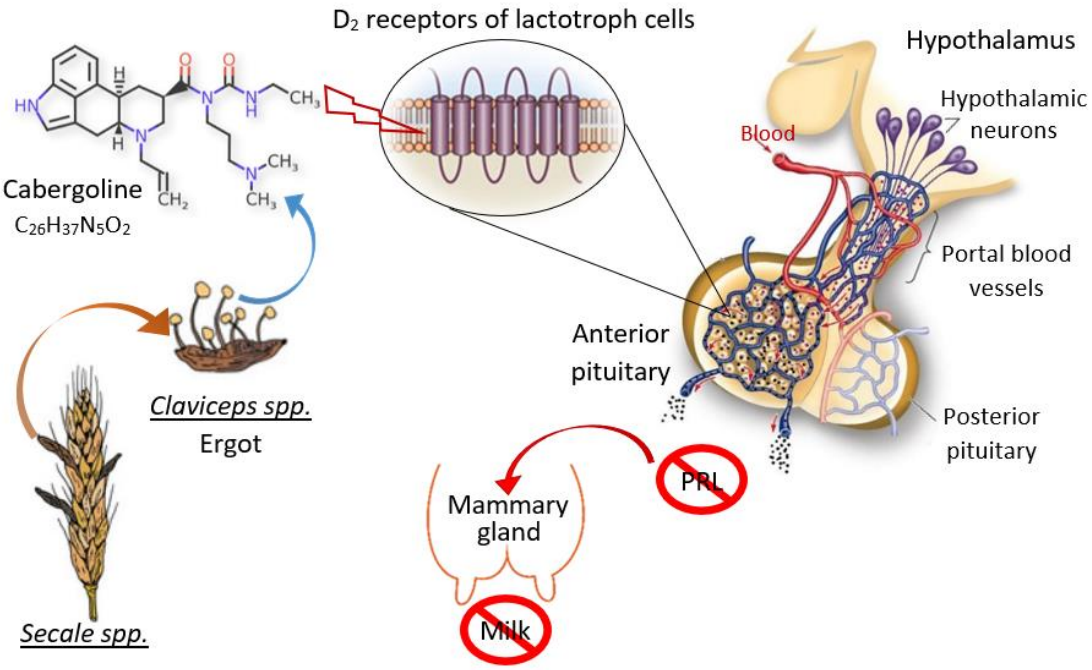


Figure 1.7. Inhibitory effect of cabergoline on prolactin secretion by pituitary gland.

Cabergoline is used in the treatment of hyperlactation by suppressing PRL secretion and dysfunctions related to hyperprolactinemia (Stolerman, 2010). It is frequently used to suppress PRL-secreting hypothalamic tumors (Becker, 2007) and as first-line agent in the management of prolactinomas (pituitary tumors) due to its less adverse effects, less rebound of mammary gland activity, and more convenient dosing schedule than bromocriptine (Webster, 1996). Moreover, cabergoline is also used to treat Parkinson's disease (Stolerman, 2010) and Cushing's Disease (Hopkins and Fleseriu, 2017). It also has shown promise as a potential drug to treat restless leg syndrome (Becker, 2007).

1.5.4. Cabergoline effects on milk secretion

Cabergoline treatment has been studied in mammals especially in humans, because of its important effect in puerperal women with hyperprolactinemic disorders (Webster, 1996). Few and controversial data are available on the use of cabergoline in small ruminants and the adequate dose for dairy ewes are unknown.

The main effects of cabergoline treatments in dairy ruminants are shown in Table 1.8 and can be summarized as:

i) Decreased PRL (range, –39 to –20%),
ii) Decreased milk yield (range, –28 to 0%),
iii) Reduced milk leakage (range, –20 to –10%),
iv) Reduced udder volume (range, –7 to 0%), and
v) Decreased udder pressure (–73%), udder pain (–2.8 times) and IMI risk (–21%).

The use of cabergoline, initially authorized by the European Medicines Agency (EMA, 2015) for facilitating the dry-off of cattle, decreased plasma PRL and accelerated udder involution reducing the secretory activity of mammary epithelial cells, udder engorgement and incidence of milk leakage in dairy cows (Bach et al., 2015; Boutinaud et al., 2016). Despite the positive effects of cabergoline and the no food safety risks for consumers, when withdrawal period is respected (i.e., 32 d during dry-off or 8 milkings during lactation; EMA, 2015), its use in high-yielding dairy cows at late pregnancy has been associated to occasional adverse events, usually in the 24-h post-injection. These were recumbency and mortality, which were related to metabolic disorders (i.e., hypocalcemia, hypothermia, ataxia, adipsia, circulatory disorder and diarrhea). Therefore, the marketing authorization of cabergoline as Velactis (Ceva Animal Health, Libourne, FR) was first suspended (EMA, 2016) and finally its use banned in Europe (EMA, 2019),

considering that the overall benefit-risk balance in dairy cows was negative.

Few and controversial data are available on the use of dopamine agonists in small ruminants. Arlt et al. (2011) reported the inefficacy of cabergoline to cease inappropriate lactations in hobby goats. On the other hand, Lacasse et al. (2016) cited no effects on milk production of repeated injections of quinagolide (1 mg/d for 4 wk; B. Ponchon, V. Lollivier and M. Boutinaud, unpublished results), whereas a single cabergoline injection (1 mg; V. Lollivier and M. Boutinaud, unpublished results) decreased milk yield (–28%) in dairy goats. The effects of dopamine agonists and the adequate dose for dairy ewes are unknown.

Finally, it should be stressed that the use of cabergoline is currently suspended in the EU for cattle and that its use in dairy sheep will require a specific approval by the EMA.

Table 1.8. Summary of the main effects of cabergoline and conventional treatments at dry-off in dairy ruminants (CAB = cabergoline; CO = control; ATB = preventive intramammary antibiotic; FR = feed restriction; MFR = gradual milking frequency reduction).

Reference	Species (Breed)	Dose	Stage	Treatments	Main effects (treatment vs. control)		
					Intake	Milk	Other
Arlt et al. (2011)	Goat (Hobby) (n = 5)	Single	Dry-off	CAB vs. CO	n/d	No effects	No effects
Bach et al. (2015)	Cattle (Holstein) (n = 199)	Single (5.6 mg)	Dry-off	CAB vs. CO	n/d	Leakage (–20%)	PRL (–20%), udder volume (–7%)
Boutinaud et al. (2016)	Cattle (Holstein) (n = 14)	Single (5.6 mg)	Dry-off	CAB vs. CO	n/d	No effect on yield. Content: SCC and fat tended to increase. No effects on α-LA, protein and citrate	PRL (–39%), udder volume (–7%)
Lacasse et al. (2016)[1]	Goat (n/d) (n = 10)	Single (1.0 mg)	Mid-lactation	CAB vs. CO	n/d	Yield (–28%)	No effects
Bertulat et al. (2017)	Cattle (Holstein & Montbeliard) (n = 234)	Single (5.6 mg)	Dry-off	CAB vs. CO	n/d	Leakage (–10%)	Udder pressure (–73%) in primiparous but not in multiparous, udder pain (–2.8 times)
Hop et al. (2019)	Cattle (Holstein & Montbeliard) (n = 840)	Single (5.6 mg)	Dry-off	CAB vs. CO	n/d	Leakage (–20%)	IMI risk (–21%)
				ATB[2] vs. CO	n/d	No effects	No effect
Steeneveld et al. (2019)	Cattle (n/d)	Single (5.6 mg)	Dry-off	CAB vs. FR	n/d	n/d	Savings per cow = €49.5
				CAB vs. MFR	n/d	n/d	Savings per cow = €21.9

[1]Cited as a personal communication from V. Lollivier and M. Boutinaud (unpublished results). [2]Cefquinome (150 mg/quarter).

CHAPTER 2

Objectives

CHAPTER 2

Objectives

The general goal of this thesis was the evaluation of the effects of shearing, melatonin implants and cabergoline injection treatments, used as management tools during lactation, on the lactational performances of 2 breeds of dairy ewes (i.e., Manchega and Lacaune), similar in body frame but differing in milk yield and milk composition.

The specific objectives were:

- To study the nutritional (i.e., feed intake) and productive (i.e., milk yield and composition, rectal temperature, body weight) responses of Manchega and Lacaune dairy ewes to shearing, as a cold stress factor, during lactation under mild-winter conditions.
- To evaluate the lactational performances (i.e., milk yield, milk composition, feed intake, body weight, blood analysis) of Manchega and Lacaune dairy ewes subcutaneously implanted with melatonin, in early-lactation and under autumn conditions.
- To identify the effective doses of cabergoline and to evaluate the effects of cabergoline on prolactin suppression, milk secretion and the time-lasting effects in Manchega and Lacaune breeds of dairy ewes in late-lactation.

CHAPTER 3

Shearing effects in lactating dairy ewes

CHAPTER 3

Effects of shearing two breeds of dairy ewes during lactation under mild winter conditions[1]

3.1. ABSTRACT

The lactational effects of shearing (CO, control unshorn; SH, shorn) were investigated in 48 dairy ewes of 2 breeds (Lacaune, LC, n = 24; Manchega, MN, n = 24) having similar stage of lactation (120 ± 6 DIM) and body frame (65.1 ± 1.5 kg BW and 2.4 ± 0.1 BCS), but differing in fleece and milk production. Ewes were penned indoors, adapted to the diet (alfalfa hay ad libitum and fixed amount of concentrate) and allocated for 30 d in 8 balanced groups to which the experimental treatments were applied. All ewes were sheared on the same day. Feed intake by pen and milk yield by ewe were recorded daily. Individual samples of milk (d −3, 3, 5, 7 and 15) and blood (d −7, 3, 7 and 15) were collected, as well as BW and BCS measured (d −15, 0 and 15), related to shearing. Pooled milk samples per pen were also collected before and after shearing for milk FA analysis (d −3 and 15). Average temperatures in the barn before (12.6 ± 0.7ºC) and after (13.7 ± 0.4ºC) shearing were mild. Fleece was heavier in MN than in LC (1.04 ± 0.10 vs. 0.75 ± 0.09 kg/ewe) and tended to cover more body surface in MN than in LC ewes. Responses to shearing varied according to breed, the rectal temperature after shearing only decreasing significantly in the MN (−0.36 ± 0.09ºC). Feed intake increased in the LC-SH (5%), when compared to LC-CO, but did not vary in the MN ewes. Ingestibility of the alfalfa hay, expressed as filling units for sheep (FUs) and monitored in 2 groups of 6 dry and unshorn ewes of each breed (73.0 ± 2.5 kg BW and 3.1 ± 0.2 BCS), was constant throughout the experiment (0.99 ± 0.03 FUs/kg DM). Regarding milk production, LC-SH ewes yielded 10% more milk (1.38 ± 0.06 vs. 1.52 ± 0.05 kg/d) than LC-CO ewes, but no differences were detected in MN ewes (0.74 ± 0.03 kg/d, on average). No differences in the concentration of major milk components by effect of the shearing treatment were detected in either breed, but LC-SH ewes yielded 9% more milk protein than did LC-CO ewes. No relevant effects of shearing were also detected on milk fatty acid profiles, although MN ewes showed lower C4:0, C6:0, C14:0, t-11 and t-12 C18:1 contents, than did LC ewes. Moreover, no changes by effect of shearing were detected in plasma glucose, NEFA, cortisol and insulin values in either breed, as well as in BW or BCS. In conclusion, shearing dairy ewes during lactation under mild-winter conditions, is a suitable management option that may increase feed intake and milk production, without deleterious effects on milk composition.

[1]This article was published in: Elhadi, A., A.A.K. Salama, X. Such, E. Albanell, P.G. Toral, G. Hervás, P. Frutos, and G. Caja. 2019. Effects of shearing 2 breeds of dairy ewes during lactation under mild winter conditions. *J. Dairy Sci.* 102:1712–1724. https://doi.org/10.3168/jds.2018-15380.

3.2. INTRODUCTION

Shearing is usually considered to be a necessary practice for flock management in order to improve sheep welfare and production (Swanson and McGlone, 2010). Shearing modifies the limits of the thermo-neutral zone of sheep, increasing the lower critical temperature and inducing adaptive responses to maintain body homeostasis (Russel et al., 1985; Symonds et al., 1988). Shearing boosts the heat transfer between the animal and its environment, especially under cold-weather conditions, resulting in a greater feed demand to cope with the increased energy demand for heat production (18 to 78%, according to temperature; Elvidge and Coop, 1974). Different degrees of cold stress can be expected by breed according to their morphological traits and their physiological and behavioral adaptations. A greater degree of cold stress would result in a greater metabolic rate, thus increasing the amount of feed needed to cope with the requirements.

In the Mediterranean countries, traditional sheep production systems involve shearing at the beginning of the summer to match the onset of hot temperatures. In Spain, sheep are usually shorn around mid-May, before mating and starting traditional grazing on cereal stubbles or transhumance. Nevertheless, intensified production systems (i.e., high milk yield and long lactation length with delayed dry-off) and out-of-season breeding (i.e., increased lambing frequency for extending the harvest of milk in the farm) resulted in the need of shearing the ewes at any time during the year. These intensification practices are currently observed in the dairy farms of many sheep's milk leading countries (Pulina et al., 2018).

When shearing is conducted during winter or early spring, shorn sheep could suffer cold stress as a consequence of the low temperatures and having lost their insulation. Piccione et al. (2002) reported an increase of over 1ºC in the core body temperature of Mediterranean dry ewes (i.e., Comisana, Barbaresca and Pinzirita) after shearing in spring (mild conditions, 16 to 28ºC), as an over-reaction of the ewes to the stress.

Despite the expected effects of environmental temperatures on the thermoregulation of the lactating animals, little is known on the effects of shearing in lactating dairy ewes. So, our hypothesis was that shearing dairy ewes during winter, when they are open and lactating, could cause a thermoregulatory response due to the removal of their fleece, which will increase the metabolic rate and feed intake of the ewes to maintain their body temperature. This catabolic effect may also modify milk yield and milk composition (e.g.,

increase milk fat or decrease milk protein) by modifying the hormonal profiles and the partitioning of nutrients between the body and the udder. To our knowledge, only Aleksiev (2008) in Tsigai and Rassu et al. (2009) in Sarda dairy ewes, have studied the specific effects of shearing during lactation, with increases in water intake and milk fat composition, respectively. It is unclear if the differences in intake and milk composition observed were breed related.

To test our hypothesis, the effects of shearing on lactational performances (i.e., milk yield and composition including milk fatty acid profile), body reserves and physiological indicators (i.e., main blood metabolites and hormones) were studied in 2 breeds of dairy ewes, similar in frame but differing in milk yield and composition, under mild-winter conditions.

3.3. MATERIALS AND METHODS

The experiment was conducted in the experimental farm of the SGCE (Servei de Granges i Camps Experimentals) of the Universitat Autònoma de Barcelona in Bellaterra (Barcelona, Spain) located at N 41º 30'20" and E 2º 05' 46" (elevation, 162 m) in mid-February and under mild-winter conditions. The ewes were sheltered in a sheep barn enclosed by 3 walls, with the other open to the West and with windbreakers. The roof was thermo-isolated and provided with stack chimneys and fans.

Animal-care conditions and management practices agreed with the Spanish Royal Decree 53/2013, on the protection of animals used for experimental purposes, the codes of recommendations for the welfare of dairy sheep of the Ministry of Agriculture, Alimentation and Environment of Spain (MAPA, 2007) and the procedures stated by the Ethical Committee of Animal and Human Experimentation of the Universitat Autònoma de Barcelona (UAB).

3.3.1. Ewes Management and Feeding

A total of 48 ewes of 2 dairy breeds (LC, Lacaune, n = 24; MN, Manchega, n = 24) were used in mid-lactation (122 ± 8 and 118 ± 7 DIM, respectively). Ewes of both breeds (LC and MN, respectively) were of similar age (2.4 ± 0.3 and 2.9 ± 0.4 yr), BW (64.6 ± 1.7 and 65.5 ± 1.6 kg) and BCS (2.19 ± 0.10 and 2.52 ± 0.14). All ewes wore plastic ear

tags (Allflex Europe, Vitré, France) and ceramic rumen mini-boluses (20 g; Datamars, Bedano, Switzerland) for visual and electronic identification that were used for automatic milk recording (Ait-Saidi et al., 2014).

Machine milking was conducted twice daily (0700 and 1700) in a double, 12-stall parallel- milking parlor (Amarre Azul I; DeLaval Equipos, Alcobendas, Madrid, Spain) with a central high milk pipeline, silicone milking clusters (DeLaval SG-TF100) and automatic milk-flow and milk-recording devices (MM25SG; DeLaval, Tumba, Sweden). Milking was performed at a vacuum of 40 kPa, 120 pulses/min, and 50% pulsation ratio. The milking routine included manual cluster attachment, machine milking and automatic cluster detachment (milk flow rate < 0.1 L/min or milking time > 3 min). Teat dipping with an iodine solution (P3-ioshield; Ecolab Hispano-Portuguesa, Barcelona, Spain) was done at the end of milking.

An adaptation period to the experimental conditions (pen and diet) was applied during 3 wk to all animals. The diet consisted of alfalfa hay fed ad libitum, 0.15 kg/d of whole-grain corn and a farm-produced concentrate (ingredients: 50.0% soybean hulls, 10.0% barley meal, 10.0% oats meal, 10.0% gluten feed, 5.0% rapeseed 00 meal, 5.0% soybean oil, 4.0% corn meal, 2.5% bi-calcium phosphate, 2.0% cane molasses, 1.0% VitafacOvino-0.3premix, 0.5% salt, as fed) fed according to requirements (LC, 0.5 kg/d; MN, 0.3 kg/d, as fed) and distributed altogether after the morning milking. Moreover, all ewes received 100 g of concentrate and 50 g of whole-grain corn in individual feeders in the milking parlor at each milking for a faster bringing in. Nutrient requirements were calculated by INRAtion v.4.06 (Educagri éditions, Dijon, France). Composition and nutritive value of the feeds used in the experiment are shown in Table 3.1. Ewes had free access to water and to commercial mineral blocks (Multi-Block; Agrària Comarcal del Vallès, Llerona, Barcelona, Spain).

Voluntary intake of the alfalfa hay was assessed to monitor the differences between breeds and the quality of hay bales used during the experiment. With this aim, 2 groups of unshorn, dry and open dairy ewes of each breed (LC, n = 6, 3.0 ± 1.1 yr, 74.4 ± 4.0 kg BW and 3.00 ± 0.19 BCS; MN, n = 6, 3.8 ± 1.6 yr, 71.6 ± 3.6 kg BW and 3.13 ± 0.27 BCS) were used as previously done by Caja et al. (1997) and Flores et al. (2008) in dairy ewes. The ewes were penned in the same building and conditions as the lactating ewes during the experiment, fed the alfalfa hay alone and their voluntary intake was used to calculate the ingestibility according to INRA (2010).

Table 3.1. Chemical composition and nutritive value of the experimental feeds.

Item, DM basis	Alfalfa hay	Concentrate mixture	Corn grain
Component, %			
DM	88.5	90.6	87.8
OM	10.7	7.5	1.2
CP	16.8	15.1	8.0
Fat	1.9	8.1	3.9
Cellulose	30.4	21.2	1.7
NDF	46.3	39.4	7.9
ADF	33.4	25.1	1.4
Nutritive value[1]			
NE_L, Mcal/kg	1.16	1.62	1.84
UFL[2]/kg	0.68	0.95	1.08
PDIN[3], g/kg	121	89	64
PDIE[4], g/kg	97	58	84

[1]Estimated according to INRA (2010) tables and PreValim 3.3 software.
[2]Feeding units for lactation (1.7 Mcal EN_L).
[3]Protein truly digested in the small intestine allowed by N.
[4]Protein truly digested in the small intestine allowed by energy.

3.3.2. Experimental Treatments

The experimental design consisted of a 2 × 2 factorial (breed × shearing treatment) to which the ewe groups were randomly allocated. Shearing treatments were: control unshorn (CO) and shorn (SH) during lactation. No ewe had been shorn since May of the previous year. Machine shearing of the SH ewes was done in mid-February by a commercial sheep-shearer on the same day. Ewes were allocated in 8 balanced groups of 6 animals according to breed, age, BW, BCS and milk yield, to which the experimental treatments were applied. After the 3-wk adaptation period to pen and diet, the experimental period lasted for approximately 4 wk (from d −15 to 15, centred by the shearing treatment).

3.3.3. Measurements, Sampling and Analyses

Fleece Extension and Wool Weight. Fleece extension at the start of the experiment was scored subjectively in all ewes by 2 operators using a 3-point scale (1, open; 2, medium; 3, extended) with an accuracy of 0.5-points. Wool weight was measured after

shearing using an electronic scale (AND FV-60K; A&D Company, Tokyo, Japan; accuracy, 20 g).

Rectal and Environmental Temperatures. Rectal temperatures were recorded at d −1, 1, 3, 7 and 15, relative to shearing, using a digital clinical thermometer (Model ICO; Technology mini color, Barcelona, Spain; reading range, 32.0 to 43.9°C; accuracy, ± 0.1°C). Environmental temperature was recorded every 10 min by using a data logger (Opus 10; Lufft, Fellbach, Germany) and the data downloaded to a computer and processed using the analysis software SmartGraph2 (Lufft).

Milk Yield. Milk yield of individual ewes was recorded daily by weight during the whole experimental period by using the milk-flow and milk-recording automatic units of the milking parlor. Data were uploaded daily using the AlPro software 7.2 (DeLaval) and weekly reviewed and updated in a spreadsheet to avoid missvalues (Nieddu and Caja, 2017).

Milk Composition. Representative milk samples (100 mL) of each ewe were taken before (d −3) and after shearing (d 3, 5, 7 and 15) for compositional analyses. Daily milk samples were composited (60:40) according to the daily milking interval (14 and 10-h), preserved with an antimicrobial tablet (Bronopol; Broad Spectrum Micro-tabs II, D&F Control Systems, San Ramon, CA) and stored at 4°C until analysis. Non-homogenized milk samples were analyzed using a near infrared spectrometer (Foss Electric; Norderstedt, Germany) for fat, total protein (N×6.38), true protein and casein contents, according to Albanell et al. (1999). Calibrations were performed using data obtained by conventional methods including the Gerber method for fat, Kjeldahl method for total protein and oven-drying at 103ºC for total solids content. Samples were also analyzed for somatic cells count (SCC) in the Dairy Herd Improvement Laboratory of Catalonia (ALLIC, Cabrils, Barcelona, Spain) using an automatic cell counter (Fossomatic 5000; Foss Electric, Hillerød, Denmark).

Milk Fatty Acid Composition. Pool samples of each treatment group, composited according to the milk yield of each ewe, were prepared from individual milk samples at d −3 and 15 for fatty acid (FA) analysis. Milk fat was separated by centrifuging 10 mL of fresh milk (2,000 × *g*, 15 min at 4ºC; Hettich Zentrifugen, Universal 32R, Tuttlingen, Germany) and the obtained fat layer was transferred to 1.5 mL Eppendorf tubes. Milk lipids were extracted from 50 mg of milk fat using diethyl ether and hexane (5:4, vol/vol)

and transesterified to FA methyl esters (FAME) using freshly prepared methanolic sodium methoxide (Shingfield et al., 2003). FAME were separated and quantified using a gas chromatograph (Agilent 7890A GC System; Agilent Technologies, Santa Clara, CA) equipped with a flame-ionization detector and a 100-m fused silica capillary column (0.25-mm i.d., 0.2-μm film thickness; CP-SIL 88, CP7489; Varian Ibérica, Madrid, Spain) and using hydrogen as carrier gas. Total FA profile was determined in a 2-μL sample with a split ratio of 1:50 using a temperature gradient program, and C18:1 isomers were resolved in a separate analysis under isothermal conditions at 170°C, according to Shingfield et al. (2003). Peaks were identified based on retention time comparisons with commercially available standards, cross referencing with chromatograms reported in the literature, and by comparison with milk samples for which the FA composition was determined based on gas chromatography analysis of FAME and GC-MS analysis of corresponding 4,4-dimethyloxazoline derivatives (Bichi et al., 2013).

Body Weight and Condition Score. The BW and the BCS of all ewes were evaluated 3 wk before the start of the experiment, to allocate the ewes in balanced groups during the adaptation period, and at d −15, 0 and 15, relative to shearing. Weighing was performed using an electronic scale (Tru-test A6500; Auckland, New Zealand) and BCS was assessed (0 to 5 points; accuracy, ±0.25 points) according to Russel et al. (1969).

Blood Measures. Blood samples were taken from the jugular vein using 10 mL vacutainer tubes with sodium heparin 170 IU (BD; Belliver Industrial Estate, Plymouth, UK) at d −7, 3, 7 and 15 before the morning feeding. Plasma was obtained by centrifugation of whole blood for 15 min at 2,000 × *g* and 4ºC, and plasma transferred to 0.5 mL Eppendorf tubes and stored at −20°C for glucose, NEFA, insulin, cortisol and IGF-1 analyses. Glucose was determined by the hexokinase method (OSR 6121; Reagent System Olympus, Beckman Coulter, Krefeld, Ireland) and NEFA by the ACS-ACOD colorimetric enzymatic test method (Wako Chemicals; Neuss, Germany), in both cases for all sampling times, using an Olympus AU400 analyzer (Olympus Europa, Hamburg, Germany) reading at 340 and 540 nm, respectively. Samples of d −7 and 3 were also analyzed for insulin by ELISA sandwich type (Ovine Insulin; Mercodia, Uppsala, Sweden) and cortisol by ELISA competitive type (Ovine salivary cortisol; DRG Instruments, Marburg, Germany). The stopped ELISA plates were read in an automatic reader (iEMS Reader MF V.2.9-0, Labsystems España, Barcelona, Spain) at 450 nm for insulin and cortisol.

Feed Intake and Sampling. Feed intake of each group of dairy ewes was assessed daily throughout the experiment by measuring the amount of feed offered and refused in the pens. No refusals of concentrate were observed in the milking parlor. Hay and concentrate offered in the pens and their refusals were sampled daily and composited for pre- and post-shearing periods and preserved at room temperature until analysis.

Ingestibility of the alfalfa hay was assessed by measuring its voluntary dry matter (DM) intake when fed alone in the groups of dry ewes. Ingestibility obtained was expressed as Fill Units for sheep (FUs) by calculating the quotient between the intake of a forage of reference (i.e., standard prairie hay) and the observed intake per metabolic weight (g DM/kg $BW^{0.75}$) according to the INRA (2010), being:

$$\mathbf{FUs} = \frac{\mathbf{75}}{\mathbf{g\ DM/kg\ BW^{0.75}}}$$

Feed Analyses. The DM content was determined by gravimetry, desiccating the sample in an air-forced stove (103ºC for 24 h) and organic matter (OM) content was measured gravimetrically by ashing samples in a muffle furnace (550ºC for 4 h) according to AOAC (1990). Total N was determined by combustion according to the Dumas method using a Leco analyzer (Leco Corporation, St. Joseph, MI), and CP was calculated as N × 6.25. Cellulose was analyzed as crude fiber according to AOAC (1990), and neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined, on an ash-free basis, by adding amylase and sodium sulfite solutions according to Van Soest et al. (1991) and using an Ankom200 Fiber Analyzer incubator (Ankom Technology, Macedon, NY). Crude fat was analyzed as ether extract by the Soxhlet method according to AOAC (1990).

3.3.4. Statistical Analyses

Data were analyzed by the MIXED procedure for repeated measurements of SAS v. 9.1.3 (SAS Institute Inc., Cary, NC). The statistical mixed model contained the breed (LC vs. MN), the shearing treatment (CO vs. SH), the sampling time, and the breed×shearing and sampling-time×shearing interactions as fixed effects, as well as the random effects of the experimental unit (either the animal -for milk yield and composition, and body and plasma indicators- or the pen -for DM intake and FA profile-), and the random residual error. For DM intake and FA profile the random effect of pen(treatment) was used

according to St-Pierre (2007). Wool weight and environmental temperature data were analyzed by the GLM procedure for single or repeated measurements of SAS, respectively. In the case of the fleece extension the CATMOD procedure of SAS was used on the basis of the categorical nature of the variable.

For lactational performances (i.e., feed intake, milk yield and composition), body indicators (i.e., rectal temperature, BW, BCS) and physiological plasma indicators (i.e., glucose, NEFA, cortisol and insulin), the individual measurements taken before shearing were used as covariates and values averaged for their respective sampling dates. Values of variables were discussed as LSM and their means separated by the PDIFF test of SAS v. 9.1.3 (SAS Institute Inc.). Pearson's correlation (r) coefficients were calculated using the CORR procedure of SAS. Significance was declared at $P < 0.05$ and a tendency was considered when $P < 0.10$.

3.4. RESULTS AND DISCUSSION

3.4.1. Environmental Temperatures.

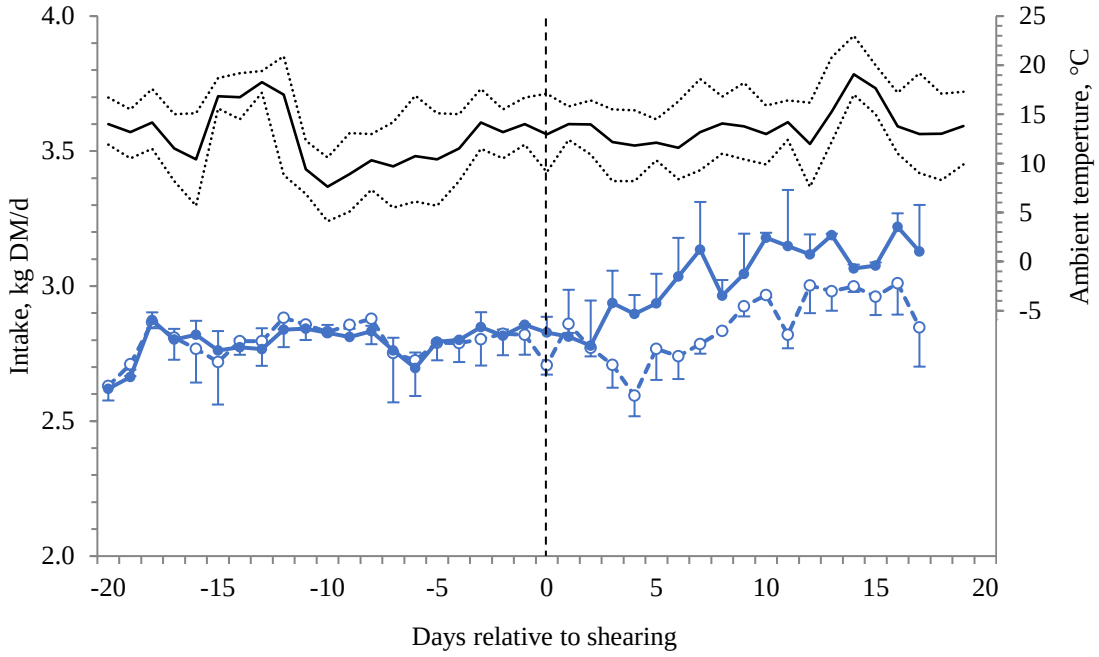
On average, mean temperatures in the sheep barn were 12.6 ± 0.7°C and 13.7 ± 0.4°C ($P = 0.08$), for the pre- and post-shearing periods, respectively. The pattern of changes was nearly symmetrical pre- and post-shearing and typical for a Mediterranean mild-winter (Figure 3.1), as has been habitual in the area over the last several years. Normal mean temperature values during winter (December to February) reported in the area (i.e., Barcelona airport, N 41º 17' and E 2º 4') are in the range of 9.2 to 10.0ºC according to the Spanish Meteorological Agency (AEMET, 2018). After shearing, the barn temperatures steadied, except for the peak reported in Figure 3.1 between d 13 and 15 (19.1ºC).

3.4.2. Wool Production

Despite having similar BW and according to the breed characteristics, clipped wool weight was lower in the LC than in the MN ewes (0.75 ± 0.09 vs. 1.04 ± 0.10 kg/ewe, respectively; $P = 0.038$). Fleece also tended to cover less body surface in LC than in MN ewes, as indicated by the extension score of the ewes before shearing (LC vs. MN, 1.39 ± 0.07 vs. 1.95 ± 0.13; $P = 0.08$). Correlations between wool weight and fleece score were positive for both breeds (r = 0.72 to 0.85; $P < 0.001$). Consequently, we expected to

induce greater cold stress by shearing the MN ewes as they had greater wool weight and fleece extension, compared to the LC ewes.

a) LC



b) MN

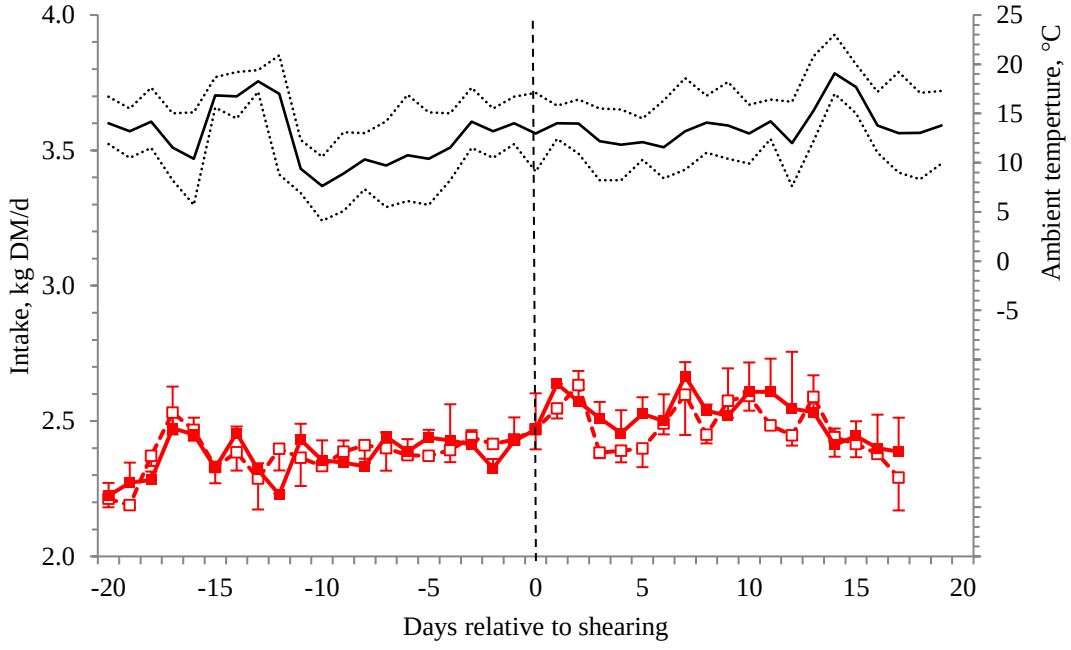


Figure 3.1. Ambient temperatures (mean in solid line, min and max in dashed lines) and voluntary feed intake recorded during mild-winter before and after shearing (CO, control; SH, shorn) in 2 breeds of dairy ewes: a) Lacaune (LC) breed (○, LC-CO; ●, LC-SH); b) Manchega (MN) breed (□, MN-CO; ■, MN-SH). Values are means with the SEM indicated by vertical bars.

3.4.3. Rectal Temperature

Agreeing with the fleece differences by breed above indicated, differences were observed in the variation of rectal temperatures before and after shearing according to breed (Figure 3.2). Rectal temperatures in the SH ewes decreased until d 3 in MN and d 7 in LC, and recovered thereafter. The mean temperature drop between CO and SH ewes was greater in the MN (−0.36 ± 0.07°C; $P < 0.001$) than in LC (−0.01 ± 0.09°C; $P = 0.93$).

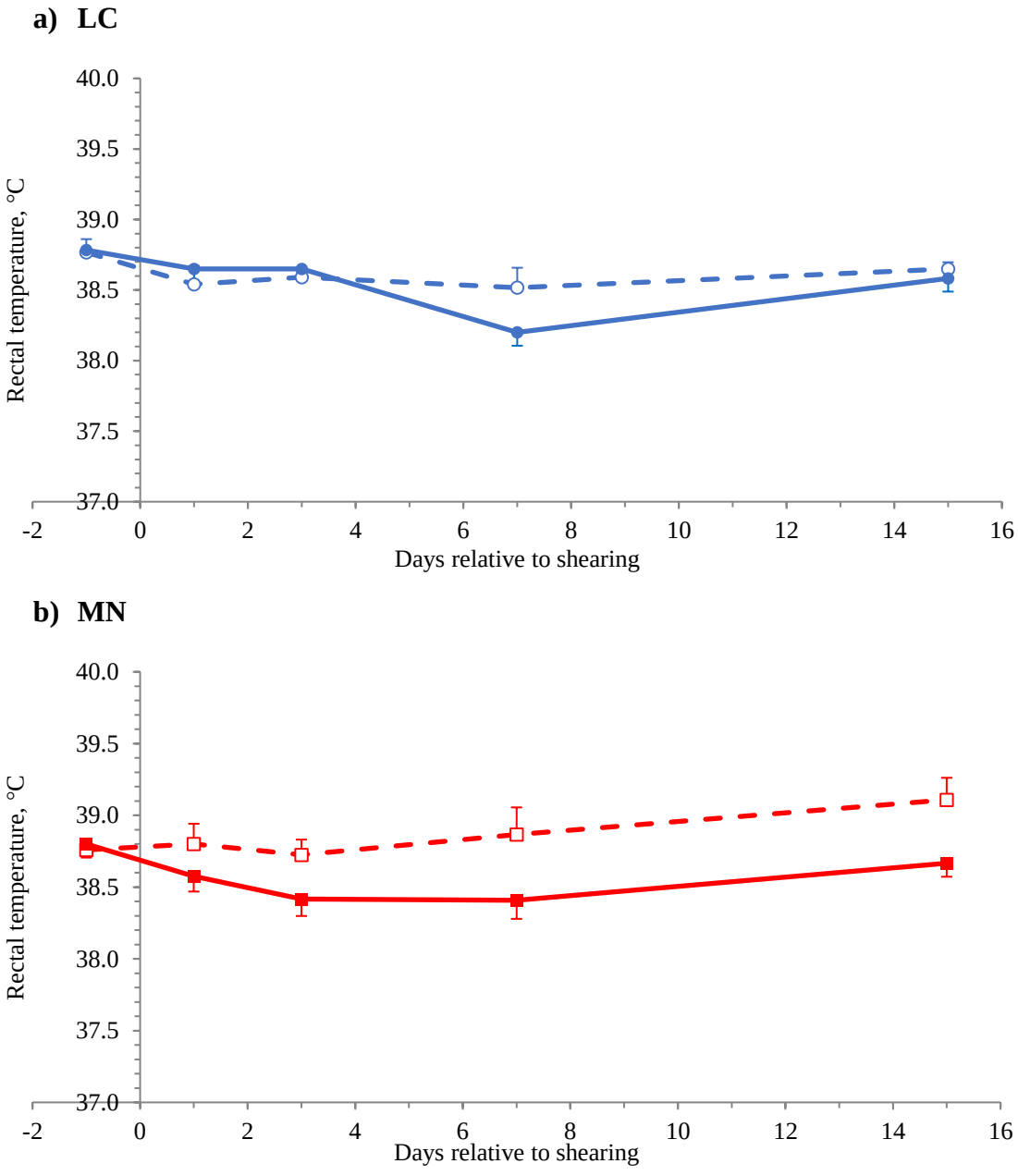


Figure 3.2. Rectal temperature before and after shearing (CO, control; SH, shorn) under mild-winter conditions in 2 breeds of dairy ewes: a) Lacaune (LC) breed (○, LC-CO; ●, LC-SH); b) Manchega (MN) breed (□, MN-CO; ■, MN-SH). Values are means with the SEM indicated by vertical bars.

The observed decrease in rectal temperatures of MN-SH was consistent, although smaller, with the results reported by Aleksiev (2008; −0.9ºC) in lactating Tsigai dairy ewes shorn during winter and maintained at mild ambient temperatures, as in our case. The greater drop in rectal temperatures reported in Tsigai ewes may be a consequence of the small frame and full fleece cover of this Balkan breed. Moreover, our results also agree with those of Leibovich et al. (2011) who sheared pregnant Assaf dairy ewes during the summer, with or without barn cooling, and found that rectal temperature decreased by −0.3°C and −0.2°C, in pregnant and lactating ewes, respectively. On the contrary, Piccione et al. (2002) reported that shearing several Mediterranean sheep breeds in mild-spring conditions (16 to 28ºC), increased their rectal temperature by 1ºC, which was considered to be a result of the hyperthermia induced by shearing stress at warmer temperatures. Although handling at shearing was done carefully in our lactating ewes, the stress-induced hyperthermia, if produced, could also have contributed to alleviate the cold effects observed in our SH-treated ewes.

3.4.4. Feed Intake

Values of voluntary feed intake of the dairy ewes during the experiment are shown in Figure 3.1. Although the temperatures of the barn varying approximately 10ºC during the pre-shearing period (d −15 to −10), DM intake values steadied in both LC and MN ewes as a result of the buffering effect of the fleece on thermoregulation.

During the post-shearing period, the buffer effect of the fleece on thermoregulation disappeared and DM intake showed a greater daily variation in both breeds (Figure 3.1). Nevertheless, the effects of shearing were only significant in the LC ewes in which DM intake increased 5% in the LC-SH ewes, when compared to LC-CO ewes (Table 3.2; P = 0.038). The intake increase found in our LC-SH ewes may have been a result of the increased energy requirements associated with the loss of insulation induced by shearing. No differences between CO and SH groups were observed in the MN ewes (Table 3.2; P = 0.38), reinforcing the importance of the breed effect in the response to shearing. Ruiz et al. (2008) also reported a 10% increase in the DM intake of lactating Latxa dairy ewes during winter, although the ewes were in this case shorn in late-pregnancy. According to Aleksiev (2008) the increase of intake after shearing, under mild-winter conditions, may not be evident despite a decrease in rectal temperature, as observed in Tsigai ewes and discussed above in the case of our MN ewes.

Table 3.2. Effect of shearing during mild-winter conditions on the lactational performances and the physiological indicators in the plasma of two breeds of dairy ewes (data are LS means).

	Lacaune		Manchega				Effect (*P*-value)		
Item	Control	Shorn	Control	Shorn	Mean	SEM	Shearing	Breed	Interaction[1]
Intake, kg DM/d	2.86[b]	3.01[a]	2.48	2.52	2.72	0.04	0.038	0.001	0.51
Milk									
Yield, kg/d	1.38[a]	1.52[a]	0.76	0.71	1.09	0.09	0.36	0.001	0.37
ECM[2], kg/d	1.33	1.43	0.87	0.82	1.11	0.09	0.58	0.001	0.67
Fat, g/d	95	101	68	65	82	7	0.64	0.001	0.89
Total protein, g/d	80[b]	87[a]	49	47	66	3	0.47	0.001	0.60
Lactose, g/d	62[b]	70[a]	35	31	50	3	0.57	0.001	0.23
SCC, $\log_{10}$/mL	5.36	5.24	5.09	5.36	5.26	0.16	0.60	0.63	0.18
Milk composition, %									
Fat	6.89	6.65	8.98	9.14	7.92	0.22	0.59	0.001	0.16
Total protein	5.80	5.74	6.50	6.59	6.16	0.21	0.55	0.001	0.15
True protein	5.63	5.43	6.37	6.84	6.07	0.25	0.51	0.001	0.13
Casein	4.31	4.15	4.92	5.27	4.66	0.18	0.56	0.001	0.11
Lactose	4.52	4.58	4.56[a]	4.39[b]	4.51	0.10	0.44	0.41	0.041
Plasma indicators									
Glucose, mg/dL	65.7	66.2	63.2[b]	65.2[a]	65.1	1.1	0.81	0.038	0.73
NEFA, mmol/L	0.125	0.095	0.115	0.100	0.109	0.015	0.47	0.61	0.52
Cortisol, ng/mL	6.5	5.4	5.6	3.8	5.4	1.1	0.73	0.84	0.97
Insulin, ng/L	0.43	0.44	0.34	0.40	0.40	0.08	0.60	0.21	0.53

[a,b,c]Within a row and breed, values with a different superscript differ ($P < 0.05$).

[1]Shearing × Breed.

[2]Energy corrected milk = Milk yield × [0.071× (Fat, %) + 0.043 × (Total protein, %) + 0.2224], according to Bocquier et al. (1993).

Breed effect was significant in our results and, on average, LC-CO ewes had 15% greater intake than did MN-CO ewes ($P < 0.001$), and LC-SH ewes had 19% greater intake than did MN-SH ewes ($P < 0.001$). Nevertheless, shearing × breed interaction was not detected on feed intake ($P = 0.51$). Apart from the differences in the fleece, the observed breed effect on intake may be related to the differences in milk production of each breed (Table 3.2).

Voluntary intake of the dry and open ewes used to monitor the ingestibility of the alfalfa hay steadied during the experimental period and was 0.99 ± 0.03 FUs/kg DM, on average. This value was close to that of the standard prairie hay used as the forage of reference (i.e., 1 FUs = 1 kg DM), and also showed the thermoregulatory buffering effects of the fleece on intake.

3.4.5. Milk Yield

Results of milk yield of the dairy ewes according to breed and shearing treatments, are shown in Table 3.2 and Figure 3.3. Milk yield slightly decreased in both breeds as lactation advanced showing small daily changes throughout the experiment. As a response to shearing during lactation the LC-SH ewes increased milk yield by 10%, when compared to LC-CO ewes ($P = 0.049$), and the effect was maintained until the end of the experiment. On the contrary, no differences were detected in the milk yield of the MN ewes by shearing ($P = 0.26$) which agreed with the results reported in Suffolk-crossbred (McBride and Christopherson, 1984) and Tsigai (Aleksiev, 2008) ewes shorn during lactation. Moreover, Ruiz et al. (2008) did not find effects of shearing in late-pregnancy on the milk yield of the following lactation in Latxa ewes. It should be stressed that some of the controversial results reported in the literature may be a consequence of the methodology used; McBride and Christopherson (1984) submitted the shorn ewes to cold conditions during lactation and estimated their milk yield by weight-suckle-weight of the lambs, whereas Ruiz et al. (2008) used the oxytocin technique and sheared the ewes in late-pregnancy. In the present study, ewes were selected after the weaning of their lambs, milk was measured directly by machine milking and the shearing took place during mid-lactation.

On the other hand, comparing our breeds of dairy ewes, LC produced on average 82% and 114% more milk than did MN before and after shearing (Table 3.2; $P < 0.001$),

respectively. The differences between our MN and LC ewes agreed with the values previously reported by Rovai et al. (2008) and Castillo et al. (2008a), under the same management conditions. Milk yield before shearing did not correlate with wool weight (r = 0.14 to 0.36; *P* = 0.68) or the fleece extension score (r = 0.07 to 0.25; *P* = 0.78) in either breed, indicating that, under our mild-winter and intensive-feeding conditions, fleece cover of dairy ewes was not relevant for thermoregulation.

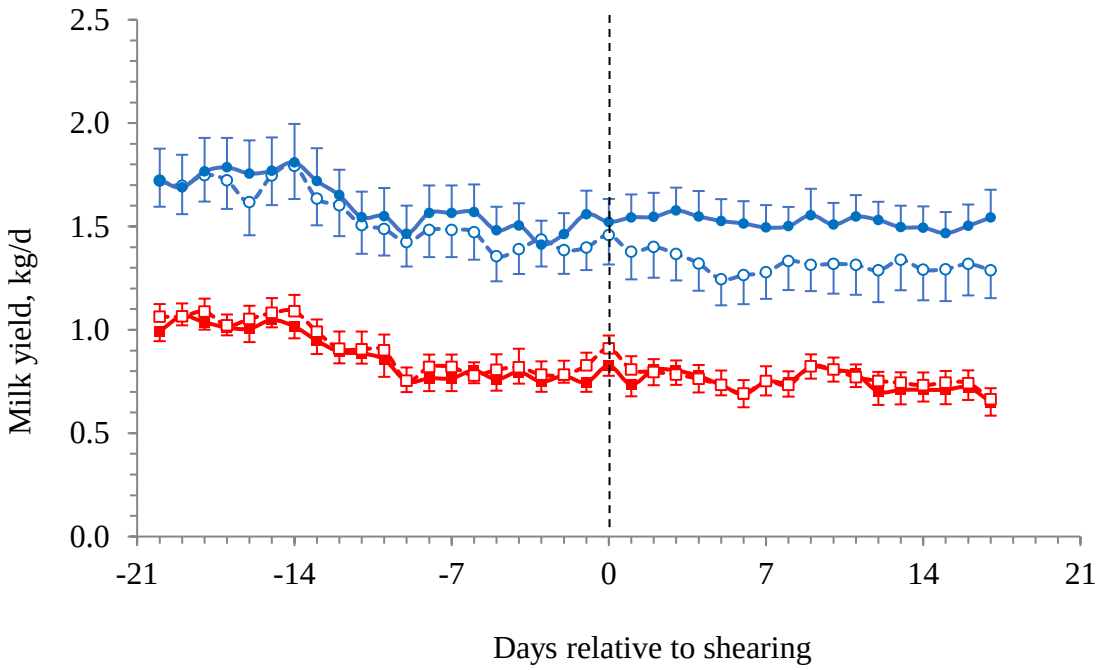


Figure 3.3. Milk yield before and after the shearing (CO, control; SH, shorn) under mild-winter conditions in 2 breeds of dairy ewes: Lacaune (LC) breed (○, LC-CO; ●, LC-SH) and Manchega (MN) breed (□, MN-CO; ■, MN-SH). Values are means with the SEM indicated by vertical bars.

3.4.6. Major Milk Components

There were no dramatic changes or differences between SH and CO treatments in the concentration of major milk components of either breed throughout the experiment (Table 3.2). Milk composition of all ewe groups steadied on the days immediately after shearing and slightly tended to decrease for fat and protein contents thereafter, whereas lactose content tended to increase. Nevertheless, LC-SH yielded more milk protein (9%; *P* = 0.044) and lactose (12%; *P* = 0.012) than did LC-CO, as a consequence of the greater milk yield of the LC-SH ewes discussed above. Our results did not agree with those

reported by McBride and Christopherson (1984), who found that shearing during suckling under cold conditions (i.e., 0ºC) improved milk fat content by 26% in Suffolk-crossbred ewes, nor with those of Rassu et al. (2009) who reported 9% increase in milk fat content of Sarda dairy ewes shorn during lactation in spring and attributed the effect to the cold nights (temperatures non available). The differences may be explained by the fact that, in our case, the lower extreme temperatures of the shelter, observed during the nights, were greater than 5ºC (Figure 3.1). No difference in milk protein content was reported by Ruiz et al. (2008) in Latxa dairy ewes shorn in late-pregnancy.

As expected, agreeing with the milk yield differences and with early reports (Castillo et al., 2008a), the breed greatly conditioned milk composition (Table 3.2). Thus, the concentration of most milk components was greater in MN than in LC but, on the other hand, the lactose content did not vary by breed (4.49 ± 0.07%, on average; $P = 0.88$).

3.4.7. Milk Fatty Acids Profile

Table 3.3 summarizes the effects of the treatments on the FA profile of the milk fat of our LC and MN dairy ewes, according to their carbon-chain length (C<16, C16 and C>16) or saturation-degree (SFA, saturated FA; MUFA, monounsaturated; PUFA, polyunsaturated) groups. No effects were detected on FA chain-length or saturation-degree groups, the milk of our ewes being characterized, on average, by high proportions of long chain (C<16:C16:C>16 = 34:28:38) and saturated FA (SFA:MUFA:PUFA = 71:23:6), respectively. All the obtained values were in the range of those reported by Ferrand-Calmels et al. (2014) in a large collection of samples from French dairy ewes mainly fed forage diets. The values also agree with those previously observed in Italian dairy ewes under grazing conditions and supplemented indoors with concentrate and oats as reported by Signorelli et al. (2008). The slightly greater MUFA contents observed in the data of our ewes, when compared to those of Signorelli et al. (2008), agreed with the fact of being supplemented with soybean oil (5% in the concentrate, as fed) as previously reported by Bouattour et al. (2008) in dairy goats.

Table 3.3. Effects of shearing during mild-winter on the major classes of fatty acids (FA) according to carbon chain lengh and saturation degree in the milk of two breeds of dairy ewes.

	Lacaune		Manchega				Effect (*P*-value)		
Item, g/100 g total FA	CO	SH	CO	SH	Mean	SEM	T[1]	B[2]	T×B[3]
Chain length									
<C16	35.9	34.3	34.2	34.6	34.8	0.5	0.47	0.28	0.22
C16	27.4	27.9	27.4	27.7	27.6	0.3	0.20	0.87	0.98
>C16	36.7	37.8	38.4	37.7	37.6	0.6	0.85	0.31	0.30
Saturation degree									
SFA[4]	70.9	70.2	70.2	70.8	70.5	0.6	0.95	0.88	0.38
MUFA[5]	22.8	23.4	23.4	22.9	23.1	0.5	0.94	0.87	0.41
PUFA[6]	6.28	6.38	6.42	6.27	6.34	0.17	0.98	0.96	0.40
Atherogenicity index[7]	2.66	2.55	2.47	2.56	2.56	0.07	0.88	0.30	0.22

[1]Shearing; [2]Breed; [3]Interaction; [4]Saturated fatty acids; [5]Monounsaturated fatty acids; [6]Polyunsaturated fatty acids; [7](12:0 + 4 ×14:0 + 16:0)/(MUFA + PUFA).

No effects of shearing were detected on the detailed SFA profile of the milk fat (Table 3.4), which mean values agreed with those reported in Assaf dairy ewes fed a diet containing 2% sunflower oil using the same analytical methodology (Toral et al., 2013). Nevertheless, small differences were found in the C14:0 and C16:0 contents that were slightly greater in our ewes than in those of Toral et al. (2013).

On the other hand, breed differences were detected in the specific profile of milk SFA for most medium- and long-chain FA (i.e., C>14:0 to C24:0) as shown in Table 3.4, the milk of LC having greater C14:0 (4%; $P = 0.044$) and lower odd- and branched-chain FA with 13 to 17 C atoms and C>18:0 than did the milk of MN. Signorelli et al. (2008) also reported similar breed effects when the milk fat SFA profile of Italian dairy breeds was compared. A breed × shearing interaction was detected for the C12:0 values ($P = 0.028$) in our data.

No effects of shearing treatment were observed on the specific unsaturated FA (MUFA and PUFA) profiles of the milk fat of our ewes (Table 3.5), with the exception of *t*-11 C18:1 (*trans*-vaccenic acid), a bioactive FA with potential healthy effects on human health that originates in the rumen (Palmquist et al., 2005). Differences in its concentration might indicate an effect on the lipid metabolism at rumen or mammary gland levels (Palmquist et al., 2005). However, the speculative approach presents challenge since almost none of the rumen biohydrogenation metabolites showed differences due to the shearing treatment.

Table 3.4. Effects of shearing during mild-winter on the saturated fatty acid (SFA) profile in the milk of two breeds of dairy ewes.

	Lacaune		Manchega				Effect (*P*-value)		
SFA, g/100 g total FA	Control	Shorn	Control	Shorn	Mean	SEM	Shearing	Breed	Interaction
4:0	3.26	3.33	2.95	2.89	3.11	0.08	0.97	0.009	0.45
5:0	0.02	0.02	0.02	0.02	0.02	0.01	0.88	0.40	0.58
6:0	2.53	2.48	2.37	2.35	2.43	0.08	0.69	0.16	0.92
7:0	0.03	0.03	0.03	0.03	0.03	0.01	0.47	0.52	0.79
8:0	2.40	2.28	2.30	2.28	2.32	0.08	0.39	0.48	0.52
9:0	0.05	0.05	0.05	0.05	0.05	0.01	0.85	0.48	0.60
10:0	7.56	7.04	7.23	7.32	7.29	0.21	0.36	0.90	0.22
11:0	0.08	0.08	0.08	0.09	0.08	0.01	0.77	0.12	0.21
12:0	4.62	4.23	4.30	4.43	4.39	0.08	0.36	0.69	0.12
13:0 *anteiso*	0.010	0.010	0.011	0.012	0.011	0.001	0.59	0.022	0.36
13:0 *iso*	0.031	0.033	0.037	0.037	0.035	0.001	0.30	0.003	0.23
14:0	11.71	11.32	10.98	11.19	11.30	0.15	0.59	0.044	0.11
14:0 *iso*	0.15	0.16	0.18	0.18	0.17	0.01	0.21	0.008	0.74
15:0	1.23	1.28	1.39	1.39	1.32	0.03	0.49	0.015	0.63
15:0 *anteiso*	0.47	0.50	0.55	0.55	0.52	0.01	0.22	0.010	0.33
15:0 *iso*	0.32	0.34	0.37	0.37	0.35	0.01	0.20	0.003	0.29
16:0	25.55	25.87	25.35	25.66	25.61	0.21	0.20	0.37	0.98
16:0 *iso*	0.28	0.29	0.33	0.34	0.31	0.01	0.27	0.002	0.50
8-oxo-16:0	0.029	0.028	0.037	0.035	0.032	0.002	0.62	0.001	0.75
17:0	0.72	0.75	0.85	0.83	0.78	0.03	0.80	0.001	0.40
17:0 *anteiso*	0.44	0.46	0.52	0.52	0.48	0.02	0.48	0.001	0.67
17:0 *iso*	0.36	0.38	0.43	0.43	0.40	0.02	0.57	0.002	0.60
18:0	7.91	8.07	8.44	8.40	8.20	0.36	0.87	0.30	0.79
18:0 *iso*[1]	0.071	0.073	0.082	0.083	0.077	0.002	0.42	0.014	0.84
10-oxo-18:0	0.04	0.04	0.05	0.05	0.05	0.01	0.68	0.10	0.17
13-oxo-18:0	0.02	0.03	0.04	0.03	0.03	0.01	0.46	0.25	0.22
19:0	0.14	0.14	0.15	0.16	0.15	0.01	0.49	0.14	0.82
20:0	0.29	0.30	0.33	0.33	0.32	0.01	0.64	0.014	0.64
21:0	0.11	0.12	0.15	0.14	0.13	0.01	0.96	0.005	0.56
22:0	0.19	0.19	0.24	0.22	0.21	0.01	0.41	0.001	0.24
23:0	0.13	0.13	0.18	0.17	0.15	0.01	0.77	0.001	0.47
24:0	0.077	0.076	0.105	0.099	0.089	0.002	0.30	0.001	0.40

[1] Contains a C17:1 isomer of indeterminate double bond position as a minor component.

Table 3.5. Effects of shearing during mild-winter on the unsaturated fatty acid (UFA) profile in the milk of two breeds of dairy ewes.

	Lacaune		Manchega				Effect (*P*-value)		
UFA, g/100 g total FA	Control	Shorn	Control	Shorn	Mean	SEM	Shearing	Breed	Interaction
MUFA[1]									
c-9 10:1	0.30	0.29	0.31	0.31	0.30	0.02	0.69	0.40	0.73
c-9 12:1	0.10	0.08	0.10	0.10	0.09	0.01	0.49	0.24	0.27
t-9 12:1	0.06	0.05	0.06	0.06	0.06	0.01	0.80	0.72	0.50
c-9 14:1	0.28	0.27	0.27	0.28	0.27	0.03	0.96	0.99	0.73
cis-12 14:1	0.09	0.07	0.09	0.10	0.09	0.01	0.37	0.16	0.16
t-5 + 6 14:1	0.02	0.02	0.02	0.02	0.02	0.01	0.36	0.27	0.72
t-9 14:1	0.01	0.01	0.01	0.01	0.01	0.01	0.11	0.94	0.59
c-9 15:1	0.01	0.01	0.01	0.01	0.01	0.01	0.96	0.09	0.72
t-5 15:1	0.19	0.19	0.23	0.22	0.21	0.01	0.85	0.001	0.62
t-6 + 7 15:1	0.023	0.023	0.028	0.027	0.025	0.001	0.66	0.001	0.49
c-7 16:1	0.24	0.25	0.27	0.26	0.25	0.01	0.76	0.020	0.28
*c*9 16:1	0.93	0.97	0.99	1.00	0.97	0.06	0.73	0.50	0.88
c-13 16:1	0.06	0.06	0.07	0.06	0.06	0.01	0.94	0.09	0.27
c-14 16:1[2]	0.17	0.14	0.16	0.18	0.16	0.01	0.68	0.29	0.24
t-6 + 8 16:1	0.08	0.08	0.08	0.08	0.08	0.01	0.35	0.27	0.31
t-9 16:1	0.10	0.10	0.09	0.08	0.09	0.01	0.59	0.13	0.68
c-9 17:1	0.28	0.29	0.33	0.32	0.30	0.01	0.80	0.012	0.39
c-9 18:1[3]	15.29	15.79	16.16	15.87	15.78	0.38	0.79	0.28	0.35
c-11 18:1	0.36	0.37	0.38	0.36	0.37	0.01	0.67	0.54	0.18
c-12 18:1	0.35	0.36	0.30	0.30	0.33	0.01	0.68	0.001	0.67
c-13 18:1	0.056	0.058	0.047	0.048	0.052	0.002	0.50	0.006	0.94
c-15 18:1	0.056	0.058	0.047	0.048	0.052	0.002	0.48	0.001	0.92
c-16 18:1	0.065	0.067	0.060	0.060	0.063	0.002	0.65	0.023	0.66
t-4 18:1	0.020	0.021	0.018	0.017	0.019	0.001	0.53	0.005	0.21
t-5 18:1	0.018	0.019	0.016	0.015	0.017	0.001	0.69	0.001	0.26
t-6 + 7 + 8 18:1	0.28	0.28	0.23	0.22	0.25	0.01	0.87	0.001	0.40
t-9 18:1	0.27	0.27	0.23	0.22	0.25	0.01	0.82	0.001	0.35
t-10 18:1	0.43	0.44	0.29	0.30	0.36	0.03	0.76	0.001	0.92
t-11 18:1	1.47	1.44	1.32	1.21	1.36	0.03	0.09	0.001	0.47
t-12 18:1	0.40	0.41	0.35	0.34	0.37	0.01	0.81	0.002	0.41
t-15 18:1	0.223	0.225	0.202	0.205	0.214	0.006	0.72	0.029	0.95
t-16 18:1[4]	0.31	0.32	0.30	0.30	0.31	0.01	0.58	0.06	0.70

Table 3.5. (Continued)

	Lacaune		Manchega				Effect (*P*-value)		
UFA, g/100 g total FA	Control	Shorn	Control	Shorn	Mean	SEM	Shearing	Breed	Interaction
c-9 20:1	0.01	0.01	0.01	0.01	0.01	0.01	0.30	0.06	0.70
c-13 22:1	0.01	0.01	0.01	0.01	0.01	0.01	0.10	0.13	0.80
c-15 24:1	0.016	0.016	0.021	0.020	0.018	0.001	0.72	0.004	0.50
PUFA[1]									
c-9, *c*-12 18:2	2.48	2.55	2.49	2.42	2.48	0.04	0.97	0.27	0.17
c-12, *c*-15 18:2	0.01	0.01	0.01	0.02	0.01	0.01	0.71	0.98	0.72
c-9, *t*-12 18:2	0.053	0.059	0.048	0.046	0.052	0.002	0.58	0.023	0.17
c-9, *t*-13 18:2	0.30	0.30	0.27	0.27	0.28	0.02	0.81	0.06	0.98
c-9, *t*-14 18:2	0.14	0.14	0.13	0.14	0.14	0.01	0.97	0.19	0.96
t-9, *c*-12 18:2	0.037	0.039	0.031	0.032	0.035	0.002	0.48	0.020	0.90
t-11, *c*-15 18:2	0.11	0.12	0.12	0.12	0.12	0.01	0.45	0.94	0.51
t-9, *t*-12 18:2	0.012	0.013	0.011	0.011	0.012	0.001	0.12	0.001	0.026
t-10, *t*-14 18:2	0.052	0.051	0.041	0.041	0.046	0.003	0.90	0.001	0.90
t-11, *t*-15 18:2	0.01	0.01	0.01	0.01	0.01	0.01	0.95	0.08	0.69
c-9, *t*-11 CLA[1]	0.952	0.945	0.850	0.785	0.883	0.060	0.58	0.09	0.65
t-9, *c*-11 CLA	0.021	0.022	0.036	0.019	0.025	0.010	0.43	0.55	0.36
t-10, *c*-12 CLA	0.008	0.009	0.007	0.009	0.008	0.001	0.11	0.39	0.76
t-11, *t*-13 CLA	0.02	0.02	0.01	0.02	0.02	0.01	0.46	0.20	0.67
other *t*-*t* CLA[5]	0.06	0.06	0.06	0.06	0.06	0.00	0.66	0.10	0.58
18:3n-3[6]	1.00	1.04	1.17	1.16	1.09	0.03	0.53	0.008	0.39
18:3n-6	0.049	0.047	0.058	0.052	0.051	0.003	0.15	0.014	0.41
20:2n-6	0.02	0.02	0.02	0.02	0.02	0.01	0.64	0.10	0.99
20:3n-6	0.027	0.028	0.038	0.033	0.031	0.002	0.35	0.015	0.20
20:4n-6	0.17	0.17	0.18	0.18	0.18	0.01	0.86	0.11	0.77
20:5n-3	0.064	0.058	0.072	0.071	0.066	0.003	0.30	0.005	0.48
22:4n-6	0.02	0.02	0.02	0.02	0.02	0.01	0.42	0.14	0.20
22:5n-6	0.01	0.01	0.01	0.02	0.01	0.01	0.21	0.19	0.25
22:5n-3	0.13	0.13	0.16	0.16	0.14	0.01	0.98	0.027	0.95
22:6n-3	0.058	0.059	0.066	0.080	0.066	0.005	0.24	0.049	0.29

[1]MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; CLA, conjugated linoleic acid.
[2]Coelutes with 3, 7, 11, 15-tetramethyl 16:0.
[3]Contains t-13 + 14 18:1 as minor components.
[4]Coelutes with *c*-14 18:1.
[5]Sum of *t*-9, *t*-11 + *t*-10, *t*-12 + *t*-8, *t*-10 CLA.
[6]Contains *c*-11 20:1 as a minor isomer.

Our results did not support those of Rassu et al. (2009) who reported that, in addition to an increase in fat content, shearing lactating dairy ewes in late spring modified the profile of milk FA by increasing the medium-length FA (i.e., C8, C10, C12 and C16) whereas it did not change the content of long-chain FA (>C18). The authors stressed that the increase in milk fat content by effect of shearing was related to the increase of the main FA synthesized in the mammary gland, and not related to fat mobilization by the expected cold stress occurred during the nights. To our knowledge, no other references are available on the effects of shearing on milk yield and composition of dairy ewes.

Regarding the effects of the breed on MUFA profiles, the *c*-12 to *c*-16 and *t*-4 to *t*-15 isomers of C18:1 were the most affected, which were 10 to 48% greater ($P = 0.001$ to $P = 0.029$; Table 3.5) in the milk of LC, when compared to MN ewes. Signorelli et al. (2008) also found differences in the milk MUFA profile according to breed in Italian dairy ewes.

Similar breed effects were observed with regard to the milk fat profile of PUFA. Although *c*-*c* C18:2 did not vary when LC and MN were compared, most *c*-*t* and *t*-*t* isomers were greater in the LC (16 to 27%; $P = 0.001$ to $P = 0.023$). No CLA contents or isomer partitioning resulted affected by shearing or breed treatments, the mean values of *c*-9, *t*-11 CLA (0.88 ± 0.06%) and *t*-10, *c*-12 CLA (0.008 ± 0.001%) being considered as high and low, respectively, as usually observed in the milk of Assaf dairy ewes using the same analytical methodology (Toral et al., 2013; Frutos et al., 2017).

It must be stressed that some of the breed differences reported in our results may have been a consequence of the different amount of concentrate, and consequently of soybean oil, fed to our ewes according to the breed and its nutritional requirements (i.e., milk yield differences). This statement is supported, for example, by the higher values of *t*-10 and *t*-11 C18:1 observed in Table 3.5 for LC, as compared to MN ewes, agreeing with the expected effects of concentrate on FA biohydrogenation (Palmquist et al., 2005).

3.4.8. Physiological Indicators

Mean values of blood metabolites and hormones measured in plasma during the experimental period in the dairy ewes, according to breed and shearing treatment, are shown in Table 3.2. There were no detectable changes on the days around shearing nor differences between SH and CO treatments of either breed. Despite the lack of differences

observed in most physiological blood indicators (glucose, NEFA, cortisol and insulin) in the LC ewes during the experiment (P = 0.12 to 0.89), a 3% increase in the glucose concentration of the MN-SH ewes when compared to MN-CO ewes (P = 0.050) was detected. This increase agreed with the decrease in rectal temperature observed in the MN-SH ewes, as discussed above, and with the increase in the concentration of blood glucose reported in pregnant ewes shorn under cold stress conditions (Thompson et al., 1982). Hargreaves and Hutson (1990) reported an acute rise in heart rate, hematocrit and plasma cortisol in Merino ewes, as a response to manipulation during the shearing procedure (i.e., restraint, up-ending, shearing noise and shearing). Nevertheless, the authors stressed that partial or total wool removal itself produced a weak effect that was unlikely to be related to thermoregulatory adjustments. According to Carcangiu et al. (2008), cortisol concentration in blood showed that shearing management also caused severe acute stress in Sarda dairy ewes under in-field conditions. Plasma levels of glucose rose in the shorn ewes, the rise being directly proportional to the level of cortisol (Carcangiu et al., 2008) and attributed to the hyperglycemic effect of this hormone which stimulates the sympathetic-adrenergic axis and increases glucose production in the liver (gluconeogenesis). These effects were not seen in our ewes that showed normal physiological indicators agreeing with and adequate plane of nutrition and positive energy balance in mid-lactation.

Mears et al. (1999) concluded that shearing itself does not elevate cortisol and β-endorphin above the levels produced by the cumulative stress of handling and processing that accompany shearing. Agreeing with this, no effects of shearing were detected in the case of our LC ewes which did not show changes of rectal temperature, had open and small fleece and are recognized to be calm dairy ewes (Pedernera-Romano et al., 2010), and calmer than MN ewes.

3.5. CONCLUSIONS

The results of the present study show that shearing high yielding dairy ewes (i.e., LC) during the milking period and under mild-winter conditions, increased feed intake and lactational performances (i.e., milk yield, protein and lactose yields) in the shorn ewes. In the case of MN ewes, no differences were detected neither in feed intake or lactational performances after shearing. On the other hand, no differences in physiological indicators

were found between shorn and unshorn ewes in either breed. Therefore, shearing dairy ewes during lactation and under mild-winter conditions, is a suitable management option for dairy ewes that may improve their lactational performances, more likely to high-yielding ewes as Lacaune, without changes in their physiological indicators or milk composition.

CHAPTER 4

Effects of melatonin in early lactating dairy ewes under short-day conditions

CHAPTER 4

Effects of melatonin implants on the lactational performances of 2 breeds of dairy ewes in early-lactation under short-day (winter solstice) conditions[1]

4.1. ABSTRACT

A total of 72 dairy ewes of 2 breeds (MN, Manchega, 72.4 ± 1.9 kg BW, n = 36; LC, Lacaune, 77.7 ± 2.3 kg BW; n = 36) were used to evaluate the lactational effects of melatonin implants in early-lactation and under short-day photoperiod conditions (centered on winter solstice). Ewes lambed in autumn and were penned indoors in 12 balanced groups of 6 ewes and randomly assigned to a 2 × 2 × 3 factorial design (treatment × breed × replicate). Treatments were: i) melatonin (MEL), which received 1 subcutaneous implant of melatonin (18 mg/ewe); in the ear base at 35 ± 1 DIM (1 wk after lamb weaning) and ii), control (CO), that did not receive any treatment. Ewes were fed a total mixed ration (forage:concentrate, 60:40) ad libitum and machine milked twice-daily. Daily milk yield was automatically recorded until 90 DIM. Milk and jugular blood were sampled for milk composition (biweekly) and plasma hormones (monthly) analyses, respectively. Body reserves (BW and BCS) were assessed biweekly. Feed intake was measured by pen during 3 separated periods after the start of the treatments (wk 2 and 3, wk 6 and 7, wk 10 and 11). Feed intake, milk yield and composition varied by breed, but no MEL effects were detected on DM intake, milk yield, milk composition or fat and protein standardized milk, in either breed. As a result of their greater BW, the melatonin treatment dose (on average, 0.24 mg/kgBW) was 6.8% lower in the LC than in the MN ewes. Basal plasmatic melatonin showed, on average, marked differences by treatment ($P < 0.001$), increasing 111% in the MEL treated ewes, and breed ($P < 0.001$), the MN having greater mean values than the LC ewes ($P < 0.001$). Moreover, the MN also responded greatly to treatment (MN and LC, 161% and 64%, respectively; $P < 0.001$). Prolactin in plasma decreased in the MEL treated ewes (–63%; $P = 0.050$), but the effect was only significant in the MN ewes. No effects were detected on plasmatic IGF-I between treatments in either breed. Body reserves did not vary by effect of MEL treatment nor breed throughout the experiment. In conclusion, the use of exogenous melatonin as MEL implants, together with the endogenous melatonin naturally produced under short-day photoperiod conditions, had no effects on the lactational performances of dairy sheep in early-lactation, despite their breed and level of production.

[1]This article was submitted to J. Dairy Sci. short-communication as: Elhadi, A., A.A.K. Salama, X. Such, and G. Caja. Effects of melatonin implants on the lactational performances of 2 breeds of dairy ewes in early-lactation under short-day (winter solstice) conditions.

4.2. INTRODUCTION

Sheep are seasonal reproduction animals conditioned by photoperiod signals (i.e., circannual and circadian rhythms) which are translated in the brain using melatonin (MEL) as neuroendocrine transmitter (Cardinali and Pevet, 1998; Borjigin et al., 1999; Tamura et al., 2008; Reiter et al., 2009). MEL has antigonadotropic and cytostatic effects, inhibits cell proliferation, stimulates immunity and acts as free-radical scavenger (Kvetnoy, 1999). MEL is mainly secreted (central) by the pineal gland and the visual system (retina, Harderian gland) into the cerebrospinal fluid, almost entirely during the night (Reiter, 1980, 1991), and it informs about light:dark pattern (Bittman et al., 1983; Karsch et al., 1984). Extrapineal secretion (peripheral) and storage of MET in endocrine (gut, liver, kidney, etc…) and non-endocrine (i.e., thymus, mast cells, leucocytes, etc…) cells has also been proved (Kvetnoy, 1999), although its secretion is unlikely to be dependent on light. Central MEL secretion mainly depends on the environmental light stimuli received by the suprachiasmatic nucleus through the retino-hypothalamic pathway (Challet, 2007; Pevet and Challet, 2011) and duration of MEL secretion during the night codes for day duration (Wayne et al., 1988). Plasmatic levels of MEL in sheep (i.e., 0 to 300 pg/mL) are highly variable among individuals, but the nocturnal MEL concentration is high (>100 pg/mL) and characteristic of each individual, which seems to be indicative of its strong genetic control (Chemineau et al., 1996; Zarazaga et al., 1998). On the contrary, MEL levels are very low (i.e., 0 to 10 pg/mL) and stable after the dawn (O'Callaghan et al., 1991).

Light-dark treatments and administration of exogenous MEL have been largely investigated, in order to advance the season and to improve the reproductive performances of small ruminants. However, there is little information on the effects of MEL on the lactational performances of different breeds of dairy ewes in different seasons and stages of lactation. Exogenous MEL administration, as continuous slow-release implants, has shown to advance the beginning of the natural breeding season in the end of summer to spring by mimicking the stimulatory effect of short-days in sheep and goats (Haresign et al., 1990; Williams et al., 1992; Zarazaga et al., 2012). Moreover, exogenous MEL do not suppress the endogenous nocturnal secretion of MEL, as reported by O'Callaghan et al. (1991) and Malpaux et al. (1997) in sheep.

On the other hand, MEL also controls the seasonal rhythm of PRL secretion (Gómez-Brunet et al., 2008), acting directly at the level of the pituitary gland (Lincoln and Clarke,

1994). The reduction of MEL secretion during long-days is accompanied by an increase in PRL secretion in sheep (Lincoln and Clarke, 1994; Misztal et al., 1997). A reverse effect is observed during short-days, where an elevated MEL secretion could inhibit PRL secretion and milk yield (Misztal et al., 2001; Molik et al., 2007). Dahl et al. (2000) concluded that milk production is negatively affected by short-day photoperiod, which is characterized by high levels of MEL and low of PRL and IGF-I in the blood of dairy cows.

With regard to dairy sheep, early results from Bocquier et al. (1997) showed that Sarda dairy ewes under short-day photoperiod decreased milk yield, while fat and protein contents increase, which was attributed to the rise of endogenous MEL secretion. Agreeing with this, Molik et al. (2007) reported lower MEL secretion and increased milk yield in Polish Longwool nursing ewes under long-day photoperiod conditions (spring).

The effects of MEL implants on the lactational performances of dairy small ruminants, used for mimicking the short-day photoperiod and improve their reproductive performances, is a nowadays a controversial topic. On one hand, Abecia et al. (2005) reported in Lacaune and Assaf dairy ewes that the use of MEL implants during lactation in spring, did not reduce their milk yield. On the other hand, Molik et al. (2010) studied the effects of MEL implants in Polish Longwool ewes in early-lactation under long-day (i.e., spring) and short-day (i.e., autumn) conditions, compared to same photoperiods control ewes. Despite the strong stimulation made by suckling, MEL ewes decreased PRL secretion, although the effect was only significant under long-day photoperiod. The lowest PRL values were observed in both MEL and control short-day ewes, that did not differ between them. Moreover, the authors reported greater GH in the plasma of long-day ewes and short-day MEL treated ewes, when compared to the other ewe groups.

In a later research, Misztal et al. (2018) revisited the issue of photoperiod and use of MEL in Polish Longwool sheep, aiming to mitigate the negative effects on milk yield and lactation length of ewes lambing in long-day (June), when compared with those naturally lambing in short-day (January) photoperiod. It should be stressed, to avoid misunderstandings with the terminology previously used, that in this case milking (started after a long suckling) was consequently performed mainly under short-day (autumn) and long-day (spring) photoperiods, respectively. Moreover, MEL was applied 6-wk before and 6-wk after lambing (2 doses with 90-d interval). So, according to Misztal et al. (2018), milk yield was greater in the long-day lactating ewes, in comparison with short-day (–

20%) and short-day treated with MEL (–32%) lactating ewes. Interestly, plasma MEL mean concentration at late-pregnancy was inverse to daily light duration, and on average greater in the long-day than in the short-day MEL treated lactating ewes.

Little information is available with regard to MEL effects on milk composition. Protein content of milk increased (15%) in Polish Longwool ewes treated with MEL implants in mid-lactation under spring conditions, as reported by Molik et al. (2012).

Based on the hypothesis that changes in MEL levels may affect milk production and composition, the aim of this study was to evaluate the lactational effects of subcutaneous MEL implants in 2 breeds of dairy sheep, differing in milk yield and milk composition, in early-lactation and under short-day conditions. With this aim, the experimental period was centered in the winter solstice, when the secretion of endogenous MEL will be maximum.

4.3. MATERIALS AND METHODS

The study was conducted in the experimental farm of the SGCE (Servei de Granges i Camps Experimentals) of the Universitat Autònoma de Barcelona (UAB) in Bellaterra (Barcelona, Spain) located at N 41°30’20” and E 2°05’46” during 14-wk centered in the winter solstice (minimum day-length, 9.25-h). Animal care conditions and management practices agreed with the Spanish Royal Decree 53/2013 on the protection of animals used for experimental purposes, the codes of recommendations for the welfare of dairy sheep of the Ministry of Agriculture, Alimentation and Environment of Spain (MAPA, 2007) and the procedures stated by the Ethical Committee of Animal and Human Experimentation of the UAB.

4.3.1. Ewe Management and Feeding

A total of 72 ewes of 2 dairy breeds (Manchega, MN, n = 36; Lacaune, LC, n = 36) in early-lactation (35 ± 1 DIM) after the weaning of the lambs (28 d) were used. The ewes (MN vs. LC, respectively) differ in body weight (BW, 72.4 ± 1.9 vs. 77.7 ± 2.3 kg BW; $P = 0.045$) but had similar body condition score (on average, 2.99 ± 0.12 units BCS) and age (on average, 4.3 ± 0.4 yr). All ewes wore plastic ear tags (Allflex Europe, Vitré, FR) and ceramic rumen mini-boluses (20 g; Datamars, Bedano, SW) for visual and electronic identification, respectively, that were used for automatic milk recording as described by

Ait-Saidi et al. (2014).

Ewes were permanently on barley straw-bedded pens and fed a TMR offered ad libitum. The TMR (forage:concentrate, 60:40%; DM basis) consisted of alfalfa hay as forage and a farm-produced concentrate [ingredients: barley grain, 37.0%; corn grain, 15.0%; soybean hulls, 15.0%; gluten feed, 10.0%; soybean meal, 5.0%; rapeseed 00 meal, 5.0%; oat grain, 4.5%; sunflower meal, 2.0%; soybean oil, 2.0%; cane molasses, 2.0%; di-calcium phosphate, 1.0%; calcium carbonate, 0.5%; VitafacOvino-0.3 premix (DSM Nutritional Products, Madrid, ES), 0.5% and salt, 0.5%; as fed], on DM basis. Moreover, all ewes received 0.10 kg of corn whole grain at each milking, in the individual feeders of the milking parlor, to facilitate their bringing in. Nutrient requirements were based on INRA (2010) and calculated by INRAtion v.4.07 (Educagri éditions, Dijon, FR). Ewes had free access to water and commercial micromineral blocks (Multi-Block, Agrària Comarcal del Vallès, Llerona, ES).

Milking machine and milking procedures were similar to those described by Elhadi et al. (2019) and milking was conducted twice daily (0700 and 1700 h) in a 2×12-stall parallel milking parlor (Amarre Azul I, DeLaval Equipos, Alcobendas, ES) with a central high-milk pipeline, silicone milking clusters (SG-TF100, DeLaval, Tumba, SE), and automatic milk-flow and milk-recording devices (MM25SG, DeLaval).

4.3.2. Experimental Treatments

After the weaning of the lambs (28-d of age), the ewes were distributed in 12 balanced groups of 6 ewes according to BW, BCS, age, milk yield and milk composition. The experiment lasted 12-wk and consisted of 1-wk of adaptation period (28 to 35 DIM) and 11-wk post-treatment period (36 to 113 DIM). The experimental design consisted of a factorial with 2 treatments × 2 breeds × 3 replicates, to which the ewe groups were randomly allocated. Treatments were: Melatonin (MEL; MN, n = 18 and LC, n = 18) that received a single MEL implant (18 mg/ewe, Melovine, Ceva Animal Health, Barcelona, ES), in the base of the ear at 35 ± 1 DIM; and control (CO; MN, n = 18 and LC, n = 18) that did not receive any treatment. At the treatment day, all the ewes were restrained in the head-lockers of the feed bunk after the p.m. milking and the MEL ewes inserted subcutaneously with the single implant in the left ear.

4.3.3. Measurements, Sampling, and Analyses

Milk Yield. Milk yield of individual ewes was recorded at each milking during the whole experiment, using the milk-flow and milk-recording automatic units of the milking parlor. Data were uploaded using the AlPro software 7.2 (DeLaval) and daily reviewed and uploaded into a spreadsheet to avoid incorrect values.

Milk Composition. Representative milk samples of each ewe were taken pre- (d −3) and post-treatment (d 15, 30, 45, 75 and 90), corresponding to 32, 50, 65, 80, 110 and 124 DIM, for compositional analysis using proportional milk samplers (MM25SG, DeLaval). Milk samples (50 mL) were composited according to the daily milking intervals (a.m.:p.m., 60:40), preserved with an antimicrobial tablet (Bronopol, Broad Spectrum Micro-tabs II, D&F Control Systems, San Ramon, CA) and stored at 4°C until analysis. Milk samples were analyzed in the Dairy Herd Improvement Laboratory of Catalonia (ALLIC, Cabrils, ES) for fat, total protein, lactose and urea (Milkoscan FT2, Foss, Hillerød, DK) and SCC (Fossomatic 5000, Foss).

Feed Intake. Feed intake of each group was assessed daily at the start (wk 2 and 3), mid (wk 6 and 7) and final (wk 10 and 11) periods post-treatment by measuring the amount of feed offered and refused in each pen of 6 ewes. No refusals of concentrate were observed in the milking parlor. The TMR offered in the pens and their refusals were sampled daily and composited weekly for pre- and post-treatment periods feed analysis.

Feed Analyses. The DM content was determined by gravimetry, desiccating the sample in an forced-air stove (103°C for 24 h) and OM content was also measured gravimetrically by ashing samples in a muffle furnace (550°C for 4 h) according to AOAC (1990). Cellulose was analyzed as crude fiber according to AOAC (1990), and NDF and ADF were determined, on an ash-free basis, by adding amylase and sodium sulfite solutions according to Van Soest et al. (1991) and using an Ankom200 Fiber Analyzer incubator (Ankom Technology, Macedon, NY). Total N was determined by combustion according to the Dumas method using a Leco analyzer (Leco Corporation, St. Joseph, MI), and CP was calculated as N × 6.25.

Basal Blood Measures. Blood samples were taken from the jugular vein using 10 mL Vacutainer tubes with sodium heparin 170 IU (BD, Belliver Industrial Estate, Plymouth, UK) at 0800 h, after the morning milking and before feeding, at d 15, 45 and 75 post-treatment (corresponding to 50, 80 and 110 DIM). This sampling time was chosen to

assess the basal level of MEL with or without the implants. Plasma was obtained by whole blood centrifugation for 15 min at 2000 × g and 4°C, transferred to 0.5 mL Eppendorf tubes and stored at −20°C for analyses. Hormone analyses in plasma were performed according to the ELISA sandwich type method by using MEL (LDN, Nordhorn, DE), PRL (DIASource Immunoassays, Louvain la Neuve, BE) and IGF-I (Mediagnost, Reutlingen, DE) kits. The stopped ELISA plates were read in an automatic reader (iEMS Reader MF V.2.9–0, Labsystems España, Barcelona, ES) at 450 nm. Sensitivity, intra- and inter-assay coefficients of variation of analyses were: MEL (1.0 ± 0.2 pg/mL, 8.5%, and 11.1%), PRL (0.35 ± 0.07 ng/mL, 4.4%, and 5.9%) and IGF-I (0.09 ± 0.01 ng/mL, 5.8%, and 8.6%), respectively.

Body Reserves. BW and BCS of all ewes were evaluated 1 wk before the start of experiment, to allocate the ewes in balanced groups during the adaptation period, and at d 15, 30, 45 and 75 post-treatment (corresponding to 28, 50, 65 and 80 DIM). Weighing was performed using an electronic scale (Tru-test A6500, Auckland, NZ), and BCS was assessed (0 to 5 points; accuracy, ± 0.25 points) according to Russel et al. (1969).

4.3.4. Statistical Analyses.

Data were analyzed by the MIXED procedure for repeated measurements of SAS v. 9.4 (SAS Institute Inc., Cary, NC). The statistical mixed model included the breed (MN and LC), the melatonin treatment (MEL and CO), the sampling time and the breed×treatment interaction as fixed effects, as well as the random effects of the experimental unit (ewe, for individual measurements, or pen for intake), and the residual error. Values of the variables were computed for their respective treatment and sampling dates, the means expressed as least squares means (LSM) and separated by pairwise comparison using the PDIFF test of SAS. Pearson correlation coefficients (r) were calculated using the CORR procedure of SAS. Significance was declared at $P < 0.05$ and a tendency was considered when $P < 0.10$.

4.4. RESULTS AND DISCUSSION

Although the dose of MEL (18 mg/ewe) was the same for the MN and LC ewes, the difference in BW between breeds (i.e., the LC ewes were 5.3 kg heavier than the MN) make that the mean MEL treatment dose (0.240 mg/kgBW) were 6.8% lower in the LC (0.232 mg/kgBW) than in the MN (0.249 mg/kgBW) ewes. This variation source has not been taken into account in previous studies and should be considered on the analysis of

our results and for further research. The main results of MEL treatment on the lactational performances of the studied dairy ewes in early-lactation are summarized in Table 4.1.

4.4.1. Feed Intake

Values of voluntary feed intake of the dairy ewes are shown in Table 4.1. No effects of the MEL implants on feed intake were detected in either breed (on average, 2.78 ± 0.07 kg DM/d; $P = 0.32$) during the post-treatment periods studied.

Although, on our knowledge, the effect of MEL implants on feed intake in dairy sheep is unknown, Bocquier et al. (1997) reported that DM intake of lactating Sarda dairy ewes submitted to short-day photoperiod treatment was lower (–16%) than ewes under long-day. On the contrary, Lacasse et al. (2014) reported an increase of DM feed intake (4%) in Holstein cows under short-day photoperiod, when compared to those MEL fed and under long-day photoperiod conditions. The effect of photoperiod length on feed intake was not significant in lactating Saanen dairy goats under short- and long-day photoperiod conditions (Mabjeesh et al., 2007). Consequently, according to these data, a small increase or no effect in DM intake should be expected when dairy ewes are treated with MEL implants during lactation.

The effect of the breed on DM intake was important in our results and, on average, MN ewes ate 33% less DM than did LC ewes ($P < 0.001$; Table 4.1). This result agrees with the greater milk yield of the LC ewes ($P < 0.001$), as later discussed, and with the results of previous studies comparing both breeds in the same stage of lactation (Molina et al., 2001; Flores et al., 2008; Elhadi et al., 2019). No MEL × breed interaction was detected on the feed intake of our ewes ($P = 0.96$; Table 4.1).

4.4.2. Milk Yield

No differences by effect of MEL treatment were detected on milk yield ($P = 0.67$) and energy corrected milk ($P = 0.68$) in both breeds (Figure 4.1). No MEL × breed interaction was detected on milk yield or milk components yield of our ewes ($P = 0.67$ to 0.92) as shown in Table 4.1 and Figure 4.1. On average, and for the same stage of lactation, LC ewes yielded 83% more milk than MN ewes (LC vs. MN, 2.53 ± 0.10 vs. 1.38 ± 0.15 kg/d; $P < 0.001$) through the experiment. The linear persistence coefficients of milk production in early lactation (35 to 95 DIM) were similar for the MN and LC ewes (–11 and –14 g/d, respectively; $P = 0.83$).

Table 4.1. Effects of melatonin implants (18 mg/ewe) under short-day conditions in 2 breeds of dairy ewes in early-lactation Values are LS means ± SEM.

	Manchega		Lacaune				Effect (*P*-value)		
Item	Control	Melatonin	Control	Melatonin	Mean	±SEM	Melatonin	Breed	Interaction[1]
Intake, kg of DM/d	2.26[b]	2.19[b]	3.37[a]	3.29[a]	2.78	0.07	0.32	0.001	0.96
Milk yield									
Yield, kg/d	1.43[b]	1.32[b]	2.54[a]	2.51[a]	1.95	0.15	0.67	0.001	0.80
ECM[2], kg/d	1.37[b]	1.28[b]	2.13[a]	2.16[a]	1.73	0.14	0.68	0.001	0.90
Fat, g/d	99[b]	93[b]	138[a]	143[a]	118	9	0.74	0.001	0.69
Protein, g/d	81[b]	76[b]	135[a]	136[a]	107	8	0.91	0.001	0.67
Lactose, g/d	70[b]	64[b]	121[a]	120	93	8	0.84	0.001	0.77
Total solids, g/d	264[b]	246[b]	419[a]	423[a]	338	26	0.75	0.001	0.92
Milk content									
Fat, %	6.92[a]	7.06[a]	5.44[b]	5.71[b]	6.28	0.18	0.27	0.001	0.72
Protein, %	5.69[a]	5.77[a]	5.34[b]	5.43[b]	5.56	0.10	0.42	0.002	0.95
Lactose, %	4.87	4.83	4.76	4.77	4.81	0.08	0.84	0.30	0.75
Total solids, %	18.5[a]	18.6[a]	16.5[b]	16.9[b]	17.6	0.2	0.27	0.001	0.68
Urea, g/L	64.9[a]	66.5[a]	56.4[b]	58.3[b]	61.5	1.5	0.26	0.001	0.94
SCC, log_{10}/mL	5.82[x]	5.30[y]	5.55[xy]	5.46[y]	5.53	0.15	0.06	0.72	0.19
Plasma hormones									
Melatonin, pg/mL	6.9[c]	17.2[a]	5.8[c]	9.4[b]	9.8	1.1	0.001	0.001	0.002
Prolactin, ng/mL	19.8[a]	9.1[b]	13.2[a]	11.1[ab]	13.3	3.4	0.050	0.49	0.19
IGF-I, ng/mL	258	283	272	294	276	27	0.41	0.66	0.97
Body reserves									
BW variation, kg/30 d	1.17	2.09	2.85	2.29	2.10	0.51	0.73	0.08	0.16
ADG, g/d	39	70	95	77	70	17	0.72	0.08	0.16
BCS variation in 30 d	0.22	0.13	0.08	0.10	0.13	0.06	0.52	0.18	0.33

[a,b]Within a row and breed, values with a different superscript differed ($P < 0.05$); [x, y]Within a row and breed, values with a different superscript tended to differ ($P < 0.10$).[1]Melatonin × Breed; [2]Energy corrected milk = Milk yield × [0.071× (Fat, %) + 0.043 × (Total protein, %) + 0.2224], according to Bocquier et al. (1993).

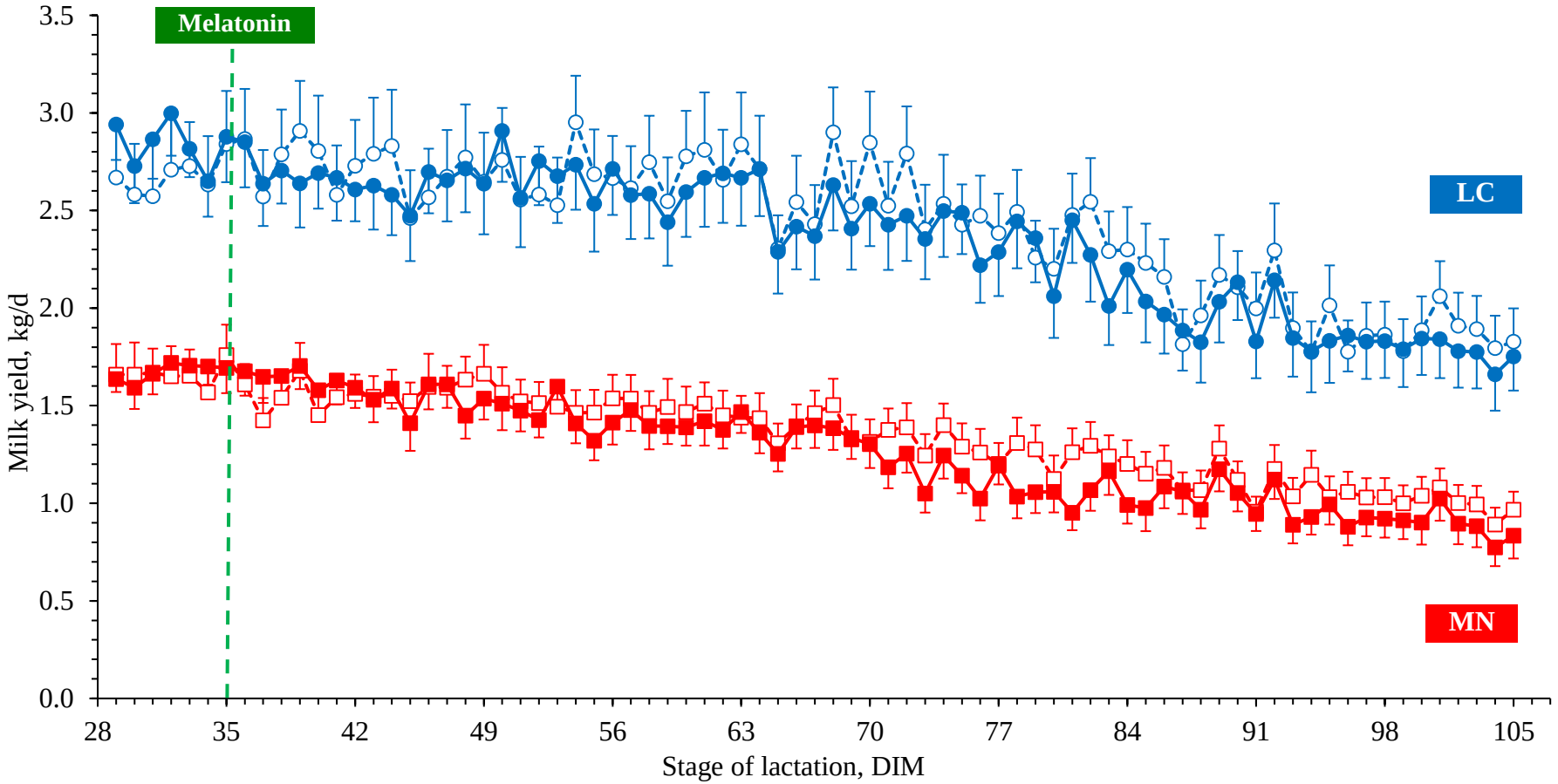


Figure 4.1. Effects of melatonin (18 mg/ewe) on milk yield of lactating Manchega (squares, □ and ■) and Lacaune (circles, ○ and ●) dairy ewes in early-lactation under short-day photoperiod conditions. Treatments: Melatonin (solid lines and closed symbols, ■ and ●) or control (broken lines and open symbols; □ and ○). Values are LS means with the SEM indicated by vertical bars.

Our results agreed with those of Abecia et al. (2005) who reported no effects on milk yield throughout the milking period of Lacaune and Assaf dairy ewes lambed in autumn and winter, respectively, and treated with MEL implants in spring (long-day) conditions. On the contrary, Molik et al. (2013) in Polish Longwool ewes milked after suckling in spring (long-day) conditions, reported that MEL treated and artificial short-day (200 lux light during 8-h) ewes yielded less milk (–16% and –38%, respectively), than control ewes under natural long-day photoperiod. Nevertheless, in this study, MEL implants were applied twice during lactation (weaning, d 57; late-lactation, d 147), although the authors did not report the plasmatic MEL values. Bocquier et al. (1997) also reported that Sarda dairy ewes produced –25% milk under short-day photoperiod (light:dark, 8:16) than those under long-day (16:8) conditions during lactation.

In a later research, Misztal et al. (2018) revisited the issue of photoperiod and the use of MEL at the end of pregnancy and during lactation in Polish Longwool sheep, aiming to mitigate the negative effects on milk yield and lactation length of ewes lambing in long-day (June), when compared to those lambing under short-day (January) photoperiod. It should be stressed, to avoid misunderstandings with the terminology previously used, that in this case milking was performed after weaning at d 56 and mainly under short-day (autumn) and long-day (spring) photoperiods, respectively. MEL was applied twice, in late-pregnancy (wk –6) and early-lactation (wk 6) with regard to lambing. According to Misztal et al. (2018), milk yield was greater in the long-day lactating ewes, in comparison with short-day (–20%) and short-day treated with MEL (–32%) lactating ewes.

The use of MEL (2 implants of 18 mg/goat) during late-pregnancy was also studied by Aviles et al. (2019) in Creole goats dried-off during the summer solstice that kidded in late August and were suckled and milked during autumn (short-day). MEL treatment, that was removed surgically at kidding, stimulated the subsequent lactational performances and increased milk yield, but not milk composition, in more than 20% during suckling (as well as kid growth) and milking. This increase in milk yield agreed with those reported in Holstein cows (Auchtung et al., 2005) and Saanen goats (Mabjeesh et al., 2013) exposed to artificial short-days (light:dark, 8:16 h) during the dry period, which produced more milk in the subsequent lactation under natural long-day photoperiod. Moreover, in the Mabjeesh et al. (2013) experiment the goats were also heat stressed in late-pregnancy, which is known to dramatically increase PRL levels, as later discussed.

Nevertheless, Dahl et al. (2000) and Lacasse et al. (2014) reported that feeding MEL during late-pregnancy had no effects on the subsequent milk yield of Holstein dairy cows, but the result may have been conditioned to the MEL source. Thus, Auldist et al. (2007) evaluate the effects of repeated MEL implants during 12-wk (6×18 mg/cow) in twin NZ Friesian dairy cows calved in spring. The cows were grazing during the summer and the twin pairs were used for treatment or control comparison. MEL implants decreased milk yield (–23%) and increased milk contents, as in late-lactation. They concluded that some of the milk changes observed in NZ throughout summer may be a consequence of the MEL increase associated to the decreasing day-length.

4.4.3. Milk Composition

No changes on milk components by effect of MEL treatment, under short-day conditions (winter solstice), were detected in our ewes throughout lactation, as well as on the daily yield of different milk components (P = 0.25 to 0.99; Table 4.1). On the other hand, marked differences in fat and protein milk contents were observed between MN and LC breeds (Figure 4.2), but not in their lactose content (P = 0.84), as previously reported for the 2 breeds under similar conditions (Flores et al., 2008; Elhadi et al., 2019). Only somatic cell count tended to decrease by effect of the MEL treatment in the case of MN ewes (P = 0.063).

On the contrary, Molik et al. (2012) reported that the administration of MEL increased the protein content (15%) of the milk of Polish Longwool ewes under long-day photoperiod. Moreover, Bocquier et al. (1997), in Sarda, and Molik et al. (2012) in Polish Longwool ewes, also reported the positive effects of short-days vs. long-days on milk protein content (4% and 15%, respectively). Nevertheless, implanting MEL to Creole goats during late-pregnancy (Aviles et al., 2019) did not change milk composition and use of short-day photoperiod during late-pregnancy in Saanen dairy goats did not change their milk composition during lactation (Mabjeesh et al., 2007).

With regard to dairy cows treated with MEL implants during summer in NZ, Auldist et al. (2007) reported increases in milk fat and protein contents (14% and 7%, respectively), while lactose content decreased (–7%), but no effects were detected by Lacasse et al. (2014) when the milk of short-day, long-day, and MEL-fed under long-day photoperiod lactating Holstein cows were compared.

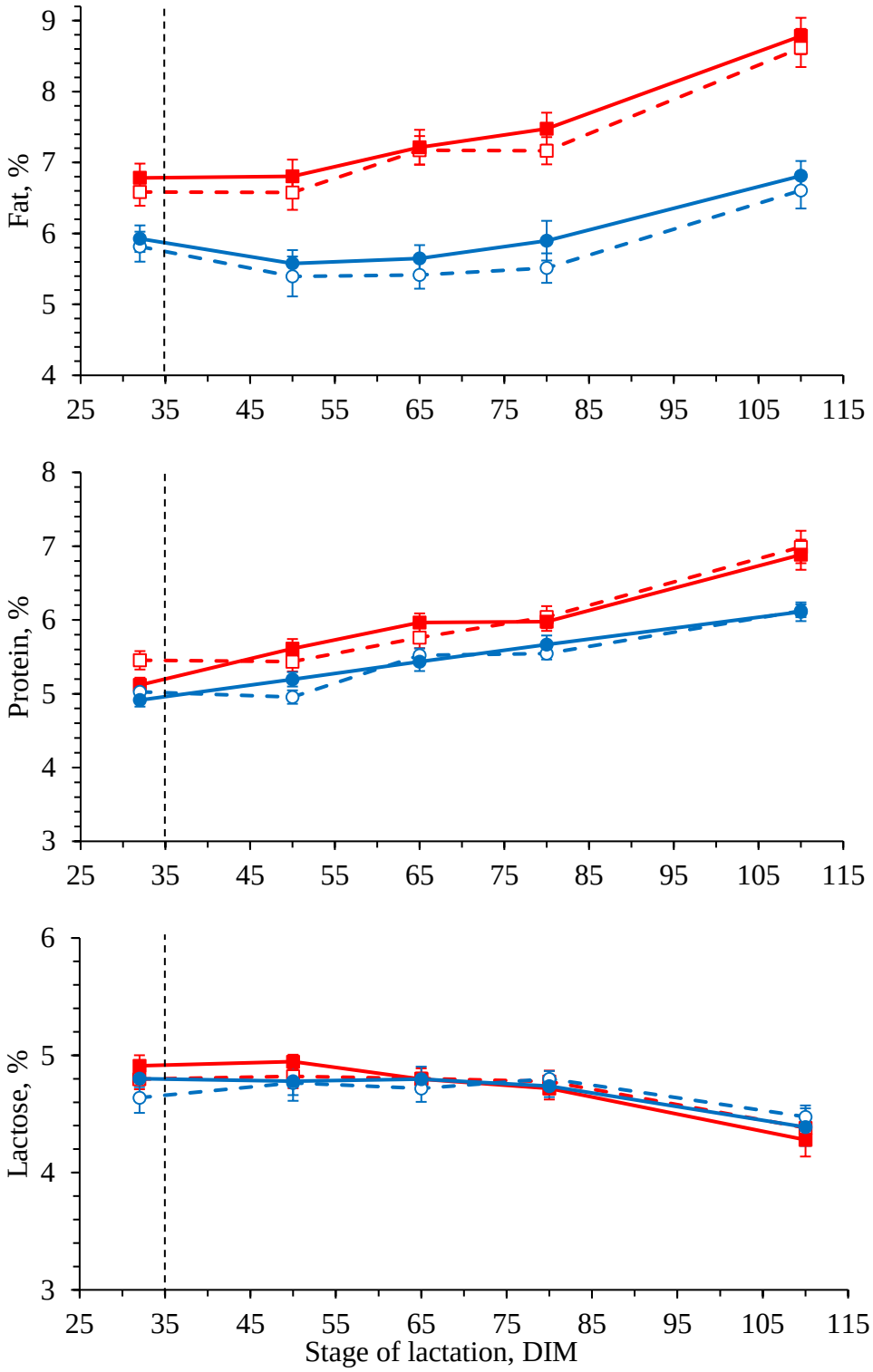


Figure 4.2. Effects of melatonin (18 mg/ewe) on milk composition (a, fat; b, protein; c, lactose) of Manchega (squares, □ and ■) and Lacaune (circles, ○ and ●) dairy ewes in early-lactation under short-day photoperiod conditions. Treatments: Melatonin (solid lines and closed symbols, ■ and ●,) or control (broken lines and open symbols; □ and ○). Values are LS means of both breeds averaged, with the SEM indicated by vertical bars.

4.4.4. Blood Hormones Concentrations

Hormonal values in the plasma of our ewes according to treatments and breed are summarized in Table 4.1. Moreover, changes on basal MEL through the experiment are shown in Figure 4.3.

Because the pineal MEL (central) is mainly secreted during the night and its concentration fall after dawn, values obtained from blood samples collected from the CO ewes after the a.m. milking represented, according to Kvetnoy (1999), the basal endogenous extrapineal MEL secretion (peripheral) during the short-day photoperiod conditions of our experiment. On the other hand, the values of the MEL-treated ewes after d 35, when the slow-release implants were applied, represented the total MEL concentration in blood (endogenous-peripheral secretion plus exogenous). As shown in Figure 4.3, no changes in the basal MEL values were observed in the CO ewes throughout the experiment (6.4 ± 1.2 pg/mL, on average; Table 4.1), although MN showed numerically greater basal MEL values than LC ewes. After the insertion of implants (18 mg/ewe), MEL values in plasma increased markedly in the treated ewes but, despite the differences in MEL doses reported in our ewes according to breed BW (MN, 0.249 mg/kgBW; LC, 0.232 mg/kgBW), the increase was greater in MN than in LC ewes (150 vs. 63%, respectively; $P < 0.001$). Plasmatic MEL values in the treated ewes reached a plateau of at least 60-d (d 50 to 110, Figure 4.3) and decreased to basal values thereafter (d 125). The difference between breeds was unexpected and we hypothesized that it may be a consequence of differences in the metabolization of exogenous MEL, which could be associated to the greater metabolic activity of the higher yielding LC ewes. Consequently, the use of a unique dose of MEL recommended in practice (a single implant of 18 mg/ewe) may be questioned and needs to be studied if it is the optimal for heavier and high yielding dairy sheep breeds (i.e., Assaf and East Friesian ewes).

After the discovery of association of MEL receptor gene MTNR 1A (MT1) polymorphisms with sheep fertility (Notter et al., 2003), Mura et al. (2010) reported stronger effects of exogenous MEL on the reproductive activity of Sarda dairy ewes homozygote to the MT1 favorable allele (+/+). This would also explain the link between genotype and photoperiod, as the –/– ewes need higher and long-lasting levels of MEL in blood to be stimulated for reproduction. It should be stressed that, in our MN and LC experimental flock, adult ewe and hogget fertility during spring was repeatedly greater in LC than in MN sheep (data not shown).

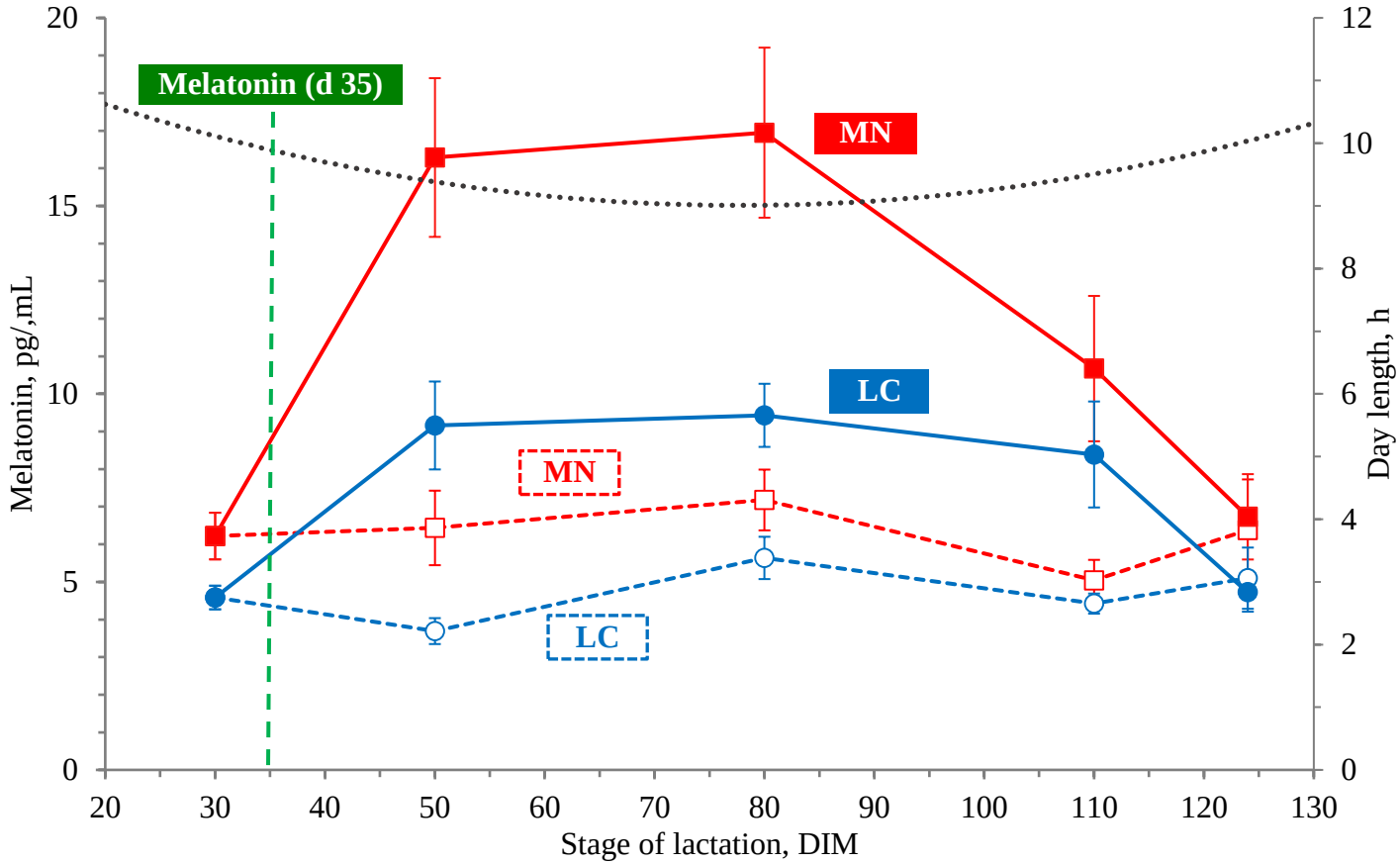


Figure 4.3. Basal values of plasmatic melatonin of Manchega (MN; squares, □ and ■) and Lacaune (LC; circles, ○ and ●) dairy ewes in early-lactation under short-day photoperiod conditions according to treatment. Treatments: Melatonin (melatonin, 18 mg/ewe; solid lines and closed symbols, ■ and ●,) or control (broken lines and open symbols; □ and ○). Grey dotted line shows the day-light duration. Values are LS means with the SEM indicated by vertical bars.

Misztal et al. (2018) reported dramatic increases of nocturnal blood MEL in Polish Longwool ewes when compared short-day photoperiod (400%) or MEL implanted under long-day conditions (425%), with long-day photoperiod as control. The increases were greater that in our results as a consequence of sampling time.

As a consequence of the greater plasmatic MEL of our MN-treated ewes, plasma concentration of PRL decreased (–54%) when compared to MN-CO ewes (Table 4.1). No effects between treatments were detected in the LC ewes. Molik et al. (2013) and Misztal et al. (2018) also reported decreases in plasma PRL concentration of the MEL treated Polish Longwool ewes (–22%). According to Misztal et al. (2018), plasma PRL in the long-day ewes increased from late pregnancy (winter) to weaning (spring), whereas in the short-day and the short-day MEL-treated ewes the direction of change was opposite, the lowest PRL concentration occurring at weaning (August).

The decreasing effect of exogenous MEL on PRL secretion was also observed during increasing photoperiod conditions, despite the strong stimulation induced by the suckling of the lambs (Molik et al., 2010). A marked PRL decrease (–46%) was also observed in lactating Saanen dairy goats under short-day photoperiod (Mabjeesh et al., 2007) and in NZ Friesian dairy cows MEL-treated (Auldist et al., 2007), compared with the control ones. In the Mabjeesh et al. (2013) experiment, all the goats were submitted to heat-stress in late-pregnancy, which is known to dramatically increase PRL leves. The positive effects of short-days during pregnancy on milk yield, were attributed to the inhibitory effects of MEL on PRL secretion, which stimulated the involution of the mammary gland and increased the expression of PRL-receptors in different tissues (Auchtung et al., 2005), such as in the mammary gland and liver. Thus, the higher the plasma MEL concentration, the lower the PRL concentration in blood, and vice versa.

No changes in plasma IGF-I values by effect of MEL treatment were detected in our ewes of either breed (P = 0.66; Table 4.1). Nevertheless, Mabjeesh et al. (2007) reported 68% decrease in plasma IGF-I values of short-day photoperiod lactating Saanen dairy goats and Auldist et al. (2007) in MEL-treated cows reported a decrease in PRL plasmatic concentrations, but values of IGF-1 did not change.

4.4.5. Body Reserves

The BW and BCS of our ewes are shown Table 4.1. MEL treatment had no effects on BW or BCS (P = 0.30 to 0.79) in either breed. On average, BW and BCS were 77.9 ± 1.1 kg BW and 3.02 ± 0.05 BCS units, for early-lactation under autumn photoperiod conditions. Bocquier et al. (1997) also found no effect of photoperiod length on body reserves of lactating Sarda dairy ewes. No differences were found within and between the short-day, long-day and the MEL with long-day treatment in lactating Polish Longwool ewes (Misztal et al., 2018).

4.5. CONCLUSIONS

In conclusion, the use of exogenous MEL, together with the effect of the endogenous MEL under decreasing photoperiod conditions, had no significant effects on milk yield and composition of medium and high-yielding dairy ewes in early lactation. Marked differences in MEL were observed between breeds that need further research.

CHAPTER 5

Cabergoline effects in lactating dairy ewes

CHAPTER 5

Suppression of prolactin and reduction of milk secretion by effect of cabergoline in lactating dairy ewes[1]

5.1. ABSTRACT

The effects of cabergoline, an ergot derivative and dopamine receptors agonist, were investigated in 30 ewes of 2 dairy breeds (Manchega, MN, n = 15; Lacaune, LC, n = 15). Ewes were in similar late-lactation stage but differed in milk yield according to breed (MN vs. LC, 1.02 ± 0.03 vs. 2.27 ± 0.05 kg/d). Treatments consisted of a single i.m. injection of cabergoline at different doses per ewe. Doses were: low (L, 0.56 mg), high (H, 1.12 mg) and control (CO, 1 mL saline). Milk yield was recorded daily (d −14 to 25), milk and blood sampled, and udder traits measured from d −2 to 14 after injection. No local reaction in the injection site, as well as on behavior and metabolic indicators of the ewes were detected after the cabergoline injection, but milk yield fell rapidly in both breeds (MN vs. LC, –54% vs. –27%), when compared to CO ewes. Cabergoline effects progressively disappeared after d 5 and no milk yield differences between treatments were detected from d 8 to 25 after injection. Milk fat and protein contents increased similarly (22% and 23%; respectively) in both breeds and at both cabergoline doses until d 5 and the effects disappeared thereafter. Plasma prolactin (PRL) decreased dramatically in the L and H treated ewes the day after injection, when compared to the CO ewes, and reached values below the detection limit of the assay between d 1 and 5, increasing similarly thereafter. On d 14, PRL values were 58% greater in the L and H treated than in the CO ewes, showing that PRL concentrations rebounded when the cabergoline effects ceased. Total udder volume correlated with milk accumulated in the udder (r = 0.77) of all groups of ewes throughout the experiment, suggesting its use as a non-invasive method for the estimation of milk stored in the udder. Udder volume was similar for the L and H ewes, but both values were lower than those of the CO ewes from d 1 to 14 after injection. No other effects on udder size were detected. In conclusion, cabergoline dramatically inhibited PRL secretion and decreased milk yield and udder volume of lactating dairy ewes. The L (0.56 mg/ewe) dose of cabergoline was as effective as the H (1.12 mg/ewe) in the 2 breeds of dairy ewes. These results suggest the interest of cabergoline to facilitate the decrease of milk production in dairy ewes (e.g., dry-off, illness care), although further research in pregnant dairy ewes and during the following lactation is still needed.

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5.2. INTRODUCTION

Late pregnancy and drying-off are challenging periods for dairy ruminants. Pregnant ewes are susceptible to ketone bodies toxemia and new intramammary infections (IMI) because of the increase of glucose demand and the decrease of immunocompetence (Shwimmer et al., 2008; Fthenakis et al., 2012; Zhao et al., 2019). The risks are greater in high-yielding and twin-bearing ewes (Oddy and Holst, 1991; Silva-del-Río et al., 2010), especially when using low energy diets or feed restriction during drying-off (Caldeira et al., 2007). Additionally, nutrient restriction at dry-off decreases 30% basal blood prolactin (PRL), as reported by Ollier et al. (2013) in dairy cows fed hay on the days preceding the dry-off.

Nutrient restriction at dry-off decrease the proliferation of mononuclear cells and compromises immunocompetence (Ollier et al., 2014, 2015; Zhao et al., 2019). Cessation of milking results in udder engorgement which leads mammary gland epithelium to apoptosis and, if excessive, induces mammary inflammation and cell necrosis (Zobel et al., 2015). In dairy sheep, where abrupt drying-off is commonly done, selective (i.e., IMI positive) or generalized antibiotic therapy is recommended at drying-off to improve udder health and milk yield in the following lactation (Gonzalo et al., 2004; Linage and Gonzalo, 2008). Consequently, reduction of udder insults during dry-off may be a strategy to decrease the use of antibiotic therapies at drying-off.

Under a physiological approach, an interesting method to facilitate the dry-off could be inducing the cessation of milk production by interfering with the transmission of hormonal signals from the pituitary gland (i.e., PRL inhibition). This approach has been proposed as a management tool in dairy husbandry (Lacasse et al., 2019) to avoid inappropriate lactation or to alleviate the nutritional stress in sick or injured lactating animals unable to support their level of production.

Dopamine has a direct effect on the lactotrophs of the anterior pituitary by binding to their D_2 receptors and reducing PRL exocytosis and gene expression (Fitzgerald and Dinan, 2008). Ergotic (e.g., bromocriptine, cabergoline and metergoline) and non-ergotic (e.g., quinagolide) derivatives also bind to D_2 receptors of the lactotrophs, and have shown to decrease PRL secretion and milk production, although with differences in affinity, half-life and side-effects (Bole-Feysot et al., 1998; Barlier and Jaquet 2006; Kvernmo et al., 2006). The use of PRL inhibitors in lactating ruminants have been deeply

reviewed by Lacasse et al. (2012, 2016, 2019) and Zhao et al. (2019).

Quinagolide injection proved to reduce plasmatic PRL and to be effective for milk reduction, both in early- (Lacasse et al., 2011; Boutinaud et al., 2012) and late-lactating (Ollier et al., 2013, 2014, 2015) dairy cows. Moreover, proliferation and survival of mammary epithelial cells after quinagolide treatment were fully restored by PRL injection (Lollivier et al., 2015; Lacasse et al., 2016), supporting the galactopoietic role of PRL in ruminants.

Cabergoline is a highly specific agonist of D_2 receptors with a long elimination half-life (Kvernmo et al., 2006; Odaka et al., 2014). The use of cabergoline, initially authorized by the European Medicines Agency (EMA, 2015) for facilitating the dry-off of cattle, decreased plasma PRL and accelerated udder involution reducing the secretory activity of mammary epithelial cells, udder engorgement and incidence of milk leakage in dairy cows (Bach et al., 2015; Boutinaud et al., 2016). A large-scale clinical study with 900 dairy cows in 63 farms (Hop et al., 2019), reported that cabergoline decreases under practical conditions the risks of milk leakage and of new IMI during the drying-off and post-calving periods. Nevertheless, despite the positive effects of cabergoline and the no food safety risks for consumers, when withdrawal period is respected (i.e., 32 d during dry-off or 8 milkings during lactation; EMA, 2015), its use in high-yielding dairy cows at late pregnancy has been associated to occasional adverse events, usually in the 24-h post-injection. These were recumbency and mortality, which were related to metabolic disorders (i.e., hypocalcemia, hypothermia, ataxia, adipsia, circulatory disorder and diarrhea). Therefore, the marketing authorization of cabergoline as Velactis (Ceva Animal Health, Libourne, FR) was first suspended (EMA, 2016) and finally its use banned in Europe (EMA, 2019), considering that the overall benefit-risk balance in dairy cows was negative.

Few and controversial data are available on the use of dopamine agonists in small ruminants. Arlt et al. (2011) reported the inefficacy of cabergoline to cease inappropriate lactations in hobby goats. On the other hand, Lacasse et al. (2016) cited no effects on milk production of repeated injections of quinagolide (1 mg/d for 4 wk; B. Ponchon, V. Lollivier and M. Boutinaud, unpublished results), whereas a single cabergoline injection (1 mg; V. Lollivier and M. Boutinaud, unpublished results) decreased milk yield (–28%) in dairy goats. The effects of dopamine agonists and the adequate dose for dairy ewes are unknown.

The objective of this work is to study the effects of 2 doses of cabergoline on PRL suppression and milk secretion to determine the suitable dose and the time-lasting effects in 2 breeds of dairy ewes in late-lactation.

5.3. MATERIALS AND METHODS

The study was conducted in the experimental farm of the SGCE (Servei de Granges i Camps Experimentals) of the Universitat Autònoma de Barcelona (UAB), in Bellaterra (Barcelona, Spain) during 2016. Animal-care conditions and management practices agreed with the Spanish Royal Decree 53/2013 on the protection of animals used for experimental purposes, the codes of recommendations for the welfare of dairy sheep of the Ministry of Agriculture, Alimentation and Environment of Spain (MAPA, 2007) and the procedures stated by the Ethical Committee of Animal and Human Experimentation of the UAB (Procedure #4992).

5.3.1. Ewe Management and Feeding

A total of 30 ewes of 2 dairy breeds (MN, Manchega, n = 15; LC, Lacaune, n = 15) in late-lactation (185 ± 11 and 186 ± 11 DIM, respectively; values are means ± SE) and kept as a single group on wood-chip bedded pens, were used. Their main characteristics (MN and LC, respectively) were: age (3.8 ± 0.5 and 2.7 ± 0.4 yr), BW (73.6 ± 2.5 and 67.6 ± 1.9 kg), BCS (3.08 ± 0.07 and 2.85 ± 0.07), and milk yield (1.02 ± 0.03 and 2.27 ± 0.05 kg/d). All ewes wore ruminal mini-boluses for electronic identification (20 g; Datamars, Bedano, SW) that were used for automatic milk recording (Ait-Said et al., 2014). Machine milking was conducted twice daily (0700 and 1700) in a 2×12 parallel stall milking parlor with automatic milk-recording devices (MM25SG; DeLaval, Tumba, SE) with similar procedures to those described by Elhadi et al. (2019).

Diet consisted of a TMR ad libitum (forage:concentrate, 55:45%; DM basis) with alfalfa hay as forage and a farm-produced concentrate [ingredients: soybean hulls, 50.0%; barley grain, 10.0%; oats grain, 10.0%; gluten feed meal, 10.0%; rapeseed 00 meal, 5.0%; soybean oil, 5.0%; corn grain, 4.0%; bi-calcium phosphate, 2.5%; cane molasses, 2.0%; VitafacOvino-0.3 premix (DSM Nutritional Products, Madrid, ES), 1.0%; salt, 0.5%; as fed]. Additionally, all ewes received 100 g of corn whole grain in the individual feeders of the milking parlor at each milking to encourage their coming in. Nutrient requirements

were calculated by INRAtion v.4.07 (Educagri éditions, Dijon, FR). Ewes had free access to water and commercial mineral blocks (Multi-Block; Agrària Comarcal del Vallès, Llerona, ES) in the pens.

5.3.2. Experimental Treatments

Ewes were blocked in 3 balanced groups of 10 animals (5 of each breed) according to age, BW, BCS, and milk yield, and submitted to a short-term lactation experiment divided in 2 periods: pre- (d –14 to –1) and post-injection (d 0 to 25). The experimental design consisted of a 2×3 factorial (breed×treatment) to which the ewe groups were randomly allocated. Treatments consisted of the dose of cabergoline (Velactis 1.12 mg/mL of cabergoline; Ceva Animal Health, Libourne, FR), intramuscularly injected into the middle of the left side of the neck after the p.m. milking, and were (cabergoline/ewe): low (L, 0.56 mg), high (H, 1.12 mg), and control (CO, 1 mL saline). The cabergoline doses used were achieved by the injection of 0.5 or 1.0 mL of Velactis per ewe, close to the EMA (2015) recommended treatment dose (RTD = 5.6 mg/cow or 7 to 10 µg/kg BW), equivalent to 0.49 to 0.70 mg of cabergoline for a standard ewe of 70 kg BW, and far from the 3×RTD (1.47 to 2.10 mg/ewe) and 5×RTD (2.45 to 3.50 mg/ewe) overdoses injected for the target animal safety studies (EMA, 2015). Although the cabergoline diluent used in the Velactis product was a lipidic solvent mixture (i.e., dimethyl sulfoxide and medium-chain triglycerides), we used sterile saline solution (0.9% NaCl; Laboratorios Grifols, Parets del Valles, ES) in our control ewes to evaluate the whole local reaction to the commercial product injection. Collected milk was discarded during the following week according to the withdrawal recommendations.

5.3.3. Measurements, Sampling, and Analyses

Reactions to the Injection. Local tolerance to cabergoline was evaluated on d 0, 1 and 7 post-injection using a severity score (0 to 3 points), according to the diameter of the adverse reaction produced by the injection (0, none; 1, <2 cm; 2, between 2 and 8 cm; 3, >8 cm; accuracy, 0.5 points). Special surveillance of the treated ewes was done at 8-h interval by a technician supervised by the veterinarian responsible of the SGCE of the UAB during the 48-h post-injection. General appearance, eating and drinking behavior

and mobility of the ewes when attending the twice-daily milking were monitored throughout the experiment.

Milk Yield. Milk yield of individual ewes was recorded by weight at each milking during the whole experimental period (d –14 to 25), using the milk-flow and milk-recording automatic units of the milking parlor (MM25SG, DeLaval). Data were uploaded using the AlPro software 7.2 (DeLaval) and daily reviewed to avoid incorrect values.

Milk Composition. Representative milk samples of each ewe were taken pre- (d −2 and −1) and post- (d 1, 2, 5, 7 and 14) cabergoline injection for compositional analysis using proportional milk samplers (DeLaval). Milk samples (50 mL) were composited according to the daily milking intervals (a.m.:p.m., 60:40), preserved with an antimicrobial tablet (Bronopol; Broad Spectrum Micro-tabs II, D&F Control Systems, San Ramon, CA) and stored at 4°C until analysis. Milk samples were analyzed in the Dairy Herd Improvement Laboratory of Catalonia (ALLIC, Cabrils, ES) for fat, total protein, lactose and urea (Milkoscan FT2; Foss, Hillerød, DK) and SCC (Fossomatic 5000, Foss).

Udder Size. Udder measurements were recorded before the p.m. milking on d −2 and −1, pre-treatment, and d 2, 5, 7 and 14 post-treatment, according to the agreed-upon FAO-M4 study protocol for dairy sheep (Labussière, 1983) with the ewes restrained by head-lockers in the milking stalls. Udder volume was estimated by water displacement using a 5-L bucket of warm water. Udder width was measured as the maximum udder width value by using a surgical thickness compass (Hauptner, Solingen, DE) and udder base-floor distance was taken by a measuring flexible tape, both from the back of the ewes.

Blood Measurements. Blood samples were taken from the jugular vein using 4-mL Vacutainer tubes with EDTA K2E 7.2 mg (BD; Belliver Industrial Estate, Plymouth, UK) 1-h after the a.m. milking (without corn supplement) and before feeding at d −1 (pre-treatment), and d 1, 2, 5, 7 and 14 (post-treatment). EDTA blood collecting tubes were used to avoid possible interactions in the hormonal enzymatic competitive analyses (Kohek et al., 2002). Plasma was obtained by blood centrifugation for 15 min at 2,000 × *g* and 4°C, transferred to 0.5 mL Eppendorf tubes and stored at −20°C for analysis.

Concentration of PRL in plasma was measured by ELISA sandwich type analysis (human PRL-ELISA KAPD1291, DIAsource Immunoassays, Leuven, BE) and the

stopped ELISA plates were read in an automatic reader (iEMS Reader MF V.2.9–0; Labsystems España, Barcelona, ES) at 450 nm. Detection limit, intra and inter assay CV were 0.35 ng/mL, 5.5 and 6.5%, respectively.

Impact of cabergoline treatments on the metabolic status of the ewes was assessed by the plasmatic concentrations of glucose, lactate, γ-glutamyl transferase (GGT), phosphorus and creatinine, using an Olympus AU480 analyzer (Olympus Europa, Hamburg, DE) with the specific Reagent System of Olympus (OSR, Beckman Coulter, Krefeld, IE); the respective analytical methods and reagents used were: hexokinase method (OSR6121), lactate oxidase method (OSR6193), γ-glutamyl-3-carboxy-4-nitroanilide method (OSR6119, with a concentration greater than 4 mmol/L), phospho molybdate method (OSR6122) and the Jaffé method (OSR6178). Changes in absorbance were read at 340 nm, except for creatinine that were read at 520 nm. Non-esterified fatty acids (NEFA) were determined by enzymatic colorimetry (ACS-ACOD-MEHA; acyl-CoA synthetase, acyl-CoA oxidase, 3-methyl-N-ethyl-N(β-hydroxy-ethyl) aniline) in the same analyzer using NEFA HR reagents (Fujifilm Wako Chemicals, Neuss, DE) and read at 410 nm. Additionally, concentration of lactose in plasma was used as indicator of the leakiness of the lactocyte tight junctions and was analyzed by difference based on two enzymatic reactions using galactose dehydrogenase and β-galactosidase, one measuring galactose and the other lactose and galactose (Boehringer Mannheim/R-Biopharm, Darmstad, DE) in the Olympus AU480 analyzer and reading at 340 nm. The use of EDTA collecting tubes did not allow the analyses of Ca, K and Na in plasma.

5.3.4. Statistical Analyses

Data were analyzed by the MIXED procedure for repeated measurements of SAS v.9.4 (SAS Institute Inc., Cary, NC). The statistical mixed model to evaluate the similarity of the ewe's groups during the pre-treatment period and the response to the treatments contained the fixed effects of breed (MN and LC), cabergoline dose (L, H and CO), sampling time and breed×treatment interaction, as well as the random effects of the experimental unit (the animal), and the residual error. Values of the variables were computed for their respective treatment and sampling dates, the means expressed as least squares means (LSM) and separated by pairwise comparison using the PDIFF ADJUST=SCHEFFE test of SAS. Pearson correlation coefficients (r) were calculated

using the CORR procedure of SAS. Significance was declared at $P < 0.05$ and a tendency was considered when $P < 0.10$.

5.4. RESULTS AND DISCUSSION

5.4.1. Cabergoline Dosage

According to the mean BW of the dairy ewes enrolled in the experiment (MN and LC, 73.6 and 67.6 kg BW, on average, respectively), the chosen cabergoline doses (L and H, respectively) were slightly lower in the MN (7.6 and 15.2 µg/kg BW) than in the LC (8.3 and 16.6 µg/kg BW) ewes. The L dose fell in the range of the EMA (2015) recommended treatment dose of cabergoline (RTD = 7 to 10 µg/kg BW) and agreed with the dosage previously used for the dry-off of dairy cows (Boutinaud et al., 2016; 8.7 µg/kg BW). On the other hand, the H dose doubled the RTD but was far from the 3×RTD (21 to 30 µg/kg BW) and 5×RTD (35 to 50 µg/kg BW) overdoses injected for target animal safety studies (EMA, 2015). As previously indicated by Lacasse et al. (2016, 2019), and despite the inconsistency of some studies done in goats, a dose of 1.0 mg cabergoline (approximately 16.6 µg/kg BW for a 60 kg BW goat) was also used in high-yielding dairy goats (i.e., 3.5 kg/d milk) in early-lactation.

5.4.2. Reaction to Cabergoline Injection

Intramuscular injection of cabergoline at both L and H doses did not produce local or adverse reactions in the right site of the ewes' neck; only 3 MN ewes, 1 from each treatment (i.e., CO, L and H), showed a slight swelling reaction (score 1) to the injection. No swelling reactions were observed in the LC ewes characterized by having open fleeces and wool-uncovered necks, in comparison to MN, which may have allowed a more precise and clean i.m. injection. As a consequence, the values of swelling scores were very low and similar between treatments (0.10 ± 0.11, on average).

Regardless of the cabergoline dose used, no general reactions or apparent changes in the behavior of the L and H treated ewes (i.e., abatement, recumbency, lack of interest to the feed bunk or drinkers after feed offering), were detected during the 48-h post-injection and throughout the experiment, when compared to the CO ewes. Moreover, no changes in the motion of the ewes, when being moved for the twice-daily milking or in their eating

behavior in the milking parlor (i.e., refusal of corn whole grain) were recorded. Unfortunately, no data on local or adverse side-effects of the high dose of cabergoline used in the goat experiments were available for comparison (Lacasse et al., 2016, 2019).

5.4.3. Prolactin in Plasma

As shown in Figure 5.1, plasma concentrations of PRL in the CO ewes ranged between 12 and 24 ng/mL (17.8 ± 1.5 ng/mL, on average) from d −1 to 14; no differences were detected in both breeds ($P = 0.99$). On the contrary, concentrations of PRL dramatically fell in the cabergoline treated ewes (Figure 5.1) which values were under the detection limit (i.e., 0.35 ng/mL) during d 1 and 2 post-injection, slightly increased on d 5, and raised rapidly thereafter. The low PRL values persisted for both L and H doses from d 1 to 5 when compared to CO ewes (–86%, on average; $P < 0.001$; Table 5.1), with no differences between breeds ($P = 0.89$; Table 5.1) and raised after d 7 (Figure 5.1). Values of plasmatic PRL of our CO ewes 1-h after milking agreed with the basal values reported by Boutinaud et al. (2016) in Holstein dairy cows before milking (approximately, 16 ng/mL). Nevertheless, the effect of cabergoline injection on the PRL concentration of our lactating ewes was greater than the decrease reported by Boutinaud et al. (2016; –39%) in Holstein dairy cows at dry-off, when compared to conventionally dried cows and both fed a dry hay diet. This lower PRL difference may have been a consequence of the negative effect of feed restriction in the control cows, as previously reported by Ollier et al. (2013). Additionally, the difference between control and cabergoline injected cows in the Boutinaud et al. (2016) study, persisted for 8-d and disappeared on d 14, likewise as it was observed in our ewes (Figure 5.1). Moreover, –20% plasma PRL after cabergoline injection was reported in the Bach et al. (2015) study, but the authors did not mention the cow's BW and the blood sampling time with regard to milking, precluding the comparison with other data.

Lacasse et al. (2011) and Boutinaud et al. (2012) also reported decreases in the concentrations of PRL released at milking (–12 to –32%) in dairy cows treated with repeated injections of quinagolide during lactation (8-wk).

Interestingly, greater PRL values in plasma were detected on d 14 post-injection in the L and H ewes (58%, on average; $P < 0.001$), when compared to the CO ewes, indicating a PRL rebound effect after ceasing the cabergoline treatment (Figure 5.1).

Table 5.1. Lactational effects of a single injection of cabergoline at different doses[1] during d 1 to 5 post-injection in two breeds of dairy ewes in late-lactation.

	Manchega			Lacaune					Effect (*P*-value)		
Item	CO	L	H	CO	L	H	Mean	±SEM	Cabergoline	Breed	Interaction[2]
Milk yields											
Milk, kg/d	0.87^{a}	0.40^{b}	0.62^{ab}	1.84^{a}	1.34^{b}	1.56^{ab}	1.11	0.12	0.001	0.001	0.99
ECM[3], kg/d	1.03^{a}	0.59^{b}	0.88^{ab}	1.88	1.55	1.82	1.29	0.13	0.050	0.001	0.95
Fat, g/d	80^{x}	48^{y}	72^{x}	138^{x}	115^{y}	137^{x}	98	11	0.07	0.001	0.93
Protein, g/d	63^{x}	37^{y}	54^{x}	116^{x}	101^{y}	116^{x}	81	8	0.10	0.001	0.97
Lactose, g/d	36^{a}	16^{b}	22^{ab}	80^{a}	52^{b}	63^{ab}	45	6	0.003	0.001	0.96
Milk contents											
Fat, %	9.15^{b}	11.74^{a}	11.64^{a}	7.49^{b}	8.60^{a}	8.82^{a}	9.57	0.54	0.001	0.001	0.36
Protein, %	7.28^{b}	9.26^{a}	8.63^{a}	6.31^{b}	7.51^{a}	7.44^{a}	7.74	0.41	0.001	0.001	0.63
Lactose, %	4.13	3.87	3.58	4.33	3.90	4.03	3.97	0.16	0.11	0.20	0.60
SCC, $\log_{10}$/mL	5.24	5.36	5.36	5.28	5.76	5.42	5.40	0.25	0.50	0.43	0.74
Plasma											
Prolactin, ng/mL	19.34^{a}	0.65^{b}	0.56^{b}	19.21^{a}	1.22^{b}	0.91^{b}	6.98	2.37	0.001	0.89	0.99
Glucose, mg/dL	62	60	60	60	64	59	61	2	0.61	0.81	0.42
NEFA, mmol/dL	0.075	0.083	0.070	0.101	0.088	0.114	0.087	0.011	0.89	0.08	0.16
Lactate, mmol/L	0.93	1.32	1.21	1.02	1.12	0.92	1.04	0.21	0.49	0.43	0.88
GGT[4], IU/L	58	60	47	77	88	67	66	12	0.53	0.12	0.80
P, mg/dL	4.23	4.57	4.28	4.73	4.44	5.03	4.50	0.31	0.89	0.49	0.34
Creatinine, mg/dL	0.68	0.71	0.71	0.64	0.67	0.65	0.68	0.03	0.69	0.08	0.98
Lactose, µmol/L	10.8	6.8	8.0	35.8	31.9	39.8	21.2	12.3	0.84	0.048	0.97
Udder traits[5]											
Volume, L	1.96^{a}	1.41^{b}	1.35^{b}	2.36	2.13	2.19	1.90	0.13	0.005	0.001	0.20
Udder tissue, L	1.28^{a}	1.24^{a}	1.09^{b}	1.52	1.43	1.57	1.35	0.11	0.74	0.002	0.36
Width, cm	13.2	12.3	12.3	15.3^{x}	14.2^{y}	14.8^{xy}	13.7	0.5	0.09	0.001	0.83
Base-floor, cm	31.9	34.0	36.4	28.4	31.8	28.5	31.2	1.9	0.53	0.001	0.47

Data are LS means ± SEM; ^{a,b}Within a row and breed, values with a different superscript differed ($P < 0.05$); $^{x, y}$Within a row and breed, values with a different superscript tended to differ ($P < 0.10$); [1]Treatments: CO, 0 mg; L, 0.56 mg; H, 1.12 mg cabergoline per ewe; [2]Cabergoline × Breed.
[3]Energy corrected milk = Milk yield × [0.071× (Fat, %) + 0.043 × (Protein, %) + 0.2224], (Bocquier et al., 1993); [4]γ-glutamyl transferase; [5]Data from d 2 to 5 post-injection.

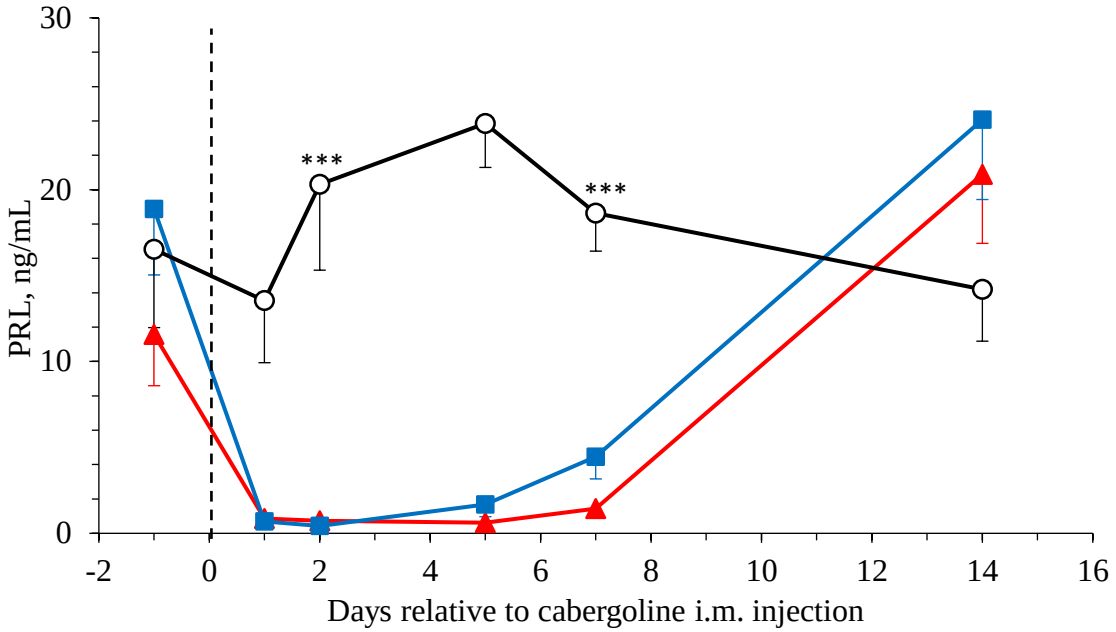

Figure 5.1. Effects of a single injection of cabergoline at different doses on plasma PRL of lactating Manchega and Lacaune dairy ewes. Doses of cabergoline: ▲ (H, 1.12 mg; n = 10), ■ (L, 0.56 mg; n = 10), and ○ (CO, 0 mg; n = 10). Values are LS means of both breeds averaged, with the SEM indicated by vertical bars. Differences between control and cabergoline i.m. injected ewes were significant from d 1 to 7 (***, $P < 0.001$).

The PRL rebound was the response (paradoxical reaction) of the ewe's metabolism to return to its basal state (homeostasis) after having been modified by the injection of cabergoline. Evidence of PRL rebound can also be observed in the results of Ollier et al. (2013) in dairy cows injected with quinagolide for 4-d before dry-off (approximately 50% increase basal value at d 14 post-injection), but it was not visible in the Bach et al. (2015) and Boutinaud et al. (2016) dairy cows injected with cabergoline at dry-off.

Given that cabergoline half-life in cows is 19-h (EMA, 2015) and its high affinity for dopamine D_2-like receptors (Kvernmo et al., 2006), the occurrence of the PRL rebound on d 14 after injection may be related to a mid-term feed-back of pituitary's lactotrophs, decreasing the release of natural dopamine, which will result in a rise of PRL. Mechanism of PRL rebound in rats after dopamine withdrawal was explained by Chen et al. (1993) and Chang and Shin (1999) who demonstrated that dopamine acts on D_2 receptors both to inhibit and to stimulate PRL release. We hypothesize that this may be related to the decrease of the activity of tyrosine hydroxylase (TH), the rate-limiting key enzyme in the biosynthesis and availability of catecholamines (i.e., dopamine, noradrenaline and adrenaline) during the cabergoline treatment. Gordon et al. (2008) showed that TH binds

to dopamine in high- and low-affinity binding sites, and dissociation of TH from dopamine markedly increases TH activity that will lead to greater PRL concentration in blood.

5.4.4. Milk Yield

All of the ewes were healthy at the start of the experiment and showed high milk yields for late-lactation before applying the treatments (Figure 5.2). Lactation persistency, estimated as the linear slope of the milk yield curve according to the stage of lactation was –18 g/d for the CO ewes (y = –0.0178x + 1.44; r = 0.92, $P < 0.001$) during the whole experiment (d –14 to 25), with differences in both breeds. Persistency was inverse to the level of production of each breed (MN, –6 g/d; LC, –28 g/d; $P = 0.004$), the lower the yield, the higher the persistency.

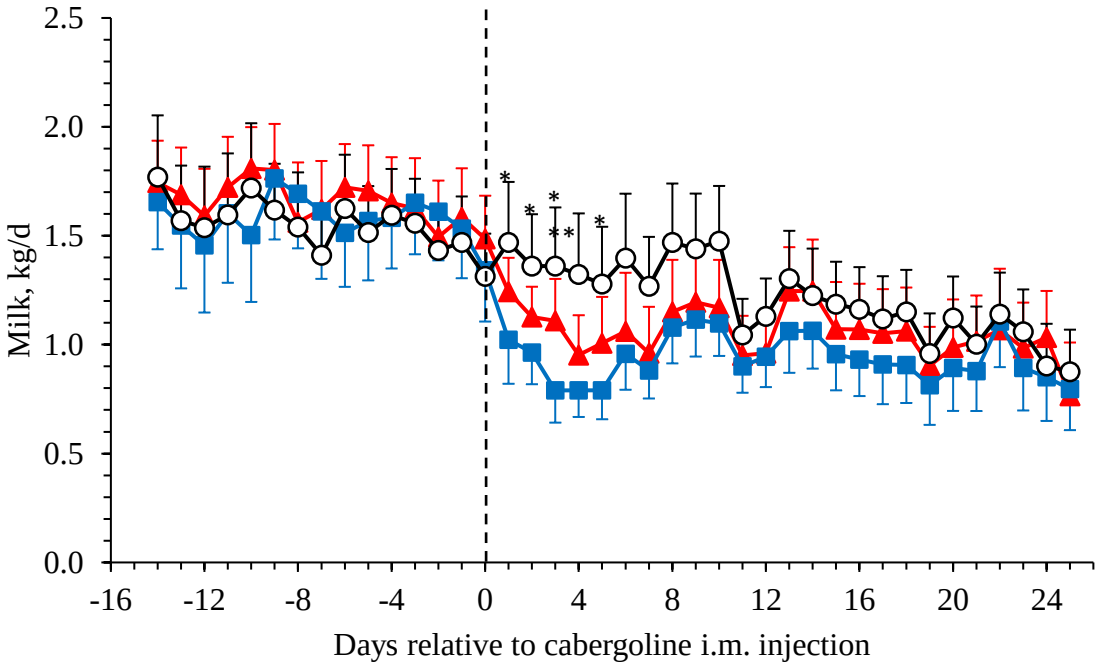

Figure 5.2. Effects of a single injection of cabergoline at different doses on milk yield of lactating Manchega and Lacaune dairy ewes. Doses of cabergoline: ▲ (H, 1.12 mg; n = 10), ■ (L, 0.56 mg; n = 10), and ○ (CO, 0 mg; n = 10). Values are LS means of both breeds averaged, with the SEM indicated by vertical bars. Differences between control and cabergoline i.m. injected ewes tended or were significant from d 1 to 5 (*, $P < 0.05$; **, $P < 0.01$).

Milk yield fell rapidly during the first 5 d after treatment (Figure 5.2 and Table 5.1) for both cabergoline doses (L vs. H, –40% vs. –22%; $P < 0.001$) and ewe breeds (MN and LC, –54% and –27%; $P < 0.001$), when compared to CO ewes. No differences were detected between the L and H doses in both breeds (Table 5.1), the cabergoline×breed interaction being not significant ($P = 0.99$). On average, the injection of cabergoline produced a sudden and marked decrease of milk yield (–31%; $P < 0.001$) immediately after the treatment and the slope (d 1 to 5) of the milk regression was –64 g/d of milk, on average. Milk yield progressively increased after d 5, and no differences were detected between the CO and the cabergoline treated ewes on d 8 after injection ($P = 0.23$) and thereafter (d 9 to 25; $P = 0.49$). Nevertheless, all ewes showed an unexpected increase in milk yield between d 8 to 10 (26%, on average), followed by a milk drop on d 11 and 12 (–20%, on average; Figure 5.2), without differences between the CO and the cabergoline treated ewes ($P = 0.90$). As a result, the persistency of the cabergoline treated ewes throughout the whole experiment (d –14 to 25) was on average –23 g/d ($y = –0.0233x + 1.35$; $r = 0.85$, $P < 0.001$), similar to the CO ewes ($P = 0.35$) and without differences between the H and L doses ($P = 0.85$). Again, persistency was inverse to the yield of each breed (MN, –16 g/d; LC, –30 g/d; $P = 0.026$).

Milk yield also declines faster in quinagolide treated cows (daily injections for 8-wk) during lactation than in control cows (Lacasse et al., 2011; approximately –15%), but no effects of a single injection of cabergoline have been tested during lactation. Milk decrease after the cabergoline injection in our ewes duplicated the above indicated value in dairy cows.

The increase in milk yield after d 11 was unlikely produced by the PRL rebound because the parallel raise in milk yield of the CO ewes and the numerically greater concentration of PRL in the plasma of the L treated ewes (Figure 5.1).

5.4.5. Milk Composition

On average, milk fat (Figure 5.3a) and milk protein (Figure 5.3b) contents of our ewes increased rapidly from d 1 to 5 (22 and 23%, respectively; $P < 0.001$) after the cabergoline treatment. Despite these increases in milk component concentrations, daily yields of milk fat and milk protein tended to decrease ($P = 0.07$ and $P = 0.10$, respectively; Table 5.1) as a result of the decrease in milk yield produced by the cabergoline injection. The effects

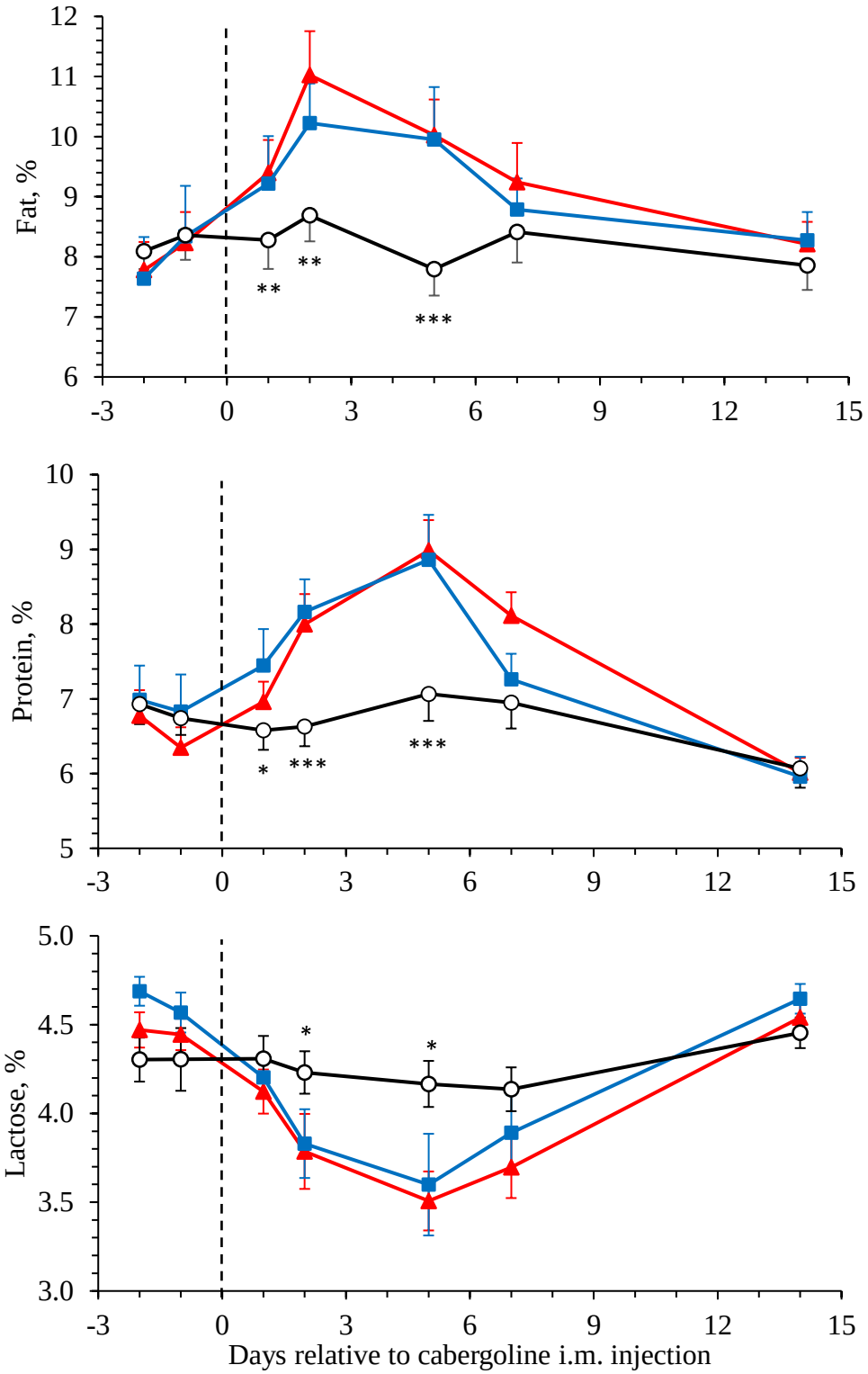

Figure 5.3. Effects of a single injection of cabergoline at different doses on milk composition (a, fat; b, protein; c, lactose) of lactating Manchega and Lacaune dairy ewes. Doses of cabergoline: ▲ (H, 1.12 mg; n = 10), ■ (L, 0.56 mg; n = 10), and ○ (CO, 0 mg; n = 10). Values are LS means of both breeds averaged, with the SEM indicated by vertical bars. Differences in fat and protein milk contents between control and cabergoline i.m. injected ewes were significant from d 1 to 5 (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$). Differences in lactose milk content were significant at d 2 and 5 (*, $P < 0.05$).

on milk fat and protein contents disappeared after d 7 post-injection, accompanying the recovery of milk yield previously discussed after d 5. On the contrary, no differences in milk lactose content were detected between treatments on average (P = 0.11), although the content of lactose in the milk of the cabergoline treated ewes was lower than in the CO ewes on d 2 and 5 (Figure 5.3c; P = 0.042). Milk lactose yield decreased by effect of cabergoline (Table 5.1; P = 0.003), although no differences were detected between the L and H treated ewes. The differences in milk composition among treatments decreased after d 5 and no differences in the concentration of milk components were detected at d 7 and 14 post-injection.

Despite the reported drop in milk yield of quinagolide treated cows during lactation (Lacasse et al., 2011), only numerically greater milk fat and milk protein contents are seen in milk; nevertheless, daily yields of both milk fat and milk protein decrease, agreeing the results obtained in our ewes. Moreover, marked decreases of milk lactose content during the last 4-wk of treatment and of lactose yield were reported by Lacasse et al. (2011) in the quinagolide treated cows, agreeing with our results in cabergoline treated ewes during lactation. Conversely, Boutinaud et al. (2016) did not find effects on the composition of mammary secretions (i.e., fat, protein and lactose) of Holstein cows treated with cabergoline at dry-off, although the composition of mammary secretions after ceasing milking is not directly comparable to that of milk obtained at milking during lactation.

No effects of cabergoline treatment were detected on the SCC of our ewes (P = 0.50; Table 5.1) that, despite being in late-lactation, showed a low SCC (5.40 ± 0.26, on average, equivalent to 250,000 cells/mL).

All effects of cabergoline on milk composition of our ewes disappeared after the first week post-treatment and no differences with the CO ewes were detectable at d 14.

5.4.6. Metabolic Indicators

Impact of cabergoline treatments on the metabolic status of the ewes was assessed by the plasmatic concentrations of several metabolic indicators during the critical period post-treatment (d 1 to 5), as well as for glucose and creatinine during the whole experiment (data not shown). No effects of cabergoline treatment were detected on glucose, NEFA, lactate and the GGT liver enzyme (related to glutathione metabolism,

amino acid absorption and protection against oxidation) as shown in Table 5.1. Moreover, glucose and creatinine in plasma were steady throughout the whole experimental period (P = 0.97). Plasmatic values of NEFA tended to be greater in the LC ewes, according to their greater milk production (P = 0.08; Table 5.1) but did not differ by cabergoline treatment (P = 0.89) indicating that cabergoline did not produce metabolic stress in our ewes. Similar results were obtained by Ollier et al. (2014) in quinagolide treated dairy cows during drying-off.

Although it was not possible to analyze Ca and Mg values because of the EDTA collecting blood tubes, no differences in P plasmatic values were detected (4.50 ± 0.31 mg P/dL, on average) between treatments (P = 0.89) or breeds (P = 0.49). According to Venjakob et al. (2017), there are positive associations between serum Ca and P concentrations in dairy cows, suggesting that no differences in blood Ca should be expected as a result of the cabergoline injection in our ewes. Lacasse et al. (2019) concluded that lowering the PRL concentration is unlikely to be responsible of a reduction in blood Ca and to cause hypocalcemia, as it was suspected in the cabergoline banning decision of EMA (2016, 2019). Nevertheless, it should be stressed that the use of cabergoline is still suspended for cattle in the EU.

No differences in plasma lactose were detected by effect of the cabergoline treatment in our ewes (P = 0.84; Table 5.1), although marked differences were observed according to breed (P = 0.048), the LC having greater values than MN ewes. This result agree with previous data in the same breeds and with the fact that LC ewes have greater milk yield and are more tolerant to milk accumulation between milkings than the MN are (Castillo et al., 2008b). Consequently, no tight junction disruption (leakiness) was produced in our ewes despite their level of production, as a result of the injection of cabergoline. Milk lactose yield reduction in our cabergoline treated ewes (Table 5.1) could be explained by the reduction of lactose synthesis in the mammary epithelial cells and not by leaking through cellular tight junctions.

5.4.7. Udder Traits

Involution of the udder induced by the cabergoline treatment was assessed by the changes in its anatomical measurements. The greater the reduction of the udder size, the better the effectivity of the dry-off facilitation treatment. Udder size will be an objective

non-invasive criterion to monitor the involution of the udder during the dry-off. Data of the udder volume (range, 1.00 to 3.42 L) and the corresponding milk yield obtained at the p.m. milking (range, 0.07 to 1.41 kg) of our ewes throughout the experiment (d –2 to 14) correlated (y = 0.4034 x – 0.226; r = 0.77, $P < 0.01$; Figure 5.4) and explained more than one-half (59%) of the variation of milk accumulated in the udder. Udder volume differed between breeds in our ewes (MN, 1.57 ± 0.07; LC, 2.23 ± 0.07; $P < 0.001$), agreeing with their differences in milk yield. Additionally, the volume of the udder tissue (non-milk volume of the udder) estimated by difference also correlated with the total volume of the udder (r = 0.82, $P < 0.001$), the udder tissue of the LC ewes being 26% greater than in the MN ewes ($P = 0.002$), as previously reported by Rovai et al. (2008). Labussière (1983), in a prospective study on the milkability of different dairy breeds in the Mediterranean, reported positive correlations between udder volume and daily milk yield (r = 0.40 to 0.71), although the reported correlations vary with the age of the ewes and the milk fraction considered, as also indicated by Fernández et al. (1983) in Manchega dairy ewes (r = 0.17 to 0.85). Similarly, udder width (MN, 12.6 ± 0.3; LC, 14.8 ± 0.3; $P < 0.001$) and base-floor distance (MN, 34.1 ± 1.1; LC, 28.3 ± 1.1; $P < 0.001$) also were, on average, different between breeds in our ewes, the greater the yield the greater the size of the udder.

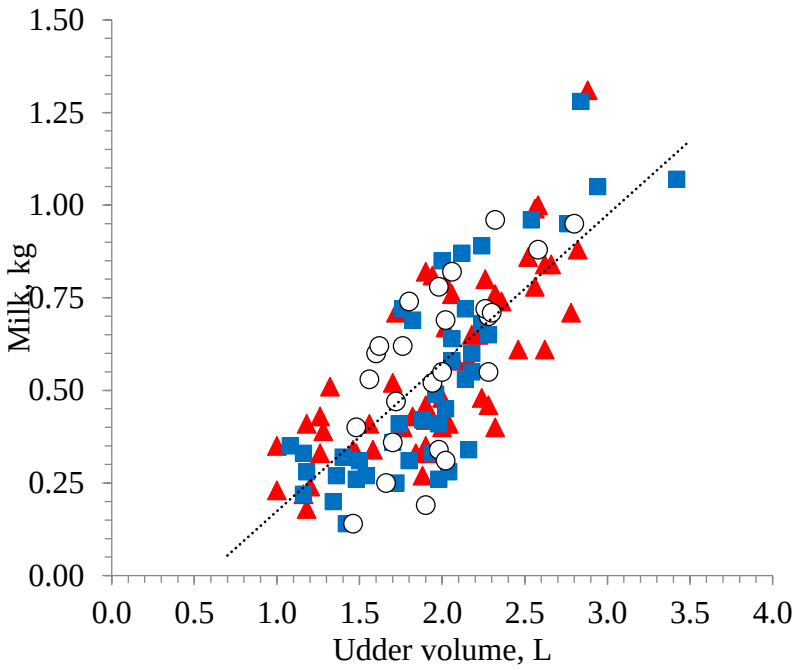

Figure 5.4. Correlation (y = 0.4034 x – 0.226; r = 0.77, $P < 0.01$; n = 180) between udder volume and milk yield at p.m. milking of lactating Manchega and Lacaune dairy ewes after a single injection of cabergoline at different doses. Doses of cabergoline: ▲ (H, 1.12 mg; n = 60, r = 0.77; $P < 0.05$), ■ (L, 0.56 mg; n = 60, r = 0.83; $P < 0.05$), and ○ (CO, 0 mg; n = 60, r = 0.66; $P < 0.05$).

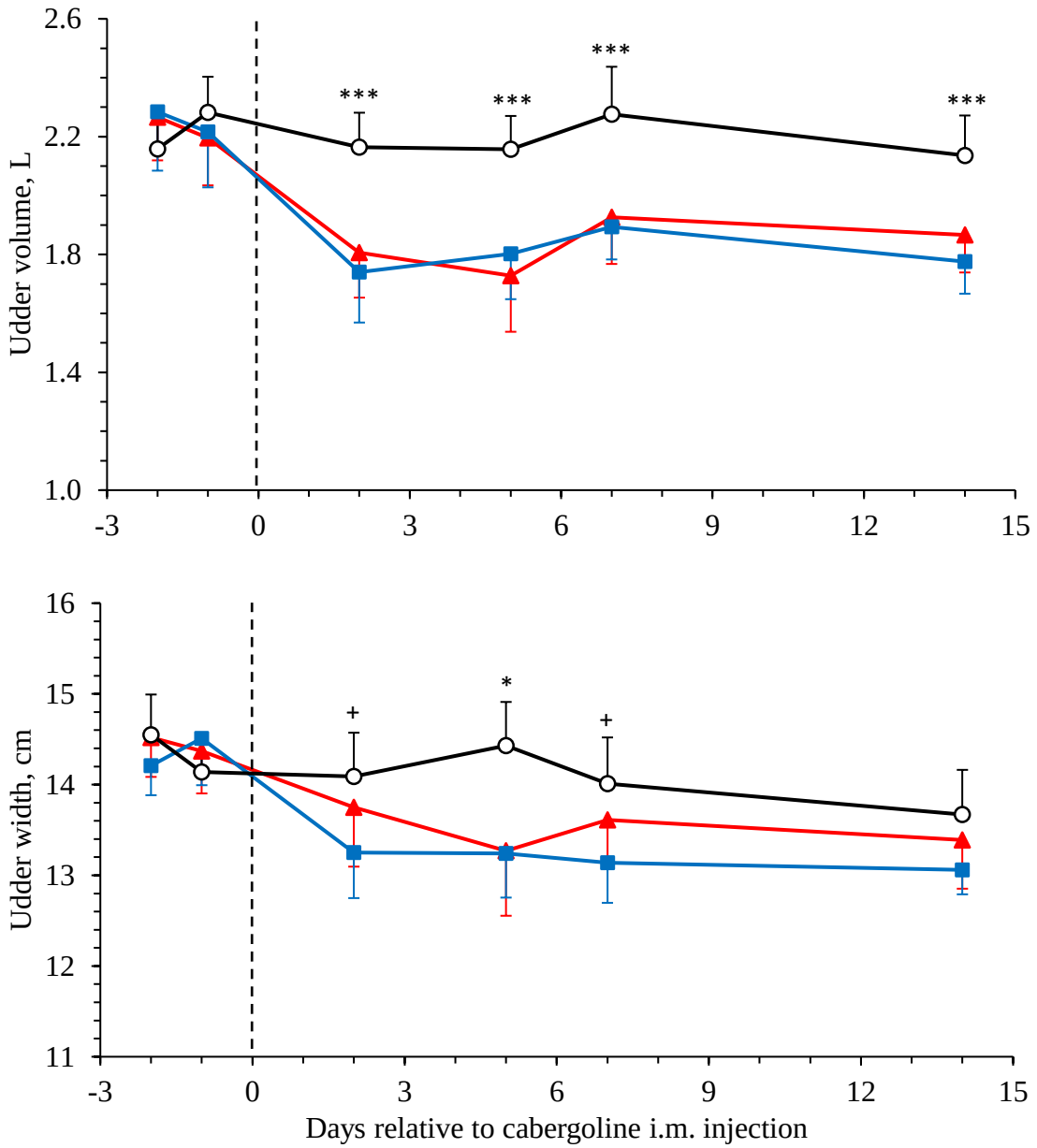

Figure 5.5. Effects of a single injection of cabergoline at different doses on the udder traits (a, volume; b, width) of lactating Manchega and Lacaune dairy ewes. Doses of cabergoline: ▲ (H, 1.12 mg; n = 10), ■ (L, 0.56 mg; n = 10), and ○ (CO, 0 mg; n = 10). Values are LS means of both breeds averaged, with the SEM indicated by vertical bars. Differences between control and cabergoline i.m. injected ewes were significant from d 2 to 14 (***, $P < 0.001$) in udder volume, and were significant at d 5 (*, $P < 0.05$) or tended to decrease (+, $P < 0.10$) at d 2 and 7 in udder width.

With regard to the cabergoline treatments, volume and width of the udder (Figure 5.5) were similar for the ewes treated with the L and H doses of cabergoline ($P = 0.36$ and $P = 0.56$, respectively) but, on average, udder volume was lower (–18%; $P < 0.005$) and udder width tended to be lower (–6%; $P = 0.09$) than those of the CO ewes from d 2 to 5

(Table 5.1). Nevertheless, no differences in udder volume between treatments were detected in the LC ewes (P = 0.57). Interestingly, despite not having differences in milk yield between CO and both L and H treatments at d 14 post-injection, udder volume of the cabergoline treated ewes remained smaller than in the CO ewes (Figure 5.5a). This result may be consequence of a reduction of the secretory tissue of the udder (i.e., mammary gland involution), which was only observed in the case of the treated MN ewes (P = 0.045), but that did not affect milk yield in the late stage of lactation of our ewes. There is also the possibility of a side-effect of the PRL rebound on oxytocin secreted at milking, agreeing the oxytocin-PRL positive feedback suggested by Kennett and McKee (2012) in rats, which may have reduced the amount of residual milk in the cabergoline treated ewes. This last hypothesis needs further research.

No effects of treatments were detected on the base-floor distance of the udder of either breed of ewes (P = 0.53; Table 5.1) indicating that pre-milking udder volume and, to a less extent the pre-milking udder width, were the only useful morphological traits to assess udder involution.

5.5. CONCLUSIONS

Cabergoline temporarily inhibited PRL and markedly decreased milk secretion and udder volume, but increased most milk components in lactating dairy ewes. The effect disappeared after 5 d and milk yield and milk composition did not differ from control values when milking was maintained for more than 20 d after injection. The effect on udder volume lasted longer than the effect on milk yield, which may have relation with the PRL rebound after cessation of cabergoline treatment and needs further research. The L dose (0.56 mg/ewe) was as effective as the H dose of cabergoline (1.12 mg/ewe) for the reduction of lactation, without differences between them with regard to udder traits. Overall, the use of 0.56 mg/ewe of cabergoline as a dry-off facilitator may be a strategy of interest in high-yielding dairy ewes in order to reduce the feed restriction stress (i.e., ketosis risk) and antibiotic therapy at dry-off.

No apparent adverse reactions were detected and our data do not support the suspicion that use of cabergoline, at the recommended treatment dose, may be related to hypocalcemia or mammary epithelial cell tight junctions disruption in lactating dairy ewes. Additionally, the results of this study may be useful to understand the use of PRL

inhibitors as a management tool in dairy small ruminants. Further research on the use of cabergoline on pregnant dairy ewes at the dry-off and during the following lactation is needed. Finally, it should be stressed that the use of cabergoline is currently suspended in the EU for cattle and that its use in dairy sheep will require a specific approval by EMA.

CHAPTER 6

General discussion

CHAPTER 6

General discussion and implications

Results of each experiment have been discussed separately in the different chapters of the present thesis. Therefore, this section discusses jointly the main results obtained in the Thesis to highlight the most interesting findings and implications to gain new insights for dairy sheep production in practice. Moreover, the conclusions of the 3 experiments of this Thesis can be used jointly in the same flock of dairy ewes.

For most farmers, shearing dairy ewes during winter is not a good practice because they consider that it may decrease milk production and deteriorate its composition as a result of exposing the animals to cold stress. It is assumed that removing the fleece alleviates the effects of heat-stress during the summer but increases cold-stress during the winter. Nevertheless, it was shown in Chapter 3 that shearing in early-lactation during mild-winter conditions has a positive effect on the dairy ewe metabolic environment, increasing milk yield in 10% in the high yielding LC ewes. These effects of shearing on milk yield were associated with the loss of insulation induced by fleece removing, which despite increasing the ewe's energy requirements, resulted in a greater feed intake (5%) and addressed the energy partitioning towards milk production. No differences by effect of shearing were detected in milk yield of the less productive but richer milk composition MN ewes. Moreover, no differences were observed in the concentration of milk components of shorn and control ewes of either breed although milk composition slightly tended to decrease for fat and protein contents, whereas lactose content tended to increase, when milk yield increased.

According to these results, dairy sheep farmers have a wide margin to choose the adequate period to perform shearing in dairy ewes, including winter and lactation, without negative effects on milk yield or milk composition. Although the response may vary according to breed, expected results of shearing on the lactational performances of dairy ewes will be positive or null. It should be stressed that, in breeds of high wool growth, it may be recommendable to shear the ewes 2 times in the same year, and with this aim, early- or mid-lactation will be adequate. Shearing the dairy ewes in mid- or late-lactation has also de advantage of reducing the occupied space (i.e., milking stalls and feed bunk),

improving fleece cleanness (e.g., udder and milk SCC) and facilitating accurate BCS assessment for the next breeding and dry-off.

MEL implants, normally used during the long-days photoperiod (summer and spring) to advance the beginning of the breeding season by imitating the stimulatory effect of short-days photoperiod, is a common management tool in dairy sheep. The use of MEL is a key strategy to maintain milking groups approximately constant through the year. In our experiment MEL implants were applied during the short-days (winter solstice) looking for the maximum production of endogenous MEL, knowing that the implants do not suppress the endogenous production of MEL on one side, and on the other side, to take advantage of early-lactation, which in our case occurs under short-days. This is the result of the out-of-season breeding system followed by our experimental dairy flock of the UAB that is based on autumn lambing and winter-spring lactation after 20 yr.

Despite the high values of MEL in blood, as shown under our experimental conditions during the day, no effects were detected by using MEL implants on the milk production and composition of the ewes of both breeds. Nevertheless, blood MEL values were greater in the Manchega than in the Lacaune dairy ewes, despite the same MEL dose used in the implants.

Further research is needed to study the pattern of night-and-day change of MEL in the implanted ewes, the effects of MEL implants under long-day photoperiod conditions and to understand the differences detected between breeds, especially when heavy and high yielding dairy ewes are used. Moreover, the use of MEL implants during late-pregnancy may be of interest to stimulate the following lactation in the case of ewes lambing during autumn and winter, as it is also de case of the out-of-season dairy ewes of the UAB flock.

Finally, high yielding dairy ewes managed and bred in groups, also need to be dried-off simultaneously, and independently of their milk yield. Most common techniques used by the farmers to dry-off high-yielding dairy ewes is basically feeding restriction, with or without antibiotic therapy, which has several limitations (i.e., pregnancy toxaemia, antibiotic resistance). The use of cabergoline as a new dry-off facilitator in dairy ewes showed interesting results to induce the cessation of milk production. Milk yield fell rapidly after cabergoline injection in both breeds, independently of their level of production. On average, cabergoline injection produced a sudden and marked decrease of milk yield and udder volume immediately after the treatment and the effect persisted

during 5 d, from which milk yield progressively increased. The cabergoline effects on milk yield in our ewes were the result of the dramatic fell of plasmatic PRL concentrations in the cabergoline-treated ewes of both breeds.

Further research will explore the effects of cabergoline on udder traits (i.e. morphological measures, ultrasonography) and behaviour of late-lactating ewes at dry-off, as well as its lactational (i.e., milk yield, milk composition, body reserves) and reproductive (i.e. litter size, lamb mortality, lamb weight at birth and at weaning) effects in the following lactation. These results would be of interest for the EMA dossier on the use of cabergoline in small ruminants and would help to improve the welfare and to reduce the risk of pregnancy toxaemia of high yielding dairy ewes at dry-off.

Based on the above conclusions and implications, it is clear that the management tools evaluated in the different chapters of this Thesis can represent important husbandry tools to improve the performance of dairy sheep farms by increasing their milk production during lactation and improving the welfare of the ewes.

CHAPTER 7

Conclusions

CHAPTER 7

Conclusions

The conclusions obtained in the different experiments carried out in this doctoral thesis are the following:

7.1. Specific conclusions

7.1.1 Shearing effects in lactating dairy ewes:

- Feed intake increased in the LC-SH (5%) ewes, when compared to LC-CO, but did not vary in the MN ewes.
- Milk yield of LC-SH ewes was 10% more milk (1.38 ± 0.06 vs. 1.52 ± 0.05 kg/d) than LC-CO ewes, but no differences were detected in MN ewes.
- No effects of shearing on the concentration of milk components in both breeds were detected, but LC-SH ewes yielded 9% more milk protein than did LC-CO ewes.
- No effects of shearing were detected on milk fatty acid profiles.
- No changes by shearing were detected in plasma values of glucose, NEFA, cortisol and insulin in either breed, as well as in body reserves.
- Body temperature after shearing only decreased (−0.36 ± 0.09ºC) in the case of the greater fleeced MN ewes.

7.1.2 Melatonin effects on lactational performances of dairy ewes:

- No melatonin effects were detected on DM intake or milk yield of both breeds studied.
- Milk composition varied by breed, but no MEL effects were detected on milk composition nor milk-fat standardized milk.
- Melatonin in plasma increased significantly in treated ewes of both breeds, however, prolactin in plasma decreased only in MN breed. No effects were detected in IGF-I between treatments.
- Body reserves did not vary by effect of melatonin treatment or breed throughout the experiment.

7.1.3 Cabergoline effects on milk secretion in dairy ewes:

- No local reaction in the injection site, as well as on behavior and metabolic indicators of the treated ewes were detected after the cabergoline injection.
- Milk yield fell rapidly in the cabergoline treated ewes of both breeds (MN vs. LC, –54% vs. –27%), when compared to CO ewes. No dose effect was detected and, after d 5, cabergoline effects progressively disappeared.
- Milk fat and protein contents increased similarly (23%) in both breeds and at both cabergoline doses until d 5 and the effects disappeared thereafter.
- Plasma prolactin decreased dramatically in the cabergoline treated ewes the day after injection, when compared to the CO ewes, reached values below the detection limit between d 1 and 5 and increased similarly thereafter.
- Udder volume decreased similarly for the both cabergoline dose used and were lower than those of the CO ewes from d 1 to 14 after injection. No other effects on udder size were detected.

7.2. General conclusions

The different management tools used in the previous experiments shown a significant importance to improve the performance of dairy ewes. They are:

- Shearing dairy ewes in mid-lactation and under mild-winter conditions, is a suitable management option for dairy ewes that may improve their milk yield, more likely in high-yielding dairy ewes, without changes in milk composition or physiological indicators.

- Treating lactating dairy ewes in early-lactation with exogenous melatonin implants and under decreasing photoperiod (autumn) conditions, despite the high level of plasmatic melatonin achieved and the milk yield of the breed, have not negative effects on the lactational performances of dairy ewes.

- Use of cabergoline as prolactin suppressor, aiming to reduce milk yield and to facilitate dry-off, could be an option of interest for dairy ewes aiming to improve their nutritive status and wellbeing. Further research in late-lactation and pregnant dairy ewes, as well as authorization from veterinary medicament agencies are specially required.

CHAPTER 8

References

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References

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