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Francisco Eduardo Canto Muñoz

Efectos de la melatonina exógena sobre la calidad del calostro, los parámetros de lactación y el desarrollo de sus crías en pequeños rumiantes

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EFFECTS OF EXOGENOUS MELATONIN ON COLOSTRUM QUALITY, LACTATION AND OFFSPRING PERFORMANCE IN SMALL RUMINANTS

Autor

Francisco Eduardo Canto Muñoz

Director

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2024

CERTIFICACIÓN DEL DIRECTOR DE TESIS

Instituto Universitario de Investigación en Ciencias Ambientales de Aragón
Universidad Zaragoza

José Alfonso Abecia Martínez, Doctor en Veterinaria, Catedrático del Departamento de Producción Animal y Ciencia de los Alimentos de la Universidad de Zaragoza e Investigador del Instituto Universitario en Ciencias Ambientales de Aragón (IUCA),

HACE CONSTAR

Que **Francisco Eduardo Canto Muñoz** ha realizado bajo mi dirección los trabajos correspondientes a su Tesis Doctoral titulada "**Effects of exogenous melatonin on colostrum quality, lactation and offspring performance in small ruminants**", que corresponde con el proyecto de Tesis aprobado por la comisión de Doctorado, y que cumple con los requisitos exigidos para optar al grado de Doctor por la Universidad de Zaragoza, por lo que autorizo su presentación por compendio de publicaciones para que pueda ser juzgada por el Tribunal correspondiente.

Lo que suscribo como director del trabajo, en Zaragoza, a 03 de octubre de 2024

José Alfonso Abecia Martínez

El doctorando ha disfrutado de una beca concedida por la Agencia Nacional de Investigación y Desarrollo (ANID) del Gobierno de Chile, correspondiente al Programas de Doctorado en el Extranjero, Becas Chile/2020 - 72210031. Además, mediante la resolución Nº 51 (09/10/2020), ha recibido la autorización del Instituto de Investigaciones Agropecuarias (INIA) para realizar sus estudios de doctorado.

La Tesis es un compendio de las siguientes publicaciones:

Publicación 1:
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JUSTIFICACIÓN DE LA CONTRIBUCIÓN DEL DOCTORANDO A LAS PUBLICACIONES DE LA TESIS

José Alfonso Abecia Martínez, Doctor en Veterinaria, Catedrático del Departamento de Producción Animal y Ciencia de los Alimentos de la Universidad de Zaragoza e Investigador del Instituto Universitario en Ciencias Ambientales de Aragón (IUCA), como Director de la Tesis Doctoral titulada “Effects of exogenous melatonin on colostrum quality, lactation and offspring performance in small ruminants”,

INFORMAN que:

- El doctorando Francisco Eduardo Canto Muñoz ha contribuido al desarrollo metodológico, la recogida y análisis de datos y muestras, el análisis estadístico, la elaboración y discusión de los resultados, y la redacción de las Publicaciones de la presente Tesis Doctoral.

Lo que suscribo como director del trabajo, en Zaragoza, a 03 de octubre de 2024

ABECIA MARTINEZ JOSE ALFONSO - DNI 25140075V

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José Alfonso Abecia Martínez

A mis queridos padres, José Javier y Victoria, por haberme formado como persona con todo el amor y comprensión del mundo. A lo largo de mi vida, me han apoyado en cada uno de mis proyectos, siempre con un cariño inmenso y una fe inquebrantable en mí. Gracias por los valores que me inculcaron, por enseñarme que con responsabilidad, trabajo, respeto y perseverancia... los sueños se hacen realidad. No tengo palabras suficientes para agradecerles por ser el pilar de todo lo que soy.

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RESUMEN

Esta Tesis Doctoral se propuso evaluar el efecto de la administración de melatonina exógena durante diferentes etapas fisiológicas de las madres sobre su producción y calidad de leche, y el rendimiento de sus crías, en pequeños rumiantes lecheros y de carne (ovejas y cabras), examinando también el impacto de la melatonina exógena en corderos durante su etapa pre-destete a lo largo de su desarrollo. Para alcanzar este objetivo general, se propusieron cinco experimentos (tres bajo condiciones comerciales y dos bajo condiciones experimentales).

En el primer experimento, 92 cabras lecheras Murciano Granadina recibieron un implante de melatonina treinta días antes del parto (grupo MEL), y las 177 cabras restantes (grupo CON) no lo recibieron. Se realizaron tres evaluaciones mensuales de la leche, incluyendo la producción de leche (kg/d) y su calidad (%Grasa=G, %Proteína=P, % Lactosa=L, Recuento Células Somáticas=RCS). Se recolectó una muestra de calostro de 165 cabras, de la cual se midió su composición y la concentración de IgG. Los resultados indicaron que MEL tuvo una producción de leche significativamente mayor (P<0,01) en el segundo mes que CON. En los tres muestreos de leche, las concentraciones de G fueron significativamente mayores (P<0,05) en las cabras MEL que en las CON.

En el segundo experimento, cuarenta días antes del parto, 246 ovejas lecheras Assaf recibieron un implante de melatonina (1M); otras 137 ovejas recibieron dos implantes (2M), y las 332 ovejas restantes no recibieron ningún implante (C). Se analizó la producción y composición láctea en base a muestreos mensuales individuales. Se recogió una muestra de calostro de 303 ovejas y se midieron las concentraciones de IgG. De media, las ovejas 1M produjeron más leche (P<0,05) que las ovejas de los otros dos grupos, y las ovejas 2M produjeron significativamente (P < 0,05) más leche que las ovejas C. En el primer y tercer control, las ovejas que recibieron dos implantes de melatonina tuvieron un SCC menor (P < 0,05) que las ovejas C y 1M, y en el segundo muestreo, las ovejas 1M y 2M tuvieron un SCC menor (P<0,01) que las ovejas C. Las ovejas que recibieron implantes de melatonina tuvieron una concentración de IgG más alta (P<0,01) que las ovejas C; las ovejas 2M tuvieron los niveles más altos de IgG.

En el tercer experimento, cuarenta días antes del parto, 457 ovejas gestantes Rasa Aragonesa recibieron o no un implante de melatonina. Posteriormente, los corderos se dividieron en dos grupos: corderos cuyas madres recibieron melatonina (MEL), y corderos cuyas madres no fueron tratadas (CTR). Los corderos fueron pesados al nacer (PV0, kg) y al destete (PVD). Además, se registraron los días hasta el destete (DD, d), lo que permitió calcular la ganancia media diaria de crecimiento (g/d) (GMD). Los corderos MEL tuvieron una media PVD significativamente (P<0,05) superior a la de los corderos CTR. En particular, los corderos MEL machos tuvieron un PVD y una GMD

significativamente mayores (P<0,05) que los corderos CTR machos. Los corderos MEL machos de parto único tuvieron mayor PV0, PVD y GMD que los demás corderos. El PV0, el PVD y la GMD se correlacionaron negativamente (P<0,05) con el intervalo implantación-destete (IID). Los corderos con el IID más corto tuvieron el mayor PVD (P<0,05) y GMD (P<0,01), y el menor DD (P<0,01).

En el cuarto experimento, cincuenta ovejas Rasa Aragonesa gestantes, recibieron un implante subcutáneo de melatonina treinta días antes del parto (M-0), en el parto (0-M), en ambos periodos (M-M), o no recibieron implante (0-0). En el experimento se midió la calidad del calostro (ºBrix e IgG) y de la leche (recogida cada dos semanas). El peso de los corderos (PV) se registró al nacimiento (PV0) y cada dos semanas hasta el destete. Al nacimiento, se midió temperatura rectal (TR) y se tomaron imágenes termográficas de los corderos (ojo=TO; hombro=TH; mitad del lomo=TM; caderas=TC). Las concentraciones de P y L en el calostro fueron significativamente mayores (P<0,05) en el grupo M-0 que en el grupo M-M. Al destete, los corderos machos criados por ovejas 0-M tuvieron significativamente (P<0,05) mayor PV que los corderos machos criados por ovejas M-0 o 0-0. Los corderos de ovejas M-0 y M-M tuvieron los mayores TM y TC, y los efectos fueron más pronunciados en los corderos machos.

En el quinto experimento, sesenta corderos se dividieron en dos grupos, uno recibió 2 implantes de melatonina a los 30 días de edad (MEL) y el otro grupo fue control (CTR). Los corderos fueron cebados desde el destete (45 d de edad) hasta su sacrificio (85 d). La tasa de conversión alimenticia (TCA) se calculó a partir del peso vivo incrementado y la cantidad de concentrado consumido. La actividad locomotora (AL) se midió semanalmente mediante actigrafía. En la última semana de cebo, se registraron el TR y la temperatura superficial (Tsur). El tratamiento no afectó a la TCA, aunque las hembras MEL consumieron significativamente (P<0,001) menos concentrado que las CTR. La AL fue significativamente (P<0.001) menor en los corderos MEL que en los corderos CTR. Los corderos MEL tuvieron un TR y Tsur significativamente (P<0.01) más bajos para todas las regiones corporales evaluadas que los corderos CTR.

En conclusión, la administración de melatonina exógena durante diferentes etapas fisiológicas tanto en pequeños rumiantes lecheros como de carne mejora los resultados productivos. En cabras y ovejas lecheras, los implantes de melatonina administrados al final de la gestación mejoran la producción y composición de la leche. En las ovejas de carne, la administración de melatonina antes del parto influyó positivamente en la temperatura superficial del cordero y en su crecimiento. Además, el tratamiento con melatonina durante la fase previa al destete en corderos, aumentó la eficiencia alimentaria en corderos de engorde, probablemente, al reducir la temperatura corporal y la actividad locomotora. Los resultados del rendimiento de los corderos estuvieron condicionados por el tamaño de la camada y el sexo de las crías.

SUMMARY

This Doctoral Thesis was proposed to evaluate the effect of exogenous melatonin administration during different physiological stages in dams on their milk production and quality, and the performance of their offspring in dairy and meat small ruminants (sheep and goats), while also examining the impact of exogenous melatonin in lambs during their preweaning stage on their life. To achieve this general objective, five experiments were proposed (three under commercial conditions and two under experimental conditions).

In first experiment, thirty days before kidding, 92 Murciano Granadina dairy goats (group MEL) received one melatonin implant, and the remaining 177 goats (group CON) did not. Three monthly milk evaluations included milk yield (kg/d) and quality (%Fat=F, %Protein=P, % Lactose=L, Somatic Cell Count=SCC). A sample of colostrum was obtained from 165 goats, from which its composition, IgG concentration were measured. Results indicated that MEL had a significantly (P<0.01) higher milk yield in the second month than CON . In the three milk samplings, F concentrations were significantly higher (P<0.05) in the MEL than in the CON does.

In the second experiment, forty days before lambing, 246 dairy Assaf ewes (1M) received a melatonin implant; another 137 ewes (2M) received two implants, and the remaining 332 ewes (C) did not receive an implant. Milk analysis was based on individual monthly milk samplings. A colostrum sample was collected from 303 ewes, and IgG concentrations were measured. Ewes implanted with melatonin had higher (P<0.01) daily milk yield in the three samplings than the C ewes. On average, 1M ewes produced more milk (P<0.05) than ewes in the other two groups, and 2M ewes produced significantly (P<0.05) more milk than C ewes. In the first and third controls, ewes that received two melatonin implants had a lower (P<0.05) SCC than C and 1M ewes, and in the second sampling, 1M and 2M ewes had a lower (P<0.01) SCC than C ewes. Ewes that received melatonin implants had a higher (P<0.01) IgG concentration than non-implanted ewes; 2M ewes had the highest IgG levels.

In third experiment, forty d before lambing, 457 pregnant ewes either did or did not receive a melatonin implant. Subsequently, lambs were divided into two groups: lambs whose mothers received melatonin (MEL), and lambs whose mothers were non-treated (CTR). Lambs were weighed (kg) at birth (LW0) and at weaning (LWW). The age at weaning (AW, d) was recorded, which allowed to calculate the average daily growth rate (g/d) (AGR). MEL lambs had a mean LWW significantly (P<0.05) higher than CTR lambs. In particular, male MEL lambs had a significantly (P<0.05) higher LWW and AGR than male CTR lambs. Singleton male MEL lambs had higher LW0, LWW, and AGR than the other lambs, and differences with singleton male CTR lambs were significant (P<0.05).

LW0, LWW, and AGR were negatively correlated (P<0.05) with the implanting- weaning interval (IWI). Lambs with the shortest IWI had the highest LWW (P<0.05) and AGR (P<0.01), and the lowest AW (P<0.01).

In the fourth experiment, fifty pregnant Rasa Aragonesa ewes, received a subcutaneous melatonin implant thirty days before lambing (M-0), at lambing (0-M), at both periods (M-M), or did not receive an implant (0-0). The experiment measured colostrum (ºBrix and IgG) and milk (collected every two weeks) quality were measured. Lamb weight (LW) was recorded at birth (LW0) and every two weeks until weaning. At birth, lamb rectal temperature (RT) and thermography images were taken (Eye=ET; shoulder=ST; mid loin=MT; hips=HT). P and L concentrations in colostrum were significantly (P<0.05) higher in the M-0 than they were in the M-M group. No significant differences were found for LW0 among groups, as well as considering litter size. However, at weaning, male lambs reared by 0-M ewes had significantly (P<0.05) higher LW than did male lambs reared by M-0 or 0-0 ewes. Lambs from M-0 and M-M ewes had the highest MT and HT, and the effects were most pronounced in male lambs.

In fifth experiment, sixty lambs were divided into two groups, one of which received 2 melatonin implants at 30 days of age (MEL) and a control group (CTR). Lambs were fattened from weaning (45 d of age) to slaughter (85 d). Feed conversion rate (FCR) was calculated based on live weight and the amount of concentrate consumed. Locomotor activity (LA) was measured weekly by actigraphy. RT and surface temperatures (Tsur) were recorded in the last week of fattening, and subcutaneous fat thickness (FT) over the longissimus dorsi muscle was measured by ultrasound scanning. Treatment did not affect FCR, although MEL female lambs consumed significantly (P<0.001) less concentrate than CTR lambs. Treatment and sex had a significant (P<0.05) interaction effect on FT; specifically, FT was significantly (P<0.05) higher in female MEL lambs than in female CTR lambs. Overall activity was significantly (P<0.001) lower in the MEL lambs than in the CTR lambs. MEL lambs had a significantly (P<0.01) lower RT and Tsur for all body regions evaluated than the CTR lambs.

In conclusion, the administration of exogenous melatonin during different physiological stages in both dairy and meat small ruminants enhances production outcomes. In dairy goats and ewes, melatonin implants administered in late pregnancy improve milk yield and composition. In meat ewes, pre-lambing melatonin administration positively influenced lamb surface temperature and growth performance. Furthermore, melatonin treatment during pre-weaning stage in lambs, increased food efficiency in fattening lambs, probably, by reducing body temperature and locomotor activity. The results of lamb performance were conditioned by litter size and the sex of the offspring.

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INTRODUCTION

LIST OF ABBREVIATIONS (English/Spanish)

-OH	Highly toxic hydroxyl radical
°C	Celsius degrees
ABCG2	ATP-binding cassette G2
ADG	Average daily gain
AGR	Average daily growth rate
AL	Actividad locomotora
AW	Age at weaning
BAT	Brown adipose tissue
BCS	Body condition score
BL	Body length
CG	Chest girth
CIDR	Controlled Internal Drug Release
CLA	Conjugated linoleic acid
CON	Control group/grupo control
CTR	Control group/grupo control
CytC	Cytochrome c
D	dark
d	Day/día
DD	Días hasta el destete
DIM	Days in milk
ECM	Energy corrected milk
ET	Eye Surface temperature
F	Fat
FCR	Feeding convertion rate
FT	Fat thickness
G	Grasa
g	Gram/gramo
GH	Growth hormone
GMD	Ganancia medía diaria
GSHPx	Glutathione peroxidase
h	Hour
HOCl	Hypochlorous acid
HT	Hips Surface temperature
IGF	Insulin growt factor
IGF	Insulin-like growth factor
IgG	Inmunoglobulin G/ Inmunoglobulina G/

IID	Intervalo implantación destete
IL-10	Interleukin 10
IL-6	Interleukin 6
IL1-β	Interleukin 1 β
IRT	infrared thermography
IWI	Implanting- weaning interval
kg	Kilogram/kilogramo
L	Light
L	Lactose/Lactosa
LA	Locomotor activity
LDPP	Long day photoperiod
LW	Liveweight
LW0	Liveweight at birth
LWW	Liveweight at weaning
m a.s.l.	Meter above sea level
MEL	Melatonin group/grupo melatonina
mg	Miligram
MT	Mid loin Surface temperature
MT1	Melatonin receptor 1
MT2	Melatonin receptor 2
MUFA	Monounsaturated fatty acids
ONOO-	Peroxynitrite anion
P	Protein/Proteína
PARP1	Polymorphism associated with radiation sensitivity
PUFA	Polyunsaturated Fatty Acid
PV	Peso vivo
PV0	Peso vivo al nacimiento
PVD	Peso vivo al destete
RBMF	Rumen bypass feeding
RCS	Recuento de Células Somáticas
resp.	Respectivily
RH	Rump height
RNA	Ribonucleic acid
RT	Rectal temperature
s.c.	Subcutaneous
SCC	Somatic cell count
SFA	Satured fatty acids
ST	Shoulder Surface temperature

SW	Shoulder width
TC	Temperatura superficial de caderas
TCA	Tasa conversión alimentaria
TGF-β	Transforming growth factor β
TH	Temperatura superficial de hombro
TM	Temperatura superficial de mitad del lomo
TNF-α	Tumor necrosis factor α
TNF-γ	Tumor necrosis factor γ
TO	Temperatura superficial del ojo
TR	Temperatura rectal
Tsur	Superficial temperature/temperatura superficial
VM	Vector magnitude
WH	Wither height
wk	week

1. Introduction

1. INTRODUCTION

1.1. Biosynthesis and role of melatonin

Melatonin (N-acetyl-5-methoxy-triptamine) is a neuroendocrine hormone derivative of tryptophan, an amino acid primarily generated in the pineal gland (Brainard et al., 1982; Cozzi et al., 1991). Tryptophan taken from the bloodstream is metabolized through four successive well-defined intracellular steps enzymatically catalyzed by tryptophan hydroxylase, aromatic amino acid decarboxylase, arylalkylamine-N- acetyltransferase and hydroxyindole-0-methyltransferase (Axelrod & Weissbach, 1960; Lovenberg et al., 1967).

This hormone was first isolated in 1958 from the bovine pineal gland (Lerner et al., 1958). Over time, it has also been proven that it is secreted in other organs, tissues and cells. They stand out among them, gastrointestinal tract (Muñoz-Pérez et al., 2016; Huether, 1993), retina (Zawilska, 1992), bone marrow (Conti et al., 2000), skin (Slominski et al., 2018) and oocytes (He et al., 2016). The main sites of melatonin synthesis at the cellular level are the mitochondria (Reiter et al., 2018), and thus, actually, all cells appear to have the ability to synthesis melatonin.

Circadian variations in the secretion of melatonin from the pineal gland are mediated by the photoperiod (Bittman et al., 1983). Considering that, at night (the dark phase), melatonin is synthesized and released, while during the day, the pineal gland's secretory activity is decreased (Arendt, 1988). The fetal pineal gland does not produce melatonin, and therefore, fetal circulating melatonin is from maternal origin (Serón-Ferré et al., 2013). In rats, sheep, and humans, the pineal gland starts producing melatonin postnatally (Deguchi, 1975; Nowak et al., 1990; Kennaway et al., 1992).

Over the past 60 years, melatonin actions in organisms have been extensively studied (Olesçuck et al., 2019). It modulates circadian and seasonal timing (Gillette & Tischkau, 1999; Reiter et al., 2010; Yawno et al., 2017); oxidative stress (Tan et al., 1993; Tan et al., 2002); immunological (Guerrero & Reiter, 2002) and cardiovascular systems (Cardinali & Pévet, 1998); fetal system nervous center (Serón-Ferré et al., 2013); neuroendocrine (Cardinali & Pévet, 1998); reproduction (Reiter, 1991; Reiter et al., 2010); pregnancy and parturition (Tamura et al., 2008; Verteramo et al., 2022) and corpus luteum function (Tamura et al., 1998), among others.

Melatonin is a multitasking molecule, showing a remarkable versatility by exhibiting diverse physiological actions in animals (Garcia-Ispierto et al., 2013). For this reason, its effects on domestic animals have been the topic of several bibliographic reviews. Thus describing the actions of melatonin on: immune system (Liebmann et al., 1997; Carrillo-Vico et al., 2005, 2013), cardiovascular (Sewerynek, 2002), brown adipose tissue (Olesçuck et al., 2019), hair follicle (Fischer et al., 2008), physiology and behavior of ungulates (Correa & Fernández, 2017), lamb neonatal mortality (Flinn et al., 2020a), male reproduction (Yu et al., 2018; Bhattacharya et al., 2019), sperm in farm animals (Ofosu et al., 2021), seasonality in sheep (Matthews et al., 1993; Williams & Helliwell, 1993), ovary activity (Abecia et al., 2008; Tamura et al., 2009); control of goat and sheep reproduction (Chemineau et al., 1992; Kennaway & Rowe, 1995; Abecia et al., 2011; Younis et al., 2019) and farm animals (Paterson & Foldes, 1994; Chemineau & Malpaux, 1998; Talpur et al., 2018; Vivid & Bentley, 2018), embryo establishment and viability in sheep (Abecia et al., 2008; Abecia et al., 2019), and production and preservation of mammalian gametes and embryos (Cruz et al., 2014).

As it can be observed, most of the research and review articles on melatonin over the 30 years after the hormone's discovery were focused on the ability to control reproduction in species sensitive to photoperiod changes (Abecia et al., 2011). In these photoperiod-dependent animals, melatonin mediates the transduction of photoperiodic information to the endocrine system inducing ovulatory activity, which leads to the precise timing of reproduction (Abecia et al., 2005; Abecia et al., 2023). Despite the deep development research of the wide range of actions of melatonin, there is still no bibliography compilation on its effects in other disciplines of animal science. It is crucial to understand the effects of exogenous melatonin supplementation in ruminants across different management scenarios, as it reveals potential implications of this hormone on productive practices. Thus, by analyzing the results in a variety of contexts, the viability and relevance of its application in various livestock farming systems can be determined. Consequently, in this section, we describe the main effects of exogenous melatonin linked to lactation, colostrogenesis, growth, and thermoregulation in ruminant livestock (Figure 1).

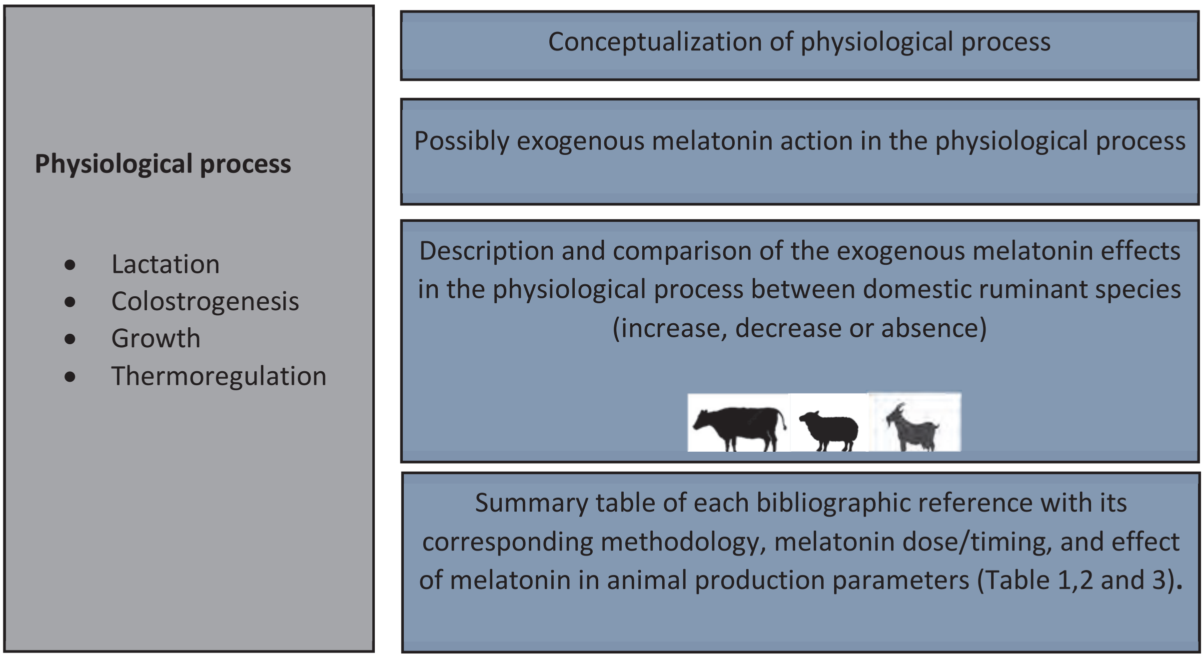

Figure 1. Diagram outlining the structure of the introduction for the influence of exogenous melatonin in lactation, colostrogenesis, growth, and thermoregulation in ruminants.

1.2. Lactation

1.2.1. Milk yield

While melatonin is primarily known for its role in regulating reproductive processes, it also plays a role in the regulation of mammary gland development and subsequent milk production in ruminants. Furthermore, there is an immunohistochemical and molecular evidence of melatonin membrane receptors MT1 and MT2 in the epithelial cells of the mammary gland in lactating dairy goats (Zhang et al., 2019); therefore, at the level of mammary tissue, melatonin can have a regulatory effect on cell proliferation (mammogenesis) and milk production (galactopoiesis). However, it is still unknown the actual role in the mammary gland as a target tissue (Dahl et al., 2000; Zhang et al., 2019), and its effects can even be contradictory in ruminant species (Abecia et al., 2005). These different melatonin responses in milk production on animals could be attributed to a wide range of factors, including significant species differences (Asher et al., 1994; Avilés et al., 2019), dosage variations (Cosso et al., 2021), or melatonin release characteristics (Yang et al., 2020).

On the one hand, several studies investigating the association between melatonin and milk production revealed that higher melatonin circulations were linked to lower milk production in dairy ruminants (Yang et al., 2020). Melatonin administration causes a decrease in prolactin secretion, and this reason could lead to a reduction in milk yield (Lacasse et al., 2019). The majority of research demonstrating a decline in milk yield has focused on the Longwool Poland ewes, which are not typical dairy animals and with a clearly preserved seasonality of reproduction. Molik et al. (2013) demonstrated that the subcutaneous implantation of exogenous melatonin twice (57 and 147 d before lambing) under long day conditions significantly decreased (15.6%) milk yields in the milking period. Two other studies showed that implanting sheep with melatonin twice (18 mg; six weeks before and after lambing, in spring and autumn, resp.) decreased milk yield. This decline was observed under long photoperiod conditions, both in the lamb-nursing period and after lamb weaning (from day 57 until the end of lactation) (Misztal et al., 2018; Molik et al., 2020). In other ruminants, Cashmere goats and dairy cows exhibited similar responses to administering one (18 mg) or six (108 mg) melatonin implants, resp., with repeated dosing during lactation (19, 23 and 27 weeks; 7 and 16 weeks, resp) (Auldist et al., 2007; Yang et al., 2020).

On the other hand, melatonin implantation during the dry period produced a stimulatory effect on milk production in the subsequent lactation in small ruminants. In nursing creole Mexican goats, two melatonin implants (36 mg) inserted seven weeks before kidding (summer solstice) increased milk production by more than 20% during the suckling and the milking period (short day) (Avilés et al., 2019). Similarly, Karagouniko ewes that received their last melatonin administration six weeks before lambing showed an increase in milk production in early lactation (40 d) (Bouroutzika et al., 2020). Concerning dairy cows, Wang et al. (2019) observed that administering subcutaneous melatonin (4.64 mg of melatonin/cow) in mid-lactation for four days in a row enhanced milk production.

Studies on the use of melatonin without modifying the milk yield have also been conducted. This lack of effect has been reported mainly with the implantation of ewes specialized in milk production during lactation and in cows that received melatonin orally throughout the dry period. Elhadi et al. (2022) and Cosso et al. (2021) reported that melatonin implants (18 mg) in early lactation (35 d or 60 d after lambing) under short-day conditions did not modify milk yield in Manchega, Lacaune and Sarda ewes. In the same manner, melatonin treatment in late lactation (fourth - seventh month) in spring (long days) did not affect the total milk and the pattern of milk production in Lacaune and Assaf ewes lambed in autumn and winter, resp. (Abecia et al., 2005). In pregnant cows and heifers, the subcutaneous melatonin administration in increasing photoperiod conditions (heifers: 162 mg, cows: 54-216 mg), and in winter or summer conditions (dairy cows: 216 mg), did not affect milk yield (Garcia-Ispierto et al., 2013; Morini et al., 2018). The absence of effects on milk production was also observed with oral administration of melatonin. Thus, when feeding cows with melatonin (22.5 mg as an oral bolus) eight weeks during the latter phases of lactation, they exhibited no effect on the amount of milk produced (Dahl et al., 2000). The lack of effects of melatonin on milk yield was also found by Petitclerc et al. (1998), Lacasse et al. (2014), and Ponchon et al. (2017) when administering melatonin orally in the dry period (25 mg/d; 25 mg in capsule/8 week; 4 mg/100 kg of LW/28 d, resp.).

1.2.2. Milk quality

The dairy industry has established a differential payment structure based on particular commercial and health factors to incentivize dairy farmers to produce high-quality milk. This payment structure involves rewards for high fat and protein content and penalties for elevated somatic cell counts (SCC) and bulk milk total bacterial count (Corrales et al., 2004). Although lactose is not included in this payment guideline because in the past it was considered a low-value component of milk, in recent years, it has gained economic interest at the international level (Costa et al., 2020). Certainly, evaluating the potential effects of melatonin on these milk parameters has been an area of research interest. However, there is still limited information available on the effect of melatonin administration on milk components and the studies conducted have not always provided homogeneous results (Cosso et al., 2021; Elhadi et al., 2022).

1.2.2.1. Milk Fat

Lipids are the most significant components of milk in terms of cost, nutrition, and physical and sensory characteristics that they impart to dairy products (Park et al., 2007). For this reason, milk fat content is an important criterion for assessing milk quality and is one of the main target traits of dairy breeding (Wang et al., 2019). Research has indicated that melatonin is a key modulator of fat synthesis and adipose differentiation (Acuña-Castroviejo et al., 2014; Yang et al., 2017a). A recent analysis of the dairy metabolome revealed that melatonin treatment reduced most metabolites linked to lipid oxidation, implying increased fat accumulation in cows. This fat accumulation might alter the concentration of fat in the milk (Fu et al., 2023). Nevertheless, the regulatory role of melatonin in fat synthesis remains unclear and needs to be further studied (Wang et al., 2019; Abecia et al., 2021).

Most of the information regarding the impact of exogenous melatonin on raising milk fat in dairy cows originates from animals that received repeated oral or subcutaneous treatment during lactation. In lactating cows, rumen bypass melatonin feeding (RBMF) at a dosage of 40 mg/d for 14 and 21 d improved milk fat content. This effect persisted even after the cessation of RBMF, the beneficial effects of this approach on milk quality have lasted, at least, for 3 d to one wk (Yao et al., 2020). However, when using unprotected melatonin at a dose of 5 g/d for 24 d, the positive effects on milk fat were only evident after the third wk (Fu et al., 2023). Auldist et al. (2007) reported that repeating subcutaneous doses of melatonin (108 mg at 19, 23 and 27 wk of lactation) increased milk fat content in spring-calving cows.

Subcutaneous melatonin implantation appears to be a promising approach for enhancing milk fat gain in ewes, offering consistent benefits across different breeds and physiological stages. Increases in milk fat concentration were achieved at the beginning of lactation by implanting dairy ewes in gestation. Meanwhile, in Rasa Aragonesa dams receiving subcutaneous melatonin treatment (18 mg) at lambing showed an increase in milk fat content, particularly near the end of lactation (Abecia et al., 2021). In addition, in Polish Longwool ewes that were implanted in lactation, or by repeated dosage in pregnancy and lactation, exhibit an increase in milk fat concentration (Molik et al., 2011, 2020).

While melatonin use in ewes has been associated with a consistent increase in milk fat concentration, the effects of decreasing milk fat have been linked only in heifers and cows. Heifers exposed to a long day and fed melatonin (25 mg; eight weeks) during the dry period produced less fat in the milk (Lacasse et al., 2014). Moreover, in mid-lactation dairy cows, the administration of subcutaneous injections of melatonin (4.64 or 9.3 mg/cow/day; four consecutive days) decreased milk fat synthesis (Wang et al., 2019; Wu et al., 2021).

1.2.2.2. Milk Protein

Total protein is one of the main quality criteria applied to cows, sheep, and goat milk payments in many countries (Pirisi et al., 2007; Popescu & Angel, 2019) . Variation of total protein content depends on genetics and non-individual factors such as stage of lactation, season, age, and feeding (Raynal-Ljutovac et al., 2008). However, studies demonstrated that exogenous melatonin can affect the synthesis of milk proteins and thus the quality and technological suitability of milk. The effects of melatonin administration reported in ruminants have only caused an increase in milk protein, particularly in dairy cows and sheep.

Milk protein increase has been achieved mainly in melatonin applications (oral or subcutaneous) during lactation of cows and sheep. In mid-lactation, cows (19 and 22 wk of lactation), subcutaneous melatonin implants (108 mg/cow) that were applied on three occasions, and oral melatonin (15 g/d for 24 d) increased milk protein (Auldist et al., 2007; Fu et al., 2023). Additionally, when rumen bypass feeding (RBMF) was given to dairy cows for 7, 14, and 21 days, the protein concentration in the milk increased. Even after the RBMF was stopped, the impact of increased milk protein persisted for a few days, particularly in the groups that received 40 mg/d (Yao et al., 2020). Concerning sheep, Molik et al. (2020) reported that milk protein content increased by 15% in Polish Longwool ewes given melatonin implants (18 mg) during mid-lactation in the spring.

1.2.2.3. Milk Lactose

The main carbohydrate in cow, sheep, and goat milk is lactose. It is synthesized from glucose in the mammary gland. Lactose is of major importance in maintaining osmotic balance between the bloodstream and the alveolar cells of the mammary gland during milk synthesis, and secretion into the alveolar lumen and the duct system of the udder (Larson & Smith, 1974). As lactose is the main compound that determines milk osmolality, its percentage presents a low variability (Costa et al., 2020). Thus, most of the research conducted on ruminants have not reported significant effects on the percentage of lactose in milk following melatonin supplementation. Despite this stability, it has been reported that exogenous melatonin can decrease milk lactose in cows and sheep.

Coherent studies have suggested that melatonin does not affect the lactose content of milk in cows, sheep, and goats. Among these ruminant species, sheep and goats exhibit a more consistent lack of response to melatonin in terms of lactose content. This consistency has been observed by implanting melatonin across various physiological states, dosing regimens and breeds. Bouroutzika et al. (2021) detected that melatonin does not significantly modify lactose in the milk of dual-purpose sheep by repeating melatonin implantations (18 mg/each) every 40 days during gestation. After lambing, single implantation in early (1 d) and mid-lactation (30 or 35 d) of meat and dairy ewes did not cause any modifications in milk lactose content (Abecia et al., 2021;Cosso et al., 2021; Elhadi et al., 2022). This lack of effect was also observed in goats, when melatonin (18 mg) was implanted into Creole dams 49 d before kidding (Avilés et al., 2019); and during lactation by single (18 mg; 3 d) or repeated (2 mg/kg; 50 and 110 d) implantation into Verata and Cashmere dams (Jiménez et al., 2009; Yang et al., 2020). Meanwhile, the absence of effects in dairy cattle was demonstrated only by oral administration of melatonin (4 mg/100 kg LW) around drying off (Ponchon et al., 2017). As previously mentioned, these results underline the stability of lactose as a component of milk composition in these species.

In spite of the low variability of lactose in milk, a decrease in this component was observed when melatonin was administered subcutaneously via implants and orally through rumen- by-pass granules in lactating dairy cows and sheep. The reduction in cows was accomplished by administering six implants (108 mg) three times during lactation, as well as by giving ruminal bypass melatonin (40 mg/d) for 14 and 21 days (Auldist et al., 2007; Yao et al., 2020). Similar results demonstrated that administering a single implant to Polish Longwool ewes at day 57 of lactation reduced the lactose content of the milk (Molik et al., 2011).

An absence of effects of exogenous melatonin on milk components (fat, protein and lactose) has been reported in dairy ewes and goats. In different dairy breeds of sheep (Manchega, Lacaune and Sarda) and goats (Creole and Verata) with various levels of milk production, the use of exogenous melatonin as a subcutaneous implant combined with naturally occurring endogenous melatonin under short-day photoperiod conditions did not affect milk components (Elhadi et al., 2022; Cosso et al., 2021; Avilés et al., 2019; Jiménez et al., 2009).

1.2.2.4. Milk Somatic Cell Counts

Another parameter to evaluate the quality of the milk is the SCC. The SCC in ruminants measures the different cells types present in milk, including immune-related lymphocytes (white blood cells) and mammary epithelial cells (Souza et al., 2012; Yao et al., 2020). Although there are marked differences between dairy ruminants concerning SCC, it is an important indicator of udder health and the raw milk quality (Gonzalo, 2018; Halasa & Kirkeby, 2020). In dairy animals, an increased in SCC, often referred as a subclinical mastitis, may be a sign of inflammation or infection in the udder (Sharma et al., 2011).

Studies have revealed the potential effects of melatonin reducing the inflammatory process in multiple ways. It suppresses the oxidative stress eliminating the highly toxic hydroxyl radical (-OH), peroxynitrite anion (ONOO-), and hypochlorous acid (HOCl) (Reiter et al., 2000). It also reduces the content of TNF-α, TNF-γ, IL-6 and increases the expression of IL-10 and TGF-β (Huang et al., 2019). In addition, according to Xia et al. (2012), melatonin has the ability to increase gene expression of anti-inflammatory enzymes and decrease gene expression of proinflammatory enzymes. Regarding to its capacity against bacterial infections, melatonin supplementation reduced elevated milk SCC induced by Staphylococcus aureus or Escherichia coli infections (Yao et al., 2020; Wu et al., 2021). The antibacterial effect of melatonin might relate to its ability to reduce intracellular substrates, lipid levels, and metal binding which are required for bacteria growth (Tekbas et al., 2008; Srinivasan et al., 2012). Considering these anti-inflammatory and antibacterial properties of melatonin, several studies have been conducted to evaluate its impact on SCC in the main dairy species.

The positive impact of melatonin (subcutaneous and oral) in reducing SCC in cows have been reported in experimental trials. In lactating dairy cows with subclinical mastitis (milk SCC greater than 3×10^5 cells/mL), the administration of subcutaneous melatonin (4.64 mg/cow/day or 9.3 mg/cow/day for 4 consecutive days) led to a decrease in SCC (Yang et al., 2017b; Wu et al., 2021). Meanwhile, Yao et al. (2020) reported that after 14 and 21 days of RBMF, milk SCC significantly reduced in both melatonin treated groups (40 or 80 mg/d). It is likely that even five to seven days after stopping melatonin administration, the residual effects on the decline of SCC will still be there.

In sheep, the beneficial effects of melatonin on mammary health by lowering SCC have been reported with melatonin implantation in lactation and pregnancy. The first observation on the effect of melatonin on the number of SCC in sheep milk has been reported by Cosso et al. (2021). They reported that implanting Sardinian sheep in the second month of lactation reduced SCC. This effect began to be evident a month and a half after implantation and was persistent in the following samplings.

In goats, there is a single study conducted in the Verata breed (dual purpose) that reveals the reduction of SCC in milk following melatonin implantation. Jiménez et al. (2009) observed that the application of melatonin implants in the beginning of lactation decreases SCC at 60 and 90 days in milk (DIM) in goats. Furthermore, they reported changes in the antioxidant activity after melatonin application, revealing significant changes in blood GSHPx activity 100,120 and 150 days after melatonin was given.

1.3. Colostrogenesis

Colostrum is the first substance secreted by the mammary gland before parturition. Its formation is through colostrogenesis, which is a complex process that can be defined as the pre-partum transfer of substances, primarily immunoglobulin G (IgG), from maternal bloodstream into mammary secretions during a specific and limited period (Barrington et al., 2001). Colostrum differs from milk in physic and chemical aspects, including density, color, and composition (Molik et al., 2012; Pecka-Kiełb, et al., 2018). It contains a source of passive immunity of newborns due to the concentration of immunoglobulins, which in cattle, sheep and goats do not get into the embryo's bloodstream (Hernández-Castellano et al., 2015). In addition to Ig for passive immunity, colostrum is also the first source of nutrition, containing proteins, fats, lactose and minerals (Grizard et al., 1995; Hernández-Castellano et al., 2014; Puppel et al., 2019).

Thus, in ruminant species, colostrum quality has a significant role in supporting the passive immune transfer of the newborn, and further determines the normal growth and muscle development effectiveness of rearing offspring (Nowak & Poindron, 2006). The quality of colostrum depends on many factors, such as breed, lactation period, length of lactation, health, and management, but predominantly on their nutrition (Moreno-Rojas et al., 1993; Nowak et al., 2012; Hyrslova et al., 2016; Agenbag et al., 2021). Beyond nutrition, hormonal factors such as melatonin may also influence immune function and colostrum quality in ruminants. Thus, there is emerging evidence suggesting that melatonin may also play a role in immune function and colostrum composition.

Several studies reviewed by Carrillo-Vico et al. (2005), demonstrate that melatonin regulate the immune system (innate and adaptive) and that it directly influences the synthesis of IgG. In ruminants, evaluations of the effects of melatonin on IgG and colostrum composition have focused on the ovine species. As the transfer of prepartum components from the maternal circulation into colostrum occurs during the last third of gestation, some studies have focused in applying melatonin during this period.

1.3.1. Colostrum IgG

On the one hand, one report indicate that IgG is positively impacted by the melatonin implantation (18 mg/implant). The study was in Rasa Aragonesa ewes, reporting that treating with one melatonin implant (18 mg/ewe) at the fourth month of pregnancy increases IgG concentrations (Abecia et al., 2021). However, on the other hand, when melatonin was implanted in pregnant ewes three times during their gestation (20, 60 and 100 d), no effect on colostral IgG concentrations was found (Bouroutzika et al., 2021).

1.3.2. Colostrum components

Apart from IgG, colostrum also provides fat, protein and lactose. In these components, the effects of melatonin administration are less predictable. Ewes that received melatonin implants at 90 d of pregnancy or that received their last melatonin implant as near as 100 d (two doses before) produced a colostrum that had higher fat concentrations (Abecia et al., 2020; Bouroutzika et al., 2021). Meanwhile, through implantation at gestation (20, 60 and 100 d gestation), an increase in protein concentration was reached (Bouroutzika et al., 2021)

1.3.3. Serum IgG in newborns

To achieve successful passive transfer, neonates must consume a sufficient mass of IgG in colostrum, and then successfully absorb a sufficient portion of IgG into their circulation. This, coupled with the beneficial effects of melatonin on colostral IgG concentrations, prompted researchers to evaluate the effects of melatonin on serum concentrations in lambs. However, supplementing pregnant twin-bearing Merino ewes with melatonin implants during the second half of pregnancy does not affect the serum IgG concentrations of lambs (Flinn et al., 2020 b, c). Moreover, repeating three times the application of melatonin throughout the gestation of ewes under heat stress caused a decrease in serum IgG levels of their lambs (Bouroutzika et al., 2021).

1.4. Growth

Animal growth is defined as an increase in tissue mass or an accretion of muscle, fat, and bone over time. In domestic ruminants, the rate of gain usually is calculated as the change in weight during a specified time interval (Owens et al., 1993, 1995). Additionally, the evaluation of body or linear dimensions is used to characterize size and shape, and has been shown to complement body weight as a measure of productivity (Afolayan et al., 2006). These performance parameters are influenced by genetic factors, as well as intrinsic factors like gender, age, and physiological status, and extrinsic factors such as environment, climate, nutrition, management, and maternal effects (Arango & Van Vleck, 2002). Within the extrinsic factors, responses to exogenous melatonin administration may alter the growth and body measurements in ruminants. Due to its precise determination and relevance to livestock farming, the majority of melatonin research has focused on the potential effects on body weight and weight gain. However, as in milk yield, research results on their impact on fetal development and postnatal growth in ruminants remain diverse and sometimes contradictory.

Melatonin has been shown to potentially enhance growth in small ruminants by the capacity to regulate hormone activity, oxidative stress, and ruminal function. The administration of exogenous melatonin caused an increase in hormones associated with muscle growth and body structural formations, including growth hormone, estradiol and testosterone plasma concentration (Molik et al., 2010; Ma et al., 2022). Additionally, as demonstrated by Bantounou et al. (2022), the antioxidative properties of melatonin may prevent muscle degradation under oxidative stress. Possibly, the mechanism used to remove aged and damaged cells during development and protect cells may involve the enrichment of genes in expressions of the apoptosis pathway, including CytC, PARP1, and IL1-β genes (Ma et al., 2022). Furthermore, Ma et al. (2022) observed that melatonin affected the rumen microbiota of lambs, potentially altering the proportion of different microflora and consequently impacting nutrient absorption.

1.4.1 Liveweight

In small ruminants, increases in body weight and growth of lambs and kids have been achieved through the subcutaneous administration of melatonin to their dams or directly to the offspring. Apparently, the effects of melatonin treatment on fetal and postnatal growth differed according to litter size and sex of the offspring. As fetal growth primarily occurs during the last third of gestation in ewes and goats, the dams were implanted at the beginning of this period to assess the effects on fetal and birth weights of the offspring. The observed increase in fetal and birth weights may be attributed to factors such as increased umbilical arterial blood flow (Lemley et al., 2012), and improved fetal oxygenation status (Sales et al., 2019). In single and twin Corriedale fetuses, the melatonin treatment (2 implants; 36 mg) of ewes at late pregnancy (100 d) increased their weight (Sales et al., 2017). Research conducted under comparable experimental conditions revealed that the highest fetal weight was found in twin male fetuses (Sales et al., 2019). Similarly, implanted Beetal goats 40 d before kidding increase in birth weight of triplets or quadruplets kids (Afzaal et al., 2023).

In pre-weaning offspring, the increased daily weight gain might have been mediated by the effects of melatonin on milk production and composition of their mothers. Melatonin administration (2 implants, 36 mg) to Creole Mexican does during the dry period resulted in an increase of milk production in the subsequent lactation. This effect improved the mean daily weight gain of their suckling kids, observed only in male (Avilés et al., 2019). Meanwhile, in Rasa Aragonesa ewes implanted (1 implant, 18 mg) at lambing increased the milk fat content (Abecia et al., 2021). This increase in total milk solids could have contributed to the higher growth rates of male lambs reared by implanted dams at parturition (Abecia et al., 2021). The improvement of growth performance was also evidenced in post-weaning male lambs by Ma et al. (2022), who showed that melatonin enhanced growth performance by the increased cross-sectional area of muscle fiber and adipose tissue cells. These structural and growth responses were achieved through melatonin implantation at weaning (2- months of age), with a dosage of 4.5 mg/kg.

Contrary to the above, the effects of melatonin on cardiovascular or insulin-like growth factor (IGF)-related pathways under chronic hypoxia gestation has been associated with lower birthweight and intrauterine growth restriction (González-Candia et al., 2016). According to Kim et al. (2002), hypotension is one of the possible effects of maternal melatonin supplementation, which can lead to uteroplacental reduced perfusion, and ultimately restrict the supply of oxygen and nutrients to the fetus. Further, it has been shown that hypoxic lambs show a decreased messenger RNA expression for

IGF that is associated with a marked decrease in fetal growth (Gentili et al., 2009). González-Candia et al. (2016), and Candia et al. (2022), found evidence supporting this hypothesis, showing that sheep supplemented with oral melatonin (10 mg/kg/day) during the last third of gestation at high altitudes (3,600 m a.s.l.), decreased birth weight of their lambs.

On the other hand, studies have suggested that melatonin has no adverse effects on fetal, birth, and prepubertal body weight. In small ruminants, the lack of effect of melatonin administration on the body weight of lambs and kids has not shown a consistent pattern, involving different stages and methods of administration. Thus, when melatonin was administered to mothers in pregnant ewes (s.c: Abecia et al., 2020; Bouroutzika et al., 2020; Flinn et al., 2020b; Bouroutzika et al., 2021) (oral: Lemley et al., 2012; Seron-Ferre et al., 2015; Flinn et al., 2020c) and lactational ewes (s.c: Elhadi et al., 2022), and goats (s.c: Yang et al., 2020), no effects were found on the weights of their offspring. Similarly, when melatonin was administered to lambs at birth (s.c: Abecia et al., 2021); kids in early postnatal (s.c: Yang et al., 2019; Diao et al., 2023); ewe lambs (s.c: Kennaway & Gilmore, 1984; Peclaris et al., 1997) (oral: Lemley et al., 2012), weight of the offspring remained unchanged. In cattle, the effects on live weight were evaluated with oral melatonin administration in growing heifers. In prepubertal Holstein Friesian (154 kg) and puberty Angus (318 kg) heifers, oral melatonin added to the concentrate (4 mg/100 kg LW/d) for two months did not affect body weight gain (Zinn et al., 1988; Sánchez-Barceló et al., 1991). The lack of impact on live weight was similarly observed in pregnant heifers, which received oral melatonin capsules (25 mg/d) two months before calving (Lacasse et al., 2014).

1.4.2. Morphometric measurements

Body measurements have been used to describe changes in size and shape and give information on differences in body proportions as well as short-term fluctuations that could be caused by weight loss and gut fill (Arthur & Ahunu, 1989). Thus, to complement weight assessments, some experiments have evaluated the effects of maternal melatonin administration during pregnancy and at lambing on biometric measurements. In models of intrauterine growth restriction induced by nutrient restriction, oral maternal melatonin supplementation (5 mg/d, from day 50 to 130 of pregnancy) increased abdominal girth, biparietal distance and kidney size (Lemley et al., 2012). The application of two melatonin implants (36 mg) on the 100th day of pregnancy caused an increased fetal thorax diameter (Sales et al., 2017). However, in gestations affected by chronic hypoxia at high altitudes (3,600 m a.s.l.), melatonin caused a decreased biparietal diameter, crown-rump length, and abdominal diameter (González-Candia et al., 2016).

1.4.3. Body condition score

The measurement of body condition score (BCS) in ruminants is considered as an indicator of fat reserves and it reflects the metabolic status of the animal (Russel et al., 1969; Fernandes et al., 2016). The BCS of an animal can be assessed in cattle visually (Moloney & McGee, 2017), or in ewes and goats by palpation of the lumbar and sternal region, respectively (Russel et al., 1969; Hervieu et al., 1991). Due to its usefulness in monitoring the different physiological states of livestock animals, this measurement has been included in the experiments performed with melatonin. A few studies have shown that the treatment with melatonin of the ewes during pregnancy or lactation did not have a significant effect on their BCS. The absence of effects could be attributed to the timing and duration of melatonin administration may not have been sufficient to elicit measurable changes in BCS. Maternal daily oral supplementation with melatonin to ewe lambs from day 50 of gestation did not affect BCS of the dams (Lemley et al., 2012). Sales et al. (2017, 2019) and Afzaal et al. (2023) did not observe differences in the BCS of the ewes and goats treated with melatonin implants (18 mg or 36 mg/ewe; 18 mg/goat) from day 100 of pregnancy. Similar results were described when administering a melatonin implant (18 mg/ewe) to ewes with 35 d of lactation (Elhadi et al., 2022).

1.5. Thermoregulation

The neonatal period is crucial for ruminants due to a high mortality rate, often attributable to hypothermia resulting from exposure or starvation (Eales & Small, 1984; Rowan, 1992). Thus, the development of thermoregulatory mechanisms that allow animals to maintain homeothermy in many environments is vital in determining neonatal survival. After birth, these mechanisms that regulate body temperature are activated by the presence of relatively large amounts of functional brown adipose tissue (BAT) that are capable of rapidly generating heat (Clarke et al., 1997; Basse et al., 2015). Several factors can influence the development and functionality of BAT, and body temperature in newborn ruminants, most of them associated with maternal husbandry practices during pregnancy. Therefore, studies have been carried out to evaluate the effect of melatonin on aspects related to the mechanism of neonatal thermogenesis through melatonin supplementation (oral or subcutaneous) of ewes during gestation, under various climatic conditions.

Maternal melatonin during gestation may play an important role in BAT development and newborn thermoregulation. Oral melatonin (12 mg/d) to ewes during late gestation enhances BAT thermogenesis in lambs. This was reflected in an increase of body temperature (central, perirenal and skin) under thermal neutrality conditions

(24°C), and under exposure to 4°C (Seron-Ferre et al., 2015). Similarly, Sales et al. (2017) reported that melatonin implants (18 mg) administered at day 100 of gestation increased BAT in Corriedale ewes, and that this increase in BAT varies according to litter size. Single fetuses of ewes that received two melatonin implants had 18% more BAT than did single fetuses whose mothers received one implant, or did not receive an implant. In ewes that had twin fetuses, BAT was 35% higher in fetuses from ewes that had received a single melatonin implant than it was in those from non-implanted ewes.

At present, in addition to the traditional tools to measure body temperature, infrared thermography (IRT) is a non-invasive method that allows measuring and monitoring the thermoregulation responses, such as body temperature patterns of heat generation and loss in newborn lambs (McCoard et al., 2014). In newborn lambs, the evaluation of lumbar region by IRT may also provide useful data, which indicates that measurements at regions such as ‘Hips’ and ‘Mid loin’ may be more sensitive to changes in thermoregulatory capacity (Labeur et al., 2017). Additionally, supporting the increase in BAT, studies conducted by Freitas-de-Melo et al. (2021) found that newborn lambs from ewes implanted with melatonin (18 mg) during gestation (at 100 and 120 d) exhibited higher hip and mid-loin body surface temperatures measured by thermography.

Birth rectal temperature is a strong indicator of the thermoregulation process in newborn ruminants. However, supplementing pregnant singleton and twin-bearing Merino ewes with melatonin implants (18 mg implant at day 70-90 of pregnancy; or at day 80 and 125) or capsules (2 mg capsule fed daily from day 80 of pregnancy until lambing) under extensive conditions does not affect birth temperature (Flinn et al., 2020 b, c). The absence of a melatonin effect was also observed when ewes were kept indoor and implanted at 100 or 120 d of gestation (Freitas-de-Melo et al., 2021; Abecia et al., 2020).

These results show that exogenous melatonin, administered orally or subcutaneously at different doses during various physiological stages, can impact milk production, milk quality, colostrum quality, growth, and thermoregulation in cows, sheep, and goats. However, the effects remain diverse and sometimes contradictory.

Table 1. Experimental studies of productive responses to exogenous melatonin administration in bovine models

Publication Cattle	Methods	Melatonin dose/timing	Effect of melatonin in animal production parameters
Zinn et al., 1988	Angus heifers exposed to 16 h light (L):8 h dark (D). **Exp 1.** 16 post puberal heifers were blocked by initial body weight (318 kg). **Exp 2.** 24 heifers were blocked by initial body weight (348 kg). LW: every two wks. Slaughtered: 59 d of treatment.	**Exp 1.** Oral melatonin (4 mg/100 kg body weight) daily for 58 d. Treatments: Four killed initially. Six received vehicle (95% ethanol). Six were melatonin fed. **Exp 2.** Oral melatonin (4 mg/100 kg body weight) daily for 63 d. Treatments: Eight killed initially. Eight received vehicle (95% ethanol). Six were melatonin fed.	**Exp 1.** - No difference in LW. -Increased fat (%) in rib, longissimus muscle, carcass fat. - Reduced protein (%) and carcass protein accretion. **Exp 2.** - No difference in LW gain or carcass measures.
Sanchéz-Barcelo et al., 1991	16 prepubertal Holstein heifers (154 ± 6 kg LW) exposed to long days (16 L: 8 D) were divided into two groups. LW: 2-week intervals. Blood sample 1 h interval: Day 67-68. Slaughtered: Day 70 -72.	Oral Melatonin (4 mg/100 kg body weight/daily) for 70 d. Treatments: Melatonin: melatonin-fed. Control: vehicle fed controls.	- No changes in LW. - Decreased Mammary parenchymal DNA content and concentration -Decreased serum prolactin.
Petitclerc et al., 1998	Cows were exposed to different photoperiod conditions during the dry period and then exposed to long day photoperiod (LDPP) after calving.	Oral melatonin (25 mg/d) to cows during the dry period. Treatments: SDPP: Short day photoperiod. LDPP: Long day photoperiod.	- No changed in milk yield - Decreased prolactin circulating concentration

		LDPP+Melatonin: Long day photoperiod + melatonin.	
Dahl et al., 2000	26 cows in late lactation (average 217 ± 27 DIM) divided in two groups. Cows were exposed to 18 h of L:6 h of D.	Cows received a daily oral bolus of 22.5 mg melatonin in the middle of the photo phase for 8 weeks. Treatments: MEL: cows received melatonin. CON: cows received a vehicle bolus.	- Suppressed prolactin secretion. - Not change the milk yield and composition.
Auldist et al., 2007	12 sets of identical twin spring lactating Friesian cows divided in two groups at 19 wk of lactation. Melatonin, IGF-1 and Prolactin: Daylight blood sampling in two occasions one week before and in seven occasions after the first implantation. Milk sampling: 6, 8, 11 and 12 weeks. after the first melatonin implantation.	Cows received 6 subcutaneous melatonin implants (108 mg/cow) applied in three occasions (19, 23 and 27 week of lactation). Treatments: Treatment group: implanted. Control group: non-implanted.	- Reduced milk yield. - Decreased lactose concentration and plasma prolactin. - Increased milk fat, protein and casein content.
Garcia-Ispierto et al., 2013	**Exp 1.** 20 multiparous lactating pregnant dairy cows selected in increasing photoperiod length. Milk production: daily. **Exp. 2.** 25 heifers and 114 pregnant dairy cows and heifers treated during the warm season. Postpartum checks: daily. Blood samples: d 120, 127, 134, 141, 148, 155, 162 and 169 of gestation. Milk production: daily. Milk for SCC: two milk samples before the dry-off period and three samples after parturition.	**Exp 1.** Subcutaneous implants on d 120 of gestation (18 mg/implant). Treatments: Untreated, three, six, nine and 12 implants. **Exp. 2.** Subcutaneous implant d 220 of gestation (18 mg/implant). Treatments: Control (C): Untreated. Melatonin (M): nine implants (heifers) or 12 (cows).	**Exp.1.** - No difference in milk production. **Exp. 2.** - No significant effects on placenta retention or metritis. - Not difference in milk production and SCC postpartum. - Suppressed prepartum prolactin concentrations.

Lacasse et al., 2014	Prepartum 29 cows and 33 heifers. Animals were allocated into three groups 8 weeks before calving. Factorial 2x3 (main effects were parity and photoperiod-melatonin treatments). Milk yield daily and milk samples: weekly. LW: weekly.	25 mg of oral melatonin by gelatin capsule (8-week prepartum-to calving). Treatments: 8L:16D: 8 h L + 16 h D. 16L:8D 16 h L + 8 h D. 16L:8D + melatonin feeding (16L:8D-melatonin.	- Reduced prolactin in cows and heifers. - Heifers exposed to a long day and fed melatonin produced less fat and tended to produce less ECM. - No effect on milk production during late lactation. - IGF-I tended to be greater in the animals fed melatonin. - No affect body weight.
Ponchon et al., 2017	Ten cows/treatment according to their milk production, parity, and SCC. Before the treatments, cows were exposed to LDPP (16 h of L: 8 h of D). Milk production: during the last 4 wk. before drying off. Milk composition: before drying-off on d −26 and −19 (before treatment) and −12, −5, and −1 d (after the start of treatments). Series of blood samples: On d −15, just before the beginning of the treatments, and on d −1, just before drying-off.	Oral supplementation of 4 mg/100 kg of BW. Each treatment started 14 d before drying-off and lasted 14 d after drying-off. Treatments: **LDPP:**16 h of L: 8 h ofD. **SDPP**:12 h of D/1 h of L/4 h of D/7 h of L). **LDPP+MEL:** LDPP supplemented with melatonin f eeding.	- LDPP+MEL cows compared with LDPP cows: - No difference in blood prolactin concentrations and milk yield and composition.
Yang et al., 2017 b	120 cows were divided into four groups based on milk SCC. Milk samples: daily, and were taken for three d before melatonin treatment, and then sampled for 18 d after.	**Exp. 1.** Subcutaneous injections of melatonin (4.64 mg/cow/d) for 4 consecutive days. In each group, half of the cows were treated with melatonin (MT) or not (NOT-MT) according to their SCC: - $<10 \times 10^4$ cells/mL - 1×10^5 to 3×10^5 cells/mL - 3×10^5 to 5×10^5 cells/mL) - 5×10^5 to 1×10^6 cells/mL)	- Reduced milk SCC.

Morini et al., 2018	120 indoor dairy cows under intensive conditions were randomly divided into four groups during winter or summer.	12 melatonin implants (18 – mg/each) administered 60 d prior calving. Treatments: Summer: Untreated. Summer: Melatonin. Winter: Untreated. Winter: Melatonin.	- Not changed on milk yield in winter or summer.
Wang et al., 2019	Ten mid-lactating Holstein dairy cows with similar parity, lactating time, milk production, milk fat, and milk protein were randomly divided into two groups. Individual milk samples: collected three times per d from d 0 to d 8.	Subcutaneous injections of 4.64 mg melatonin (Sigma) for four consecutive days at 8:00 am. Treatments: Melatonin: (Melatonin). Control: (NC) cows received the vehicle (absolute ethyl alcohol) only.	- Increase prolactin serum concentration. - Milk yield was slightly increased. - Milk fat concentration decreased. - No difference in the concentration of milk lactose and milk protein.
Yao et al., 2020	35 Holstein cows with high milk SCC (0.4~0.7 million cells/ml) semi-enclosed with natural L were divided in groups with two doses of melatonin and three different days of feeding. Milk: every two d. After the end of the melatonin treatment, milk samples were collected daily for one wk.	Rumen bypass melatonin granules was feeding in granules. Treatments: - 40 mg/cow/d (feeding 7, 14 or 21 d). - 80 mg/cow/d (feeding 7, 14 or 21 d). - Control: untreated.	- Decreased SCC. - Increased milk fat and protein. - Decreased milk lactose.

Wu et al., 2021	Holstein cows with SCC 0.3–1 million/mL were selected for the studies based on the monthly dairy herd improvement. Milk yield and composition: 15 days before and after melatonin injection.	Subcutaneous injections of 9.3 mg/cow/d for 4 consecutive days at 10:00 am in different lactation period (early, middle and late) season (spring, summer, autumn, winter) and age (2-7). Treatments: MT: Melatonin treated. Control: Untreated.	- Decreased milk fat and SCC. - Increased milk protein and lactose.
Fu et al., 2023	Twenty Holstein cows in 152 ± 12 d of lactation were divided into two groups under semi-enclosed conditions. Milk collected: in the morning on d 0, 7, 14, and 21.	Oral 15 g/d for each cow (10^{-3} mol/L × rumen volume (67 L) × melatonin molarmass (232 g/moL) ≈ 15 g oral powder melatonin for 24 d. Treatments: Control: No melatonin. MT: Oral melatonin.	-Increased fat at d 21. -Increased protein at d 7, 14 and 21. -No difference in lactose and urea milk concentration.

Table 2. Experimental studies of productive responses to exogenous melatonin administration in ovine models

Publication Sheep	Methods	Melatonin dose/timing	Effect of melatonin in animal production parameters
Kennaway and Gilmore 1984	Pinealectomized and unoperated ewes were exposed to constant light before and after lambing. Ewe lambs born to pinealectomized ewes were divided into two groups (Implanted or non-implanted). Ewe lambs born to unoperated ewes were kept in the same group. Lambs were weaned at 16 weeks. LW: every 8 wks for 72 wks. Blood: weekly for 69 wks.	Subcutaneous implants with melatonin (crystalline) sachets prepared from Silastic medical-grade sheeting. Implants release 100-150 ug melatonin per d. Ewes lambs were implanted at 3-4 weeks and 13-14 weeks. Ewes lamb treatments: Ce: Intact dam and empty sachet. Pe: Pinealectomized dam and empty sachet. Pm: Pinealectomized dam and melatonin sachet.	- No change in LW and prolactin.
Plecaris et al., 1997	80 Boutziko female lambs from single lambing assigned at weaning (63.2±O.4 d old, January 5) to 4 groups. Experiment end (160 d old, April 12). Low plane nutrition: 70 to 75% of the ad libitum. High plane nutrition: ad libitum. Lamb LW: biweekly. Growth hormone (GH): 3 d blood samples one week before the end of the experiment. Mammary gland was taken from seven slaughtered lambs/group.	Subcutaneous melatonin implants (18 mg) on January 10 and March 1 to ewes' lambs. Treatments: HC: High plane nutrition non implanted. HM: High plane nutrition implanted. LC: Low plane nutrition non-implanted. LM: Low plane nutrition implanted.	No effect of melatonin treatment on: - LW. - GH. - Mammary gland.
Abecia et al., 2005	**Exp 1.** 188 lactating Lacaune ewes lambed between September and November (one lambing/year). Artificial rearing. **Exp 2.** 124 lactating Assad ewes lambed between November and January (three lambing/two years). Weaned at 20 d. Milk records: monthly.	Subcutaneous melatonin implant (18 mg of melatonin) to lactating ewes On march (Exp 1) and February (Exp 2) ewes divided in two groups with similar milk production. Treatments: M: Implanted group. C: Non implanted group.	Exp 1 and 2: - No changes in milk production.
Molik et al 2011	60 Polish Longwool ewes mated on September. Lambs were reared with mothers for 56 days. On day 57 (LDPP) the ewes were milked twice daily and divided into three group. Milk yield: every 10 days.	The ewes were implanted s.c with melatonin (18 mg of melatonin/implant) at d 57 of lactation. Treatments: Group I: natural day length.	- Increased milk fat concentration. - Decreased lactose level after only 30 d of lactation. - Decreased the content of monounsaturated fatty acid (MUFA),

	Milk composition: every 28 d. Fatty acid content was determined by gas chromatography.	Group II: natural day length and implanted subcutaneously with melatonin. Group III: exposed to an artificially short photoperiod.	polyunsaturated fatty acids (PUFA) and conjugated linoleic acid (CLA).
Lemley et al., 2012	31 singleton Western White Face ewe lambs were allocated into four groups on d 50 of gestation. Ewes LW, BCS, back fat thickness and longissimus muscle area : d 48, 88, and 124 of gestation. Fetal growth parameters by ultrasound: 50, 60, 70, 80, 90, 100, and 110 of gestation. Fetal LW, crown rump length, biparietal distance, and abdominal girth: d 130 of gestation.	5 mg of oral powder melatonin dissolved in 95% ethanol orally from d 50 to 130 of pregnancy. Treatments: - Adequate diet (100% nutrients requirements). CON-ADQ: no melatonin. MEL-ADQ: melatonin. - Restricted diet (60% nutrients requirements). CON-RES: no melatonin. MEL-RES: melatonin.	- No difference in LW, BCS, loin muscle area, and back fat thickness of the ewes. - No difference in fetal growth parameters. - Increased parietal diameter. - MEL-ADQ increased abdominal girth.
Molik et al., 2013	60 Polish Longwool ewes were divided on three equal groups (n=20) postweaning (57th d of lactation). Rearing period: lambs were weaned at 56 d of lactation. Milk production was estimated based on the lamb's weight gain from 2 to 28 d of age. Milking period: from d 57 of lactation to the dry period (milk yield was less than 50 ml/ewe/d). Milk yield: at 10-d intervals. Blood sampling: every 28 d for prolactin and growth hormone.	Melatonin implants (18 mg of melatonin) were inserted into each ewe twice, at 90- d intervals. Treatments: LDC: natural long-day control group. LDM: natural long-day melatonin-treated group. ASD: artificial short-day with 16 h of D and 8 h of L.	In LDM compared with the LDC group: - Shorter length of lactation and milking days. - Milking period: decreased milk yield, prolactin and growth hormone secretion.
Seron-Ferre et al., 2015	18 newborns lambs aged 5–6. The lambs were allocated in three groups according to the treatment their mothers received during pregnancy. Lamb LW: Birth and 5 d old. Lambs were exposed for 1 h at 24°C (basal period), followed by 1 h at 4°C (cold period) and 1 h at 24°C (recovery period). In each period: central, skin, and perirrenal fat temperature were evaluated.	Ewes received oral daily 12 mg during from 63% gestational until lambing. Treatments: LD ewes: kept under photoperiod 12h L:12h D. LL ewes: kept under continuous L from 60% gestation. LL + Mel ewes: were kept in continuous L an received an oral melatonin.	- No difference in LW. - Increased BAT. - Increased central, perirenal and skin temperature.
González-Candia et al., 2016	12 singleton ewes maintained under high altitude conditions were allocated into two groups. Lambs LW, biparietal diameter, abdominal diameter, and crown-rump length: birth.	10 mg/kg/d of melatonin in 5 mL 1.4% ethanol orally from d 100 of gestation to lambing. Treatments: MM: melatonin-treated. MC: vehicle- treated (control).	- Reduced neonatal LW and biometry.

Sales et al., 2017	24 pregnant singleton and 24 twin-bearing Corriedale ewes were allocated into three groups at 100 d of gestation. Fetal perirenal fat BAT (weight) and fetal biometrics: at 140 d of gestation.	One (18 mg) or two (36 mg) melatonin implanted s.c at 100 d of gestation. Treatments: Single bearing: SM0: non implanted. SM1: one implant. SM2: two implants. Twin bearing: TM0: non implanted. TM1: one implant. TM2: Two implants.	- Increased BAT - SM2 and TM2 increased fetal weight and thorax diameter.
Misztal et al., 2018	Thirty-six Polish Longwool sheep (rearing twins). Mating September and January. After weaning (from the 57th d of lactation), all ewes were milked twice a day. Milk production: every 10 d. Mammary gland biopsy: 3–4 d before lambing.	Subcutaneous melatonin implant (18 mg of melatonin) in third month of pregnancy and the second implant repeated after 90 d. Treatments: SD treatment: Lambed in January. LD treatment: Lambed in June. LDM treatment: lambed in June and treated with melatonin implanted.	- Decreased milk yield. - Increases number of milking days. -Sheep mammary gland is qualitatively at the same level of preparation.
Abd-Allah and Daghash 2019	34 non-pregnant Ossimi ewes were divided into four groups after weaning (autumn). The 60-d experimental treatment period starts at the mating season. Ewes LW: before mating and after parturition. Lambs LW: at birth and at weaning (90 d).	18 mg/h Melatonin throughout the mating season for 60 d. Treatments: NL: natural daylight. AR:16-hour artificial lighting. NL+MEL: natural daylight + 18 mg/h melatonin. AL+MEL: 16-hour artificial lighting + 18 mg/h melatonin.	- Ewes: higher body weights changing (positive) in NL+ME and AL+MEL. - Lambs: tended to increase weaning, net weight, and average daily gain.
Sales et al., 2019	24 pregnant singleton and 24 twin-bearing Corriedale ewes were allocated into three groups at 100 d of gestation. LW and BCS of ewes: 140 d of gestation. LW of lambs: 140 d of gestation.	One (18 mg) or two (36 mg) melatonin implanted s.c at 100 d of gestation. Treatments: Single bearing: S-0MEL: non implanted. S-18 mg MEL: one implant. S-36 mg MEL: two implants Twin bearing: T-0MEL: non implanted. T-18 mg MEL: one implant. T-36 mg MEL: two implants.	- No difference in LW and BCS of the ewes. - T-18 mg and T-36 mg increased the male fetus weight.
Abecia et al., 2020	60 pregnant Rasa Aragonesa mated on June were allocated into three groups.	Ewes were implanted s.c with melatonin (18 mg). Treatments:	- No difference in LW at birth or rectal temperature of the lambs. - 4M increased IgG in colostrum

	LW of lambs, rectal temperature and colostrum quality (IgG, %Fat, %Protein): immediately after lambing.	Control: non-implanted. 3M: melatonin implant at the third month of pregnancy. 4M: melatonin implant at the fourth month of pregnancy.	- 3M increased % fat in colostrum. - No difference in % protein in colostrum.
Bouroutzika et al., 2020	68 Karagouniko ewes were allocated into two groups 16 d before mating. All pregnant ewes were exposed to heat stress (first 100 d of pregnancy). Lamb LW: birth. Milk: 5, 10 and 40 d.	Ewes were implanted s.c with melatonin (18 mg): before mating and during pregnancy every 40 d (in total 4 melatonin implants; 72 mg). Treatments: M: implanted. C: non-implanted.	- No difference in lambs LW. - Increased milk yield.
Flinn et al., 2020b	164 twin-bearing Merino ewes were allocated into three groups at gestational d 70-90. Lambs LW: Birth, marking (30 d) and weaning (71 d). Rectal temperature: Birth. Lambs serum IgG: 24 h of life.	The ewes were implanted s.c with melatonin (18 mg of melatonin/implant) at gestational d 70-90. Treatments: CTL: non-implanted control group. M1: One melatonin implant. M2: Two melatonin implants.	- No difference in birth, marking and weaning liveweight. - No difference in serum IgG and birth rectal temperature.
Flinn et al., 2020c	181 pregnant singleton and twin-bearing Merino ewes lambing in autumn and spring. Ewes were allocated into three groups after pregnancy scan (65 d after removing CIDR). Lambs LW: 4, 24 and 72 h, 7 d, marking (49 d) and weaning (129 d). Lambs serum IgG and rectal temperature: 4 and 24 h of life.	Two lambing seasons Treatments: CTL: control non-melatonin group. Mel-FED: oral melatonin via 2-mg capsule fed daily from gestation d 80 until lambing. Mel-IMP: subcutaneous melatonin via 18-mg implant at gestation d 80 and 125.	- No difference in LW. - No difference in serum IgG and rectal temperature.
Molik et al., 2020	Sixty Polish Longwool ewes (rearing twins). Mating: September and January. Rearing period: 0-56 d of lactation. Milk production estimated based on weight gain of the lambs. Milking period: From d 57 to dry period (milk yield < 50 mL of milk/d). Milk yield: every 10 d and milk quality. F, P, L, Ca, P and milk fatty acids: every 28 d.	Subcutaneous melatonin implanted (18 mg) in third month of pregnancy and the second implant repeated after 90 d. Treatments: G1: lambing in February. G2: lambing in June. G3: lambing in June + one melatonin implant .	- Decreased milk yield in rearing and milking period. - Higher content of dry matter, protein, and fat. - Increased content of SFAs. - Reduced MUFA and PUFA and CLAS.
Abecia et al., 2021	**Exp. 1:** 53 Rasa Aragonesa lambs borned on November reared by their dams. **Exp. 2:** 55 Rasa Aragonesa lambs borned on October reared by their dams.	Subcutaneous melatonin (18 mg) in the base of the ear at 24 h after lambing (dam or lamb). Treatments: **Exp 1**:	**Exp. 1:** **-** No differences in LW and ADG. **Exp. 2:**

	Milk samples: 15 d, 30 d, and 45 d post-lambing. **Exp. 3:** 16 twin lambs borned on October reared by artificial lactation. Lamb weight: birth and weekly until weaning (Exp. 1=7 week; Exp 2= 6 week; Exp 3=5 week).	m: implanted lambs. c: control group. **Exp 2:** cC: control group. cM: implanted dams. mC: implanted lambs. mM: implanted dams and lambs. **Exp 3:** m: implanted lambs. c: control group.	- Lambs: Increased the LW and ADG of lambs reared by melatonin-implanted ewes, especially in males. - Ewes: Increased milk solids and fat content. - No difference in milk protein and lactose. **Exp. 3:** - No differences in LW and ADG.
Bouroutzika et al., 2021	31 Karagkouniko ewes were allocated into two groups 16 d before mating. All pregnant ewes were exposed to heat stress (first 100 d of pregnancy). Lamb LW: birth, 15 and 40 (weaning) d. Lamb blood: at birth, 24 and 48 h after lambing. Colostrum: at lambing, 24 and 48 h after lambing. Milk: 5, 10 and 40 d.	Ewes were implanted s.c with melatonin (18 mg): before mating and during pregancy every 40 d (in total 4 melatonin implants; 72 mg). Treatments: MEL: implanted. CON: non-implanted.	- No difference in lambs LW. - Decreased plasma IgG in lambs. - Increased protein content in colostrum. - No difference in colostrum IgG. - Increased fat content in colostrum (24h after lambing).
Cosso et al., 2021	100 lactating Sarda Dairy sheep lambing between December and January. On March 1, were allocated to two groups. Milk yield and composition, and Interleukin 2 and 6: every 15 d.	One subcutaneous implant (18 mg) of melatonin to dairy ewes on March 1 (~two month of lactation). Treatments: M: Melatonin implanted. C: Non-implanted.	- No effect in milk yield, fat, protein and lactose concentration. - Reduced SCC 3rd, 4rd and 5th samplings. - No changes in cytokines levels.
Freitas de Melo et al., 2021	93 single-bearing ewes were allocated into two groups at d 100 of gestation. LW, rectal and surface temperature: Birth. Ewe–lamb behaviours: 6–17 h after lambing.	Ewes were implanted s.c with melatonin (18 mg) at d 100 of gestation. Mel: implanted Con: non implanted	- No difference in birth LW and rectal temperature or ewe–lamb behaviours - Increased hip minimum surface temperature
Candia et al., 2022	10 singleton ewes high-altitude pregnant were allocated into two groups. Lambs LW and biparietal diameter: 2 and 11 d.	10 mg/kg/d of melatonin in 5 mL 1.4% ethanol orally from d 100 of gestation to lambing (100–150 d). Treatments: MM: melatonin-treated. MC: vehicle- treated (control).	-Reduced LW at 2 and 11 d. -Reduced biparietal diameter at d 2.
Elhadi et al., 2022	72 dairy ewes of 2 breeds (Manchega; n=36 and Lacaune; n=36) lambed in autumn (short-day photoperiod). After the weaning of the lambs (28 d), the ewes were distributed in 12 balanced groups of 6. Milk yield: daily. Milk composition: 32, 50, 65, 80, and 110 DIM. Body weight and BCS: 28, 50, 65, and 80 DIM.	One melatonin implant (18 mg/ewe) at 35 d of lactation (SDPP). Treatments: MN CO: non-implanted Manchega. MN MEL: implanted Manchega. LA CO: non-implanted Lacaune. LA MEL: implanted Lacaune.	- No changes in milk yield and composition - Decreased prolactin in MN ewes. - No effects on plasma IGF-I, BW and BCS.

Ma et al., 2022	120 healthy male lambs were allocated into three groups at 2 month of age (19 kg). Body height, oblique length, chest circumference, chest width, chest depth, anterior duct and posterior duct: 2, 3 and 4th month of age. *Longissimus dorsi* muscle and back adipose tissue, and intestinal microbiome sequencing: 4th month.	Lambs were implanted s.c with melatonin in the neck at 2 months of age. Treatments: Control: 0 mg of melatonin. 3 mg/kg: 3mg/kg of melatonin. 4.5 mg/kg: 4.5 mg/kg of melatonin.	- 4.5 mg/kg group: Increased body weight, body length, chest circumference, chest width and chest depth. - Increased the size of the muscle and fat cells. - Modified rumen microbiota.

Table 3. Experimental studies of productive responses to exogenous melatonin administration in goat models

Publication Goats	Methods	Melatonin dose/timing	Effect of melatonin in animal production parameters
Jiménez et al., 2009	60 Verata breed (dual purpose) goat were allocated into two groups at the third d after kidding. Kids were separated from their mothers 72 h after birth. Blood samples: 3 d after kidding and then 4 times more at monthly intervals. Milk samples: 3, 30, 60, 90 and 120 d in milk.	Goats were implanted s.c with melatonin (18 mg/goat) at the third d after kidding. Treatments: Control: non implanted. Implanted: melatonin implanted.	- Decreased SCC in milk. - No difference in milk composition.
Avilés et al., 2019	25 Creole does were kept under naturally long day conditions. At dry period, does were divided into 2 groups. Suckling period: 21 d postpartum, milk yield was estimated by suckle-weigh-suckle method. Milking period: 22-91 d postpartum. Milk yield: estimated by hand milking each week. Milk composition: every milk yield evaluation. Plasma T3: 2, 6, 10, and 14 wk of lactation. LW of kids: at birth, 1, 2, and 3 wk of life.	Two subcutaneous melatonin implants (36 mg of melatonin/doe) at the dry period (49 d before kidding). Implants were removed at kidding. Treatments: CONT: non-implanted group. MEL: melatonin implanted group.	- Increased milk yield during the suckling and the milking period. - No difference in milk composition. - Improved the mean daily weight gain in kids, especially in males.
Yang et al., 2019	16 Inner Mongolian Arbace cashmere goat kids borned in spring. At 12 d of age, kids were allocated to two groups. The weaning of the kids was at 124 d. Cashmere fiber sample: 4th month and at the year of life. Skin biopsies, blood samples and LW: 2, 6, 10, 14, 18, 22 wk of life.	The kids were implanted s.c with melatonin (2 mg/kg live weight) at first week and the second month of age. Treatments: Control: non-implanted. Melatonin: implanted.	- No difference in LW. - Increased cashmere yield and improves fiber quality of kids (length, density and fiber). - Increased the total secondary follicle population in the skin of early postnatal kids.
Yang et al., 2020	24 Inner Mongolian cashmere goats and their kids (single-female) were assigned into two groups at d 50 of lactation (spring). Dams: Milk yield and composition: 0, 2, 4, 6 and 8 weeks of lactation. Blood samples: 0, 4 and 8 weeks of lactation. Dam cashmere samples: the following year. Kids: Kids LW: 0, 2, 4, 6 and 8 wks of age. Kids cashmere samples: yearling. Kids skin biopsies: 0, 8 and 12 wks; and 14 months of age.	Ewes were implanted s.c with melatonin (2 mg/kg live weight) at days 50 and 110 of lactation. Treatments: MEL: implanted. CON: non-implanted.	Dams: - Decreased milk yield. - Increased fat milk concentration. - No difference in milk protein (%), lactose (%) and SCC. - Decreased serum prolactin. - Increased cashmere yield, staple length and density. - Fine-tuned fiber diameter. Kids: - No difference in LW and cashmere parameters.

Afzaal et al., 2023	46 pregnant Beetal goats at 100 d of gestation (kidding from February to march) were divided in two groups. Then, on 7th d postpartum, goats in each group were subdivided randomly into two subgroups based on suckling treatment. LW kids: Birth. LW and BCS goats: 40 d before and 28 d after kidding.	Goats were implanted s.c with melatonin (18 mg/goat) at 100 d of gestation. Treatments: MCS: melatonin implanted continuous suckling. MRS: melatonin implanted restricted suckling. CCS: control continuous suckling. CRS: control restricted suckling.	- Increase in birth weight of triplets or quadruplets kids. - No difference in LW and BCS of the goats.
Diao et al., 2023	32 newborn cashmere goats were allocated to two groups. LW and cashmere fiber sample: 1 d of age, at 3 and 6 months of age, and at 1 and 2 years of age. Skin sample: 1 d of age, at 2, 4, and 6 months of age, and in the second year.	The kids were implanted s.c with melatonin pellets (2 mg/kg) on d 1 from birth and at 2 and 4 months of age. Treatments: Control: non-implanted. Melatonin: implanted.	- No difference in LW. - Increased cashmere yield and improves fiber quality of kids (length, density and fiber). - Increases the number of secondary hair.

2. Objectives

2. OBJECTIVES

This PhD Thesis was proposed to evaluate the effect of exogenous melatonin administration during different physiological stages in dams on their milk production and quality, and the performance of their offspring in dairy and meat small ruminants (sheep and goats), while also examining the impact of exogenous melatonin in lambs during their preweaning stage on their life.

To achieve this general objective, the following specific objectives were proposed:

- Evaluate the effect of melatonin implants during the last third of gestation (30 d before kidding) on colostrum quality, milk production, and milk quality in Murciano Granadina dairy goats under commercial farm conditions.
- Evaluate the effect of melatonin implants during the last third of gestation (40 d before lambing) on colostrum quality, milk production, and milk quality in Assaf dairy ewes under commercial farm conditions.
- Evaluate the effects of melatonin implants during the last third of gestation (40 d before lambing) using meat ewes (Rasa Aragonesa) on live weight and daily weight gain of their lambs from birth to weaning under commercial farm conditions.
- Evaluate the effects of melatonin implants during the last third of gestation (30 d before lambing), immediately after lambing, or at both moments using meat ewes (Rasa Aragonesa) on colostrum and milk composition, lamb's live weight from birth to weaning, daily gain, body dimensions, and rectal and surface temperatures under indoor conditions.
- Evaluate the effects of melatonin implants before weaning (30 days of age) on locomotor activity, body temperature, and growth of fattening female and male lambs under indoor conditions.

3. Material and methods

3.1 Animals

For each of the specific objectives, an experiment was proposed. Therefore, five experiments were designed to analyze the effects of exogenous melatonin administration at different physiological stages on colostrogenesis, lactation and lamb performance in small ruminants. The experiments were performed in different areas of Spain, using small ruminants (sheep and goats) from various breeds and their corresponding production systems:

Specific objective 1: The experiment 1 involved 269 Murciano Granadina goats from four commercial dairy farms in Granada.

Specific objective 2: The experiment 2 involved 715 Assaf ewes from five commercial sheep dairy farms in Castilla y León.

Specific objective 3: The experiment 3 involved 457 Rasa Aragonesa ewes that gave birth to 575 lambs (294 males, 281 females; 199 singles, 307 twins, 69 triplets) from two commercial sheep farms in Zaragoza (Farm1, n=354; Farm2, n=221).

Specific objective 4: The experiment 4 involved 50 Rasa Aragonesa ewes that gave birth to 67 lambs (31 males, 36 females; 27 singles, 40 twins) at the experimental farm of the University of Zaragoza.

Specific objective 5: The experiment 5 involved 60 Rasa Aragonesa lambs (31 males, 29 females; 40 singles, 20 twins) born at the experimental farm of the University of Zaragoza.

In experiments 1 - 4, the groups were managed as a single flock. Meanwhile in experiment 5, the groups were housed separately from weaning until they slaughter. Throughout each experiment, the experimental groups were subjected to the same nutritional and husbandry practices, under an intensive system, with permanent stabling. They were fed to meet their live weight requirements, either during pregnancy, lactation, growing and fattening, in conformity with the standards of the AFRC (1993).

In experiment 1 and 2, after parturition, offspring were separated from their dams and reared on an artificial lactation program until commercialization. Meanwhile, in experiment 3 and 4, lambs remained with their respective dams until weaning (45 and 42 d postpartum, resp.) and suckled freely.

3.2 Treatments

Animals were assigned to one of the treatments groups, that either did or did not receive subcutaneous melatonin implant(s) in the base of the ear (18 mg melatonin; Melovine, CEVA Salud Animal, Barcelona, Spain). The groups were balanced in LW, BCS and parity number. The specific treatments of each experiment, detailing the timing and the doses of melatonin implantation were (Table 4).

3.2.1. Experiment 1

- Group MEL: Goats received a subcutaneous melatonin implant 28.5 ± 0.9 d before kidding (n = 92 goats).

- Group CON: Control, non-implanted goats (n = 177 goats).

3.2.2. Experiment 2

- Group 1M: Ewes received a subcutaneous melatonin implant 45 ± 0.6 d before lambing (n = 246 ewes).

- Group 2M: Ewes received a subcutaneous melatonin implant 40 ± 0.7 d before lambing (n = 137 ewes).

- Group C: Control, non-implanted ewes (n = 332 ewes).

3.2.3. Experiment 3

- Group MEL: Ewes received a subcutaneous melatonin implant 39 ± 7 d before the expected time of lambing (n = 248 lambs, 166 males, 161 females).

- Group CTR: Control, non-implanted ewes (n = 327 lambs, 128 males, 120 females).

3.2.4. Experiment 4

- Group M-0: Ewes received a subcutaneous melatonin implant 32 ± 0.7 d before lambing (n = 14 ewes; 19 lambs, 7 males, 12 females).

- Group 0-M: Ewes received a subcutaneous melatonin implant immediately after lambing (n = 14 ewes; 21 lambs, 10 males, 11 females).

- Group M-M: Ewes received a subcutaneous melatonin implant on both occasions (32.8 ± 0.5 d before lambing, and after lambing)(n = 10 ewes; 13 lambs, 6 males, 7 females).

- Group 0-0: Control, non-implanted ewes (n = 12 ewes; 14 lambs, 8 males, 6 females).

3.2.5. Experiment 5

- Group MEL: Lambs received two subcutaneous melatonin implants at 30 ± 2 d of age (14 d pre-weaning) (n = 15 males, 15 females).

- Group CTR: control, non-implanted lambs (n = 16 males, 14 females).

Table 4. Timing of melatonin implantation relative to different physiological stages (pregnancy, parturition, lactation/rearing, and fattening) and across different months of the year for each experiment.

	Pregnancy	Parturition	Lactation*/ Rearing**	Lactation*/ Rearing**	Fattening
Experiment 1	Jul./Aug.	Aug./Sept.	Sept./Oct.	Oct./Nov.	
	MEL				
Experiment 2	Apr	Jun.	Jul.	Aug.	
	1M, 2M				
Experiment 3	Apr./May.	May./Jun.	Jun./Jul.	Jul./Aug.	
	MEL				
Experiment 4	Sept.	Oct.	Nov.	Dec.	
	M-0, M-M	**0-M, M-M**			
Experiment 5	Sept./Oct.	Oct./Nov.	Nov./Dec.	Dec./Jan.	Jan./Feb.
				MEL	

* Experiment 1 and 2.

** Experiments 3 – 5.

3.3. Methodology

3.3.1. Milk records

Experiment 1 and 2: Three monthly milk samplings were performed on each farm. Milk yield were recorded by volumetric meters integrated in the milking system such that the milk production of each animal is recorded on a daily basis, alternating between mornings and afternoons (ICAR 2016).

3.3.2. Milk sampling and analysis

Experiment 1 and 2: Three monthly milk quality analysis included fat (F), protein (P), and lactose (L) content measurements based on method used by the International Dairy Federation (IDF, 2010). SCC was analyzed using an electronic fluorescence-based cell-counter following the guidelines of the International Dairy Federation (IDF, 2013).

Experiment 4: Milk samples were collected by hand-milking at 2, 4, and 6 wk postpartum for F, P, and L, and analyzed by an automatic milk analyzer calibrated for sheep following the manufacturer's instructions (Milkotronic Ltd., Tsentar, Nova Zagora, Bulgaria).

3.3.3. Colostrum sampling and analysis

Colostrum samples were obtained from goats and ewes immediately after birth (Experiment 1: CON, n= 88, MEL, n= 77; Experiment 2: 1M, n = 118; 2M, n = 73; and C, n = 112; Experiment 4: M-0, n=,14, 0-M, n=14, M-M, n=10, 0-0, n=12).

Colostrum concentrations of IgG were analyzed using a direct enzyme immunoassay, sandwich ELISA Calokit–Cabra Test (Experiment 1, ZEULAB, Zaragoza, Spain) and Calokit–Sheep Test (Experiment 2 and 4, ZEULAB, Zaragoza, Spain). A digital Brix refractometer (Deltatrak, Pleasanton, CA, USA) was used for Brix refractometry. Colostrum quality (F, P and L) were measured using a milk analyzer (Lactoscan SP+) that we calibrated for goat and sheep in accordance with the manufacturer's instructions (Milkotronic Ltd., Tsentar, Nova Zagora, Bulgaria).

3.3.4. Liveweight, average daily gain, body measurements and feeding convertion rate

Experiment 3: Lambs were weighed (kg) at birth (LW0) and at weaning (47 ± 8 d of age) (LWW). Age at weaning (AW, d) was recorded, and the average daily growth rate (g/d) (AGR) was calculated as [(LWW-LW0)/AW].

Experiment 4: Lambs were weighed at birth, week 2, week 4, and at weaning (week 6). Average daily gain (ADG) was based on the intervals between the sampling days. At birth and at weaning, five features (body length=BL, chest girth=CG, wither height=WH, rump height=RH and shoulder width=SW) were measured to define the body size of the animals (Idris et al., 2011).

Experiment 5: Lambs were weighed at 45 (weaning), 60, 75 and 85 (slaughter) d. Average daily gain (ADG) was based on the intervals between the sampling days. The feed conversion rate (FCR) was calculated as kilogram concentrate consumed per kilogram LW gained.

3.3.5. Rectal and surface temperatures

Experiment 4: At birth, the rectal temperature of the lambs was recorded by a conventional thermometer, and the surface temperature was recorded by a thermographic camera (Teso 880, Teso SE & Co. KGaA, Titisee- Neustadt, Germany). For eye temperature (ET), the camera was positioned 40 cm from the lamb's head. The other body surface temperatures were measured 1 m from the dorsal midline of the lamb. Temperature data were analyzed by Testo IRSoft software, where three fixed-size equidistant areas were identified: shoulder (ST), mid-loin (MT), and hips (HT) (Labeur et al., 2017).

Experiment 5: One week before slaughter, rectal temperature was recorded by a conventional thermometer, and surface temperatures were recorded by a thermographic camera (Teso 883, Teso SE & Co. KGaA, Titisee-Neustadt, Germany). In the lateral image, the hotspot surface temperature was identified, and the mean surface temperatures of specific areas (area 1=leg, ribs, shoulder; area 2=rump and leg; and area 3=loin) were calculated. In the dorsal image, the mean surface temperatures of the hind saddle and back area were calculated.

3.3.6. *Longissimus dorsi* and back fat thickness scanning

Experiment 5: One week before slaughter, a real-time ultrasound was used to measure the subcutaneous fat thickness (FT) over the *longissimus dorsi* muscle cross-section (between the 12th and 13th ribs on left side). All measurements were taken using an ExaGo (IMV imaging Angoulême, France) scanner connected to a 7.5 MHz linear probe. Muscle depth and width were taken at the same points and the area was calculated.

3.3.7. Locomotor activity and circadian rhythms

Experiment 5: During the five weeks of the experiment, lambs were fitted with commercially available sensors that record high-resolution raw acceleration data (ActiGraph wGT3X-BT; ActiGraph, FL, USA) (46 mm × 33 mm × 15 mm in size, mass = 19 g), which were attached to the dorsal side of a neck collar. At the end of each week, the activity data were downloaded by the ActiLife software (ActiGraph, LLC, Pensacola, FL, USA). Actigraph produces three columns of data, that is, activity in the x-, y-, and z-axes. The activity counts for the three axes were used to create minute-by-minute activity (counts per 1-minute intervals) data values (vector magnitude [VM]), which represent the combined accelerations from the three axes). Circadian rhythms in VM were graphed by fitting the time-series measurements of each lamb to the cosine curve of a 24 hour activity rhythm, which was obtained by the cosinor method at the Cosinor on-line platform (Molcan, 2019). Midline estimating statistic of rhythm (MESOR, or the average around which the variable oscillates), amplitude (the difference between the peak and the mean value of a wave), and acrophase (the time of peak activity) were calculated for each individual.

The statistical analysis of the results is detailed in each of the corresponding publications.

4. Publications

4.1. Using melatonin implants in late pregnancy increases milk production and improves milk quality in dairy goats. Canadian Journal of Animal Science. Canto F., Peña-Delgado, V., Noya, A., Manenti, I., José Abecia, J.A. Article sent to: Canadian Journal of Animal Science el 20/11/2023 (reference number: CJAS-2023-0123). In revision

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Melatonin implants in late pregnancy increase yield and enhance milk quality in dairy goats

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Melatonin implants in late pregnancy increase yield and enhance milk quality in dairy goats

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ABSTRACT

This study investigated the effects of melatonin implants in pregnancy on milk production and composition, and the quality of colostrum in dairy goats. Thirty days before kidding, 92 goats (group MEL) received one melatonin implant, and the remaining 177 goats (group CON) did not. Three monthly milk evaluations included milk yield (kg/d), composition (%Fat, %Protein, % Lactose), daily yield (Fat, g/d Protein, g/d Lactose). A sample of colostrum was obtained from 165 goats, from which its composition, IgG concentration were measured. MEL had a significantly (P<0.01) higher milk yield in the second month (3.16 ± 0.10 kg/d) than CON (2.78 ± 0.07 kg/d). In the three milk samplings, fat concentrations were significantly higher (P<0.05) in the MEL than in the CON does. In the second milk sampling, does that had received a melatonin implant produced higher (P<0.05) daily milk yield components than did non-implanted (Fat: 144 ± 6.0 vs 115 ± 3.4; Protein: 104 ± 3.4 vs 91 ± 2.5; Lactose: 148 ± 5.8 vs 131 ± 4.3 g/d). In conclusion, melatonin implants administered 30 d before kidding increased milk production, the amounts of milk daily components in the second month of lactation, and the concentration of fat milk.

Keywords: goat, melatonin, dairy, milk composition, fat, colostrum

Introduction

Since the 1960s, the world's goat population has increased, mainly because of changes in human food preferences and incomes, and climate change, which limits the areas suitable for livestock farming. There has been a continuous increase in dairy goat numbers globally, with dramatic increases in the 1990s, particularly in Asia and Africa, although with a minor net decrease observed in Europe (–0.9%) and Americas (–0.7%) (Miller and Lu 2019). In spite of this figures, dairy goat production is more popular in Europe, particularly in the Mediterranean basin, where it is vital economically, environmentally, and socially for the Mediterranean countries of Spain, France, Italy, and Greece (Dubeuf et al. 2004). Goat milk production in the world, from over one billion head of goats, amounts to about 18.6 million tons, of which 15.14% in Europe (2.8 million tons). There are over 15 million dairy goats in the EU. In particular, the total production of goat milk in the EU in the amount of 2.2 million tons, the largest share belongs to Greece (26.16%), followed by France (27.45%), followed by Spain (22.85%) (Sredojević et al. 2020).

Cheese yield is a crucial economic target for dairy goat farmers, specially in the EU, which has emerged as the leading cheese-producing region, contributing 10.39 mmt in 2023 (Langat, 2024). Cheese yield depends on milk fat and protein concentrations (Pazzola et al. 2019), therefore, improving these parameters can enhance profitability in dairy farmers. Although goat colostrum is not commercialized, it is crucial for immune system development of the kids (Hernández-Castellano et al. 2014) as it provides essential nutrients, such as protein, lactose, fat, vitamins, minerals and several biologically active compounds (Kráčmar et al. 2005). Currently, new management practices have been adopted that reduce the use of chemical inputs and medications (antibiotics) in food animal production (Miller and Lu 2019; More 2020), which can impact in both goat milk and colostrum quality. In this context, exogenous melatonin can be an alternative for improving productive and health indicators in dairy goats.

Melatonin is a natural neuro-endocrine hormone, and since its discovery, most research has focused on its effects on reproduction in photoperiod-dependent breeding animals. Recently, the potential of melatonin to enhance lactation and colostrogenesis in small ruminants has been investigated (Avilés et al. 2019; Abecia et al. 2020; Yang et al. 2020; Abecia et al. 2021; Canto et al. 2022). In a study of Creole goats, two melatonin implants administered in the dry period increased milk yield during the first 14 weeks of lactation, but melatonin treatment did not have a significant effect on milk components (Avilés et al. 2019). Conversely, in Cashmere goats that received melatonin implants twice during early lactation, daily milk yield, milk protein and milk lactose decreased, while milk fat content increased (Yang et al. 2020). Additionally, melatonin has been shown to protect against oxidative damage (Andrés et al. 2009; Bouroutzika et al. 2021), improve mammary health (Jiménez et al. 2009; Canto et al. 2022) and enhance immune colostrum quality in meat and dairy ewes (Abecia et al. 2020; Canto et al. 2022). Although, the role of melatonin in the mammary gland as a target tissue has not been identified (Zhang et al. 2019), and the effects of melatonin on milk production in several ruminant species have been contradictory (Asher et al. 1994; Abecia et al. 2005) and with limited evidence of its role in goats.

The objective of this study was to measure the effects of melatonin treatment at pregnancy on milk production and composition, and colostrum quality in dairy goats reared under commercial farm conditions. The rational for choosing the last month of gestation is that the transfer of prepartum components from the maternal circulation into colostrum occurs during this period, and is a critical period for mammary gland development. Additionally, a single subcutaneous implant is an effective method for slow-release melatonin, which has demonstrated positive effects on colostrogenesis and lactation, as demonstrated in previous studies conducted by our group with sheep (Abecia et al. 2020, 2021; Canto et al. 2022). Thus, we support the hypothesis that exogenous melatonin administered in late pregnancy can affect the function of the mammary gland, and consequently, lactogenesis and colostrogenesis.

Material and methods

Experimental treatments

Four commercial dairy goat farms that raise the Murciano Granadina breed in Spain participated in this study. Approximately 30 (±S.D) d (28.5 ± 0.88 d) before the expected onset of the kidding date, goats were randomly chosen from among 269 multiparous animals with no diseases, and a comparable body condition score (BCS). At that time, 92 goats (group MEL) (parity number: 3.78 ± 0.20; BCS: 3.43 ± 0.24) were administered subcutaneously at the base of the ear one melatonin implant (18 mg melatonin; Melovine, CEVA Salud Animal, Barcelona, Spain). Those implants provide a continuous release of melatonin for approximately 10 wk and high plasma concentrations are maintained throughout the day (Delgadillo, 2011). The remaining 177 goats (parity number: 3.61 ± 0.17; BCS: 3.41 ± 0.21) did not receive an implant and constituted the control group (CON).

Animal management

In each farm, the two groups were managed as a single flock, and were subjected to the same nutritional and husbandry practices throughout the experiment, under an intensive system, with permanent stabling. Kidding occurred indoors over seven weeks in Aug and Sep. After parturition, kids were separated from their does and reared on an artificial lactation program until commercialization. In lactation, goats were milked twice per day and were offered a total mixed ration (TMR) of concentrates and forage to meet their liveweight maintenance and milk production requirements. Water was provided *ad libitum.*

Milk records

In the experiment, three monthly milk samplings were performed on each farm. The first milk sampling was performed two weeks after kidding, and subsequent milk collections occurred every 30 d. Table 1 presents the mean (±SEM) Days In Milk (DIM) at each sampling day, and the interval between melatonin implantation and milk sampling. In each sampling, measurements of individual milk yield were recorded by volumetric meters integrated in the milking system such that the milk production of each animal is recorded on a daily basis, alternating between mornings and afternoons (ICAR 2016). The Official Milk Control technique, which has been certified by the International Committee for Animal Recording (ICAR 2016), was used to measure daily milk production, and was calculated as follows:

Daily milk yield = (Registered milk × 24)/(Time between milk records)

Monthly milk productions were calculated based on the ICAR Method, which takes a monthly alternate milk sample (ICAR 2016).

Sample collection

At the time that milk yield was measured, a milk sample was collected and preserved at 4°C for subsequent laboratory analysis. For the SCC, an aliquot of each milk sample was conserved in bronopol (0.1%). Colostrum samples were obtained from 165 goats immediately after birth (CON: n= 88, MEL: n= 77), which were frozen and kept at -20° C until assayed for immunological and nutritional composition.

Milk and colostrum quality analysis

Fat, protein, and lactose contents were measured based on the methods used by the IDF (2010a). Milk samples were subjected to direct reference analysis for fat content (Gerber method), crude protein content (Kjeldahl method), and lactose content in accordance with International Dairy Federation Standard 105 (IDF 2008), International Dairy Federation Standard 020-5 (IDF 2001), and International Dairy Federation Standard 214 (IDF 2010b), respectively. The milk samples were analyzed for SCC by an electronic fluorescence-based cell-counting Fossomatic 5000 (Foss Electric, Hillerød, Denmark) following the guidelines of the International Dairy Federation's (IDF 2013).

A digital Brix refractometer (Deltatrak, Pleasanton, CA, USA), which had a detection range of 0%-53% was used for Brix refractometry at room temperature. Before testing, colostrum samples were completely homogenized following the manufacturer's instructions. Total colostral IgG concentration was measured by a direct enzyme immunoassay, sandwich ELISA Calokit–Cabra Test (ZEULAB, Zaragoza, Spain).

Samples were diluted to fit the operating range of IgG quantification (mg/ml) in the ELISA test, and the results were read by a 450-nm absorbance Multiskan microplate reader (Labsystems, Helsinki, Finland). The concentration of goat IgG in the colostrum sample was calculated by interpolating the absorbance reading on the calibration curve. The samples were diluted to 1:2 before the nutritional analysis. Fat, protein, and lactose concentrations in the colostrum samples were estimated by an indirect method that involved an ultrasonic analyzer (Lactoscan SP+), which had been calibrated for goats following the guidelines provided by the manufacturer (Milkotronic Ltd., Tsentar, Nova Zagora, Bulgaria).

Statistical analysis

Daily yield (g/d) of each milk component was calculated for individual goats as follows: (milk yield × component content (%) of milk) / 100. To compare statistically significant differences in milk yield, colostrum and milk composition, and daily milk yield components, a multifactorial model based on the Least Squares Method of the GLM procedure in SPSS v.26 (IBM, Chicago, Il, USA) was used. The analysis included farm and melatonin treatment as fixed effects, and their interaction. A general representation of the model is as follows:

$$Y_{ij} = \mu + F_i + M_j + F_i \times M_j + E_{ij},$$

where Y_{ij} is the analyzed parameters (milk yield, colostrum and milk composition, and daily milk yield components); μ is the overall mean; F_i is the effect of the farm (*i*= Farm 1, Farm 2, Farm 3, Farm 4); M_j is the effect of the melatonin treatment (*j*= MEL and CON group); $F_i \times M_j$ is the effect of the interaction between farm and melatonin treatment; and E_{ij} is the random residual effect. Within fixed effects, significant differences in milk and daily yield components, and colostrum and milk composition were identified by an ANOVA. Statistical significance was defined as a P-value ⩽ 0.05.

Results

Milk yield, composition and quality

In each of the three milk samplings, farm had a significant ($P < 0.05$) effect on milk yield. In the first sampling, the treatment x farm interaction had a significant ($P < 0.0001$) effect on milk yield. Goats that had received a melatonin implant 30 d before kidding had a significantly ($P < 0.01$) higher milk yield (3.16 ± 0.10 kg/d) than did the CON goats (2.78 ± 0.07 kg/d) in the second month of lactation (Fig. 1); however, in the first and the third month of sampling, melatonin treatment did not have a significant effect (Fig. 1).

Farm had a significant ($P < 0.05$) effect on the content of fat, protein, and lactose in milk in each of the three milk samplings. The effects of the farm x melatonin treatment interaction on the concentrations of fat and protein in the milk were significant ($p < 0.0001$) in the first sampling, only. In the experiment, fat concentration was significantly higher in the MEL than it was in the CON does (Month 1: $P < 0.05$; Month 2: $P < 0.001$; Month 3: $P < 0.05$). Melatonin treatment did not have a significant effect on the concentrations of protein and lactose in the milk (Fig. 2).

In the second sampling, farm had a significant ($P < 0.0001$) effect on each of the daily milk components, and melatonin treatment had a significant ($P < 0.05$) effect on daily fat yield. In the third sampling, farm had a significant effect on daily fat ($P < 0.001$) and protein ($P < 0.05$) yields, and the farm x melatonin treatment interaction with had a significant ($P < 0.05$) effect on daily lactose yield. Does that receive a melatonin implant had a significantly higher daily fat ($P < 0.001$), daily protein ($P < 0.001$), and daily lactose ($P < 0.05$) milk yield than did non-implanted goats (Fat: 144 ± 6.0 vs 115 ± 3.4; Protein: 104 ± 3.4 vs 91 ± 2.5; Lactose: 148 ± 5.8 vs 131 ± 4.3 g/d, resp.) in the second milk sampling, but not in the first and the third milk samplings.

Farm ($P < 0.05$) and melatonin treatment ($P < 0.01$) had significant effects on SCC in the first sampling. In the second and third month, melatonin treatment did not have a significant effect on SCC, although the SCC in goats that had received a melatonin implant tended ($P = 0.17$) to be lower than in those that did not (Table 2).

Colostrum quality

Farm had a significant effect on the components of goat colostrum (°Brix, IgG, Fat: $P < 0.0001$; Protein, Lactose: $P < 0.001$), and MEL goats tended ($P = 0.17$) to have higher colostrum protein concentrations than did CON goats. Brix degree, IgG, fat, and lactose concentrations did not differ significantly between the two groups (Table 3).

Discussion

In our experiment, goats that had received one melatonin implant 30 d before kidding significantly increased milk production in the second month of lactation, approximately 75 d post-melatonin implantation. These findings are consistent with studies by Mabjeesh et al. (2013), who exposed dairy goats to a short photoperiod (60 d prepartum), and Avilés et al. (2019), who administered two melatonin implants to Creole goats during the drying-off phase (21 d prepartum), increasing milk production throughout lactation. Similarly, in our study of Assaf sheep conducted under commercial conditions, one or two melatonin implants administered 40 d before lambing increased milk production in the first three months of lactation (Canto et al. 2022). This suggests that melatonin treatment, or exposure to a short-day photoperiod during the dry period, increases milk yield in the subsequent lactation of small ruminants. Mabjeesh et al. (2013) proposed that the sensitivity to prolactin, particularly during the transition to lactation, may be the mechanism behind this effect. Supporting this hypothesis, Wall et al. (2005) found that cows exposed to a short-day photoperiod (linked to a high endogenous melatonin secretion) during the dry period, underwent more extensive mammary remodeling and cell renewal. However, our results were in contrast with the inhibitory effects of a short daily photoperiod or melatonin treatment during lactation on milk yield reported for sheep (Molik et al. 2013), goat (Yang et al. 2020; Zhang et al. 2019) and cattle (Auldist et al. 2007). This reduction in milk production could be caused by melatonin administration, which decreases prolactin secretion by protecting and stimulating dopaminergic neurons (Lacasse et al. 2019; Li et al. 2020; Liu et al. 2023). Additionally, these different melatonin responses in milk production on animals could be attributed to a wide range of factors, including significant species differences (Asher et al. 1994; Avilés et al. 2019), dosage variations (Cosso et al. 2021), or melatonin release characteristics (Yang et al. 2020).

In our study, providing melatonin to Murciano Granadina goats in late pregnancy increased the concentration of fat in their milk. This increase in milk fat content appears to be more consistent, as it has been observed in cows that received three repeated doses of melatonin during mid-lactation (Auldist et al. 2007), or in goats that received two doses at mid and late lactation (Yang et al. 2020), or in dairy ewes that received two melatonin implants 40 d before lambing (Canto et al. 2022); moreover, meat-type ewes that received a melatonin implant 24 h after lambing also presented an increment of fat proportion in milk (Abecia et al. 2021). There is an evidence that melatonin affects the gut microbiota in mice through mechanisms of AMP induction (Kim et al. 2020), and the rumen microflora of cows and lambs (Ouyang et al. 2021; Ma et al. 2022). These alterations in the bacterial biota can affect the fat content of milk, thereby significantly affecting ruminal lipid metabolism in both goats and cows (Toral et al. 2016). In addition, research involving bovine intramuscular preadipocytes demonstrated that melatonin regulates adipose differentiation via the melatonin receptor MT2 (Yang et al. 2017). This regulatory effect on fat cells aligns with findings from a recent analysis of the dairy metabolome, which revealed that melatonin treatment reduced most metabolites linked to lipid oxidation, implying increased fat accumulation in cows. This fat accumulation might alter the concentration of fat in the milk (Fu et al., 2023).

In our study, melatonin implants did not have a significant effect on protein and lactose concentrations in goat milk. Similarly, in other studies, melatonin implants administered 49 d prepartum in goats did not affect protein and lactose concentrations (Avilés et al. 2019), and implants given 40 d prepartum in sheep did not affect the lactose concentrations in milk (Canto et al. 2022). In small ruminants, under various farming and feeding systems, protein content is more stable than fat content in milk (Barillet 2007; Morand-Fehr et al. 2007) and milk lactose concentrations do not vary appreciably (Zervas and Tsiplakou 2013).

In our study of goats, melatonin implants did not reduce the SCC of milk, in contrast to findings from studies on cows and sheep (Yang et al. 2017; Canto et al. 2022), which might reflect species-specific differences (Avilés et al. 2019). These specie specific differences are mainly due to the higher SCC in uninfected goat halves, the higher apocrine component of goat milk secretion compared to sheep (Paape et al. 2001; Contreras et al. 2007). Moreover, there is a distinction in SCC profiles reflected in the predominant cell types present. Polymorphonuclear neutrophilic leukocytes (PMNs) were the predominant cell type in milk from healthy uninfected goats, comprising 40 to 80% of the SCC. In contrast, milk from healthy uninfected sheep primarily contains macrophages, accounting for 45–88% of the SCC, while PMNs constitute 10–35%, lymphocytes 10–17%, and epithelial cells 2–3% (see review Kaskous et al. 2023).

Although the primary husbandry and feeding practices across the flocks were consistent, there was a significant effect of the farm ($P<0.05$) on milk yield and composition. Therefore, the high variability across different farms may hinder the interpretation of the results. In addition, Morand-Fehr et al. (2007) highlighted the complexity of different farming systems, indicating that not all variables can be controlled. In our study, uncontrolled factors that might have contributed to farm effect variability include environmental factors, such as temperature and humidity (Zhu et al. 2020); genetic factors, including individual genetic effects (Goetsch et al. 2011), intra-breed variability (Idowu and Adewumi, 2017), and the presence of genomic single nucleotide polymorphisms (SNP) (Mucha et al. 2018); and sociodemographic characteristics (Lianou and Fthenakis 2021).

In our study, the immunity and chemical quality of colostrum did not differ significantly between treated and control goats. Concerning the immunity quality, IgG levels were elevated and exceeded the 28.2 mg/ml recorded in the same goat breed (Romero et al. 2013), which suggested that the animals in the two groups in our study were in good nutritional and immunological condition (Agenbag et al. 2021). The observed level of colostrum fat aligns with findings from other studies on goats. However, in our study, protein and lactose concentrations were lower and higher, respectively, than previously reported in the literature (Argüello et al. 2006; Keskin et al., 2007; Yang et al., 2009; Moreno-Indias et al., 2012; Romero et al., 2013). These variations may be related to the goat breed and the intensive concentrated feeding method used in the study. Sánchez-Macías et al. (2014) indicated that the lower protein concentrations might be linked with highly productive goat breeds, while, higher lactose concentrations seem consistently associated with increased starch and energy in the diet (Banchero et al., 2015; Hare et al., 2021). Moreover, a similar trend (low protein and high lactose) was described by Agradi et al. (2023) in Oborica goats, where the authors highlighted that these results were anticipated due the osmotic effects of lactose. Consequently, a higher percentage of lactose leads to an increased water influx, which in turn results in a higher dilution of the protein.

Conclusion

In conclusion, the administering of subcutaneous melatonin implants 30 d before kidding had a significant effect on milk yield and composition in goats reared under commercial farm conditions. Specifically, exogenous melatonin increased milk yield and milk daily components in the second month of lactation and fat milk concentration at the beginning and at mid-lactation. Factor such as fat and protein milk yield might be the best for price incentives, particularly, if the milk is used to produce cheese, and administering exogenous melatonin in goats at the end of pregnancy might be a means of increasing income in dairy goat production systems.

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Ethical Considerations Statement

The Ethics Committee for Animal Experiments at the University of Zaragoza approved all of the procedures performed in the study. The care and use of animals were in accordance with the Spanish Policy for Animal Protection RD1201/05, which meets the European Union Directive 2010/63 on the protection of animals used for experimental and other scientific purposes.

Declaration of Competing Interest

The authors declare no conflict of interest.

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Table 1

Mean (±SEM) Days In Milk (DIM) in the first three monthly milk samplings, and the interval between melatonin implantation and day of milk sampling in Murciano Granadina dairy goats that either did (MEL) or did not (CON) receive a melatonin implant 30 d before kidding.

	Numbers of goats/farm (F)	Days In Milk (DIM)			Interval implant-sampling (d)		
	F1 F2 F3 F4	Month 1	Month 2	Month 3	Month 1	Month 2	Month 3
CON (n=177)	97 27 24 29	13.67±0.59	42.64±0.67	75.08±0.72	--	--	--
MEL (n=92)	20 24 20 28	16.99±0.90	47.51±0.91	79.33±0.89	45.34±0.79	76.17±0.80	107.17±0.62

Table 2

Mean (± SEM) daily fat, protein, and lactose yield (g/d), and somatic cell count (SCC) in Murciano Granadina dairy goats that either did (MEL) or did not (CON) receive a melatonin implant 30 d before kidding in early- and mid-lactation under commercial farms conditions ([a,b]: different superscripts indicate significant differences between groups, within each month, $P < 0.05$) ([x,y]: indicate differences among groups, $p = 0.10$) .

	CON	MEL
Milk daily yield Month 1		
Fat (g/d)	104 ± 3.7	113±5.3
Protein (g/d)	85±2.7	89±3.9
Lactose (g/d)	117±3.9	122±5.5
Milk daily yield Month 2		
Fat (g/d)	115±3.4[a]	144±6.0[b]
Protein (g/d)	91±2.5[a]	104±3.4[b]
Lactose (g/d)	131±4.3 [a]	148±5.8 [b]
Milk daily yield Month 3		
Fat (g/d)	112±3.0	118±5,2
Protein (g/d)	94±2.2	94±3.6
Lactose (g/d)	134±3.6	139±6.5
Milk quality		
SCC month 1 (10^3/ml)	1.487±195[x]	1.094±163[y]
SCC month 2 (10^3/ml)	1.906±420	1.360±417
SCC month 3 (10^3/ml)	1.240±154	1.151±144

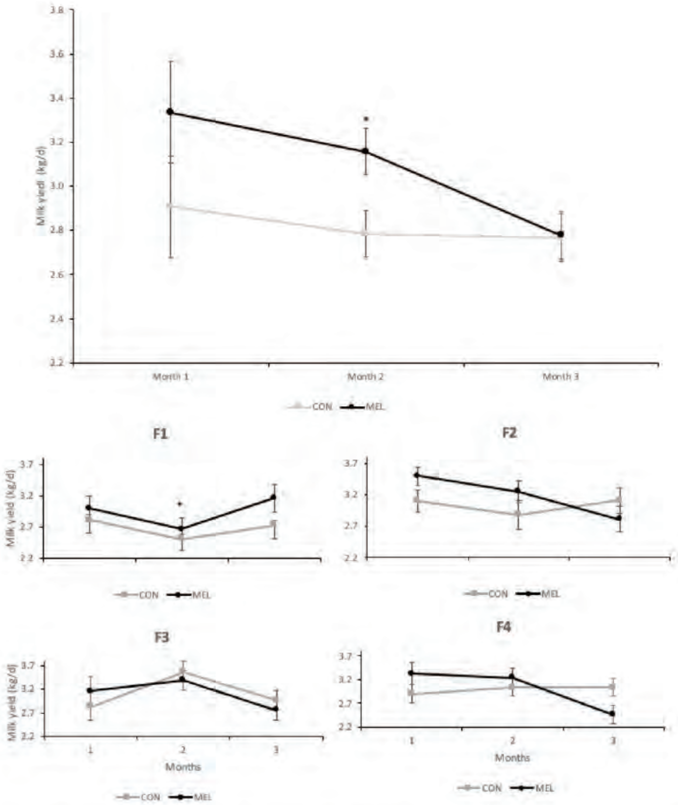
Table 3

Brix (°), IgG (mg/ml), fat (%), protein (%), and lactose (%) in colostrum from goats that either did (MEL) or did not (CON) receive a melatonin implant (18 mg) 30 d before kidding that had been collected immediately after parturition in Murciano Granadina dairy goats on commercial farms (Mean ± SEM) (x,y: indicate differences between groups, p = 0.10).

	CON	MEL
°Brix (%)	23.73±0.57	24.11±0.68
IgG (mg/ml)	51.06±3.04	52.27±3.84
Fat (%)	8.82±0.42^{x}	9.69±0.49^{y}
Protein (%)	6.42±0.13	6.59±0.17
Lactose (%)	9.72±0.21	9.98±0.25

Fig. 1. Mean (±SEM) daily milk yield (kg/d) in the first three months of lactation in Murciano Granadina dairy goats that either did (MEL) or did not (CON) receive a melatonin implant 30 d before kidding by treatment (upper panel), and the interaction treatment x farm 1 (F1), farm 2 (F2), farm 3 (F3) and farm 4 (F4) (lower panel). *: significant difference between groups, within the month, at P ⩽ 0.05.

Fig. 2. Mean (± SEM) daily fat, protein, and lactose milk content (%) in Murciano Granadina dairy goats that either did (MEL) or did not (CON) receive a melatonin implant (18 mg) 30 d before kidding by treatment (upper panel), and the interaction treatment x farm 1 (F1), farm 2 (F2), farm 3 (F3) and farm 4 (F4) (lower panel), reared under commercial farms conditions. * and** indicate significant differences between groups, within month (P ⩽ 0.05 and 0.01, resp.).



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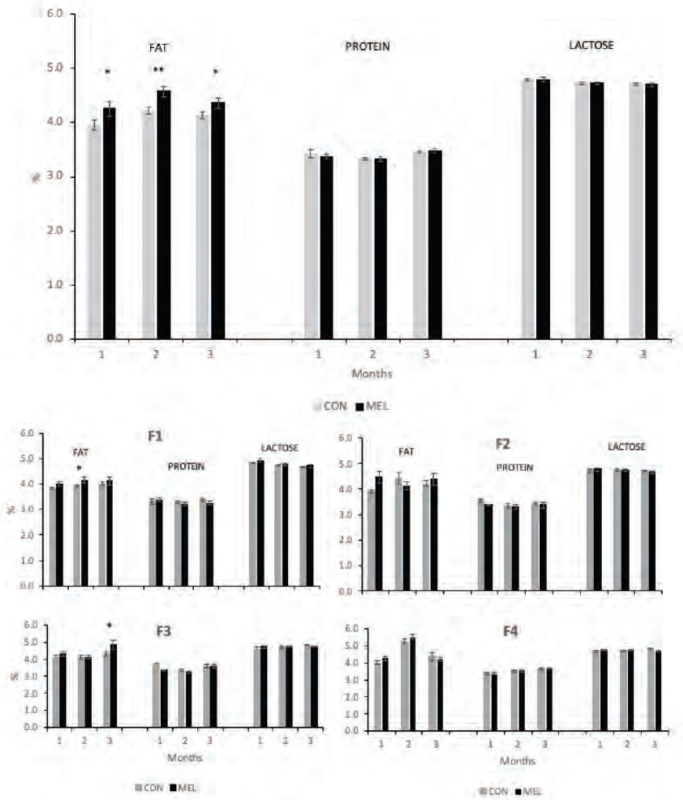

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4.2. Effects of Implanting Exogenous Melatonin 40 Days before Lambing on Milk and Colostrum Quality. Canto, F., González, E., Abecia, J.A. (2022). Animals 2022, 12, 1257. https://doi.org/10.3390/ani12101257

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Effects of Implanting Exogenous Melatonin 40 Days before Lambing on Milk and Colostrum Quality

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Simple Summary: Colostrum is the first product produced by mammals in the mammary gland immediately after parturition. It contains immunoglobulins, which are essential for the survival of the newborn. Because some evidence exists of a positive effect of melatonin on colostrum quality in sheep, we studied the effect of implanting melatonin 6 weeks before lambing in five dairy farms, and simultaneously on milk yield and quality during the first three monthly milk samplings after lambing. We compared one vs. two implants, and a control, nonimplanted group. Ewes that received a melatonin implant 40 d before lambing produced colostrum that had a higher IgG concentration than the colostrum from nonimplanted ewes, and produced more milk which, had a lower somatic cell count (SCC). The effect on SCC was prolonged if the sheep received a second melatonin implant.

Abstract: The effects of exogenous melatonin implanted before lambing on the quality of colostrum and milk yield were quantified in 715 ewes. Forty days before lambing, 246 ewes (1M) received a melatonin implant; another 137 ewes (2M) received two implants, and the remaining 332 ewes (C) did not receive an implant (control). Milk analysis was based on individual monthly milk samplings (June, July, and August) after lambing. A colostrum sample was collected from 303 ewes (118 1M; 73 2M; and 112 C), and IgG concentrations were measured. Ewes implanted with melatonin had higher ($p < 0.01$) daily milk yield (DMY) in the three samplings than the C ewes. On average, 1M ewes produced more milk ($p < 0.05$) than ewes in the other two groups, and 2M ewes produced significantly ($p < 0.05$) more milk than C ewes. In the first and third controls, ewes that received two melatonin implants had a lower ($p < 0.05$) SCC than C and 1M ewes, and in the second sampling, 1M and 2M ewes had a lower ($p < 0.01$) SCC than C ewes. Ewes that received melatonin implants had a higher ($p < 0.01$) IgG concentration (21.61 ± 1.03 mg/mL) than non-implanted ewes (16.99 ± 1.13 mg/mL); 2M ewes had the highest IgG levels. In conclusion, ewes that received a melatonin implant 40 d before lambing produced colostrum that had a higher IgG concentration than the colostrum from nonimplanted ewes, and produced more milk, which had a lower SCC. The effect on SCC was prolonged if the sheep received a second melatonin implant.

Keywords: sheep; melatonin; colostrum; milk

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1. Introduction

Colostrum is the first substance secreted by the mammary gland immediately after parturition, and differs from milk in its organoleptic structure, density, color, and chemical composition [1]. Colostrum is produced by colostrogenesis, in which immunoglobulin (Ig) is transferred from the maternal bloodstream to mammary secretions for a limited time within the postpartum period [2].

Factors such as age, breed, maternal nutrition, prolificacy, and health status can affect colostrum composition [3]. However, we demonstrated that treating ewes with melatonin implants in the fourth month of pregnancy increased colostrum quality as measured by IgG concentrations [4].

Melatonin is synthesized in the pineal gland, and transfers the day/night signals to the reproductive neuroendocrine axis [5], facilitated by enzymes that are controlled by the photoperiod perception. Subcutaneous implants of melatonin were used to advance the breeding season in sheep [6], and the effects of melatonin on the ovary, maternal recognition mechanism, and embryo viability were reviewed by Abecia et al. [7,8]. Carrillo-Vico et al. [9] summarized the role of melatonin in adjusting the seasonality of the immune system because it is an immunomodulatory component secreted by immunocompetent cells. In mice [10] and rats [11], melatonin has a stimulatory effect on IgG-producing cells and prevents the immunosuppression observed in aged animals because it increases IgG and IgM levels.

Melatonin implants inserted at the start of lactation do not appear to affect the milk yield of milking ewes. Melatonin implants at day 35 of lactation did not have a significant effect on milk yield or composition in Manchega and Lacaune ewes [12], and a melatonin implant at midmilking did not have a significant effect on milk yield throughout the subsequent milking period in Lacaune or Assaf dairy ewes [13]. Cosso et al. [14] did not detect a significant difference between melatonin-implanted and control Sarda ewes if the implants were inserted about two months after lambing, at the time lambs were weaned. However, the milk of ewes treated with melatonin 6 weeks before lambing presented a higher content of dry matter, protein, and fat than nontreated ewes [15].

Following experimental evidence that melatonin implants inserted once [4,15] or repeatedly [16,17] during pregnancy in ewes improved the antioxidant capacity [16] and the IgG concentration [4] in the colostrum of treated ewes, as well as the quality and the quantity [15,17] of milk, the objective of this study was to validate and quantify the effects of melatonin treatments (one or two implants) before lambing on the composition of colostrum and milk yield from ewes in a higher number of ewes, managed under normal commercial farm management procedures.

2. Materials and Methods

2.1. Animal Management and Milk Records

The experiment involved 715 Assaf ewes in their last third of pregnancy (in May) from five sheep farms in Spain. Forty days before the expected date of the onset of lambing, 246 ewes (group 1M) were implanted with a subcutaneous melatonin pellet (18 mg melatonin; Melovine, CEVA Salud Animal, Barcelona, Spain) under the left ear through a Melovine/Regulin implanter device (CEVA Salud Animal, Barcelona, Spain); another 137 ewes (group 2M) received two melatonin implants (36 mg melatonin), and the remaining 332 ewes (group C) did not receive an implant (control group). Melatonin implants provide a continuous release of melatonin and high levels of circulating plasma melatonin during the daytime for 100 days after treatment [18].

Milk analysis was based on 2145 individual monthly milk samplings (June, July, and August) after lambing. During the sampling period, data from the milk yield of each animal were collected, and a sample of milk was taken, cooled at 4 °C, then taken to the laboratory. The mean (±SEM) interval between melatonin implantation and the date of lambing, and the mean interval between melatonin implantation and the date of milk sampling are presented in Table 1. The quantity of milk produced was measured by volumetric meters inserted into the milking system and calculated using the alternated morning and afternoon records [19]. The official milk record method [19] was applied to calculate daily milk yield (DMY) through the following formula:

$$\text{DMY} = (\text{Registered milk} \times 24)/(\text{Time between milk records})$$

Milk production per month was calculated based on the ICAR method, which uses alternating monthly sampling [19]. The mean days in milk (DIM) of each experimental group on the day of milk sampling are shown in Table 1.

Table 1. Mean (±SEM) interval between melatonin implantation and day of lambing, mean interval between melatonin implantation and day of milk sampling, and days in milk (DIM) for the milk sampling of Assaf ewes that received either one (1M), two (2M) or no (C) melatonin implants 40 days before lambing.

Group	Number of Ewes/Farm	Interval Implant–Lambing (Days)	Interval Implant–Milk Sampling (Days)			DIM (Days)		
	F1 F2 F3 F4 F5		June	July	August	June	July	August
C	35 87 27 30 153	–	–	–	–	12.6 ± 0.4	54.7 ± 1.1	95.7 ± 1.0
1M	28 91 33 23 41	44.6 ± 0.6	57.3 ± 0.8	100.3 ± 1.0	134.0 ± 0.8	15.9 ± 0.5	55.8 ± 0.9	89.5 ± 0.9
2M	32 40 34 20 41	40.0 ± 0.7	48.1 ± 0.9	96.9 ± 1.7	133.0 ± 1.4	12.0 ± 0.7	57.4 ± 1.4	93.5 ± 1.3

Immediately after lambing, a sample of colostrum was collected from 303 ewes (1M, n = 118; 2M, n = 73; and C, n = 112). Colostrum samples were frozen and stored at −20 °C until the analysis.

Nutritional and milking normalized management systems were applied in the farms; in particular, the sheep were raised in an intensive production system, housed permanently indoors, and, after lambing, were weaned from their lambs and milked immediately twice per day. A unifeed mixture of concentrates and forage was offered. The lambs were reared on artificial lactation until they were sold.

2.2. Milk and Colostrum Analyses

Fat, protein, and lactose percentage (%), and somatic cell count (SCC) were analyzed, following the IDF 020-5 [20], the FIL 105 [21], and the IDF 79-1.2/ISO 5765-1.2 [22] Standards for protein, fat and lactose content, respectively. Aliquots of each milk sample were conserved in bronopol (0.1%) to estimate the SCC by a Fossomatic 5000 (Foss Electric, Hillerød, Denmark), which we calibrated with recognized standards [23].

Colostrum concentrations of IgG were analyzed using the Calokit–Sheep Test (ZEU-LAB, Zaragoza, Spain) [24]. Samples were diluted to adapt the IgG concentrations to the ELISA test working range, which was read under a 450-nm absorbance Multiskan microplate reader (Labsystems, Helsinki, Finland). The minimum detection threshold for sheep colostrum was 0.82 mg/mL. The IgG concentration in colostrum samples was calculated by interpolation of a quadratic calibration curve, which we obtained by plotting the concentrations of IgG standards against the absorbance readings.

Colostrum quality was analyzed by a milk analyzer (Lactoscan SP+) that we calibrated for sheep following the manufacturer's instructions (Milkotronic Ltd., Tsentar, Nova Zagora, Bulgaria) for measuring the fat, protein, and lactose in colostrum. Samples were 1:2 diluted before the analysis, and colostrum quality was estimated by a Brix refractometer (Deltatrak, Pleasanton, CA, USA).

2.3. Statistical Analysis

A multifactorial model using the least squares method of the GLM procedure in SPSS v.26 (IBM, Chicago, Il, USA) [25] was applied to compare IgG concentration in colostrum, colostrum and milk composition, and DMY, including farm and melatonin treatment as fixed effects. After that, colostrum IgG levels and colostrum and milk quality variables were statistically evaluated by an ANOVA within fixed effects. A general representation of the model is as follows: $y = xb + e$, where y is the $N \times 1$ vector of records, b denotes the fixed effect in the model within the association matrix x, and e is the vector of residual effects. To assess the statistical significance of the effects of melatonin treatment (0 vs. 1 vs. 2 implants), a post hoc Fisher's least significant difference (LSD) test was performed. A p-value < 0.05 was considered statistically significant.

3. Results

3.1. Milk Production and Quality

Farm ($p < 0.0001$), treatment with melatonin ($p < 0.0001$), and their interaction ($p < 0.0001$) had a significant effect on the DMY and SCC in each of the three milk samplings. Ewes implanted with melatonin had a significantly ($p < 0.01$) higher DMY in the three months than the C ewes (June: 3.29 ± 0.05 vs. 2.98 ± 0.06 kg; July: 3.37 ± 0.05 vs. 2.98 ± 0.07 kg; August: 2.89 ± 0.05 vs. 2.55 ± 0.06 kg). In addition, ewes in the 1M group produced significantly ($p < 0.05$) more milk than the ewes in the other two groups (Table 2), and 2M ewes produced significantly ($p < 0.05$) more milk than the C ewes. In the first and third samplings, ewes that received two melatonin implants had an SCC that was significantly ($p < 0.05$) lower than that of ewes in the C and 1M groups. In July, 1M and 2M ewes had significantly lower SCCs ($p < 0.01$) than the C ewes (Table 2).

Table 2. Mean (±SEM) daily milk yield (DMY) and somatic cell count (SCC) of Assaf ewes that received either one (1M), two (2M), or no (C) melatonin implants 40 days before lambing (a,b,c: different superscripts indicate significant differences among groups, within the month, $p < 0.05$).

Group	DMY (kg)			SCC (×1000)		
	June	July	August	June	July	August
C	2.98 ± 0.06^{a}	2.99 ± 0.07^{a}	2.55 ± 0.06^{a}	$1597 \pm 213^{a,b}$	1420 ± 216^{a}	$1355 \pm 173^{a,b}$
1M	3.36 ± 0.07^{b}	3.47 ± 0.07^{b}	2.98 ± 0.06^{b}	1491 ± 230^{a}	884 ± 133^{b}	1220 ± 183^{a}
2M	3.19 ± 0.08^{c}	3.21 ± 0.07^{c}	2.75 ± 0.08^{c}	960 ± 202^{c}	623 ± 159^{b}	715 ± 187^{c}

In each of the three milk sampling periods, farm ($p < 0.001$) and the interaction farm × treatment ($p < 0.001$) had a significant effect on the fat content of the milk. Farm, treatment with melatonin, and their interaction had significant ($p < 0.001$) effects on the protein content of the milk. In the first and third controls, ewes in the 2M group had a higher fat content in their milk than the ewes in the other groups ($p < 0.05$), and the groups significantly differed in the second control (Figure 1). Protein content differed among the groups in the three controls, and treatment with melatonin did not have a significant effect on the lactose content of the milk.

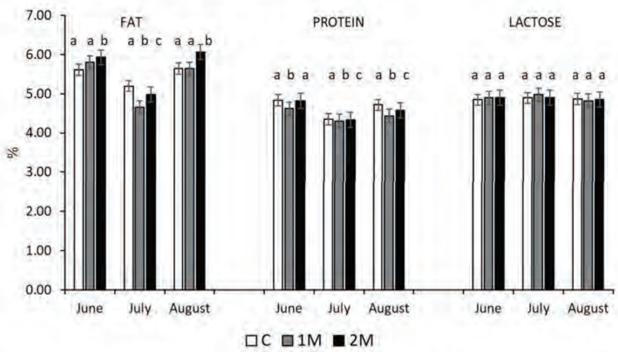

Figure 1. Mean (±SEM) fat, protein, and lactose content in milk samples collected in June, July, and August, of Assaf ewes that received either one (1M), two (2M), or no (C) melatonin implants before lambing (a,b,c: different superscripts indicate significant differences among groups, within the month, $p < 0.05$).

3.2. Colostrum Quality

Farm ($p < 0.0001$), treatment with melatonin ($p < 0.0001$), and their interaction ($p < 0.0001$) had significant effects on colostrum IgG concentrations. Ewes implanted with melatonin had a significantly ($p < 0.01$) higher IgG concentration (21.61 ± 1.03 mg/mL) than nonimplanted ewes (16.99 ± 1.13 mg/mL), and 2M ewes had the highest IgG levels ($p < 0.05$) (Table 3). Farm had a significant ($p < 0.001$) effect on the degrees Brix, fat, protein, and lactose content of the colostrum. The protein content of the colostrum of 2M ewes trended higher than that in the colostrum of C ewes ($p = 0.10$).

Table 3. Mean (±SEM) concentration of IgG, degrees Brix, and fat, protein, and lactose content in colostrum immediately collected after lambing from Assaf ewes that received either one (1M), two (2M), or no (C) melatonin implants before lambing (a,b,c: different superscripts indicate significant differences among groups, $p < 0.05$) (x,y: different superscripts indicate differences among groups, $p = 0.10$).

Group	IgG (mg/mL)	°Brix (%)	Fat (%)	Protein (%)	Lactose (%)
C	16.99 ± 1.13 ^{a}	21.70 ± 0.49 ^{a}	7.02 ± 0.34 ^{a}	7.06 ± 0.11 ^{a,x}	6.71 ± 0.11 ^{a}
1M	19.88 ± 1.39 ^{b}	22.06 ± 0.38 ^{a}	7.09 ± 0.28 ^{a}	7.18 ± 0.13 ^{a}	6.89 ± 0.13 ^{a}
2M	24.41 ± 1.46 ^{c}	22.57 ± 0.42 ^{a}	7.65 ± 0.29 ^{a}	7.39 ± 0.16 ^{a,y}	7.06 ± 0.16 ^{a}

4. Discussion

In this experiment, melatonin implants increased milk production, reduced the SCC of the milk, and increased the IgG concentrations in colostrum, which is consistent with our earlier finding that exogenous melatonin implanted one month before lambing had a positive effect on colostrum quality as reflected in an increase in IgG concentrations [4]. In Australian Merino lambs, the melatonin treatment of ewes at midpregnancy increased survival and growth rates from birth to weaning [26]. Flinn et al. [27,28] reported an increase in the survival rate of second-born twin lambs if mothers were treated with melatonin in the second half of pregnancy, although no differences in serum IgG of the lambs were found [27]. Increased lamb survival is probably mediated by the high colostrum quality caused by exogenous melatonin and/or by an increase in brown adipose tissue and birth weight if maternal melatonin implants are inserted at day 100 of gestation [29]. Although Bouroutzika et al. [16] did not observe differences in the IgG concentration of the colostrum of pregnant ewes treated or not with melatonin, they found a better redox status in lambs born from melatonin-treated ewes, as well as higher antioxidant capacity in the colostrum of melatonin-treated ewes compared with that of the untreated ones.

Most of the evidence of the effects of exogenous melatonin on milk production in sheep has been derived from animals that were treated during lactation rather than several weeks before lambing, as in our experiment. In goats, melatonin implants inserted seven weeks before kidding had a significant effect on milk production in the subsequent lactation and improved the daily weight gain of their suckling kids [30]. Melatonin membrane receptors MT1 and MT2 are persistently expressed in the mammary glands of dairy goats throughout lactation [31], which suggests that melatonin has a direct role in the regulation of mammary physiology.

In our experiment, exogenous melatonin provided using two melatonin implants had an effect on the fat content of ewe milk in June and August, and, in a study of a meat breed of sheep, melatonin implants at lambing increased the fat content of the milk, especially at the end of lactation, and increased the growth rate of their lambs [32]. Exogenous melatonin simulates a short-day photoperiod and has significant effects on the levels of solids, protein, fat, lactose, and the fatty acids in sheep milk [33]; however, the specific role of melatonin in the regulation of milk fat synthesis, particularly in sheep, requires further study. Using bovine mammary epithelial cells, exogenous melatonin suppressed milk fat synthesis by inhibiting the signaling pathway via the melatonin-1 receptor [34]. Furthermore, exogenous melatonin significantly increased the differentiation of bovine intramuscular preadipocytes into adipocytes, in vitro, with large lipid droplets and high cellular triacylglycerol levels [35].

In our experiment and that of Cosso et al. [14], exogenous melatonin reduced the SCC in the milk of sheep. In the latter experiment, ewes had received implants about two months after lambing. Evidently, melatonin influenced the immune response in the mammary gland of the implanted ewes, although the ewes in our experiment received the exogenous hormone approximately 40 d before lambing. Yang et al. [35] reported that a subcutaneous injection of melatonin significantly reduced the SCC in the milk of cows that had subclinical mastitis, which they attributed to the anti-inflammatory and immune enhancement actions of melatonin. Furthermore, the cows that had mastitis had reduced cortisol levels and upregulated levels of IgG, IgM, lymphocytes, and neutrophils after they received melatonin administration. Blood glutathione reductase, glutathione peroxidase, and superoxide dismutase activities increased in a group of goats implanted with melatonin at the beginning of lactation [36], and SCC was significantly lower in the implanted group than in the control group at midlactation.

The reduction in somatic cells after melatonin administration is very important because the SCC of milk is a sensitive indicator of udder health (see review [37]). In addition, it is a useful characteristic for evaluating the relationship between intramammary infection and changes in milk characteristics, and the effect of a high SCC on the yield, quality, and price of milk and dairy products. Cosso et al. [14] suggested that exogenous melatonin can be a therapy for treating subclinical mastitis in sheep. In our experiment, in the 2M group, low SCC was observed until the third milk sampling (August), which was 133 days after melatonin implantation. Ewes that received one melatonin implant, however, presented an effect on SCC in the second sampling (July), 100 d after implantation. Melatonin implants can release the hormone for up to 100 d [18]; therefore, apparently, one implant can have an effect on the mammary gland up until the implant is exhausted. In ewes that received two implants, the effects of exogenous melatonin persisted for an additional month. The mechanisms involved in the reduction in SCC may be mediated either through the ability of this hormone to reduce neutrophil infiltration and the inflammatory reaction [38] or through the antioxidative effect of melatonin [39]. Moreover, it was reported that melatonin suppressed and enhanced anti-inflammatory cytokines under different pathophysiological conditions [40], which in turn may have facilitated the reduction in the SCC observed in this experiment.

5. Conclusions

In conclusion, ewes that received one or two melatonin implants 40 d before lambing produced colostrum that had a higher IgG concentration than that produced by nonimplanted ewes, and produced more milk, which had a lower SCC. The effect on SCC was prolonged if the sheep received two melatonin implants.

Author Contributions: Conceptualization, J.A.A.; methodology, J.A.A., E.G. and F.C.; investigation, J.A.A., E.G. and F.C.; resources, J.A.A.; data curation, F.C.; writing—original draft preparation, J.A.A.; writing—review and editing, J.A.A. and F.C.; project administration, J.A.A.; funding acquisition, J.A.A. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The experiment was performed following procedures approved by the Ethics Committee for Animal Experiments of the University of Zaragoza. Animals were cared for following the Spanish Policy for Animal Protection RD1201/05, which meets the European Union Directive 2010/63 on the protection of animals.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

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Conflicts of Interest: The authors declare no conflict of interest.

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Melatonin-Implanted Pregnant Ewes Produce Lambs that have Higher Average Daily Growth Rates and Live Weights at Weaning

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Summary

The aim of this study was to quantify differences between lambs from birth to weaning born from ewes that received a melatonin implant before lambing and those that were not implanted. Forty d before lambing, 457 pregnant ewes either did or did not receive a melatonin implant. Subsequently, lambs were divided into two groups: lambs whose mothers received melatonin (MEL, n=248, 166 males, 161 females), and lambs whose mothers were non-treated (CTR, n=327, 128 males, 120 females). Lambs were weighed (kg) at birth (LW0) and at weaning (LWW) and age at weaning (AW, d) was recorded. Average daily growth rate (g/d) (AGR) was calculated as [(LWW-LW0)/AW]. MEL lambs had a mean LWW significantly ($P<0.05$) higher than CTR lambs. In particular, male MEL lambs had a significantly ($P<0.05$) higher LWW and AGR than male CTR lambs. Singleton male MEL lambs had higher LW0, LWW, and AGR, and lower AW at the day of weaning than the other lambs, and differences with singleton male CTR lambs were significant ($P<0.05$). LW0, LWW, and AGR were negatively correlated ($P<0.05$) with the implanting-weaning interval (IWI). Lambs with the shortest IWI had the highest LWW ($P<0.05$) and AGR ($P<0.01$), and the lowest AW ($P<0.01$). Treatment of pregnant ewes with melatonin before lambing increased lamb performance until weaning, and the effect was most pronounced in singleton male lambs, which had the highest growth rate. It remains to be elucidated what is the minimal interval between implantation in the pregnant ewes and parturition that maximizes the growth of lambs during lactation.

Key Words: Lambs, Melatonin, Growth, Live Weight

Introduction

Sheep productivity is constrained by sexual seasonality, which is governed by photoperiod (Yeates, 1949); i.e., the endocrine system receives photoperiodic information from the hormone melatonin, which dictates the timing of reproduction. Melatonin is secreted at night, and subcutaneous melatonin implants can be used to artificially manage estrus in sheep, which causes a brief daytime-like response without inhibiting endogenous production (O'Callaghan et al. 1991; Malpaux et al. 1997). In Spain, the anestrous period covers the late winter/early spring (Feb-Mar) to early- or mid-summer period (June-July); this seasonal breeding pattern results in a clear period of lambing, which in turn causes a seasonal pattern of product prices, with prices being lowest when the supply of meat is the highest (late spring to early fall) and vice versa. If farmers were able to produce products "out of-season", they could take advantage of higher prices for these during the winter by inducing estrous cycles during the seasonal anestrus. In this context, melatonin implants play an important role to obtain out-of-season lambs at high prices. Spain has reached the highest market share of the melatonin implants for sheep in the world, with more than 500,000 treatments per year applied in the ovine population, which is about 14 million heads; it means that around 1 out of 30 ewes in Spain have been treated with melatonin.

Recently, various uses of melatonin have been used in small ruminants, apart from the traditional reproductive control, with most of the focus on the survival and growth of offspring, and the improvement of colostrum and milk quality. Melatonin implants given between 70 and 120 d of pregnancy reduced neonatal mortality and increased survival rates at weaning, which were associated with increases in survival of twins and tolerance for prolonged parturition in sheep flocks that had been intensively managed (Flinn et al. 2020a, 2020b). Melatonin rapidly crosses the blood-brain barrier and the ovine placenta (Yellon and Longo 1987; Aly et al. 2015), which allows for maternal supplementation as a means for providing melatonin to the fetus before birth. Elsewhere (Abecia et al., 2020), we demonstrated that, in the fourth month of pregnancy, melatonin implants in ewes improved the quality of the colostrum produced, and lambs born of ewes that received exogenous melatonin had more IgG than did lambs from ewes that did not receive an implant. Furthermore, treatment with melatonin in ewes at lambing increased the growth rates in their lambs and the fat content of the milk (Abecia et al., 2021), and newborn lambs from ewes that had received a subcutaneous melatonin implant at day 120 of pregnancy had higher rectal temperatures and higher average and minimum body surface temperatures of the shoulder, mid loin, and hips than did control lambs (Canto et al., 2023). Recently, we showed that ewes that received a melatonin implant 40 d before lambing produced colostrum that had higher IgG concentrations, produced more milk, which had a lower somatic cell count (SCC), than did non-implanted ewes. A second melatonin implant prolonged the effect on SCC (Canto et al., 2022). In Lacaune dairy sheep, although exogenous melatonin treatment in late pregnancy did not have an effect on milk yield, it did affect milk composition; specifically, increasing milk fat concentrations and decreasing milk protein and lactose (Canto and Abecia, 2022). Collectively, the evidence indicates that administering melatonin implants to pregnant sheep might increase their economic return by improving the performance of their lambs through an increase in milk quantity and quality, and or an increase in lamb survival.

The Spanish sheep and goat sector accounts for approximately 10% of Spain's final livestock production, when considering the entire meat and dairy subsector. With a sheep population of around 14 million in the last five years, our country holds the top position in importance within the European Union (MAPA, 2023).

The objective of this study was to quantify the differences from birth to weaning between Rasa Aragonesa lambs born from ewes that had received an exogenous melatonin implant before lambing and those that were not.

Material and Methods

The experiment followed a protocol (PI29/21) approved by the Ethics Committee of the University of Zaragoza, which met the requirements of the European Union for Scientific Procedure Establishments.

The study involved 575 Rasa Aragonesa lambs (294 males, 281 females; 199 singles, 307 twins, 69 triplets) born on two commercial sheep farms (Farm1, n=354; Farm2, n=221) in Zaragoza, Spain. The farms were members of the Cooperative "Oviaragón", which produce the local lamb "Ternasco de Aragón" under the European Protected Geographical Indication (PGI). The Rasa Aragonesa sheep breed is a native breed of Spain that has been traditionally raised in Northeastern Spain; this breed has been recognized for its resistance, adaptability to various environments and its role in meat and wool production. After weaning in the farms, at an age of 45 d, lambs are housed in feed lots to achieve the slaughter weight (18-24 kg; 70-90 d of age). The farms applied the same management of the animals for the breed (Rasa Aragonesa), because farms that are involved in the production of that PGI lamb must meet several quality standards.

Approximately 40 d (mean ± S.D.= 39 ± 7 d; range 18-60 d) before the expected time of lambing (lambing season: 2 May-9 Jun), 457 pregnant ewes were either treated or not with a single melatonin implant (18 mg melatonin; Melovine, CEVA Salud Animal, Barcelona, Spain), which produced lambs that were assigned to one of two groups for analytical purposes: lambs whose mother had received a melatonin implant (group MEL, n=248) and lambs whose mothers were non-treated (group CTR, n=327). Lambs were weighed (kg) at birth (LW0) and at weaning (47 ± 8 d of age) (LWW). Age at weaning (AW, d) was recorded, and the average daily growth rate (g/d) (AGR) was calculated as [(LWW-LW0)/AW]. The weaning date is decided by the farmer when the lambs begin to reach a LW of about 12 kg, and the whole group of lambs is weaned. The effects of farm, sex of the lamb, type of parturition (single or multiple), and treatment with melatonin (MEL or CTR), were evaluated statistically based on a multifactorial model. It included farm, sex of the lamb, type of parturition, and treatment as fixed effects, and the Least Squares Method of the GLM procedure in SPSS v.26 (IBM

Corp. Released, 2019) was used. Sire effects were not considered since no information about mating is available in our farms when natural mating is used. Within fixed effects, significant differences were identified by an ANOVA. Pearson correlation coefficients among the implantation-weaning interval (IWI) and the lamb performance (LW0, LWW, AGR and AW) were calculated. A regression analysis was conducted between IWI and AGR.

To identify the optimal time before parturition to insert a melatonin implant in the ewes, the interval between melatonin implantation and lambing (IIL) was divided into four quartiles based on 'visual binning' (SPSS), which provides an interactive means of choosing how to transform a quantitative variable into a categorical variable. Differences in LW0, LWW, AGR, and AW among quartiles and the control group were assessed statistically by an ANOVA and the Least Squares Method.

Results and Discussion

Farm, sex of the lamb, type of parturition, and treatment with melatonin of the mothers had a significant (at least $P<0.05$) effect on LW0 and LWW (Table 1), and the interaction between type of parturition and treatment with melatonin of the mothers had a significant ($P=0.01$) effect on LW0. Farm, type of parturition, and their interaction had a significant ($P<0.001$) effect on AGR, and the interaction between sex and treatment was significant ($P=0.05$). Farm and type of parturition, but not melatonin treatment of the mothers, had a significant effect on AW.

MEL lambs had a higher mean (± S.E.) LWW (12.26 ± 0.10 g/d) than did CTR lambs (12.00 ± 0.08 g/d) ($P<0.05$). In particular, male MEL lambs had a higher LWW and AGR than did male CTR lambs ($P<0.05$), but there were no significant differences between female MEL and CTR lambs (Table 2). Singleton male MEL lambs had the highest LW0, LWW, and AGR, and the lowest AW, and the differences with singleton male CTR lambs were significant ($P<0.05$) (Table 3). Male MEL and CTR lambs with littermates, and female MEL and CTR lambs with or without littermates did not differ significantly (Table 3).

LW0, LWW, and AGR were significantly negatively correlated with IIW ($P<0.01$ for LW0, AGR, and AW, and $P<0.05$ for LWW) (Table 4). The linear regression analysis between IIW and AGR had a high coefficient of determination with AGR (0.3512), and a negative slope (-2.3459) (Figure 1), which reflected the negative relationship between the IIW and the AGR of the lambs. The lambs that had the shortest IIW had the highest LWW ($P<0.05$) and AGR ($P<0.01$), and the lowest AW ($P<0.01$) (Table 5).

The experiment in this study demonstrated that lambs born from ewes that had received a melatonin implant in the last third of pregnancy had the highest LW during lactation and grew faster than did lambs born of non-implanted ewes. Their mother's milk was the lamb's only source of nourishment; therefore, the melatonin implants increased the quantity and or quality of the milk. Previously, we (Canto et al., 2022) demonstrated that melatonin implants in pregnancy had a significant effect on milk quality; specifically, ewes that had received a melatonin implant 40 d before lambing produced the most milk, which had the highest fat content. In another study (Abecia et al., 2021), ewes that had received a melatonin implant at lambing produced milk that had the fattest content, and their offspring had the highest growth rate In goats, melatonin implants inserted seven weeks before kidding had a significant effect on milk production in the subsequent lactation and improved the daily weight gain of their suckling kids (Avilés et al., 2019). Melatonin membrane receptors MT1 and MT2 are expressed in the mammary glands of goats throughout lactation (Zhang et al., 2019), which suggests that melatonin has a direct role in the regulation of mammary physiology.

In our study, the effects of treating with melatonin ewes in pregnancy were

Table 1. *P*-values in each of the factors affecting live weight at birth (LW0) and weaning (LWW), and the average daily growth rate (g/d) (AGR) and age at weaning (AW) in Rasa Aragonesa lambs.

	LW0	LWW	AGR	AW
Farm	0.046	<0.001	<0.001	<0.001
Sex	0.002	0.002	0.169	0.638
Type of parturition	<0.001	<0.001	<0.001	<0.001
Treatment	0.049	0.029	0.181	0.681
Farm x Sex	0.787	0.259	0.092	0.240
Farm x Type of parturition	0.579	0.198	<0.001	0.130
Farm x Treatment	0.733	0.565	0.092	0.806
Sex x Type of parturition	0.946	0.637	0.356	0.130
Sex x Treatment	0.918	0.100	0.050	0.956
Type of parturition x Treatment	0.010	0.175	0.397	0.217

Table 2. Mean (±S.E.) live weight at birth (LW0) and at weaning (LWW) (kg), average growth rate (AGR) (g/d), and age at weaning (AW) (d) of male and female Rasa Aragonesa lambs that were born of ewes that either did (MEL) or did not (CTR) receive a melatonin implant in the last third of pregnancy.

Sex	Group	LW0 (kg)	LWW (kg)	AGR (g/d)	AW (d)
Male	CTR (128)	4.15±0.05	12.13±0.12[a]	174.31±3.33[a]	46.9±0.6
	MEL (166)	4.20±0.07	12.62±0.15[b]	188.59±4.12[b]	46.2±0.8
Female	CTR (120)	3.93±0.06	11.87±0.11	173.82±3.45	47.0±0.7
	MEL (161)	3.94±0.07	11.87±0.12	170.29±3.25	47.4±0.6

Means within an effect with no common superscript are different $P<0.05$.

Table 3. Mean (±S.E.) live weight at birth (LW0) and at weaning (LWW) (kg), average growth rate (AGR) (g/d), and age at weaning (AW) (d) of singleton and multiple Rasa Aragonesa lambs born of ewes that either did (MEL) or did not (CTR) receive a melatonin implant in the last third of pregnancy.

		Singleton				Multiple			
Sex	Group	LW0 (kg)	LWW (kg)	AGR (g/d)	AW (d)	LW0 (kg)	LWW (kg)	AGR (g/d)	AW (d)
Male	CTR (128)	4.49±0.07[a]	12.36±0.19[a]	184.82±5.91[a]	43.7±0.9[a]	3.93±0.07	11.98±0.14	167.38±3.78	49.1±0.8
	MEL (166)	4.79±0.11[b]	13.19±0.25[b]	211.83±6.69[b]	40.6±0.8[b]	3.87±0.08	12.29±0.18	175.55±4.66	49.3±0.9
	Total	4.61±0.06[a]	12.70±0.16[a]	195.91±4.59[a]	42.4±0.6	3.90±0.0[a]	12.12±0.1[a]	171.06±2.9[a]	49.2±0.6
Female	CTR (120)	4.38±0.09	12.12±0.22	188.97±7.25	42.6±1.2	3.73±0.06	11.75±0.13	166.32±3.28	49.2±0.7
	MEL (161)	4.53±0.10	12.22±0.27	173.82±6.09	44.9±1.0	3.71±0.05	11.73±0.13	168.92±3.85	48.4±0.8
	Total	4.43±0.07[b]	12.12±0.11[b]	183.22±5.10b	43.5±0.8	3.72±0.0[b]	11.74±0.0[b]	167.46±2.4[b]	48.9±0.5
	CTR (327)	4.44±0.06[a]	12.25±0.15	186.68±4.59	43.2±0.7	3.82±0.05	11.86±1.00	166.83±2.48	49.2±0.5
	MEL (248)	4.68±0.08[b]	12.78±0.19	195.95±5.09	42.4±0.7	3.79±0.05	12.01±0.11	172.18±3.02	48.8±0.6

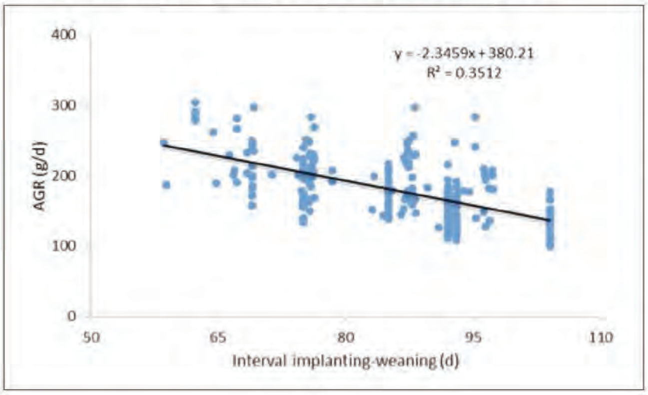
Means within an effect with no common superscript are different $P<0.05$.

strongest in male lambs. Similarly, Abecia et al. (2021) reported that the effects of melatonin implants in the mothers was significant in male lambs, only; specifically, male lambs reared by melatonin-treated ewes had significantly higher LW at weeks 2, 3, and 4 than did male lambs that had been reared by untreated ewes. In goats, melatonin implants in the dry period increased milk yield and the weight gain of male offspring, only (Avilés et al., 2019). Wallace et al. (2014) reported that, in early postnatal life, lamb sex had a significant effect on adipose tissue gene expression in favor of male lambs because female lambs had lower IGF1, IGF2, IGF1R, IGF2R, and hormone-sensitive lipase mRNA expression levels, which are associated with growth and reflect the sexual dimorphism in body composition.

Table 4. Matrix of correlations between the interval between the insertion of a melatonin implant in Rasa Aragonesa ewes in the last third of pregnancy and weaning (IIW), live weight at birth (LW0) and at weaning (LWW) (kg), average growth rate (AGR) (g/d), and age at weaning (AW) (d) of lambs (*$P<0.01$; ** $P<0.001$).

	IIW	LW0	LWW	AGR	AW
IIW		-0.240**	-0.162*	-0.593**	0.763**
LW0	-0.240**		0.293**	0.183**	-0.492**
LWW	-0.162*	0-293**		0.727**	-0.102*
AGR	-0.593**	0.183**	0.727**		-0.627**
AW	0.763**	-0.492**	-0.102*	0.627**	

Figure 1. Linear regression between the interval between the insertion of a melatonin implant in Rasa Aragonesa ewes in the last third of pregnancy and weaning (d) and the average growth rate of their lambs (AGR) (g/d).



The effects of melatonin implants in the mothers on the growth of male lambs might have been because they consumed the most colostrum, or the colostrum had the best quality. Elsewhere (Canto et al., 2022), we showed that ewes that received a melatonin implant 40 d before lambing produced colostrum that had higher IgG concentrations than did the colostrum from non-implanted ewes, and that ewes that had singleton male lambs had higher colostrum IgG concentrations (54.57 ± 5.37 mg IgG mg/mL) than ewes that had singleton female lambs (34.66 ± 4.30 mg/mL) (Abecia et al., 2020). In sheep, colostrum is important in the development of the immune system, post-natal growth, and thermoregulation, and mediates the formation of the ewe-lamb bond (Agenbag et al., 2021). In addition to increasing neonate survival, access to colostrum in the neonatal period can have a positive effect on future production, development, and reproductive efficiency of lambs through growth factors that facilitate neonatal growth and development. Öztabak and Özpinar (2006) reported that, from the

Table 5. Mean (±S.E.) live weight at birth (LW0) and at weaning (LWW) (kg), average growth rate (AGR) (g/d), and age at weaning (AW) (d) of Rasa Aragonesa lambs born of ewes that either did (MEL) or did not (CTR) receive a melatonin implant in the last third of pregnancy, and the interval between implantation and lambing (IIL) (IIW: interval between implantation with melatonin and weaning).

Group	IIL (d)	AW (d)	IIW (d)	LW0 (kg)	LWW (kg)	AGR (g/d)
CTR (n=329)	--	47.0±0.5[a]	--	4.05±0.04	12.00±0.08[a]	174.07±2.36[a]
MEL 1 (n=63)	30.34±0.34	44.1±1.3[b]	74.48±1.23[a]	3.94±0.11[a]	12.49±0.20[b]	200.37±5.92[b]
MEL 2 (n=61)	36.77±0.12	47.4±1.1[a]	84.19±1.11	4.04±0.09	12.24±0.21	177.64±5.07[a]
MEL 3 (n=62)	40.31±0.17	48.3±0.7[a]	88.64±0.65	4.09±0.09	12.13±0.19	169.71±4.64[a]
MEL 4 (n=60)	47.61±0.68	47.2±0.8[a]	94.83±0.86[a]	4.22±0.10[b]	12.16±0.21	170.87±5.09[a]

Means within an effect with no common superscript are different $P<0.05$.

second week onward, rearing method has an effect on body weight gain; specifically, lambs that were reared with their mothers and received colostrum had a higher mean body weight in lactation than did lambs that were reared without colostrum or artificially.

In our study, the correlations between implantation-weaning interval and the LW and AGR of lambs, indicated that the efficacy of the melatonin implants in improving lamb performance was greatest for individuals in which the melatonin implant was inserted closest to parturition. However, the later in pregnancy that the ewe was implanted, the smaller the effect on the development of mammary tissue because milk fat and total solid content were higher in ewes that had been implanted immediately after parturition than they were in control ewes at day 45 of lactation, only, which was close to weaning, and had no effect on the amount of milk produced. Although milk production and quality was not assessed, apparently, a single melatonin implant can affect the mammary gland of the ewes until the implant is exhausted. The implants can release melatonin for up to 100 d (Forcada et al., 2002), therefore, probably, the poorer performances of the lambs of mothers that had been implanted > 30 d before lambing was due to the earlier absorption of the implant such that the beneficial effects of melatonin on milk production diminished earlier in lactation.

Conclusions

Melatonin treatment of pregnant ewes before lambing increased lamb performance until weaning and, in particular, the effects were observed in singleton male lambs, who had the highest LW at birth and weaning, and the highest growth rate. These results, and our previous findings on the effect of melatonin treatment at the end of pregnancy, open new possibilities to optimize lamb performances during lactation. It remains to be elucidated what is the minimal interval between implantation in the pregnant ewes and parturition that maximizes the growth of the lambs during lactation.

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Effects of melatonin implants in late gestation and at lambing on colostrum and milk quality of ewes, birth temperature and growth performance of their lambs

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ABSTRACT

The objective of this study was to measure the effects of melatonin implants in meat ewes at late pregnancy, at lambing, or both, on colostrum and milk composition, growth of their lambs, and lamb surface and rectal temperatures under intensive conditions. Pregnant Rasa Aragonesa ewes, that were previously synchronized (mean parity number (± SD): 2.50 ± 0.82, mean live weight: 55 ± 4 kg; mean body condition: 3.00 ± 0.25) received a subcutaneous melatonin implant thirty days before lambing (M-0, n = 14), at lambing (0-M, n = 14), at both periods (M-M, n = 10), or did not receive an implant (0–0, n = 12). Mean (± SD) prolificacy (number of lambs born/lambing) was 1.43 ± 0.14; 1.57 ± 0.13; 1.40 ± 0.16, and 1.42 ± 0.14 for the M-0, 0-M, M-M and 0–0 groups, resp. The distribution of lambs born according to their sex was: M-0, 7 males, 12 females; 0-M, 10 males, 11 females; M-M, 6 males, 7 females, and 0–0, 8 males, 6 females. Fat (F), protein (P), and lactose (L) content were measured in colostrum (collected at lambing) and milk (collected every two weeks), and °Brix and IgG were quantified in colostrum. Lamb weight (LW) was recorded at birth and every two weeks until weaning (day 42 of age). At birth, lamb rectal temperature (RT) and thermography images were taken (Eye=ET; shoulder=ST; mid loin=MT; hips=HT). P and L concentrations in colostrum were significantly ($P < 0.05$) higher in the M-0 (P: 9.01 ± 0.45, L: 8.53 ± 0.42%) than they were in the M-M group (P: 7.38 ± 0.34, L: 6.99 ± 0.32), with no significant differences among groups for F content. No significant differences were found for birth weight among groups, as well as considering litter size. However, at weaning, male lambs reared by 0-M (12.31 ± 0.57 kg) ewes had significantly ($P < 0.05$) higher LW than did male lambs reared by M-0 (9.43 ± 1.01) or 0–0 (9.65 ± 0.99 kg) ewes. Lambs from M-0 and M-M ewes had the highest MT and HT, and the effects were most pronounced in male lambs. In conclusion, melatonin implants during pregnancy had positive effects on ewes by improving colostrum quality, and increased the MT and HT in lambs. Implants at lambing enhanced the productive performance of ewes and male lambs, but implants at both moments did not provide beneficial effects.

1. Introduction

In order to maintain the efficiency of a sheep meat system, it is crucial to develop management practices that improve neonate survival (Refshauge et al., 2016). Management practices for maximizing the survival rate of lambs include the availability of high-quality colostrum, reducing the risk of hypothermia, a birthweight near the optimum for the breed, an easy birth, and maximizing opportunities for establishing a strong ewe-lamb bond (Hinch and Brien, 2014). Additionally, it is important to obtain appropriate growth rates and produce the most weaned lambs (Notter et al., 2018).

Colostrum is an essential source of nutrition; immunoglobulins and sufficient amount of colostrum greatly improves the neonate's chances of survival until weaning (Banchero et al., 2004). In addition, body heat production is crucial to avoiding hypothermia at birth and maximizing lamb survival. After birth, mechanisms that regulate body temperature are activated by the presence of relatively large amounts of functional brown adipose tissue (BAT) (Basse et al., 2015).

Furthermore, in the pre-weaning stage, lambs depend on milk for their nutrition in their first weeks of life (Mekoya et al., 2009), and increasing the ewe's milk yield and improving its composition are ways to increase lamb growth rate (Galvani et al., 2014; Abecia et al., 2021).

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Recently, studies have evaluated the use of exogenous melatonin as a means of improving ewe and lamb performance in sheep. Melatonin, a hormone that is synthesized and released primarily during the dark phase of the day (Arendt, 1988), has diverse physiological effects on animals (García-Ispierto et al., 2013). Melatonin freely crosses the ovine placenta and the fetal blood–brain barrier (Miller et al., 2005), and can be transferred into maternal milk (Reppert and Klein, 1978). Thus, maternally administered melatonin could have positive effect on newborn lambs and their productive performance in pre-weaning stages (Abecia et al., 2021; Davis et al., 2021).

Exogenous melatonin seems to have effect on colostrum composition. Thus, melatonin implants inserted in the fourth month of gestation, increased immunoglobulin G (IgG) concentrations of sheep colostrum (Abecia et al., 2020; Canto et al., 2022; Tekin and Akkuş, 2023).

Maternal melatonin in gestation influences the amount and normal function of BAT in the neonate (Seron-Ferre et al., 2015). Sales et al. (2017) reported that melatonin implants administered to Corriedale ewes (dual purpose breed) at day 100 of gestation increased BAT in fetuses. In addition, 5–6-day old lambs born to mothers that were subjected to constant light and were supplemented with oral melatonin from day 92 of gestation until birth, had higher temperatures (central, skin and perirenal fat) than those whose mothers were not supplemented with melatonin (Seron-Ferre et al., 2015).

In Assaf ewes, fat concentration increased at day 15 and 90 of lactation, when two melatonin implants were inserted 40 d before lambing (Canto et al., 2022). In Rasa Aragonesa ewes, melatonin implants inserted 24 h after lambing increased milk fat content at the end of lactation, 45 d after lambing (Abecia et al., 2021). Elhadi et al. (2022) reported that melatonin implants at day 35 of lactation did not significantly affect milk composition in Manchega and Lacaune ewes.

The effects of melatonin in lambs can also be reflected in their growth and body development performance. Lambs reared by ewes that had been implanted in the second half of pregnancy (Davis et al., 2021), or 24 h after lambing, had the highest growth rates (Abecia et al., 2021). Furthermore, implanting melatonin in lambs (3.0 and 4.5 mg/kg) at two months of age increased body length, and chest circumference, width and depth (Ma et al., 2022).

It is obvious that, there are different responses to exogenous melatonin in ewes, depending on the period in which it is administered (pregnancy or lactation), or on the breed (dairy or meat), conditioned by the lactation curve and the frequency of milking (Pulina et al., 2007). Consequently, the present study aimed to evaluate the effects of melatonin implants 30 d before lambing, immediately after lambing, or at both moments using meat ewes (Rasa Aragonesa) on colostrum and milk composition, lamb's live weight from birth to weaning, daily gain, body dimensions, and rectal and surface temperatures under indoor conditions.

2. Materials and methods

2.1. Animals and treatments

The flock was housed at the experimental farm of the University of Zaragoza, Spain (41°40′ N 0°53′ W). Fifty healthy pregnant Rasa Aragonesa ewes (mean parity number (± SD): 2.50 ± 0.82; mean live weight 55 ± 4 kg; mean body condition: 3.00 ± 0.25), were involved in the experiment. Ewes were either artificially inseminated on May 8 after a synchronized estrus cycle treatment using intravaginal sponges impregnated with progestogens, and 480 IU eCG (Sincropart, CEVA Salud Animal, Barcelona, Spain), or naturally mated at the second estrus after progestogen treatment. They were maintained indoors, under natural photoperiod conditions. Straw bedding material was laid to a depth of approximately 5 cm over a concrete floor. Fresh straw was laid daily throughout the trial. Sheep were fed to meet their live weight requirements, both during pregnancy and lactation, in conformity with the standards of the AFRC (1993). The diet comprised barley straw ad libitum and 0.45 kg/d per ewe per day of concentrate offered at 08:00 and 13:00 h, which provided 7.8 MJ of metabolizable energy per ewe. The pelleted diet consisted of barley (85%) and soy bean (15%). The animals had unlimited access to water and mineral salts.

Ewes were randomly assigned to one of four groups that either did or did not receive a subcutaneous melatonin implant in the base of the ear (18 mg melatonin; Melovine, CEVA Salud Animal, Barcelona, Spain), at late pregnancy (30 d before lambing) and/or immediately after lambing; specifically, Group M-0 (n = 14; mean (± SEM) liveweight (LW) = 55.1 ± 4.1 kg; mean body condition score (BCS) = 3.00 ± 0.30) implanted before lambing; Group 0-M (n = 14; 54.9 ± 4.5 kg; 2.95 ± 0.23), implanted immediately after lambing: Group M-M (n = 10; 54.5 ± 3.9 kg; 2.98 ± 0.23), implanted on both occasions, and a non-implanted control group (0−0) (n = 12; 54.8 ± 3.7 kg; 3.02 ± 0.29). Implantation date (Sep 11 or Sep 28) was calculated as 30 days before the expected date of lambing (Oct 11 or Oct 28, resp.) (date of insemination or date of natural estrus + 146 days) for those ewes pregnant that were from the insemination or from the natural mating, resp.

Lambing occurred indoors within three weeks in Oct. Just before lambing (140 d of pregnancy), ewes were allocated to individual pens (2 × 2 m) so that the precise moment of lambing could be recorded. Sixty-seven lambs were born, which were distributed in the M-0 (n = 19 lambs; 7 males, 12 females), 0-M (n = 21 lambs; 10 males, 11 females), M-M (n = 13 lambs; 6 males, 7 females), and 0–0 (n = 14 lambs; 8 males, 6 females) groups. The prolificacy (± SD, number of lambs born/lambing) was 1.43 ± 0.14 (8 singles, 11 twins); 1.57 ± 0.13 (6 singles, 15 twins); 1.40 ± 0.16 (6 singles, 7 twins), and 1.42 ± 0.14 (7 singles, 7 twins) for the M-0, 0-M, M-M and 0–0 groups, resp. To ensure a strong ewe-lamb bonding, the ewes remained with their lamb(s) in individual pens for three days. The lambs remained with their respective dams until weaning (42 ± 1 d postpartum) and suckled freely. Intervals (mean ±SEM) between day of melatonin implantation in M-0 (32.15 ± 0.74, 49.00 ± 0.00, 60.53 ± 1.26 and 73.70 ± 0.84 d) and M-M group (32.77 ± 0.54, 49.00 ± 0.00, 62.20 ± 1.20, and 74.90 ± 0.73 d) at pregnancy and the day of lambing, the weeks 2, 4, and 6 (weaning), respectively, or in 0-M group (17.57 ± 0.59, 30.00 ± 0.98 and 42.92 ± 0.74 d) and in M-M group (16.30 ± 0.47, 29.50 ± 1.08 and 42.09 ± 0.66 d) at lambing and weeks 2, 4 and 6 (weaning) respectively, did not differ.

2.2. Colostrum and milk sample collection

Colostrum samples (20 ml) were collected immediately after lambing, and the nutritional composition of the fresh sample was analyzed. Thereafter, colostrum samples were frozen and stored at − 20 °C until the IgG analysis. Milk samples (10 ml) were collected by hand-milking at 2, 4, and 6 wk postpartum, and the milk composition analysis was performed immediately.

2.3. Colostrum and milk analysis

°Brix were estimated by a refractometer (Deltatrak, Pleasanton, CA, USA) immediately after colostrum samples had been collected. IgG colostrum concentrations were measured based on the Calokit-Sheep Test (ZEULAB, Zaragoza, Spain) (Galán-Malo et al., 2014). All samples were diluted in distilled water to ensure that they were within the working range of the test. A Multiskan microplate reader (Labsystems, Helsinki, Finland), under an absorbance of 450-nm, provided the reading. To quantify the concentration of IgG in the colostrum samples, a polynomial calibration curve derived from the absorbance values of the standards was used.

Colostrum and milk samples were analyzed for fat (F), protein (P), and lactose (L) by an automatic milk analyzer (Lactoscan SP+) calibrated for sheep following the manufacturer's instructions (Milkotronic Ltd., Tsentar, Nova Zagora, Bulgaria). To ensure that the colostrum samples were within the measurement range of the equipment, they were diluted 1:2 before the analysis.

2.4. Liveweight, average daily gain, and body measurements

A digital dynamometer recorded lamb LW at birth, week 2, week 4, and at weaning (week 6). Average daily gain (ADG) was based on the intervals between the sampling days. At birth and at weaning, five features were measured to define the body size of the animals (Idris et al., 2011). Body length (BL) was measured from the anterior shoulder point to *tuber ischiadicum*, chest girth (CG) was the shortest circumference around the animal just behind the shoulder, wither height (WH) was the vertical distance behind the forelegs to the ground, rump height (RH) was from the surface of a platform to the rump, and shoulder width (SW) was the horizontal distance between the processes on the left and right shoulder blades.

2.5. Rectal and surface temperatures

At birth, the rectal temperature of the lambs was recorded by a conventional thermometer, and the surface temperature was recorded by a thermographic camera (Teso 880, Teso SE & Co. KGaA, Titisee-Neustadt, Germany). The camera specifications were as follows: temperature range − 30 to + 650 °C, resolution focus (manual) 320 × 240, image refresh rate 27 Hz, emissivity 0.01 to 1, with automatic recognition of emissivity and determination of reflected temperature. For eye temperature (ET), the camera was positioned 40 cm from the lamb's head. The other body surface temperatures were measured 1 m from the dorsal midline of the lamb. Temperature data were analyzed by Testo IRSoft, where three fixed-size equidistant areas were identified: shoulder (ST), mid-loin (MT), and hips (HT) (Labeur et al., 2017). For each image, minimum, average, and maximum temperatures were recorded. Mean ambient temperature and relative humidity (± SD) when the images were recorded were 7.86 ± 0.52 °C and 85.6 ± 2.67%, respectively.

2.6. Statistical analysis

Colostrum composition, rectal temperature, and body surface thermography data were evaluated statistically by a multifactorial model that was based on the least square's method of the General Linear Mixed (GLM). Colostrum composition (IgG, F, P and L), lamb's rectal temperature and body surface thermography were evaluated. The model included melatonin treatment, litter size (single or multiple lambs), offspring sex (male, female, or male + female) and their interactions as fixed effects.

Milk quality, LW, ADG, and body measurement data were analyzed based on a General Linear Mixed (GLM) Model procedure for repeated measurements. The model for milk quality included melatonin treatment, week, and their interactions as fixed effects. The model for LW, ADG, and body measurements included melatonin treatment of their dam, week, litter size and sex, and their interactions as fixed effects. The dam was included as a random effect. Thereafter, variables were evaluated statistically within fixed effects by an ANOVA. Individual ANOVA were performed to compare differences among the periods, within the groups. If significant differences were detected, differences among means were tested by least significant differences. Statistical analyses were performed by SPSS Statistics v.26 (IBM Corp, Released, 2019). Results are expressed as mean ± SEM, or least square mean ± SEM, and a p-value ≤ 0.05 was considered statistically significant.

3. Results

3.1. Colostrum

The GLM procedure indicated that melatonin treatment had a significant effect on the P ($P = 0.026$) and L ($P = 0.026$) content of the colostrum, but neither litter size nor offspring sex, nor their interactions had a significant effect on colostrum composition. Protein and L concentrations of the colostrum were highest in the M-0 group, and lowest in M-M, with the other two groups showing intermediate values. Melatonin treatments did not have a significant effect on the °Brix, IgG concentrations, or F concentrations of the colostrum (Table 1).

3.2. Milk quality

Melatonin treatment had no significant effect on milk composition. Week of lactation had a significant effect on the F ($P < 0.001$), P ($P < 0.05$), and L ($P < 0.001$) concentrations of the milk (Table 2). At the last milk sampling (week 6), the F of the M-0, 0-M, and 0–0 groups was significantly ($P < 0.05$) higher than it was in week 2 or week 4 (Fig. 1). Protein content of the milk from the M-M group increased significantly ($P < 0.05$) between weeks 4 and 6, but the L content of the milk from the M-0 and 0–0 groups decreased significantly ($P < 0.05$) between weeks 4 and 6. Nevertheless, F (M-M group), P (M-0, 0-M and 0–0 groups) and L (0-M and M-M groups) content did not differ significantly among weeks (Fig. 1).

3.3. LW, ADG and body measurements

Melatonin treatment had no significant effect on LW, ADG and body lamb dimensions. The litter size had significant effect ($P < 0.001$) on LW, ADG, BL, CG, WH, RH and SW. The interaction between treatment and offspring sex had significant effects ($P < 0.05$) on LW, ADG, CG, WH and SW. For BL, that interaction effect was close to significance ($P = 0.16$). The dam effect significantly affected the RH ($P < 0.01$) and SW ($P < 0.05$) (Table 2).

Melatonin treatments did not have a significant effect on lamb LW at birth, 2 weeks and 4 weeks of age, or weaning LW, and ADG and daily weight gains from birth to weaning (Table 3), or body measurements.

From birth up to week 2 of age, LW of male lambs did not differ between groups. At week 4, male lambs reared by 0-M dams (12.31 ± 0.57 kg) had significantly ($P < 0.05$) higher LW than did male lambs from M-0 or 0–0 dams (9.43 ± 1.01 and 9.65 ± 0.99 kg, respectively), and a similar tendency ($P = 0.13$) in LW occurred at weaning. In the second week, female lambs reared by M-0 (7.54 ± 0.40 kg) or 0–0 (7.91 ± 0.62 kg) ewes had a significantly ($P < 0.05$) higher LW than did female lambs from 0-M (6.18 ± 0.48 kg) ewes. In the last two records, female lambs reared by 0–0 ewes tended ($P = 0.12$) to have a higher LW than did female lambs from 0-M ewes (Table 3).

At weaning, the CG of male lambs differed significantly ($P < 0.05$) among groups. In female lambs, CG ($P = 0.011$), WH ($P < 0.05$), RH ($P < 0.05$), and SW ($P = 0.01$) differed significantly among groups at birth, and in WH ($P = 0.01$) and RH ($P = 0.01$) at weaning. At birth, 0-M female lambs showed the lowest CG, WH, RH and SW, and continued to be the lowest at weaning for WH and RH. At weaning, 0-M male lambs showed the highest CG, meanwhile female lambs of the same group tended ($P = 0.17$) to have the lowest values (Fig. 2).

3.4. Rectal and surface temperatures

Melatonin treatment had a significant effect on minimum and

Table 1
Mean (± SEM) concentration of IgG, °Brix, fat, protein, and lactose content in colostrum collected immediately after lambing from Rasa Aragonesa ewes that received a melatonin implant either in late pregnancy (M-0), at lambing (0-M), in late pregnancy and at lambing (M-M) or did not receive an implant (0–0) (a,b: different superscripts indicate significant differences among groups, $p < 0.05$).

	M-0	0-M	M-M	0-0
°Brix (%)	28.74 ± 1.36	26.60 ± 1.93	28.84 ± 1.96	25.71 ± 1.73
IgG (mg/ml)	52.80 ± 7.19	46.33 ± 9.17	33.05 ± 6.64	43.12 ± 8.96
Fat (%)	8.58 ± 1.17	9.34 ± 0.87	9.17 ± 1.39	7.93 ± 1.18
Protein (%)	9.01 ± 0.45^{a}	8.24 ± 0.41^{ab}	7.38 ± 0.34^{b}	8.19 ± 0.55^{ab}
Lactose (%)	8.53 ± 0.42^{a}	7.80 ± 0.39^{ab}	6.99 ± 0.32^{b}	7.75 ± 0.52^{ab}

Table 2
Least square means (± SEM) of fat, protein, and lactose concentrations in milk from Rasa Aragonesa ewes that received a melatonin implant either in late pregnancy (M-0), at lambing (0-M), in late pregnancy and at lambing (M-M) or did not receive an implant (0–0) and the live weight (LW, kg), average daily gain (ADG, kg) and body dimensions (body length, chest girth, whither height, rump height and shoulder width, cm) of their respective lambs for fixed and random effects (repeated measures analyses).

		Least squares mean (± SEM) per treatment				Effect (P-value)						
	Item	M-0	0-M	M-M	0-0	Mel-Treat.	Week	Litter size	Sex	Mel- Treat*Litter size	Mel- Treat* Sex	Dam
Milk	Fat (%)	5.19 ± 0.33	5.50 ± 0.365	5.50 ± 0.40	5.17 ± 0.40	0.82	< 0.001	-	-	-	-	-
	Protein (%)	5.01 ± 0.07	5.00 ± 0.07	5.09 ± 0.08	4.92 ± 0.08	0.57	0.037	-	-	-	-	-
	Lactose (%)	5.03 ± 0.10	4.92 ± 0.10	4.97 ± 0.11	4.81 ± 0.11	0.54	< 0.001	-	-	-	-	-
Lambs	Liveweight (kg)	8.43 ± 0.41	9.17 ± 0.42	8.64 ± 0.62	8.66 ± 0.546	0.62	< 0.001	< 0.001	0.40	0.98	0.05	0.89
	ADG (kg)	0.196 ± 0.15	0.218 ± 0.16	0.198 ± 0.23	0.197 ± 0.20	0.66	0.50	< 0.001	0.58	0.98	0.026	0.84
	Body length (cm)	40.61 ± 1.04	42.37 ± 1.08	39.62 ± 1.55	40.30 ± 1.38	0.34	< 0.001	< 0.001	0.72	0.24	0.16	0.65
	Chest girth (cm)	50.11 ± 0.80	51.33 ± 0.83	51.24 ± 1.20	50.99 ± 1.07	0.65	< 0.001	< 0.001	0.42	0.98	0.05	0.83
	Whiters height (cm)	48.04 ± 0.67	49.19 ± 0.69	48.20 ± 0.99	48.79 ± 0.88	0.75	< 0.001	< 0.001	0.43	0.50	0.03	0.32
	Rump height (cm)	48.42 ± 0.72	49.81 ± 0.74	48.24 ± 1.07	50.12 ± 0.95	0.62	< 0.001	< 0.001	0.68	0.90	0.20	0.005
	Shoulder width (cm)	14.15 ± 0.25	13.84 ± 0.26	14.16 ± 0.39	13.90 ± 0.34	0.89	< 0.001	< 0.001	0.55	0.39	0.01	0.016

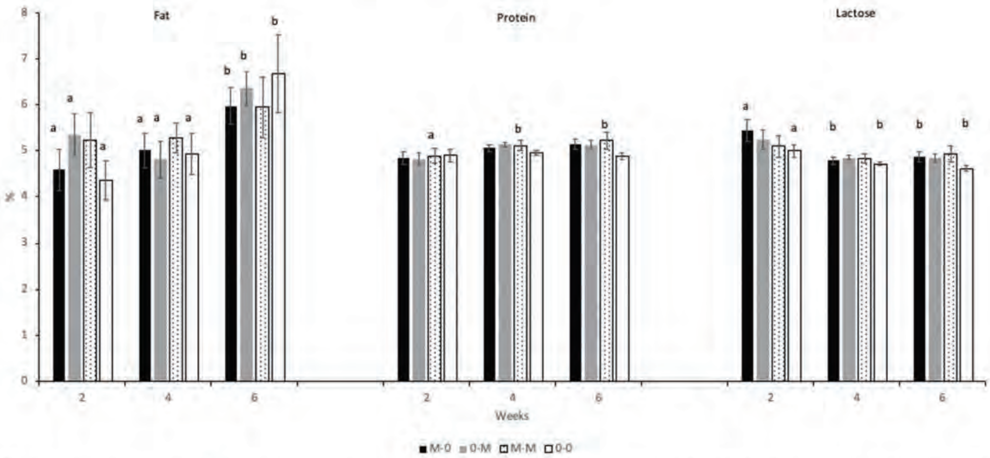

Fig. 1. Least square means (± SEM) fat, protein, and lactose concentrations in milk samples collected in weeks 2, 4, and 6 from Rasa Aragonesa ewes that received a melatonin implant either in late pregnancy (M-0), at lambing (0-M), in late pregnancy and at lambing (M-M) or did not receive an implant (0–0) (a,b: indicate significant differences among the weeks, within the groups, $p < 0.05$).

maximum MT, maximum HT ($P < 0.05$), and minimum HT ($P < 0.001$), and might ($P = 0.10$) have had an effect on minimum and average HT. In addition, the interaction treatment x sex x litter size, and sex x litter size had a significant ($P < 0.05$) effect on minimum ST and rectal temperature, respectively.

Lambs from ewes that received a melatonin implant in late pregnancy (M-0 and M-M groups) had the highest minimum and maximum MT ($P < 0.05$), and the highest minimum, average, and maximum HT ($P < 0.01$) (Table 4). The effects of melatonin implants in the mothers on rectal and surface temperatures were most pronounced in male lambs; specifically, male lambs born from M-0 and M-M ewes presented the highest rectal temperature, average MT, maximum MT, and maximum HT (Table 5). Male lambs from the M-0 ewes had significantly higher average and maximum ST, and higher average HT than did male lambs born to non-treated control ewes. Minimum HT was significantly higher in male lambs from the M-0 group than it was in the 0-M and 0–0 groups. However, these effects of melatonin implants in rectal and body surface temperatures were not detected in females' lambs (Table 5).

4. Discussion

In small ruminants, the transfer of prepartum components from the maternal circulation into colostrum occurs during the last weeks of gestation (Castro et al., 2011); therefore, the effects of melatonin on

Table 3
Least square means (± SE) live weight (LW, kg) average daily gain (kg/d) from birth-to weaning of Rasa Aragonesa lambs whose mothers received a melatonin implant either in late pregnancy (M-0), at lambing (0-M), in late pregnancy and at lambing (M-M) or did not receive an implant (0–0) by whole lambs (n = 67), litter size (singles, n = 27; twins, n = 40) and progeny sex (males, n = 31; females, n = 36).) (a, b indicates significant differences among groups, $p < 0.05$); (x,y,z indicates differences among groups, $p = 0.10$).

	Item	M-0	0-M	M-M	0-0
	LW Birth	4.17	3.94	4.32	4.26
		± 0.19	± 0.24	± 0.24	± 0.23
	LW 2	7.35	7.49	7.30	7.64
	weeks	± 0.37	± 0.48	± 0.45	± 0.53
	LW 4	9.47	10.04	9.74	10.12
	weeks	± 0.52	± 0.63	± 0.66	± 0.74
	LW	11.85	12.46	12.21	12.61
	Weaning	± 0.71	± 0.81	± 0.87	± 0.99
	ADG birth-	0.19	0.20	0.19	0.19
	weaning	± 0.01	± 0.02	± 0.02	± 0.02
Singles	LW Birth	4.84	4.80	4.94	4.75
		± 0.12	± 0.22	± 0.18	± 0.34
	LW 2	8.22	9.88	8.46	8.63
	weeks	± 0.64	± 0.75	± 0.73	± 0.82
	LW 4	11.46	13.07	11.54	11.64
	weeks	± 0.57	± 0.65	± 0.91	± 1.08
	LW	14.64	16.38	14.52	14.66
	Weaning	± 0.80	± 0.90	± 1.21	± 1.38
	ADG birth-	0.24	0.27	0.23	0.24
	weaning	± 0.02	± 0.02	± 0.03	± 0.03
Twins	LW Birth	3.69	3.61	3.78	3.77
		± 0.22	± 0.29	± 0.29	± 0.19
	LW 2	6.72	6.54	6.31	6.66
	weeks	± 0.36	± 0.41	± 0.17	± 0.47
	LW 4	8.03	8.83	8.20	8.60
	weeks	± 0.42	± 0.62	± 0.41	± 0.66
	LW	9.83	10.89	10.23	10.56
	Weaning	± 0.53	± 0.77	± 0.62	± 1.01
	ADG birth-	0.15	0.17	0.15	0.16
	weaning	± 0.01	± 0.01	± 0.01	± 0.02
Males	LW Birth	4.37	4.70	4.63	4.20
		± 0.29	± 0.17	± 0.26	± 0.37
	LW 2	7.02	8.95	7.800.83	7.44
	weeks	± 0.79	± 0.62		± 0.83
	LW 4	9.43	12.3	10.12	9.65
	weeks	± 1.02^{b}	± 10.58^{a}	± 1.04^{ab}	± 1.00^{b}
	LW	11.73	15.25	12.71	11.97
	Weaning	± 1.36^{x}	± 0.88^{y}	± 1.45^{xy}	± 1.37^{x}
	ADG birth-	0.18	0.25	0.19	0.19
	weaning	± 0.03	± 0.02	± 0.03	± 0.03
Females	LW Birth	4.06	3.26	4.05	4.34
		± 0.25	± 0.32	± 0.37	± 0.25
	LW 2	7.54	6.18	6.87	7.91
	weeks	± 0.39^{a}	± 0.48^{b}	± 0.45^{ab}	± 0.62^{a}
	LW 4	9.50	7.98	9.42	10.75
	weeks	± 0.61^{xy}	± 0.61^{x}	± 0.90^{xy}	± 1.16^{y}
	LW	11.93	9.91	11.79	13.46
	Weaning	± 0.85^{xy}	± 0.72^{x}	± 1.13^{xy}	± 1.52^{y}
	ADG birth-	0.19	0.15	0.19	0.20
	weaning	± 0.02	± 0.01	± 0.02	± 0.03

colostrum quality should occur in sheep that received melatonin before lambing (in our study, M-0 and M-M groups), rather than in those that received the hormone implant at lambing (0-M group) or those that did not receive an implant (0–0 group). In our experiment, however, colostrum composition did not differ significantly between those groups (M-0 and M-M vs. 0-M and 0–0). Melatonin implants affected the protein and lactose concentrations of the colostrum; specifically, the colostrum from ewes that received a melatonin implant in late pregnancy (M-0) had higher P and L concentrations than did the colostrum from ewes that received an implant in pregnancy and at lambing (M-M). It is likely that the increase of lactose concentration in the colostrum of the group M-0 is the result of a greater energy availability in the form of glucose, a precursor of lactose (Banchero et al., 2004). Recent research findings have suggested the involvement of melatonin in glucose metabolism in mammals (Watanabe et al., 2023). Therefore, this melatonin effect on energetic metabolism could justify the higher levels of lactose observed in colostrum. On the other hand, as melatonin plays a role in mediating ruminal microbes and their metabolic pathway (Fu et al., 2023), it is likely that the increase in colostrum protein in the M-0 group could be attributed to a change in rumen microorganisms.

In our study, we did not find effects of melatonin implants on the concentration of IgG and °Brix of colostrum. In previous studies, the main effect of exogenous melatonin in ewes in late pregnancy was on colostrum IgG concentrations. In the fourth month of pregnancy, one (Abecia et al., 2020, Canto et al., 2022, Tekin and Akkuş, 2023) or two (Canto et al., 2022) melatonin implants increased colostrum IgG. Contradictions in results may be affected by differences in analytical procedures and sampling protocols in colostrum (Kessler et al., 2019). In relation to the °Brix, which is a tool for assessing colostrum quality, there are few studies that have evaluated sheep colostrum quality on-farm by this way (Kessler et al., 2021). In our study, the °Brix of colostrum did not differ significantly between treated and control ewes. Similar results were reported by Canto et al. (2022), when implanting ewes with melatonin (18 or 36 mg) 40 d before lambing. It should be mentioned that the °Brix were within the values reported by Santiago et al. (2020) and Kessler et al. (2021).

The effects of melatonin on milk composition have been heterogeneous due to the modulation of lipid metabolism by melatonin in different species (Le Gouic et al., 1997; Bartness et al., 2002). Furthermore, studies have shown that melatonin plays important roles in regulating adipose differentiation and fat synthesis (Acuna-Castroviejo et al., 2014; Yang et al., 2017). However, in our study, milk composition did not differ between treatment groups. Similarly, in other studies, milk composition was unaffected by pre-lambing melatonin administration in dairy goats (Avilés et al., 2019), melatonin administration at weaning in two dairy breeds (Elhadi et al., 2022), and exposure to a short photoperiod in dairy cows (Miller et al., 2000; Auchtung et al., 2005).

Similar to the studies of Flinn et al. (2020a, b), we did not detect an effect of melatonin treatment on birth weight. In another study, however, one (18 mg melatonin/ewe) or two (36 mg melatonin/ewe) melatonin implants administered to ewes at day 100 of gestation increased the birth weight of twin male lambs (Sales et al., 2019). The nutritional status of the dams could explain the different effects on birth weight; thus, in nutrient-restricted rats, oral melatonin enhanced the production of placental antioxidant enzymes and may improve placental nutrient transfer capacity, fetal organ development, birth weight and fetal growth (Richter et al., 2009).

In our study, the effects of melatonin implants on lamb LW differed based on the sex and the moment the implant was inserted. The highest LW was observed in 0-M males and in M-0 female lambs. Abecia et al. (2021) reported that, in the second, third, and fourth week of suckling period, development was highest in pre-weaning male lambs of ewes that had received exogenous melatonin (18 mg melatonin/ewe) 24 h after parturition.

In our study, the number of days since implantation of the ewe influenced the effects of exogenous melatonin on the LW of male and female lambs; i.e., in male and female lambs, the effects were apparent at 30 d and 49 d after implantation of the dam, respectively. Zúñiga et al. (2002) showed that melatonin implants in ewes (18 mg melatonin) gradually release the hormone to various tissues and plasma melatonin concentrations were highest 37 d after implantation. Although the lambs did not receive a melatonin implant, they were supplemented indirectly through maternal circulation (M-0) or breast milk (M-0 and 0-M). The transplacental transfer of melatonin from the maternal circulation to the fetus has been documented in ewes (Zemdegs et al., 1988). Recently, in vitro experiments have shown that mechanisms of the efficient transport of melatonin into milk are mediated by the ovine ATP-binding cassette G2 (ABCG2) (Álvarez-Fernández et al., 2023). This indirect melatonin supplementation, according to our data, enhanced the growth of lambs. Similarly, Ma et al. (2022) reported that the effect of melatonin in lambs

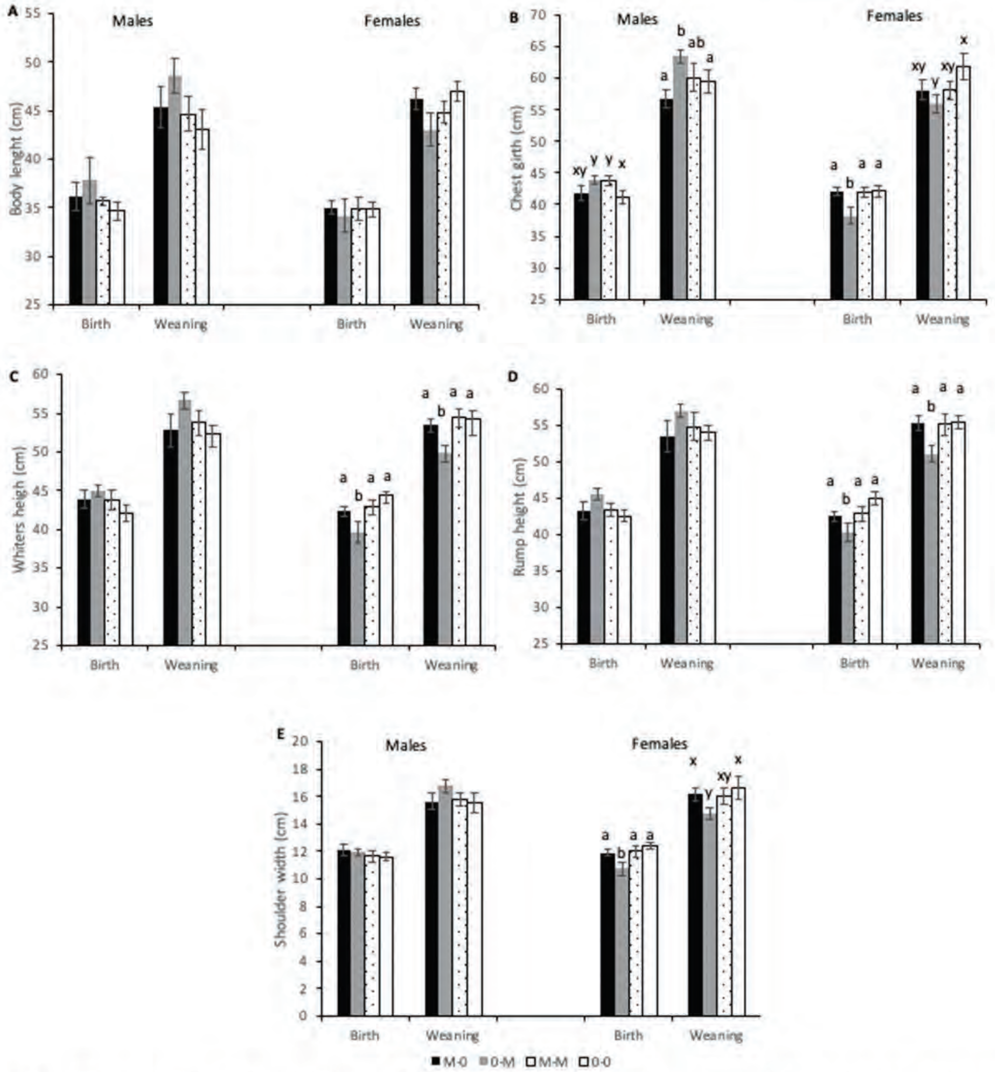

Fig. 2. Least square means (± SE) body length (A, cm), chest girth (B, cm), whiters height (C, cm), rump height (D, cm) and shoulder width (E, cm) at birth and weaning of male and female Rasa Aragonesa lambs whose mothers received a melatonin implant either in late pregnancy (M-0), at lambing (0-M), in late pregnancy and at lambing (M-M) or did not receive an implant (0–0) (a,b: indicate significant differences among groups, within the sex, $p < 0.05$) (x,y: indicate differences among groups, within the sex, $p = 0.10$).

was improving muscle growth and development, by increasing the cross-sectional area of muscle fiber and adipose tissue cells, possibly by being involved in gene expression of apoptotic signaling pathway.

In our study, CG, WH, RH, and SW were shortest in the female lambs that were born from ewes that had received a melatonin implant at lambing, which reflected that those lambs were the smallest at birth. However, those ewes (0-M) should not have had an effect on their offspring at birth because the bioavailability of the hormone is not immediate at the time of application (Zúñiga et al., 2002). Therefore, other factors might have influenced the size and conformation of the body at birth and its development; e.g., genetic potential (dam and sire), litter size, and environmental conditions. Studies on the maternal heritability

Table 4
Mean (± SE) rectal temperature, and minimum, average, and maximum body surface temperature (°C) measure by infrared thermography (Eye, Shoulder, Mid loin, Hips) of Rasa Aragonesa lambs whose mothers received a melatonin implant either in late pregnancy (M-0), at lambing (0-M), in late pregnancy and at lambing (M-M), or did not receive an implant (0–0) (a,b indicates significant differences among groups, $p < 0.05$, * indicates differences among groups, $p < 0.001$); (x,y,z indicate differences among groups, $p = 0.10$).

	M-0	0-M	M-M	0-0
Rectal temperature				
Mean	39.17 ± 0.08^{y}	38.77 ± 0.13^{xy}	38.98 ± 0.30^{xy}	38.69 ± 0.13^{x}
Eye temperature				
Minimum	30.53 ± 0.42^{y}	29.64 ± 0.43^{x}	30.56 ± 0.49^{y}	29.46 ± 0.43^{x}
Average	33.97 ± 0.54^{z}	32.68 ± 0.41^{y}	33.83 ± 0.37^{xz}	32.82 ± 0.48^{xy}
Maximum	35.67 ± 0.45	34.81 ± 0.51	35.90 ± 0.39	35.46 ± 0.51
Shoulder surface temperature				
Minimum	19.97 ± 0.37^{y}	19.08 ± 0.42^{xy}	19.53 ± 0.50^{xy}	18.45 ± 0.53^{x}
Average	23.46 ± 0.58^{y}	22.10 ± 0.48^{x}	23.08 ± 0.48^{x}	21.73 ± 0.54^{x}
Maximum	25.91 ± 0.75	24.62 ± 0.81	24.98 ± 0.60	23.81 ± 0.64
Mid loin surface temperature				
Minimum	19.99 ± 0.37^{a}	18.80 ± 0.33^{b}	19.93 ± 0.38^{a}	18.70 ± 0.45^{b}
Average	23.65 ± 0.55^{y}	22.47 ± 0.50^{xy}	23.66 ± 0.46^{y}	22.11 ± 0.57^{x}
Maximum	25.97 ± 0.56^{a}	24.23 ± 0.58^{b}	25.64 ± 0.44^{a}	23.76 ± 0.64^{b}
Hips surface temperature				
Minimum	$20.93 \pm 0.55^{a*}$	$18.95 \pm 0.41^{b*}$	$20.38 \pm 0.52^{a*}$	$18.76 \pm 0.52^{b*}$
Average	$23.97 \pm 0.59^{a*}$	$22.10 \pm 0.48^{b*}$	$23.64 \pm 0.38^{a*}$	$21.97 \pm 0.65^{b*}$
Maximum	$25.84 \pm 0.62^{a*}$	$23.90 \pm 0.55^{b*}$	$25.78 \pm 0.42^{a*}$	$23.66 \pm 0.71^{b*}$

of CG and WH indicate that genetic effects are important for these traits at birth (Mandal et al., 2008). Thus, some caution is required in the interpretation of our results, since the influence of the sire on lamb's birth weight has not been considered in the present experiment. However, it should be considered that the growth of lambs before weaning is also affected by the maternal ability of their mothers, as well as the variation of milk produced and its direct impact on the growth of lambs until weaning (Abdullah, 2023). In relation to litter size, there is a stronger compensatory growth after weaning for multiple-born lambs compared to single-born lambs (Kelman et al., 2022). In our study, the rearing period was short (42.21 ± 0.29 d); therefore, probably, the persistence until weaning of the lower development in the WH and RH of the lambs from mothers that received a melatonin implant at lambing is because of the dimensions at birth, and weights at birth and at weaning are correlated (Abdullah and Tabbaa, 2011). Male lambs from mothers that received an implant at lambing had the largest chest girth at weaning. Ma et al. (2022) found that exogenous melatonin in doses of 3.5 or 4.0 mg/kg increased chest width in two-month-old lambs beginning 30 d after implantation.

In our study, melatonin implants in the dam did not affect rectal temperature in whole newborn lambs. Similar results have been reported in sheep reared under extensive conditions that had received subcutaneous (18 or 36 mg melatonin/ewe) or oral melatonin (140 mg/ewe) in gestation (Flinn et al., 2020a, b), and in sheep reared in permanent housing implanted (18 mg melatonin/ewe) at the third or fourth month of pregnancy (Abecia et al., 2020). In our study, however, thermography indicated that MT and HT were highest in lambs born of dams that had received a melatonin implant in pregnancy. Freitas-de-Melo et al. (2021) reported that the surface temperature of the hip was highest in lambs from ewes that received a melatonin implant at 100 d of gestation. The improvement in the primary mechanism for neonatal thermogenesis might be because of an increase in brown adipose tissue caused by melatonin supplementation of pregnant sheep (Sales et al., 2017, 2019). In addition, fetal hypoxia increases the concentration of plasma norepinephrine, which causes peripheral vasoconstriction and a reduction in surface temperature (Sahni, 2017). Prenatal melatonin supplementation can protect the fetus against acute hypoxia in sheep (see review by Flinn et al., 2020c); therefore, lambs from implanted mothers might indirectly display vasodilation in the peripheral blood system (which increases surface temperature). Nevertheless, the absence of treatment effects on the other thermographic body regions (ET and ST) might have been caused by the high variability in surface temperatures across body regions measured by thermography, which might have been caused by the heterogeneous distribution of blood flow in peripheral tissues (Vicente-Pérez et al., 2019). The interaction between

Table 5
Mean (± SE) rectal temperature, and minimum, average, and maximum body surface temperature (°C) measure by infrared thermography (Eye, Shoulder, Mid loin, Hips) temperature of male and female Rasa Aragonesa lambs whose mothers received a melatonin implant either in late pregnancy (M-0), at lambing (0-M), in late pregnancy and at lambing (M-M) or did not receive an implant (0–0) (a,b: indicate significant differences among groups, $p < 0.05$) (x,y,z: indicate differences among groups, $p = 0.10$).

	Males				Females			
	M-0	0-M	M-M	0-0	M-0	0-M	M-M	0-0
Rectal temperature								
Mean	39.11 ± 0.16^{a}	39.11 ± 0.13^{a}	39.31 ± 0.12^{a}	38.69 ± 0.21^{b}	39.20 ± 0.09	38.51 ± 0.18	38.69 ± 0.56	38.69 ± 0.16
Eye temperature								
Minimum	31.00 ± 0.32^{xy}	29.51 ± 0.80^{x}	31.26 ± 0.63^{y}	29.33 ± 0.64^{x}	30.28 ± 0.62	29.74 ± 0.48	29.93 ± 0.69	29.60 ± 0.61
Average	33.87 ± 0.64^{xy}	32.70 ± 0.67^{x}	34.28 ± 0.55^{y}	32.33 ± 0.71^{x}	34.02 ± 0.77	32.66 ± 0.54	33.43 ± 0.50	33.31 ± 0.64
Maximum	35.74 ± 0.72	34.96 ± 0.66	36.29 ± 0.60	34.99 ± 0.81	35.62 ± 0.59	34.69 ± 0.76	35.56 ± 0.52	35.93 ± 0.63
Shoulder surface temperature								
Minimum	20.87 ± 0.35^{y}	18.90 ± 0.52^{x}	19.29 ± 0.73^{xy}	18.53 ± 0.74^{x}	19.48 ± 0.50	19.22 ± 0.64	19.74 ± 0.73	18.38 ± 0.80
Average	24.25 ± 0.78^{a}	22.22 ± 0.58^{bc}	23.60 ± 0.63^{ac}	21.31 ± 0.69^{b}	23.02 ± 0.78	22.02 ± 0.74	22.62 ± 0.72	22.15 ± 0.84
Maximum	26.98 ± 1.52^{a}	24.28 ± 0.70^{bc}	25.91 ± 0.80^{ac}	23.21 ± 0.79^{b}	25.32 ± 0.81	24.88 ± 1.35	24.16 ± 0.82	24.41 ± 1.02
Mid loin surface temperature								
Minimum	20.64 ± 0.52	18.82 ± 0.53	$20,34 \pm 0.60$	$18,40 \pm 0.47$	19.64 ± 0.48	18.78 ± 0.44	19.57 ± 0.50	19.00 ± 0.79
Average	24.41 ± 0.59^{a}	22.53 ± 0.71^{b}	23.81 ± 0.55^{a}	21.69 ± 0.72^{b}	23.24 ± 0.77	22.42 ± 0.73	23.53 ± 0.74	22.54 ± 0.91
Maximum	26.41 ± 0.75^{a}	24.20 ± 0.79^{b}	26.09 ± 0.33^{a}	23.10 ± 0.77^{b}	25.73 ± 0.78	24.25 ± 0.87	25.24 ± 0.79	24.41 ± 1.01
Hips surface temperature								
Minimum	21.87 ± 0.81^{a}	19.27 ± 0.72^{b}	20.04 ± 0.77^{ab}	18.66 ± 0.46^{b}	20.42 ± 0.70	18.71 ± 0.48	20.69 ± 0.74	18.85 ± 0.96
Average	24.78 ± 0.71^{a}	22.08 ± 0.77^{bc}	23.81 ± 0.55^{ac}	21.49 ± 0.74^{b}	23.52 ± 0.82	22.12 ± 0.64	23.49 ± 0.55	22.45 ± 1.10
Maximum	26.51 ± 0.83^{a}	23.86 ± 0.78^{b}	26.18 ± 0.49^{a}	23.05 ± 0.82^{b}	25.47 ± 0.84	23.94 ± 0.80	25.43 ± 0.66	24.28 ± 1.17

melatonin treatment and sex indicated that the effects of exogenous melatonin were most prominent in male lambs, and males from pregnant implanted ewes had higher surface temperatures in most of the areas measured (RT, ST, MT and HT) than did control male lambs. Clarke et al. (2012) stated that there is a sexual dimorphism in the regulation of thermogenesis at the following three levels: 1) direct effects on peripheral tissues, 2) intrinsic central regulation of sympathetic outflow, and 3) modulation of the central actions of metabolic factors such as leptin.

5. Conclusions

In conclusion, the administration of melatonin to ewes in late pregnancy (30 d before lambing) increased the concentration of protein and lactose in the colostrum of Rasa Aragonesa, a meat sheep breed. Implants inserted immediately after lambing, only, tended to increased lamb performance (especially, the LW of male lambs). The addition of a melatonin implant in pregnancy and lambing did not add to the effects that exogenous melatonin had on lamb growth. Thermography indicated that the application of melatonin in pregnancy might influence thermoregulation in the lamb at birth, which can increase in the temperatures of the mid-loin and the hip surface, particularly, in male lambs under intensive conditions. Further studies involving a higher number of animals should be considered.

Ethics approval and consent to participate

The experiment followed a protocol (PI47/21) that was approved by the Ethics Committee of the University of Zaragoza (21/09/2023), which met the requirements of the European Union for Scientific Procedure Establishments.

CRediT authorship contribution statement

Canto Francisco: Writing – review & editing, Writing – original draft, Methodology, Data curation, Conceptualization. **Abecia José-Alfonso:** Writing – review & editing, Writing – original draft, Validation, Supervision, Project administration, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of Competing Interest

The authors declare no conflict of interest.

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Effects of melatonin implants on locomotor activity, body temperature, and growth of lambs fed a concentrate-based diet

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ABSTRACT

Melatonin is involved in the regulation of circadian rhythms and is implicated in seasonal reproduction in sheep. In several physiological mechanisms, it acts as an antioxidant and an anti-inflammatory molecule, regulating energy metabolism. This work investigated the effects of melatonin implants at 30 days of age on locomotor activity (LA), body temperature, and growth of fattening female and male lambs. Sixty lambs were divided into two groups: one of which received melatonin (MEL, 15 males, 16 females) and a control group (CTR, 16 males, 14 females). In the melatonin group, two 18 mg melatonin implants were placed at 30 days of age. Lambs were fattened for 6 weeks from weaning (45 days of age) to slaughter (85 days). The feed conversion rate (FCR) was calculated based on live weight and the amount of concentrate consumed. LA was measured weekly by actigraphy, and circadian rhythmicity was calculated. Rectal (Trec) and surface temperatures (Tsur) were recorded in the last week of fattening, and subcutaneous fat thickness (FT) over the *longissimus dorsi* muscle was measured by ultrasound scanning. Treatment did not affect FCR, although MEL female lambs consumed significantly ($P < 0.001$) less concentrate than CTR lambs. Treatment and sex had a significant ($P < 0.05$) interaction effect on FT; specifically, FT was significantly ($P < 0.05$) higher in female MEL lambs (3.22 ± 0.21 mm) than in female CTR lambs (2.57 ± 0.24 mm), but FT in males did not differ between MEL (2.77 ± 0.21) and CTR (2.94 ± 0.24 mm) lambs. Overall activity was significantly ($P < 0.001$) lower in the MEL lambs (72.22 ± 0.10 counts/min) than in the CTR lambs (78.89 ± 0.12 counts/min). MEL lambs had a significantly ($P < 0.01$) lower Trec (CTR: 39.00 ± 0.07; MEL: 38.68 ± 0.10) and Tsur for all body regions evaluated than the CTR lambs. In conclusion, treatment with exogenous melatonin at 30 days of age increased food efficiency in fattening female lambs, probably, because of the lower metabolism in treated lambs, which was reflected by the lower body temperature and LA exhibited by these animals. In addition, the study has demonstrated the effect of exogenous melatonin in the growth performances of post-weaning lambs, and that its effects depend on animal sex, which suggests that treatments that target females might be most appropriate in the fattening period.

Introduction

The development and growth of lambs play a crucial role in sheep husbandry because they influence the quality of the meat and the productive efficiency of the farms (Fogarty et al., 2000). For that reason, an integrative approach involving veterinary knowledge and the use of new technologies for recording physiological parameters provides an opportunity to improve sheep management.

Melatonin is involved in the regulation of circadian and seasonal rhythms (Pévet, 2003) and is implicated in seasonal reproduction in sheep (Palacin et al., 2011), and in several physiological mechanisms because it acts as an antioxidant and an anti-inflammatory molecule and regulates energy metabolism (Ma et al., 2022). Studies have demonstrated that melatonin has several roles in the intra-uterine and neonatal life of lambs (Flinn et al., 2020), and melatonin implants in pregnant ewes improve uterine blood flow and fetal oxygen supply, which reduces neonatal mortality and increases birth weight (Flinn et al., 2020). Melatonin treatment of ewes at lambing increases the fat content of milk throughout lactation, which increases growth rate and weight of lambs (Abecia et al., 2021), and ewes implanted with melatonin in the last third of pregnancy show an improvement in colostrum quality (Abecia et al., 2020).

Cagnacci et al. (1997) reviewed the relationships between melatonin and core body temperature in humans and showed that the rhythms in both variables are caused by the circadian pacemaker located in the hypothalamic suprachiasmatic nuclei, and its effect on thermoregulatory centers, heat loss, and probably heat production are likely involved.

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Giannetto et al. (2016) reported that the daily rhythms of melatonin and rectal temperature in sheep and goats were synchronized, although they differed consistently from the rhythm in locomotor activity (LA).

Exogenous melatonin has been shown to induce circadian rhythmicity (CR) in the LA of several species, such as rats (Redman et al., 1995; Martinet et al., 1996) and sparrows, which facilitates the synchronization of circadian rhythms to light (Hau and Gwinner, 1994). In addition, exogenous melatonin can reduce LA in humans (Zhdanova, 2005) and some birds (Mintz et al., 1998).

In the last 20 years, new technologies have been used to collect real-time data on the behavior of farm animals. For instance, accelerometer sensors are noninvasive devices for collecting LA, which can indicate a well-functioning system. A triaxial accelerometer provides data on body motion and physical state continuously (Barwick et al., 2018) and has been used to measure posture in cattle for monitoring feeding and grazing activity and for detecting calving (Robert et al., 2009; González et al., 2015). Those devices have been used to monitor activities in adult sheep (Giovanetti et al., 2017) and to detect lambing time in pasture-based sheep (Fogarty et al., 2020). In addition, actigraphy has confirmed that, in lambs, activity in the first week of life did not follow a circadian rhythm, and that twin-born lambs had stronger associations with their littermates than did singletons with other lambs (Abecia et al., 2022). Furthermore, actigraphy was useful in documenting the locomotion and feeding behavior of artificially reared lambs, which detected a reduction in CR and the number of suckling sessions as the lambs aged (Abecia and Canto (2023).

In small ruminants, the effect of gender on growth performances is mainly related to the quantity of fat deposited, deposition site, growth rate, and carcass yield, so that females are more affected than males due to their higher precociousness (Guerrero et al., 2013). In light of those findings, we hypothesized that melatonin implants affect growth and locomotor behavior in post-weaning lambs, and that accelerometers might be useful in confirming this hypothesis. The aim of this study was to compare the LA, body temperature, and growth of fattening lambs treated or not with melatonin implants from 30 to 85 days of age. We also determine if the effect of melatonin treatment varies according to the sex of the lambs.

Material and methods

Animals and experimental procedures

The study was conducted at the experimental farm of the University of Zaragoza, Spain (41°40′N, 0°53′W). Sixty Rasa Aragonesa lambs (31 males, 29 females; 40 singles, 20 twins), born between October 13 and November 6 (mean live weight [LW] ± SD = 4.19 ± 0.83 kg) from a synchronized mating in May, were assigned to one of two groups: one in which the animals received two subcutaneous melatonin implants (36 mg melatonin, Melovine, CEVA Animal Health, Barcelona, Spain) at 30 ± 2 days of age (MEL) (n = 15 males, 15 females) and one in which they did not (CTR, non-implanted, control (n = 16 males, 14 females). From weaning (44 ± 2 days of age) until the age of 86 ± 3 days, the groups were housed separately within the same building, in 25 m^2 paddocks, and were fed a 14.8% crude protein concentrate (Cadecor-2, Agroveco, Zaragoza, Spain) and barley straw ad libitum. Lambs were managed in two batches, depending on the day of birth, so that implanting, weaning, and slaughter dates were November 14, November 28, and January 12, and December 1, December 15, and January 29, respectively. Twin lambs were always separated into the two experimental groups. After weaning, lambs were fitted with commercially available sensors that record high-resolution raw acceleration data (ActiGraph wGT3X-BT; ActiGraph, FL, USA) (46 mm × 33 mm × 15 mm in size, mass = 19 g), which were attached to the dorsal side of a neck collar that remained in place until slaughter, 5 weeks later. At the end of each week, the recorded activity data were downloaded by the ActiLife software (ActiGraph, LLC, Pensacola, FL, USA). Actigraph produces three columns of data, that is, activity in the x-, y-, and z-axes. The activity counts for the three axes were used to create minute-by-minute activity (counts per 1 minute intervals) data values (vector magnitude [VM]), which is the magnitude of the vector that is formed from the combination of the sampled accelerations from the three axes on any device.

One week before slaughter, rectal temperature (Trec) was recorded by a conventional thermometer, and surface temperatures were recorded by a thermographic camera (Teso 883, Teso SE & Co. KGaA, Titisee-Neustadt, Germany). The areas delineated from the images are described in Figure 1. The camera specifications are temperature range −30 °C to +650 °C, resolution focus (manual) 320 × 240, image refresh rate 27 Hz, emissivity 0.01-1, with automatic recognition of emissivity and determination of reflected temperature. Images were taken at a distance of 1 m. Temperature data were analyzed by the IRSoft software. Ambient temperature and relative humidity when the images were recorded were 11 °C and 52%, respectively. At the same time, a real-time ultrasound was used to measure the subcutaneous fat thickness (FT) over the *longissimus dorsi* muscle between the 12th and 13th ribs and over the third and fourth lumbar vertebrae. Scanning gel was applied to the scanning sites to improve conduction between the skin and the transducer. All measurements were taken on the left side by a portable ultrasound scanner (7.5 MHz transducer; EXAGO, France). Muscle depth and width were taken at the same points and the area was calculated. At the end of the finishing period, the concentrate residuals were weighed to calculate the total amount of concentrate

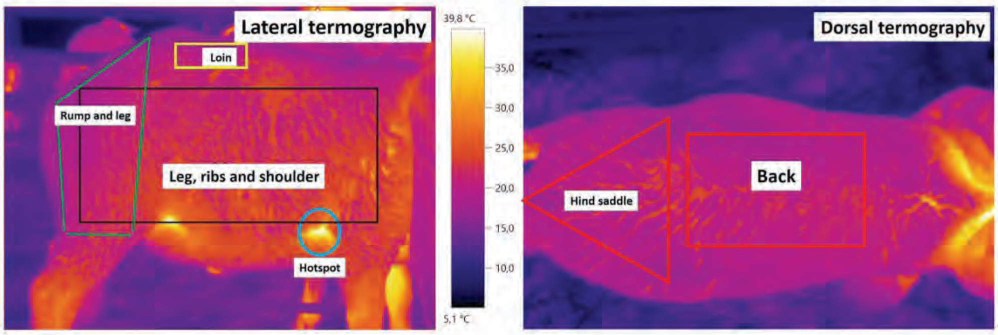

Figure 1. Body regions thermographed in Rasa Aragonesa lambs.

consumed per group, and the feed conversion rate (FCR) was calculated as kilogram concentrate consumed per kilogram LW gained.

Statistical analysis

For each of the 5 weeks of data collection, mean (±SE) VM for the 7 days of each week that the lambs wore the sensors was calculated at hourly intervals. Circadian rhythms in VM were graphed by fitting the time-series measurements of each lamb to the cosine curve of a 24 hours activity rhythm, which was obtained by the cosinor method at the Cosinor on-line platform (Molcan, 2019). Midline estimating statistic of rhythm (MESOR, or the average around which the variable oscillates), amplitude (the difference between the peak and the mean value of a wave), and acrophase (the time of peak activity) were calculated for each individual. To test for rhythmicity, an F-test compared the (reparameterized) cosine model and the nonrhythmic model. A $P < 0.05$ indicated that the time series fit a 24 hours rhythm. Thereafter, the data were pooled and the mean 24 hours cosinor curve for each of the three parameters was calculated for each group, and the cosinor values of MEL versus CTR groups were compared statistically by an ANOVA. To detect significant differences among the 5 weeks of fattening, a repeated-measures *t*-test was used.

The effects of week, lamb sex, type of parturition (single or twin), and time of day (day or night) on activity-related variables were evaluated statistically based on a multifactorial model that included these parameters as fixed effects, and the least-squares method of the GLM procedure in SPSS v.26 (IBM Corp. Released, 2019) was used. Within fixed effects, significant differences were identified by an ANOVA. A general representation of the model is as follows: $y = xb+e$, where y is the N × 1 vector of records, b denotes the fixed effect in the model within the association matrix x, and e is the vector of residual effects. Before the statistical tests, a Kolmogorov-Smirnov test was performed to confirm the normality of the data.

Results

LW, growth rate, and FCR

The melatonin treatment did not have a significant effect on LW and growth rates throughout the finishing period (Tables 1 and 2); however, most of the LW and growth variables evaluated differed significantly between the sexes (Table 3), with no interaction between sex and treatment. Regarding type of parturition, single lambs had higher LWs than twins ($P < 0.05$) (Table 3), with significant differences only for the growth rate between birth and weaning ($P < 0.05$), with no interaction between type of parturition and treatment.

In the finishing period, melatonin-treated lambs consumed significantly ($P < 0.001$) less concentrate per lamb per day (0.99 ± 0.01 kg) than did nontreated lambs (1.03 ± 0.01 kg), although the two groups had a similar FCR (3.24 vs. 3.34, respectively); however, there was a significant ($P < 0.001$) effect of the sex of the lamb and a significant ($P < 0.05$) interaction between treatment and sex on FCR. Specifically, males had a significantly ($P < 0.001$) lower FCR (2.94) than did females (3.66), and there was a significant ($P < 0.001$) effect of melatonin treatment on FCR in female (MEL: 3.47, CTR: 3.85) but not in male (MEL: 3.00, CTR: 2.89) lambs. No differences between single and twin lambs were observed either for the amount of concentrate consumed per day (single: 1.02 ± 0.01; twins: 1.00 ± 0.01) or the FCR (single: 3.25; twins: 3.33). Considering the interaction between type of parturition and treatment, twin MEL lambs presented a significantly lower concentrate consumption (0.99 ± 0.01 kg) than single MEL lambs (1.00 ± 0.01 kg) ($P < 0.05$), with no differences between single and twins for the CTR group.

Table 1
Mean (±SE) age (d) and live weight (LW, kg) of Rasa Aragonesa lambs that did (MEL, n = 30) or did not (CTR, n = 30) receive two 18 mg melatonin implants at day 30 of age and were fattened for 5 weeks from weaning (45 d of age) to slaughter (85 d of age).

	CTR		MEL	
	Age	LW	Age	LW
Birth	0.00 ± 0.00	4.25 ± 0.16	0.00 ± 0.00	4.13 ± 0.15
Implant	29.80 ± 0.36	9.69 ± 0.43	30.10 ± 0.36	9.70 ± 0.41
Weaning	44.57 ± 0.34	14.47 ± 0.57	44.90 ± 0.36	14.39 ± 0.56
60 days	60.03 ± 0.39	19.41 ± 0.75	60.30 ± 0.37	19.56 ± 0.74
75 days	74.27 ± 0.44	23.56 ± 1.10	74.50 ± 0.40	23.95 ± 0.83
Slaughter	85.73 ± 0.55	27.61 ± 0.86	85.90 ± 0.49	27.36 ± 0.0.86

Table 2
Mean (±SE) fattening parameters (kg/d) of Rasa Aragonesa lambs that did (MEL, n = 30) or did not (CTR, n = 30) receive two 18 mg melatonin implants at day 30 of age and were fattened for 5 weeks from weaning (45 d of age) to slaughter (85 d of age).

	CTR	MEL
Finishing period (d)	41.17 ± 0.39	41.00 ± 0.37
Growth birth-weaning	0.23 ± 0.10	0.23 ± 0.01
Growth weaning-60 d	0.32 ± 0.02	0.34 ± 0.02
Growth weaning-75 d	0.31 ± 0.03	0.32 ± 0.01
Growth weaning-slaughter	0.32 ± 0.01	0.32 ± 0.01
Growth birth-slaughter	0.27 ± 0.01	0.27 ± 0.01

Longissimus dorsi and back FT scanning

Neither treatment nor sex of the lamb was correlated with the measurements of the *longissiums dorsi* (Table 4); however, a significant interaction between treatment and sex ($P < 0.05$) was detected for back FT; specifically, MEL (3.22 ± 0.21 mm) and CTR (2.57 ± 0.24 mm) females differed significantly ($P < 0.05$), but male lambs did not (MEL: 2.77 ± 0.21, CTR: 2.94 ± 0.24 mm).

Locomotor activity

Week, sex of the lamb, time of day, and treatment with melatonin had a significant ($P < 0.001$) effect on LA of lambs in the finishing period, and there were significant ($P < 0.001$) interaction effects between treatment, time of day, and sex of the lamb. Overall activity was significantly ($P < 0.001$) higher in the CTR (78.89 ± 0.12 counts/min) than it was in the MEL group (72.22 ± 0.10 counts/min), particularly, from week 1 to week 4 (week 1: 56.65 ± 0.14; week 2: 66.51 ± 0.16; week 3: 76.19 ± 0.17; week 4: 81.21 ± 0.18; week 5: 97.82 ± 0.21). The differences between the MEL and CTR groups were more pronounced in the male lambs (CTR: 81.52 ± 0.17; MEL: 69.20 ± 0.14; $P < 0.001$) than they were in the female lambs (CTR: 76.01 ± 0.17; MEL: 75.26 ± 0.14; $P < 0.001$) (Figure 2). Lambs in the MEL group were less active than were those in the CTR group (Figure 3). Lambs increased their activity from week 1 to week 5 ($P < 0.001$), and female lambs (75.58 ± 0.11 counts/min) exhibited more movements than did male lambs (75.58 ± 0.11 counts/min). Activity was significantly ($P < 0.001$) higher in the day (90.10 ± 0.13 counts/min) than it was at night (63.18 ± 0.09 counts/min), and the differences between groups were greater in the day (CTR: 99.51 ± 0.21; MEL: 82.57 ± 0.17; $P < 0.001$) than they were at night (CTR: 62.20 ± 0.14; MEL: 63.95 ± 0.12; $P < 0.001$). No differences between single and twin lambs were observed, and no interaction between type of parturition and treatment was detected.

The 24 hours evolution of activity each week is presented in Figure 4. Both groups had peaks in activity at 0800 hours and at 1800 hours, which were significantly ($P < 0.05$) higher in the control group than in the MEL group in week 4.

Table 3
Mean (± SE) live weight (LW, kg) of Rasa Aragonesa male (n = 31) and female (n = 29) lambs that were fattened for 5 weeks from weaning (45 d of age) to slaughter (85 d of age).

	Males	Females	Single	Twins
LW	n = 31	n = 29	n = 40	n = 20
Birth	4.42 ± 0.16[a]	3.94 ± 0.13[b]	4.56 ± 0.12[a]	3.70 ± 0.15[b]
Weaning	14.97 ± 0.61	13.84 ± 0.49	15.50 ± 0.51[a]	13.02 ± 0.52[b]
60 d	20.50 ± 0.76[a]	18.41 ± 0.66[b]	20.58 ± 0.72[a]	18.06 ± 0.66[b]
75 d	25.69 ± 0.83[a]	21.69 ± 0.97[b]	24.70 ± 1.03[a]	22.52 ± 0.76[b]
Slaughter	29.33 ± 0.87[a]	25.51 ± 0.67[b]	28.63 ± 0.84[a]	25.98 ± 0.78[b]
Growth				
Birth-weaning	0.24 ± 0.01	0.22 ± 0.01	0.24 ± 0.01[a]	0.21 ± 0.01[b]
Weaning-60 d	0.36 ± 0.02[a]	0.29 ± 0.02[a]	0.33 ± 0.19[b]	0.32 ± 0.01
Weaning-75 d	0.36 ± 0.01[a]	0.26 ± 0.02[a]	0.31 ± 0.02[b]	0.32 ± 0.01
Weaning-slaughter	0.32 ± 0.01[a]	0.32 ± 0.01[a]	0.32 ± 0.01[b]	0.31 ± 0.01
Birth-slaughter	0.29 ± 0.01[a]	0.25 ± 0.01[a]	0.28 ± 0.01[b]	0.26 ± 0.01

[a,b] Indicates significant differences at $P < 0.05$.

Table 4
Mean (± SE) dimensions (mm) of the *longisimus dorsi* muscle of Rasa Aragonesa lambs that did (MEL, n = 30) or did not (CTR, n = 30) receive two 18 mg melatonin implants at day 30 of age and were fattened for 5 weeks from weaning (45 d of age) to slaughter (85 d of age), as measured by real-time ultrasound between the 12th and 13th ribs and over the third and fourth lumbar vertebrae.

	CTR	MEL
Depth	18.62 ± 0.47	19.23 ± 0.40
Width	42.55 ± 2.99	47.52 ± 2.50
Area	614.50 ± 0.17	676.29 ± 33.01
Fat thickness	2.77 ± 0.17	3.00 ± 0.16

Circadian rhythms

All lambs exhibited a circadian rhythm in activity on every day of the fattening period, and melatonin treatment did not have a significant effect on mean MESOR or amplitude. Acrophase differed significantly ($P < 0.05$) between MEL and CTR groups in week 3 only (Figure 5). MESOR in the CTR group increased from week 1 to week 3 ($P < 0.01$), and in the MEL group from week 1 to week 5. In the MEL group, only, amplitude increased significantly ($P < 0.01$) from week 4 to week 5. In every week, CTR lambs experienced a significant ($P < 0.001$) delay in acrophase, although significant changes in acrophase in the MEL group occurred from week 1 to week 3, only (Figure 5).

Thermography

Trec was significantly ($P < 0.01$) lower in the MEL lambs (38.68 ± 0.10) than it was in the CTR lambs (39.00 ± 0.07). In all the regions analyzed, surface temperatures were significantly ($P < 0.01$) lower in the MEL group than they were in the CTR group (Table 5). Body temperatures did not differ significantly between the sexes.

Discussion

In this study, subcutaneous melatonin implants inserted at day 30 of age had a significant effect on the growth and activity in Rasa Aragonesa lambs in the fattening period. In addition, exogenous melatonin differentially affected the feeding conversion rate, locomotion, and body temperature of female and male lambs. Melatonin implants increased significantly the feeding conversion rate and FT in female lambs but not in males.

In post-pubertal beef heifers fed melatonin, the amount of fat on ribs, the growth of the longissimus muscle, and carcass fat increased significantly, which suggested that melatonin partitions nutrients toward fat accretion in later-maturing fat depots (i.e., im fat), an effect that is similar to that from exposure to short-day photoperiods (Zinn et al., 1988). An effect of melatonin treatment on adipose tissue metabolism and an increase in the cross-sectional area of longissimus muscle and back adipose tissue has been observed in lambs (Ma et al., 2022). Melatonin did not affect liver and kidney function but did increase superoxide dismutase blood levels (Ma et al., 2022) as expressed by its antioxidant action (Rodriguez et al., 2004), which has a positive effect on muscle and adipose tissue development. Furthermore, melatonin seemed to ameliorate the rumen microflora in lambs by enhancing nutrient absorption and increasing muscle and fat cell size (Ma et al., 2022). In a previous study, we observed an increase in the fat content of milk from melatonin-

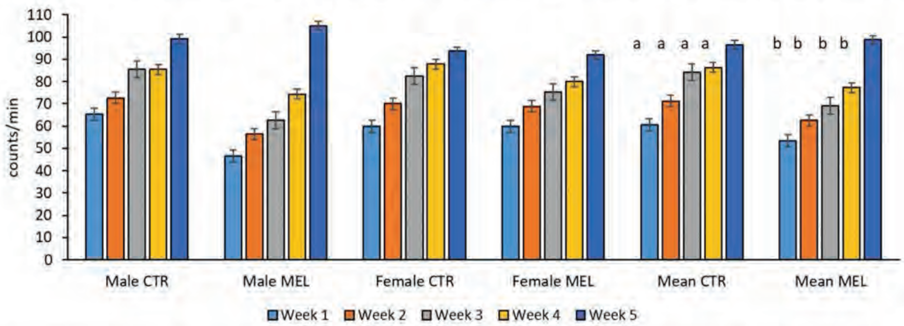

Figure 2. Mean (± SE) vector of magnitude (counts/min) of Rasa Aragonesa lambs that did (MEL, n = 30) or did not (CTR, n = 30) receive two 18 mg melatonin implants at day 30 of age and were fattened for 5 weeks from weaning (45 days of age) to slaughter (85 days of age) (a and b indicate significant differences at $P < 0.05$).

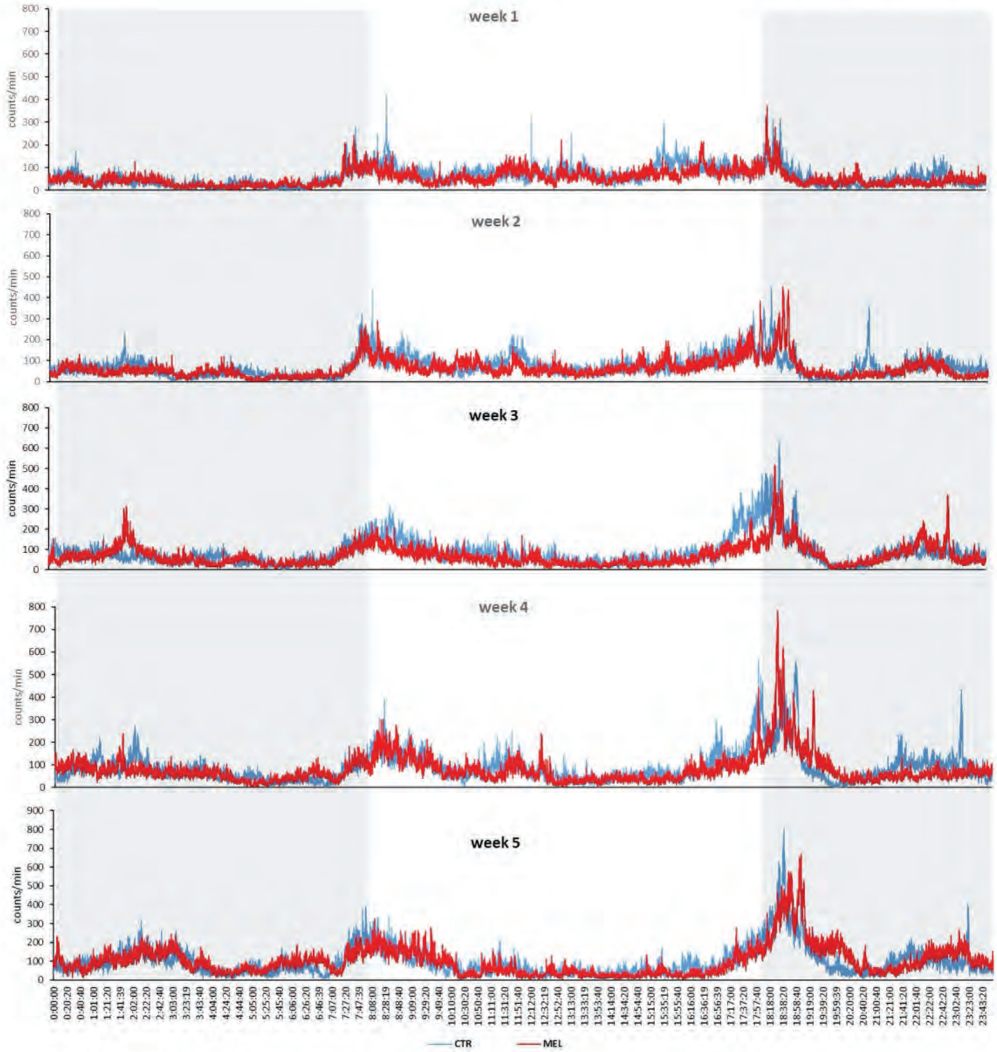

Figure 3. Mean 24 hours locomotor activity (counts/min) of Rasa Aragonesa lambs that did (MEL, n = 30) or did not (CTR, n = 30) receive two 18 mg melatonin implants at day 30 of age and were fattened for 5 weeks from weaning (45 days of age) to slaughter (85 days of age) (gray areas represent night).

treated ewes, even though a direct effect on growth rate in implanted-newborn lambs was not observed (Abecia et al., 2021).

In a study designed to determine whether melatonin and light-dark schedule influence energy metabolism and performance in broiler chicken, Apeldoorn et al. (1999) found a reduction in energy expenditure for physical activity caused by melatonin supplements in the diet, which might have caused the increase in the FCR in melatonin-supplemented chickens. To our knowledge, our study is the first to demonstrate a direct effect of melatonin implants on post-weaning lambs and an interaction effect between melatonin treatment and animal sex, although it remains uncertain why it impacted adipose tissue development more in females than it did in males. Clearly, melatonin implants influenced energy balance in growing female lambs.

In our experiment, body temperature was lower in melatonin-treated lambs than it was in untreated lambs. In addition to its role in the timing of seasonal reproduction, melatonin affects seasonal thermoregulation; in particular, acting as a transducer in mediating information about energy balance (Saarela and Reiter, 2005). Considering that the temperature to which the animals were exposed at the time of the experiment, when the thermography was performed, was low (11 °C), lambs were losing body heat to the environment to

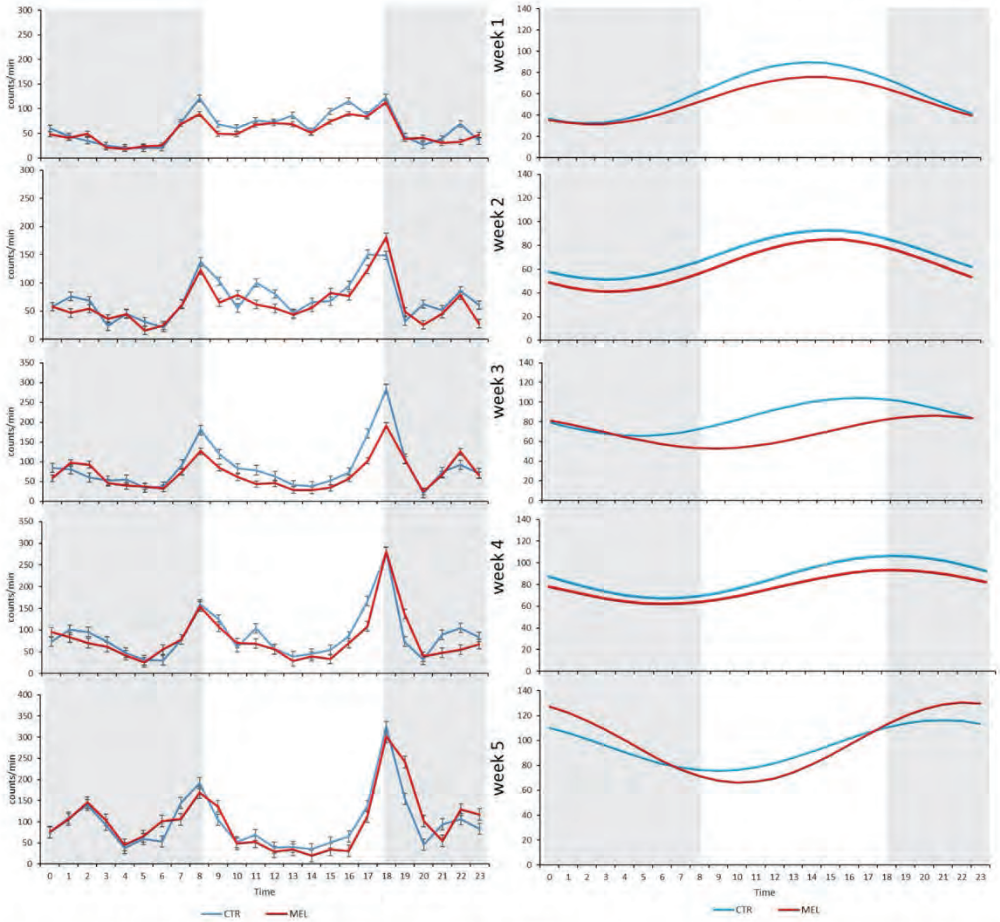

Figure 4. Mean (±SE) daily activity (counts/min/h) (left panel) of Rasa Aragonesa lambs that did (MEL, n = 30) or did not (CTR, n = 30) receive two 18 mg melatonin implants at day 30 of age, in the 5 weeks fattening period as measured by actigraphy, and the corresponding cosinor curves (right panel) of a 24 hours activity rhythm (gray areas represent night).

compensate cold temperatures, so that it is likely that MEL lambs have a higher ability to thermoregulate their bodies under cold ambient temperatures. In the vasculature, melatonin predominantly acts through two membrane-bound receptors (MT1 and MT2), whose opposite effects have been demonstrated: the activation of MT1 elicits vasoconstriction and MT2 elicits vasodilatation (Doolen et al., 1998). When ingested as a supplement in humans, melatonin blunts the cutaneous vasoconstrictor response during cooling (Aoki et al., 2008). As treated lambs presented a lower surface temperature, they likely had a greater level of vasoconstriction and therefore, a better capacity to retain body heat. Moreover, a greater level of vasoconstriction may be related to the fact that MEL lambs consumed less concentrate than CTR lambs, and thus, a lower need to increase metabolic rate by consuming more food, which in turn can be related to the lower activity exhibited by MEL lambs.

In a study of the effects of exogenous melatonin on rectal and body surface temperatures in donkeys subjected to packing in the hot-dry season in Nigeria, Ake et al. (2023) demonstrated that melatonin had hypothermic effects on the rectal and surface temperatures of the donkeys and neutered the circadian rhythms of these parameters through its biphasic effect, viz., its hypothermic effect in photophase and hyperthermic effect in scotophase. Ake et al. (2023) recommended that melatonin be administered to donkeys in the hot-dry season to improve the well-being and performance of working donkeys under heat stress conditions.

Environmental interference has been reported as a limitation of infrared thermography, so that inaccurate results caused by direct sunlight, humidity, or wind have been described (McManus et al., 2016). However, in the present study, rectal and body surface temperature measurements were well related—they were lower in treated lambs compared with the control animals—so that it is likely that the ambient conditions where the images were taken allowed a good quality of the thermographic pictures.

In a study by Bouroutzika et al. (2020), pregnant ewes under heat stress treated with melatonin had a consistently lower body temperature than did untreated ewes. In addition, melatonin

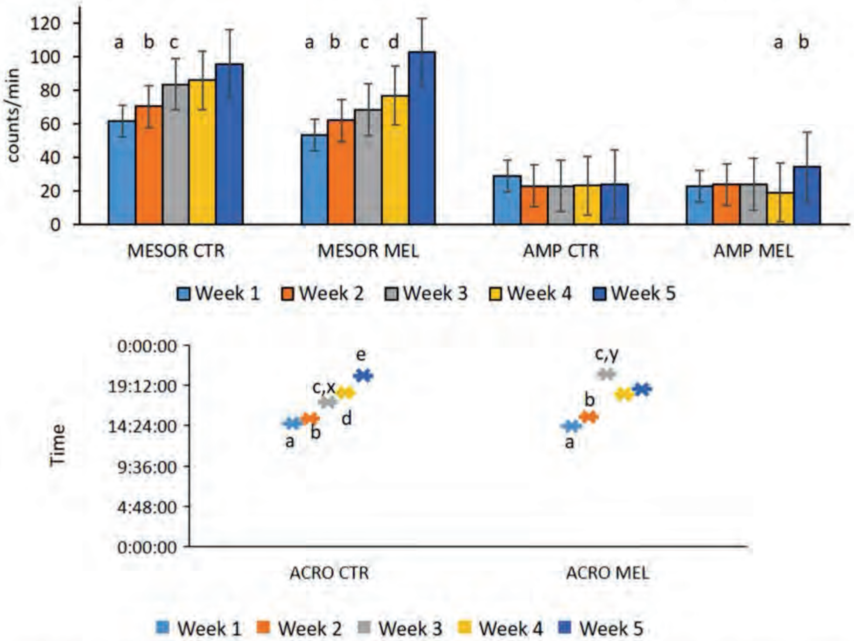

Figure 5. Mean (± SE) midline estimating statistic of rhythm (MESOR), amplitude (the difference between the peak and the mean value of a wave), and acrophase (the time of peak activity) of the cosine curve of a 24 hours locomotor activity rhythm of Rasa Aragonesa lambs that did (MEL, n = 30) or did not (CTR, n = 30) receive two 18 mg melatonin implants at day 30 of age and were fattened for 5 weeks from weaning (45 days of age) to slaughter (85 days of age) (a, b, and c indicate significant differences at $P < 0.05$ within groups; x and y indicate significant differences at $P < 0.05$ between groups in the same week).

Table 5
Mean (± SE) temperature (°C) of Rasa Aragonesa lambs that did (MEL, n = 30) or did not (CTR, n = 30) receive two 18 mg melatonin implants at day 30 of age and were fattened for 5 weeks from weaning (45 d of age) to slaughter (85 d of age).

	CTR	MEL
Hotspot (armpit)	38.51 ± 0.41[a]	36.79 ± 0.53[b]
Leg, ribs, and shoulder	20.14 ± 0.26[a]	18.32 ± 0.14[b]
Rump and leg	19.16 ± 0.29[a]	17.34 ± 0.13[b]
Loin	18.30 ± 0.27[a]	16.73 ± 0.16[b]
Hind saddle	17.39 ± 0.30[a]	16.51 ± 0.14[b]
Back	18.51 ± 0.32[a]	17.35 ± 0.17[b]

[a,b] Indicates significant differences at $P < 0.05$.

inhibits thyroid, adrenal, and corticosteroid hormones, which are linked indirectly to thermic modulation (Saarela and Reiter, 2005; Ma et al., 2022). In humans, the nocturnal peak in melatonin levels closely correlates with the nadir in core body temperature, and treatment with exogenous melatonin lowered it by 0.2 °C-0.4 °C (Cagnacci et al., 1992).

In our experiment, melatonin-treated lambs were less active than were nontreated lambs, which might have been related to the lower body temperatures exhibited by the implanted lambs because elevated melatonin levels from either endogenous nocturnal production or exogenous daytime administration are associated with effects such as increased sleepiness, reduced core temperature, increased heat loss, and other generally anabolic physiological changes in humans (van den Heuvel et al., 2005). The earliest evidence of a direct effect of the pineal hormone on the locomotor activities of vertebrates was found when the removal of the pineal gland from house sparrows maintained in constant darkness eliminated the free-running circadian rhythm in LA (Zimmerman and Menaker, 1975), and when a subcutaneous injection of protein-free pineal extract reduced running wheel activity in rats, and a pinealectomy increased activity (Ozaki et al., 1976). Furthermore, melatonin reduces pain and anxiety, which puts rodents into a tranquilization state (Golombek et al., 1996). Although cortisol levels were not measured in our study, it is plausible that a similar calming effect might have occurred in lambs. Given that locomotion is a large proportion of the energy budget in mammals (Bertram, 2016), and a small lowering of body temperature increases metabolic rate, probably, the reduction in temperature and LA caused by melatonin in our experiment was responsible for the reduction in concentrate ingested by treated lambs and, in females, an increase in food conversion rate. Melatonin treatments significantly reduced heat production in 2-week-old and 3-week-old broiler chickens and improved FCR, especially in younger chickens, which suggests that melatonin reduces heat production by lowering body temperature and regulating heat dissipation (Zeman et al., 2001).

Conclusion

In conclusion, our study demonstrated that new-generation devices for monitoring daily activity can increase our understanding of lamb behavior in the fattening period and, in particular, detect the effects of exogenous melatonin on LA. Treatment with exogenous melatonin at 30 days of age in fattening lambs improved food efficiency rate, especially in females, probably, because of a reduction in the metabolism of treated lambs as indicated by the lower body temperature and LA exhibited by these animals. Furthermore, the reduction in the body temperature in lambs that received melatonin implants suggests the possibility of improving lamb performance in the summer fattening period, when lambs are exposed to heat stress. In addition, our study confirmed that melatonin plays an important role in post-weaning lamb metabolism, and that its effects depend on animal sex, which suggests that treatments that target females might be most appropriate in the fattening period.

Authorship statement

The idea for the paper was conceived by José Alfonso Abecia. The experiment was designed by José Alfonso Abecia, Irene Viola, and Francisco Canto. The experiments were performed by Irene Viola and Francisco Canto. The data were analyzed by Irene Viola and José Alfonso Abecia. The paper was written by Irene Viola and José Alfonso Abecia. The final version to be submitted was approved by Irene Viola, José Alfonso Abecia, and Francisco Canto.

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Ethical Considerations

The Ethics Committee for Animal Experiments at the University of Zaragoza approved all of the procedures performed in the study. The care and use of animals were in accordance with the Spanish Policy for Animal Protection RD1201/05, which meets the European Union Directive 2010/63 on the protection of animals used for experimental and other scientific purposes.

Conflict of Interest

The authors declare no conflict of interest.

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5. General Discussion

5. General discussion

Domestic small ruminant species are adapted to intensive production systems, and convert their feed into highly nutritious milk and meat efficiently. However, when applying any management strategy, including exogenous melatonin administration, it is necessary to quantify the productive, performance, and quality impact of the products derived from these farming systems. This PhD thesis aimed to analyze the effects of exogenous melatonin administration during different physiological states of small ruminants on several parameters involved in the colostrogenesis, lactation, and the performance of the offspring. The study involves two representative dairy breeds, the Murciano Granadina goats and Assaf sheep, and a meat sheep breed, the Rasa Aragonesa.

The main results and considerations obtained throughout this study were:

5.1. Milk yield

In the dairy experiments (Exp. 1 and 2), melatonin implants increased milk production in early lactation. Specifically, goats that had received a melatonin implant 30 d before kidding had a significantly ($P < 0.01$) higher milk yield than did the control, non-implanted goats in the second month of lactation (44.3 ± 9.3 DIM). Similarly, ewes in the 1M group, which received a melatonin implant 40 days before lambing, produced significantly ($P < 0.05$) more milk during the three-months than ewes that received two implants or were non-implanted. This was in agreement with previous studies (Avilés et al., 2019), demonstrating that administering two melatonin implants to Creole goats in the drying-off phase (21 d prepartum) increased milk production throughout lactation, including the suckling (0 - 21 d postpartum) and milking (22 - 98 d postpartum) periods. These modulations in milk yield could be attributed to the persistent expression of melatonin membrane receptors MT1 and MT2 in the mammary glands of dairy goats throughout lactation (Zhang et al., 2019), which suggests that melatonin has a direct role in the regulation of mammary physiology.

The timing of melatonin treatment may influence the effects on milk yield. Most of the evidence on the effects of exogenous melatonin on milk production in sheep has been derived from studies where the animals were treated during lactation, showing a decreased in milk yield. In contrast, in this thesis, the animals were treated during the dry period. Supporting this approach, Wall et al. (2005) found that cows exposed to a short-day photoperiod (linked to a high endogenous melatonin secretion) during the dry period underwent more extensive mammary remodeling and cell renewal. They proposed that these changes could lead to an increase in milk production in the subsequent lactation.

5.2. Milk quality

The effects of melatonin on milk fat have varied between dairy-type and meat-type small ruminants. Providing melatonin to Murciano Granadina goats (Exp. 1) in late pregnancy increased the concentration of fat in their milk during the first three months of lactation. Similarly, Assaf ewes (Exp. 2) treated with two melatonin implants in late pregnancy showed an increase in milk fat concentration during the first and third months of lactation. This increase in milk fat appears to be more consistent, as it has been observed in cows with three repeated doses of melatonin during mid-lactation and in goats with two doses at mid and late lactation (Auldist et al., 2007; Yang et al., 2020). There is evidence that melatonin affects the gut microbiota in mice through mechanisms of AMP induction (Kim et al., 2020), and the rumen microflora of cows and lambs (Ouyang et al., 2021; Ma et al. 2022). These alterations in the bacterial biota can affect the fat content of milk, thereby significantly affecting ruminal lipid metabolism in both goats and cows (Toral et al., 2016). In addition, research involving bovine intramuscular preadipocytes demonstrated that melatonin regulates adipose differentiation via the melatonin receptor MT2 (Yang et al., 2017a).

Meanwhile, in the experiment conducted with Rasa Aragonesa meat-type ewes implanted before lambing (Exp. 3), at lambing or at both times (Exp. 4), milk fat concentration was not affected. This heterogeneity of effects on milk fat could be due to the fact that the modulation of lipid metabolism by melatonin varies across different species (Le Gouic et al., 1997; Bartness et al., 2002). Furthermore, the specific role of melatonin in controlling the synthesis of fat in milk remains unclear (Wang et al., 2019; Abecia et al., 2021).

In both goat and dairy sheep experiments (Exp. 1 and 2), no effects on protein and lactose concentrations were observed after the application of exogenous melatonin in late gestation. Similarly, there were no differences in these milk components when melatonin was implanted in meat ewes either in late pregnancy, at lambing, or at both times (Exp. 4). The absence of effects of exogenous melatonin on milk protein and lactose has also been reported in mid lactating cows (s.c injections of 4.64 mg melatonin for 4 d), mid and late lactating goats (s.c implants; 2 mg/kg live weight at 50 and 110 DIM) and lactating ewes (s.c melatonin implant of 18 mg at 24 h after lambing) (Wang et al., 2019; Yang et al., 2020; Abecia et al., 2021). These findings suggest a pattern with low variability in milk protein a lactose content in different species and physiologic stages.

In small ruminants, under various farming and feeding systems, protein content is more stable than fat content in milk (Barillet 2007; Morand-Fehr et al., 2007). Likewise, milk lactose concentrations exhibit minimal variability, as lactose is the primary compound influencing milk osmolality (Costa et al., 2020).

In addition to the nutritional parameters of the milk, the SCC was also measured to evaluate the effect of melatonin on the status of udder health in dairy small ruminants. On the one hand, in the study of goats (Exp. 1), melatonin implants administered 30 d before kidding did not improve this indicator of mammary health in any of the three sampling periods (45 -107 d after implantation). Similar results were reported by Yang et al. (2020) in lactating Mongolian Cashmere Goats implanted with melatonin (2 mg/kg live weight) at d 50 and 110 of lactation.

On the other hand, in the experiment involving dairy ewes (Exp. 2), exogenous melatonin provided by melatonin implants 40 d before lambing reduced the SCC in milk. In the group that received two melatonin implants, low SCC was observed until the third milk sampling (August), which was 133 d after melatonin implantation. Ewes that received one melatonin implant, however, presented an effect on SCC in the second sampling (July), 100 d after implantation. Melatonin implants can release the hormone for up to 100 d in sheep (Forcada et al., 2002); therefore, apparently, one implant can have an effect on the mammary gland up until the implant is exhausted. In ewes that received two implants, the effects of exogenous melatonin persisted for an additional month. A similar decrease in SCC was observed in Sardinian sheep implanted with melatonin in the second month of lactation. This effect became evident a month and a half after implantation, and persisted in the following samplings (Cosso et al., 2021). Additionally, cows exhibited a decrease in SCC after 14 and 21 d of rumen bypass melatonin feeding (40 or 80 mg/d). Even five to seven days after stopping melatonin administration, the residual effects on the decline of SCC are likely to remain (Yao et al., 2020). These results may be a consequence of the enhancement of the udder immune system. The mechanisms involved in the reduction in SCC may be mediated either through the ability of this hormone to reduce neutrophil infiltration and the inflammatory reaction (Shin et al., 2015) or through the antioxidative effect of melatonin that is more prepared for protecting leucocytes from oxidative damage (Jiménez et al., 2009; Najafi et al., 2017). Moreover, it was reported that melatonin suppressed and enhanced anti-inflammatory cytokines under different pathophysiological conditions (Yu et al., 2017), which in turn may have facilitated the reduction in the SCC observed in the experiment of dairy ewes.

In experiments with small dairy ruminants, the effects of melatonin on SCC may reflect species-specific differences (Avilés et al. 2019). The different responses to melatonin implantation in the experiment of goats and sheep could be due to factors associated with the specific characteristics of SCC linked to each species. These species-specific differences are mainly due to the higher SCC in uninfected goat halves, the higher apocrine component of goat milk secretion compared to sheep (Paape et al., 2001; Contreras et al., 2007). Moreover, there is a distinction in SCC profiles reflected in the predominant cell types present. Polymorphonuclear neutrophilic leukocytes (PMNs) were the predominant cell type in milk from healthy uninfected goats, comprising 40 to 80% of the SCC. In contrast, milk from healthy uninfected sheep primarily contains macrophages, accounting for 45–88% of the SCC, while PMNs constitute 10–35%, lymphocytes 10–17%, and epithelial cells 2–3% (see review Kaskous et al., 2023).

5.3. Colostrum

In the study of dairy goats (Exp. 1) and meat-type ewes (Exp. 4), the immunity quality of colostrum did not differ significantly between melatonin-treated groups and control animals. Similarly, Bouroutzika et al. (2021) did not observe differences in the IgG concentration of the colostrum of pregnant ewes treated or not with melatonin, but they found a better redox status in lambs born from melatonin-treated ewes, as well as higher antioxidant capacity in the colostrum of melatonin-treated ewes compared with that of the untreated ones.

In the dairy ewe experiment (Exp. 2), melatonin implants increased the IgG concentrations in colostrum, which is consistent with earlier finding that exogenous melatonin implanted one month before lambing had a positive effect on colostrum quality as reflected in an increase in IgG concentrations (Abecia et al., 2020). Studies by Flinn et al. (2020 b,c) on Merino ewes treated with melatonin in the second half of pregnancy have not directly evaluated the effects on colostrum quality. Furthermore, although no differences in serum IgG of the lambs were found, exogenous administration of melatonin produced an increase in the survival rate of second-born twin lambs. Considering the critical importance of colostrum for newborn survival (Hernández-Castellano et al., 2016), this suggests that melatonin may enhance lamb survival by improving immunological colostrum quality.

Furthermore, the effects of melatonin on humoral immune responses could be related to the immune status of the animals at the time of melatonin administration. In particular, the immunomodulatory action of melatonin in vivo was most evident during a state of immune depression (Bouroutzika et al. 2021). In the present PhD thesis, dairy goats (Exp. 1) and meat ewes (Exp. 4) appear to have a good immune condition shown by higher colostrum IgG levels (dairy goat: 51.06–52.27 mg/ml; meat-type ewes: 25.71–28.84 mg/ml), which resulted in an absence of response to melatonin treatment. However, it is likely that dairy ewes (Exp. 2) had a low immunity, indicated by their lower colostrum IgG concentrations (16.99–24.41 mg/ml), and responded to melatonin treatment by improving the immunological quality of their colostrum.

In relation to the ºBrix, which is considered as a tool for assessing colostrum quality, there are few studies that have evaluated sheep and goat colostrum quality on-farm by this way (Kessler et al., 2021). In the present thesis, the ºBrix of colostrum did not differ significantly between treated and control animals. It should be mentioned that the ºBrix were within the values reported for goat (21.6 ± 5.3 ºBrix) and sheep (28.5 ± 6.8 ºBrix) by Kessler et al. (2021).

Regarding the effects of melatonin on the nutrient composition of colostrum, experiments with small dairy ruminants did not show any effect on its components. However, in the Rasa Aragonesa meat-type experiment (Exp. 4), protein concentrations of the colostrum were highest in those ewes implanted 30 d before lambing (M-0). In agreement with these findings, Bouroutzika et al. (2021) observed that sheep receiving their last melatonin implant similarly toward the end of the third month of gestation (100 d) had higher colostrum protein concentrations. As melatonin plays a role in mediating ruminal microbes and their metabolic pathway (Fu et al., 2023), it is likely that the increase in colostrum protein could be attributed to a change in rumen microorganisms. Additionally, in the same experiment with Rasa Aragonesa ewes (Exp. 4), an increase in the concentration of lactose in the colostrum was observed in those ewes implanted during gestation (M-0). Although there are no reports of this effect in ruminant species, it is likely that the increase of lactose concentration in the colostrum of the group is the result of a greater energy availability in the form of glucose, a precursor of lactose (Banchero et al., 2004). Recent research findings have suggested the involvement of melatonin in glucose metabolism in mammals (Watanabe et al., 2023). Therefore, this melatonin effect on energetic metabolism could justify the higher levels of lactose observed in colostrum.

5.4. Growth

Similar to the studies of Flinn et al. (2020b, c), the Rasa Aragonesa breed experiment conducted at the University of Zaragoza (Exp. 4) did not detect an effect of melatonin treatment on birth weight. In the study with the same breed under farm conditions (Exp. 3), however, melatonin treatment increased the birth weight of single male lambs. The nutritional status of the dams could explain the different effects on birth weight; thus, in nutrient-restricted rats, oral melatonin enhanced the production of placental antioxidant enzymes and may improve placental nutrient transfer capacity, fetal organ development, birth weight and fetal growth (Richter et al., 2009).

This thesis found that melatonin implants have differential effects on Rasa Aragonesa lambs, varying across various developmental periods, litter size, sex of the lamb, and the timing of implantation. In the study conducted during the fattening period (Exp. 5), subcutaneous melatonin implants inserted at day 30 of age had no significant effect on the LW, ADG or ultrasound evaluation of the *longissiums dorsi* in Rasa Aragonesa lambs. However, in other experiments during the rearing period, the effects of melatonin implants were significant on lamb LW. The experiment under farm conditions (Exp. 3) demonstrated that lambs born from ewes that had received a melatonin implant in the last third of pregnancy had the highest LW during lactation and grew faster than did lambs born of non-implanted ewes. Additionally, under experimental conditions (Exp. 4), implants inserted before and at lambing increased the LW of lambs. These increasing effects of melatonin on LW are in accordance with Avilés et al.(2019) and Abecia et al. (2021), who reported that during suckling period, development was highest in pre-weaning kids and lambs of dams that had received exogenous melatonin implant in the dry period or 24 hours after parturition, respectively.

Although in the rearing experiments (Exp. 3 and 4) lambs did not receive a melatonin implant, they were supplemented indirectly through the maternal circulation or the milk. The transplacental transfer of melatonin from the maternal circulation to the fetus has been documented in ewes (Zemdegs et al., 1988). Recently, in vitro experiments have shown that mechanisms of the efficient transport of melatonin into milk are mediated by the ovine ATP-binding cassette G2 (ABCG2) (Álvarez-Fernández et al., 2023). This indirect melatonin supplementation has been shown to potentially enhance growth in small ruminants by the capacity to control hormone levels, mitigate oxidative stress, and regulate ruminal function. Melatonin has been associated with the increased of growth hormone, estradiol and testosterone plasma concentration, which are crucial for muscle growth and body structural formations (Molik et al., 2010; Ma et al., 2022). The antioxidative properties of melatonin may prevent muscle degradation under oxidative stress (Bantounou et al., 2022). Possibly, the mechanism used to remove aged and damaged cells during development, and protects cells, is by enriching the expressions of genes in the apoptosis pathway, especially in CytC, PARP1 and IL1-β genes (Ma et al., 2022). Furthermore, melatonin seemed to ameliorate the rumen microflora in lambs by enhancing nutrient absorption and increasing muscle and fat cell size (Ma et al., 2022).

On the other hand, the improvement of live weight and growth may be through the beneficial effect of melatonin on colostrum and milk. The effects of melatonin implants in the mothers on the growth of male lambs might have been because they consumed more colostrum. In addition to increasing neonate survival, access to colostrum in the neonatal period can have a positive effect on future production, development, and reproductive efficiency of lambs through growth factors that facilitate neonatal growth and development. Previously, Abecia et al. (2021) demonstrated that melatonin implant at lambing had a significant effect on milk quality, specifically, increasing fat content in milk. In another study in goats (Avilés et al., 2019), melatonin implants inserted seven weeks before kidding had a significant effect on milk production in the subsequent lactation and improved the daily weight gain of their suckling kids.

A sex effect of melatonin implants on LW was observed. The effects of treating with melatonin ewes in pregnancy were strongest in single male (Exp. 3) and female (Exp. 4), while administration at lambing specifically enhanced LW in male lambs (Exp. 4). Abecia et al. (2021) reported that the effects of melatonin implants in the mothers was significant in male lambs, only; specifically, male lambs reared by melatonin-treated ewes had significantly higher LW at weeks 2, 3, and 4 than did male lambs that had been reared by untreated ewes. In goats, melatonin implants in the dry period increased the weight gain of male offspring, only (Avilés et al., 2019). Wallace et al. (2014) reported that, in early postnatal life, lamb sex had a significant effect on adipose tissue gene expression in favor of male lambs because female lambs had lower IGF1, IGF2, IGF1R, IGF2R, and hormone-sensitive lipase mRNA expression levels, which are associated with growth and reflect the sexual dimorphism in body composition.

Our studies indicate that the effects of melatonin on the LW of lambs were influenced by the pattern of delivery from the implant and its timing of administration. Specifically, the impact was related to the onset of release and duration of hormone availability. In the experiment 4, the number of days since implantation of the ewe influenced the effects of exogenous melatonin on the LW of male and female lambs; i.e., in male and female lambs, the effects were apparent at 30 d and 49 d after implantation of the dam, respectively. Zúñiga et al. (2002) showed that melatonin implants in ewes (18 mg melatonin) gradually release the hormone to various tissues, and plasma melatonin concentrations were highest 37 d after implantation. Meanwhile, under farm conditions (Exp. 3), the correlations between implantation-weaning interval and the LW and AGR of lambs, indicated that the efficacy of the melatonin implants in improving lamb performance was greatest for individuals in which the melatonin implant was inserted close to parturition. The implants can release melatonin for up to 100 d (Forcada et al., 2002), therefore, probably, the poorer performances of the lambs from ewes implanted > 30 d before lambing was due to the earlier absorption of the implant, so that the beneficial effects of melatonin on milk production diminished earlier in lactation.

5.5. Body measurements

In experiment 4, the morphometrics measurements demonstrated that male lambs from melatonin-treated ewes at lambing showed the largest chest girth at weaning. Supporting this, Ma et al. (2022) found that exogenous melatonin in doses of 3.5 or 4.0 mg/kg increased chest width in two-month-old lambs beginning 30 d after implantation. In our study, CG, WH, RH, and SW were shortest in the female lambs that were born from ewes that had received a melatonin implant at lambing. Despite the melatonin implant, which should not have immediately influenced the lambs at birth due to the hormone's delayed bioavailability (Zúñiga et al., 2002), other factors like genetic potential, litter size, and environmental conditions likely contributed birth size and development.

5.6. Feeding convertion rate

To our knowledge, the fattening experiment (Exp. 5) is the first to demonstrate a direct effect of melatonin implants on FCR in post-weaning lambs, and an interaction effect between melatonin treatment and animal sex. Specially, this experiment found that female implanted lambs had a significantly lower FCR (3.47) than non-implanted female lambs (3.85). In a study designed to determine whether melatonin and light- dark schedule influence energy metabolism and performance in broiler chicken, Apeldoorn et al. (1999) found a reduction in energy expenditure for physical activity caused by melatonin supplements in the diet, which might have caused the improvement in the FCR in melatonin-supplemented chickens. However, it remains uncertain why it impacted adipose tissue development more in females than it did in males. Clearly, melatonin implants influenced energy balance in growing female lambs.

5.7. Rectal and surface temperatures

In this PhD thesis, different effects of exogenous melatonin on rectal and surface temperature were observed in experiments 4 and 5. These effects are likely influenced primarily by the action of melatonin on thermogenic tissues such as BAT. Additionally, the role of melatonin receptors in the vascular regulation may account for variations in thermoregulatory responses in lambs.

Melatonin implants in the dam did not affect rectal temperature in newborn lambs (Exp. 4). Similar results have been reported in sheep reared under extensive conditions that had received subcutaneous (18 or 36 mg melatonin/ewe) or oral melatonin (140 mg/ ewe) in gestation (Flinn et al., 2020b, c), and in sheep reared in permanent housing implanted (18 mg melatonin/ewe) at the third or fourth month of pregnancy (Abecia et al., 2020). However, in the lambs implanted at 30 d of age (Exp.

5), the rectal temperature was significantly lower in the MEL lambs than it was in the CTR lambs.

In our study of melatonin-treated ewes (Exp. 4), thermography indicated that MT and HT were highest in lambs born of dams that had received a melatonin implant in pregnancy. Freitas-de-Melo et al. (2021) similarly reported that the surface temperature of the hip was highest in lambs from ewes that received a melatonin implant at 100 d of gestation. The improvement in the primary mechanism for neonatal thermogenesis might be because of an increase in BAT caused by melatonin supplementation of pregnant sheep (Sales et al., 2017, 2019). In addition, fetal hypoxia increases the concentration of plasma norepinephrine, which causes peripheral vasoconstriction and a reduction in surface temperature (Sahni, 2017). Prenatal melatonin supplementation can protect the fetus against acute hypoxia in sheep (see review by Flinn et al., 2020a); therefore, lambs from implanted mothers might indirectly display vasodilation in the peripheral blood system (which increases surface temperature). Furthermore, the interaction between melatonin treatment and sex indicated that the effects of exogenous melatonin were most prominent in male lambs, and males from pregnant implanted ewes had higher surface temperatures in most of the areas measured (RT, ST, MT and HT) than did control male lambs. Clarke et al. (2012) stated that there is a sexual dimorphism in the regulation of thermogenesis at the following three levels: 1) direct effects on peripheral tissues, 2) intrinsic central regulation of sympathetic outflow, and 3) of metabolic factors such as leptin.

However, in the experiment involving melatonin implantation of lambs at 30 d of age (Exp. 5), body temperature was lower in melatonin-treated lambs than it was in untreated lambs. In a study of the effects of exogenous melatonin on rectal and body surface temperatures in donkeys subjected to packing in the hot-dry season in Nigeria, Ake et al. (2023) demonstrated that melatonin had hypothermic effects on the rectal and surface temperatures of the donkeys. In our experiment (Exp. 5), the effects of melatonin on BAT would be limited, as these older animals do not produce or have significant amounts of BAT. This is because BAT develops during the fetal stage (Bienboire-Frosini et al., 2023). Additionally, as lambs mature, thermogenesis decreases rapidly during the first weeks after birth, and BAT is replaced by white adipose tissue (Thompson & Jenkinson, 1969). Considering that the temperature to which the animals were exposed at the time of the experiment, when the thermography was performed, was low (11 °C), lambs were losing body heat to the environment to compensate cold temperatures, so that it is likely that MEL lambs have a higher ability to thermoregulate their bodies under cold ambient temperatures. When ingested as a supplement in humans, melatonin blunts the cutaneous vasoconstrictor response during cooling (Aoki et al., 2008). As treated lambs presented a lower surface temperature, they likely had a greater level of vasoconstriction and therefore, a better capacity to retain body heat. Moreover, a greater level of vasoconstriction may be related to the fact that MEL lambs consumed less concentrate than CTR lambs, and thus, a lower need to increase metabolic rate by consuming more food, which in turn can be related to the lower activity exhibited by MEL lambs.

As observed, in experiment 4, the melatonin effects were vasodilatory, while in experiment 5, they were vasoconstrictive. These contradictory effects could be explained by the diverse actions of melatonin on vascular system. Melatonin predominantly acts through two membrane-bound receptors (MT1 and MT2), whose opposite effects have been demonstrated: the activation of MT1 elicits vasoconstriction and MT2 elicits vasodilatation (Doolen et al., 1998).

5.8. Locomotor activity (LA) and circadian rhythms

In our experiment (Exp. 5), melatonin-treated lambs were less active than were non-treated lambs. The earliest evidence of a direct effect of the pineal hormone on the locomotor activities of vertebrates was found when the removal of the pineal gland from house sparrows, maintained in constant darkness, eliminated the free-running circadian rhythm of LA (Zimmerman & Menaker, 1975), and when a subcutaneous injection of protein-free pineal extract reduced running wheel activity in rats, and a pinealectomy increased activity (Ozaki et al., 1976). Furthermore, melatonin reduces pain and anxiety, which puts rodents into a tranquilization state (Golombek et al., 1996). Although cortisol levels were not measured in our study, it is plausible that a similar calming effect might have occurred in our lambs. Despite these effects, all lambs exhibited a circadian rhythm in activity on every day of the fattening period, and melatonin treatment did not have a significant effect on mean MESOR or amplitude.

6. Conclusions

6.1. CONCLUSIONS

The conclusions derived from the results obtained in the five experiments carried out during this PhD thesis are presented below:

1) The administering of subcutaneous melatonin implants 30 d before kidding had a significant effect on milk yield and composition in dairy goats reared under commercial farm conditions. Specifically, exogenous melatonin increased milk yield and milk daily components in the second month of lactation and fat milk concentration at the beginning and at mid-lactation.

2) Ewes that received one or two melatonin implants 40 d before lambing produced colostrum that had a higher IgG concentration than that produced by non-implanted dairy ewes, and produced more milk, which had a lower SCC. The effect on SCC was prolonged if the sheep received two melatonin implants.

3) Melatonin treatment of pregnant meat-type ewes before lambing increased lamb performance until weaning and, in particular, the effects were observed in singleton male lambs, who had the highest LW at birth and weaning, and the highest growth rate.

4) The administration of melatonin to ewes in late pregnancy (30 d before lambing) increased the concentration of protein and lactose in the colostrum of Rasa Aragonesa, a meat sheep breed. Implants inserted immediately after lambing, only, tended to increased lamb performance (especially, the LW of male lambs). The addition of a melatonin implant in pregnancy and lambing did not add to the effects that exogenous melatonin had on lamb growth. Thermography indicated that the application of melatonin in pregnancy might influence thermoregulation in the lamb at birth, which can increase in the temperatures of the mid-loin and the hip surface, particularly, in male lambs under intensive conditions.

5) Treatment with exogenous melatonin at 30 days of age in fattening lambs improved food efficiency rate, especially in females, probably, because of a reduction in the metabolism of treated lambs, as indicated by the lower body temperature and LA exhibited by these animals. Furthermore, the reduction in the body temperature in lambs that received melatonin implants suggests the possibility of improving lamb performance in the summer fattening period, when lambs are exposed to heat stress.

6.2. CONCLUSIONES

A continuación, se presentan las conclusiones derivadas de los resultados obtenidos en los cinco experimentos realizados durante esta tesis doctoral:

1) La administración de implantes subcutáneos de melatonina 30 d antes del parto tuvo un efecto significativo sobre la producción y composición de la leche en cabras lecheras criadas bajo condiciones comerciales. En particular, la melatonina exógena aumentó la producción de leche y los componentes diarios de la leche en el segundo mes de lactación, así como la concentración de la grasa láctea al inicio y a mitad de lactación.

2) Las ovejas lecheras que recibieron uno o dos implantes de melatonina 40 d antes del parto produjeron un calostro con mayor concentración de IgG en comparación con las ovejas no implantadas, además produjeron más leche con un menor RCS. El efecto sobre el RCS fue más prolongado cuando las ovejas recibieron dos implantes de melatonina.

3) El tratamiento con melatonina en ovejas de carne gestantes antes del parto aumentó el rendimiento de los corderos hasta el destete. En particular, los efectos fueron más evidentes en los corderos machos únicos, que tuvieron un mayor PV al nacimiento y al destete, además de una mayor tasa de crecimiento.

4) La administración de melatonina a ovejas al final de la gestación (30 días antes del parto) incrementó la concentración de proteína y lactosa en el calostro de la raza ovina de carne Rasa Aragonesa. Los implantes administrados inmediatamente después del parto, únicamente, tendieron a aumentar el rendimiento de los corderos (especialmente, el PV de los corderos machos). Sin embargo, la combinación de implantes de melatonina tanto en la gestación y en el parto no sumó a los efectos de la melatonina exógena sobre el crecimiento de los corderos. La termografía sugiere que la aplicación de melatonina en la gestación podría influir en la termorregulación del cordero al nacer, al aumentar las temperaturas superficiales del lomo medio y de la cadera, en particular en los corderos machos bajo condiciones intensivas.

5) El tratamiento con melatonina exógena a los 30 días de edad en corderos de cebo mejoró la eficiencia de conversión alimentaria, especialmente en las hembras, probablemente debido a una reducción en el metabolismo de los corderos tratados,

como lo indica la menor temperatura corporal y LA observada. Además, la disminución de la temperatura corporal en los corderos que recibieron implantes de melatonina sugiere la posibilidad de mejorar el rendimiento de los corderos en el periodo estival de cebo, cuando están expuestos a estrés térmico.

6.3. GENERAL CONCLUSION AND FUTURE RESEARCH

The results of these PhD thesis, and our previous findings on the effect of melatonin at the end of pregnancy, open new possibilities to optimize lactation and the lamb performances during the rearing period. In addition, melatonin plays an important role in post-weaning lamb metabolism, and that its effects depend on animal sex, which suggests that treatments targeting females might be most appropriate in the fattening period.

The use of exogenous melatonin in small ruminants presents a potential strategy to enhance the efficiency and competitiveness of animal production systems, particularly by improving milk, colostrum and lamb growth parameters. However, in terms of basic science, detailed knowledge of the metabolic pathway of melatonin is essential to understand the various physiological processes associated with mechanism of actions on sex, litter size, and effects on different tissues and organs. From an applied animal science perspective, it remains to be elucidated what is the optimal interval between implantation in the pregnant dams and parturition that maximizes the lactation parameters and the growth of the lambs during the rearing period. Additionally, comprehensive bioeconomic evaluations are necessary to assess the costs of melatonin administration in relation to its potential benefits.

This represents a challenge for future basic and applied research. Thus, melatonin should be exploited as sustainable alternative management strategy in animal production, with multiple potential applications.

7. Appendix

7. Appendix

The impact factor of the journals and the thematic areas corresponding to the articles included in the thesis are presented in brackets below (except for the publication 3). This information has been obtained from the Journal Citation ReportsTM (JCR) database.

1. Canto F., Peña-Delgado, V., Noya, A., Manenti, I., José Abecia, J.A. (2024). Using melatonin implants in late pregnancy increases milk production and improves milk quality in dairy goats. Article sent to: Canadian Journal of Animal Science el 20/11/2023 (reference number: CJAS-2023-0123). Under review **[Journal Impact Factor (2023): 1.2; Subject areas: Agriculture, Dairy & Animal Science (Q3)].**

2. Canto, F., González, E., Abecia, J.A. (2022). Effects of Implanting Exogenous Melatonin 40 Days before Lambing on Milk and Colostrum Quality. Animals. 12, 1257. https://doi.org/10.3390/ani12101257 **[Journal Impact Factor (2021): 3.0; Subject areas: Agriculture, Dairy & Animal Science (Q1); Veterinary Sciences (Q1)].**

3. Canto F., Riaguas L., Fantova E., Abecia J.A. (2024). Melatonin-Implanted Pregnant Ewes Produce Lambs that have Higher Average Daily Growth Rates and Live Weights at Weaning. Sheep & Goat Research Journal 39, 1-6.

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