Impacto de la alimentación y nivel de exposición al ambiente sobre la ingestión, digestión, metabolismo y producción en vacas lecheras de parición otoñal y primaveral

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Tabla de contenido

Resumen

La alta carga animal y la suplementación en sistemas lecheros intensivos mixtos (SM) aumentan el uso de la estabulación y, ante eventos climáticos extremos cada vez más frecuentes, la exposición ambiental y las instalaciones cobran relevancia. A su vez, algunos productores lecheros en Uruguay están reemplazando los SM de bajo costo, pero complejos, por sistemas de estabulación total (alimentados con ración totalmente mezclada; TMR) para ejercer mayor control sobre la exposición ambiental y el consumo, y optimizar la producción. Se sometieron vacas Holstein de parición otoñal (ACS, n = 48) y primaveral (SCS, n = 48) a tres tratamientos durante una lactancia completa: a) pastoreo + ración mezcla (MR) durante la estabulación parcial en corrales al aire libre con suelo de tierra y tosca y sombra (OD-GRZ), b) pastoreo + MR durante la estabulación parcial en un galpón con cama de compost y sistema de enfriamiento (CB-GRZ) o c) estabulación total con TMR *ad libitum* en un galpón con cama de compost y sistema de enfriamiento (CB-TMR). Durante toda la lactancia, se determinaron periódicamente la ingesta, la producción y composición de leche, la condición corporal y los ácidos grasos no esterificados y beta-hidroxibutirato en suero. Específicamente en la lactancia temprana, se determinaron el comportamiento, la ingesta, la utilización de nutrientes y síntesis ruminal de proteína microbiana. Los resultados muestran que las vacas en sistemas OD-GRZ con diseño y manutención apropiados, y en condiciones climáticas moderadas, compensaron las condiciones de alojamiento menos favorables mediante adaptaciones en su comportamiento y lograron una ingesta, síntesis ruminal de proteína microbiana, utilización de nutrientes y potencial de producción de leche equiparable al de vacas con infraestructura mejorada (CB-GRZ). Sin embargo, ante condiciones climáticas adversas más severas (SCS), se observó menor producción de leche en vacas OD-GRZ respecto a CB-GRZ (5 % durante toda la lactancia y de hasta

20 % en episodios puntuales de lluvias intensas o estrés por calor). Las vacas CB-TMR superaron ampliamente a los SM, alcanzando 20-35 % más producción de leche y 8-18 % más eficiencia alimenticia, sin impacto en los componentes de la leche. Los niveles de producción superaron estudios previos en la región y se alinearon con los reportados en sistemas lecheros de California, EE. UU.

Palabras clave: producción de leche, metabolismo, comportamiento, síntesis de proteína microbiana

Behavior, intake, digestion, metabolism and milk yield of autumn and spring calved Holstein dairy cows with two levels of environmental exposure and feeding strategy

Summary

High stocking rates and supplemental feeding in intensified pasture-based dairy systems (mixed systems, MS) increases the use of confinement, and with extreme weather events becoming more frequent, environmental exposure and facilities become more relevant. Meanwhile, some dairy producers in Uruguay are replacing low-cost, but complex, MS with total confinement systems (fed with total mixed rations; TMR) to exert greater control over environmental exposure and intake, optimizing production. Holstein cows with autumn calving (ACS, n=48) and spring calving (SCS, n=48) were subjected to three treatments during a full lactation: (a) grazing + mixed ration (MR) during partial confinement in outdoor soil-bedded pens with shade (OD-GRZ); (b) grazing + MR during partial confinement in a compost-bedded pack barn with cooling (CB-GRZ); or (c) total confinement in a compost-bedded pack barn with cooling fed a TMR *ad-libitum* (CB-TMR). Throughout lactation, intake, milk production and composition, body condition, and serum non-esterified fatty acids and beta-hydroxybutyrate were determined periodically. In early lactation, specifically, behavior, intake, nutrient utilization, and ruminal microbial synthesis were measured. The results show that cows in properly designed and maintened OD-GRZ systems, under moderate climatic conditions, compensated for less favorable housing conditions through behavioral adaptations and achieved an intake, microbial flow, nutrient utilization, and milk production potential comparable to cows in enhanced infrastructure (CB-GRZ). However, when faced severe adverse climatic conditions (*i.e.,* in SCS), lower production was observed in OD-GRZ cows compared to CB-GRZ (5% throughout lactation and up to 20% during specific episodes of intense rainfall or heat stress). CB-TMR cows greatly outperformed the MS, achieving 20–35% higher milk production and 8–18% better feed efficiency, with no impact on milk components. Production levels surpassed previous studies in the region and aligned with those reported in California dairy systems, USA. The results show that cows in well-managed OD-GRZ systems, under moderate climatic conditions, compensated for less favorable housing conditions through behavioral adaptations and achieved an intake, microbial flow, nutrient utilization, and milk production potential comparable to that of cows in enhanced infrastructure (CB-GRZ).

Keywords: milk yield, metabolism, behaviour, microbial crude protein synthesis

1. Introducción

1.1. Fundamentación general

Uruguay exporta más del 70 % de la leche que produce. La competitividad y la sostenibilidad del sector lácteo uruguayo, como en otros sistemas de producción de leche pastoril, se deben en gran medida a los menores costos de producción asociados con la alta inclusión de forraje de cosecha directa en las dietas (Fariña y Chilibroste, 2019).

Si bien las pasturas templadas de buena calidad son ampliamente utilizadas en los sistemas de producción de leche y constituyen una excelente fuente de nutrientes para alimentar las vacas en ordeñe, sus altos contenidos de humedad y de fibra (National Research Council, 2001) pueden resultar en bajos consumos de materia seca (MS) y energía (Kolver y Muller, 1998), lo que condiciona la producción individual de leche. Estos factores, en adición a las grandes fluctuaciones en la disponibilidad de forraje en las diferentes épocas del año, han llevado a que los sistemas de alimentación incluyan niveles crecientes de suplemento (reservas de forraje o concentrados) en la dieta con el fin de aumentar y estabilizar la producción de leche a lo largo del año. De hecho, la producción de leche nacional tuvo un crecimiento importante hasta el ejercicio 2014-2015 gracias al aumento de los niveles de suplementación individual y el incremento de la carga animal del sistema, que permitió aumentar la productividad por hectárea en un contexto de reducción del área nacional destinada a la lechería (Fariña y Chilibroste, 2019; MGAP-DIEA, 2012, 2023). El aumento de la carga animal del sistema permite a su vez cosechar más forraje fresco por hectárea, con reducción de los costos productivos y mejora del margen sobre los costos de alimentación, un indicador de beneficio económico parcial. No obstante, existe una variación considerable del margen entre sistemas con similar carga animal y estrategia de alimentación que indicaría que existen otros factores en juego (Chilibroste y Battegazzore, 2014, 2019).

Estudios de simulación y resultados de experimentos en *farmlets* realizados en Uruguay muestran que el aumento de carga animal del sistema conlleva una reducción del tiempo en la pastura y un mayor porcentaje de tiempo en las áreas de estabulación durante el año, dada la necesidad de suplementar y necesidad de descansar la pastura para recuperación de *stock* de forraje del sistema (Ortega et al., 2024). Aunque la mayor ingesta de forraje cosechado directamente los hace más competitivos, los sistemas uruguayos que están explorando las fronteras en cuanto a carga animal enfrentan el problema de no estar preparados —en términos de infraestructura y logística— para soportar largos o repetidos períodos de estabulación. Esto expone a los animales a condiciones climáticas extremas como alta temperatura, humedad o radiación solar, así como al contacto con el barro generado tras precipitaciones copiosas (Aguerre et al., 2018; Aguerre y Chilibroste, 2018). La última encuesta lechera del Instituto Nacional de la Leche (INALE) arrojó que, si bien los tambos que cuentan con plazas de alimentación poseen en mayor parte comederos de hormigón y piso de hormigón (esto es más frecuente en los estratos más altos de remisión de leche anual que en estratos de mediana y baja remisión), solamente el 30 % de los predios encuestados poseen plaza de alimentación diseñada específicamente para dicho fin (INALE-MGAP, 2021), lo que deja en evidencia la falta de estrategias de contención de los animales en momentos que no se encuentran en la pastura.

En este sentido, los comportamientos naturales que más influyen en la salud, el bienestar y la productividad animal son descansar, comer y rumiar; las vacas tienden a mantener una cantidad relativamente fija de descanso que alteran solo cuando otras necesidades se ven muy afectadas o cuando las condiciones ambientales para echarse no son lo suficientemente cómodas (Cook et al., 2004; Kilgour, 2012; Krawczel y Grant, 2009; Schütz et al., 2019). Es por ello que la eficiencia de uso de la energía y nutrientes consumidos para la producción de leche depende no solo de la composición de la dieta, del genotipo animal y del estado fisiológico, sino también del ambiente productivo (confort) y su influencia sobre la expresión del comportamiento natural de las vacas (De Ondarza y Tricarico, 2017; Fregonesi et al., 2007; La Manna et al., 2015; Tucker et al., 2007, 2020).

Por otra parte, el 65 % de los sistemas lecheros comerciales nacionales concentran los partos de marzo a septiembre y el 40 % ocurre durante los meses de otoño (Chilibroste y Battegazzore, 2019). La concentración de partos en otoño tiene la desventaja del incremento de la carga animal del sistema en momentos de menor *stock* de forraje, lo que acentúa la necesidad de suplementar y encerrar los animales al menos durante un turno diario a inicio de lactancia. Esta restricción es superada en aquellos sistemas con parición primaveral (fin de invierno con pico de producción en primavera), pero, en contrapartida, la probabilidad de que las vacas sufran estrés térmico durante la lactancia temprana es considerablemente superior (Román et al., 2019), lo que podría perjudicar la producción, con efectos residuales sobre todo el ciclo productivo (Arcos, 2008; Ubios y Mendoza, 2024). Trabajos de caracterización climática de Uruguay indican que el ambiente atmosférico (humedad y temperatura ambiente) podría ser una limitante productiva para los rodeos lecheros durante el verano (Cruz y Saravia, 2008; Cruz y Urioste, 2009). El calentamiento global ha provocado un aumento de las temperaturas mínimas anuales, especialmente en verano y otoño, y, por lo tanto, una disminución del número de noches frías y tendencia a mayor frecuencia de noches cálidas (Renom, 2009). A su vez, la atmósfera se ha vuelto cada vez más inestable, con tendencia a aumentos en la frecuencia de ocurrencias climáticas adversas, tanto sequías como precipitaciones copiosas e inundaciones (Barreiro et al., 2019; Bernabucci et al., 2010; Giménez et al., 2009).

Si bien se ha generado información nacional (Acosta et al., 2010; Fajardo et al., 2015; Meikle, Cavestany, et al., 2013) y regional (Salado et al., 2018, 2020) sobre los efectos benéficos de la estabulación en el desempeño animal (producción de leche y sólidos, estado corporal, metabolismo), estos trabajos experimentales, realizados por cortos períodos de tiempo y sobre estructuras a cielo abierto, han mostrado resultados muy variables. Ante un aumento en la frecuencia de condiciones climáticas extremas y en un escenario de volatilidad creciente de precios de insumos y productos (Fariña, 2016), nos cuestionamos la sustentabilidad y la permanencia de la implementación de sistemas estabulados a escala comercial por períodos prolongados. Se considera fundamental incorporar estos factores en el análisis de sistemas y contabilizar las pérdidas que generan las pobres condiciones ambientales para la producción en el corto, mediano y largo plazo.

Se vuelve necesario, entonces, comparar el desempeño animal de vacas sometidas a sistemas estabulados, con bajo nivel de exposición al ambiente, recibiendo una ración o dieta totalmente mezclada (TMR de ahora en más por sus siglas en inglés), con el desempeño de vacas en sistemas pastoriles mixtos (SM de ahora en más) con altos niveles de suplementación con concentrados y forrajes mezclado (MR de ahora en más por sus siglas en inglés) y alta carga animal, bajo condiciones de infraestructura y ambiente productivo similares a los animales estabulados completamente o, por el contrario, a cielo abierto, como ocurre normalmente a escala comercial.

A su vez, resta también explorar los límites productivos alcanzables con rodeos uruguayos en ambientes similares a los de referencia internacional, es decir, bajo estabulación total y consumiendo TMR, pero con los nutrientes aportados por alimentos disponibles en nuestro contexto agrícola regional. Será necesario caracterizar el valor nutritivo de las dietas consumidas durante la lactancia y compararlas con las utilizados en los ya probados sistemas de estabulación (por ejemplo, *free-stalls*, *dry lots*, *compost barns*) vigentes en otras zonas del mundo (Swanepoel et al., 2016).

1.2. Antecedentes bibliográficos

1.2.1. Intensificación de la producción en sistemas lecheros pastoriles

La producción de leche en Uruguay incrementó de 1300 a 2200 millones de litros al año, aproximadamente, entre 2001 y 2014, y se ha mantenido en un nivel estable desde entonces (MGAP-DIEA, 2010, 2023). Este crecimiento estuvo dado por un mayor nivel de suplementación con concentrados y reservas forrajeras que permitió aumentar la producción de leche por vaca y el nivel de carga animal del sistema y mejoró, por ende, la producción por hectárea. No obstante, la inclusión de forraje de cosecha directa continúa siendo el componente principal de la dieta de vacas lecheras de Uruguay (Chilibroste y Battegazzore, 2019; Fariña y Chilibroste, 2019), ya que es el componente alimenticio de menor costo y es esencial para la competitividad y sustentabilidad de dichos sistemas lecheros (Dillon et al., 2008; Doyle et al., 2001).

Las pasturas templadas de Uruguay, tanto gramíneas como leguminosas, se caracterizan por un elevado valor nutritivo, ya que presentan alto contenido de proteína cruda (PC, ≈19 %) de alta solubilidad y rápida degradación en rumen (Repetto et al., 2005), con niveles de fibra neutro detergente (FND) aproximados al 40 %, de muy buena degradabilidad (50-60 %; Cajarville et al., 2003, 2006), y un porcentaje de azúcares solubles variable entre 6 y 10 % (Cajarville et al., 2015). Estas cualidades redundan en una elevada fermentescibilidad y, en consecuencia, alta síntesis de proteína microbiana y producción de ácidos grasos volátiles (Cajarville et al., 2006). La digestibilidad de la materia orgánica de nuestras pasturas se aproxima al 70 % tanto para leguminosas y gramíneas como para sus mezclas (Aguerre et al., 2009; Cajarville et al., 2006; Félix et al., 2017; Pérez-Ruchel et al., 2017, 2023; Trujillo et al., 2010).

Sin embargo, las pasturas poseen restricciones físicas que limitan la tasa de pasaje de alimento en el tracto digestivo, como el bajo contenido de materia seca y su alto aporte de fibra, que demandan largos períodos de cosecha y rumia, y podrían limitar capacidad de consumo de los animales (Bargo et al., 2003; Doyle et al., 2001; Mertens, 1994; Wales y Kolver, 2017). A su vez, el aporte energético de las pasturas varía según su manejo, clima y estado fisiológico (1,3-1,7 Mcal ENL/kg MS) y podría condicionar la producción de leche en vacas de alto potencial (Bargo et al., 2003; Chilibroste et al., 2003; Mieres, 2004). El bajo aporte de carbohidratos de rápida fermentación, en conjunto con la alta concentración de PC de alta degradabilidad, provoca un desbalance ruminal entre energía y proteína que deriva en baja utilización del nitrógeno y altas concentraciones instantáneas de N-NH3 ruminal (Cajarville et al., 2006; Repetto et al., 2005), lo que implica un costo extra de reciclaje del nitrógeno excedente. Por otra parte, las grandes fluctuaciones de disponibilidad de forraje en las diferentes estaciones del año y entre años, y las condiciones climáticas adversas que impiden el acceso a la pastura exigen a los sistemas comerciales el aporte de suplementos en la dieta para asegurar una producción estable a lo largo del ciclo productivo (Chilibroste et al., 2003; Doyle et al., 2001; Wales y Kolver, 2017).

El consumo de materia seca (MS) de pastura no solo está condicionado por sus cualidades nutricionales, sino que depende en gran medida de la estructura del tapiz vegetal (altura, disponibilidad) y estado fisiológico, así como de la oferta de forraje diaria por animal (Chilibroste, 2015; Chilibroste et al., 2005). Hace veinte años las condiciones para la cosecha directa de pasto por las vacas eran muy restrictivas, dada la baja disponibilidad y asignación de forraje (promedio anual por debajo de 1500 kg MS/ha y 15 kg MS/VO/d, respectivamente), condiciones que se volvían más críticas durante el otoño-invierno, cuando el área disponible para pastoreo se reduce por la implantación de nuevos verdeos y praderas concomitante a la mayor demanda de alimento por parte de vacas en etapa temprana de lactación (Chilibroste et al., 2004 a, b, c, d). Asimismo, el consumo de pastura representaba más del 70 % de la dieta, sobre una carga animal de 0,85 vaca masa (VM)/ha, con un promedio de cosecha anual de forraje en la hectárea de 3200 kg MS/ha VM. El aporte de forraje fresco era complementado por una oferta de concentrado y reservas que variaba entre de 3,5-2 y 4,5-1 kg MS/VO/d, respectivamente, con lo que se lograba bajos consumos de MS (CMS) total y, por ende, baja producción individual (cercano a 3200 L anuales/VM, MGAP-DIEA, 2010). En la actualidad, las restricciones en las condiciones para la cosecha de forraje en pie han sido ampliamente superadas, incluso en las estaciones del año más críticas como otoño e invierno, con valores medios anuales de 31 cm de altura y 2400 kg MS/ha de disponibilidad, y asignaciones de forraje promedio de 31 kg MS/vaca/día (Méndez et al., 2019). No obstante, la inclusión de pastura de cosecha directa en la dieta cayó a un 60 %, acompañada de una suplementación promedio anual de 4,5 y 3,7 kg MS/VO diarios de concentrado y reservas, respectivamente, para el año 2018 (Chilibroste y Battegazzore, 2019). A pesar de que el CMS de pastura por animal fue en cierta medida reemplazado por el uso de suplementos, la cosecha de forraje en la hectárea ha aumentado gracias al incremento en la dotación de animales a 1,07 VM/ha VM, lo que resultó en 3800 kg MS/ha VM de pastoreo directo para el mismo año (Chilibroste y Battegazzore, 2019).

Si bien la creciente inclusión de concentrados o reservas en la dieta ha permitido superar ciertas limitaciones de la pastura, diversos trabajos han demostrado que la mejora de la producción de leche se debe al aumento en la capacidad de consumo de los animales y no a mejoras en la utilización del NH3 ruminal o en la eficiencia de síntesis de proteína microbiana (Auldist et al., 2013; García et al., 2000). De hecho, la suplementación puede provocar alteraciones en la digestión ruminal de la fibra aun a niveles de inclusión bajos cuando la pastura posee alta capacidad fermentativa, por descensos precipitados de pH ruminal (Cajarville et al., 2006). Estos resultados invitan a reconsiderar el rol de la suplementación, considerando la pastura templada -siempre que sea manejada correctamente y no pierda calidad- como un alimento completo que debe ser acompañado de otros alimentos que aporten todos los nutrientes necesarios de forma equilibrada (Cajarville et al., 2012; Mendoza et al., 2011; Repetto y Cajarville, 2010).

En este sentido, la utilización de fuentes de fibra en simultáneo al suministro de concentrados en MR podría permitir mayores CMS y mejor producción de leche y sólidos (Wales et al., 2013). Los trabajos de investigación nacional sobre este tipo de sistemas de alimentación (SM), con los recursos forrajeros disponibles en la región, son relativamente recientes y falta aún dilucidar la relación óptima MR:forraje fresco que permita maximizar la producción de leche con la mayor inclusión de pastura posible. Mendoza et al. (2016) reportaron que, en vacas estabuladas, el suministro de MR y pastura fresca cortada ofrecidos por separado, ambos *ad libitum*, no tuvo efecto sobre el CMS, la digestibilidad aparente de los nutrientes y su utilización, ni sobre la producción de leche y sólidos cuando se incluyó pastura fresca en un 11 % del total de la dieta, con un consumo total de 25 kg MS/día y una producción diaria de 34,5 L. Asimismo, Pastorini et al. (2015) y Pomiés et al. (2014) trabajaron con mayores niveles de inclusión de forraje fresco cortado, también ofrecido a animales en estabulación, y no observaron cambios en el consumo total (24,7 kg MS/vaca/día), en la digestibilidad aparente ni en la eficiencia de utilización de los nutrientes hasta niveles de inclusión de pastura fresca de 28 % respecto al tratamiento MR; consecuentemente, sin efectos sobre la producción de leche (30,5 L/vaca/día) y sus componentes. Sin embargo, un 50 % de inclusión de forraje fresco provocó disminuciones del CMS total (2 kg MS/vaca/día) y de la producción de leche (2,5 L/día) así como de sólidos, sin afectar la digestibilidad de fracciones fibrosas ni nitrogenadas, ni su eficiencia de utilización (Pastorini et al., 2015).

No obstante, la combinación de MR con pastura fresca que debe ser cosechada por los animales implica un aporte diferido de los componentes de la dieta, consumo de alimento en períodos acotados de tiempo y mayor demanda energética asociada a la caminata y actividad de pastoreo. Esto podría repercutir en el resultado productivo final en comparación con animales que se alimentan solamente de TMR. En este sentido, Fajardo et al. (2015) observaron que una inclusión de 28 % de pastura por cosecha directa en la dieta, disminuyó el CMS total en comparación con sistemas TMR a cielo abierto (21,8 vs. 26,1 kg MS/día), lo que redundó en una producción de leche 10 % menor (33,9 vs. 37,2 L/vaca) y un balance energético más negativo respecto al sistema TMR (Astessiano et al., 2015). Jasinsky et al. (2019) estudiaron el desempeño animal entre sistemas TMR a cielo abierto y SM con 30 % de pastura por cosecha directa en la dieta y observaron una tendencia a mayor producción de leche (28,1 vs. 26,3 L/vaca/día), sin diferencias en el contenido de sólidos, pero acompañado de una mayor energía retenida en leche y tejidos en el tratamiento TMR. Salado et al. (2018) observaron que vacas consumiendo dietas con 21 %, 44 % y 70 % de inclusión de pastura de cosecha directa produjeron 6,5 %, 20,4 % y 27,6 % menos leche que vacas en sistemas TMR (32,1, 28,4 y 26,8 vs. 34,2 L/vaca/día con CMS de 22,4 21,0 y 19,7 vs. 24,1 kg MS/vaca/día, respectivamente). El análisis de regresión lineal de dicho estudio arrojó un incremento de 0,7 kg MS/vaca/día y 1,1 L/vaca/día por cada 10 % de incremento en la proporción de MR de la dieta.

Por otro lado, el manejo del pastoreo modifica la estructura del tapiz vegetal y su calidad nutricional, lo que puede afectar la capacidad de consumo y el resultado productivo final. Vibart et al. (2008) demostraron que la inclusión de pastura en estado vegetativo en hasta un 41 % de la dieta no significó diferencias en producción de leche entre SM y TMR, mientras que la misma pastura en un estado más avanzado de madurez ante inclusiones de 35 % y 21 % de pastura redujo el consumo y la producción de leche en comparación al sistema TMR (19,6 y 19,5 vs. 24,9 kg MS/vaca/día, y 31,9 y 32,7 vs. 36,6 L/vaca/día respectivamente). Meikle et al. (2013) contrastaron el desempeño de sistemas TMR y SM con diferente asignación de pastura (30, 15 y 7,5 kg MS de pasto por vaca y día) y observaron producciones de leche en los primeros sesenta días posparto de 25,4 vs. 24,1, 23,0 y 19,1 L/vaca/día para el tratamiento TMR y aquellos con diferentes asignaciones de forraje, respectivamente. Las vacas con mayor asignación de forraje obtuvieron similar condición corporal y estatus metabólico que el tratamiento estabulado, mientras que los demás tratamientos mostraron peor desempeño.

Si bien los sistemas estabulados a cielo abierto alimentados con TMR presentan mayor consumo, producción y balance que sistemas netamente pastoriles, los sistemas mixtos tienen la ventaja de capitalizar parte de los beneficios de las TMR mientras mantienen niveles relativamente bajos de costos de alimentación, basados en la inclusión de pasturas (Wales et al., 2013), e incluso igualar el resultado económico de sistemas totalmente estabulados (Fontaneli et al., 2005; Soriano et al., 2001; White et al., 2002). Son pocos los trabajos que estudian el efecto de los diferentes sistemas de alimentación sobre la curva de producción de leche y el estatus metabólico en la lactancia completa.

1.2.2. Estrategias de distribución de partos en sistemas lecheros comerciales

El momento y distribución de los partos a lo largo del año es una estrategia con efectos importantes en el patrón de oferta y demanda de alimentos y, en consecuencia, en la remisión de leche a industria y flujo de caja de la empresa durante el año (Arcos, 2008). Según un monitoreo realizado en más de ochocientos sistemas lecheros comerciales durante el período 2013-2018 (Chilibroste y Battegazzore, 2019), el 65 % de estos concentran los partos de marzo a septiembre y casi el 40 % de los partos anuales ocurren durante el otoño. Estos valores son coincidentes con los resultados de la Encuesta Lechera 2019 del Instituto Nacional de la Leche (INALE-MGAP, 2021), que representa el 89 % de los establecimientos lecheros y el 81 % del total de la leche producida en el país. Mediante un análisis de la base de datos del Instituto Nacional para el Control y Mejoramiento Lechero (31.981 registros), Arcos (2008) evidenció que vacas paridas en otoño-invierno logran mayor producción total de leche que aquellas paridas en primavera, así como mayor duración de la lactancia y menor intervalo parto-concepción, de acuerdo con lo reportado por García y Holmes (2001) en el ámbito internacional. Sin embargo, la disminución del área efectiva de pastoreo en otoño-invierno debido a la siembra de praderas y verdeos y el consecuente aumento de la carga animal redunda en bajas asignaciones de forraje por vaca (Zibil et al., 2016), lo que se superpone con los altos requerimientos de nutrientes durante la lactancia temprana. A su vez, durante estas estaciones es más frecuente la ocurrencia de precipitaciones copiosas que impiden el acceso a las pasturas debido a la anegación del suelo y se ha identificado una tendencia positiva en la ocurrencia de eventos extremos de lluvias diarias (Barreiro et al., 2019). En consecuencia, los partos otoño-invernales exigen un mayor uso de forrajes conservados y de concentrados, lo que implica un mayor costo de producción. Por otro lado, aunque los partos de primavera evitan el problema del bajo *stock* de pastura y alcanzan un pico de producción de leche más alto que las vacas paridas en otoño, estas vacas son desafiadas a mantener la normotermia bajo condiciones climáticas de estrés calórico (índice de temperatura y humedad > 68; Collier et al., 2011) durante el período de posparto temprano, lo que aumenta los requerimientos energéticos y podría comprometer su bienestar y productividad.

1.2.3. Influencia del ambiente productivo en el comportamiento, ingestión, digestión, producción y estado metabólico de vacas lecheras

1.2.3.1. Efecto del estrés calórico en el desempeño animal

El estrés calórico (EC) se define como la suma de fuerzas externas al animal que actúan elevando la temperatura corporal desde la normotermia e inducen respuestas conductuales y fisiológicas (Dikmen y Hansen, 2009; Yousef et al., 1986). El índice de temperatura y humedad (ITH), desarrollado por Thom (1959) y modificado por Armstrong (1994), permite detectar la presencia de EC. El umbral o valor crítico del ITH en el que la producción de leche comienza a verse afectada depende del nivel productivo y de la susceptibilidad de los animales, variando entre 72 (Armstrong, 1994; Ravagnolo et al., 2000) y 68 (Collier et al., 2011).

Los cambios en el comportamiento incluyen menor actividad física (Tapki y Şahin, 2006), búsqueda de sombra y lugares frescos, mayor consumo de agua, menor ingesta voluntaria de MS a una mayor tasa (Kadzere et al., 2002), mayor tiempo de pie (Allen et al., 2015; Cook et al., 2007) y menor tiempo de descanso (Tucker et al., 2007). Una mayor proporción de tiempo en pie, como mecanismo de convección para disipar el calor corporal (Allen et al., 2015; Cook et al., 2007), implica un menor tiempo de rumia (Kilgour, 2012; Schirmann et al., 2012), así como un menor flujo sanguíneo a la glándula mamaria (Rulquin y Caudal, 1992), en comparación con menores tiempos en pie y mayores tiempos tumbadas. A su vez, durante el EC el patrón diario de rumia se altera, con una mayor proporción durante la noche a medida que aumenta el ITH (Collier et al., 1981; Soriani et al., 2013).

El tiempo total de rumia está relacionado negativamente con el ITH y la frecuencia respiratoria y positivamente con la producción de leche, por lo que se considera un marcador de EC (Moallem et al., 2010; Soriani et al., 2013). Además, la rumia desempeña un papel fundamental en la descomposición física del material vegetal, la motilidad ruminal, la tasa de pasaje, la distensión ruminal y, en consecuencia, el consumo total de alimento (Allen, 1996; Silanikove, 1992). En simultáneo, la activación de mecanismos termorreguladores como la sudoración y el aumento de la frecuencia respiratoria provocan un desequilibrio en los niveles de sodio y potasio y reducen el aporte de bicarbonato al rumen a través de la saliva (Baumgard y Rhoads, 2013; Das et al., 2016; Schneider et al., 1986; West, 2003). Los cambios en los patrones de ingestión, que conducen a menos contracciones ruminales (Collier et al., 1981), se suman a la menor irrigación del epitelio ruminal y alteraciones metabólicas que redundan en menor aporte de bicarbonato al rumen y conducen al desequilibrio del ambiente ruminal, y perjudican el consumo de alimento y absorción de nutrientes (Baumgard et al., 2014; Collier et al., 1981, 2014; Schneider et al., 1986). Esto explica un 35 % a 50 % de la disminución en la producción de leche bajo condiciones de EC (Bernabucci et al., 2010; Wheelock et al., 2010).

Por otro lado, la sudoración, el aumento de la frecuencia respiratoria y el mayor tiempo en pie incrementan los requerimientos de EN de mantenimiento hasta un 25 % (Polsky y von Keyserlingk, 2017; West, 2003), por lo que parte de los nutrientes consumidos se desvían de su destino original -la glándula mamaria- para cubrir estas funciones. Al mismo tiempo, el flujo sanguíneo a los tejidos periféricos aumenta para disipar el calor y, en contrapartida, disminuye el flujo a la glándula mamaria y el aporte de nutrientes para la lactogénesis (Rulquin y Caudal, 1992). En consecuencia, la producción de leche puede verse disminuida hasta un 35 % ante situaciones de EC (Collier et al., 2011; Davis y Collier, 1985; Rhoads et al., 2009; Saravia et al., 2011).

1.2.3.2. Efecto de la exposición al frío y barro en el desempeño animal

Si bien la exposición al calor suele tener repercusiones inmediatas y claramente visibles y medibles sobre los animales, estos también están sujetos a la influencia de las condiciones ambientales durante la época otoño-invernal, cuyas consecuencias no son tan evidentes ni fácilmente cuantificables. Los factores externos de mayor influencia sobre la termorregulación en condiciones invernales son el viento, las precipitaciones y el espesor de la capa protectora animal (pelaje). Por ejemplo, ante precipitaciones de 10 mm/d es necesaria una temperatura ambiente menor a -4 ºC para provocar estrés por frío cuando el viento no supera los 5 km/h y el espesor del pelaje es mayor a 3 cm; sin embargo, ante vientos de 20 km/h, una temperatura menor a 14 ºC ya induce la termorregulación y, a menor espesor de pelaje, puede ser inducida a temperaturas por debajo de los 18 ºC (Freer et al., 2007).

A su vez, en condiciones invernales adversas, las vacas pasan más tiempo de pie en posturas que pueden reducir la cantidad de superficie expuesta al viento y la lluvia (Tucker et al., 2007; Webster et al., 2008), así como también lo hacen ante la presencia de barro en zonas de comedero y descanso (Chen et al., 2017). Estas posturas significan un aumento del gasto energético para mantenimiento (Tucker et al., 2007; West, 2003). La exposición a superficies húmedas o fangosas no solo impacta el tiempo total en que las vacas permanecen echadas, sino también la frecuencia con que se echan, la posición y la calidad del descanso (Chen et al., 2017; Schütz et al., 2019). De hecho, existe una fuerte tendencia a que la mayor parte del descanso se realice mientras las vacas están echadas (Kilgour, 2012). Esto se vuelve especialmente crítico para animales de alta producción que maximizan el tiempo destinado a la alimentación para cumplir con sus requerimientos, lo que limita aún más el tiempo disponible para descansar y otras actividades (Botheras, 2007). Como las vacas están muy motivadas para echarse incluso después de pequeños períodos de privación (dos a cuatro horas, Botheras, 2007), se podría suponer que, si no tuvieran la oportunidad de echarse durante la estabulación, usarían el tiempo de pastoreo para hacerlo (Cooper et al., 2007; Schütz et al., 2019). Esto podría alterar el comportamiento (tasa de ingesta y duración de la primera comida) y desestabilizar el ambiente ruminal, la fermentación y el crecimiento microbiano (Chilibroste et al., 2007, 2008). A su vez, como ya fue mencionado, un menor tiempo echadas podría afectar el tiempo de rumia (Cooper et al., 2007; Kennedy et al., 2005; Schirmann et al., 2012) y perjudicar aún más el CMS. Por último, un mayor esfuerzo para caminar sobre áreas fangosas provoca la resistencia de los animales a moverse a través de estas (Dickson et al., 2022), lo que puede hacer que coman con menos frecuencia y más cantidad por comida (Meyer, 2015), y por ende perjudicar el ambiente ruminal e incrementar el riesgo de trastornos ruminales (Mattiauda et al., 2018). En este sentido, una profundidad de barro de cuatro a ocho pulgadas puede reducir la ingesta de alimento en ganado en estabulación entre un 5 % y un 15 %, mientras que une profundidad entre doce y veinticuatro pulgadas puede disminuir la ingesta entre un 15 % y un 30 % según el National Research Council (1981).

1.2.3.3. Alteraciones hormonales y metabólicas bajo estrés

Además de los cambios de comportamiento y fisiológicos específicos para restablecer el equilibrio térmico, la exposición a condiciones ambientales adversas provoca estrés, lo que desencadena una cascada hormonal asociada a este, altera la secreción y regulación del eje pituitario-adrenal y, en consecuencia, perjudica el metabolismo energético (Fisher et al., 2002). Las interacciones complejas entre múltiples hormonas dificultan la comprensión de las vías implicadas (Moberg y Mench, 2000).

Las principales hormonas implicadas son el cortisol, la somatotropina (GH) y el factor insulino-símil (IGF-1), que instalan un estado catabólico y un redireccionamiento de nutrientes hacia la función de supervivencia en lugar de crecimiento, producción o reproducción (Moberg y Mench, 2000; Sapolsky et al., 2000). No obstante, las adaptaciones homeoréticas dependen de la naturaleza del factor estresante. Durante el EC, las vacas exhiben niveles elevados de insulina basal (Bernabucci et al., 2010) y reducción de los niveles de hormona tiroidea, leptina e IGF-1 (Baumgard y Rhoads, 2013; West, 2003), con consecuente reducción de la glucemia y aumento de β-hidroxibutirato (Soriani et al., 2013). Sin embargo, pareciera existir un cambio en las fuentes de energía endógenas gluconeogénicas hacia la utilización del tejido muscular en lugar de adiposo, debido al menor calor metabólico producido (Wheelock et al., 2010), por lo que no se acompaña de un aumento de los niveles de ácidos grasos no esterificados (AGNE; Bernabucci et al., 2010; Rhoads et al., 2009; Soriani et al., 2013; Wheelock et al., 2010), pero sí de mayor nivel de nitrógeno ureico plasmático, dada la proteólisis y desaminación consecuente (Cowley et al., 2015). Estos cambios en los sustratos gluconeogénicos podrían repercutir sobre las concentraciones de los componentes lácteos. Por otro lado, Tucker et al. (2007) observaron concentraciones más altas de cortisol, hormona tiroidea y AGNE en vacas expuestas al clima frío y húmedo en comparación con vacas alojadas en refugios interiores. Durante el estrés por frío, aunque el cortisol y la insulina aumentan, la leptina se regula a la baja con el fin de aumentar el CMS, lo que sugiere que la sensibilidad a la insulina en la secreción de leptina en ambientes fríos está deprimida (Asakuma et al., 2003).

1.2.4. Síntesis

En resumen, la intensificación de la producción lechera de base pastoril ha implicado mayor exigencia metabólica animal, mayores niveles de suplementación y concentración de animales en espacios reducidos por períodos de tiempo crecientes. A su vez, la mayor exposición a las condiciones ambientales durante la estabulación, ya sea calor, humedad o frío, causa alteraciones tanto en el comportamiento como en las respuestas endócrino-metabólicas de los animales que finalmente afectan la ingesta de nutrientes, su gasto energético y el desempeño productivo. Para comprender los efectos de la exposición ambiental sobre los animales como parte de un sistema lechero integral, es esencial estudiar diferentes niveles de exposición en diferentes situaciones ambientales durante distintos momentos de la lactancia (parición otoñal vs. primaveral).

1.3. Hipótesis

En SM a cielo abierto, la mayor exposición al ambiente afectará negativamente el comportamiento animal, reduciendo el consumo de alimentos y la eficiencia del uso de nutrientes para la producción de leche. Como consecuencia, las vacas presentarán menor producción de leche y sólidos, menor condición corporal (CC), y mayores concentraciones de metabolitos sanguíneos asociados a la lipomovilización.

Los sistemas productivos estabulados con TMR darán como resultado mayor producción de leche y sólidos, así como mayor CC y niveles más bajos de indicadores de lipomovilización, en comparación con los SM que ofrecen pastoreo directo + MR. Dichas diferencias entre sistemas de alimentación serán menores bajo condiciones similares de infraestructura durante su estabulación en comparación a los SM en condiciones de infraestructura más precarias y con mayor exposición a las condiciones ambientales.

1.4. Objetivos

1.4.1. Objetivo general

Evaluar el comportamiento, consumo, síntesis ruminal de proteína microbiana, estatus metabólico (reservas corporales, indicadores de lipomovilización) y desempeño animal (producción de leche y sólidos), durante la lactancia completa de vacas lecheras paridas en otoño y primavera alimentadas con un pastoreo diario + MR (SM) sometidas a diferentes niveles de exposición al medioambiente durante la estabulación parcial en comparación con el desempeño de vacas en SM con vacas totalmente estabuladas alimentadas con TMR *ad libitum*, utilizado como control positivo.

1.4.2. Objetivos específicos

Estudiar el efecto de dos condiciones contrastantes de alojamiento durante la estabulación parcial (corrales a cielo abierto sobre piso de tierra y tosca y estructuras para sombra versus galpón totalmente techado sobre cama compost y con dispositivos de ventilación y aspersión) sobre

a) comportamiento en pastoreo: duración de la primera sesión, tasa de bocados, probabilidad de estar comiendo, rumiando, descansando echadas o descansando paradas;
b) comportamiento ingestivo durante el consumo de MR y TMR en las primeras seis horas de suministrado el alimento: probabilidad de estar comiendo, rumiando, descansando echadas o descansando paradas;
c) CMS de pastura y alimento total;
d) síntesis ruminal de proteína microbiana;
e) desempeño animal: producción y composición de leche;
f) condición corporal e indicadores de movilización de reservas lipídicas (beta hidroxibutirato y ácidos grasos no esterificados)
g) eficiencia de conversión del alimento a leche corregida por grasa y proteína

en vacas alimentadas con MR + 1 turno de pastoreo de 7 horas (SM).

Estudiar el efecto del sistema de alimentación y su interacción con condiciones contrastantes de alojamiento (sistema TMR alojado en galpón totalmente techado sobre cama compost y con dispositivos de ventilación y aspersión versus dos SM bajo las dos condiciones de alojamiento —previamente mencionadas—) sobre

a) CMS de alimento total,

b) síntesis ruminal de proteína microbiana,

c) desempeño animal: producción y composición de leche,

d) condición corporal e indicadores de movilización de reservas lipídicas (beta hidroxibutirato y ácidos grasos no esterificados) y

e) eficiencia de conversión del alimento a leche corregida por grasa y proteína.

Estudiar el valor nutricional de las pasturas y TMR/MR consumidas en el experimento mediante

a) cinética de fermentación y

b) degradación de la MO y concentración energética.

2. Estructura general de la tesis

Para alcanzar los objetivos planteados, se llevaron a cabo dos experimentos: uno en vacas paridas a mediados de marzo (partos otoñales) y otro en vacas paridas a mediados de agosto (partos comúnmente denominados primaverales). Ambos experimentos siguieron un diseño factorial incompleto, con tres tratamientos combinando dos estrategias de alimentación y dos niveles de exposición ambiental. Se midieron variables de forma repetida a lo largo de gran parte de la lactancia (≈ 280 días) y otras variables fueron determinadas específicamente en un período limitado (dos semanas) durante la lactancia temprana (≈ 45 días posparto). La información generada en el desarrollo de este trabajo doctoral, derivada de los experimentos realizados, se presenta en formato de artículos científicos. El primer artículo se titula «Performance of Autumn and Spring Calving Holstein Dairy Cows with Different Levels of Environmental Exposure and Feeding Strategies» (Méndez et al., 2023) y analiza los efectos de los tratamientos aplicados a lo largo de la lactancia sobre las curvas de producción y composición de leche, el CMS total y de pastura estimado por balance energético en SM y oferta-rechazo en el tratamiento 100 % confinado, la evolución de la condición corporal y de metabolitos indicadores de estatus energético (BHOB y NEFA). El segundo artículo, titulado «Behavior, Intake, Digestion and Milk Yield of Early Lactation Holstein Dairy Cows With Two Levels of Environmental Exposure and Two Feeding Strategies» (Méndez et al., 2024), presenta los resultados de las variables de producción y composición de leche, CMS de pastura y total mediante estimación por técnica de doble marcaje en SM y oferta-rechazo en el tratamiento 100 % confinado, síntesis de proteína microbiana en rumen y comportamiento ingestivo en pastoreo y en estabulación en la lactancia temprana. Posteriormente, se incluye una discusión general sobre los temas abordados y las conclusiones e implicancias del trabajo doctoral para el sector productivo.

Es relevante aclarar que durante el experimento de parición de otoño surgieron inconvenientes con el manejo de la cama compost, como la filtración de aguas pluviales y dificultades con adquisición de materiales para reposición, lo que resultó en alta humedad de la cama e imposibilitó su correcto compostaje, especialmente en la zona de mayor intensidad de uso de la cama y deposición de excreciones, como es el caso del tratamiento totalmente confinado con TMR *ad libitum*. Esto redundó en problemas sanitarios en el rodeo (mastitis). En consecuencia, este tratamiento debió ser eliminado del ensayo otoñal luego de los noventa días de experimento. Por dicho motivo, el primer artículo, que analiza el desempeño animal y estatus metabólico a lo largo de toda la lactancia, no lo incluye, pero sí se compara en el segundo artículo que contempló un período acotado de la lactancia temprana, cuando aún no habían ocurrido dichos inconvenientes.

Por convención internacional, los términos *dieta mezclada* (utilizada para SM con pastoreo y suplementación) y *dieta totalmente mezclada* (utilizada para el sistema totalmente confinado) están abreviados por sus siglas en inglés MR y TMR (*mixed ration* y *total mixed ration*, respectivamente), mientras que las demás abreviaturas mantendrán sus siglas en español.

3. Impacto de la alimentación y nivel de exposición al ambiente sobre la producción de leche y estado metabólico en la lactancia completa en vacas de parición otoñal y primaveral

animals

MDPI

Article

Performance of Autumn and Spring Calving Holstein Dairy Cows with Different Levels of Environmental Exposure and Feeding Strategies

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Simple Summary: Since there has been an increase in the frequency of extreme weather events (i.e., heavy rains, heat stress) due to climate change, the interaction between feeding and management issues and the required facilities to alleviate environmental effects on animal performance has become relevant. Although there is extensive literature on the implementation of confined vs. mixed (grazing + mixed ration) feeding strategies in dairy systems, most are short-term studies and there is little information on their effects on cow full lactation performance, associated with environmental exposure stress throughout their productive cycle, which depends on their calving season. This manuscript determines and interprets its effects on milk production and composition and energy balance (i.e., body condition score, non-esterified fatty acids, and beta-hydroxybutyrate) during a full lactation in two calving seasons, addressing whole-herd (extensive to whole-farm) feeding and management issues. The results demonstrate that outdoor soil-bedded milk production systems, when well-managed, have a very high milk production potential that could equate to the productive response of improved infrastructure systems (i.e., a compost-bedded pack barn with cooling capacity) under moderately unfavorable environmental conditions (i.e., infrequent heavy rains), but in worse situations (i.e., severe heat waves and frequent heavy rains), performance could be compromised.

Abstract: Environmental exposure during confinement and feeding strategy affects cow behavior, nutrient utilization, and performance. Milk production and composition, body condition score, non-esterified fatty acids, and beta-hydroxybutyrate were determined during a full lactation in cows submitted to (a) grazing + partial confinement in outdoor soil-bedded pens with shade structures (OD-GRZ); (b) grazing + partial confinement in a compost-bedded pack barn with cooling capacity (CB-GRZ); or (c) total confinement (same facilities as CB-GRZ) and fed TMR ad libitum (CB-TMR). Autumn (ACS) and spring (SCS) calving season cows were used for each treatment, except for CB-TMR (only SCS). In ACS, treatments did not differ in any variable, possibly due to mild weather. In SCS, milk production was higher in CB-TMR than CB-GRZ, which in turn produced more milk than OD-GRZ. Differences coincided with heat waves and/or heavy rains (similar grazing conditions and mixed ration DM intake). Milk fat, protein and lactose yield, protein content, and BCS were higher in CB-TMR, without differences between CB-GRZ and OD-GRZ. Cows in OD-GRZ had impaired energy metabolism. Under moderately unfavorable environmental conditions (ACS), when well-managed, OD-GRZ systems could equate to the productive response of CB-GRZ. However, in worse climatic conditions (SCS), performance could be compromised, especially when compared to TMR systems.

Keywords: grazing; mixed ration; confinement; heat stress; heavy rain; full lactation performance

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3.1. Introduction

Cows in totally confined milk production systems fed total mixed rations (TMR) have higher intake, production, and energy balance than those in pastoral systems that harvest their forage. Nevertheless, mixed systems (grazing + a MR; MS) can capture some benefits of confined systems while maintaining relatively low feeding costs, which could improve the economics of totally confined systems [1–3].

Intensification of pasture-based milk production systems through stocking rate increments results in higher inclusion of supplements in the diet. Indeed, use of conserved forage in conjunction with concentrates in a MR results in higher dry matter (DM) intake and higher milk and solids production [1,2]. This production strategy involves cows being out of the paddock 40 to 60% of the time in an area where feed supplements are provided [3]. Furthermore, high pasture grazing intensity associated with high stocking rates leads to longer periods without access to pasture in order to recover forage in the system, thereby generating the need for facilities to confine the cows [4].

Calving distribution through the year has important effects on the annual milk remittance pattern [5] and, consequently, on milk industry supply. Autumn/winter calved cows achieve more total milk production than those calved in spring. They also have longer lactation and a shorter calving to conception interval [6]. However, in Uruguayan winters, a lower pasture growth rate and heavy rains can prevent pasture access, resulting in less directly harvested forage inclusion in the diet of autumn calving cows (ACS) during early lactation. This results in higher feed supplementation and more time in confinement facilities. Although spring calving cows (SCS) achieve higher pasture DM intake and lower production costs, cows experience the challenge of maintaining normothermia in addition to the stress of early lactation [7], which can compromise welfare and productivity. Each dairy system must be evaluated for the opportunities and weaknesses of each calving strategy and adopt the one that best suits its productive objectives.

When confinement time becomes considerable, the negative effects of exposure to environmental conditions on cow comfort, behavior, and performance are accentuated [8]. During heat stress (HS), energy demand for the thermoregulation of immune system hyperactivation diverts the energy supply to the mammary gland [9,10]. This, together with reduced nutrient absorption and lower udder nutrient uptake [11,12], causes milk production decreases of up to 35% [10,13]. On the other hand, under adverse winter conditions, with cold and mud in feeding and resting areas, cows spend more time standing and less time lying [14,15]. Lying time is critical not only because it is associated with rumination time [16], but it is also important for cows to achieve a sufficient amount of quality rest time [17]. Lying deprivation during confinement periods has an impact on behavior during next period activity, which in MS corresponds to grazing [15,16,18]. In addition, cow resistance to moving through muddy areas can cause cows to eat less overall, with fewer larger meals, which negatively impacts ruminal fermentation [19]. As a consequence, nutrient utilization and performance are impaired.

There is scarce literature on pasture-based cow performance under different housing conditions and environmental exposure stress (i.e., cold, mud, or heat stress) at different stages of lactation, depending on calving season strategy. Research on the effects of expo-sure to the environment on intensive grazing systems is not only original but also highly relevant from an economic and environmental perspective for the intensification of dairy systems in the southern hemisphere [4]. Although the implementation of confined vs. MS feeding strategies has research antecedents, the existent literature refers to short-term studies [1,2,20–24], and according to our knowledge, no research has been conducted throughout the productive cycle. Furthermore, compost-bedded pack barns are novel facilities to house animals that allow cows freedom to move and a soft place to lye, thus improving animal welfare while enabling manure recycling, consequently diminishing environmental pollution and productive costs [25,26].

The objectives of this study were to: (a) measure and evaluate the effect of different levels of environmental exposure on the performance of cows consuming MR + grazing in two different strategies of calving season (autumn and spring); (b) compare high and low environmental exposure MS with a 100% confined TMR system when detrimental heat stress effects are most pronounced (i.e., SCS strategy). It was hypothesized that milk cows partially confined in a compost-bedded pack barn with a cooling capacity would improve full lactation milk and solids production and energy balance when compared to cows partially confined in outdoor soil-bedded pens with shade structures. Further, a totally confined system would improve milk and solids production, as well as energy balance, compared to MS-fed cows, obtaining more contrasting responses when compared to cows housed in outdoor soil-bedded pens than when compared to cows housed in a compost-bedded pack barn.

3.2. Materials and Methods

3.2.1. Cows and Experimental Design

A total of 80 Holstein cows (2.8 ± 1.25 lactations, 640 ± 85 kg body weight; BW) were used in 2 calving experiments, 1 in each calving season, conducted at the Estación Experimental Dr. M. A. Cassinoni (EEMAC) of the Facultad de Agronomía (Paysandú, Uruguay) of the Universidad de la República (UdelaR). Experimental periods lasted from March 2019 to January 2020 for ACS cows and from August 2019 to May 2020 for SCS cows. The experimental protocol was evaluated and approved by the Comisión Honoraria de Experimentación Animal (CHEA) from UdelaR (Montevideo, Uruguay). All cows were managed equally during the dry and prepartum periods and confined and fed a pre-partum TMR for ~3 weeks before expected calving dates. Autumn-calving cows had calving dates of 18 March 2019 ± 14.5 days and spring-calving cows had calving dates of 16 August 2019 ± 8.2 days. Cows were blocked by BW, lactation number, pre-calving body condition score (BCS) and expected calving date, randomly assigned to treatment, and grouped in 4 pens of 4 cows each (i.e., 16 cows/treatment).

Treatments consisted of: (a) ACS and SCS cows subjected to 8 h in a grazing paddock + supplemental MR in outdoor soil-bedded pens with shade structures during confinement (OD-GRZ; high environmental exposure MS); (b) ACS and SCS cows subjected to 8 h in a grazing paddock + supplemental MR in a compost-bedded pack barn with cooling capacity during confinement (CB-GRZ; low environmental exposure MS); (c) SCS cows subjected to a totally confined system with cows in the same facilities as CB-GRZ but fed a TMR twice daily ad libitum (CB-TMR).

3.2.2. Management and Feeding

The CB-TMR and CB-GRZ cows were confined in a fully roofed, compost-bedded pack barn divided into pens, each containing four cows. Compost-bedded pack area was of 13.5 m^2 per cow and was continued by a concrete area of 6.7 m^2 per cow with access to a feed bunk per pen with a length of 0.75 m per cow and automatic drinkers to ensure ad libitum access to water. A 15 cm layer of new, fresh bedding material (i.e., rice husks and wood chips) was supplied every 15–20 days. Compost was superficially labored twice a day with a chisel plough in order to remove water vapor, allow oxygen entry, and maintain small, homogeneous particles. The concrete area was cleaned three times a week by trawling with a tractor carrying rubber. The barn had cooling capacity, with continuous-operation fans and sprinklers with automatic operation over 25 °C.

The OD-GRZ cows were confined in outdoor soil-bedded pens made up of 2 paddocks, alternately occupied according to soil moisture and surface deterioration, with an area of 48 m^2 per cow. Paddocks had a slight slope for water and manure runoff. Each pen had shade structures of 4.8 m^2 per cow (nylon roofed at 4.5 m height with a slope of 15%), close to automatic drinkers (same as previous). Feeders were located at the other end of the paddocks, with a length of 1.10 m per cow and a feeding area of 10 m^2 per cow.

The milk parlor was built 100 m from pens in order to minimize cow activity and long waiting periods during milking.

The TMR/MR were the same for all treatments. It varied over time according to available conserved forage as well as market available grains and by-products for the commercial concentrate, with a total of 7 combinations used in the 15 months of the experiments (Table 1). Diets were formulated based on the recommendations of the National Research Council (NRC [27]) for 620 kg cows producing 45 L/d of 4% fat-corrected milk. In MS, both pasture and MR were considered nutritionally balanced diets, with MR used as a pasture complement (limited amounts) to achieve the desired DM intake, which was dependent on current pasture stock of the system.

The MS (i.e., OD-GRZ and CB-GRZ) were high stocking rate systems (i.e., 2.5 lactating cows and/or 1550 kg BW/ha grazing platform), where cows had 7 h of daily access to weekly grazing plots, if allowed by weather conditions and/or grazing platform available herbage mass (HM). Both grazing treatments accessed different grazing plots (all pens of the same treatment together) with similar herbage allowances (HA). From March to October 2019, cows grazed between 7 a.m. and 2 p.m., while from November 2019 to April 2020, cows grazed from 6 p.m. to 2 a.m., in order to minimize heat stress and its negative impact during grazing. The pastures used were: tall fescue (*Festuca arundinacea*), lucerne (*Medicago sativa*) + orchard grass (*Dactylis glomerata*), oat (*Avena sativa*), annual raygrass (*Lolium multiflorum*), and soybean (*Glycine max*). Forage management and chemical composition are summarized by season in Table 2. Herbage mass was determined weekly using the double sampling technique [28] and then calculated pasture growth in order to adjust HA, taking into account sward condition (i.e., number of leaves or axillary buds) and available HM in the total grazing platform. Supplementation with TMR/MR was adjusted to ensure cow requirements and productivity goals were met and to achieve the appropriate pasture rotation length depending on pasture growth rate.

3.2.3. Data collection, Measurements and Estimates

Climatic conditions (i.e., ambient temperature, relative humidity, rain) records were obtained from the meteorological agency of the experimental station. Heat stress was calculated using a temperature humidity index (THI) as: (1.8 × ET + 32) − (0.55 − 0.55 × RH/100) × (1.8 × ET − 26.8), where ET is environmental temperature and RH is relative humidity [29]. It was considered a mild heat wave when 2 of the 3 following criteria occurred at least 3 days in a row: daily THI average > 72, maximum daily temperature was >32 °C and/or minimum daily temperature was >23 °C. When all three conditions occurred simultaneously, it was considered a severe heat wave [30].

Cows were milked at 4 a.m. and 5 p.m. during spring/summer and at 3 a.m. and 4 p.m. during autumn/winter in order to minimize heat stress and its negative impact during grazing, as previously mentioned. Individual cow production was recorded at each milking. Milk samples were collected weekly from calving to 90 days in milk (DIM), biweekly from 91 to 180 DIM, and then monthly to the end of the lactation to determine milk fat, protein, and lactose levels (MilkoScan FossElectric FT2®, Drachten, The Netherlands).

The offered and refused TMR/MR were measured weekly, as well as sampled, weighed, and oven-dried at 55 °C for 48 h to calculate dry matter intake. Samples were also analyzed for crude protein (CP), neutral detergent fiber (NDF), and acid detergent fiber (ADF), according to AOAC [31]. Total N for CP estimation used the Kjeldahl method of AOAC [32], which involves sulfuric acid digestion with subsequent distillation and titration. NDF used α-amylase, and, as for ADF, an ANKOM200 Fiber Analyzer (ANKOM Tech. Corp., Fairport, NY, USA) was used. Pasture was also sampled and chemically analyzed weekly.

Daily pasture DM intake (kg DM/cow) was estimated by energy balance according to NRC [27], as the kg of pasture necessary to provide the remaining energy to achieve cow net energy (NE) requirements did not come from MR. Cow NE requirements were estimated as the sum of maintenance, pregnancy, and milk production requirements, taking into account energy contributed or required from losing or gaining BW and BCS [29]. Maintenance requirements were calculated as 80 kcal of NE/kg BW0.75, with a 20% increase for grazing activity in mixed systems [27]. Cow BW was measured monthly for this purpose. The amount of NE/kg BW was calculated according to actual BCS, adjusted for when it was used to support milk production or body deposition [29]. The BCS was assessed biweekly until 120 DIM and monthly from 120 to 305 DIM based on a 5-point scale, ac-cording to [33]. Pregnancy requirements were calculated from 190 to 279 days of gestation as: NEL (Mcal/day) = (0.00318 × D − 0.0352) × (CBW/45)/0.218, where D is day of gestation and CBW is calf birth BW in kilograms. Milk NEL concentration for productive requirements (i.e., energy in milk) was calculated from milk production and composition as milk NEL (Mcal/kg) = 0.0929 × Fat% + 0.0547 × CP% + 0.0395 × Lactose%. The NEL provided by pasture and TMR was calculated according to NRC [34] as Pasture NEL (Mcal/kg) = 2.149 − (0.0223 × ADF) and TMR NEL (Mcal/kg) = 1.909 − (0.017 × ADF).

Blood samples were collected biweekly until 120 DIM and monthly from day 120 to 210 DIM, by venipuncture of the coccygeal vein, using 10 mL Vacutest® tubes (Vacutest Kima, Arzergrande, Italy) with heparin. Refrigerated samples were centrifuged at 3000× g for 15 min, and plasma was stored at −20 °C until analysis. Non-esterified fatty acids (NEFA) and beta hydroxybutyrate (βHB) concentrations were determined spectrophotometrically using commercial kits (Wako NEFA-HR (2) from Wako Pure Chemical Indus-tries Ltd., Osaka, Japan, and Oxidase/Peroxidase, UREA/BUN-UV, Ureasa/Glutamate De-hydrogenase from BioSystems SA, Barcelona, Spain, respectively).

Table 1. Composition, chemical analysis, and nutritional value of mixed diets fed to lactating cows (% dry matter).

From **To**	**February-19** **May-19**	**June-19**	**July-19** **August-19**	**August-19** **October-19**	**October-19** **February-20**	**March-20** **April-20**	**April-20** **May-20**
Experiment	ACS [1]	ACS [1]	ACS [1]	ACS [1] SCS [2]	ACS [1] SCS [2]	SCS [2]	SCS [2]
Ingredient							
Forage							
Corn silage	24.6	35.3	35.3	-	-	26.0	23.0
Sorghum silage	-	-	-	37.5	37.5	-	-
Lucerne silage	-	-	-	-	-	9.0	8.0
Ryegrass silage	21.1	-	-	-	6.5	9.0	8.0
Fescue hay	-	2.0	6.0	6.5	-	-	-
Commercial concentrate mix [3]	54.3	62.7	58.8	56.0	56.0	56.0	61.0
Dry matter (DM)	43.4	51.8	59.7	55.9	50.4	37.0	41.0
Nutrient							
NE_L (Mcal/kg DM) [4]	1.63	1.68	1.68	1.68	1.65	1.64	1.64
Crude Protein	15.9	15.1	16.4	16.5	15.8	14.6	16.7
Neutral Detergent Fiber	33.1	31.0	28.0	29.5	29.6	36.3	34.4
Acid Detergent Fiber	16.5	13.6	13.5	13.5	15.1	15.7	15.9
Ether Extract	3.8	3.5	4.1	3.8	4.6	3.1	3.5
Starch	22.0	27.0	20.0	18.0	17.0	17.0	18.0

[1] Autumn calving season. [2] Spring Calving Season. [3] Based on ground corn grain, wheat bran, soybean meal, sunflower meal, cottonseed meal, canola meal, rumen inert fat, urea, yeast, and minerals. [4] Net Energy of lactation, calculated as 1.909 − (0.017 × ADF) according to NRC [34].

Table 2. Forage management and chemical composition in each season in mixed systems with low (CB-GRZ) or high (OD-GRZ) environmental exposure.

		Herbage			**Chemical Composition**			
		Allowance [1]	**Mass** [2]	**DM**	**NE_L** [3]	**CP** [4]	**NDF** [4]	**ADF** [4]
Autumn 2019	CB-GRZ	16.3	2378	34.7	1.45	9.7	62.0	32.2
	OD-GRZ	15.1	2411	32.4	1.47	13.8	57.9	29.5
Winter 2019	CB-GRZ	17.9	2213	22.7	1.60	19.4	47.9	22.0
	OD-GRZ	19.7	2429	21.7	1.64	18.2	47.8	20.0
Spring 2019	CB-GRZ	23.8	3198	24.0	1.54	14.5	51.5	25.5
	OD-GRZ	23.5	3022	24.5	1.65	16.7	51.2	19.3
Summer 2020	CB-GRZ	26.5	3804	35.7	1.56	14.5	41.0	24.6
	OD-GRZ	26.7	3566	30.3	1.55	17.9	40.1	25.1
Autumn 2020	CB-GRZ	21.1	2497	38.0	1.61	22.4	43.1	21.6
	OD-GRZ	19.8	2337	37.8	1.60	21.3	44.5	22.3

[1] Expressed as kg DM/cow/day. [2] Expressed as kg DM/hectare, estimated at ground level. [3] Calculated as (3.2 − 0.028 × ADF) × 0.62 [35]. Expressed as Mcal/kg DM. [4] Expressed as % of DM.

3.2.4. Statistical Analysis

Data were analyzed using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC, USA) with the model: Yij = μ + Ti + WOSj + Bk+ Ti × WOSj + eijk, where Yij is the response variable, Ti is treatment, WOSj is week of study (WOS), Bk is block fixed effect, Ti × WOSj is treatment by WOS interaction effect, and eijk is residual error. Treatments were compared with each other. The cow was considered the experimental unit for milk production and composition, BCS, and metabolite concentrations, while the pen was the experimental unit for TMR and pasture DM intake. Data were analyzed as repeated measures over time. Equidistant distribution was considered for milk production (daily) as well as DM intake (weekly), while uneven distribution was considered for milk solids production, BCS, NEFA, and βOHB. Values at calving were included in the model as co-variables to compare BCS, NEFA, and βOHB between treatments throughout lactation. Each calving season was analyzed separately. Mean comparisons were performed by Tukey-Kramer analysis. Mean differences were considered significant if $p \leq 0.05$. Results are shown as least square means ± standard error of the mean (SEM).

3.3. Results

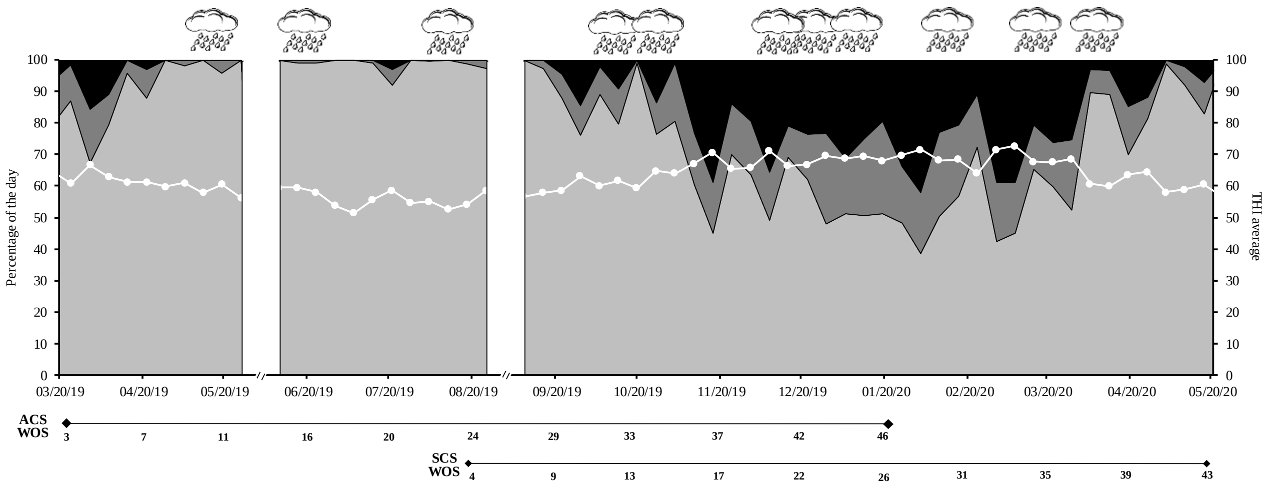

Figure 1. Percentage of the day in which a temperature humidity index (THI) was observed that was less than 68 (light grey), between 68 and 72 (dark grey), and higher than 72 (black). The continuous white line with dots indicates the THI weekly average. Cloudy icons indicate moments of heavy rain (>50 mm per week during consecutive weeks or >80 mm in a week). There were no records during part of May and August 2019 due to technical problems at the meteorological station (blank spaces).

The daily average THI and percentage of the days in which THI was <68, between 68 and 72, and >72, and times of heavy rains (i.e., >50 mm/week during at least 3 consecutive weeks or >80 mm in a week) through the 15 months of both experiments are shown in Figure 1. Table 1 shows the ingredients and chemical composition of the 7 TMR/MR fed in both experiments (ACS and SCS). Table 2 shows HA and HM during each season of the experiment, as well as the chemical composition of all MS.

3.3.1. Autumn Calving Season

Heavy rain occurred during WOS 10, 15, 23, 32 to 35, 41 to 43, and 45. Mild heat waves occurred in WOS 37, 40, and 45, and a severe heat wave occurred at WOS 43 (Figure 1). The MR intake averaged 11.7 ± 3.4 kg DM/cow/day, while pasture was 7.2 vs. 7.3 ± 3.2 kg DM in CB-GRZ and OD-GRZ during periods without total confinement (Table 3). Cows were confined during

WOS 6–7, 14–15, and 42–43 due to low HM. During these periods, DMI averaged 21.5 ± 4.3 kg/cow/day.

Table 3. Total mixed ration (TMR) and pasture dry matter intake (DMI) per cow (kg DM/day) in the autumn and spring calving seasons in confined (CB-TMR) and mixed systems with low (CB-GRZ) or high (OD-GRZ) environmental exposure during complete lactation.

		Autmn Calving Season		Spring Calving Season		
		CB-GRZ	OD-GRZ	CB-TMR	CB-GRZ	OD-GRZ
Autumn 2019	Pasture	7.8	7.6	-	-	-
	TMR	14.0	13.6	-	-	-
Winter 2019	Pasture	4.3	4.1	-	5.9	4.7
	TMR	14.5	14.3	24.1	14.0	14.6
Spring 2019	Pasture	9.2	9.3	-	7.2	6.1
	TMR	8.2	8.2	28.0	11.2	11.5
Summer 2020	Pasture	8.8	11.3	-	5.9	5.0
	TMR	8.5	8.5	27.1	12.8	12.8
Autumn 2020	Pasture	-	-	-	9.3	8.9
	TMR	-	-	21.9	10.9	10.9

All milk production response variables were affected by WOS, but none were affected by treatment (Table 4). Milk production had a T×WOS interaction (Figure 2). Although fat and protein content and protein had overall T×WOS interaction effects, there were no specific WOS in which the treatment outcomes differed.

Table 4. Milk production and composition (means ± SEM) per cow in autumn and spring calving seasons in confined (CB-TMR) and mixed systems with low (CB-GRZ) or high (OD-GRZ) environmental exposure during complete lactation.

		Treatment				*p*-Value		
		CB-TMR	CB-GRZ	OD-GRZ	SEM	TRT	WOS	TRT × WOS
Autumn calving season								
L/cow/day		-	26.2	26.5	0.16	0.14	<0.01	<0.01
Fat	%	-	3.55	3.57	0.04	0.74	<0.01	<0.01
	kg/d	-	1.02	1.01	0.02	0.58	<0.01	0.07
Protein	%	-	3.40	3.44	0.02	0.21	<0.01	<0.01
	kg/d	-	0.98	0.98	0.01	0.80	<0.01	<0.01
Lactose	%	-	4.82	4.87	0.03	0.28	<0.01	0.06
	kg/d	-	1.42	1.41	0.03	0.73	<0.01	0.14
Spring calving season								
L/cow/day		35.9 ^{a}	27.4 ^{b}	26.0 ^{c}	0.17	<0.01	<0.01	<0.01
Fat	%	3.59 ^{a}	3.55 ^{ab}	3.43 ^{b}	0.04	0.03	<0.01	<0.01
	kg/d	1.34 ^{a}	1.04 ^{b}	1.01 ^{b}	0.02	<0.01	<0.01	<0.01
Protein	%	3.29 ^{a}	3.17 ^{b}	3.19 ^{b}	0.04	0.01	<0.01	<0.01
	kg/d	1.23 ^{a}	0.96 ^{b}	0.95 ^{b}	0.02	<0.01	<0.01	<0.01
Lactose	%	4.89 ^{a}	4.78 ^{b}	4.87 ^{a}	0.04	<0.01	<0.01	<0.01
	kg/d	1.82 ^{a}	1.44 ^{b}	1.44 ^{b}	0.02	<0.01	<0.01	<0.01

^{a,b,c} Means within season with different superscripts differ ($p < 0.05$). TRT—treatment; WOS—weeks of study; TRT×WOS—interaction.

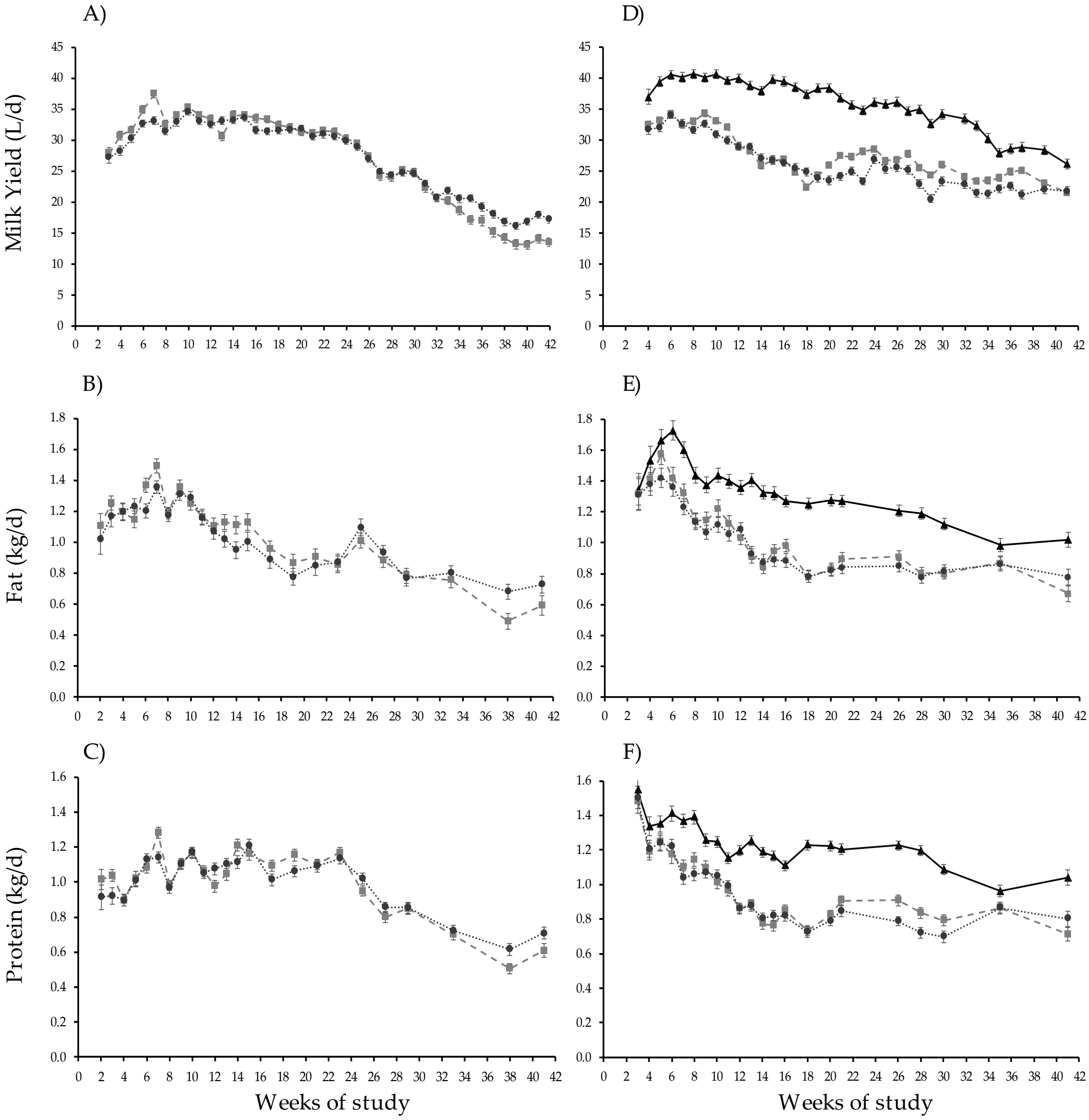

Figure 2. Milk (L/day), fat, and protein (kg/day) yield per cow in autumn (**A**–**C**) and spring (**D**–**F**) calving seasons in confined (—▲—) and mixed systems with low (- ■ -) or high (···●···) environmental exposure during complete lactation.

The BCS patterns during complete lactation adjusted for BCS at calving (3.05 ± 0.27) were affected by WOS for all treatments (Figure 3) but did not differ between treatments (2.67 vs. 2.69 ± 0.02).

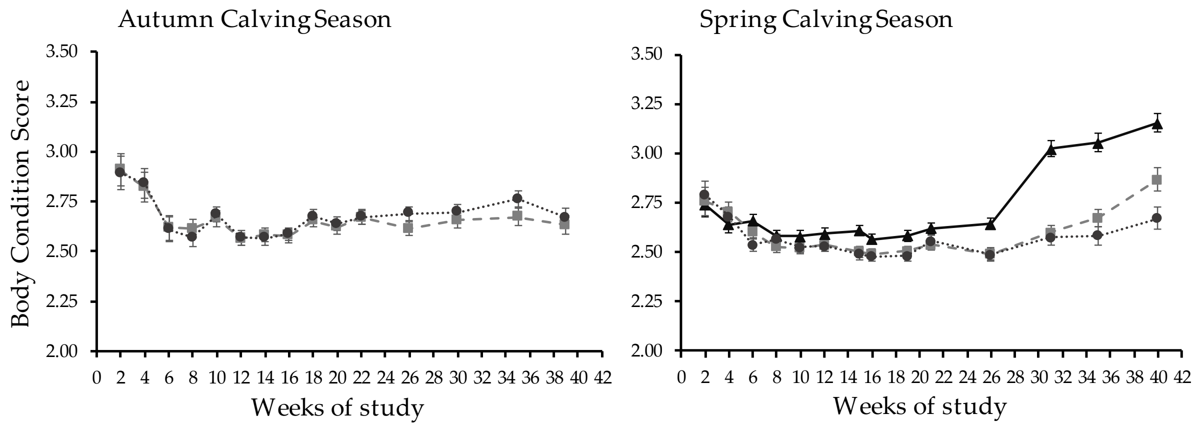

Figure 3. Patterns of body condition score in autumn and spring calving seasons in confined (▲) and mixed systems with low (■) or high (●) environmental exposure during complete lactation.

Plasma levels of NEFA and βHB were only affected by WOS (Figure 4). The mean levels throughout WOS 2 to 30 were 0.35 ± 0.13 mmol/L of NEFA and 0.72 ± 0.07 mmol/L of βHB.

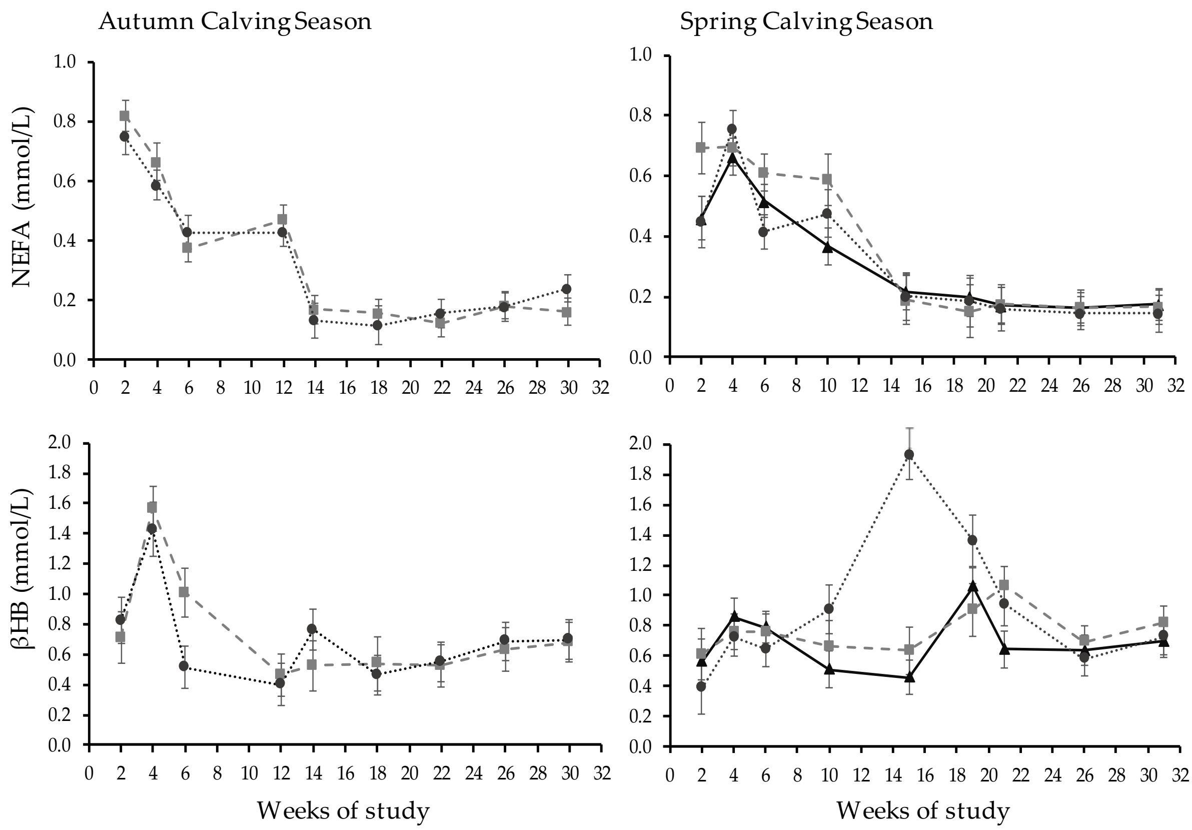

Figure 4. Patterns of non-esterified fatty acids (NEFA, mmol/L) and beta-hydroxybutirate (βHB, mmol/L) plasma levels in autumn and spring calving seasons in confined (▲) and mixed systems with low (■) or high (●) environmental exposure.

3.3.2. Spring Calving Season

Heavy rain occurred during WOS 12 to 15, 21 to 23, 25, 30, 34, and 37. Mild heat waves occurred in WOS 17, 20, 25, 28, and 32 to 34, and severe heat waves occurred in WOS 23 and 29 (Figure 1).

The MR intake averaged 12.3 ± 2.3 kg DM/cow/day, while pasture averaged 6.8 vs. 5.8 ± 2.5 kg DM in CB-GRZ and OD-GRZ, not including periods of total confinement (Table 3). Cows were confined during WOS 22–23, 35–38, and 43–44, always as a consequence of low HM, averaging 20.0 ± 1.4 kg DM/cow/day. The totally confined system for SCS cows averaged a daily TMR intake of 25.7 ± 2.8 kg DM/cow/day.

All production responses were affected by WOS treatment and had a T×WOS interaction (Table 4). Milk production was higher in CB-TMR than MS, and CB-GRZ produced more milk than OD-GRZ. Confinement system cows produced more milk than both MS in all WOS except 4, 36, and 37, whereas CB-TMR equaled CB-GRZ and was higher than OD-GRZ. The MS groups were similar, except in WOS 23, 29, and 37, when CB-GRZ milk production was higher than OD-GRZ. Fat content was higher in CB-TMR than OD-GRZ and intermediate in CB-GRZ. Fat, protein, and lactose yields, as well as protein content, were higher in CB-TMR compared to both MS, which were similar. Lactose content was higher in TMR and OD-GRZ than CB-GRZ. While fat content had a T×WOS interaction in SCS, no treatment differences occurred in any WOS. Protein content was only higher in CB-TMR than OD-GRZ and CB-GRZ in WOS 14. Lactose, protein, and fat yield were higher overall in CB-TMR than OD-GRZ and CB-GRZ.

The BCS was affected by WOS treatment and had a T×WOS interaction (Figure 3). The confined system had a higher BCS than CB-GRZ and OD-GRZ ($p < 0.01$), which did not differ (2.76 vs. 2.62 and 2.59 ± 0.02, respectively). At WOS 15 and 26 onwards, CB-TMR had a higher BCS than MS. During WOS 40, OD-GRZ had a lower BCS than CB-GRZ, which in turn was lower than CB-TMR, with a BCS of 2.67, 2.87, and 3.15 ± 0.05, respectively ($p < 0.01$).

Plasma NEFA was only affected by WOS ($p < 0.01$, Figure 4, mean 0.35 ± 0.03 mmol/L during the first 30 WOS). The plasma βHB was affected by treatment ($p = 0.01$), as well as WOS and the T*WOS interaction ($p < 0.01$). The OD-GRZ had the highest plasma βHB throughout, while the confined system had the lowest mean and CB-GRZ was intermediate (0.91 vs. 0.69 vs. 0.77 ± 0.05 mmol/L, respectively). During WOS 10, OD-GRZ had higher βHB plasma levels than CB-TMR, while CB-GRZ was intermediate (0.91 vs. 0.51 and 0.66, ± 0.15 mmol/L respectively, $p < 0.01$). At WOS 15, OD-GRZ had the highest βHB plasma levels of all WOS and treatments, 1.93 mmol/L, without differences between CB-TMR and CB-GRZ. In WOS 21, OD-GRZ βHB plasma levels declined and were intermediate between CB-GRZ and CB-TMR (0.94, 1.07, and 0.65 mmol/L, respectively, $p < 0.05$).

3.4. Discussion

3.4.1. Autumn Calving Season

In the autumn calving season, the MS subjected to different levels of environmental exposure did not differ from each other in any analyzed variable (i.e., milk production, milk composition, energy metabolism). Although June, October, and December rainfall values exceeded historical averages, during the rest of the year they were below historical values (the cumulative value of the 3 months was 598 vs. 307 mm, and the remaining months were 965 vs. 1369 mm for experimental station and the national historical average, respectively, Instituto Uruguayo de Meteorología) [36]. In addition, heat stress only occurred for 2 weeks at the end of the study at an advanced stage of lactation, when cows are less susceptible to heat stress due to lower intake and production, and therefore lower metabolic heat output [11]. Although the OD-GRZ treatment was more exposed to environmental conditions than the CB-GRZ, good maintenance of the infrastructure in the feeding and resting areas (i.e., cleaning after periods of rain, mound construction), as recommended [37], in addition to correct shade sizing [7] and ad libitum access to fresh water, likely mitigated the negative effects of such exposure. The open-air conditions to which the OD-GRZ treatment cows were subjected were judged to be better than those on commercial Uruguayan dairy farms. In general, commercial dairy farm animal facilities maintenance is less frequent and rigorous than in our study, such that cows face longer and muddier conditions, which are detrimental to their well-being and performance [14,16]. As well, the number of cows per pen is usually much higher than that used in our study, with higher deposition of excreta and higher ground pressure, causing higher moisture and surface deterioration.

The seasonal HM and HA values, as well as paddock access time, indicate that cows had no l imitations [38,39] to reaching forage harvest levels close to 11 kg DM/cow/day [40]. Herbage allowance reached in our MS, characterized by high stocking rates and supplementation, was 38.2 vs. 38.5 for CB-GRZ and OD-GRZ, respectively. It should be taken into account that pasture DM intake values were estimated according to the difference between the energetic contribution of the TMR and estimated production and maintenance requirements, plus a fixed extra maintenance cost of 20% for grazing activity, making it an approximate estimate that allows values to be compared to other studies. Notwithstanding, milk fat content did not differ between groups. In general, increasing pasture inclusion causes a higher acetic:propionic ratio in the rumen [20,41], thereby increasing precursors for de novo milk fat synthesis as opposed to diets with a higher inclusion of concentrate. This result confirms the lack of difference in estimated pasture intake and pasture inclusion diet between our treatments [42].

In ACS, the lack of treatment differences with no T×WOS interaction in BCS is consistent with similar levels of indicators of energetic metabolism (i.e., NEFA and βHB). Calving BCS was at the lower limit of what is considered desirable (3.03 and 3.00 ± 0.27 for both MS) according to Roche et al. [43]. However, cows mobilized tissue to a nadir of 2.58 in WOS 8 and 12, a value that is not critically low, which is supported by NEFA values, which decreased to <0.6 mmol/L in WOS 6, stabilizing in 0.16 ± 0.05 mmol/L between WOS 14 and 30.

3.4.2. Spring Calving Season

In SCS mixed systems, differences occurred in milk production during full lactation among treatments subjected to different levels of environmental exposure. This was mainly due to the cumulative numerical differences between WOS 20 and 37, which was summer. As MR quantity (15.4 ± 3.9 kg DM) and composition, as well as HM and HA, were similar between treatments during this period (3783 ± 2610 kg DM/ha and 28.4 ± 15.9 kg DM/cow, respectively), the lower cow performance in the OD-GRZ treatment (1.5 L less than CB-GRZ) could be due to differences in environmental exposure. It was during this period that the 6 mild waves and the 2 severe heat waves were concentrated, and, during WOS 23, both treatments were in confinement, consuming equal amounts of MR. Thus, it seems that the productive difference was due to differences in environmental exposure, as the first severe heat wave occurred in that WOS. The next significant difference in WOS 29 coincides with the 2nd severe heat wave. At this time, cows were under similar grazing conditions: 16 vs. 18 kg DM/cow of HA and 2150 vs. 2500 kg DM/ha of HM for OD-GRZ and CB-GRZ, respectively. The third significant difference in milk production between MS and WOS 37 was observed. Moreover, during WOS 36 and 37, CB-GRZ milk production did not differ from CB-TMR, but it did from OD-GRZ. Although the conditions to rate WOS 36 as a heat wave were not reached, it averaged a THI of 73.2, which means that cows suffered heat stress. In addition, there was a heavy rain event in WOS 37 that could have affected milk production, as moisture content is aversive to cows' willingness to lie down. The fact that both

MS were in total confinement entails that OD-GRZ cows could not trade off confinement resting behavior deprivation during grazing time [16,18], impairing their welfare and performance.

The higher energy demand due to the activation of adaptation mechanisms [9,10,44], together with the reduction of nutrient absorption and lower udder nutrient uptake [45] cause milk production to decrease by up to 35% [7,13]. Thus, milk production differences between levels of environmental exposure were ~5%, a lower difference that may be due to this being a long-term study that encompasses a full lactation, with part of the experiment occurring in thermoneutral conditions. During summer, the mean difference amounted to 10%, with peak differences of 20% in the weeks where heat waves occurred (i.e., WOS 23 and 29).

No differences in milk fat and protein content occurred between MS subjected to contrasting environmental exposure, although milk lactose content was higher in OD-GRZ. Smith et al. [46] showed increased milk fat content (3.5 to 3.7%) and decreased milk protein content (3.2 to 3.1%) in heat stressed cows from mild to moderate HS without changes between moderate and severe HS. This is consistent with Wheelock et al. [9], who did not report an effect on milk fat or protein content in heat-stressed cows. In contrast, Cowley et al. [47] observed that HS had a strong influence on milk protein concentration but no effect on that of milk fat or lactose, hypothesizing that HS could provoke specific downregulation of mammary protein synthesis. Quantifying direct physiological effects on milk production is difficult, and inconsistent results have been reported, as it is also strongly affected by behavioral factors that affect nutrient ingestion and absorption, which is even more complex in grazing conditions. The numerically lower pasture DM intake and its inclusion in the diet in OD-GRZ, and therefore higher TMR inclusion, suggests a relatively higher starch passage to the small intestine and higher glucose availability in the mammary gland in comparison to CB-GRZ, which consumed more herbage, which could explain the higher milk lactose content [22,41].

The treatment with the highest environmental exposure expressed an energy metabolism imbalance, evidenced by higher values of βHB between WOS 15 and 19 (~85 to 125 DIM), indicating situations of subclinical ketosis (>1.2 mmol/L [48]), which resulted in lower BCS recovery at the end of the study, compared to treatments less exposed to the environment (CB-GRZ) and/or with a higher feeding level (CB-TMR). Climatic conditions during WOS 12–15 were associated with cold rather than heat stress, as the ambient temperature averaged 18.5 °C and heavy rain occurred. Cows subjected to wet surfaces exhibit less lying and a lower quality of rest [16], in addition to the extra energy expended on thermoregulation [49]. The lack of a NEFA surge in OD-GRZ cows coupled to β-hydroxybutyrate may have occurred because of differential tissue utilization of NEFA due to increased physical activity [50,51]; as muscle shivering for thermoregulation [52], less lying and resting time [16], more walking time, and standing in postures that may reduce the amount of surface area exposed to wind and rain [14,49]. These metabolic differences allowed CB-GRZ cows to have different BCS from OD-GRZ cows at WOS 40 of almost a fourth point on the scale of Edmonson et al. [33].

The CB-TMR treatment had 6.4 and 7.1 kg more DM intake than CB-GRZ and OD-GRZ, respectively, which reached pasture inclusion levels of 35.9 and 31.4% of the diet [27]. In consequence, the confined group exceeded milk production by 31% and 38% compared to the MS of CB-GRZ and OD-GRZ, respectively, and reached an average of 28% more milk solids production than the MS, while maintaining higher and more stable production levels throughout the full lactation compared to systems including grazing. Indeed, MS production levels sharply declined (~31.0 to 23.5 L) from WOS 11 to 18, throughout spring, as daily PA incremented from 18 to 27 kg DM/cow and supplementation decreased from 15 to 11 kg DM/cow. Once summer began, PA diminished again, and therefore supplementation increased, showing a production improvement although not recovering initial values. The early difference in milk production in favor of CB-TMR (~8 L at 30 DIM) leads to the assumption that a short confinement at the beginning of lactation (i.e., the first 3 weeks) with ample TMR supply in conditions of low environmental exposure might be a management alternative in MS in order to maintain high production levels during full lactation.

The higher milk production of CB-TMR vs. MS cows was due in part to higher DM intake, in agreement with other studies that indicate a higher DM intake in more nutrient-rich diets [1,2,42]. Bargo et al. [20] studied the performance of cows in early to mid-lactation during 21 weeks in spring consuming 100% TMR or 32% pasture + 68% MR and housed overnight in a free-stall barn and found that totally confined cows had higher DMI and milk production compared to MS (26.7 vs. 25.2 kg DM and 38.1 vs. 32.0 kg milk/day for each treatment, respectively), which represented an advantage of 19% in milk production during the study. Otherwise, neither milk fat nor true protein content differed between treatments [20]. Similar results were obtained by White et al. [53] in a 4-year study that showed 11% more milk production per lactation in cows fed TMR than MS cows but found no differences between treatments for milk fat or protein content in pasture + concentrate (occasionally fed with pasture hay or haylage) compared to confined cows fed TMR. Vibart et al. [54] showed that increasing the pasture proportion of the diet caused a quadratic decrease in DM and CP intake, which resulted in lower milk production in cows consuming 35% of the diet as pasture compared to TMR-fed cows (32.7 vs. 36.6 kg/d) during mid-lactation, housed in free-stall barns in an 8-week study during spring, although there were no differences when consuming pasture during autumn at up to 41% of the diet inclusion of pasture. Again, no differences occurred in milk fat content between feeding systems (3.68 vs. 3.31%) or in CP (2.86 vs. 2.84%) with increased pasture proportion. Salado et al. [24], in a 9-week study in autumn-winter during early lactation, found no change in milk fat (3.88%) or protein (3.43%) content as TMR proportion increased from 25 to 100% of the diet, with gradual increases in productive levels in response to higher DM and energy intake as pasture proportion in the diet decreased. Subsequently, Salado et al. [55] observed higher milk production (33.7 vs. 32.3 kg/d) during early to mid-lactation in TMR fed cows compared to 75% TMR + 25% oat pasture during autumn–winter, with similar milk fat content (3.90%) but higher milk protein content (3.53 vs. 3.47%). In our study, milk protein content was higher in TMR-fed cows than MS-fed cows, in accordance with the latter experiment and with expectations, as DM and energy intake are associated with milk protein content [23]. Contrary to mentioned previous reports, milk fat content was lower in OD-GRZ cows relative to CB-TMR cows, although it was similar between CB-TMR and CB-GRZ cows. High environmental exposure during HS alters ingestive patterns, prompting less frequent and longer meals at a higher intake rate [11], thereby altering rumination patterns and saliva supply to the rumen [56,57]. Finally, fewer ruminal contractions and decreased blood flow to the rumen epithelium in response to HS [58] impair ruminal stability, fermentation, and nutrient absorption [59], which may cause a lower contribution of fatty acids to support the synthesis of milk fat in the mammary gland in OD-GRZ cows.

In SCS, calving BCS was below recommended values for successful transition to early lactation [43], with treatment means of 2.80, 2.83, and 2.72 ± 0.21 for CB-TMR, CB-GRZ, and OD-GRZ, respectively. For the confined system, the BCS nadir was at WOS 10 (2.58 ± 0.03), while MS continued losing BCS until WOS 16, with a minimum of 2.48 ± 0.03. The confined treatment had a change in BCS slope from WOS 26 onward, which corresponds to 164 DIM, when BCS started recovering, although it was slow compared to that reported for these systems [43], probably due to the low BCS at calving. However, the confined treatment overcame the negative energy balance in less time than the MS, supported by lower levels of βHB in the OD-GRZ cows, and started increasing BCS earlier than in both MS, although there were no differences in plasma NEFA between treatments. The final BCS of MS cows was more than a quarter point lower in the scale [33] than the CB-TMR treatment cows, staying below 3.00 until the end of the study. These results are not consistent with those of Bargo et al. [20], where the final BCS did not differ between CB-TMR and MS, probably because this experiment started at 110 DIM, when the period of greatest lipid mobilization had already occurred. Fajardo et al. [2] did not find differences in BCS when comparing feeding strategies, similar to our results, since the experiment was completed in the 1st 10 weeks of lactation, when cows on both treatments were still in early lactation, as in our study.

3.5. Conclusions

In autumn calving cows, CB-GRZ and OD-GRZ failed to differentiate from each other in any measured response. This was likely due to the lower rainfall compared to the historical average, limited and mild heat stress only at lactation's end, and good infrastructure design and maintenance in the feeding and resting areas. Spring-calving cows in a fully confined TMR system had the highest milk production and overcame the early lactation negative energy balance in less time than MS. In grazing spring-calving cows, lower milk production and worse indicators of energy metabolism (i.e., higher βHB and lower BCS recovery by the end of the study) showed that heat stress impaired the performance of grazing cows with high environmental exposure.

Overall, results show that outdoor soil-bedded milk production systems, when well-managed, have a very high milk production potential that could equate to the productive response of improved infrastructure systems (i.e., a compost-bedded pack barn with cooling capacity) under moderately unfavorable environmental conditions, but in worse situations (i.e., the presence of medium or severe heat waves and heavy rain), performance could be compromised.

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4. Impacto de la alimentación y nivel de exposición al ambiente sobre el comportamiento, ingestión, digestión y producción en vacas lecheras Holando de parición otoñal y primaveral en lactancia temprana

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Article

Behavior, Intake, Digestion and Milk Yield of Early Lactation Holstein Dairy Cows with Two Levels of Environmental Exposure and Feeding Strategy

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Simple Summary: Higher stocking rates and supplemental feed intake in intensified pasture-based dairy systems (mixed systems) result in more confinement time. Thus, housing design is more important in minimizing environmental effects on animal performance. In addition, some Uruguayan dairy farmers are replacing low-input, but complex, mixed systems with total confinement (i.e., total mixed-ration systems) to better balance animal energy demand and exert more control over environmental exposure in order to optimize milk production. This study aims to determine the magnitude of the effects of implementing a partially confined grazing system with access to outdoor soil-bedded pens or a compost-bedded pack barn and a fully confined compost-bedded pack barn system (both of the latter with cooling capacity) on behavior, intake, nutrient utilization and microbial outflow, as well as milk production and composition, during early lactation in two calving seasons. The results demonstrate that under our relatively benign weather conditions, the cows in the outdoor soil-bedded system adapted some behaviors to compensate for their poorer living conditions but had a similar nutrient intake, utilization and microbial crude protein synthesis than the cows in the enhanced confinement systems. The fully confined cows greatly

outperformed those in the other systems, enhancing milk production by 20–35% and feed efficiency by 8–18%, with no impact on milk component levels.

Abstract: Eighty-four autumn (ACS, n = 45)- and spring (SCS, n = 39)-calved multiparous early lactation Holstein cows were assigned to groups of either: (a) grazing + mixed ration (MR) during partial confinement in outdoor soil-bedded pens with shade (OD-GRZ); (b) grazing + MR during partial confinement in a compost-bedded pack barn with cooling (CB-GRZ); or (c) total confinement fed a totally mixed ration (CB-TMR) in a compost-bedded pack barn. Data were analyzed using the SAS MIXED procedure with significance at $p \leq 0.05$. In both seasons, despite behavioral differences ($p < 0.05$) between the OD-GRZ and CB-GRZ groups (i.e., standing, first grazing meal length, bite rate), the milk and component yields, DM intake, microbial CP output (MCP) and NE efficiency were unaffected by the housing conditions, possibly due to mild weather conditions. The milk yield was substantially higher in the CB-TMR group versus the OD-TMR and CB-TMR groups ($p < 0.01$) in both ACS (~35%) and SCS (~20%) despite there being no intake differences, without any impact on milk component levels. In ACS, this was associated with a higher MCP, likely due to the higher nutritional value of TMR compared to pasture, which was not the case in SCS. In conclusion, the OD-GRZ group achieved the same milk production as the CB-GRZ group through behavior adaptation, under mild weather conditions, in both calving seasons. The CB-TMR group outperformed the grazing systems in both calving seasons, regardless of the MCP.

Keywords: grazing; mixed ration; confinement; behavior; microbial crude protein; milk yield

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4.1. Introduction

The sustainable intensification of pasture-based dairy farms implies a balanced increase in individual cow milk production and stocking rates in order to increase non-pasture feed intake (supplement, i.e., mixed ration; MR) without losing the potential to harvest home-grown forages by cows, thus allowing for low production costs and facilitating international competitiveness [1]. In this sense, Ortega et al. [2] reported that farmlets with a stocking rate of 1.5 cows per hectare had access to two grazing turns per day for 56% of the lactation days and one grazing turn per day for 40% of the lactation days. In contrast, farmlets with a stocking rate of 2.0 cows per hectare had access to two grazing turns per day for 42% of the lactation days and one grazing turn per day for 28% of the lactation days. The higher stocking rates of mixed systems (i.e., pasture + supplement; MS) result in more confinement time at feeders or in resting areas when the pasture is inaccessible (i.e., during heavy rains or when there is a low herbage mass) [2]. Hence, the design of housing facilities is important in intensified MS dairy farms.

Although higher levels of directly harvested forage dry matter intake (DMI) make systems more competitive, Uruguayan systems exploring the borders of stocking rates (mostly open-door, dry-lot) face the problem of not being prepared, largely due to poor infrastructure and management, to withstand long and/or repeated confinement periods, thereby exposing cows to extreme weather conditions such as high temperature, humidity and solar radiation or mud after heavy rainfall [3]. In this sense, the efficiency of nutrient use for milk production depends not only on the composition of the diet, cow genotype and physiological state but also on the weather and its influence on cow behaviors [4]. Housing conditions determine cow comfort as well as their quantity and quality of rest, which in turn define cow behaviors and activities [5,6], especially when they have to graze, as is the case in MS. Unmet needs for rest, lying and rumination affect cows' motivation to search for grazing and harvest [7,8], altering their ingestive patterns, ruminal conditions and digestive processes [9]. These situations can occur when cows are exposed to extreme weather, as the facilities provided may not mitigate the environmental effects, with cows having to regulate their body temperature through behavior and physiological changes [10,11], or when conditions are too wet to stimulate cows to lie down, such as when there is mud in resting areas [8,12], as can be the case in the intensive Uruguayan pastoral systems. Thus, it becomes imperative to determine the extent to which these conditions limit cow performance. The first approach of our group to addressing this problem revealed that enhanced housing facilities allowed for better performance during specific periods of environmental stress or even during the whole lactation period, dependent on the lactation period when the cows faced climatic challenges, which is linked to the calving season [13]. As a second step, it was necessary to determine which mechanisms cows use to compensate for impaired environmental conditions and/or how inefficiencies occur when they fail to overcome them.

In Uruguay, 46% of annual calvings are concentrated in the autumn and 24% in the spring [14]. The distribution of calvings has a direct effect on the instantaneous stocking rate of the system and the total feed demand in relation to supply, which determines the need for supplementation to compensate for insufficient pasture intake. In this sense, rumen utilization and productive performance depend on the level of inclusion and quality of each component of the diet [15]. On the other hand, feeding management in the first weeks after parturition is crucial in establishing the milk production potential, metabolic health and overall productivity of the cow throughout its lactation cycle [16–18], so nutritional and herd management in early lactation is a key point. During the fall–winter period, the pasture growth rate and available pasture in the rotation are minimal, so supplementation is maximized. At the same time, excessive rainfall, either in quantity or frequency, can impede pasture access and compromise animal welfare. In the spring, pasture use is maximized while environmental factors such as heat stress are present, which could affect productive performance during the most demanding period of the productive cycle [19,20].

In addition, some Uruguayan dairy farmers are replacing low-input, but managerially complex, MS with total confinement (i.e., total mixed-ration—TMR—systems) to overcome the gap between feed demand and supply and to exert more control over environmental exposure in order to optimize milk production [15,21,22]. Although this issue has been previously studied [23–25], it is necessary to quantify the productivity gap between the two feeding systems (MS vs. TMR) but with different levels of environmental exposure under local environmental conditions and with available feed.

Our goal was to study ingestive behavior, DM intake and ruminal microbial CP synthesis as factors associated with feed efficiency and milk yield in two mixed-management systems (i.e., cows grazed in pasture and those fed a mixed ration) with high or low environmental exposure compared to a reference TMR system in autumn- (ACS) and spring-calved (SCS) early lactation cows.

4.2. Materials and Methods

4.2.1. Cows and Experimental Design

Two experiments with autumn- and spring-calved Holstein dairy cows were conducted at the Estación Experimental Dr. M. A. Cassinoni (EEMAC) of the Facultad de Agronomía (Paysandú, Uruguay) of Universidad de la República (UdelaR). The autumn-calved cows were as follows: Experiment 1: n = 45, 2.9 ± 1.4 lactations, 654 ± 99 kg body weight (BW), calving dates of 16 March 2019 ± 10 days at 3.0 ± 0.27 points of BCS. The spring-calved cows were as follows: Experiment 2, n = 39, 2.7 ± 0.9 lactations, 624 ± 61 kg BW, calving dates of 9 August 2019 ± 13 days at 2.8 ± 0.22 points of BCS. All cows were managed similarly during their dry and prepartum periods when they were confined and fed a prepartum TMR for 3 weeks before their expected calving date. The cows were blocked by BW, expected calving date, lactation number and pre-calving and body condition score (BCS) according to [26] before random assignment to treatments and grouping into pens of 4 cows each (i.e., 16 cows/treatment). The cows were assigned to their corresponding treatments immediately after calving. The sampling periods consisted of 5 days of measurements and samplings during week 6 of the study for ACS (44 ± 10 days in milk) and week 8 for SCS (50 ± 13 days in milk), which corresponded to late April and late September, respectively. Some cows were removed from the experiment due to calving or postpartum illnesses and were replaced with non-study cows in order to maintain equal conditions in all pens. The experimental protocol was evaluated and approved by the Comisión de Ética en el Uso de Animales de la Facultad de Agronomía (ID 682- Exp020300-000602-18), UdelaR (Montevideo, Uruguay).

In both experiments, the cows were assigned to one of three treatments, which consisted of: (1) high environmental exposure in a mixed-management system (MS: i.e., outdoor soil-bedded pens with shade structures) when not out to pasture (OD-GRZ); (2) low environmental exposure in an MS (i.e., compost-bedded pack barn with cooling capacity) when not out to pasture (CB-GRZ); or (3) a totally confined system with cows in the same facilities as the CB-GRZ group but fed a TMR twice daily ad libitum (**CB-TMR,** similar chemical composition to MS).

4.2.2. Management and Feeding

This study was part of a larger experiment [13] in which cow performance (i.e., DM intake, milk production and composition, BW, BCS, energy metabolism) were measured over a full lactation to evaluate cows' treatment responses (from 0 to 290 days in milk, which corresponded to from March 2019 to January 2020 for ACS cows and from August 2019 to May 2020 for SCS cows).

The OD-GRZ cows were confined in outdoor soil-bedded pens (48 m^2 per cow) with shade structures (nylon roof at 4.5 m height with a slope of 15%, 4.8 m^2 per cow). The pens had a slight slope for water and manure runoff and were divided in half, and they were alternately occupied according to soil moisture and surface deterioration. Feeders were located at an end of the paddocks at a feeding area of 10 m^2/cow, and they had a length of 1.10 m/cow (for a detailed description of these facilities, see [27]).

The CB-TMR and CB-GRZ cows were confined in a fully roofed compost-bedded pack barn (13.5 m^2/cow) with a concrete floor (6.7 m^2/cow) with cooling capacity (i.e., fans and with sprinklers fitted with an automatic operation, over 25 °C, of 3 min on and 10 min off. Surface compost was removed twice daily with a chisel plough to remove water vapor, limit oxygen entry and maintain small homogeneous particles. The temperature and humidity of the compost bed were assessed weekly, with new material (i.e., wood chips, rice husks) added every 20 days. The concrete feeding area was cleaned thrice weekly by a tractor-mounted rubber scraper. The feeders were inside the barn but separated from the composted area by a concrete cow standing area (area: 6.7 m^2/cow; length; 0.75 m/cow).

The cows were confined in groups of 4 in separate pens when in pens, whereas when outside, they were co-mingled. Water was available ad libitum to all cows by automatic waterers. The cows were milked twice daily at 3:00 and 16:00 h in a milk parlor 100 m from the pens. The TMR fed to the CB-TMR cows was also the mixed ration (**MR**) fed to the grazing cows but at lower levels, depending on the available pasture. Due to changes in the availability of the stored feed, the ACS and SCS MRs differed in the available conserved forage, which consisted of corn and ryegrass silage for the MR in the ACS experiment and sorghum silage plus fescue hay in the SCS experiment. The diets were formulated based on the guidelines in [28] for 620 kg cows producing 45 L/d of 4% fat-corrected milk. In the MS group, the MR was a pasture complement to achieve the desired DM intake. The ingredients, chemical composition and calculated nutritional value of the TMR/MR are shown in Table 1.

The OD-GRZ and CB-GRZ groups were high-stocking-rate systems (i.e., 2.5 lactating cows and/or 1550 kg BW/ha of grazing platform). The cows grazed between 7:00 and 14:00 h in grazing plots, rotated weekly. The cows with grazing in their treatment accessed various grazing plots with similar herbage allowance (**HA**). The cows in the MS in ACS grazed on a 2nd-year permanent sward composed of Tall Fescue (*Lolium arundinaceum* (Schreb.) Darbysh.)) + Birdsfoot Trefoil (*Lotus corniculatus* L.), and the cows in the SCS MS grazed on a 1st-year permanent sward composed of Lucerne (*Medicago sativa* L.) + Orchard Grass (*Dactylis glomerata* L.). Herbage mass (**HM**) was determined using a double sampling technique [29], where 5 scale points of biomass availability representative of the field were selected and three replicates of each were cut in the field. The forage management and its nutritional value during the sample periods are shown in Table 2.

4.2.3. Data collection, Measurements and Estimates

Climatic conditions (i.e., ambient temperature, relative humidity, wind speed, rain) during the sampling periods were obtained from the meteorological agency of the experimental station. Heat stress was determined by a temperature humidity index (**THI**) as follows:

$$(1.8 * ET + 32) − (0.55 * RH/100) * (1.8 * ET − 26)$$

where ET is the environmental temperature and RH is the relative humidity [30].

Individual cow milk production was recorded at each milking during fecal sampling days. Milk samples were collected to determine the fat, crude protein (**CP**) and lactose contents (MilkoScan FossElectric FT2®). Milk (L) was converted to a standard of 40 g/L fat and 33 g/L protein (kg, fat-/protein-corrected milk, **FPCM**) using the following formula:

$$(0.337 * \text{milk production, kg}) + (1.16 * \text{fat in g/L} + (0.60 \times \text{protein in g/L})$$

according to [31] in order to calculate the feed efficiency (kg FPCM/kg DMI).

The energy retained in the milk (Mcal NE/cow/day, ERM) was calculated as follows:

$$(0.929 * \text{fat in g/L} + 0.547 * \text{protein in g/L} + 0.395 * \text{lactose in g/L}) * \text{milk yield, L/d}$$

The amounts of MR offered and refused per pen were recorded daily during the sampling periods in order to determine the DMI. Refused feed was removed and weighed prior to the morning feeding during the sampling periods. Pasture samples were collected every hour by hand clipping, according to [32], using two cows per treatment during scan sampling, which occurred during the grazing sessions on days 1, 3 and 5 of each feces collection period. Each pasture sample was composed of 42 subsamples. The samples were collected and stored at −20 °C. The pasture and TMR/MR samples were weighed and dried in a forced air oven at 60 °C for 48 h. The samples were reweighed after drying to determine the DM content and finally ground through a 1 mm sieve for chemical analysis and in vitro gas production. The mixed-ration samples per pen were pooled to create a composite sample for each treatment. Chemical analysis consisted of the DM, ash (to calculate the organic matter content; **OM**) and CP as well as neutral detergent (**aNDFom**) and acid detergent fiber (**ADFom**) according to [33]. The NDF used α-amylase, and both were assayed using an ANKOM200 Fiber Analyzer (ANKOM Tech. Corp., Fairport, NY, USA). Total N for CP calculation was calculated using the Kjeldahl method [34].

In vitro gas production was used to determine the feed nutritional value (i.e., OM digestibility, net energy, fermentation kinetics). Approximately 200 mg of DM of each feed composite sample was incubated in duplicate in rumen fluid in glass syringes following the procedures of [35]. Rumen fluid was collected from 2 donor non-lactating dairy cows fed a diet containing 500 g/kg hay and 500 g/kg concentrate twice daily at a level sufficient to meet their estimated maintenance NE requirements. The syringes were pre warmed at 39 °C before injection of 10 mL of rumen fluid and 20 mL of reducing agent/buffer mineral medium mixture into each syringe, followed by incubation in a water bath at 39 °C. Gas production was recorded at 2, 4, 6, 8, 24, 30, 48, 72 and 96 h of incubation. The gas values were corrected for blank incubation in order to correct for gas production from the fermentation of residual feed in the rumen fluid and for the inter-run standard (i.e., an alfalfa hay standard with a known gas production history) in order to standardize the gas readings among runs. Values were converted to ml gas/g OM.

The cumulative gas production data were fitted to the model of [36] as follows:

$$y = a + b\,(1 - e^{-ct})$$

where y is the gas produced at time ‘t’, a is the gas produced (ml) from the immediately soluble fraction, b is the gas produced (ml) from the insoluble fraction (ml), c is the gas production rate constant for the insoluble fraction b, a + b is the potential gas production (ml) and t is the incubation time (h). There was no lag term used in the model, as the gas production was essentially instantaneous (Figure 1), and the ‘a’ values are not reported as they were essentially zero.

The energy value of the TMR/MR and pastures was calculated from the amount of gas produced (GP) at 24 h of incubation and the ether extract (**EE**) content, according to the following [37]:

$$\text{ENL (Mcal/kg DM)} = 0.689 + 0.0134\text{GP}_{24} + 0.0771\text{EE}$$

The total DMI per cow in the mixed systems was estimated with a double marker technique, which consisted of an indigestible external marker (i.e., chromium oxide, Cr_2O_3) to estimate the fecal output [38,39] and an internal marker (i.e., acid-insoluble ash) to estimate the DM digestibility [40]. Fecal output was estimated as Cr intake/Cr concentration in the feces. For this purpose, each cow received an oral bolus of 7.5 g of Cr_2O_3 at each milking (chromium III oxide, G-105M, 98% purity; Ferro Colombia S.A.S., Colombia) for 12 days, and fecal samples were collected from the rectum for the last 5 days, when the cows returned from AM and PM milking. The cows were observed after dosing to ensure no bolus regurgitation. All the samples were preserved at −20 °C. The samples were dried in a forced air oven at 60 °C to a constant weight and then milled through a 1 mm screen. Subsamples from each cow, day and shift were used to generate a composite sample by cow and shift for analysis. The chromium content was determined according to [41]. The AIA contents of the pasture, TMR/MR and feces were determined according to [42]. The DM digestibility was estimated as follows:

$$\text{(g AIA/kg DM feces} - \text{g AIA/kg MR)/(g AIA/kg DM feces).}$$

Finally, the intake (kg DM/cow/day) was determined as reported by [43] as follows:

$$\text{fecal output}/(1 - \text{diet DM digestibility})$$

The daily pasture DM intake per cow was estimated as the difference between the total DMI and DMI of the MR.

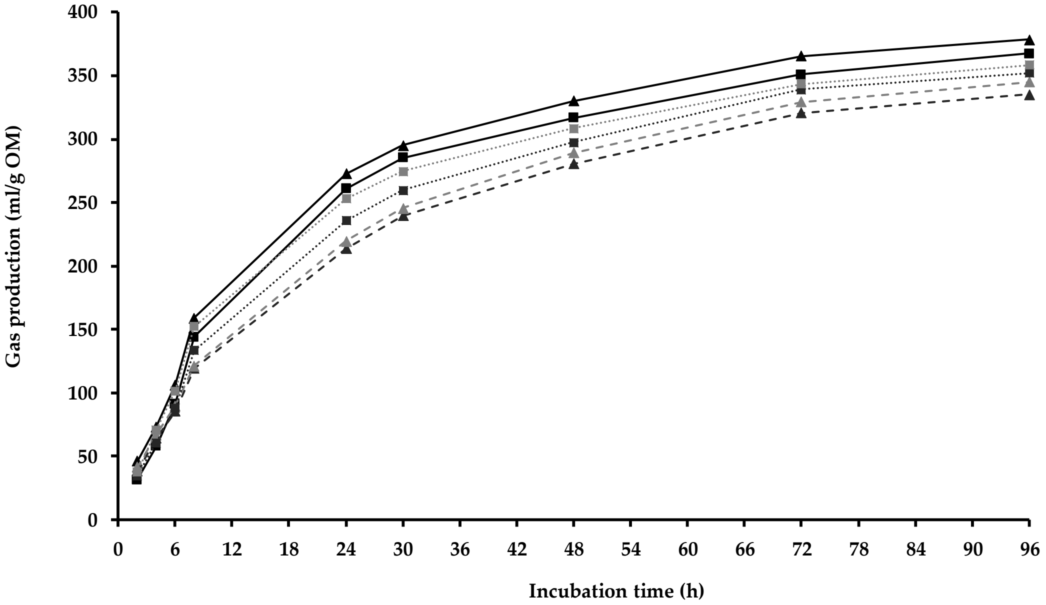

Figure 1. Gas production in mixed diet (continuous line) and pasture (dashed lines) offered to autumn (triangles, ACS)- and spring (squares, SCS)-calving-season cows with low (dark gray, CB-GRZ) or high (light gray, OD-GRZ) environmental exposure.

Behavioral evaluation was conducted during the fecal sampling periods in both MS (i.e., CB-GRZ and OD-GRZ). The activity pattern of each cow was recorded from direct real-time observation using instantaneous scan sampling on days 2, 4 and 6 of the weekly grazing paddock occupation (day 1, 3 and 5 of the sampling period). For each day, scan recordings were conducted every 10 min throughout grazing (07:00 to 14:00 h) and part of confinement (16:00 to 23:00 h). Access to the offered feed (i.e., pasture and TMR/MR, respectively) determined the start time. In each scan, the cows were as recorded as eating (i.e., grazing or standing with her head in the feeder during confinement), ruminating, lying, standing or other (i.e., drinking, walking, allogrooming). Ruminating vs. eating as well as lying vs. standing were mutually exclusive, but ruminating and lying and ruminating and standing could be simultaneous. During grazing observations, bites/minute were counted, if the cows were eating, in order to determine the bite rate (**BR**). It was assumed that each eating occurred over the entire 10 min observation in order to determine the first grazing meal length (**FGML**). Data are presented as the probability of the cows eating, ruminating, lying and standing during the first 90 min, as an expected length for the first active grazing bout [44], and during the total time at grazing or confinement.

The allantoin (**AL**) content, a derivative of absorbed microbial nucleic acid purines, was determined in urine samples according to Chen and Gomes [45] as an indirect measure to estimate the ruminal microbial CP (**MCP**) output to the duodenum. Individual urine samples were collected following the fecal sampling days over three consecutive days at six times (i.e., 3:00, 7:00, 11:00, 15:00, 19:00 and 23:00 h), as suggested by [46], in order to obtain a 'super-sample' per cow that included the within-day variation. Urine was collected by manual urinary bladder compression by perineal massage into 100 mL flasks with 5 mL of hydrochloric acid (6 N HCl), and the pH was measured prior to freezing at −20 °C to ensure that all values were below 5.0 in order to prevent bacterial destruction of the AL. These frozen samples were later thawed, and aliquots of 7 mL were combined with 1 mL of HCl and diluted with 25 mL of deionized water to a total of 33 mL and re-frozen. Finally, these urine samples were thawed and centrifuged at 1200 x*g* for 15 min at 20 to 22 °C and diluted 60 times in order to fit the standard curve. Each sample was analyzed in duplicate. Standards of increasing concentrations (i.e., 20, 40, 60, 80 and100 mg AL/L) were run at the start and end of each run to generate the reference curve. The AL values were corrected for the blank and an inter-run standard in duplicate according to [47,48]. Analytical results were corrected by days of frozen storage (i.e., by microbial destruction during storage) using an equation obtained through analyzing a group of samples repeatedly over the time of storage time as follows:

$$y = -0.082\ln(x) + 0.8954\ (r^2 = 0.9992)$$

where y is a correction factor depending on x, which is the number of days of pre-dilution.

In order to estimate the total urinary AL output and ruminal MCP flow to the small intestine, the urine volume was determined by measuring the urine creatinine content, a metabolite of phospho-creatine (energy storage in muscle) that is excreted at a relatively constant rate by the kidneys, according to [46], using a commercial colorimetric assay kit (Item No. 500701, © Cayman Chemical Company, MI, USA). Samples were also corrected by blanks, standards and days prior to dilution as follows:

$$y = -0.0002x^2 - 0.0246x + 99.999\ (r^2 = 0.9985)$$

where y is a correction factor depending on x, which is the number of days of pre-dilution.

4.2.4. Statistical Analysis

Data were analyzed using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC, USA) using the following model:

$$Y_{ij} = \mu + T_i + e_{ijk}$$

where Y_{ij} is the response variable, T_i is the treatment and e_{ijk} is the residual error. Cow was accepted as the experimental unit for milk production and composition, behavior and microbial CP yield, with pen as a random effect. For the TMR and pasture DM intake, pen was the experimental unit. The model (co)variance structure was AR (1), selected based on the smallest Bayesian information criterion (BIC) value. For all variables except behavior, normality was assumed and tested. For behavior, a binomial distribution was assumed and tested. The probability of cows grazing, ruminating or being engaged in other activities was calculated using a mixed model that included the fixed effects of the treatments and the residual error. Mean comparisons were performed by Tukey–Kramer's analysis. Mean differences were considered significant if $p \leq 0.05$. Results are shown as least square means ± standard error of the mean (**SEM**).

4.3. Results

The daily mean temperatures during the sampling weeks were 16.4 and 19.0 °C, with a THI daily mean of 61 (0% of daily time > 72) and 64 (19% of daily time > 72) in ACS and SCS, respectively. The wind speed averaged 3.1 and 4.3 km/h, and the accumulated rain was 12 and 2 mm, respectively, for each calving season, with a total rainfall of 71 and 37 mm in April and September 2019 (ACS and SCS, respectively. Please refer to Supplementary Table S1).

The ACS MR/TMR included a commercial feed concentrate plus corn and ryegrass silage, while the SCS MR/TMR was the same concentrate plus sorghum silage and fescue hay (Table 1). The MR/TMR diets differed numerically in DM content but had a similar chemical composition, estimated NE concentration, fermentability (i.e., mL gas produced/200 mg DM at 24 h of incubation, Figure 1), ml gas produced/g OM at 30 h of incubation, potentially degraded fraction (i.e., 'b') and rate of gas appearance (i.e., 'c'). The OM digestibility was judged as higher in the ACS vs. SCS MR/TMR.

Table 1. Composition and predicted nutritional value of the mixed diets fed to autumn (ACS)- and spring (SCS)-calved cows.

	ACS	SCS
Ingredient (g/kg DM)		
Forage		
Corn silage	246	-
Sorghum silage	-	375
Ryegrass silage	214	-
Fescue hay	-	65
Concentrate mixture [1]	540	560
Dry matter (DM; g/kg)	434	559
Nutrient (g/kg DM)		
Crude protein	159	165
Neutral detergent fiber	331	295
Acid detergent fiber	165	135
Ether extract	38	38
Starch	25	29
NE_L (Mcal/kg OM) [2]	1.66	1.62
Gas production [3]		
24 h (ml/200 mg DM)	51	47
30 h (ml/g OM)[3]	295	285
Rumen kinetics [4]		
b (mg/g OM)	367	360
c (h^{-1})	0.058	0.053
OM digestibility (g/kg) [5]	609	582

[1] Based on ground corn grain, wheat bran, soybean meal, sunflower meal, cottonseed meal, canola meal, rumen inert fat, urea, yeast and minerals. [2] Net energy of lactation, determined from accumulated gas production at 24 h (ml/200 mg DM) and ether extract content, as described by [38]. [3] In vitro following the Menke and Steingass [36] procedure. [4] According to the model of Ørskov and McDonald [37]. [5] Apparent, in whole tract, according to Menke and Steingass [36].

Both the ACS MS cow treatments grazed a permanent sward with a similar chemical composition, NE concentration, fermentability and OM digestibility, although the HM was lower in the CB-GRZ group than in the OD-GRZ group (Table 2). For the SCS group, the MS had similar HA and DM contents, though there was an apparent higher OM digestibility in the OD-GRZ pasture.

Table 2. Characteristics of pasture grazed by autumn (ACS)- and spring (SCS)-calved cows with low (CB-GRZ) or high (OD-GRZ) environmental exposure.

	ACS		SCS	
	CB-GRZ	OD-GRZ	CB-GRZ	OD-GRZ
Herbage mass (kg DM/ha)	1550	2200	3400	2500
Herbage allowance (kg DM/cow/day)	18	18	23	21
Herbage dry matter (g/kg)	238	227	279	277
Nutrient (g/kg DM)				
Crude protein	149	147	156	139
Neutral detergent fiber	517	481	610	548
Acid detergent fiber	246	241	315	262
Ether extract	42	40	31	39

Ash	111	110	108	114
NE_L (Mcal/kg OM) [1]	1.53	1.53	1.49	1.60
Gas production [2]				
24 h (ml/200 mg DM)	39	40	42	45
30 h (ml/g OM)	240	245	260	275
Rumen kinetics [3]				
b (mg/g OM)	329	338	346	345
c (h^{-1})	0.047	0.047	0.049	0.058
OM digestibility (g/kg) [4]	504	515	535	564

[1] Net energy of lactation, determined from accumulated gas production at 24 h (mL/200 mg DM) and ether extract content, as described by [38]. [2] In vitro following the Menke and Steingass [36] procedure. [3] According to the model of Ørskov and McDonald [37]. [4] Apparent, in whole tract, according to Menke and Steingass [36].

4.3.1. Autumn Calving Season

During confinement, the OD-GRZ cows had a 46% lower likelihood of eating during the first 90 min than the CB-GRZ cows did ($p < 0.01$), they tended to have a 24% lower probability of lying at confinement ($p = 0.06$), they were 65% more likely to be standing during the total time ($p = 0.05$) and they had triple the probability of standing in the first 90 min at confinement ($p < 0.01$, Table 3).

Table 3. Confinement behavior of autumn- and spring-calved cows with low (CB-GRZ) or high (OD-GRZ) environmental exposure.

			CB-GRZ	**OD-GRZ**	**SEM**	***p*-Value**
Autumn calving						
Probability of						
	Eating	90 min	0.82	0.56	0.039	<0.01
		Total	0.43	0.37	0.036	0.25
	Ruminating	90 min	0.01	0.03	0.012	0.19
		Total	0.16	0.21	0.027	0.20
	Lying	90 min	0.01	0.01	0.012	0.90
		Total	0.33	0.25	0.023	0.06
	Standing	90 min	0.10	0.33	0.022	<0.01
		Total	0.20	0.33	0.038	0.05
Spring calving						
Probability of						
	Eating	90 min	0.79	0.77	0.061	0.84
		Total	0.25	0.25	0.027	0.99
	Ruminating [1]	90 min	-	-	-	-
		Total	0.36	0.38	0.016	0.36
	Lying	90 min	0.02	0.02	0.010	0.52
		Total	0.48	0.51	0.023	0.27
	Standing	90 min	0.14	0.15	0.059	0.90
		Total	0.27	0.21	0.013	0.03

[1] Did not converged due to insufficient number of observations.

During grazing, both treatments had similar first grazing meal lengths ($p = 0.29$, Table 4), but the OD-GRZ cows tended ($p = 0.09$) to have a higher bite rate in this period (GM_1), and they had a higher bite rate (+16%, $p < 0.01$) during the rest of the time in the paddock (GM_0). The probability of the cows eating during the first 90 min at pasture was 42% higher in the OD-GRZ cows ($p < 0.01$), while their probability of ruminating during the first 90 min was 4 times lower compared to the CB-GRZ cows ($p < 0.01$). There was no effect of the level of environmental exposure on the lying time during grazing, but the CB-GRZ cows were 7 times more likely to stand during the first 90 min at pasture ($p < 0.01$, Table 4).

Table 4. Grazing behavior of autmn- and spring-calved cows with low (CB-GRZ) or high (OD-GRZ) environmental exposure

		CB-GRZ	OD-GRZ	SEM	*p*-Value
Autumn calving					
FGML (min)[1]		84	71	9.1	0.29
BR (bites/min)[2]	GM_1	45	50	1.7	0.09
	GM_0	44	51	1.1	<0.01
Probability of					
Eating	90 min	0.55	0.78	0.044	<0.01
	Total	0.45	0.52	0.034	0.18
Ruminating	90 min	0.33	0.08	0.025	<0.01
	Total	0.31	0.25	0.017	0.03
Lying	90 min	0.23	0.12	0.049	0.19
	Total	0.41	0.39	0.040	0.74
Standing	90 min	0.14	0.02	0.025	<0.01
	Total	0.10	0.06	0.016	0.12
Spring calving					
FGML (min) [1]		139	115	6.1	<0.01
BR (bites/min) [2]	GM_1	48	54	1.5	<0.01
	GM_0	41	53	1.8	<0.01
Probability of					
Eating	90 min	0.90	0.89	0.017	0.69
	Total	0.56	0.63	0.022	0.06
Ruminating	90 min	0.01	0.05	0.012	0.01
	Total	0.17	0.23	0.013	0.01
Lying	90 min	0.01	0.08	0.032	0.05
	Total	0.19	0.26	0.035	0.22
Standing	90 min	0.04	0.00	0.011	<0.01
	Total	0.18	0.07	0.014	<0.01

[1] FGML = first grazing meal length. [2] BR = bite rate. GM_1 = grazing meal 1, considered as first 85 min for ACS and 140 min for SCS. GM_0 = all scan samplings not considered in GM_1.

For ACS, neither the total nor pasture DM intake differed among the treatments, although the CB-TMR cows were ~20% more efficient in converting feed into milk than the MS cows, expressed as kg FPCM/kg DM ($p = 0.03$) or MCal NEl milk/Mcal of diet NEl ($p = 0.02$, Table 5).

Table 5. Intake and efficiency of autumn- and spring-calved cows in confined (CB-TMR) and mixed systems with low (CB-GRZ) or high (OD-GRZ) environmental exposure.

	CB-TMR	CB-GRZ	OD-GRZ	SEM	*p*-Value
Autumn calving					
Intake					
Pasture (kg DM/cow/day)	-	9.3	9.5	1.29	0.94
Total					
(kg DM/cow/day)	24.6	22.4	22.6	1.08	0.33
% BW	3.44	3.31	3.27	0.12	0.60
Efficiency					
kg FPCM/kg DM [1]	1.70 [a]	1.41 [b]	1.40 [b]	0.07	0.02

	CB-TMR	CB-GRZ	OD-GRZ	SEM	p-value
NE Milk/NEl diet	0.77 [a]	0.66 [b]	0.65 [b]	0.03	0.03
Spring calving					
Intake					
Pasture (kg DM/cow/day)	-	15.4	13.9	2.01	0.61
Total					
(kg DM/cow/day)	28.8	27.0	25.5	1.73	0.43
% BW	4.39	4.08	3.93	0.24	0.42
Efficiency					
kg FPCM/kg DM [1]	1.33	1.19	1.23	0.09	0.49
NE Milk/NEl diet	0.62	0.57	0.58	0.04	0.58

[a,b] Means within season with different superscripts differ ($p < 0.05$). [1] FPCM = fat–protein-corrected milk, according to FAO [32].

The ruminal microbial CP output was 32% higher in the CB-TMR cows than in the MS cows ($p < 0.01$), with no differences in grams of CP per kg digestible OM (Table 6).

Table 6. Microbial crude protein synthesis of autumn- and spring-calved cows in confined (CB-TMR) and mixed systems with low (CB-GRZ) or high (OD-GRZ) environmental exposure.

		CB-TMR	CB-GRZ	OD-GRZ	SEM	*p*-Value
Autumn calving						
Creatinine	(mg/L urine)	989 [b]	1211 [a]	908 [b]	47.9	<0.01
Allantoine	(mg/L urine)	2960 [a]	2924 [a]	2442 [b]	136	0.02
Microbial Crude Protein						
	(g CP/cow/day)	1840 [a]	1304 [b]	1480 [b]	80	<0.01
	(g CP/kg DOM/day) [1]	134	115	127	6.4	0.12
Spring calving						
Creatinine	(mg/L urine)	687 [a]	519 [b]	609 [ab]	31.1	<0.01
Allantoine	(mg/L urine)	2762 [a]	2106 [b]	2286 [b]	115	0.01
Microbial Crude Protein						
	(g CP/cow/day)	2169	2167	1905	110	0.21
	(g CP/kg DOM/day) [1]	144	161	146	9.0	0.20

[a,b] Means within season with different superscripts differ ($p < 0.05$). [1] DOM = digestible organic matter.

The CB-TMR cows had a 35% higher milk yield ($p = 0.02$), 32% higher energy retained in the milk ($p = 0.03$) and 33% and 35% higher protein and lactose yields (kg/cow/day) compared to the MS cows ($p < 0.01$) without differences in milk composition (Table 7).

Table 7. Milk yield per cow of autumn- and spring-calved cows in confined (CB-TMR) and mixed systems with low (CB-GRZ) or high (OD-GRZ) environmental exposure.

	CB-TMR	CB-GRZ	OD-GRZ	SEM	*p-value*
Autumn calving					
L/cow/day	42.8 [a]	31.9 [b]	31.7 [b]	2.39	0.02
kg FPCM[1]/cow/day	42.1 [a]	32.2 [b]	32.0 [b]	2.53	0.03
Mcal NE/cow/day	31.7 [a]	24.1 [b]	24.0 [b]	1.86	0.03

Fat	g/kg	38.1	38.3	40.0	0.148	0.72
	kg/day	1.64	1.26	1.28	0.116	0.08
Protein	g/kg	33.5	33.2	33.0	0.067	0.89
	kg/d	1.43 [a]	1.09 [b]	1.06 [b]	0.069	<0.01
Lactose	g/kg	51.0	50.1	50.3	0.063	0.59
	kg/d	2.19 [a]	1.64 [b]	1.61 [b]	0.113	<0.01
Spring calving						
L/cow/day		40.7 [a]	34.1 [b]	33.7 [b]	1.15	<0.01
kg FPCM[1]/cow/day		38.5 [a]	30.6 [b]	30.2 [b]	1.35	<0.01
Mcal NE/cow/day		28.4 [a]	22.4 [b]	22.3 [b]	1.03	<0.01
Fat	g/kg	34.4	34.2	32.00	0.120	0.34
	kg/d	1.44 [a]	1.14 [b]	1.09 [b]	0.060	<0.01
Protein	g/kg	30.3	29.6	30.1	0.052	0.70
	kg/d	1.27 [a]	1.00 [b]	1.02 [b]	0.051	<0.01
Lactose	g/kg	49.0	47.8 [y]	49.4 [x]	0.044	0.06
	kg/d	2.05 [a]	1.61 [b]	1.68 [b]	0.079	<0.01

[1] Fat–protein-corrected milk, according to [32]. [a,b] Means within season with different superscripts differ ($p < 0.05$). [x,y] Means within season with different superscripts tend to differ ($p < 0.10$).

4.3.2. Spring Calving Season

During confinement, the probability of standing was the only variable that differed between the treatments ($p = 0.03$), with it being 29% higher in the OD-GRZ cows compared to the CB-GRZ cows (Table 3). However, during grazing, the OD-GRZ cows spent 24 min less time eating at the first grazing meal ($p < 0.01$) at a bite rate that was 13% higher during GM_1 and 29% higher during GM_0 ($p < 0.01$). In addition, during the first 90 min at pasture, the OD-GRZ cows had a higher probability of ruminating ($p < 0.01$), accompanied by a lower probability of standing ($p < 0.01$) and a higher probability of lying ($p = 0.05$, Table 4).

There was no effect of the feeding system nor environmental exposure on the pasture and total DM intake, feed efficiency (Table 5) and microbial CP output (Table 6).

For the SCS cows, the CB-TMR cows had a 20% higher milk yield ($p < 0.01$) and 27% energy retained in the milk ($p < 0.01$), and they had higher fat, protein and lactose yields (kg/cow/day) compared to the CB-GRZ and OD-GRZ cows ($p < 0.01$, Table 7), although no differences in the milk component contents were observed.

4.4. Discussion

4.4.1. Weather Conditions during Both Calving Seasons

The daily mean temperature during the sampling weeks were within the historical values for these periods of the year. However, the monthly accumulated rainfall levels were about 50% below the historical averages (Instituto Uruguayo de Meteorología [49]). Thus, it is clear that the cows experienced relatively benign weather during the sampling weeks, with no heavy or accumulated rainfall or extreme heat (i.e., daily THI mean above 72) in either experiment.

4.4.2. Autumn Calving Season

The drier weather experienced by the autumn-calved cows prevented mud formation in the pens (feeders and/or rest area) of the OD-GRZ cows and/or heat stress, which would have been detrimental to the cows' well-being and performance [5,7,8,50,51]. This, combined with good infrastructure design and maintenance in the feeding and resting areas of the OD-GRZ cows, likely explains why the CB-GRZ and OD-GRZ cows did not differ in most of the measured variables. However, the behavioral differences suggest that the conditions for the expression of behaviors (i.e., a lesser lying and a greater standing likelihood) were not optimal for the OD-GRZ cows, meaning that they likely adapted to compensate during the grazing period, as evidenced by the CB-GRZ cows spending more time on other activities (i.e., ruminating and standing) than the OD-GRZ cows during the first 90 min of grazing. Contrary to expectations, the OD-GRZ cows were not more likely to lie down and/or ruminate during grazing as a way to compensate for the lower rest time in confinement [5,8,12,28]. It seems clear that with the benign weather conditions, the time that the cows were able to lie down and ruminate during confinement was enough to fulfill their needs, while the energetic requirements of standing caused greater hunger/motivation to eat when arriving at pasture [52], thereby achieving similar pasture DMI and MCP outflows as the CB-GRZ cows. This also suggests that the grazing behavior differences were not predictors of productive outcomes for the CB-GRZ and OD-GRZ cows.

The CB-TMR cows had a higher (~35%) milk yield than the MS cows, whose diet (DM) consisted of 58% MR and 42% directly harvested pasture. The combination of TMR and pasture that had to be harvested by the cows implies a deferred contribution of diet components to the intake in these limited periods of time and a higher energy demand associated with walking and grazing activity, which could have impacted the productive outcome compared to the cows fed TMR alone. Fajardo et al. [53] observed that a 28% inclusion of grazed grass in the diet decreased the total DMI (20.0 kg DM/day) compared to open-door TMR (26.1 kg DM/day), which resulted in a 10% lower milk production (33.7 vs. 37.2 L/cow) and a more negative energy balance for the MS [54]. Jasinsky et al. [55] studied cow performance in open-door TMR and MS with 30% (DM) direct-harvested pasture included in the diet, and they noted a trend towards differences in milk production (28.1 vs. 26.3 L/cow/day), and there were no differences in the solids content, but it was accompanied by a higher level of energy retained in the milk and tissues in the TMR-treated cows. Salado et al. [22] observed that cows consuming diets with 21, 44 and 70% inclusions of direct-harvested pasture produced 6.5%, 20.4% and 27.6% less milk than cows in TMR systems (32.1, 28.4 and 26.8 vs. 34.2 L/cow/day, with a DMI of 22.4, 21.0 and 19.7 vs. 24.1 kg DM/cow/day, respectively). Their linear regression analysis showed an increase of 0.7 kg DM/cow/day and 1.1 L/cow/day for each 10% increase in TMR proportion in the diet [22]. In our study, as previously mentioned, the MS group had a 42% pasture inclusion in the diet, but a higher gap in milk production occurred with respect to the confined system, and there were higher absolute milk production values in all the treatments in all the studies, although with similar DMI levels. The higher milk yield of the confined cows was associated with a ~32% higher MCP outflow from the rumen and a ~18% increase in feed conversion efficiency (milk NE/diet NE) compared to the MS group. While the nutritional value of the pastures was lower than that of the mixed diet (as evidenced by the gas production at 24 and 30 h of incubation, the potentially degraded OM fraction, the rate of OM disappearance, the OM digestibility and the energy content), the pasture inclusion could have resulted in a lower intake of DOM and therefore a lower MCP. This, together with the unsynchronized supply of protein and energy and, on the other hand, the likely higher energy requirements for walking and grazing in the MS group, resulted in a lower milk performance and feed efficiency compared to the confined-system cows.

4.4.3. Spring Calving Season

The average temperature was thermoneutral [56]. Although 19% of the early afternoon time was above a THI of 72, other times with lower values may have allowed for the OD-GRZ cows to dissipate their body heat load (i.e., below 20 °C, nighttime recovery) without impairing the cow's performance compared to the CB-GRZ cows [19,57], which were allocated in better housing conditions during confinement time. Notwithstanding the higher FGML and total number of bites (6672 vs. 6210; not statistically analyzed) in the CB-GRZ group during the first grazing meal, the OD-GRZ cows had a higher probability of lying and ruminating and spending less time standing during the first 90 min at pasture. This is consistent with Pons et al. [28], who evaluated similar treatments but during summer when extreme environmental situations occurred, and they observed that OD-GRZ cows were less likely to be lying during confinement and were more likely to be lying in the paddock than CB-GRZ cows, which spent more time grazing. Although the OD-GR cows had a lower probability of standing during confinement, this was not accompanied by a higher probability of lying down, suggesting that the cows were reluctant to perform either of these behaviors, probably because the soil surface was not comfortable to lie on [51,58]. Lying deprivation during confinement resulted in the cows attempting to recoup lost resting time at pasture [5], thus leading to more intense grazing periods in an attempt to maximize resting time.

The confined cows had a similar intake to the MS cows, as well as a similar MCP outflow and MCP synthesis efficiency, although the CB-TMR cows produced 20% more milk than the MS cows. Swanepoel et al. [48] also found no correlation between the MCP flow from the rumen and milk production during early lactation. The cows in SCS (all treatments) started lactation with a BCS below that desired (2.78 ± 0.218) according to Roche et al. [59], which seems a frequent issue in MS SCS as a likely consequence of low pasture availability and quality during late lactation in late summer/early autumn [60]. As a result, the milk synthesis relied more on nutrients from the diet (~4.1% BW DMI) and less on nutrients from body reserves (¼ point of BCS mobilized in 50 days [13]), which diluted the efficiency of converting the feed into milk relative to the ACS cows, who had a higher feed efficiency at a DMI of ~3.4% BW, sustained by a drop of ½ a point of BCS in 40 days (BCS at calving: 3.05 ± 0.265).

The CB-TMR cows had a higher milk yield vs. both mixed systems, with no impact on milk components. White et al. [24] reported low milk responses in TMR cows vs. MS cows during spring, although the absolute values were lower than in our study. Although the differences between the feeding systems in this period responded to a 56% pasture inclusion in the diet of the MS cows, the productive gap with TMR feeding resembled that reported by Salado et al. [22] between TMR and MS with a pasture inclusion in the diet of 44% and that reported by Bargo et al. [61] with a 30% pasture inclusion in the diet. According to Salado et al.'s [22] regression analysis, with the actual pasture inclusion levels, our MS cows should have ingested almost 4 kg total DM less than those in the TMR system, which did not occur. The MCP outflow was not affected by the feeding system, and the TMR fed cows were only 8% more efficient at converting feed into milk (milk NE/diet NE) than the MS cows despite the high pasture inclusion in the MS diet. This could be due to the similar pasture and TMR nutritional value as well as the total DMI, with a possibly similar total digestible nutrient input for microbial growth in all treatments, as found by Mendoza et al. [62] and Pastorini et al. [63]. As part of the consumed energy was directed to incremental maintenance functions such as walking and grazing activity in the MS cows compared to the TMR-fed cows [55,64], a lower milk yield and feed efficiency was achieved.

4.5. Conclusions

No significant differences were observed between MS cows subjected to different levels of environmental exposure in terms of milk yield, feed efficiency, DMI or MCP in any calving season. The results demonstrate that under non-detrimental environmental conditions (i.e., no frequent heavy rains or severe heat waves), well-managed outdoor soil-bedded milk production systems can achieve the same high milk production potential as improved infrastructure systems (i.e., compost-bedded pack barns with cooling capacity). Notwithstanding, the observed differences in behavior between the treatments during confinement and grazing suggest that the OD-GRZ cows may have resorted to adaptive mechanisms to compensate for the worse conditions to express their behavior during confinement.

The autumn-calved TMR-fed cows had a substantially higher (~35%) milk yield with no impact on milk component levels vs. both mixed systems, regardless of environmental exposure. This was associated with a higher MCP outflow from the rumen (~32%) and an ~18% increase in milk NE/diet NE, which was likely due to the higher nutritional value of the TMR diet compared to the pasture. The spring-calved TMR-fed cows also had a higher milk yield vs. both mixed systems, with no impact on milk component levels regardless of environmental exposure. However, the MCP outflow was not impacted, and the TMR-fed cows were only 8% more efficient at converting feed into milk than the MS cows (milk NE/diet NE), which was likely due to the similar total DMI as well as the pasture's and TMR's nutritional value.

Further research should focus on measuring 'hidden costs' not evaluated in this study, such as impaired energy–metabolic status and immune functions, which could impact reproductive efficiency and long-term variables like the lifetime productivity of mixed systems subjected to different environmental exposures.

Supplementary Materials: The following supporting information can be downloaded at: www.mdpi.com/xxx/s1, Table S1: Meteorological conditions in autumn (ACS) and spring (SCS) calved season cows during sampling periods.

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5. Discusión general

5.1. Sistemas mixtos con dos niveles de exposición ambiental

En contra de la hipótesis planteada, no se observaron diferencias entre SM de parición otoñal con diferentes condiciones de alojamiento y exposición al ambiente en el consumo de MS, síntesis de proteína microbiana, eficiencia de conversión, producción de leche, condición corporal e indicadores de lipomovilización. Los resultados de este trabajo demuestran que los sistemas de producción de leche parcialmente estabulados a cielo abierto, cuando están bien diseñados y gestionados y bajo condiciones ambientales neutras a moderadamente desfavorables (sin grandes acumulaciones de barro o estrés calórico), tienen un potencial de producción de leche muy elevado que podría equipararse a la respuesta productiva de SM con infraestructuras mejoradas, como corrales techados sobre cama caliente, con ventilación y aspersión. Si bien no se encontraron diferencias en el consumo de forraje entre SM con diferentes niveles de exposición ambiental a través de ninguno de los métodos de estimación, sí se observaron divergencias en el comportamiento ingestivo que adoptaron las vacas para alcanzar dicho consumo. Esto probaría la existencia de diferentes mecanismos compensatorios para mantener los niveles de consumo necesarios para dicha producción de leche, que probablemente fueron factibles gracias a un ajustado manejo del pastoreo y buenas condiciones de disponibilidad, asignación y tiempo en la pastura.

Sin embargo, condiciones ambientales más desfavorables o en momentos de la lactancia de mayor susceptibilidad perjudicaron el desempeño de vacas sometidas a mayores niveles de exposición ambiental (OD-GRZ) en vacas de parición primaveral, de acuerdo a nuestra hipótesis. Si bien la diferencia promedio en producción de leche entre SM con distinta exposición ambiental para todo el período experimental fue de solo 5 %, durante el verano el contraste ascendió a 10 %, con diferencias puntuales máximas del 20 % en las semanas en las que se produjeron olas de calor severas (semana 23 y 29) o que ocurrieron precipitaciones copiosas (semana 23 y 37). Las diferencias productivas observadas entre vacas en diferentes condiciones de infraestructura significaron unos ~380 L/vaca menos de leche en todo el período experimental. Durante las semanas 23 y 37, los SM se encontraban en encierro total debido a un bajo *stock* de forraje del sistema. Esto implicó que las vacas OD-GRZ no tuvieran la oportunidad de compensar la privación de ciertos comportamientos durante la estabulación en otro momento o lugar, como podría haber ocurrido al acceder al pastoreo, y perjudicó su bienestar y desempeño. Este aspecto adquiere relevancia en la medida en que los sistemas productivos incrementan la carga animal como estrategia de intensificación y, en consecuencia, aumentan la proporción de tiempo en estabulación total.

Por otra parte, si bien no hubo una repercusión sobre la producción de leche previo a la semana 23, las vacas del tratamiento OD-GRZ de parición primaveral expresaron un desequilibrio en el metabolismo energético (evidenciado por altos valores de βHB) que no fue observado en el SM con mejores condiciones de alojamiento y que fue coincidente con episodios repetidos de lluvias intensas y situaciones puntuales de frío (dado por la combinación de lluvia, viento y bajas temperaturas relativas). Consecuentemente, las vacas de este tratamiento lograron una menor recuperación de reservas corporales al final del estudio (~280 días en lactancia), permaneciendo por debajo de 2,75 puntos de CC, cuando lo ideal es que haya superado esa condición a los doscientos días a modo de prepararse para el siguiente ciclo productivo, por lo que el estatus energético estuvo comprometido hasta el final del período experimental (Roche et al., 2009).

Estos resultados poseen implicancias prácticas sobre los sistemas de producción lecheros de Uruguay, en los que en general las condiciones de infraestructura suelen ser precarias, ya sea por ausencia de diseños estratégicamente creados con fines de mitigación de los efectos ambientales o por falta de mantenimiento periódico de estos (Aguerre et al., 2018; INALE-MGAP, 2021; Pereira et al., 2017). Si bien los sistemas mixtos de producción de leche con encierros a cielo abierto bien diseñados y gestionados lograron equiparar la respuesta productiva de SM con mejor infraestructura bajo condiciones ambientales neutras a moderadamente desfavorables, los sutiles cambios detectados en el comportamiento ingestivo (pastoreo, descanso, rumia) dan indicios de la expresión de mecanismos compensatorios en respuesta a condiciones ambientales contrastantes durante el encierro. Sin embargo, estos mecanismos adaptativos no fueron suficientes para sostener los niveles productivos cuando las condiciones climáticas se volvieron más severas. A su vez, debe considerarse el aumento en la frecuencia de eventos climáticos extremos, como olas de calor y lluvias intensas, que perjudican el bienestar y desempeño de los animales; por lo que se torna imprescindible contar con instalaciones adecuadas para contenerlos en dichos momentos. En otro orden, los sistemas a cielo abierto implementados comúnmente en Uruguay no contemplan la acumulación excesiva de nutrientes en el suelo, producto de las deyecciones de las vacas, lo que puede generar contaminación de cuerpos de agua y ecosistemas circundantes (Clay et al., 2020). Por ello, es esencial diseñar estrategias sostenibles que no solo garanticen el bienestar animal, sino que también minimicen el impacto negativo sobre el ambiente (Stirling et al., 2024).

5.2. Sistemas totalmente estabulados y su comparación con sistemas mixtos con dos niveles de exposición ambiental

El tratamiento en estabulación total con dieta *ad libitum* (CB-TMR) de parición otoñal alcanzó un valor promedio diario de 42,8 ± 2,4 L/vaca a la octava semana de lactancia. El nivel productivo obtenido en este ensayo supera los valores alcanzados en estudios anteriores en nuestro país (Acosta et al., 2010; Fajardo et al., 2015; Mendoza et al., 2016; Pastorini et al., 2019) o en la región (Salado et al., 2018) con vacas de parición otoñal totalmente estabuladas a cielo abierto con similares días en lactancia (20-100 días), que resultaron entre 31 y 37 L/vaca/día. Los valores de consumo reportados en ensayos previos se encuentran en el rango de 24,1-26,3 kg MS/vaca/día, similares a los obtenidos en el presente estudio en la etapa temprana de lactancia para vacas de parición otoñal (24,6 kg MS/vaca/día), pero menores a los alcanzados con vacas de parición primaveral (28,8 kg MS/vaca/día). En cuanto a la composición química de las TMR utilizadas en dichos estudios, fueron reportados rangos de 38-59 % MS, 15-18 % PC, 34-41 % FDN, 18-25 % FDA, 1,8-5,4 % EE, 31-38 % CNE y 1,55-1,70 Mcal ENL/ kg MS. Las TMR suministradas en el ensayo actual, tanto para el experimento de parición de otoño como el de primavera, presentaron valores medios de MS, PC, EE y Mcal ENL/kg MS, valores superiores de CNE (38-41 %) y valores inferiores de FDN (30-33 %) y FDA (14-17 %). Probablemente, el menor contenido de fibra del actual trabajo, en línea con lo sugerido por NRC (2001) para vacas de alta producción, junto al mayor contenido de CNE, permitió alcanzar el mayor consumo observado en vacas de parición primaveral respecto a estudios previos.

A su vez, el tratamiento CB-TMR de parición otoñal logró superar en un ≈35 % la producción obtenida por los SM (con 42 % de inclusión de pastura en la dieta) a la octava semana de lactancia, independientemente de la infraestructura en la que se encontraban los SM. Las diferencias productivas entre sistemas de parición otoñal completamente estabulados y SM observadas por Salado et al. (2018), a un nivel de inclusión de pastura fresca cosechada por los animales de 44 %, fueron de 20 % a favor de vacas alimentadas con TMR. Estas estuvieron dadas por un mayor consumo total de alimento (24,1 vs. 21,0 kg MS/vaca/día respectivamente), aunque con una eficiencia de conversión del alimento a leche similar, a diferencia del actual trabajo en el que los valores de consumo no difirieron entre CB-TMR y SM, pero sí la eficiencia de conversión del alimento a leche. Si bien la diferencia productiva entre sistemas de parición otoñal con diferente estrategia de alimentación en el presente estudio fue mayor en comparación con los resultados de Salado et al. (2018), cabe mencionar que también se observó un mayor nivel productivo de los SM (31,8 L/d vs. 28,4 kg/d en SM) ante un consumo de pastura similar (~9 kg MS/vaca/día), pero un nivel de consumo total superior en los SM del presente trabajo (22,5 vs. 21,0 kg MS/vaca/día).

Las diferentes respuestas observadas en los niveles de consumo y eficiencia en las distintas estaciones podrían estar asociadas a diferencias en la calidad nutritiva entre la TMR/MR y pastura ofrecida. En este sentido, en el tratamiento CB-TMR de parición de otoño se observó un mayor flujo de PC microbiana ruminal diaria total (~32 %) respecto a SM y un aumento de ~18 % en la eficiencia de conversión alimenticia (EN leche/EN dieta) en comparación con SM. El valor nutricional de la pastura fue inferior al de la TMR/MR, evidenciado por la producción de gas a las 24 h y 30 h de incubación, la fracción potencialmente degradada, la tasa de desaparición, la digestibilidad de la MO y el contenido energético, por lo que la inclusión de pastura podría haber dado lugar a un menor aporte de nutrientes digestibles totales y, por tanto, a una menor síntesis de PC microbiana diaria por vaca. Sin embargo, en el experimento de parición primaveral no se observaron diferencias en el consumo de alimento ni en la eficiencia de conversión entre CB-TMR y SM, coherente con un similar flujo de PC microbiana ruminal diaria total entre estrategias de alimentación, posiblemente dada una similar calidad nutritiva entre la TMR/MR y pastura ofrecida en cuanto a nutrientes disponibles para la fermentación ruminal. En este segundo experimento, el tratamiento CB-TMR de parición primaveral superó solamente en un ≈20 % la producción obtenida por los SM (sin diferencias entre SM bajo diferente nivel de exposición ambiental), similar a Salado et al. (2018), pero con niveles de inclusión de pastura en la dieta superiores al 50 %. Nuevamente, la similar calidad nutricional entre TMR/MR y la pastura, así como la similar cantidad de alimento total consumido, pueden haber acortado la brecha productiva entre sistemas de alimentación. A su vez, los diferentes contrastes entre sistemas de alimentación observados en ensayos previos respecto al presente estudio podrían deberse también a diferentes necesidades energéticas de mantenimiento asociadas a la caminata y actividad de pastoreo (Jasinsky et al., 2019), así como a las diferentes condiciones ambientales de alojamiento en que se encontraron los diferentes tratamientos de otros estudios (anteriormente todos a cielo abierto).

Si bien no se comprobaron diferencias en el CMS entre tratamientos de parición otoñal ni primaveral en los períodos de medición de consumo con marcadores externos e internos (lactancia temprana), podríamos esperar mayor aporte energético en forma de almidón de sobrepaso en el tratamiento CB-TMR respecto a SM, considerando que los valores de almidón en pasturas no superan un 5 % de la MS (The National Academies of Sciences, Engineering and Medicine, 2021), por lo que, más allá de la síntesis de proteína microbiana, la dieta total podría haber aportado más nutrientes precursores de la síntesis de lactosa, en concordancia con el mayor volumen de leche producida (Reis y Combs, 2000;

Reynolds, 2006). Un consecuente ahorro de aminoácidos glucogénicos podría haber ocurrido, acompañando el aumento en litros con mayor producción de kilogramos de proteína, que explicaría la falta de efecto de dilución de proteína láctea (Auldist et al., 2016; Bauman et al., 2006). La falta de diferencia en la proporción de grasa láctea resultante de las mediciones realizadas en lactancia temprana entre sistemas de alimentación es coherente con los similares valores de producción de gas y aporte de lípidos de la pastura y TMR/MR e indicaría que la degradación de la fibra en rumen no fue perjudicada por una mayor inclusión de almidón en vacas del tratamiento CB-TMR (Oba y Allen, 2003).

Las vacas paridas en primavera en un sistema TMR totalmente confinado tuvieron la mayor producción de leche a lo largo de la lactancia y superaron el balance energético negativo en menos tiempo que los SM. Las diferencias productivas entre estrategias de alimentación en el ensayo extendido significaron 2240 y 2610 L/VO más en CB-TMR respecto a CB-GRZ y OD-GRZ a las 42 semanas en ensayo (aproximadamente 270 días en lactancia), con una producción de 9670 L/VO en el sistema totalmente confinado en dicho período. Adicionalmente, el tratamiento 100 % confinado superó el balance energético negativo en menos tiempo que los SM, con un cambio en la tendencia de la curva de CC antes que ambos SM, y alcanzó una CC de 2,75 a la semana 30, mientras que las vacas CB-GRZ recién lo lograron a la semana 40. En comparación con los tratamientos menos expuestos al medioambiente (CB-GRZ) o con un mayor nivel de alimentación (CB-TMR), las vacas pertenecientes a OD-GRZ nunca superaron dicho valor durante el estudio. Al final de este, las vacas pertenecientes al sistema totalmente confinado ya contaban con una CC adecuada para su próxima transición (Roche et al., 2009), mientras que los SM aún deberían recuperar CC, especialmente las vacas de OD-GRZ.

En comparación con sistemas estabulados de parición otoñal de otras partes del mundo, por ejemplo, sistemas comerciales de leche tipo *free stall* de California (EE. UU.), la producción de leche diaria en lactancia temprana de CB-TMR fue ligeramente menor en nuestro ensayo (42,8 vs. 44,8 L/vaca/día) respecto a lo reportado por Swanepoel et al. (2014, 2015, 2016). Sin embargo, el valor de energía retenida en leche fue numéricamente superior (31.7 vs. 30.5 Mcal ENL/vaca/día en este ensayo vs. anteriores, respectivamente), dado el mayor contenido de grasa y proteína láctea por unidad. Los valores de CMS total, gas producto de la fermentación, síntesis ruminal de PC microbiana y eficiencia de conversión alimentaria reportados por Swanepoel et al. (2014, 2015, 2016) se asimilan a los del presente ensayo. Los valores promedio de composición química de las dietas de los veinte predios comerciales de California estudiados por Swanepoel et al. (2016) fueron 57,8 ± 12,0 % MS, 16,5 ± 2,3 % PC, 29,9 ± 5,1 % FDN y 40,3 ± 5,9 % CNE, con valores de producción de gas a las veinticuatro y treinta horas de incubación de 264 ± 3,4 (52,8 ml/200 mg MO) y 278 ± 3,4 ml/g MO, respectivamente, y 1350-2200 g PC/vaca/día (promedio, 1700 g PC/vaca/día) de síntesis de proteína microbiana en rumen, valores similares a lo reportado en el trabajo actual. Esto indica que es factible generar dinámicas de fermentación ruminal y niveles de biosíntesis de leche cercanos a los obtenidos en otros sistemas del mundo con TMR elaborada a partir de alimentos disponibles en nuestra región.

6. Conclusiones e implicancias

Si bien los sistemas mixtos de producción de leche con encierro a cielo abierto, bien diseñados y gestionados, lograron equiparar el desempeño (producción, movilización de reservas) de aquellos con mejor infraestructura bajo condiciones ambientales neutras a moderadamente desfavorables (parición otoñal), se observaron cambios en el comportamiento que dan indicios de implementación de mecanismos compensatorios en respuesta a las condiciones ambientales durante el encierro y, cuando las condiciones climáticas se volvieron más severas (parición primaveral), estos mecanismos adaptativos no fueron suficientes para sostener los niveles productivos. En Uruguay, las condiciones de infraestructura a menudo suelen ser deficientes, ya sea por ausencia de diseños estratégicamente creados con fines de mitigación de los efectos ambientales o por escaso mantenimiento periódico de estos. Además, cada vez son más frecuentes las situaciones de eventos climáticos extremos que pueden desestabilizar el sistema y disminuir el bienestar y desempeño de los animales, como olas de calor y lluvias puntuales intensas. Este estudio destaca la importancia de contar con instalaciones adecuadas para contener a los animales en dichos momentos para minimizar los efectos perjudiciales sobre ellos. Aunque una infraestructura a cielo abierto puede ser suficiente en condiciones neutras a moderadamente desfavorables, es crucial dimensionar correctamente los espacios para permitir que las vacas expresen su comportamiento y asegurar el bienestar animal, y además considerar en el diseño posibles eventos climáticos extremos.

El tratamiento totalmente confinado superó la producción obtenida por los SM en un ≈35 % para vacas de parición otoñal y ≈20 % para vacas de parición primaveral durante la lactancia temprana. Las vacas del tratamiento totalmente confinado de parición primaveral superaron el balance energético negativo en menos tiempo que los SM y alcanzaron valores aceptables de CC al final del período de ensayo, en contraste a los SM, especialmente respecto a vacas OD-GRZ, que presentaron los niveles más bajos de CC al final del ensayo. Por otra parte, los valores de síntesis ruminal de PC microbiana diaria total y eficiencia de conversión alimenticia fueron mayores para el tratamiento CB-TMR respecto a SM cuando el valor nutricional de la pastura fue menor respecto al de la MR (otoño), pero no se observaron diferencias cuando la calidad nutricional fue similar entre ambos alimentos (primavera). A su vez, los valores de gas fermentado, síntesis ruminal de PC microbiana y eficiencia de conversión alimentaria del presente ensayo en vacas del tratamiento CB-TMR se asimilan a los obtenidos en sistemas estabulados de otras partes del mundo, como los de California (EE. UU.), con respuestas productivas similares a las obtenidas en el actual trabajo, las cuales fueron ampliamente superiores a trabajos previos locales. Queda demostrado que es factible alcanzar parámetros de fermentación ruminal y biosíntesis de leche cercanos a los de otros sistemas del mundo con una TMR *ad libitum* elaborada a partir de alimentos disponibles en nuestra región, en animales alojados en un galpón totalmente techado con cama de compost, equipados con sistema de ventilación y aspersión para mitigación del estrés calórico, y con comederos y bebederos acordes a las necesidades. Esto no solo impactó positivamente en la producción individual total del período evaluado, sino que también favoreció la recuperación de reservas corporales, lo que permite una preparación adecuada para el próximo ciclo productivo.

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