UNIVERSIDAD DE LA REPÚBLICA

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Programa de Posgrados

MANEJOS DE ALIMENTACIÓN Y AMBIENTE EN LOS SISTEMAS LECHEROS DE BASE PASTORIL EN VACAS CON PARTOS DE PRIMAVERA: IMPACTOS EN EL BIENESTAR ANIMAL, PRODUCCIÓN, COMPOSICIÓN Y PERFIL DE ÁCIDOS GRASOS EN LA LECHE Y QUESO

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TESIS DE DOCTORADO EN PRODUCCIÓN ANIMAL

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La Dra Grille presentó una tesis muy bien redactada, basada en 2 experimentos muy complejos, y que está apoyada en 3 artículos publicados en revistas arbitradas. Realizó una presentación oral clara y ajustada a los tiempos. Posteriormente, en la defensa de su tesis demostró un cabal conocimiento de sus resultados, mostrando las implicancias que los mismos tienen a nivel de los sistemas de producción. Por todo lo anterior, es que se considera que la tesis ha sido aprobada con mención.

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APÉNDICE

Artículos de I a III

La presente Tesis está basada en los siguientes artículos, a los cuales se referirá en el texto por su numeración romana (I-III):

I. Grille L., Adrien M.L., Olmos M., Chilibroste P., Damián J.P. Diet change from a system combining total mixed ration and pasture to confinement system (total mixed ration) on milk production and composition, blood biochemistry and behavior of dairy cows. (2019). *Animal Journal Science,* 90(11), 1484-1494. DOI: 10.1111/asj.13288

II. Grille L., Adrien M.L., Méndez M.N., Chilibroste P., Olazabal L., Damián JP. Milk fatty acid profile of Holstein cows when changed from a mixed system that combines total mixed ration and double grazing to a single confinement system (total mixed ration) or to a mixed system with only one night grazing. (2022). *International Journal of Food Science*, 1-9. DOI: https://doi.org/10.1155/2022/5610079

III. Grille L., Escobar D., Méndez M.N., Adrien M.L. Olazabal L., Rodriguez V., Pelaggio R., Chilibroste P., Meikle A., Damián J.P. Different conditions during confinement (compost-bedded pack barn vs. outdoor soil-bedded pen) in mixed systems (grazing plus total mixed ration) and feeding systems (mixed vs. confined) affect fatty acid profile in milk and cheese of Holstein dairy cows (2023). *Animals*, 13(8), 1426. DOI: https://doi.org/10.3390/ani13081426

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RESUMEN

Los sistemas de base pastoril presentan un perfil de ácidos grasos (PAG) más saludable para los consumidores y mejor bienestar que los sistemas confinados (SC). En sistemas mixtos (SM: pastura+suplementación) las vacas están más expuestas a las condiciones ambientales que en SC, y por tanto estos últimos han sido utilizados para atenuar los efectos negativos de la exposición ambiental. Las vacas en SM están gran parte del tiempo en los lugares donde reciben la suplementación (encierros), por tanto las condiciones de estos encierros adquieren gran importancia. El primer objetivo fue determinar el efecto del cambio brusco desde un SM a SC (GCHD) en comparación con vacas en SC (GTMR) sobre el comportamiento, bioquímica sanguínea, producción de leche (PL) y composición (Artículo I). Las vacas del GCHD tuvieron menor PL, lactosa y proteína (kg/d) antes del cambio de dieta que las del GTMR ($p<0,01$), pero estas diferencias desaparecieron después del cambio. La frecuencia de rumia y echado disminuyó en GCHD después del cambio ($p<0,03$) e incluso tuvieron menor frecuencia de estos comportamientos que las del GTMR ($p<0,02$). La concentración de BHB ($p<0,0001$) y AST ($p=0,03$) disminuyó en GCHD luego del cambio mientras que en GTMR estas variables aumentaron. El segundo objetivo fue determinar el PAG de la leche del GCHD y de vacas que cambian desde un SM a un solo pastoreo nocturno en verano (GTMR+P), ambos en comparación con GTMR (Artículo II). A diferencia de GTMR+P, GCHD mejoró la PL después del cambio, pero el PAG de la leche empeoró. En M3, el porcentaje de ácido linoleico conjugado (CLA) en GTMR y GCHD fue menor que en GTMR+P ($p<0,05$) y C18:3 y n-3 fue menor en GCHD que GTMR+P. El último objetivo fue comparar el contenido de grasa y PAG de leche y quesos en SM con diferentes condiciones de encierro [baja (CB-GRZ) y alta (OD-GRZ) exposición ambiental] en comparación con SC con baja exposición al ambiente (CB-TMR) (Artículo III). Los AG saturados en leche y n-6/n-3 en leche, leche de pool (MilkP) y queso fueron mayores en CB-TMR ($p<0,0001$) que en SM, mientras que los AG insaturados y monoinsaturados (MUFA) en leche fueron menores en CB-TMR que en SM ($p<0,001$). El porcentaje de n-3, C18:3 y CLA en leche fue menor en CB-TMR que en SM ($p<0,001$). Entre SM, n-3 y C18:3 en leche fueron mayores en CB-GRZ que en OD-GRZ ($p<0,01$). En conclusión, el cambio abrupto desde un SM a un SC mejoró la PL y variables en sangre, pero afectó negativamente el PAG en leche y el comportamiento, dado que las vacas tuvieron problemas de adaptación al corto plazo. Mantener un SM con pastoreo nocturno en encierros a cielo abierto en verano sería una buena alternativa de manejo, permitiendo que los animales puedan pastorear y mejorar la calidad de la grasa láctea. En comparación con SC, los SM presentaron mejor PAG en leche y quesos. Dentro de los SM, aquellos con mejores condiciones durante el encierro presentaron mejor PAG.

ABSTRACT

Pasture-based systems present a healthier fatty acid profile (FAP) for consumers and allows better cow welfare than confined systems (SC). Mixed systems (MS: pasture + supplementation) are more exposed to environmental conditions than SC, and therefore the latter are used to mitigate environmental exposure negative effects. Mixed systems cows spend a large part of the time in places where they receive supplementation (confinement); therefore, facility conditions acquire great importance. The first objective was to determine the effect of an abrupt change from MS to SC (GCHD) compared to cows in SC (GTMR) on behavior, blood biochemistry, milk production (PL) and composition (Article I). Before the diet change, GCHD cows had lower PL, lactose and protein (kg/d) than GTMR cows ($p<0.01$), but these differences disappeared after the change. After the diet change, GCHD cows decreased rumination and lying frequency ($p<0.03$) and had a lower frequency of these behaviors than GTMR cows ($p<0.02$). Also, after the change, GCHD cows decreased BHB ($p<0.0001$) and AST ($p=0.03$) concentration, while GTMR cows had an increase. The second objective was to compare milk FAP in GCHD with GTMR and with cows that changed from MS to overnight grazing in summer (GTMR+P) (Article II). Unlike GTMR+P, GCHD improved PL after the change, but FAP was impaired. In M3, conjugated linoleic acid (CLA) percentages in GTMR and GCHD were lower than GTMR+P ($p<0.05$) and C18:3 and n-3 were lower in GCHD than GTMR+P. The last objective was to compare fat content and FAP of milk and cheeses from MS with different facility conditions during confinement [low (CB-GRZ) and high (OD-GRZ) environmental exposure] compared to SC with low environmental exposure (CB -TMR) (Article III). Saturated fatty acid (FA) in milk and n-6/n-3 in milk, pooled milk (MilkP) and cheese were higher in CB-TMR ($p<0.0001$) than in MS, while unsaturated and monounsaturated FA in milk were lower in CB-TMR than in MS ($p<0.001$). Milk percentage of n-3, C18:3 and CLA were lower in CB-TMR than in MS ($p<0.001$). In MS, n-3 and C18:3 in milk were higher in CB-GRZ than in OD-GRZ ($p<0.01$). In conclusion, the abrupt change from MS to SC improved PL and blood variables, but impaired milk FAP and cow behavior (indicating adaptation problems in the short term). Furthermore, MS with overnight grazing in summer allows cows to access grazing and therefore improves milk fat quality. Thus, these systems would be a good management alternative in dairy cow systems. Mixed systems showed better FAP in milk and cheese compared to SC. In MS with different facilities conditions during confinement, those with better conditions (CB-GRZ) showed better FAP.

INTRODUCCIÓN

Situación lechera y sistemas de producción a nivel nacional

La producción nacional se realiza en 3100 establecimientos lecheros que ocupan un total de 735 mil hectáreas. De estos establecimientos un 80,3% remiten a planta y los restantes elaboran productos en el propio establecimiento (DIEA, 2022). En el 2021 la producción lechera alcanzó los 2274 millones de litros, de los cuales 2118 millones son remitidos a la industria (93% de la leche producida). Desde el año 1992 hasta la fecha la producción de leche tuvo un aumento del 84,8% (365-2401 L/día/remitente) con una disminución del 60% de los establecimientos remitentes, pérdida de 3581 remitentes (de 5998 a 2417) (DIEA, 2022). Este aumento de la producción es explicado por un incremento productivo a nivel individual (litros por vaca) y en menor medida por un incremento en la carga animal (Fariña & Chilibroste, 2019). Según Fariña & Chilibroste (2019), datos recabados a partir del relevamiento de 2013 a 2017 (Fuente: producción competitiva CONAPROLE) "la media productiva fue de 8831 L/ha de plataforma de pastoreo" (área total del predio potencialmente pastoreable por la vaca lechera). Para acompañar este aumento de producción, la dieta de los animales presenta un mayor aporte de concentrados y reservas forrajeras (fardos y ensilados) en comparación con décadas anteriores. Igualmente, en nuestros sistemas productivos, el pastoreo sigue siendo clave para mantener un bajo costo de producción y así poder ser competitivos a nivel internacional (Fariña & Chilibroste, 2019). En este sentido, los sistemas lecheros predominantes en Uruguay, se caracterizan por ser de base pastoril con suplementación (sistemas mixtos: SM), los que presentan en general la siguiente estructura en su dieta: 55% pastura, 19% suplemento y 25% concentrados, con una carga animal promedio de 1,15 vacas en ordeñe por hectárea (Fariña & Chilibroste, 2019). Esta estrategia productiva implica que cuando las vacas no están pastoreando, se encuentran en áreas destinadas para el suministro del suplemento. En sistemas pastoriles intensivos (alta carga animal) el tiempo que las vacas se encuentran pastoreando podría afectarse con más facilidad frente a los cambios climáticos y ambientales. Por ejemplo, en un experimento realizado en el Centro Regional Sur (CRS; Facultad de Agronomía, Uruguay) reportaron que en sistemas con alta carga animal (2 vacas por ha) los tiempos de pastoreo fueron menores (las vacas pasaron menos tiempo en la pastura y más en los encierros con suplementación) en comparación con los sistemas de carga media (1,5 vacas por ha) (Custodio, 2018; Ortega et al., 2019). Lo cual, a su vez, fue relacionado a temporadas lluviosas o sequía. En relación a esto, las áreas destinadas a la suplementación, en su mayoría son praderas viejas, campo natural o monte en donde las vacas están muy expuestas a las condiciones climáticas externas (barro, estrés calórico, etc) (INALE, 2019). Además, a pesar que el 63% de los establecimientos tienen acceso a agua y el 75% tiene acceso a sombra natural, en general las vacas están muy expuestas a sufrir estrés por calor, principalmente en el verano y más en los establecimientos localizados al norte del país. Es importante destacar que la sombra en la mayoría de los casos está localizada en caminos y no en potreros o áreas de descanso (INALE, 2019).

En los establecimientos lecheros de Uruguay, el 65% de los partos se dan en los meses de marzo a setiembre, con concentraciones en otoño (marzo-abril) y en menor medida en primavera (agosto-setiembre) (Chilibroste & Battegazzore, 2019). Esta concentración de partos en momentos puntuales del año se justifica en la necesidad de optimizar recursos del establecimiento, sobre todo en SM de alta carga animal (Fariña & Chilibroste, 2019). En este sentido, los partos en primavera se realizan para hacer coincidir la época de mayor disponibilidad de pasturas con la lactancia temprana, donde se dan los mayores requerimientos en la vaca lechera (Van Knegsel et al., 2007). Una de las desventajas de esta estrategia es el estrés calórico que sufren las vacas durante la lactancia temprana y media (verano), afectando negativamente el bienestar y por lo tanto la producción y componentes de la leche. En los meses cálidos, debido a la combinación de temperatura y humedad alta, se ve sobrepasada la capacidad para disipar calor por parte de los animales. Esto genera que salgan de su zona de confort, resultando de un menor consumo voluntario y por lo tanto una menor producción en litros de leche, materia grasa y proteína (Allen et al., 2015; Bernabucci et al., 2015; Kadzere et al., 2002; Román et al., 2019; Zimbelman et al., 2010). A su vez, estos efectos podrían tener mayor relevancia en la región norte del país, ya que las temperaturas máximas alcanzadas en verano son mayores en comparación con la cuenca lechera del sur (Dirección Nacional de Meteorología, 2020). En Uruguay, Saravia et al. (2011), han realizado una caracterización climática de ITH en el período estival (promedios históricos mensuales de la serie 1961 - 1990 de los meses de diciembre a marzo), encontrando probabilidades mayores al 55% de que se presenten valores por encima del valor crítico en el norte del Río Negro para el mes de enero. En este escenario, los productores deben tener presente alternativas de manejo (ambiente y alimentación) para disminuir el estrés calórico y mantener un buen desempaño productivo.

Por otro lado, en Uruguay, el 70% de la leche que se produce se destina a la exportación. A enero de 2023, los principales productos de exportación fueron leche en polvo entera: 70,41%; leche en polvo descremada: 5,5%, quesos: 9,3% y manteca: 7,5% (INALE, 2023). Es importante resaltar que la mayor parte de la leche producida se destina a la industrialización (90%). El sector industrial lechero, integrado por empresas nacionales y extranjeras ha expandido continuamente su capacidad, diversificando su producción en el mercado interno y exportando diversos productos a diferentes mercados. Las empresas cooperativas captan la mayor proporción de la leche, contando con un 77% del total, siendo Conaprole la principal industria, captando el 73% de la leche producida en el país (INALE, 2021). Por lo tanto, dado que Uruguay es un país básicamente exportador de leche, resaltar las ventajas de los sistemas lecheros con respecto a la calidad de los productos lácteos lo posicionaría de forma ventajosa frente a los mercados internacionales. Esto sustentado además, en que a nivel internacional hay un gran interés en valorizar los componentes lácteos con aspectos saludables y terapéuticos en relación a la salud humana (Haug et al., 2007; Schroeder et al., 2003).

Sistemas de base pastoril (pastura más suplementación): ventajas y desventajas

Uno de los desafíos de la lechería en Uruguay, es lograr altas producciones de leche por hectárea (proveniente de forraje), para así lograr mejores márgenes de alimentación y por lo tanto menores costos de producción y de esa manera ser competitivos a nivel internacional (Fariña, 2016). En relación a esto, los sistemas de base pastoril (característicos de Uruguay) presentan ventajas con respecto a los sistemas estabulados, como ser menores costos de producción, mejores condiciones de bienestar y mejor calidad de la leche (Barca et al., 2018; Bargo et al., 2006; Olmos et al., 2009; White et al., 2001, 2002).

En cuanto a la leche, es interesante destacar las características de la misma sobre los beneficios a la salud de los consumidores. La leche es uno de los alimentos líquidos más completos de origen animal en la naturaleza y junto con el resto de los productos lácteos son considerados de los más importantes en la alimentación humana (Lambrini et al., 2021; German y Dillard, 2010). Esto se debe a su mezcla compleja de grasas, proteínas (caseínas y proteínas del suero), carbohidratos (principalmente lactosa), minerales y vitaminas (Walstra et al., 2001). La leche es una excelente fuente de proteínas de alta calidad, conteniendo 9 aminoácidos esenciales, que sirven como base estructural para el crecimiento y desarrollo del organismo (Fox, 2003). Además, se ha demostrado que la leche presenta péptidos bioactivos con propiedades antihipertensivas, antitrombóticas e inmunomoduladoras (Mills et al., 2011; Steijns, 2001). Las proteínas en leche también están presentes como lipoproteínas asociadas al glóbulo graso (MFGM), con similares funciones benéficas que las nombradas anteriormente (Manoni et al., 2020). En cuanto a la lactosa (b-D-galactosil-D-glucosa), es el principal carbohidrato encontrado en leche. Es un disacárido que se encuentra exclusivamente en la leche de los mamíferos. Presenta bajo poder edulcorante, bajo índice calórico y glucémico. Posee propiedades prebióticas (similar a la fibra dietética) y mejora la absorción de calcio y magnesio a nivel intestinal (Schaafsma, 2008), por todo esto tiene gran importancia tanto desde el punto de vista de la salud como a nivel industrial. En relación a las grasas, por décadas la leche de rumiantes tuvo una imagen negativa por su alto contenido de ácidos grasos saturados (AGS) y su relación con las enfermedades cardiovasculares (Astrup et al., 2020; Yakoob et al., 2016). Sin embargo, se ha demostrado que la leche contiene ácidos grasos (AG) beneficiosos para la salud humana, tales como el ácido oléico (OA; C18:1 cis 9), vaccenico (VA; C18:1 trans 11) y el ácido linoléico conjugado (CLA) (Drouin-Chartier et al., 2016; Elgersma et al., 2006). Recientemente también han sido reportadas funciones benéficas en algunos AG saturados de la leche (AG de cadena ramificada: BCFA) como C14:0, C15:0 y C17:0 (Bainbridge et al., 2016).

Si bien la producción y composición de la leche depende de muchos factores, es la nutrición animal uno de los que más influye en la composición de la misma, principalmente en el contenido de grasa (Artegoitia et al., 2013; Dewhurst et al., 2006). En este sentido, en comparación con sistemas con baja o nula proporción de pastura en la dieta, los sistemas pastoriles presentan un perfil de ácidos grasos en leche (PAG) más saludable para el consumo humano (Mordenti et al., 2015). En relación a esto, se ha demostrado que la inclusión de pasturas en sistemas de confinamiento (100% DTM) aumentan los ácidos grasos monoinsaturados: MUFA, polinsaturados: PUFA, omega 3 y CLA, mejorando el PAG desde el punto de vista de la salud humana (Gagliostro, 2004; Schroeder et al., 2003). En este sentido, el aumento en la ingesta de la pastura fresca resulta en un aumento de 2 a 3 veces en el contenido de CLA en la leche (Kelly et al., 1998; Stanton et al., 1997). En algunos trabajos realizados en nuestro país, se observó que en sistemas mixtos, el aumento en las horas de pastoreo o diferentes proporciones de DTM en sistemas mixtos, también mejora el PAG, siempre a favor de los sistemas con mayor inclusión de pasturas (Barca et al., 2018; Mendoza et al., 2016; Pastorini et al., 2019). Por lo tanto, el PAG en los sistemas de base pastoril difiere al de los sistemas estabulados, determinado principalmente por la dieta y el ambiente productivo en el que se encuentran los animales. Asimismo, los sistemas de base pastoril presentan ventajas en la calidad de los productos industrializados, muchas de las cuales están dadas por las diferencias en el PAG de la leche. Por ejemplo, quesos obtenidos de sistemas pastoriles presentan características deseables sobre la calidad nutricional, textura y calidad sensorial (sabores, olores y colores más intensos) (Girard et al., 2016; Martin et al., 2005; O'Callaghan et al., 2017) en comparación con sistemas estabulados (sin pastura en la dieta). En tal sentido, la leche proveniente de sistemas pastoriles presenta menor relación palmítico/oleico que aquella proveniente de heno o ensilaje (Villeneuve et al., 2013), lo que influye en una disminución en la percepción de la firmeza en quesos y mayor derretimiento en boca (Agabriel et al., 2004; Cornu et al., 2009). Por lo tanto, el sistema de base pastoril predominante en Uruguay, lo posicionaría de forma ventajosa frente a los mercados internacionales, dado que la leche y quesos provenientes de sistemas pastoriles presentan mejores atributos para el consumidor en comparación con los sistemas sin acceso al pasto (Kilcawley et al., 2018; Martin et al., 2005).

Los sistemas pastoriles también presentan algunas desventajas en relación a los sistemas confinados o estabulados. En relación a las variables productivas y metabólicas, las vacas en los sistemas pastoriles consumen menos materia seca y producen menos leche que en sistemas confinados (Fontaneli et al., 2005; Kolver & Muller, 1998). De hecho, algunos trabajos han mostrado la respuesta de las vacas lecheras cuando cambian de sistemas 100% DTM a sistemas de base pastoriles, encontrando aumento en la concentración de los ácidos grasos no esterificados (NEFA) y beta-hidroxibutirato (BHB) en suero, una disminución de la concentración de insulina, así como menor producción de leche, consumo de material seca y condición corporal (Astessiano et al., 2017; Schären et al., 2016).

Desde el punto de vista del bienestar, definido como el estado de un individuo con respecto a los intentos de hacer frente y/o adaptarse a su entorno (Broom, 1986) y según las cinco libertades definidas por la Farm Animal Welfare Council (1992), los sistemas de producción basados en pasturas son más propicios a que las vacas encuentren un mejor estado de bienestar. Estos sistemas ofrecen un entorno natural para el ganado lechero (Clutton-Brock., 1999) permitiendo la expresión del comportamiento "normal" de la especie como, por ejemplo, el pastoreo (Olmos et al., 2009). En este sentido, en pasturas las vacas se encuentran entre el 90 y el 95% del día pastando, rumiando y descansando (Kilgour, 2012), siendo estos comportamientos considerados indicadores positivos del bienestar animal (Krawczel et al., 2009; Olmos et al., 2009; Roca-Fernández et al., 2013). Sin embargo, en estos sistemas las vacas están más expuestas a variaciones ambientales extremas, que pueden afectar negativamente su bienestar, lo que se evidencia mediante el uso de indicadores fisiológicos, de salud, comportamentales y productivos (Arnott et al., 2017; Charlton & Rutter, 2017; Schütz et al., 2010). Un ejemplo de esto serían las altas temperaturas ambientales y humedad que pueden afectar a la vaca lechera durante el verano (estrés calórico), repercutiendo negativamente en la producción, composición de la leche, la salud y la reproducción (Bernabucci et al., 2010, 2015; Cowley et al., 2015; Jordan, 2003), lo que también podría considerarse una desventaja de estos sistemas.

Estrategias de manejo en los sistemas de base pastoril (sistemas mixtos: pastoreo más suplementación)

En muchos países se utilizan los sistemas de confinamiento (encierros) con acceso a sombra como medida de manejo alternativo en los sistemas de base pastoril, especialmente en momentos de baja disponibilidad de forraje (Bargo et al., 2002; Chilibroste et al., 2007; Wales et al., 2013). En Uruguay, como forma de minimizar el desequilibrio entre los aportes y los requerimientos nutricionales en las vacas lecheras, dado por las épocas de parto y estacionalidad de las pasturas (Naya et al., 2002), se ha incorporado desde hace algunos años, el uso de confinamientos (encierros totales con DTM) en SM, principalmente durante la lactancia temprana (Cajarville et al., 2012; Fajardo et al., 2015). Este manejo en los SM logra mejorar los niveles de producción de leche y sólidos en lactancia temprana (Bargo et al., 2002; Soriano et al., 2001). Por lo tanto, el uso de DTM de forma estratégica, como medida de manejo en diferentes momentos del año en los sistemas de base pastoril, podría ser una alternativa para mejorar los niveles de producción en el ganado lechero. Estas medidas de manejo alternativo también son utilizadas para disminuir los efectos negativos sobre el bienestar de las vacas lecheras, dados por condiciones climáticas adversas, los que se ven reflejados en pérdidas de producción y salud de los animales (Charlton & Rutter, 2017; Schütz et al., 2010). En relación a esto último, otro punto importante a tener en cuenta son las condiciones en las que se realizan dichos confinamientos. El ambiente (infraestructura) en el que se encuentran las vacas durante los encierros juegan un rol clave. Encierros con techo (sombra), adecuado tamaño de los corrales, buena ventilación, aspersores, ventiladores, suelo y camas confortables, así como el enriquecimiento del lugar para disminuir el estrés, logran un adecuado estado de confort de los animales y por tanto mejores resultados productivos (Black et al., 2013; Charlton & Rutter, 2017; Mandel et al., 2016).

Cambios de sistemas (base pastoril vs estabulados) y su efecto en la calidad de la grasa láctea

Con respecto a la composición de la grasa de la leche, cuando las vacas cambian desde un sistema de base pastoril (SM) a 100% DTM, los AGS de la leche aumentan en detrimento de los AG benéficos (Khanal et al., 2008; Rego et al., 2016). Mientras tanto, cuando las vacas cambian desde un sistema 100% DTM a un sistema de base pastoril, aumentan los AG saludables (Barca et al., 2018; Elgersma, 2015; Khanal et al., 2008; Rego et al., 2016). Si bien se ha evaluado el perfil de ácidos grasos (PAG) cuando las vacas cambian de sistemas (desde un SM a confinamiento y viceversa), según nuestro conocimiento, a la fecha no se ha estudiado las consecuencias sobre el PAG al cambiar desde un SM con doble pastoreo a un pastoreo nocturno en el verano (ambos sistemas mixtos), ni la comparación de este manejo (pastoreo nocturno) con el cambio desde un SM a un sistema estabulado.

En resumen, las vacas con partos de primavera en SM presentan un gran desafío en la estación de verano, dado que están muy expuestas al estrés calórico. Para disminuir los efectos negativos de esta estación se han ensayado diferentes manejos, como cambios desde SM (doble pastoreo) a confinamiento total (100% DTM) o cambios en los horarios de pastoreo y encierro de los animales, por ejemplo, pastoreo nocturno, con el fin de evitar la exposición de las vacas en las horas de mayor radiación solar. Este manejo también se sustenta en la actividad de pastoreo que presentan las vacas, con sesiones más largas e intensas durante las primeras horas de la mañana y fundamentalmente en las últimas horas de la tarde (Arnott et al., 2017; Gibb et al., 1998; Mattiauda et al., 2013). De acuerdo a nuestro conocimiento, hasta el momento no hay trabajos que hayan evaluado como la vaca lechera es capaz de adaptarse a los cambios repentinos de manejo desde SM a confinamiento con DTM. Un diseño que permita evaluar no sólo aspectos productivos y fisiológicos, sino también el comportamiento, podrá brindar información sobre la manera en que los animales responden a estos cambios y/o sus posibles dificultades de adaptación a corto o mediano plazo, y por tanto sus repercusiones en el bienestar animal.

Por otro lado, como se comentó anteriormente, los sistemas de base pastoril presentan ventajas en comparación con los sistemas estabulados 100% DTM, entre las que se destacan mejor calidad de grasa láctea (mayor proporción de ácidos grasos saludables) que repercuten en mejor calidad de los productos lácteos. Por lo tanto, mantener SM en el verano, atenuando los efectos negativos de esta estación es un gran desafío para los sistemas de producción de Uruguay. En sistemas mixtos, el cambio de doble pastoreo a un pastoreo nocturno podría ser una alternativa para mantener los beneficios de estos sistemas (calidad de la grasa láctea y comportamiento en pastoreo), reduciendo los efectos negativos (exposición a altas temperaturas diarias) durante el verano. En relación a lo anterior, si bien el pastoreo nocturno en los SM sería una buena estrategia para mantener las ventajas de estos sistemas en el verano, las condiciones de las instalaciones de los encierros en los SM (lugares donde se encuentran las vacas durante el día) adquiere gran importancia en esta estación.

En Uruguay se han estudiado varios tipos de encierros, los cuales pueden ser encierro total (100% DTM) (Cajarville et al., 2012; Fajardo et al., 2015) o encierro parcial (diario), donde las vacas alternan tiempos de pastoreo y tiempo de encierro (SM) (Barca et al., 2018; Bargo et al., 2002; Fajardo et al., 2015; García-Roche et al., 2021; Jasinsky et al., 2019). Los más frecuentes en nuestro país son los denominados sistemas a "cielo abierto" (INALE, 2019) o como se denomina en otros países sistemas *dry lot* o "parcialmente abiertos". Este tipo de encierro tiene gran interés a nivel internacional debido a su bajo costo de inversión en comparación con sistemas *free stall* o *compost barn* (Marcondes et al., 2020). En los sistemas a cielo abierto las vacas se encuentran sobre piso de tierra o tosca y en general cuentan con sombra artificial. En muchos casos los comederos se encuentran techados, mientras que en el resto de las áreas los animales están expuestos al clima (lluvia, estrés calórico, etc.; INALE, 2019). Este tipo de sistemas pueden asociarse (sobre todo en el largo plazo) con problemas de salud y bienestar de la vaca lechera debido a la exposición a la radiación solar, lluvia y barro (Marcondes et al., 2020). En relación a esto, en los últimos años en Uruguay, han empezado a aumentar los encierros donde las vacas se encuentran en galpones totalmente techados con bebederos, comederos y diferentes tipos de cama. Este tipo de encierros se caracterizan porque las vacas tienen baja exposición al clima exterior (mejor control del ambiente, mejor confort). Un ejemplo de este tipo de encierro es el sistema con *compost barn* o también llamado "cama de compost" (Leso et al., 2020). Ha sido demostrado que este tipo de cama mejora el confort de las vacas, con menores índices de mastitis, laminitis y mayor longevidad (Black et al., 2013). Como desventaja de estos sistemas es que requieren una gran inversión inicial y personal capacitado para mantener una adecuada calidad de las condiciones de la cama (Bewley et al., 2017). En este sentido, se requiere un monitoreo constante de la temperatura y humedad de la cama, parámetros claves para mantener estos sistemas de forma eficiente (Barberg et al., 2007; Janni et al., 2007). Para lograr la eliminación de los microorganismos patógenos (potenciales patógenos de mastitis) y una adecuada degradación de la celulosa (con una mayor durabilidad de la cama) la temperatura debe estar entre 40 y 65°C y no debe superar el 60% de humedad (Black et al., 2013). Por lo tanto, contar con encierros en buenas condiciones de infraestructura en SM minimizaría los efectos negativos de la exposición climática de las vacas lecheras, como por ejemplo estrés calórico en el verano. Esto permitiría que las vacas durante el encierro se encuentren en buenas condiciones de bienestar (confort), permitiendo alcanzar buenos niveles productivos (según alimentación y genética). Así como también obtener leche y quesos con grasas más saludables desde el punto de vista de la salud humana (por presencia de pastura en la dieta) en comparación con los sistemas confinados (100% DTM). Es interesante resaltar que hasta la fecha no se ha evaluado como la infraestructura de los encierros (exposición al clima) en SM intensivos afecta la producción y calidad de la grasa láctea, desde una mirada integral (producción primaria e industria).

Con respecto al efecto del ambiente productivo sobre la calidad de los quesos, si bien existen algunos trabajos que estudiaron diferentes características de los quesos elaborados de sistemas en confinamiento vs. sistemas de base pastoril, estos sistemas no evaluaron el efecto del ambiente independientemente del sistema de alimentación. En el trabajo realizado por Esposito et al. (2014), evaluaron granjas lecheras que cambiaron el manejo según la estación del año (pastoreo: PS o confinamiento: CS). El cambio de sistema involucró un cambio en el tipo de alimento (pastura vs dieta en base a cereales: harina de avena, cebada y trigo y heno: campo natural y trébol). Estos autores encontraron que los quesos elaborados en los meses que los animales estaban pastoreando (PS) tuvieron mayor proporción de C18:3, CLA (cis9-trans11) y C18:1 trans-11 y mejores características organolépticas en comparación con los meses en confinamiento (CS). La principal limitante en este estudio es que no se pudo evaluar cómo la dieta, el ambiente y la estación de año afectó el PAG de los quesos en forma separada, dado que los 3 efectos cambiaron en forma conjunta. En el caso de O'Callaghan et al. (2017), estudiaron 3 sistemas con diferente alimentación y ambiente: 2 sistemas pastoriles (un grupo raigrás y otro raigrás más trébol blanco) y un sistema estabulado 100% DTM. Los principales resultados estuvieron relacionados al tipo de dieta (diferentes sistemas pastoriles vs. confinamiento), teniendo como resultado que los sistemas pastoriles presentaron mejor calidad de grasa en el queso que los estabulados. La limitante que se puede observar en este caso, es que ambos tratamientos manejan ambos elementos: ambiente y dieta en forma conjunta. Por lo que al igual que en el anterior, existen efectos confundidos, dado que no se podrían adjudicar los resultados a cada efecto independiente (dieta – ambiente), sino que ambos efectos afectaron de forma conjunta los AG de los quesos. En el caso del trabajo de Bonanno et al. (2013), estudiaron la calidad del queso de 2 tipos de granjas con diferentes sistemas (extensivo vs. intensivo). Esta clasificación se hizo en base a la alimentación y manejo (sistema básicamente pastoril vs. dieta a base de heno y concentrado con baja proporción de pastura en la dieta) y razas (vacas "autóctonas" vs. vacas de raza especializadas en la producción de leche: "Brown cows"). En este caso los efectos confundidos involucraron el tipo de dieta, el ambiente y las razas. Los principales resultados de los 3 estudios, fue la mayor proporción de AG saludables en leche y quesos, así como mejores características sensoriales en los quesos de sistemas de base pastoril en comparación con las vacas que se encuentran en confinamiento. Pero en ninguno de ellos se pudo comprobar cómo las condiciones climáticas (de forma independiente) afectan el PAG en leche y quesos.

Por lo tanto, según nuestro conocimiento, hasta el momento no hemos encontrado trabajos que hayan evaluado cómo el ambiente (condiciones climáticas externas) durante el encierro (momentos donde el animal no está pastoreando) afectan el PAG de leche y quesos bajo un mismo sistema de alimentación en SM. En relación a esto, esta tesis generaría un aporte novedoso, sobre cómo las condiciones de infraestructura que presentan los SM durante el encierro podrían afectar la calidad de la grasa láctea y como esto repercutiría en la calidad de los quesos elaborados.

HIPÓTESIS

A. El cambio brusco de manejo desde un sistema mixto (pastura más encierro con DTM) hacia un sistema de confinamiento en el verano mejora la producción y composición de leche y perfiles metabólicos, pero afecta negativamente algunos indicadores de comportamiento en vacas con partos de primavera **(Artículo I)**

B. El cambio brusco de manejo desde un SM con doble pastoreo a un SM con un pastoreo nocturno durante el verano mejora la calidad de la grasa láctea en comparación con vacas que cambian desde un sistema mixto a confinamiento y con las que se mantienen siempre en confinamiento **(Artículo II)**

C. En vacas con partos de primavera, los SM (con baja y alta exposición al ambiente) alcanzan un perfil de ácidos grasos más saludable, pero menor producción de leche y contenido de grasa en comparación con un sistema confinado (100% DTM y baja exposición al ambiente) **(Artículo III)**

D. En vacas con partos de primavera, diferentes condiciones (alta vs. baja exposición al ambiente) durante el confinamiento en SM en el verano afecta el perfil de ácidos grasos de leche de pool (MilkP) y quesos en comparación con un sistema confinado (100% DTM y baja exposición al ambiente) **(Artículo III)**

OBJETIVOS GENERALES

1. El primer objetivo general es determinar si el cambio desde SM (pastoreo + DTM) a sistemas confinados (100% DTM) o SM (con pastoreo nocturno) afecta la producción y composición de leche, PAG, bioquímica sanguínea y comportamiento en vacas con partos de primavera en comparación con un sistema en confinamiento (100% DTM).

2. El segundo objetivo general es determinar si diferentes condiciones ambientales (alta y baja exposición al ambiente) durante el encierro en SM afecta el contenido en grasa, el PAG en leche y la calidad de los quesos en vacas con partos de primavera y en comparación con un sistema DTM con baja exposición al ambiente.

ESTRATEGIA DE INVESTIGACIÓN

La siguiente tesis se desarrolla en base a 2 grandes capítulos dentro de los cuales se presentan los objetivos específicos, materiales y métodos, principales resultados y discusión particular de cada uno de ellos. Con respecto a los nombres de los tratamientos, se describirán en español y entre paréntesis se pondrá la nomenclatura tal cual está en los artículos. De esta manera se mantienen las mismas siglas en ambos documentos (tesis y artículo).

El **primer capítulo** se enmarcó en el proyecto ***"Estrategias nutricionales y de manejo para maximizar la calidad de la leche y atenuar el estrés calórico en vacas con partos en primavera"***. El experimento en el cual se enmarcó dicho proyecto fue financiado a través de los siguientes proyectos y fuentes de financiación: 1) *"Sistemas de producción de leche competitivos, sostenibles y simples: el desafío de la lechería uruguaya"* (RTS_1-2014_1_03: Fondo Alianzas ANII-2014) y 2) *"Factores de riesgo e inmunosupresión en el periparto de vacas lecheras: búsqueda de indicadores en sistemas de producción sobre pastoreo controlado"* (FSA_1_2013_1_12442: fondo INNOVAGRO ANII-2013). El mismo fue aprobado por CEUA-FVET (Facultad de Veterinaria CHEA ID149).
Enmarcado en el proyecto *"Estrategias nutricionales y de manejo para maximizar la calidad de la leche y atenuar el estrés calórico en vacas con partos en primavera"* se desarrollaron los objetivos específicos 1 y 2 (Artículos I y II) de esta tesis. Para responder estos objetivos se realizó un experimento en el establecimiento comercial "La Armonía", de la empresa PILI S.A. Del total del rodeo lechero (1300 vacas en ordeñe), fueron seleccionadas 90 vacas multíparas con fecha promedio de parto 15 de setiembre ± 13 días (partos de primavera). Se implementaron tres tratamientos nutricionales y de manejo en función de dos estaciones del año (primavera y verano). Cada tratamiento fue una estrategia diferente para transitar ambas estaciones del año. De la primavera al verano en los SM se realizaron cambios en el manejo de alimentación y ambiente para evaluar diferentes formas de mitigar el estrés calórico. Durante los primeros 3 meses desde el parto se determinó la producción y composición de la leche, variables de comportamiento y metabolitos sanguíneos. Al mes (30 días posparto, dpp) y a los 3 meses (100 dpp) se hizo un muestreo de leche para la evaluación del PAG en leche (Figura 1).

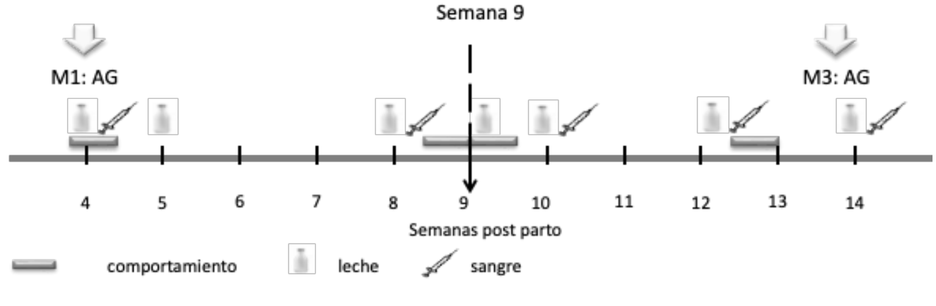

Figura 1: Diseño experimental. Días de evaluación de comportamiento, extracción de muestras de leche (producción y composición) y sangre. Extracción de muestras de leche para perfil de ácidos grasos.

El **segundo capítulo** se enmarcó en el proyecto "¿Cuánto cuesta y cuánto paga el control del ambiente productivo en sistemas comerciales de producción de leche? Análisis a lo largo de la cadena de valor" el que fue financiado por (RTS_1-2014_1_03: Fondo Alianzas ANII-2014). El mismo fue aprobado por CEUA-FAGRO (Facultad de Agronomía, CHEA: ID 682). En este experimento se desarrollaron los objetivos específicos 3 y 4 (Artículo III). Para cumplir con dichos objetivos se seleccionaron 48 vacas (multíparas y primíparas) con partos de primavera. La estrategia de investigación se realizó en 2 etapas siguiendo el diseño experimental mostrado en la Figura 2.

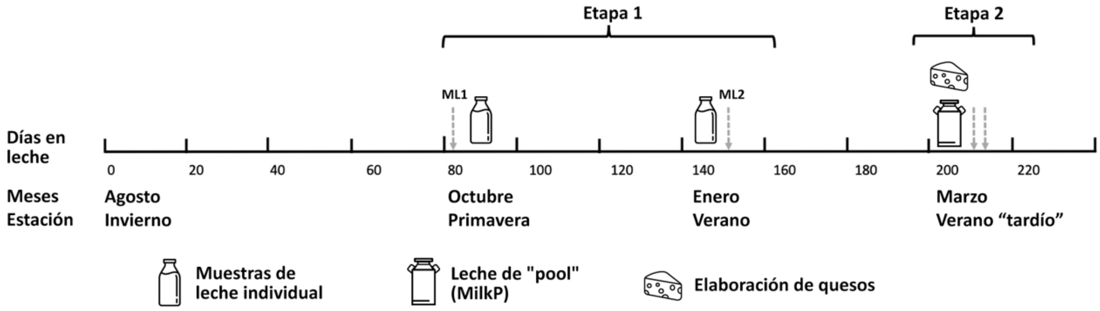

Figura 2: Diseño experimental: momento de extracción de muestras de leche individual, leche de pool (MilkP) y elaboración de quesos. ML1: momento de lactancia (70-90 días post parto:dpp); ML2: momento de lactancia (140-180 dpp).

En una primera etapa (Etapa 1) correspondiente al objetivo específico 3, se evaluó si la alta o baja exposición al ambiente durante la suplementación en los SM afectó el PAG de la leche en dos momentos de la lactancia: ML1: primavera - Octubre (70-90 dpp) y ML2: verano - Enero (140-180 dpp) y comparar ambos SM con vacas que estuvieron siempre con baja exposición al clima (100% DTM) (la unidad experimental es la vaca). En una segunda etapa (Etapa 2) (objetivo específico 4), se evaluó como la alta o baja exposición al ambiente repercutió en el perfil de ácidos grasos de los quesos. Se realizaron elaboraciones de quesos en el verano tardío- Marzo (195-225 dpp). Dichas elaboraciones fueron realizadas a partir de muestras de leche de pool (MilkP) de cada corral (4 corrales) para cada tratamiento (la unidad experimental es el corral). De cada vaca individual se obtuvo el total de leche del ordeño completo, la que fue mezclada y homogeneizada en tanques, obteniendo el total de la leche producida de cada corral (n=4). A partir de la leche homogeneizada de cada tanque, se extrajeron 60 L de leche en bolsas de 20 L, la que fue inmediatamente refrigerada en cámara para luego ser enviada a la planta piloto del LATU (en camión refrigerado a 4°C) para su posterior procesamiento (Figura 3).

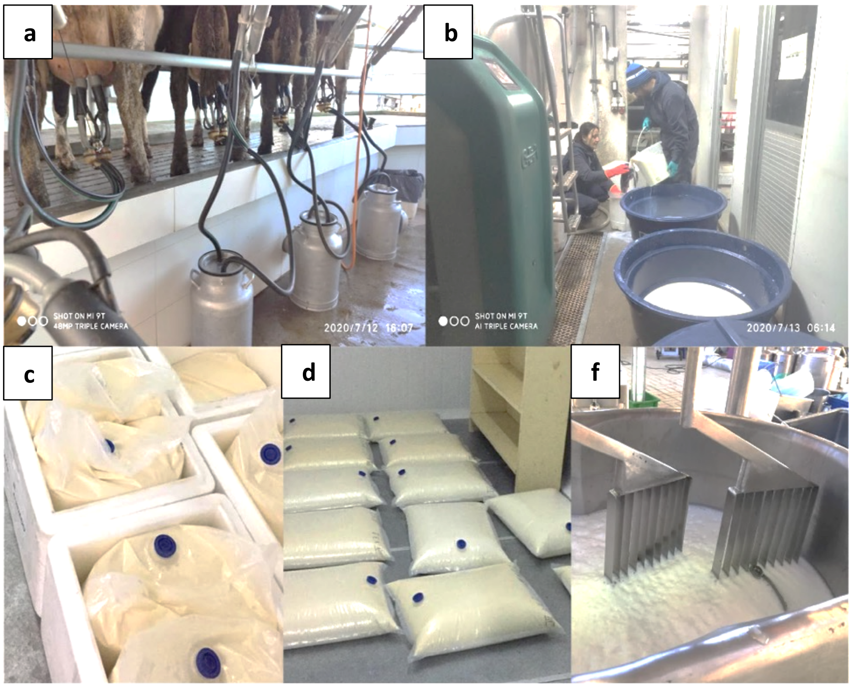

Figura 3: a). Ordeñe completo de cada vaca individual (al tarro); b). Mezcla de leche del ordeño completo de cada vaca individual de cada corral (n=4): leche de pool (MilkP) y llenado de bolsas para almacenamiento y traslado de leche; c-d). Almacenamiento y enfriado de la leche previo al traslado a planta industrial; f. Elaboración de quesos en la planta industrial (tina quesera).

CAPÍTULO 1

El primer capítulo fue desarrollado en base a un único experimento. En esta tesis para un mejor entendimiento de los tratamientos y mejor visualización de los resultados serán presentados en forma separada según los siguientes objetivos específicos:

Objetivos específicos

1. Determinar el efecto del cambio brusco desde un SM (pastoreo + DTM) a un sistema de confinamiento (100% DTM) sobre el comportamiento, bioquímica sanguínea, producción y composición de la leche en vacas de partos de primavera en comparación con un sistema en confinamiento (100% DTM).

2. Determinar el perfil de ácidos grasos de la leche cuando las vacas cambian desde un SM (pastoreo + DTM) a 100% DTM y cuando cambian desde un SM a uno con un solo pastoreo nocturno en verano y en comparación con vacas mantenidas desde el inicio en encierro con 100% DTM

Materiales y métodos - Artículo I

Para cumplir con el **objetivo específico 1** se planteó el siguiente diseño experimental (Figura 1):

Se utilizaron 30 vacas multíparas en dos tratamientos (n=15):
1) GTMR: vacas alimentadas con DTM *ad libitum* durante todo el estudio
2) GCHD: vacas en sistema mixto: doble pastoreo en primavera en el horario de 7:30 a 11:30 y 18:30 a 6:00 h más suplemento con DTM. En verano cambiaron a un sistema de manejo y alimentación igual que el tratamiento GTMR.
Las vacas del GCHD tuvieron acceso a pasturas de Festuca (*Festuca arundinacea*) y Dactylis (*Dactylis perseo*) en los meses de primavera. La asignación de forraje fue de 40 kg MS/vaca/día, en parcelas de ocupación diarias. La proporción de DTM ofrecida como suplemento al GCHD en primavera fue del 25 % de la oferta de DTM del tratamiento 1 (GTMR).
Los corrales donde se encontraban las vacas en confinamiento (encierro total) y donde permanecian las vacas del GCHD en los momentos donde no estaban pastoreando (encierro parcial) tuvieron las siguientes caracteristicas: comederos cubiertos (70 × 3 m) con piso de concreto y techo metálico y un espacio con piso de tierra sin techo pero con un área con sombra (50 × 35 m): corral a “cielo abierto” (Figura 4). Ambos tratamientos se alojaban en el mismo ambiente pero en corrales diferentes (Ver Figura 1 - Artículo I). Para ambos tratamientos la dieta total mezclada (DTM) fue administrada en dichos corrales.

Figura 4: a). Comederos cubiertos con piso de concreto y techo metálico; b). Área con piso de tierra sin techo pero con un espacio de sombra.

Se evaluó comportamiento, producción, composición de la leche y bioquímica sanguínea. Los eventos de comportamiento se evaluaron en forma individual (15 animales por grupo) usando un muestreo de *scan* cada 10 min, mediante tres observadores entrenados, uno para cada grupo, los cuales alternaron entre tratamientos, turnos (am: 08:00 a 11:00 y pm: 12:00 a 15:00) y días. Una vez por mes durante la lactancia se evaluó el comportamiento durante un período de 3 a 6 días, según los meses. Al mes (1M) y a los 3 meses (3M) las evaluaciones fueron realizadas durante 3 días consecutivos. A los 2 meses (2M), momento en el cual se realizó el cambio de estrategia, el comportamiento fue evaluado durante 6 días consecutivos: 3 días antes del cambio (2MB) y 3 días después del cambio (2MA). Los comportamientos registrados fueron: rumiando, echado, caminando, comiendo (pastando o DTM) (Damián et al., 2013). La producción y composición (proteínas totales, grasa, lactosa) de leche se registró semanalmente mediante el equipo LactoScope, que utiliza la metodología de interferograma de transformado Fourier (FTIR), en el laboratorio COLAVECO (Nueva Helvecia-Colonia). Los indicadores bioquímicos séricos (BHB, NEFA, Colesterol, proteínas totales, albúmina, globulina, urea y CK) se determinaron en sangre cada 15 días.

Todas las variables (comportamiento, leche y sangre) fueron analizaron mediante medidas repetidas utilizando el PROC MIXED de SAS (SAS Institute Inc., Cary, NC, EE. UU., 2004). Las variables de comportamiento se analizaron por separado en cada mes (1M, 2M y 3M) y los resultados se presentan como el promedio de 3 días en cada período. En octubre (1M) y diciembre (3M) se consideraron bloque y tratamiento (GTMR o GCHD) como efectos fijos y en noviembre se consideraron bloque, tratamiento, período (GTMR o GCHD), e interacción tratamiento - período (2M-A y 2M-B) como efectos fijos. Los animales en cada tratamiento se consideraron como efecto aleatorio. Los resultados fueron considerados como significativos con un valor de $\alpha \leq 0,05$. Los datos se presentaron como media ± error estándar de la media (eem).

Resultados principales - Artículo I

A continuación, se presentan los principales resultados obtenidos en relación al objetivo específico 1. En cuanto al comportamiento se observó que las vacas del GCHD después del cambio (2M-A) rumiaron (13,8 ± 1,3 %) y se echaron (17,2 ± 1,5 %) con menor frecuencia que antes del cambio (2M-B) (31,3 ± 1,3 % y 23,5 ± 2,1 %, p<0,0001 y p=0,03; respectivamente). A su vez, las vacas del GCHD luego del cambio (2M-A) rumiaron y se echaron incluso con menor frecuencia que las del GTMR en el mismo periodo (19,6 ± 1,3 %; y 26,6 ± 1,5 %, p=0,001 y p=0,02; respectivamente). Al evaluar el comportamiento un mes luego del cambio (GCHD un mes de encierro total), no hubo diferencias en ninguna de las variables, mostrando un patrón de comportamiento similar a las del GTMR. Sin embargo, las del GCHD mantuvieron tendencia a rumiar con menor frecuencia que las del GTMR (p=0,08) al mes luego del cambio.

Con respecto a las variables productivas, en el GTMR la producción de leche (PL) incrementó desde la semana 4 a la 9 (p<0,0001) y disminuyó desde la 9 a la 14 (p<0,0001), mientras que el GCHD incrementó su PL desde la semana 9 a la 10 (p<0,0001), luego del cambio de dieta y se mantuvo constante hasta la semana 14. Una semana previa al cambio, las vacas del GCHD produjeron menos leche, proteína y lactosa (kg/d) en comparación con las del GTMR (p=0,009, p=0,01 and p=0,002; respectivamente), pero luego del cambio de sistema las diferencias entre los grupos desaparecieron (2M-A).

A su vez, estas modificaciones en la producción de leche y sólidos en el GCHD fueron acompañadas por algunos cambios metabólicos, como la disminución en la concentración de BHB (p<0,0001) y AST (p=0,03) desde la semana 4 hasta el final del periodo (semana 14), mientras que en el GTMR los niveles se incrementaron desde la semana 4 a la 8 y se mantuvieron altos hacia el final del experimento. Por otro lado, estos cambios en las vacas del GCHD fueron acompañados por una caída temprana (semana 8) de la concentración del colesterol (p<0,001), en comparación con las vacas del GTMR.

Discusión - Artículo I

Con respecto a los resultados obtenidos correspondientes al objetivo 1 se observó que el cambio de dieta de SM (doble pastoreo + DTM) a un sistema de confinamiento único (100% TMR) en vacas lecheras afectó la producción y composición de la leche, el comportamiento y algunos parámetros metabólicos en sangre. Alrededor de una semana antes del cambio de dieta, las vacas de GCHD produjeron menos leche y menor kg/d de proteína y lactosa que las vacas de GTMR, pero tales diferencias desaparecieron después del cambio de sistema. Estos resultados concuerdan con la información disponible sobre las ventajas del sistema en confinamiento, donde las vacas que consumen dieta DTM producen más leche que las vacas en pastoreo o en SM, lo que se asocia con un aumento en el consumo de materia seca y una mayor disponibilidad de energía (Bargo et al., 2002; Fajardo et al., 2015; Kolver & Muller, 1998; Vibart et al., 2008; Wales et al., 2013). Además, el cambio desde el SM al confinamiento con DTM mejoró la producción y composición de la leche en un corto período de tiempo, lo que se evidenció al observar los cambios desde una semana antes del cambio hasta una semana después. Por otro lado, estos cambios en la producción de leche y composición estuvieron acompañados de algunas modificaciones metabólicas, como una disminución continua en la concentración de BHB y AST hacia el final del período experimental y una caída temprana en la concentración de colesterol, lo que sugiere una menor movilización de reservas corporales y actividad hepática después del cambio de sistema (García et al., 2011; Noro et al., 2013). Aunque las vacas en SM durante el primer mes de lactación sufrieron un mayor balance energético negativo en comparación con las vacas de GTMR [evidenciado por mayores valores de AGNE y menor condición corporal (CC)], no lograron recuperar su CC después del cambio. Esto podría deberse a que durante la lactancia temprana la energía generada por la vaca lechera prioriza la glándula mamaria para la producción de leche en lugar de las reservas corporales (Gross et al., 2011). Por lo tanto, en términos de producción de leche, composición y metabolismo las vacas lecheras responden favorablemente al cambio desde un SM a un confinamiento con DTM. Si bien las vacas del GCHD presentaron una mejora en la PL luego del cambio (dieta 100% DTM y menor desgaste energético), se hubiera esperado que alcanzaran mayores niveles de PL en base a la formulación de la dieta y requerimientos. Las posibles limitantes pudieron ser principalmente el tipo y condiciones del encierro (a cielo abierto) lo que pudieron haber limitado la mitigación del estrés calórico. Por otro lado, las vacas del GCHD (SM al inicio de la lactancia) mostraron un mayor balance energético negativo y menor PL que las vacas del GTMR. Esto pudo haber generado un efecto residual negativo en la PL a lo largo del de la lactancia (Jorgensen et al., 2016; al Ibrahim et al., 2013; Bargo et al., 2002; Fajardo et al., 2015).

Con respecto al comportamiento, se observó que después del cambio (de SM a confinamiento 100% DTM), las vacas del GCHD rumiaban y se echaban con menos frecuencia que antes, e incluso realizaban estos comportamientos con menos frecuencia que las vacas mantenidas siempre en DTM (GTMR). El hecho de que ambos comportamientos (rumiar y echarse) disminuyeran tras el cambio de sistema podría deberse a la falta de acceso a la pastura. Posiblemente, la pérdida de la posibilidad de pastar sea un factor clave en las respuestas de comportamiento evidenciadas por las vacas luego del cambio de sistema y manejo, lo cual coincide con lo reportado por (Arnott et al., 2017; Beauchemin, 2018; Kilgour, 2012; Pollock et al., 2022). Dado que estos comportamientos junto con el pastoreo en la vaca lechera se asocian con un buen bienestar y comodidad (Arnott et al., 2017; Higashiyama et al., 2007; Kilgour, 2012; Tucker et al., 2004), es posible que el cambio de sistema y la dificultad de adaptación durante los primeros días hayan redundado en un peor bienestar. El comportamiento de rumia y estar echada están asociados entre sí, y ocurren cuando las vacas están en buenas condiciones (Schirmann et al., 2012). En condiciones de encierro más cómodas las vacas se echan con más frecuencia (Charlton & Rutter, 2017), lo cual resalta la importancia de estos comportamientos como indicadores de bienestar. Por lo tanto, la reducción de la rumia tras el cambio de sistema puede ser un indicador de respuesta emocional negativa, como fue sugerido por Herskin et al. (2004). En resumen, las vacas expresaron dificultades para adaptarse rápidamente (primeros 3 días) al cambio abrupto desde un SM a un sistema de confinamiento con DTM (GCHD). Incluso, al mes luego del cambio las vacas del GCHD tendieron a rumiar menos, por lo que se evidencia que tuvieron dificultades para adaptarse totalmente al cambio de sistema al menos durante el periodo de un mes. Los resultados coinciden con los de Enriquez-Hidalgo et al. (2018), quienes reportaron que las vacas necesitaron más de 10 días para adaptar su comportamiento al cambio de manejo de un sistema de pastoreo a uno de confinamiento. Por lo tanto, las vacas necesitan una ventana de tiempo de algunas semanas para adaptar su comportamiento a un nuevo entorno después de un cambio abrupto desde un sistema de base pastoril a un sistema de confinamiento. Dado que las vacas lecheras no se adaptan fácilmente a cambios bruscos de sistema, sería interesante evaluar cómo los animales pueden adaptar su comportamiento a cambios graduales en estos sistemas de producción. Por lo que la decisión de confinar (alimentación 100% DTM) a las vacas lecheras en el verano como medida de manejo para disminuir el efecto del estrés calórico, tendría ventajas desde el punto de vista productivo y metabólico, sin embargo, según las variables de comportamiento evaluadas, las vacas tendrían algunas dificultades para adaptarse a los cambios repentinos de sistema.

Materiales y métodos - Artículo II

Para cumplir con el **objetivo específico 2** se utilizaron los dos mismos grupos mencionados en el primer objetivo específico (GTMR y GCHD) pero con el agregado de un tercer grupo (GTMR+P).

A continuación se describen las características de los respectivos grupos:

Se utilizaron 45 vacas multíparas en tres tratamientos (n=15):
1) GTMR: vacas alimentadas con DTM *ad libitum* durante todo el estudio
2) GCHD: vacas en sistema mixto: doble pastoreo en primavera en el horario de 7:30 a 11:30 y 18:30 a 6:00 horas [oferta de 40 kgMS/vaca/d; (4 cm por encima del suelo) y disponibilidad de 1500 a 1700 kgMS/ha], más suplemento con DTM (encierro en los momentos de no pastoreo). En verano cambiaron a un sistema de manejo y alimentación igual que el tratamiento GTMR.
3) GTMR+P con igual manejo que el anterior (2) durante primavera (misma asignación y disponiblidad que GCHD, y en verano un régimen de un pastoreo en el turno vespertino/nocturno en horario de 19:00 a 6:00 h) más suplemento con DTM durante el turno matutino (encierro en los momentos de no pastoreo).
Las vacas del GTMR+P tuvieron acceso a pasturas de Festuca (*Festuca arundinacea*) y Dactylis (*Dactylis perseo*). La asignación de forraje fue la misma que para GCHD (40kgMS/vaca/d).
La proporción de DTM ofrecida como suplemento a ambos SM en primavera fue del 25 % de la oferta de DTM del tratamiento 1 (GTMR), mientras que la suplementación ofrecida al GTMR+P en verano fue 35% de lo ofrecido al GTMR. Las caracteristicas de los corrales donde se administraba la DTM y donde permanecian las vacas mientras no estaban pastoreando eran las mismas que las descriptas para el tratamiento GCHD en el apartado materiales y métodos I.

La producción de leche se registró individualmente con medidores Waikato®. Las muestras de leche fueron recolectadas en dos momentos: al mes de lactancia (M1: semana 4, 35±15 dpp) y a los tres meses de lactancia (M3: semana 13, 98±14 dpp) para composición de leche y PAG (muestra compuesta y representativa de ambos ordeños diarios). La determinación de ácidos grasos se realizó por cromatografía gaseosa en leche, pasturas, ensilaje en el Laboratorio Tecnológico del Uruguay (LATU) (metodología detallada en Artículo II)

Resultados principales - Artículo II

En este apartado se presentan los principales resultados obtenidos a partir del objetivo 2. Las vacas cambiaron desde un sistema mixto (doble pastoreo + DTM) a un encierro total (100% DTM-a cielo abierto) o desde un sistema mixto (doble pastoreo + DTM) a un solo pastoreo nocturno + DTM. Ambos cambios se compararon con vacas que siempre se mantuvieron en confinamiento alimentadas con 100% DTM.

En cuanto a las variables productivas se observó que las vacas que estaban en sistema de confinamiento en M3 (verano), tanto las que cambiaron (GCHD) como las que siempre se mantuvieron en este sistema (GTMR) mostraron mayor producción de leche que las que se mantuvieron en sistema mixto (GTMR+P) ($p=0.04$). En M1 no hubo diferencia entre los tratamientos. En relación a la grasa láctea en M1, GTMR tuvo menor porcentaje de grasa que GCHD y GTMR+P ($p=0{,}0006$) y en M3 GTMR+P tendió a ser mayor que GTMR ($p=0{,}08$), pero no hubo diferencias entre GCHD y los otros tratamientos. Cuando se evaluó el efecto del cambio de manejo en estas variables se observó que las vacas que pasaron desde un sistema mixto a uno en confinamiento en el verano (GCHD) aumentaron la producción de leche ($p=0{,}05$), mientras que las vacas que mantuvieron un pastoreo nocturno (GTMR+P), disminuyeron desde M1 a M3 ($p=0{,}004$). Por otro lado, el GCHD disminuyó el porcentaje de grasa desde M1 a M3 ($p=0{,}0005$), pero GTMR y GTMR+P no mostraron diferencias entre los meses. Sin embargo, la grasa (kg/d) en GCHD y GTMR no cambió entre los meses, mientras que en GTMR+P disminuyó ($p=0{,}0001$).

En cuanto al perfil de ácidos grasos de la leche, cuando las vacas fueron sometidas al cambio de manejo se observó que las vacas que accedieron a un pastoreo nocturno en el verano (GTMR+P) alcanzaron mayor contenido de n-3, C18:2 (CLA) y C18:3 ($p=0{,}02$; $p<0{,}05$) y menor relación n-6/n-3 ($p<0{,}0001$) en comparación con las que cambiaron a un sistema de confinamiento (GCHD). En M1 la relación n-6/n-3 fue mayor en GTMR ($p < 0{,}0001$) en comparación con ambos SM (GCHD y GTMR+P), mientas que no hubo diferencias entre ellos en esta variable. En M3 el GTMR+P (un turno de pastoreo) tuvo la menor relación n-6/n-3 ($p<0{,}0001$) en comparación con GCHD (cambio a estabulación completa) y GTMR, sin diferencias entre ellos (ambos grupos en estabulación). El ácido linoleico conjugado (C18:2) y linolenico [C18:3 (n-3)] no mostraron diferencias entre tratamientos en M1, mientras que en M3 ambos AG fueron menores (%) en GCHD que en GTMR+P ($p<0{,}05$). Al estudiar el PAG de la leche luego del cambio en cada grupo, se observó que en la leche de las vacas del GCHD disminuyó el porcentaje de n-3 desde M1 a M3 ($p=0,0001$), mientras que en GTMR y GTMR+P no cambió entre los meses. La relación n-6/n-3 en GCHD se incrementó ($p<0,0001$) desde M1 a M3, mientras que en GTMR+P se mantuvo sin cambios. En el caso del C18:3 (n-3) y C18:2 (CLA) disminuyeron desde M1 a M3 ($p<0,01$) en ambos grupos (GCHD y GTMR+P), pero en GTMR no hubo diferencias entre los meses.

Discusión - Artículo II

En relación a los resultados obtenidos en base al objetivo 2 se puede afirmar que las vacas en SM, incluso durante el verano y con un solo pastoreo nocturno, tuvieron mayor concentración de CLA y linolénico (n-3) en la leche que las vacas que cambiaron al sistema de confinamiento (GCHD). Estos resultados ratifican que la inclusión de pastura en la dieta [principal fuente de C18 como linolénico (n-3) y AG linoleico; Elgersma, 2015], podría aumentar los intermediarios de AG (VA y RA) y por lo tanto la proporción de CLA en la leche (Bauman & Griinari, 2001; Chilliard et al., 2007). Estos resultados también son consistentes con los de Barca et al. (2018) y Morales-Almaráz et al. (2010), quienes encontraron mayores cantidades de n-3 en sistemas que incluyen pastura en la dieta en comparación con sistemas 100% DTM. Además, la leche de los SM (GCHD y GTMR+P en primavera y GTMR+P en verano) tuvieron menor n-6/n-3 en comparación con las vacas en confinamiento (GCHD en verano y GTMR). En relación a esto, el GCHD luego del cambio aumentó la relación n-6/n-3 a valores considerados inadecuados para el consumo humano (superiores a 4/1), mientras que GTMR+P mantuvo valores adecuados para la salud humana (Simopoulos, 2008). Por lo tanto, este trabajo destaca la importancia de mantener el pastoreo nocturno en SM durante el verano, ya que conduce a un PAG considerado beneficioso para la salud humana, como un alto contenido de n-3 y C18:2 (CLA), y baja relación n-6/n-3 (Elgersma, 2015; Ruxton et al., 2004).

CAPÍTULO 2

Objetivos específicos

3. Comparar el perfil de ácidos grasos en leche entre SM con diferentes condiciones ambientales (alta y baja exposición al ambiente) durante el encierro, en comparación con un sistema 100% estabulado con baja exposición al ambiente, de vacas con partos de primavera.

4. Evaluar como las diferentes condiciones ambientales (alta y baja exposición al ambiente) durante el encierro en SM repercute en el perfil de ácidos grasos de leche de pool (MilkP) y quesos y compararlos con los obtenidos de un sistema 100% estabulado con baja exposición al ambiente, en vacas con partos de primavera

Materiales y métodos – Artículo III

El estudio se realizó en la Plataforma Experimental de Lechería de la Estación Experimental Dr. Mario A. Cassinoni, Facultad de Agronomía, Paysandú. Desde un rodeo de 100 vacas, se utilizaron 48 vacas con partos de primavera 2019, las que se evaluaron hasta los 300 días de lactación.
Fueron constituidos, luego del parto, 3 tratamientos de 16 vacas cada uno, las cuales se distribuyeron en 4 corrales, cada uno con 4 vacas.

Los tratamientos conformados fueron los siguientes:
CB-TMR: Sistema confinamiento con alimentación 100% DTM, en establo con cama caliente (Baja exposición al ambiente)
CB-GRZ: Sistema mixto: pastoril intensivo más DTM, en encierro en establo cama caliente durante la suplementación (Baja exposición al ambiente)
OD-GRZ: Sistema mixto: pastoril intensivo más DTM, en encierro a cielo abierto durante la suplementación (Alta exposición al ambiente).
En los SM la pastura y la DTM se consideraron alimentos balanceados nutricionalmente y la DTM se utilizó como complemento de la pastura para lograr el consumo deseado de materia seca (MS). El horario de pastoreo en los SM desde octubre (31/10) hasta marzo fue de 17:30 a 3:30 h (pastoreo nocturno).
La pastura ofrecida para ambos grupos (GRZ) estaba compuesta por Festuca (*Festuca arundinacea*) en Octubre-Primavera (ML1) con una asignación de 28kgMS/vaca/d. En enero-Verano (ML2) las vacas accedieron a pastoreo de Soja (*Glycine max*) con una asignación de 46 kgMS/vaca/d. Al momento de la elaboración de quesos (marzo-verano tardío) la pastura ofrecida fue alfalfa y dactylis con una asignación de 31 kgMS/vaca/d. Las vacas pastoreaban en una parcela con ocupación de 7 días. El sistema de pastoreo tuvo una carga anual de 2,5 vacas/hectarea de pastoreo.
En CB-TMR y CB-GRZ las vacas fueron confinadas en un establo completamente techado con cama de compost (CB), ventiladores y aspersores (activados cuando la temperatura supera los 25°C), protegidas de la radiación solar, lluvia, barro y viento (exposición ambiental baja). Cada corral tenía 6 m de ancho por 13,5 m de largo (9 m de

cama y 4,5 m de espacio de piso de cemento). El área con cama de compost tenía una superficie de 13,5 m2/vaca y el área con piso de cemento de 6,7m2/vaca (Figura 5, a y b). En el tratamiento OD-GRZ, las vacas se confinaron en corrales al aire libre "cielo abierto" con piso de tierra y sombra. Los corrales a cielo abierto (OD) tenían 46 m de largo por 10,4 de ancho. Estaban compuestos por 2 potreros cada uno (que se alternaban según las condiciones de barro) con las siguientes medidas cada uno: 5,2 m de ancho por 46 m de largo (área sombreada: 4,8 m de ancho por 4 m de largo). El agua se dispuso *ad libitum* para todos los tratamientos (Figura 5, c).

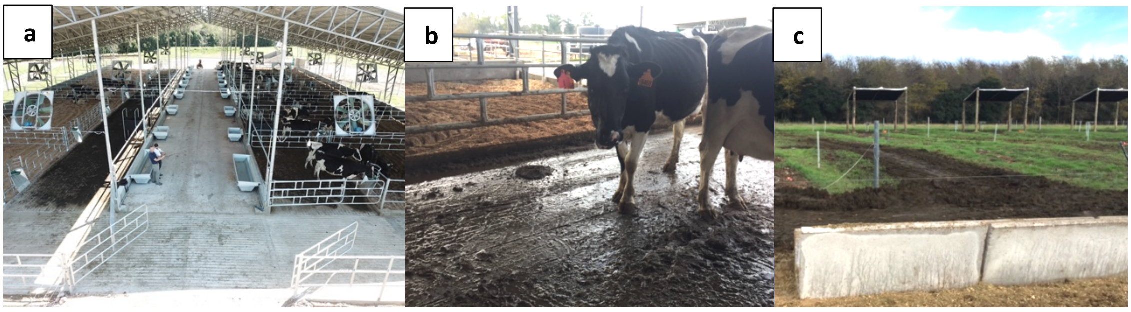

Figura 5: a). Galpón con corrales de cama de compost, ventiladores y aspersores (CB); b). Vacas en el corral con piso de cemento y cama de compost; c). Corrales a cielo abierto (OD).

Para cumplir con el **objetivo específico 3** se realizaó un experimento en 2 etapas. En la primera etapa se extrajeron muestras individuales de leche en primavera y verano. Todas las vacas fueron ordeñadas 2 veces al día (5:00 am y 3:00 pm) y la producción de leche fue registrada individualmente. En 2 momentos específicos de la lactancia: ML1: 80 ± 15 dias post parto (dpp) (Octubre: primavera) y ML2: 155 ± 15 dpp (Enero: verano) fueron colectadas muestras de leche individuales (muestras compuestas representativas de ambos ordeñes: am y pm), muestras de dieta totalmente mezclada (DTM) y pastura. La producción de leche individual fue registrada diariamente en cada turno (am – pm) por el programa "dairy plan" (GEA). A partir de estos datos se calculó la producción en kg/d. De las muestras obtenidas se analizó el contenido y rendimiento de grasa total (% y kg/d) mediante LactoScope FT infrarrojo (FTIR) (Delta Instruments, Drachten the Netherlands) en COLAVECO in Colonia Suiza, Uruguay. La metodología para la determinación del PAG en leche fue detallada en el capítulo 1- Artículo II.

Para cumplir con el **objetivo específico 4** a partir del diseño experimental descripto para el objetivo 3, en una segunda etapa se realizaron elaboraciones de quesos tipo Dambo (Figura 2). Este tipo de queso está clasificado dentro de los quesos de pasta semidura (MSP, 1994). El diseño experimental consistió en realizar elaboraciones de quesos a partir de las muestras de leche obtenidas de 2 corrales por cada tratamiento, y en 2 semanas consecutivas, constituyendo el total de los 4 corrales de cada tratamiento (Unidad experimental=corral). En este proyecto se siguió una metodología similar a la utilizada por otros trabajos que han evaluado efectos del sistema de producción sobre las características del queso (Bonanno et al., 2013; Esposito et al., 2014; O'Callaghan et al., 2017). A diferencia de los trabajos antes mencionados en el diseño para este proyecto se utilizaron mayor número de unidades experimentales (n=4). Cada elaboración se realizó con muestras de leche del corral "leche de pool" (MilkP) obtenidas del ordeñe completo de la mañana y de la tarde (am y pm) (4 elaboraciones por tratamiento). Previa homogenización, se extrajo la cantidad de 60 L, los que fueron mantenidos a 4°C hasta su posterior traslado a la planta piloto del LATU, donde se realizaron las elaboraciones (el transporte se realizó desde Paysandú a Montevideo en camión refrigerado a 4°C). Al total de la leche recibida en planta (360 L), en el día 1 se la sometió a un proceso de pasteurización HTST (high temperature-short time: 72°C durante 15 seg). Las elaboraciones se realizaron de acuerdo con el siguiente esquema de elaboración (Tabla 1).

Tabla 1: Diseño experimental de las elaboraciones Queso. CB: alto control ambiente; OD: bajo control ambiente; TMR: dieta total mezclada; GRZ: sistema mixto (pastura más TMR).

Día	Semana 1		Semana 2	
	tratamiento	corral	tratamiento	corral
1	CB-TMR	1	CB-TMR	3
1	CB-GRZ	1	CB-GRZ	3
1	OD-GRZ	1	OD-GRZ	3
2	CB-TMR	2	CB-TMR	4
2	CB-GRZ	2	CB-GRZ	4
2	OD-GRZ	2	OD-GRZ	4

En cada semana se realizaron 6 elaboraciones (12 en total) y el diseño se bloqueó por semana. Se determinó el perfil de ácidos grasos en la leche estandarizada (previo a la elaboración) y en los quesos luego de 30 días de maduración.

La producción de leche (kg/d), grasa (kg/d) y porcentaje de grasa (%) y perfil de ácidos grasos (PAG) (%) se analizaron con un ANOVA para medidas repetidas utilizando el GLIMMIX de SAS (SAS Studio). El modelo estadístico para producción de leche, grasa (producción y porcentaje) y PAG incluyó como efectos fijos el tratamiento (CB-TMR, CB-GRZ y OD-GRZ), momentos de lactancia (ML1y ML2) e interacción entre tratamiento y momento de lactancia, la vaca se consideró como un efecto aleatorio dentro de cada tratamiento. Para datos individuales de leche (objetivo 3), la unidad experimental considerada fue la vaca, mientras que para MilkP y queso (objetivo 4) se consideró como unidad experimental el corral y el tratamiento como efecto fijo. Para el objetivo 4, la semana fue considerada como efecto aleatorio. En todas las variables se incluyeron como co-variable los días post parto (dpp). Las comparaciones post hoc se realizaron con la prueba de Tukey-Kramer. Para una mejor visualización de los resultados, cuando se analiza el conjunto de datos de ambos períodos (ML1 y ML2) en cada tratamiento, se expresan como la media del tratamiento (AT). El conjunto de datos analizados de los tres tratamientos (CB-TMR, CB-GRZ y OD-GRZ) en cada momento de lactancia (ML) se expresa como el promedio de ambos momentos de lactación (AML), ya sea para el momento de lactancia 1 (AML1) o ML2 (AML2). Las diferencias se consideraron significativas con un $\alpha \leq 0,05$ y tendencia cuando el valor de α se encontraba entre 0,05 y 0,10. Los datos se presentan como media ± eem

Resultados principales- Artículo III

En este capítulo se presentan los principales resultados correspondientes a los objetivos 3 y 4. Con respecto al objetivo 3, en leche individual las vacas de ambos SM (CB-GRZ y OD-GRZ) tuvieron mayor porcentaje de UFA, MUFA (p<0,001), C18:2 y C18:3 AG (p<0,0001) y menor SFA, relación n-6/n-3, índice aterogénico y trombocitogénico (AI y TI) (p<0,001) que las del sistema estabulado en cama de compost (CB-TMR). Los principales cambios en SFA fueron debidos a C4:0; C6:0; C8:0; C10:0 y C12:0, los cuales fueron más altos en CB-TMR que en CB-GRZ y OD-GRZ. En relación a los momentos de lactación, en ML1 (primavera) el porcentaje de n-3 en CB-GRZ fue mayor que en CB-TMR (p=0,02), mientras que OD-GRZ no mostró diferencias con CB-TMR. En ML2 (verano), las vacas del CB-GRZ tuvieron mayor porcentaje de n-3 que las del CB-TMR (p=0,04). En ambos ML, la leche del CB-TMR tuvo mayor porcentaje de n-6 que los SM (CB-GRZ y OD-GRZ; p<0,0001, p=0,001). Cuando se evaluó el efecto del ambiente durante el confinamiento en los SM se observó que las vacas del CB-GRZ tuvieron mayor porcentaje de n-3 y C18:3 que las del OD-GRZ (p=0,005 y p=0,001). Al comparar entre estos sistemas en ambos ML, se observó que el CB-GRZ tuvo mayor porcentaje de n-3 que OD-GRZ (p=0,004) en ML1 (primavera), mientras que en ML2 no hubo diferencias entre ambos sistemas. No se encontraron diferencias entre los SM en ninguno de los otros AG estudiados. Por otro lado, ambos SM (CB-GRZ y OD-GRZ) presentaron mayor proporción de AG de origen mixto (C16:0 y C16:1) en ML2 con respecto a ML1 (p=0,002 y p=0,0007; respectivamente), de los cuales el C16:0 fue el que predominó en esas diferencias entre ambos ML para los SM (p=0,002 y p=0,004, respectivamente).

En una segunda etapa (objetivo 4) se analizó como las diferentes condiciones ambientales durante el encierro en los SM afectaron la calidad de la grasa en MilkP, su repercusión en los quesos y a su vez se compararon ambos grupos con el sistema CB-TMR. La leche de MilkP y quesos del tratamiento CB-TMR tuvo mayor porcentaje de n-6 y relación n-6/n-3 que CB-GRZ y OD-GRZ (p<0,01). En MilkP el porcentaje de n-3 fue menor en CB-TMR que en CB-GRZ y OD-GRZ (p<0,0001), pero en quesos si bien en CB-TMR el porcentaje de n-3 fue menor que CB-GRZ no hubo diferencias con el OD-GRZ (p=0.03). No hubo diferencias en ningún AG entre ambos sistemas mixtos, tanto en MilkP como en los quesos.

Discusión – Artículo III

Los resultados confirman que tanto la leche como los quesos obtenidos de SM presentan un PAG más saludable para el consumo humano en comparación con vacas en confinamiento en acuerdo con (Alothman et al., 2019; Hirigoyen et al., 2018; Maniaci et al., 2021; O'Callaghan et al., 2016, 2017). Estas diferencias en el PAG entre SM y CB-TMR podría deberse al mayor aporte de precursores de AG en leche en los SM, como C18:2 y C18:3, los cuales son proporcionados por la pastura (Elgersma, 2015; Moscovici Joubran et al., 2021).

Por otro lado, en los SM con diferente exposición al ambiente durante el confinamiento se observó que las vacas que tuvieron menor exposición (CB-GRZ) lograron mejor calidad de la grasa láctea (mayor porcentaje de n-3 y C18:3) en comparación con las vacas con alta exposición (OD-GRZ). Sin embargo, en verano no fueron observadas diferencias entre los tratamientos en leche individual, MilkP, ni quesos. En primavera (ML1), cuando las vacas estuvieron expuestas a la lluvia y barro durante el encierro (OD-GRZ), se observó un impacto negativo en el PAG de la leche (menor contenido de n-3) en comparación con CB-GRZ. Como fue evidenciado por Pons et al. (2021) y Pons (2022), (datos relevados en este mismo experimento), los corrales de OD-GRZ presentaron altos niveles de barro en toda la superficie en el momento que coincide con el muestreo de leche en primavera (ML1). Debido a las malas condiciones ambientales, se puede asumir que las vacas disminuyeron el tiempo que se encontraban echadas durante el encierro en OD-GRZ compensando el mismo durante el pastoreo. Esto se sustenta en los datos también hallados en este mismo experimento, que cuando ocurrieron condiciones extremas (lluvia acumulada) las vacas del OD-GRZ se echaron con menor frecuencia durante el encierro y lo hicieron con mayor frecuencia en el pastoreo en comparación con las vacas del CB-GRZ (Pons, 2022). La implicancia de las malas condiciones de los encierros a cielo abierto (ej: exceso de barro por lluvias) sobre las variables de comportamiento (disminución de la frecuencia de rumia y echado) coincide con lo reportado en estudios previos (Cook et al., 2005; Krawczel et al., 2009) e incluso con lo observado en el primer objetivo de esta tesis. En base a lo anteriormente mencionado y en relación a los resultados, se puede especular que el cambio de estrategia de las vacas del OD-GRZ (por las malas condiciones del encierro) pudo disminuir el consumo de pastura (Cooper et al., 2007; Schütz et al., 2019) lo que repercutió en menor contenido de n-3 en la leche, en comparación con CB-GRZ en primavera.

Contrariamente a la hipótesis original, no se encontraron diferencias en PAG en leche entre ambos SM durante el verano (ML2), lo que podría deberse o explicarse por algunas razones. En primer lugar, no hubo ITH altos, como sería de esperar en la región en esta temporada, ni precipitaciones abundantes durante los días previos al muestreo de leche individual (ML2) (ITH = 67; promedio 10 días previos). En segundo lugar, las instalaciones de OD-GRZ contaban con suficiente sombra por animal y agua *ad libitum*, lo que podría haber contribuido a mitigar los efectos negativos del verano en OD-GRZ, tal como fue reportado por Román et al., (2019), sin repercusiones negativas en el PAG. Es importante destacar que si bien el tratamiento OD-GRZ intentó simular las condiciones de los SM a cielo abierto del país, tenía características de infraestructura que ofrecía mejores condiciones para el confort de la vaca, en comparación a muchos de los encierros a cielo abierto de los sistemas lecheros del país. Cada potrero tenía un espacio de sombra por vaca de 4,8m^2 (techo de nylon a una altura de 4,5 metros); bebederos debajo del techo con agua fresca *ad libitum* y comederos de concreto con un área de 1,3 m/vaca. Además, cada corral estaba dividido en 2 sub corrales, que se alternaban cuando las condiciones del piso (exceso de barro) no eran las adecuadas. Por lo que, la buena gestión de la infraestructura, tareas de mantenimiento de áreas de comedero y descanso, buena sombra y agua disponible *ad libitum*, pudo haber atenuado los efectos negativos de dicha exposición. Con base en todo lo anterior, bajo las condiciones realizadas en este estudio y las particularidades ambientales del encierro a cielo abierto, los efectos negativos del estrés calórico en las vacas OD-GRZ probablemente no fueron tan extremas, no reflejándose cambios en el PAG de la leche en verano.

En relación a las diferencias en el PAG en MilkP y queso entre los SM (objetivo 4), se hipotetizaba que si las vacas del OD-GRZ sufrían más estrés por calor durante el verano (por mayor exposición al clima durante el encierro), esta mayor respuesta de estrés tendría un impacto negativo no solo en la leche, sino también en el producto final, el queso. Sin embargo, en línea con lo mencionado anteriormente para leche individual (objetivo 3), la mayor exposición al ambiente durante el encierro en SM en verano tampoco afectó el PAG en MilkP, ni en queso. Probablemente, como se comentó para el objetivo 3, las buenas condiciones del confinamiento a cielo abierto y las características del ambiente en los momentos cercanos al muestreo, no generaron grandes cambios en los animales que afectaran la calidad de la leche y queso. En relación a esto, cuando se tomaron las muestras de MilkP para las elaboraciones de quesos, los valores de ITH no fueron considerados “severos” [fluctuaron entre 71 a 62; (Bernabucci et al., 2014; Kic, 2022)]. Estas fluctuaciones en valores considerados “no críticos” pudo haber mitigado el estrés calórico en las vacas y por lo tanto no haberse afectado el PAG en los quesos de los diferentes SM. A su vez, otra posible explicación podría ser que los quesos fueron elaborados al final de la lactancia, y según lo reportado por Román et al. (2019), el estrés calórico en esta etapa de la lactación, tendría menor efecto sobre los sólidos en comparación con el inicio de la lactancia. Por lo tanto, el PAG en leche, MilkP y quesos no difirió en el verano, lo cual pudo estar influenciado principalmente por el buen manejo y las condiciones de las instalaciones de OD-GRZ a pesar de ser un encierro a cielo abierto.

DISCUSIÓN GENERAL

Las medidas de manejo que se realizan en el verano en los sistemas lecheros son claves para mitigar los efectos del estrés por calor que sufren principalmente las vacas en los SM con partos de primavera (lactancia temprana - media). Por lo tanto, en el primer capítulo se evaluó cómo las vacas se adaptan a los cambios desde un manejo de SM (doble pastoreo) a un encierro total (100% DTM) con cielo abierto (objetivo específico 1) o a un SM con un solo pastoreo nocturno (objetivo específico 2), ambos en comparación con vacas en encierro permanente a cielo abierto (GTMR).

En el primer objetivo (Artículo I) se pudo demostrar que el cambio desde SM (con doble pastoreo) hacia un encierro total a cielo abierto, fue positivo desde el punto de vista de la producción y metabolismo, pero mostraron cambios negativos en el comportamiento, lo que sugiere que las vacas tuvieron dificultades en adaptarse a los cambios abruptos del sistema en el corto periodo de tiempo. En tal sentido, este trabajo resalta que aunque el cambio desde un SM a un encierro 100% DTM durante el verano presentó algunas ventajas en las variables productivas y metabólicas, estos indicadores no necesariamente implican mejoras en el bienestar. De hecho, algunos comportamientos se afectaron negativamente, lo que permitió inferir problemas en el bienestar de las vacas, al menos en los primeros días luego del cambio. Estas dificultades de adaptación de las vacas observadas en el GCHD (cuando cambiaron al encierro total), también podrían ser causa de las condiciones de la infraestructura del encierro. Dado que al tratarse de un encierro a cielo abierto, la exposición a las condiciones ambientales en este tipo de instalaciones (lluvia, barro, radiación solar) pudieron generar algunas dificultades cuando las vacas dejaron de acceder a la pastura.

Con respecto al GTMR (Capítulo 1), también se podría especular que este tipo de encierro tuvo algunas limitantes, no siendo del todo eficiente en la mitigación del estrés calórico en el verano. Esto se podría sustentar en que la PL comenzó a disminuir a partir de la semana 9 lo que coincidió con los momentos que el ITH empezó a aumentar (Artículo I). Dado que en este sistema las otras condiciones: alimentación (DTM) y el lugar donde se encontraban las vacas (confinamiento a cielo abierto) no fueron cambiadas, se podría inferir que las vacas pudieron estar afectadas por el estrés calórico, lo que pudo haber repercutido negativamente en la PL. Esto coincide con lo reportado por Becker et al. (2020) y Kumar et al. (2020), quienes afirman que el estrés calórico afecta la PL y sólidos.

En base a la información generada en el primer Capítulo, en el segundo Capítulo se implementó el sistema de encierro en cama de compost, con el objetivo de lograr un mejor control del ambiente y alimentación y una menor exposición de las vacas al clima exterior (baja exposición) durante el encierro (tanto para el encierro total: CB-TMR como para el sistema mixto: CB-GRZ). En relación a esto último, es claro que las condiciones de la infraestructura de los encierros en los sistemas lecheros son clave para lograr mitigar los efectos negativos de las condiciones climáticas (sobre todo en verano) en las vacas lecheras. Al mejorar estas condiciones, las vacas logran mejor bienestar lo que incide en las variables respuestas de interés para los productores e industria (PL, sólidos, calidad de leche).

Existen extensos reportes que han demostrado que los sistemas en confinamiento (encierros totales 100% DTM), presentan desventajas comparativas en relación a los SM. Estas desventajas han sido enfocadas básicamente en que poseen mayores costos de producción (Fariña & Chilibroste, 2019), peores condiciones de bienestar (Arnott et al., 2017) y menor calidad de leche, principalmente relacionada a la calidad de la grasa (Alothman et al., 2019; Barca et al., 2018; Mendoza et al., 2016; Pastorini et al., 2019). Es por esto que para el segundo objetivo se ensayó como medida de manejo, mantener un SM con un solo pastoreo nocturno en el verano (GTMR+P) con el fin de maximizar las ventajas de los sistemas pastoriles (en comparación con el cambio de SM a encierro total realizado en el primer objetivo). Como era de esperar, contar con presencia de pastura en la dieta en las vacas, resultó en un PAG más saludable en comparación con las estuvieron siempre confinadas tanto en sistemas a cielo abierto (GTMR) como con las que cambiaron de SM a confinamiento en el verano (GCHD; Artículo II). Está misma tendencia fue observada al comparar SM con pastoreo nocturno en el verano (CB-GRZ y OD-GRZ) vs. vacas encerradas en sistema con cama de compost (CB-TMR, artículo III). En concordancia con lo anterior, se pudo demostrar que los quesos elaborados de SM (CB-GRZ o OD-GRZ) presentaron mejor calidad de la grasa láctea en comparación con vacas en encierro total (CB-TMR), coincidiendo con Esposito et al. (2014). Por lo tanto, según estos resultados, el SM con pastoreo nocturno sería una buena alternativa de manejo en el verano por presentar mayor proporción de AG considerados saludables para la salud humana en comparación con vacas en confinamiento, tanto en encierros a cielo abierto (GTMR)- Capítulo 1 como en cama de compost (CB-TMR)- Capítulo 2. Esta mejor calidad de la grasa láctea de los SM estaría determinada por mayor concentración de MUFA, PUFA, C18:2 (CLA) y C18:3 (n-3), menor proporción de SFA y n-6/n-3, así como menor AI y TI (Ferlay et al., 2017; Mollica et al., 2021) en comparación con CB-TMR.

Como se comentó anteriormente el tipo y estado de la infraestructura en los sistemas lecheros repercute en el bienestar animal, en las variables productivas y en la calidad de leche. En relación a esto y en base a la importancia que tienen los SM en Uruguay, se evaluó cómo la infraestructura de los encierros en SM (encierros parciales en sistemas intensivos con alta carga animal) afectaría la calidad de la leche y quesos. En este sentido, en nuestro trabajo sugerimos que la menor proporción de n-3 en leche que presentaron las vacas del OD-GRZ en comparación con CB-GRZ en ML1 (primavera) pudo deberse a que las condiciones climáticas (lluvias) empeoraron las condiciones del corral a cielo abierto (alta exposición). En consecuencia, las vacas debieron cambiar su estrategia de comportamiento (meno echadas en el corral compensando ese comportamiento en la pastura), lo que pudo afectar el consumo de pasto y por lo tanto el PAG de la leche (menor n-3 en leche que el CB-GRZ). Esto coincide con lo reportado por Tucker et al. (2021), quienes reportan que si las vacas están en condiciones desfavorables cuando no están pastoreando, al momento del pastoreo sacrifican tiempo de comiendo a favor del echado, dado que prefieren descansar que comer. Sin embargo, en verano, no se observaron diferencias en ningún AG, ni en leche individual, MilkP, ni quesos (Artículo III). En leche, estos resultados podrían explicarse en primer lugar por las buenas condiciones de las instalaciones en los corrales del OD-GRZ a pesar de ser un encierro a cielo abierto. Además, en los días previos a la extracción de leche en ML2 no se registraron condiciones climáticas extremas (ITH por encima de 72). Con respecto a los quesos, además de las condiciones de los corrales, se suma el hecho que fueron elaborados en lactancia tardía. Por lo tanto, si bien la lluvia durante la primavera afectó el PAG de la leche de forma negativa, en el verano no hubo diferencias entre los SM. Por lo que se podría pensar que esta infraestructura de los encierros a cielo abierto (agua *ad libitum,* sombra y una buena gestión de los corrales) permitió a las vacas transitar el verano sin repercusiones en la calidad de la grasa láctea en leche y quesos, no así cuando se presentaron precipitaciones que afectaron negativamente la infraestructura de los corrales (barro).

Implicancias

Es claro que los sistemas en confinamiento (100% DTM) tienen ventajas desde el punto de vista de la producción de leche (cantidad de litros y sólidos), pero presentan un PAG menos saludable para los consumidores, en comparación con los sistemas con pastoreo. Esto se observa tanto en leche como en los quesos. En base a los resultados de este proyecto, mantener un pastoreo nocturno como medida de manejo en los sistemas lecheros en vacas con partos de primavera en verano, fue una mejor alternativa en comparación con el cambio de SM (doble pastoreo + DTM) hacia un encierro total a cielo abierto (confinamiento 100% DTM). El SM con un único pastoreo nocturno mantuvo las ventajas de los SM como calidad de leche superior (perfil de ácidos grasos más saludable) (comprobado en este trabajo), además de mejor bienestar para las vacas y menores costos productivos, como ha sido ampliamente reportado (Charlton y Rutter, 2017; Arnott, 2016). Si bien en esta tesis no se evaluó el bienestar en el GTMR+P en el verano, se puede afirmar que las vacas que cambian a encierro total sufren algunos problemas de bienestar en el corto periodo de tiempo. Por lo tanto, en situaciones en que sea necesario la estabulación completa a cielo abierto, deberán tenerse en cuenta las instalaciones para garantizar el descanso de las vacas y en consecuencia un adecuado bienestar.

Una de las desventajas de los SM, es la exposición a condiciones meteorológicas que son sometidos los animales, sobre todo cuando estas son extremas (olas de calor, precipitaciones copiosas y formación de barro). Las condiciones de las instalaciones donde se encuentran las vacas cuando no están pastoreando en SM (encierros parciales) son claves para mitigar los efectos del clima. En el caso de las vacas con partos de primavera esas instalaciones deben fundamentalmente atenuar el efecto del estrés calórico al que están propensas y por lo tanto disminuir las repercusiones negativas en las variables productivas, bienestar y calidad de leche. Las condiciones a cielo abierto de este ensayo (OD-GRZ) resultaron en un PAG en leche y quesos saludables (sin diferencias con el encierro controlado: CB-GRZ) y sin repercusiones negativas en el verano tanto en leche como en quesos. Esto sugiere que mantener SM con un solo pastoreo nocturno en el verano, con encierros a cielo abierto podría ser una buena alternativa en relación a la calidad de la grasa láctea de leche y quesos. Esta afirmación se sostiene siempre y cuando las instalaciones estén correctamente dimensionadas (agua *ad libitum*, sombra suficiente por animal) y se realice un buen manejo del corral (condiciones de suelo, acceso a comederos).

CONCLUSIONES

1. El cambio de manejo de SM a encierro total (100% DTM) de forma abrupta fue favorable en las variables productivas y fisiológicas, como producción y composición de la leche y metabólicas. Sin embargo, en relación al comportamiento, las vacas expresaron dificultades en adaptarse rápidamente a los cambios en un corto periodo de tiempo. A su vez, este cambio de sistema, tuvo efectos negativos en el PAG (disminución del contenido de AG saludables). Por otro lado, se concluye que mantener un pastoreo nocturno en el verano fue favorable en el PAG en comparación con el grupo que cambió a encierro total. Por lo tanto, mantener SM con pastoreo nocturno podría ser una mejor alternativa de manejo en el verano, para mantener una calidad de grasa láctea saludable desde el punto de vista de los consumidores en comparación con el cambio a confinamiento total.

2. Los cambios más importantes en el PAG de la leche, MilkP y quesos fueron debidos a la presencia de pastura en la dieta más que por las condiciones de las instalaciones durante el encierro en SM, sobre todo en verano. Esto fue evidenciado por mayor contenido en AG insaturados (principalmente monoinsaturados), menor contenido de AG saturados y menor relación n-6/n-3 en leche en vacas de SM en comparación con CB-TMR. Esta misma tendencia se observó en MilkP y quesos (mayor n-3 y menor n-6 y relación n-6/n-3) en SM en comparación con CB-TMR. Con respecto a las condiciones durante el encierro en los SM, la leche de las vacas del CB-GRZ presentaron altos porcentajes de n-3 y C18:3 (benéficos para el consumo humano) en comparación con OD-GRZ, aunque no se observó efecto de las condiciones del encierro en MilkP ni en quesos en los SM.

REFERENCIAS BIBLIOGRÁFICAS

Agabriel, C., Martin, B., Sibra, C., Bonnefoy, J. C., Montel, M. C., Didienne, R., & Hulin, S. (2004). Effect of dairy production systems on the sensory characteristics of Cantal cheeses: A plant-scale study. *Animal Research*, *53*(3), 221–234. https://doi.org/10.1051/animres:2004013

Al Ibrahim, R. M., Whelan, S. J., Pierce, K. M., Campion, D. P., Gath, V. P., & Mulligan, F. J. (2013). Effect of timing of post-partum introduction to pasture and supplementation with Saccharomyces cerevisiae on milk production, metabolic status, energy balance and some reproductive parameters in early lactation dairy cows. *Journal of Animal Physiology and Animal Nutrition*, *97*(1), 105–114. https://doi.org/10.1111/jpn.12048

Allen, J. D., Hall, L. W., Collier, R. J., & Smith, J. F. (2015). Effect of core body temperature, time of day, and climate conditions on behavioral patterns of lactating dairy cows experiencing mild to moderate heat stress. *Journal of Dairy Science*, *98*(1), 118–127. https://doi.org/10.3168/jds.2013-7704

Alothman, M., Hogan, S. A., Hennessy, D., Dillon, P., Kilcawley, K. N., O'Donovan, M., Tobin, J., Fenelon, M. A., & O'Callaghan, T. F. (2019). The "grass-fed" milk story: Understanding the impact of pasture feeding on the composition and quality of bovine milk. *Foods*, *8*(8). https://doi.org/10.3390/foods8080350

Arnott, G., Ferris, C. P., & O'connell, N. E. (2017). Review: welfare of dairy cows in continuously housed and pasture-based production systems. *Animal*, *11*(2), 261–273. https://doi.org/10.1017/S1751731116001336

Artegoitia, V., Meikle, A., Olazabal, L., Damián, J. P., Adrien, M. L., Mattiauda, D. A., Bermudez, J., Torre, A., & Carriquiry, M. (2013). Milk casein and fatty acid fractions in early lactation are affected by nutritional regulation of body condition score at the beginning of the transition period in primiparous and multiparous cows under grazing conditions. *Journal of Animal Physiology and Animal Nutrition*, *97*(5), 919–932. https://doi.org/10.1111/j.1439-0396.2012.01338.x

Astessiano, A. L., Meikle, A., Chilibroste, P., Mattiauda, D. A., Fajardo, M., & Carriquiry, M. (2017). Metabolic Adaptations Due to the Inclusion of Pasture in the Diet of Dairy Cows Fed Total Mixed Ration during Early Lactation. *Open Journal of Animal Sciences*, *07*(02), 127–140. https://doi.org/10.4236/ojas.2017.72011

Astrup, A., Magkos, F., Bier, D. M., Brenna, J. T., de Oliveira Otto, M. C., Hill, J. O., King, J. C., Mente, A., Ordovas, J. M., Volek, J. S., Yusuf, S., & Krauss, R. M. (2020). Saturated Fats and Health: A Reassessment and Proposal for Food-Based Recommendations: JACC State-of-the-Art Review. *Journal of the American College of Cardiology*, *76*(7), 844–857. https://doi.org/10.1016/j.jacc.2020.05.077

Bainbridge, M. L., Cersosimo, L. M., Wright, A. D. G., & Kraft, J. (2016). Content and composition of branched-chain fatty acids in bovine milk are affected by lactation stage and breed of dairy cow. *PLoS ONE*, *11*(3). https://doi.org/10.1371/journal.pone.0150386

Barberg, A. E., Endres, M. I., & Janni, K. A. (2007). COMPOST DAIRY BARNS IN MINNESOTA: A DESCRIPTIVE STUDY. *Applied Engineering in Agriculture*, *23*(2), 231–238.

Barca, J., Carriquiry, M., Olazabal, L., Fajardo, M., Chilibroste, P., & Meikle, A. (2018). Milk fatty acid profile from cows fed with mixed rations and different access time to pastureland during early lactation. *Journal of Animal Physiology and Animal Nutrition*, *102*(3), 620–629. https://doi.org/10.1111/jpn.12826

Bargo, F., Delahoy, J. E., Schroeder, G. F., Baumgard, L. H., & Muller, L. D. (2006). Supplementing total mixed rations with pasture increase the content of conjugated linoleic acid in milk. *Animal Feed Science and Technology*, *131*(3–4), 226–240. https://doi.org/10.1016/j.anifeedsci.2006.04.017

Bargo, F., Muller, L. D., Delahoy, J. E., & Cassidy, T. W. (2002). Performance of high producing dairy cows with three different feeding systems combining pasture and total mixed rations. *Journal of Dairy Science*, *85*(11), 2948–2963. https://doi.org/10.3168/jds.S0022-0302(02)74381-6

Bauman, D. E., & Griinari, J. M. (2001). Regulation and nutritional manipulation of milk fat: low-fat milk syndrome. *Livestock Production Science*, *70*, 15–29. www.elsevier.com/locate/livprodsci

Beauchemin, K. A. (2018). Invited review: Current perspectives on eating and rumination activity in dairy cows. *Journal of Dairy Science*, *101*(6), 4762–4784. https://doi.org/10.3168/jds.2017-13706

Becker, C. A., Collier, R. J., & Stone, A. E. (2020). Invited review: Physiological and behavioral effects of heat stress in dairy cows. *Journal of Dairy Science*, *103*(8), 6751–6770. https://doi.org/10.3168/jds.2019-17929

Bernabucci, U., Basiricò, L., Morera, P., Dipasquale, D., Vitali, A., Piccioli Cappelli, F., & Calamari, L. (2015). Effect of summer season on milk protein fractions in Holstein cows. *Journal of Dairy Science*, *98*(3), 1815–1827. https://doi.org/10.3168/jds.2014-8788

Bernabucci, U., Biffani, S., Buggiotti, L., Vitali, A., Lacetera, N., & Nardone, A. (2014). The effects of heat stress in Italian Holstein dairy cattle. *Journal of Dairy Science*, *97*(1), 471–486. https://doi.org/10.3168/jds.2013-6611

Bernabucci, U., Lacetera, N., Baumgard, L. H., Rhoads, R. P., Ronchi, B., & Nardone, A. (2010). Metabolic and hormonal acclimation to heat stress in domesticated ruminants. *Animal*, *4*(7), 1167–1183. https://doi.org/10.1017/S175173111000090X

Bewley, J. M., Robertson, L. M., & Eckelkamp, E. A. (2017). A 100-Year Review: Lactating dairy cattle housing management. *Journal of Dairy Science*, *100*(12), 10418–10431. https://doi.org/10.3168/jds.2017-13251

Black, R. A., Taraba, J. L., Day, G. B., Damasceno, F. A., & Bewley, J. M. (2013). Compost bedded pack dairy barn management, performance, and producer satisfaction. *Journal of Dairy Science*, *96*(12), 8060–8074. https://doi.org/10.3168/jds.2013-6778

Bonanno, A., Tornambè, G., Bellina, V., De Pasquale, C., Mazza, F., Maniaci, G., & Di Grigoli, A. (2013). Effect of farming system and cheesemaking technology on the physicochemical characteristics, fatty acid profile, and sensory properties of Caciocavallo Palermitano cheese. *Journal of Dairy Science*, *96*(1), 710–724. https://doi.org/10.3168/jds.2012-5973

Broom, D. M. (1986). Indicators of Poor Welfare. *British Veterinary Journal*, *142*(6), 524–526. https://doi.org/https://doi.org/10.1016/j.rvsc.2017.07.005

Cajarville, C., Mendoza, A., Santana, A., & Repetto. (2012). En tiempos de intensificación productiva... Cuánto avanzamos en el conocimiento de los nuevos sistemas de alimentación de la vaca lechera? *Veterinaria*, *48*(1), 35–39.

Charlton, G. L., & Rutter, S. M. (2017). The behaviour of housed dairy cattle with and without pasture access: A review. *Applied Animal Behaviour Science*, *192*, 2–9. https://doi.org/10.1016/j.applanim.2017.05.015

Chilibroste, P., & Battegazzore, G. (2019). Proyecto Producción Competitiva 2013 - 2018: Dinámica bio-económica de los sistemas de producción de leche. *Conaprole*, 31.

Chilibroste, P., Soca, P., Mattiauda, D. A., Bentancur, O., & Robinson, P. H. (2007). Short term fasting as a tool to design effective grazing strategies for lactating dairy cattle: A review. *Australian Journal of Experimental Agriculture*, *47*(9), 1075–1084. https://doi.org/10.1071/EA06130

Chilliard, Y., Glasser, F., Ferlay, A., Bernard, L., Rouel, J., & Doreau, M. (2007). Diet, rumen biohydrogenation and nutritional quality of cow and goat milk fat. *European Journal of Lipid Science and Technology*, *109*(8), 828–855. https://doi.org/10.1002/ejlt.200700080

Clutton-Brock., J. (1999). A Natural History ofDomesticated Mammals. In *Oryx* (Issue 04). https://doi.org/10.1017/S0030605300022432

Cook, N. B., Bennett, T. B., & Nordlund, K. v. (2005). Monitoring indices of cow comfort in free-stall-housed dairy herds. *Journal of Dairy Science*, *88*(11), 3876–3885. https://doi.org/10.3168/jds.S0022-0302(05)73073-3

Cooper, M. D., Arney, D. R., & Phillips, C. J. C. (2007). Two- Or four-hour lying deprivation on the behavior of lactating dairy cows. *Journal of Dairy Science*, *90*(3), 1149–1158. https://doi.org/10.3168/jds.S0022-0302(07)71601-6

Cornu, A., Rabiau, N., Kondjoyan, N., Verdier-Metz, I., Pradel, P., Tournayre, P., Berdagué, J. L., & Martin, B. (2009). Odour-active compound profiles in Cantal-type cheese: Effect of cow diet, milk pasteurization and cheese ripening. *International Dairy Journal*, *19*(10), 588–594. https://doi.org/10.1016/j.idairyj.2009.04.008

Cowley, F. C., Barber, D. G., Houlihan, A. V., & Poppi, D. P. (2015). Immediate and residual effects of heat stress and restricted intake on milk protein and casein composition and energy metabolism. *Journal of Dairy Science*, *98*(4), 2356–2368. https://doi.org/10.3168/jds.2014-8442

Custodio, D. , O. G. , L. Y. , N. T. , M. R. , & C. P. (2018). Effect of stocking rate on feeding strategies and individual milk production of autumn calving grazing dairy cows. *American Dairy Science Association Annual Meeting* , 291.

Dewhurst, R. J., Shingfield, K. J., Lee, M. R. F., & Scollan, N. D. (2006). Increasing the concentrations of beneficial polyunsaturated fatty acids in milk produced by dairy cows in high-forage systems. *Animal Feed Science and Technology*, *131*(3–4), 168–206. https://doi.org/10.1016/j.anifeedsci.2006.04.016

DIEA. (2022). *Anuario Estadistíco Agropecuario*.

Drouin-Chartier, J. P., Côté, J. A., Labonté, M. É., Brassard, D., Tessier-Grenier, M., Desroches, S., Couture, P., & Lamarche, B. (2016). Comprehensive review of the impact of dairy foods and dairy fat on cardiometabolic risk. *Advances in Nutrition*, *7*(6), 1041–1051. https://doi.org/10.3945/an.115.011619

Elgersma, A. (2015). Grazing increases the unsaturated fatty acid concentration of milk from grass-fed cows: A review of the contributing factors, challenges and future perspectives. *European Journal of Lipid Science and Technology*, *117*(9), 1345–1369. https://doi.org/10.1002/ejlt.201400469

Elgersma, A., Tamminga, S., & Ellen, G. (2006). Modifying milk composition through forage. *Animal Feed Science and Technology*, *131*(3–4), 207–225. https://doi.org/10.1016/j.anifeedsci.2006.06.012

Enriquez-Hidalgo, D., Teixeira, D. L., Lewis, E., Buckley, F., Boyle, L., & O'Driscoll, K. (2018). Behavioural responses of pasture based dairy cows to short term management in tie-stalls. *Applied Animal Behaviour Science*, *198*, 19–26. https://doi.org/10.1016/j.applanim.2017.09.012

Esposito, G., Masucci, F., Napolitano, F., Braghieri, A., Romano, R., Manzo, N., & Di Francia, A. (2014). Fatty acid and sensory profiles of Caciocavallo cheese as affected by management system. *Journal of Dairy Science*, *97*(4), 1918–1928. https://doi.org/10.3168/jds.2013-7292

Fajardo, M., Mattiauda, D. A., Motta, G., Genro, T. C., Meikle, A., Carriquiry, M., & Chilibroste, P. (2015). Use of mixed rations with different access time to pastureland on productive responses of early lactation Holstein cows. *Livestock Science*, *181*, 51–57. https://doi.org/10.1016/j.livsci.2015.09.023

Fariña S. (2016). Desafíos futuros para los sistemas de producción de leche de Uruguay. *XLIV Jornadas Uruguayas Buiatría*, 65–69.

Fariña, S. R., & Chilibroste, P. (2019). Opportunities and challenges for the growth of milk production from pasture: The case of farm systems in Uruguay. *Agricultural Systems*, *176*. https://doi.org/10.1016/j.agsy.2019.05.001

Ferlay, A., Bernard, L., Meynadier, A., & Malpuech-Brugère, C. (2017). Production of trans and conjugated fatty acids in dairy ruminants and their putative effects on human health: A review. *Biochimie*, *141*, 107–120. https://doi.org/10.1016/j.biochi.2017.08.006

Fontaneli, R. S., Sollenberger, L. E., Littell, R. C., & Staples, C. R. (2005). Performance of Lactating Dairy Cows Managed on Pasture-Based or in Freestall Barn-Feeding Systems. *Journal Dairy Science* , *88*, 1264–1276.

Fox, P. F. (2003). Milk Proteins: General and Historical Aspects. In Springer (Ed.), *Advanced Dairy Chemistry—1 Proteins*. https://doi.org/https://doi.org/10.1007/978-1-4419-8602-3_1

Gagliostro. (2004). Nutritional control of conjugated linoleic acid (CLA) content in milk and natural functional foods. 1. Effects on human health. *Revista Argentina de Producción Animal* , *24*, 3–4.

García, A. M. B., Cardoso, F. C., Campos, R., Thedy, D. X., & González, F. H. D. (2011). Metabolic evaluation of dairy cows submitted to three different strategies to decrease the effects of negative energy balance in early postpartum 1. *Pesquisa Veterinária Brasileira*, *31*(1), 11–17.

García-Roche, M., Cañibe, G., Casal, A., Mattiauda, D. A., Ceriani, M., Jasinsky, A., Cassina, A., Quijano, C., & Carriquiry, M. (2021). Glucose and Fatty Acid Metabolism of Dairy Cows in a Total Mixed Ration or Pasture-Based System During Lactation. *Frontiers in Animal Science*, *2*. https://doi.org/10.3389/fanim.2021.622500

Gibb, M. J., Huckle, C. A., & Nuthall, R. (1998). Effect of time of day on grazing behaviour by lactating dairy cows. *Grass and Forage Science*, *53*(1), 41–46.

Girard, M., Dohme-Meier, F., Wechsler, D., Goy, D., Kreuzer, M., & Bee, G. (2016). Ability of 3 tanniferous forage legumes to modify quality of milk and Gruyère-type cheese. *Journal of Dairy Science*, *99*(1), 205–220. https://doi.org/10.3168/jds.2015-9952

Gross, J., Van Dorland, H. A., Bruckmaier, R. M., & Schwarz, F. J. (2011). Milk fatty acid profile related to energy balance in dairy cows. *Journal of Dairy Research*, *78*(4), 479–488. https://doi.org/10.1017/S0022029911000550

Haug, A., Høstmark, A. T., & Harstad, O. M. (2007). Bovine milk in human nutrition - A review. *Lipids in Health and Disease*, *6*(1). https://doi.org/10.1186/1476-511X-6-25

Higashiyama, Y., Nashiki, M., Narita, H., & Kawasaki, M. (2007). A brief report on effects of transfer from outdoor grazing to indoor tethering and back on urinary cortisol and behaviour in dairy cattle. *Applied Animal Behaviour Science*, *102*(1–2), 119–123. https://doi.org/10.1016/j.applanim.2006.03.007

Hirigoyen, D., De Los Santos, R., Calvo, M. F., González-Revello, A., & Constantin, M. (2018). Chemical composition and seasonal changes in the fatty acid profile of Uruguayan "Colonia" Cheeses. *Grasas y Aceites*, *69*(2). https://doi.org/10.3989/gya.1217172

INALE. (2019). *https://www.inale.org/estadisticas/encuesta-lechera-2019-resultados-definitivos/*.

INALE. (2021). *Situación y Perspectivas de la Lechería Uruguaya. Período Enero-Diciembre 2021*.

INALE. (2023). *https://www.inale.org/exportaciones-mejora-de-38-comparadas-con-2022-con-aumento-de-precios/*.

Janni, K. A., Endres, M. I., Reneau, J. K., & Schoper, W. W. (2007). Compost dairy barn layout and management recommendations. *Applied Engineering in Agriculture*, *23*(1), 97–102. http://www.exten-

Jasinsky, A., Mattiauda, D. A., Ceriani, M., Casal, A., & Carriquiry, M. (2019). Heat production and body composition of primiparous Holstein cows with or without grazing pastures in early lactation. *Livestock Science*, *225*, 1–7. https://doi.org/10.1016/j.livsci.2019.04.017

Jordan, E. R. (2003). Effects of heat stress on reproduction. *Journal of Dairy Science*, *86*(1). https://doi.org/10.3168/jds.S0022-0302(03)74043-0

Kadzere, C. T., Murphy, M. R., Silanikove, N., & Maltz, E. (2002). Heat stress in lactating dairy cows: a review. *Livestock Production Science*, *77*, 59–91. www.elsevier.com/locate/livprodsci

Kelly, M. L., Berry, J. R., Dwyer, D. A., Griinari, J. M., Chouinard, P. Y., Van Amburgh, M. E., & Bauman, D. E. (1998). Nutrient Metabolism Dietary Fatty Acid Sources Affect Conjugated Linoleic Acid Concentrations in Milk from Lactating Dairy Cows 1,2,3. *The Journal of Nutrition*, *128*(5), 881–885. https://academic.oup.com/jn/article-abstract/128/5/881/4722392

Khanal, R. C., Dhiman, T. R., & Boman, R. L. (2008). Changes in fatty acid composition of milk from lactating dairy cows during transition to and from pasture. *Livestock Science*, *114*(2–3), 164–175. https://doi.org/10.1016/j.livsci.2007.04.020

Kic, P. (2022). Influence of External Thermal Conditions on Temperature–Humidity Parameters of Indoor Air in a Czech Dairy Farm during the Summer. *Animals*, *12*(15). https://doi.org/10.3390/ani12151895

Kilcawley, K. N., Faulkner, H., Clarke, H. J., O'Sullivan, M. G., & Kerry, J. P. (2018). Factors influencing the flavour of bovine milk and cheese from grass based versus non-grass based milk production systems. *Foods*, *7*(3). https://doi.org/10.3390/foods7030037

Kilgour, R. J. (2012). In pursuit of "normal": A review of the behaviour of cattle at pasture. *Applied Animal Behaviour Science*, *138*, 1–11. https://doi.org/10.1016/j.applanim.2011.12.002

Kolver, E. S., & Muller, L. D. (1998). Performance and nutrient intake of high producing Holstein cows consuming pasture or a total mixed ration. *Journal Dairy Science*, *81*(5), 1403–1411.

Krawczel, P., Grant, R., & Miner, W. H. (2009). Effects of cow comfort on milk quality, productivity and behavior. *NMC Annual Meeting Proceedings*, 15–24. www.certifiedhumane.org

Kumar, G., Devi, P., Sharma, N., Yallappa, I., Somagond, M., & Somagond, Y. M. (2020). Impact of thermal stress on milk production, composition and fatty acid profile in dairy cows: A review. ~ *1278 ~ Journal of Entomology and Zoology Studies*, *8*(5), 1278–1283. http://www.entomoljournal.com

Leso, L., Barbari, M., Lopes, M. A., Damasceno, F. A., Galama, P., Taraba, J. L., & Kuipers, A. (2020). Invited review: Compost-bedded pack barns for dairy cows. *Journal of Dairy Science*, *103*(2), 1072–1099. https://doi.org/10.3168/jds.2019-16864

Mandel, R., Whay, H. R., Klement, E., & Nicol, C. J. (2016). Invited review: Environmental enrichment of dairy cows and calves in indoor housing. *Journal of Dairy Science*, *99*(3), 1695–1715. https://doi.org/10.3168/jds.2015-9875

Maniaci, G., Di Grigoli, A., Bonanno, A., Giosuè, C., Ilardi, V., & Alabiso, M. (2021). Fatty acids as biomarkers of the production season of caciocavallo palermitano cheese. *Animals*, *11*(9). https://doi.org/10.3390/ani11092675

Manoni, M., Di Lorenzo, C., Ottoboni, M., Tretola, M., & Pinotti, L. (2020). Comparative proteomics of milk fat globule membrane (MFGM) proteome across species and lactation stages and the potentials of mfgm fractions in infant formula preparation. *Foods*, *9*(9), 1251. https://doi.org/10.3390/foods9091251

Marcondes, M. I., Mariano, W. H., & De Vries, A. (2020). Production, economic viability and risks associated with switching dairy cows from drylots to compost bedded pack systems. *Animal*, *14*(2), 399–408. https://doi.org/10.1017/S1751731119001848

Martin, B., Verdier-Metz, I., Buchin, S., Hurtaud, C., & Coulon, J. B. (2005). How do the nature of forages and pasture diversity influence the sensory quality of dairy livestock products? *Animal Science*, *81*(2), 205–212. https://doi.org/10.1079/ASC50800205

Mattiauda, D. A., Tamminga, S., Gibb, M. J., Soca, P., Bentancur, O., & Chilibroste, P. (2013). Restricting access time at pasture and time of grazing allocation for Holstein dairy cows: Ingestive behaviour, dry matter intake and milk production. *Livestock Science*, *152*(1), 53–62. https://doi.org/10.1016/j.livsci.2012.12.010

Mendoza, A., Cajarville, C., & Repetto, J. L. (2016). Short communication: Intake, milk production, and milk fatty acid profile of dairy cows fed diets combining fresh forage with a total mixed ration. *Journal of Dairy Science*, *99*(3), 1938–1944. https://doi.org/10.3168/jds.2015-10257

Mills, S., Ross, R. P., Hill, C., Fitzgerald, G. F., & Stanton, C. (2011). Milk intelligence: Mining milk for bioactive substances associated with human health. *International Dairy Journal*, *21*(6), 377–401. https://doi.org/10.1016/j.idairyj.2010.12.011

Mollica, M. P., Trinchese, G., Cimmino, F., Penna, E., Cavaliere, G., Tudisco, R., Musco, N., Manca, C., Catapano, A., Monda, M., Bergamo, P., Banni, S., Infascelli, F., Lombardi, P., & Crispino, M. (2021). Milk fatty acid profiles in different animal species: Focus on the potential effect of selected pufas on metabolism and brain functions. *Nutrients*, *13*(4), 1111. https://doi.org/10.3390/nu13041111

Morales-Almaráz, E., Soldado, A., González, A., Martínez-Fernández, A., Domínguez-Vara, I., De La Roza-Delgado, B., & Vicente, F. (2010). Improving the fatty acid profile of dairy cow milk by combining grazing with feeding of total mixed ration. *Journal of Dairy Research*, *77*(2), 225–230. https://doi.org/10.1017/S002202991000004X

Mordenti, A. L., Brogna, N., Merendi, F., Canestrari, G., Dall'olio, M., Biagi, G., & Formigoni, A. (2015). Effect of feeding whole soybean and linseed on milk and Parmigiano Reggiano cheese lipid fraction. *Progress in Nutrition* , *17*(3), 220–230.

Moscovici Joubran, A., Pierce, K. M., Garvey, N., Shalloo, L., & O'Callaghan, T. F. (2021). Invited review: A 2020 perspective on pasture-based dairy systems and products. *Journal of Dairy Science*, *104*(7), 7364–7382. https://doi.org/10.3168/jds.2020-19776

Naya H, Urioste J.I., & Chilibroste P. (2002). Evaluación cuantitativa de curvas de lactancia de vacas holando en Uruguay. Ajuste de un modelo bifásico. *Revista Argentina de Producción Animal*, *22*, 357–358.

Noro, M., Cid, P. T., Wagemann, C. F., Arnés, V. V, Med, T., & Wittwer, F. M. (2013). Diagnostic assessment of liver enzymes in blood profiles from dairy cows. *Journal of the Faculty of Veterinary Medicine and Zootechny of University of Cordoba*, *18*, 3474–3479.

O'Callaghan, T. F., Hennessy, D., McAuliffe, S., Kilcawley, K. N., O'Donovan, M., Dillon, P., Ross, R. P., & Stanton, C. (2016). Effect of pasture versus indoor feeding systems on raw milk composition and quality over an entire lactation. *Journal of Dairy Science*, *99*(12), 9424–9440. https://doi.org/10.3168/jds.2016-10985

O'Callaghan, T. F., Mannion, D. T., Hennessy, D., McAuliffe, S., O'Sullivan, M. G., Leeuwendaal, N., Beresford, T. P., Dillon, P., Kilcawley, K. N., Sheehan, J. J., Ross, R. P., & Stanton, C. (2017). Effect of pasture versus indoor feeding systems on quality characteristics, nutritional composition, and sensory and volatile properties of full-fat Cheddar cheese. *Journal of Dairy Science*, *100*(8), 6053–6073. https://doi.org/10.3168/jds.2016-12508

Olmos, G., Boyle, L., Hanlon, A., Patton, J., Murphy, J. J., & Mee, J. F. (2009). Hoof disorders, locomotion ability and lying times of cubicle-housed compared to pasture-based dairy cows. *Livestock Science*, *125*(2–3), 199–207. https://doi.org/10.1016/j.livsci.2009.04.009

Ortega, G., Garrido, J. M., & Chilibroste, P. (2019). Pasture access time in dairy grazing high stocking rate systems. *Farming System Design Annual Meeting*.

Pastorini, M., Pomiés, N., Repetto, J. L., Mendoza, A., & Cajarville, C. (2019). Productive performance and digestive response of dairy cows fed different diets combining a total mixed ration and fresh forage. *Journal of Dairy Science*, *102*(5), 4118–4130. https://doi.org/10.3168/jds.2018-15389

Pollock, J. G., Gordon, A. W., Huson, K. M., & McConnell, D. A. (2022). The Effect of Frequency of Fresh Pasture Allocation on the Feeding Behaviour of High Production Dairy Cows. *Animals*, *12*(3). https://doi.org/10.3390/ani12030243

Pons, V. (2022). *Impacto del control del ambiente durante el encierro en sistemas mixtos sobre el bienestar de las vacas lecheras: Indicadores comportamentales, bioquímicos y fisiológicos* . Facultad de Veterinaria .

Pons, V., Damián, J. P., Mattiauda, D. A., Meikle, A., Adrien, M. L., & Chilibroste, P. (2021). ¿Como afectan las caracteristicas del encierro el comportamiento de vacas lecheras en sistemas mixtos? *IV Congreso de Colegio Latinoamericano de Bienestar Animal y Medicina Del Comportamiento*.

Rego, O. A., Cabrita, A. R. J., Rosa, H. J. D., Alves, S. P., Duarte, V., Fonseca, A. J. M., Vouzela, C. F. M., Pires, F. R., & Bessa, R. J. B. (2016). Changes in milk production and milk fatty acid composition of cows switched from pasture to a total mixed ration diet and back to pasture. *Italian Journal of Animal Science*, *15*(1), 76–86. https://doi.org/10.1080/1828051X.2016.1141330

Roca-Fernández, A. I., Ferris, C. P., & González-Rodríguez, A. (2013). Short communication. behavioural activities of two dairy cow genotypes (Holstein-Friesian vs. Jersey × Holstein-Friesian) in two milk production systems (grazing vs. confinement). *Spanish Journal of Agricultural Research*, *11*(1), 120–126. https://doi.org/10.5424/sjar/2013111-2682

Román, L., Saravia, C., Astigarraga, L., Bentancur, O., & La Manna, A. (2019). Shade access in combination with sprinkling and ventilation effects performance of Holstein cows in early and late lactation. *Animal Production Science*, *59*(2), 216–224. https://doi.org/10.1071/AN16571

Ruxton, C. H. S., Reed, S. C., Simpson, M. J. A., & Millington, K. J. (2004). The health benefits of omega-3 polyunsaturated fatty acids: a review of the evidence. *Journal of Human Nutrition and Dietetics*, *17*(5), 449–459. https://doi.org/10.1111/j.1365-277X.2004.00552.x

Saravia, C., Astigarraga, L., Van Lier, E., & Bentancur, O. (2011). Impacto de las olas de calor en vacas lecheras en Salto (Uruguay). *Agrociencia Uruguay*, *15*(1), 93–102.

Schaafsma, G. (2008). Lactose and lactose derivatives as bioactive ingredients in human nutrition. *International Dairy Journal*, *18*(5), 458–465. https://doi.org/10.1016/j.idairyj.2007.11.013

Schären, M., Jostmeier, S., Ruesink, S., Hüther, L., Frahm, J., Bulang, M., Meyer, U., Rehage, J., Isselstein, J., Breves, G., & Dänicke, S. (2016). The effects of a ration change from a total mixed ration to pasture on health and production of dairy cows. *Journal of Dairy Science*, *99*(2), 1183–1200. https://doi.org/10.3168/jds.2015-9873

Schirmann, K., Chapinal, N., Weary, D. M., Heuwieser, W., & von Keyserlingk, M. A. G. (2012). Rumination and its relationship to feeding and lying behavior in Holstein dairy cows. *Journal of Dairy Science*, *95*(6), 3212–3217. https://doi.org/10.3168/jds.2011-4741

Schroeder, G. F., Delahoy, J. E., Vidaurreta, I., Bargo, F., Gagliostro, G. A., & Muller, L. D. (2003). Milk fatty acid composition of cows fed a total mixed ration or pasture plus concentrates replacing corn with fat. *Journal of Dairy Science*, *86*(10), 3237–3248. https://doi.org/10.3168/jds.S0022-0302(03)73927-7

Schütz, K. E., Cave, V. M., Cox, N. R., Huddart, F. J., & Tucker, C. B. (2019). Effects of 3 surface types on dairy cattle behavior, preference, and hygiene. *Journal of Dairy Science*, *102*(2), 1530–1541. https://doi.org/10.3168/jds.2018-14792

Schütz, K. E., Rogers, A. R., Poulouin, Y. A., Cox, N. R., & Tucker, C. B. (2010). The amount of shade influences the behavior and physiology of dairy cattle. *Journal of Dairy Science*, *93*(1), 125–133. https://doi.org/10.3168/jds.2009-2416

Simopoulos, A. P. (2008). The importance of the omega-6/omega-3 fatty acid ratio in cardiovascular disease and other chronic diseases. *Experimental Biology and Medicine*, *233*(6), 674–688. https://doi.org/10.3181/0711-MR-311

Soriano, F. D., Polan, C. E., & Miller, C. N. (2001). Supplementing Pasture to Lactating Holsteins Fed a Total Mixed Ration Diet. *Journal Dairy Science*, *84*(11), 2460–2468.

Stanton, C., Lawless, F., Kjellmer, G., Harrington, D., Devery, R., Connolly, J. F., & Murphy, J. (1997). Dietary Influences on Bovine Milk cis-9,trans-11-Conjugated Linoleic Acid Content. *Journal of Food Science, 62*(5), 1083–1086.

Steijns, J. M. (2001). Milk ingredients as nutraceuticals. *International Journal of Dairy Technology*, *54*(3), 81–88. https://doi.org/10.1046/j.1364-727x.2001.00019.x

Tucker, C. B., Weary, D. M., & Fraser, D. (2004). Free-stall dimensions: Effects on preference and stall usage. *Journal of Dairy Science*, *87*(5), 1208–1216. https://doi.org/10.3168/jds.S0022-0302(04)73271-3

Van Knegsel, A. T. M., Van Den Brand, H., Dijkstra, J., Van Straalen, W. M., Heetkamp, M. J. W., Tamminga, S., & Kemp, B. (2007). Dietary energy source in dairy cows in early lactation: Energy partitioning and milk composition. *Journal of Dairy Science*, *90*(3), 1467–1476. https://doi.org/10.3168/jds.S0022-0302(07)71632-6

Vibart, R. E., Fellner, V., Burns, J. C., Huntington, G. B., & Green, J. T. (2008). Performance of lactating dairy cows fed varying levels of total mixed ration and pasture. *Journal of Dairy Research*, *75*(4), 471–480. https://doi.org/10.1017/S0022029908003361

Villeneuve, M. P., Lebeuf, Y., Gervais, R., Tremblay, G. F., Vuillemard, J. C., Fortin, J., & Chouinard, P. Y. (2013). Milk volatile organic compounds and fatty acid profile in cows fed timothy as hay, pasture, or silage. *Journal of Dairy Science*, *96*(11), 7181–7194. https://doi.org/10.3168/jds.2013-6785

Wales, W. J., Marett, L. C., Greenwood, J. S., Wright, M. M., Thornhill, J. B., Jacobs, J. L., Ho, C. K. M., & Auldist, M. J. (2013). Use of partial mixed rations in pasture-based dairying in temperate regions of Australia. *Animal Production Science*, *53*(11), 1167–1178. https://doi.org/10.1071/AN13207

Walstra, P., Geurts T.J., Noomen A., Jellema A., & Van Boekel M.A.J.S. (2001). *Ciencia de la leche y tecnología de los productos lácteos* (E. S. A. Acribia, Ed.).

White, S. L., Benson, G. A., Washburn, S. P., & Green, J. T. (2002). Milk production and economic measures in confinement or pasture systems using seasonally calved holstein and jersey cows. *Journal of Dairy Science*, *85*(1), 95–104. https://doi.org/10.3168/jds.S0022-0302(02)74057-5

White, S. L., Bertrand, J. A., Wade, M. R., Washburn, S. P., Green, J. T., & Jenkins, T. C. (2001). Comparison of fatty acid content of milk from jersey and holstein cows consuming pasture or a total mixed ration. *Journal of Dairy Science*, *84*(10), 2295–2301. https://doi.org/10.3168/jds.S0022-0302(01)74676-0

Yakoob, M. Y., Shi, P., Willett, W. C., Rexrode, K. M., Campos, H., Orav, E. J., Hu, F. B., & Mozaffarian, D. (2016). Circulating Biomarkers of Dairy Fat and Risk of Incident Diabetes Mellitus Among Men and Women in the United States in Two Large Prospective Cohorts. *Circulation*, *133*(17), 1645–1654. https://doi.org/10.1161/CIRCULATIONAHA.115.018410

Zimbelman, R. B., Baumgard, L. H., & Collier, R. J. (2010). Effects of encapsulated niacin on evaporative heat loss and body temperature in moderately heat-stressed lactating Holstein cows. *Journal of Dairy Science*, *93*(6), 2387–2394. https://doi.org/10.3168/jds.2009-2557

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ORIGINAL ARTICLE

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Diet change from a system combining total mixed ration and pasture to confinement system (total mixed ration) on milk production and composition, blood biochemistry and behavior of dairy cows

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Abstract

This study aimed to determine if a diet change from a mixed system to a confinement system affects the milk production and composition, behavior and blood biochemistry of dairy cows. Cows were assigned randomly to one of the two treatments: cows fed with TMR (total-mixed-ration) (confined) throughout the period group fed TMR (GTMR, $n = 15$) and cows that changed their diet from pasture plus TMR to exclusive TMR at 70 ± 14 DIM (GCHD, $n = 15$). GTMR cows produced more milk and greater lactose and protein yield before the change of diet than GCHD cows ($p \le .01$), but these differences disappeared after the change. GCHD cows decreased the frequency of rumination and lying from before to after the change ($p \le .03$), but in GTMR cows no changes were observed. After diet change, GCHD cows had lower frequency of rumination and lying than GTMR cows ($p \le .02$). Before the change, GCHD cows had greater NEFA (non esterified fatty acids) concentrations than GTMR cows ($p = .002$). Abrupt change from a mixed system to a confined system was favorable on blood biochemical and milk variables of dairy cows. However, in relation to behavior, the cows expressed difficulties to adapt quickly to the abrupt change of system.

KEYWORDS
dietary change, housed system, pasture-based system, rumination behavior, welfare

1 | INTRODUCTION

Different dairy production systems influence milk composition and production, physiology, behavior, and animal welfare. Pasture-based dairy systems have productive advantages compared with confinement systems, such as low production costs and improvements in milk quality, e.g.: fatty acid profile (Barca et al., 2018; Bargo, Delahoy, Schroeder, Baumgard, & Muller, 2006; White, Benson, Washburn, & Green, 2002; White et al., 2001) and industrialization products, such as cheeses (Martin, Verdier-Metz, Buchin, Hurtaud, & Coulon, 2005). From the animal welfare point of view, pasture-based dairy systems are considered a natural environment for dairy cattle (Clutton-Brock, 1987) since it allows the expression of the normal behavior of the species, such as grazing (Olmos et al., 2009). Overall, the cows spend between 90% and 95% of the day grazing, ruminating, and resting in pastoral systems (Kilgour, 2012) and all these behaviors are considered as positive indicators of animal welfare (Krawczel & Grant, 2009; Olmos et al., 2009; Roca-Fernández, Ferris, & González-Rodríguez, 2013). In this sense and in comparison with confinement systems, pasture-based system has advantages on the animal welfare.

On the other hand, with regard to productive disadvantages, cows in pasture-based systems consume less dry matter and have less milk yield than in confinement systems (Fontaneli, Sollenberger, Littell, & Staples, 2005; Kolver & Muller, 1998). Besides, cows in pasture conditions are more exposed to extreme environmental variations, which can affect their physiology, production and welfare (Schütz, Rogers, Poulouin, Cox, & Tucker, 2010). For example, in summer, high environmental temperatures and humidity (heat stress) affects the production and milk composition, health and reproduction (Bernabucci et al., 2010, 2015; Cowley, Barber, Houlihan, & Poppi, 2015; Jordan, 2003). Thus, due to the fluctuation in the availability of forage that occurs at different times of the year (Bargo, Muller, Delahoy, & Cassidy, 2002; Chilibroste, Soca, Mattiauda, Bentancur, & Robinson, 2007; Wales et al., 2013) and adverse weather conditions (heat stress), confinement system with access to shade is used as an alternative management in dairy cattle to reduce the negative effects on milk production and improve animal welfare (Charlton & Rutter, 2017; Schütz et al., 2010;). In this sense, total mixed ration (TMR) has been strategically incorporated to increase total dry matter (DMI) and energy intake to ensure an adequate balanced supply of nutrients in quality and quantity throughout the year. In this way, it is easier to meet the nutritional requirements of dairy cows and therefore achieve a better productive level (Bargo, Muller, Kolver, & Delahoy, 2003; Cajarville, Mendoza, Santana, & Repetto, 2012; Charlton, Rutter, East, & Sinclair, 2011; Fajardo et al., 2015; Soriano, Polan, & Miller, 2001). Then, the use of confinement system as strategies of management could be an alternative to mitigate the climatic conditions in summer and minimize the negative effects on milk yield. Therefore, achieving the best use of the different production systems according to environmental variations and respecting the welfare is a challenge.

Due to the fact that the "welfare of an individual is its state as regards its attempts to cope with its environment" (Broom, 1986) changes in systems and environments can affect animal welfare. However, there is little information, about how cows adapt to these changes of management and feeding. In addition, the available information was focused on a change of diet from TMR to pasture. In these studies, the way cows responded to these changes (from TMR to pasture system) was evidenced through the concentrations of several blood parameters (insulin concentration decrease, nonesterified fatty acids (NEFA) and beta-hydroxybutyrate (BHB) concentration increase), lower milk production, and by decreases in DMI and body conditional score (BCS; Agenäs, Holtenius, Griinari, & Burstedt, 2002; Astessiano et al., 2017; Schären et al., 2016). If the change is made gradually (per 10 weeks), the cows show a negative effect on blood parameters and milk during the first week; but after, they adapted to the new system (recovery of body weight, lower concentration of NEFA, greater DMI and decrease in energy deficit), although they maintained negative energy balance and high levels of BHB (Schären et al., 2016). They evaluated how the gradual inclusion of pasture in cows maintained in TMR systems affects blood parameters and milk. However, according to our knowledge, there is no information on how the cows adapt to the abrupt change from a mixed system (grazing plus TMR) to a single system in confinement (TMR) with an integrated perspective that includes/covers blood, milk and behavioral parameters. We hypothesize that the abrupt change from a mixed to a confinement system improves milk production and composition and metabolic profile, but negatively affects some behavioral indicators of welfare in dairy cows. Therefore, the objective of this study was to determine if a diet change, from a mixed system (grazing plus TMR) to confinement system (100% TMR) affects milk production and composition, behavior, and blood biochemistry of dairy cows.

2 | MATERIALS AND METHODS

2.1 | Location, animals, and treatments

Experimental protocol was evaluated and approved by the Comisión Honoraria de Experimentación Animal (CHEA), Universidad de la República, Montevideo, Uruguay. The study was conducted at the commercial farm located in the department of Paysandú, Uruguay.

Thirty multiparous Holstein dairy cows with mean date of calving on September 15th± 13 days, number of lactations of 3.1 ± 1.2 and an average live weight of 660 ± 82.1 kg were used. All cows were under the same management and feeding conditions throughout the 21days before expected calving date (prepartum diet). Cows were blocked by calving date, number of lactation, pre-calving body condition and live weight and randomly assigned to one of the two following treatments immediately after calving: cows confined and fed with TMR ad libitum (GTMR, n = 15) throughout the period and cows that changed their diet (GCHD, n = 15) from a mixed system (pasture plus TMR) to a single TMR system, in the same way as GTMR.

Diet change in GCHD was carried out from November 16th, according to historical records of Temperature-humidity index (THI) values in the region. This change of management from mixed systems to systems in confinement, which is usually done at the beginning of summer, is called in some farms of the region as "summer management". This date (November 16th) corresponds to the second month of calving (2M), 70 ± 14 DIM (week 9).

The TMR was offered from 11:00 to 15:00 in both groups (ad libitum), in open stalls with and access to water. Feeders covered (70 × 3 m) with concrete floor and metal roof and an area with dirt floor without roof but with shade in each group (50 × 35 m). Both groups were in the same environment and confinement system but in different and adjacent pens. The drinking troughs were plastic-made (3.76 × 0.76 × 0.44 m; Figure 1). GCHD group cows were grazing in two sessions (08:00–11:00 and 19:00–06:00 hr) after each milking and fed with TMR in one session (11:00–15:00 hr, until milking in the afternoon) equivalent to 25% of that received by the GTMR. The pasture used was composed of *Festuca arundinacea* and *Dactylis perseo*. The herbage allowance was ample over 40 kg/cow of dry matter (4 cm above ground level).

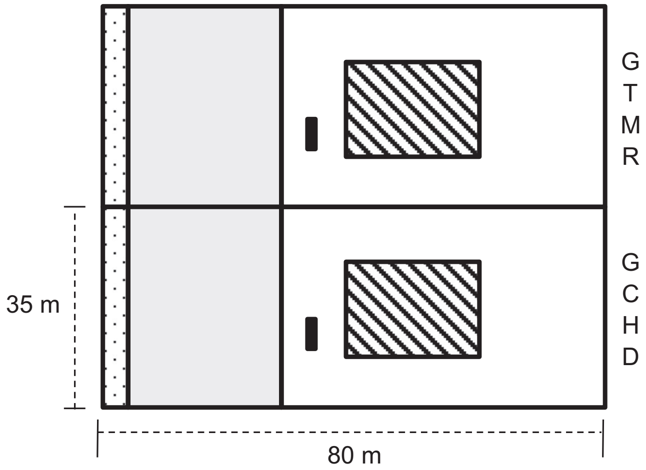

FIGURE 1 Barn diagram. GTMR: barn where cows were in confinement system during all experiment. GCHD: barn where cows were fed with TMR before change (mixed system) and after diet change with only TMR. : Feeders covers, : concrete floor, : drinking troughs, : shade. Feeders covers and concrete floor were covers with metal roof. The others places were "open" with dirt floor and without roof. Both barns were separate with wire fence

2.2 | Feed chemical composition

Ration components were analyzed by near-infrared spectro- scopy (methods 167.03, 42.05, and 984.13; AOAC, 1990). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were measured sequentially (Van Soest, Robertson, & Lewis, 1991) without sodium sulphite in the neutral detergent solution) using an ANKOM200 Fibre Analyzer (ANKOM Technology Corp.). NDF was assayed without a heat stable amylase. Both fiber contents were expressed inclusive of residual ash.

Total Mixed Ration (TMR) consisted of silage of whole sorghum plant (33%), dry grain of sorghum (12.5%), citrus pulp (10%), canola expeller (16.5%), sorghum burlanda (10%), and soybean husk (16%). In addition, a premix of minerals and vitamins formulated to measure (1.3%) and urea (0.2%) was added (Table 1).

Prepartum diet consisted of whole sorghum plant silage (51%), Corn grain (26%), Canola meal (21%), CaCo3 (0.4%), Insalmix® (0.6%), Prepartum Bovigold® (1.0%). The chemical composition of prepartum diet was: crude protein (CP): 19.9%; ether extract (EE): 2.4%; neutral detergent fiber (NDF): 36%: acid detergent fiber (ADF): 19%; dry matter (DM): 88.5%; Calcium (Ca):0.5%; Phosphorus (P): 0.5%.

2.3 | Consumption of TMR

The TMR consumption was estimated by difference between the feed offered and rejected (each groups) in two consecutive days each week. Consumption of TMR (kgDM/cows) was in week 8 (before change): GTMR: 27.6kgDM, GCHD: 8.4kgDM; in week 10 (after change) GTMR: 26.4kgDM, GCHD: 27.1kgDM and in week 14 (one month after change) GTMR: 25.2kgDM, GCHD: 25.2kgDM.

2.4 | Temperature-humidity index

Mean and maximum THI value, were recorded during study period. The mean THI values recorded in each month were in October: 59.8 ± 3.2, November: 64.2 ± 2.4, and December: 67.8 ± 2.5. The maximum THI values registered for each month (October, November and December; Figure 2).

2.5 | Body condition score, sampling procedures, and laboratory analysis

The body condition score (BCS) was registered every 15 days (scale 1 = emaciated, 5 = fat) by one trained observed using the scale of Edmonson, Lean, Weaver, Farver, and Webster (1989).

All cows were milked twice a day at 06:30 and 15:30 hr. Milk production was individually recorded with Waikato® meters. Milk samples were taken at one month of lactation (week 4 and 5), at two month of lactation (before changing: week 8 and after changing: week 9 and 10) and three month of lactation (week 12 and 14; Figure 3). Analyzes for the determination of milk composition (fat, total protein, lactose) were performed using: LactoScope FT infrared (FTIR; Delta Instruments, Drachten the Netherlands). Milk yield and composition were analyzed as an average of the two daily milkings.

Blood samples were taken from coccygeal vein into 5-ml, after morning milking, at one month of calving (week 4), at two month of calving (before changing: week 8 and after changing: week 10), and three month of calving (week 12 and 14; Figure 3). The samples were centrifuged at 2,500 × *g*, 10 min to obtain the serum. The serum was aliquotted and stored at −20°C. Blood biochemistry was analyzed in the Laboratorio de Endocrinología y Metabolismo Animal, Facultad de Veterinaria, Universidad de la República according to the following colorimetric methodologies: total proteins (TP): Biuret reaction; albumin: Bromocresol green, creatine kinase (CK): immunoinhibition; urea: UV GIDH; cholesterol: CHOD-PAP,

TABLE 1 Mean chemical composition of samples of the total mixed diet (TMR) and herbage hand clipped in the months of the experiment (%DM)

Feed	Month	CP	EE	NDF	ADF	C
Festuca + Dactylis	October	13.56	2.29	48.94	24.01	10.05
Festuca + Dactylis	November	12.63	1.23	55.1	27.31	10.5
TMR	October	16.8	3.48	40.4	22.34	6.5
TMR	November	17.35	3.75	38.41	20.99	5.44
TMR	December	16.59	3.14	35.66	20.51	6.03

Abbreviations: ADF, acid detergent fiber; C, Ash; CP, crude protein; EE, ether extract; NDF, neutral detergent fiber.

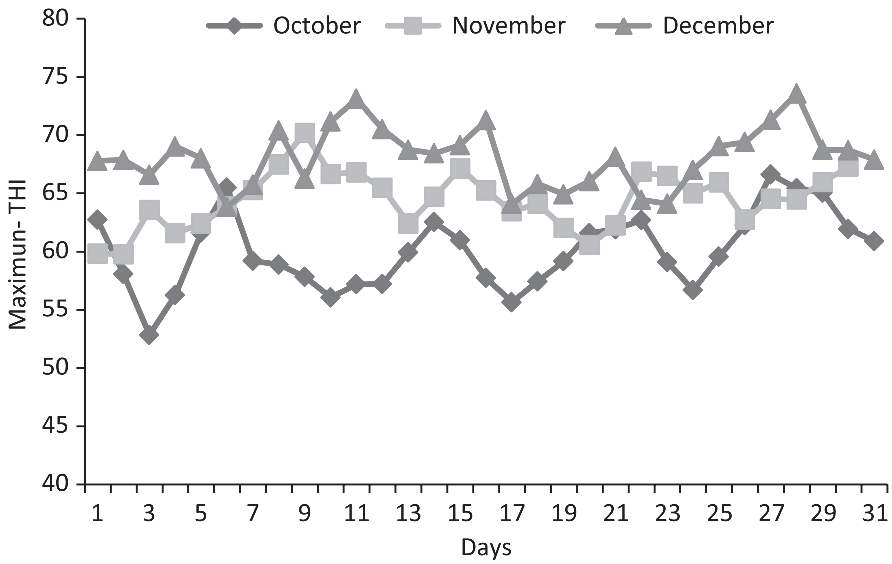

FIGURE 2 Maximum ITH value in each day of October, November, and December

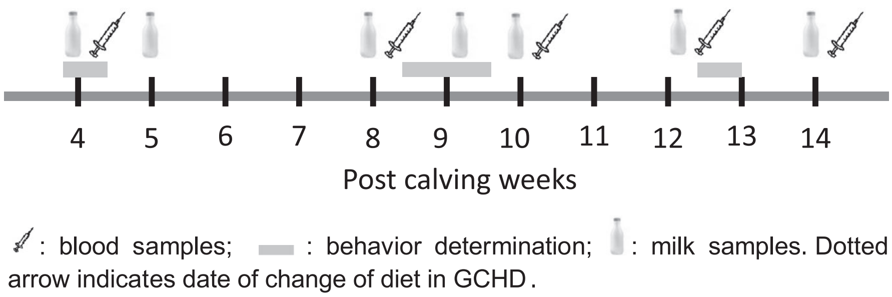

FIGURE 3 Experimental design. Moments (post calving weeks) in which the determinations of blood, behavior, and milk samples were made

calcium: o-cresolphtaleine; phosphorus: phosphomolybdate UV; aspartate aminotransferase (AST); and alanine aminotransferase (ALT): IFCC optimized (37°C). For all these determinations commercial kits were used from the Wiener laboratory (Rosario, Argentina). For CK, BioSystem commercial kits were used. The equipment used was the Vitalab Selectra 2 automatic autoanalyzer (Vital Scientific, Dieren, Netherlands). The concentration of NEFA was determined by the ACS-ACOD method (NEFA-C kit; Wako Chemicals) and BHB by the d-3-hydroxybutyrate kit (Randox Laboratories Ltd.). The controls used were those included in the kit and internal laboratory controls. The concentration of globulins was determined by the difference between the concentration of total proteins and albumin. The interassay and intraassay CV for all determinations was less than 10%

2.6 | Animal behavior

Cows of each treatment were identified with different color (red and blue) collars and numbers drawn on the body to individualize each animal, two days before starting the evaluation. Behavior of individual cows (ruminating, lying, walking, and eating (grazing or TMR; Table 2) were recorded with instantaneous scan sampling every 10 min, performed by two observers simultaneously one in each group (Schütz et al., 2010). Data were recorded during two observation periods daily (08:00–11:00 and 12:00–15:00 hr) for three consecutive days to first and third month after calving (1M and 3M respectively) and for six consecutive days to second month after calving (2M): 3 days before (2M-B) and 3 days after (2M-A) change diet made in GCHD (Figure 3).

2.7 | Statistical analyses

Data of BCS, behavior, milk production, milk composition, and blood biochemistry were analyzed by repeated measures using the PROC MIXED of SAS (SAS Institute Inc., 2004). The BCS, milk yield, milk composition (percentage and yield), and blood biochemistry were analyzed as fixed effects of treatment (GTMR or GCHD), time (weeks) and interaction between treatment and time. The cows in each treatment

TABLE 2 Behaviors observed and their respective descriptions

Behavior	Description
Lying	Lying down in any resting position
Walking	All four legs are moved with head raised or not
Eating: Grazing/ TMR	Picking or consuming pasture/TMR, with the head above ground, still or moving slowly
Ruminating	Chewing movements without feed in the mouth, regurgitation of feed, or both

were considered as a random effect. In BCS, milk and blood variables, post calving days was included as co-variable. Behavior variables were analyzed separately in each month (1M, 2M, and 3M) and the results are presented as average of 3 days in each period. In October (1M) and December (3M) the model considered the treatment (GTMR or GCHD) as fix effect and in November the treatment (GTMR or GCHD) and interaction between treatment and period were considered (2M-B and 2M-A) as fix effects. Post hoc comparisons were performed with least significant difference (LSD). Results were considered significant at $\alpha \leq 0.05$. Data are presented as mean ± *SEM*.

3 | RESULTS

3.1 | Milk production, milk composition, and body condition score

There was not effect of the treatment in milk, lactose, and protein yield ([kg/d] ($p > .30$). All these variables showed an interaction between treatment and time ($p < .0001$, Figure 4). Milk yield in week 9 was greater in GTMR than in GCHD ($p = .009$, Figure 4a). Milk yield increased from week 4 to 9 ($p < .0001$) and decreased from week 9 to 14 ($p < .0001$) in GTMR, while that in GCHD increased from week 9 to 10 ($p < .0001$) and then remained stable until week 14 (Figure 4a). The GTMR cows had greater lactose yield in week 8 and 9 than GCHD cows ($p = .01$ and $p = .002$, respectively, Figure 4b). In GCHD, lactose yield decreased from week 4 to 9 ($p = .01$) and increased from 9 to 10 ($p < .0001$) and then remained at high level until week 14. In GTMR, lactose yield increase from week 4 to 9 ($p = .004$) and decreased from week 10 to 14 ($p = .001$). Protein yield had similar patterns to the lactose yield (Figure 4c). Fat yield did not show effect of treatment ($p = .40$) or interaction between treatment and time ($p = .12$). The differences found between groups (GTMR vs. GCHD) in milk, lactose and protein yield one week before change disappeared after diet change ($p > .05$). The percentage of fat was greater in GCHD than in GTMR (3.2 ± 0.1% vs. 2.5 ± 0.1%, respectively, $p = .01$, Table 3). The GCHD cows had greater percentage of fat than GTMR cows on weeks 4 (3.8 ± 0.2% vs. 2.1 ± 0.2%, $p < .0001$), 5 (3.7 ± 0.2% vs. 2.5 ± 0.2%, $p = .0001$), 8 (3.0 ± 0.2% vs. 2.1 ± 0.2%, $p = .004$) and 9 (3.3 ± 0.2% vs. 2.4 ± 0.2%, $p = .004$). The percentage of fat in GTMR cows increased from week 8 to 10 (2.1 ± 0.2% and 2.8 ± 0.2%, $p = .004$) and remained with high levels and in GCHD decreased from week 4 to 14 (3.8 ± 0.2% and 2.8 ± 0.2%, $p < .0001$). The GTMR cows presented greater percentage of protein than GCHD cows on week 8 (GTMR: 3.1 ± 0.04% vs. GCHD: 2.9 ± 0.04%; $p = .0009$), and lower on week 12 (GTMR: 3.1 ± 0.04% vs. GCHD: 3.3 ± 0.04%; $p = .01$). In GTMR, the percentage of protein decreased from week 4 to 10 (3.2 ± 0.03% and 3.1 ± 0.03%, $p = .02$), while in GCHD decreased from week 4 to 8 (3.1 ± 0.04% and 2.9 ± 0.04%, $p < .0001$) and then increased from week 8 to 14 (2.9 ± 0.04% and 3.2 ± 0.04%, $p < .0001$). In GTMR cows the percentage of lactose increased from week 10 (4.7 ± 0.1%) to 12 (4.9 ± 0.1%, $p = .007$). In GCHD cows the percentage of lactose increased from week 8 to 12 (4.6 ± 0.1% and 5.1 ± 0.1%, respectively, $p < .0001$) and decreased from week 12 to 14 (5.1 ± 0.1% and 5.0 ± 0.1%, respectively, $p = .02$).

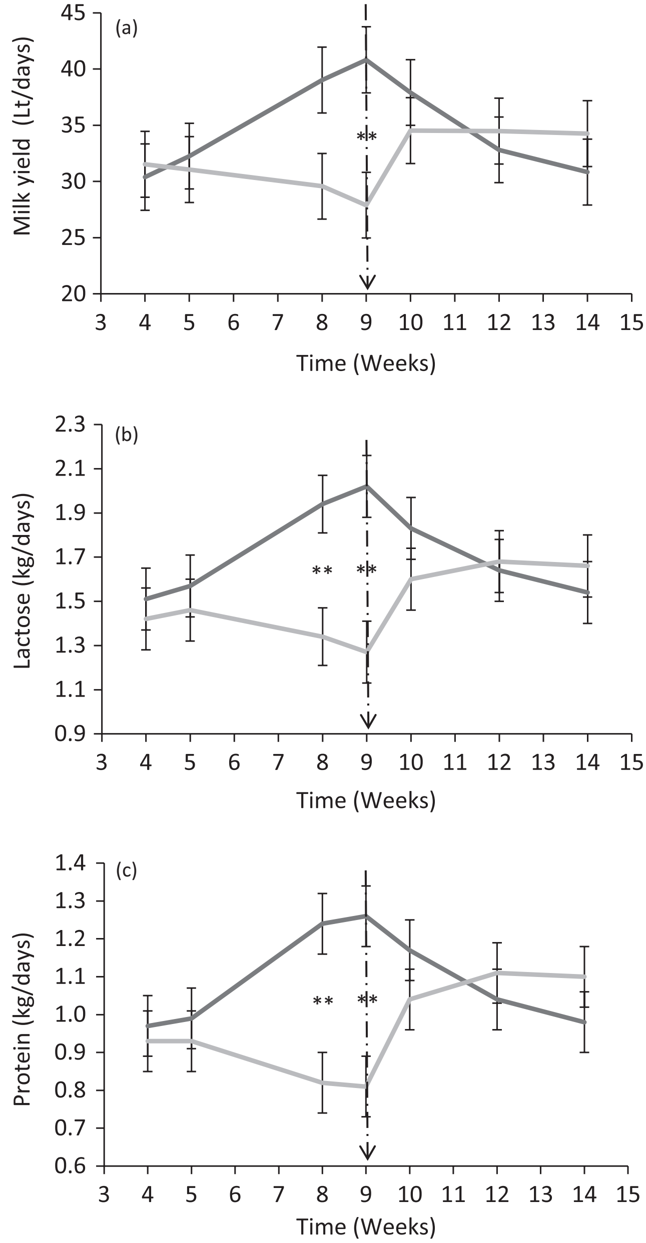

FIGURE 4 Milk yield (Lt/d) (a), Lactose (kg/d) (b), Protein (kg/d) (c) (mean ± *SEM*) in milk of GTMR (line black) and GCHD (line gray). The dotted arrow indicates date of change of diet in GCHD. Asterisks indicate differences between treatment in the same week: $^{**}p < .01$

The BCS was greater in GTMR cows than in GCHD cows ($p = .04$; Table 3).

3.2 | Animal behavior

3.2.1 | First month of lactation

Frequency in which cows were observed ruminating was greater in GCHD than in GTMR ($p < .001$, Figure 5a). There was not difference between groups in the frequency in which cows were observed eating (Figure 5d), lying (Figure 6a), and walking (Figure 6d).

TABLE 3 Effect of treatment (T: GTMR vs. GCHD), week (W) and interaction between treatment and week (T×W) on BCS; percentage of Fat; Protein and Lactose (mean ± *SEM*) during the experimental period

	Treatment			*p* value		
	GTMR	GCHD	*SEM*	T	W	T×W
Fat (%)	2.5	3.2	0.11	.01	.005	.0006
Protein (%)	3.1	3.1	0.13	.49	<.0001	<.0001
Lactose (%)	4.8	4.8	0.2	.96	<.0001	.003
BCS	3.0	2.5	0.11	.04	.0003	.11

Abbreviations: BCS, body condition score; GTMR, group fed TMR; GCHD, group diet change.

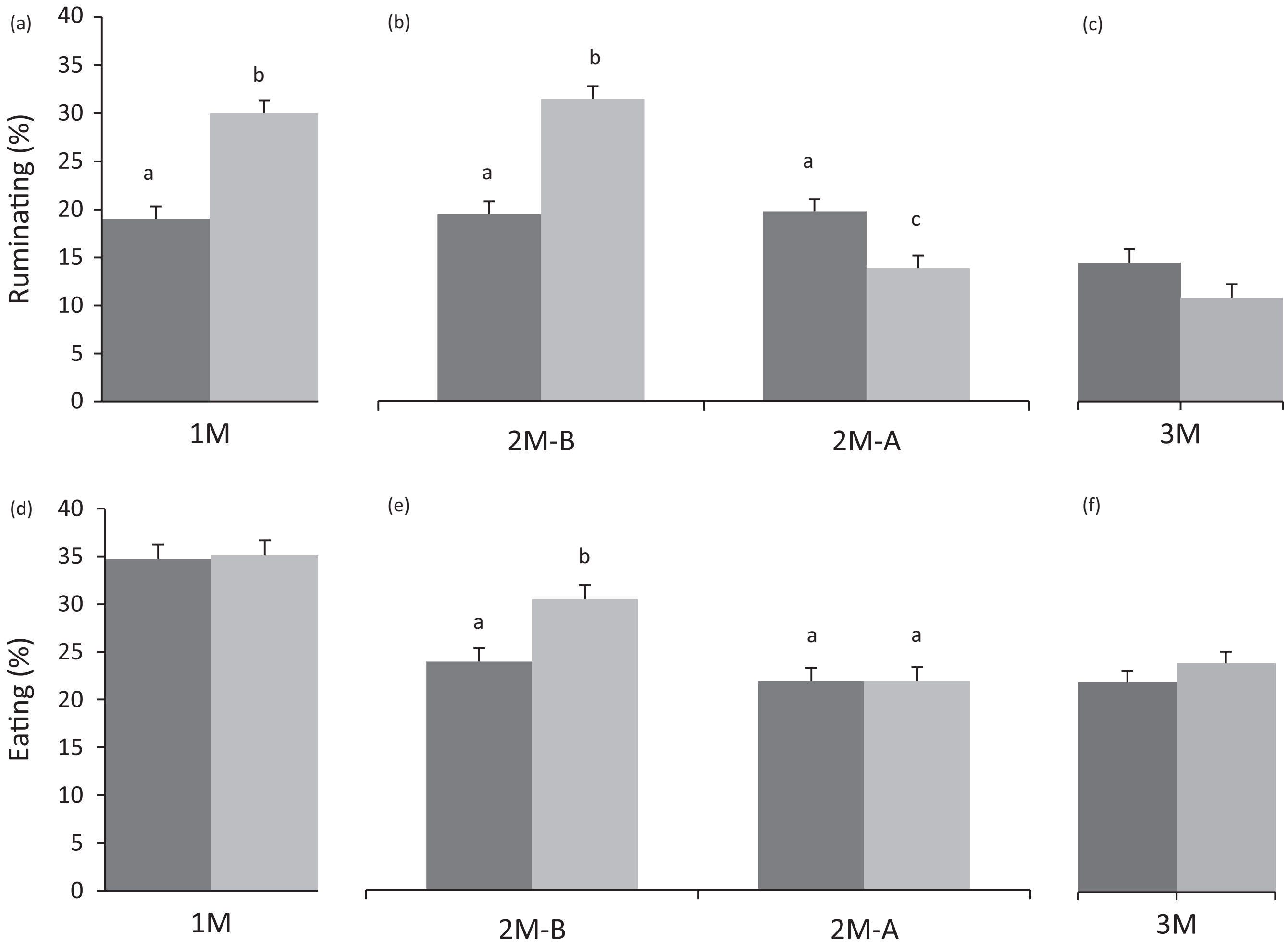

FIGURE 5 Frequency (mean ± *SEM*) in which cows were observed ruminating (%) (a, b, and c) and eating (%) (d, e, and f) in GTMR (black bar) and GCHD (grey bar) at first month from calving (1M), second month from calving: before change feeding (2M-B) and after change feeding (2M-A), and at third month from calving (3M). Different small letters show differ between treatment and between period (2M-B and 2M-A) in second month ($p < .05$)

3.2.2 | Second month of lactation

The frequency in which cows were observed ruminating was greater in GCHD than in GTMR (22.6 ± 0.9% vs.19.5 ± 0.9%; $p = .02$; respectively). There was interaction between treatment and periods ($p < .0001$) in this variable (Figure 5b). During period 2M-B, GCHD cows were ruminating with greater frequency than GTMR cows ($p < .0001$). After the diet change (2M-A) GCHD cows had lower frequency of rumination than GTMR cows ($p = .001$; Figure 5b). The frequency of rumination in GCHD cows decreased from 2M-B (31.4 ± 1.3%) to 2M-A (13. 8 ± 1.3%; $p < .0001$). In GTMR cows there was no difference in the frequency of rumination between periods (2M-B vs. 2M-A; Figure 5b).

The frequency in which cows were eating was greater in GCHD (25.8 ± 0.9%) than in GTMR (22.5 ± 0.9%; $p = .02$). There was an interaction between treatment and period ($p = .02$): before diet

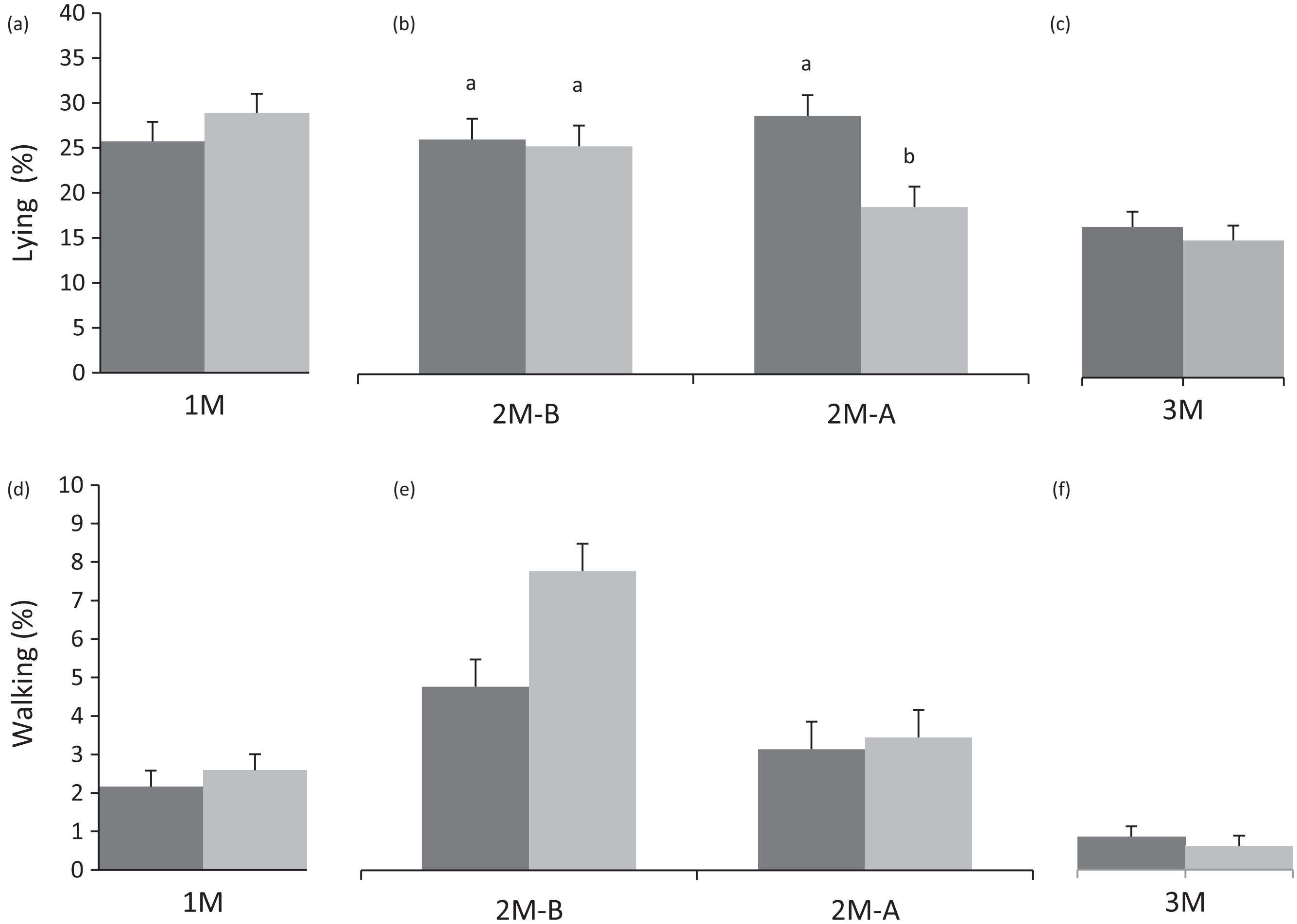

FIGURE 6 Frequency (mean ± *SEM*) in which cows were observed lying (%) (a,b,c) and walking (%) (d, e, f) at one month from calving (1M), two month from calving: before change feeding (2M-B), and after change feeding (2M-A) and at three month from calving (3M), in the groups GTMR (black bar) and GCHD (bar Gray). Different small letters show differ between treatment and period (2M-B and 2M-A) in second month ($p < .05$)

change, GCHD cows had greater frequency of eating than GTMR cows ($p = .001$). The GCHD cows had greater frequency of eating during 2M-B (39.0 ± 1.6%) than during 2M-A (21.6 ± 1.6%; $p < .0001$; Figure 5e).

The GTMR cows were lying with greater frequency than GCHD cows (25.4 ± 1.5% and 20.3 ± 1.5%, respectively; $p = .02$). There was an interaction between treatment and period ($p = .04$; Figure 6b): GCHD cows had greater frequency of lying before diet change (2M-B; 23.5 ± 2.1%) than after diet change (2M-A; 17.3 ± 2.1%; $p = .03$). After diet changing (2M-A) frequency of cows lying was lower in GCHD (17.2 ± 1.5%) than in GTMR (26.6 ± 1.5%; $p = .02$; Figure 6b).

The frequency in which cows were walking was greater in GCHD (5.5 ± 0.5%) than in GTMR (3.9 ± 0.5%; $p = .02$). There was a tendency of interaction between treatment and period in the frequency in which cows were walking ($p = .06$; Figure 6e).

3.2.3 | Third month of lactation

There was no difference in the frequency in which cows were observed eating lying and walking between treatments (Figures 5f, 6c and f) The GTMR cows tended to ruminate more than GCHD cows (14.6 ± 1.5% and 11.0 ± 1.4% respectively, $p = .06$; Figure 5c).

3.3 | Blood biochemistry

Nonesterified fatty acids concentration did not show effects of the treatment ($p = .7$). There was an interaction between treatment and time ($p < .001$) for NEFA concentration. In week 4, GCHD cows had greater NEFA concentrations (0.8 ± 0.1 mmol/L) than GTMR cows (0.4 ± 0.1 mmol/L; $p = .002$; Figure 7a). There was interaction between treatment and time ($p = .03$) for BHB concentration. In GCHD, BHB concentration decreased from week 4 to 14 ($p < .0001$) and in GTMR decreased from 4 to 8 ($p = .0005$; Figure 7b). Cholesterol concentration showed interaction between treatment and time ($p < .001$). In week 10, GTMR cows had greater concentration of cholesterol (5.4 ± 0.39 mmol/L) than GCHD cows (4.0 ± 0.4 mmol/L), ($p = .049$; Figure 7c). The cholesterol concentration increased from week 4 to 10 ($p < .0001$) and decreased from week 10 to 12 ($p < .0001$) in GTMR cows and increased from week 4 to 8 ($p < .0001$) and decreased from week 8 to 12 ($p < .001$) in GCHD cows (Figure 7c). The

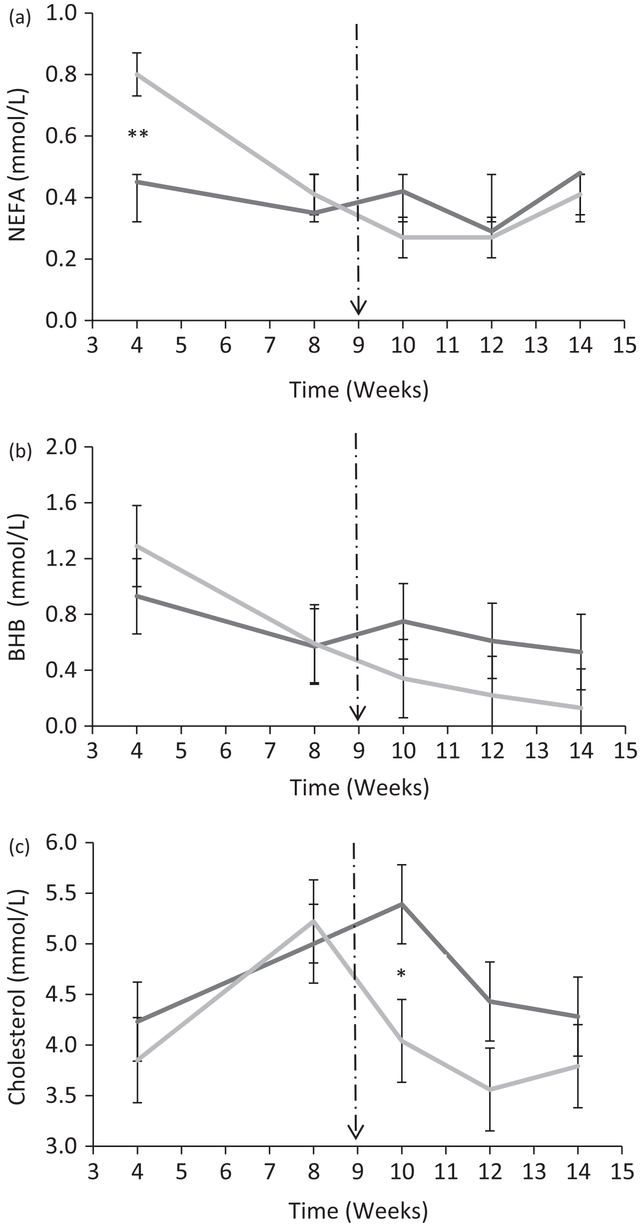

FIGURE 7 Non-esterified fatty acids (NEFA) (a); Beta-hydroxybutyrate (BHB) (b); Cholesterol (c) (mean ± *SEM*) in GTMR (black line) and GCHD (gray line). Dotted arrow indicates date of change of diet in GCHD. Asterisks indicate differences between treatments in the same week: *$p < .05$, **$p < .01$

AST concentration showed interaction between treatment and time ($p = .01$). The AST concentration was greater in GTMR cows than in GCHD cows (114.9 ± 9.1 U/L and 103.2 ± 9.5 U/L respectively; $p = .05$) in week 14. Their AST concentration decreased from week 4 to 14 ($p = .03$) in GCHD cows, in contrast to GTMR cows, whose level increased from week 4 to 8 ($p = .03$) and remained the same until the end of the experiment. There was no effect of the treatment in other blood variables, except for a tendency in ALT (Table 4). There was no interaction between treatment and time for the other blood variables (Table 4), except for a tendency in the concentration of globulins ($p = .08$).

4 | DISCUSSION

The diet change from a mixed system (pasture grazing plus TMR) to single confinement system (100% TMR) in dairy cows affected the milk production and composition, behavior, and some metabolic parameters in blood. On one hand, the change from a mixed system to a single system in confinement was positive in terms of milk production and composition and metabolism. However, the abrupt change from one system to the other negatively affected the behavior of cows, evidencing the difficulty to adapt quickly during the first days. After the change, cows were ruminating and lying with less frequency than before, and they even performed these behaviors less frequently than those cows kept in TMR. The fact that both behaviors (ruminating and lying) decreased after the change of system could be due to the lack of access to the pasture or the factors associated with it. Since these behaviors along with the grazing in the dairy cow are associated with good well-being and comfort (Arnott, Ferris, & O'Connell, 2017; Higashiyama, Nashiki, Narita, & Kawasaki, 2007; Kilgour, 2012; Tucker, Weary, & Fraser, 2004), it is possible that the change of system and the difficulty to adapt during the first days resulted in worse welfare. Ruminating and lying are associated behaviors and occur when cows are at rest (Schirmann, Chapinal, Weary, Heuwieser, & von Keyserlingk, 2012), and in more comfortable housing conditions cows lie more frequently (Charlton & Rutter, 2017), highlighting the importance of these behaviors as welfare indicators. In our study, before the change, cows were in a mixed system, and possibly preferred to perform behaviors of ruminating and lying more in pasture than in confinement, where they do not find a comfortable ground to lie on, as it has been reported in other works (Arnott et al., 2017; Charlton & Rutter, 2017). Nevertheless, after the change of system the cows lost access to the pasture, and possibly in an uncomfortable environment to rest during confinement, they reduced the frequency of lying. Rumination activity is influenced mainly by diet characteristics (Erina et al., 2013; Welch & Smith, 1970) therefore the decrease in rumination activity after the change of system may be due to changes in the composition of the diet. But also, rumination activity is negatively affected by different stressors, such as high stocking density, heat stress, and acute stress (Grant & Albright, 2001; Herskin, Munksgaard, & Ladewig, 2004; Kadzere, Murphy, Silanikove, & Maltz, 2002; Moallem, Altmark, Lehrer, & Arieli, 2010; Moretti, Biffani, Chessa, & Bozzi, 2017). In addition, more stressed cows (with greater serum cortisol concentration) spent less time ruminating (Bristow & Holmes, 2007; Lindström, Redbo, & Uvnäs-Moberg, 2001). Therefore, the reduction in rumination after the change of system may be an indicator of negative emotional response as was suggested by Herskin et al. (2004). In short, the cows expressed difficulties to adapt quickly in a short time window to the abrupt change from a mixed system to a single confinement system with TMR.

One month after the system change, cows of both groups displayed a pattern of similar behavior, which could suggest that cows were adapted to the system. However, even one month after the change, cows of GCHD tended to ruminate less than those of GTMR, suggesting that the process of adapting cows to change was gradual, and that animals need a longer window of time to fully adapt to a

TABLE 4 Effect of treatment (T: GTMR vs. GCHD), week (W) and interaction between treatment and week (T×W) on blood metabolites (mean ± *SEM*) during the experimental period

	Treatment			*p* value		
Blood metabolites	GTMR	GCHD	*SEM*	T	W	T×W
BHB (mmol/L)	0.7	0.5	0.3	.7	<.001	.03
NEFA (mmol/L)	0.4	0.4	0.1	.7	<.001	<.001
Cholesterol (mmol/L)	4.7	4.1	0.4	.8	<.001	<.001
TP (g/L)	79.0	80.6	1.2	.4	<.0001	.16
Albumin (g/L)	34.5	33.5	0.7	.4	<.0001	.4
Globulin (g/L)	44.1	47.1	1.0	.2	.0008	.08
CK (U/L)	225.6	331.1	89.6	.5	.8	.3
Urea (mmol/L)	6.3	5.9	0.1	.8	.02	.4
Calcium (mmol/L)	2.2	2.3	0.1	.6	.01	.3
Phosphorus (mmol/L)	2.2	2.3	0.1	.6	.01	.3
AST (U/L)	117.5	103.6	6.8	.3	.1	.01
ALT(U/L)	44.1	31.8	3.6	.08	.08	.8

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BHB, Beta-hydroxybutyrate; CK, creatine-kinase; GCHD, group diet change; GTMR, group fed TMR; NEFA, Non-esterified fatty acids; TP, Total protein.

system change. Our results match those of Enriquez-Hidalgo et al. (2018) who reported that cows needed more than 10 days to adapt their behavior to the change of management from a pasture system to one of confinement. Therefore, the cows need a window of time of some weeks to adapt their behavior to a new environment after abrupt change from a pasture-based system to a confinement system. Given that dairy cows do not adapt easily to abrupt changes of system, it would be interesting to evaluate how animals can adapt their behavior to gradual changes in these systems of production.

Around one week before the diet change, cows of GCHD produced less milk, and less protein and lactose yield than the cows of GTMR, but such differences disappeared after the change of system. These results are in agreement with the available information on the advantages of the system in confinement, where cows consuming TMR diet produce more milk than cows grazing or in mixed systems, which is associated with an increase in DMI and greater availability of energy (Bargo et al., 2002; Fajardo et al., 2015; Kay, Mackle, & Auldist, 2002; Kolver & Muller, 1998; Meikle, Adrien, Mattiauda, & Chilibroste, 2013; Vibart, Fellner, Burns, Huntington, & Green, 2008; Wales et al., 2013). In addition, the change from a mixed system to a single system in confinement with TMR improved the production and composition of milk in a short period of time, evidenced from one week before the change to one week after. These changes in milk production and composition were accompanied by some metabolic changes, such as a continuous decrease in the concentration of BHB and AST toward the end of the experimental period and an early fall in cholesterol concentration, suggesting less mobilization of body reserves and liver activity after the change of system (Garcia, Cardoso, Campos, Thedy, & Gonzalez, 2001; Noro, Cid, Wagemann, Arnés, & Wittwer, 2013). Although cows in a mixed system during the first month of lactation had greater negative energy balance compared to cows of GTMR (evidenced by greater values of NEFA and lower BCS), they failed to recover their BCS after the change. This could be due to the fact that during early lactation the energy generated by the dairy cow prioritizes the mammary gland for milk production rather than the body reserves (Gross, van Dorland, Bruckmaier, & Schwarz, 2011). Therefore, in terms of milk production, milk composition and metabolism, dairy cows respond favorably to change of diet and environment from mix system to TMR system.

It is interesting to note that according to our knowledge there are few studies that evaluated the adaptation of dairy cows to changes in diet and management considering behavioral variables. This study reinforces the concept of the need to evaluate behavior along with other variables (productive or physiological) for a comprehensive analysis of animal welfare in dairy production systems.

5 | CONCLUSION

Abrupt change from a mixed system to confinement system (100% TMR) was favorable in productive and physiological variables, such as milk production, milk composition, and metabolism of dairy cows. However, in relation to behavior, the cows expressed difficulties to adapt quickly in a short time window to the abrupt change of system.

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REFERENCES

Agenäs, S., Holtenius, K., Griinari, M., & Burstedt, E. (2002). Effects of turnout to pasture and dietary fat supplementation on milk fat composition and conjugated linoleic acid in dairy cows. *Acta Agriculturae Scandinavica, A - Animal Science*, *52*, 25–33. https://doi.org/10.1080/09064700252806399

AOAC. (1990). *AOAC official methods of analysis* (Vol. 15th, pp. 136–138). Washington, D.C: Association of Official Agricultural Chemists.

Arnott, G., Ferris, C. P., & O'Connell, N. E. (2017). Review: Welfare of dairy cows in continuously housed and pasture-based production systems. *Animal*, *11*, 261–273. https://doi.org/10.1017/S1751731116001336

Astessiano, A. L., Meikle, A., Chilibroste, P., Mattiauda, D. A., Fajardo, M., & Carriquiry, M. (2017). Metabolic adaptations due to the inclusion of pasture in the diet of dairy cows fed total mixed ration during early lactation. *Open Journal of Animal Science*, *7*, 127–140. https://doi.org/10.4236/ojas.2017.72011

Barca, J., Carriquiry, M., Olazabal, L., Fajardo, M., Chilibroste, P., & Meikle, A. (2018). Milk fatty acid profile from cows fed with mixed rations and different access time to pastureland during early lactation. *Journal of Animal Physiology and Animal Nutrition*, *102*, 620–629. https://doi.org/10.1111/jpn.12826

Bargo, F., Delahoy, J. E., Schroeder, G. F., Baumgard, L. H., & Muller, L. D. (2006). Supplementing total mixed rations with pasture increase the content of conjugated linoleic acid in milk. *Animal Feed Science and Technology*, *131*, 226–240. https://doi.org/10.1016/j.anifeedsci.2006.04.017

Bargo, F., Muller, L. D., Delahoy, J. E., & Cassidy, T. W. (2002). Performance of high producing dairy cows with three different feeding systems combining pasture and total mixed rations. *Journal of Dairy Science*, *85*, 2948–2963. https://doi.org/10.3168/jds.S0022-0302(02)74381-6

Bargo, F., Muller, L. D., Kolver, E. S., & Delahoy, J. E. (2003). Invited review: Production and digestion of supplemented dairy cows on pasture. *Journal of Dairy Science*, *86*, 1–42. https://doi.org/10.3168/jds.S0022-0302(03)73581-4

Bernabucci, U., Basiricò, L., Morera, P., Dipasquale, D., Vitali, A., Piccioli Cappelli, F., & Calamari, L. (2015). Effect of summer season on milk protein fractions in Holstein cows. *Journal of Dairy Science*, *98*, 1815–1827. https://doi.org/10.3168/jds.2014-8788

Bernabucci, U., Lacetera, N., Baumgard, L. H., Rhoads, R. P., Ronchi, B., & Nardone, A. (2010). Metabolic and hormonal acclimation to heat stress in domesticated ruminants. *Animal*, *4*, 1167–1183. https://doi.org/10.1017/S175173111000090X

Bristow, D. J., & Holmes, D. S. (2007). Cortisol levels and anxiety-related behaviors in cattle. *Physiology & Behavior*, *90*, 626–628. https://doi.org/10.1016/j.physbeh.2006.11.015

Broom, D. M. (1986). Indicators of poor welfare. *British Veterinary Journal*, *142*, 524–526. https://doi.org/10.1016/0007-1935(86)90109-0

Cajarville, C., Mendoza, A., Santana, A., & Repetto, J. L. (2012). En tiempos de intensificación productiva… ¿Cuánto avanzamos en el conocimiento de los nuevos sistemas de producción. *Veterinaria*, *48*, 35–39.

Charlton, G. L., & Rutter, S. M. (2017). The behaviour of housed dairy cattle with and without pasture access: A review. *Applied Animal. Behaviour Science*, *192*, 2–9. https://doi.org/10.1016/j.applanim.2017.05.015

Charlton, G., Rutter, S. M., East, M., & Sinclair, L. A. (2011). The motivation of dairy cows for access to pasture. *Journal of Dairy Science*, *96*(7), 4387–4396. https://doi.org/10.3168/jds.2012-6421

Chilibroste, P., Soca, P., Mattiauda, D. A., Bentancur, O., & Robinson, P. H. (2007). Short term fasting as a tool to design effective grazing strategies for lactating dairy cattle: A review. *Australian Journal of Experimental Agriculture*, *47*, 1075–1084.

Clutton-Brock, J. (1987). *The natural history of domesticated mammals*. London, UK: Cambridge University Press

Cowley, F. C., Barber, D. G., Houlihan, A. V., & Poppi, D. P. (2015). Immediate and residual effects of heat stress and restricted intake on milk protein and casein composition and energy metabolism. *Journal of Dairy Science*, *98*, 2356–2368. https://doi.org/10.3168/jds.2014-8442

Edmonson, A. J., Lean, I. J., Weaver, L. D., Farver, T., & Webster, G. (1989). A body condition scoring chart for holstein dairy cows. *Journal of Dairy Science*, *72*, 68–78. https://doi.org/10.1016/j.compag.2018.09.039

Enriquez-Hidalgo, D., Teixeira, D. L., Lewis, E., Buckley, F., Boyle, L., & O'Driscoll, K. (2018). Behavioural responses of pasture based dairy cows to short term management in tie-stalls. *Applied Animal Behaviour Science*, *198*, 19–26. https://doi.org/10.1016/j.applanim.2017.09.012

Erina, S., Cziszter, L. T., Acatinc, S., Baul, S., Tripon, I., Gavojdian, D., … Buzam, G. (2013). Research on rumination time according to administration order of forages in Romanian Black and White Cows. *Animal Science and Biotechnologies*, *46*, 302–305.

Fajardo, M., Mattiauda, D. A., Motta, G., Genro, T. C., Meikle, A., Carriquiry, M., & Chilibroste, P. (2015). Use of mixed rations with different access time to pastureland on productive responses of early lactation Holstein cows. *Livestock Science*, *181*, 51–57. https://doi.org/10.1016/j.livsci.2015.09.023

Fontaneli, R. S., Sollenberger, L. E., Littell, R. C., & Staples, C. R. (2005). Performance of lactating dairy cows managed on pasture-based or in freestall barn-feeding systems. *Journal of Dairy Science*, *88*, 1264–1276. https://doi.org/10.3168/jds.S0022-0302(05)72793-4

Garcia, A., Cardoso, F., Campos, R., Thedy, D., & Gonzalez, F. (2001). Metabolic evaluation of dairy cows submitted to three different strategies to decrease the effects of negative energy balance in early postpartum. *Pesquisa Vetetinaria Brasileira*, *31*, 11–17. https://doi.org/10.1590/S0100-736X2011001300003

Grant, R. J., & Albright, J. L. (2001). Effect of animal grouping on feeding behavior and intake of dairy cattle. *Journal of Dairy Science*, *84*, E156–E163. https://doi.org/10.3168/jds.S0022-0302(01)70210-X

Gross, J., van Dorland, H. A., Bruckmaier, R. M., & Schwarz, F. J. (2011). Performance and metabolic profile of dairy cows during a lactational and deliberately induced negative energy balance with subsequent realimentation. *Journal of Dairy Science*, *94*, 1820–1830. https://doi.org/10.3168/jds.2010-3707

Herskin, M. S., Munksgaard, L., & Ladewig, J. (2004). Effects of acute stressors on nociception, adrenocortical responses and behavior of dairy cows. *Physiology & Behavior*, *83*, 411–420. https://doi.org/10.1016/j.physbeh.2004.08.027

Higashiyama, Y., Nashiki, M., Narita, H., & Kawasaki, M. (2007). A brief report on effects of transfer from outdoor grazing to indoor tethering and back on urinary cortisol and behaviour in dairy cattle. *Applied Animal Behaviour Science*, *102*, 119–123. https://doi.org/10.1016/j.applanim.2006.03.007

Jordan, E. R. (2003). Effects of heat stress on reproduction. *Journal of Dairy Science*, *86*, E104–E114. https://doi.org/10.3168/jds.S0022-0302(03)74043-0

Kadzere, C. T., Murphy, M. R., Silanikove, N., & Maltz, E. (2002). Heat stress in lactating dairy cows: A review. *Livestock Production Science*, *77*, 59–91. https://doi.org/10.1016/S0301-6226(01)00330-X

Kay, J., Mackle, T., & Auldist, M. (2002). Endogenous synthesis and enhancement of conjugated linoleic acid in pasture-fed dairy cows. *Proceedings of the New Zealand Society of Animal Production*, *62*, 12–15.

Kilgour, R. J. (2012). In pursuit of "normal": A review of the behaviour of cattle at pasture. *Applied Animal Behaviour Science*, *138*, 1–11. https://doi.org/10.1016/j.applanim.2011.12.002

Kolver, E. S., & Muller, L. D. (1998). Performance and nutrient intake of high producing holstein cows consuming pasture or a total mixed ration. *Journal of Dairy Science*, *81*, 1403–1411. https://doi.org/10.3168/jds.S0022-0302(98)75704-2

Krawczel, P., & Grant, R. (2009). Effects of cow comfort on milk quality, productivity and behavior. In 48th Annual Meeting, Managing and Monitoring the Environment for Milk Quality. Symposium conducted at the National Mastitis Council, Charlotte, NC.

Lindström, T., Redbo, I., & Uvnäs-Moberg, K. (2001). Plasma oxytocin and cortisol concentrations in dairy cows in relation to feeding duration and rumen fill. *Physiology & Behavior*, *72*, 73–81. https://doi.org/10.1016/S0031-9384(00)00381-4

Martin, B., Verdier-Metz, I., Buchin, S., Hurtaud, C., & Coulon, J. B. (2005). How do the nature of forages and pasture diversity influence the sensory quality of dairy livestock products? *Animal Science*, *81*, 205–212. https://doi.org/10.1079/ASC50800205

Meikle, A., Adrien, M. L., Mattiauda, D. A., & Chilibroste, P. (2013). Effect of sward condition on metabolic endocrinology during the early postpartum period in primiparous grazing dairy cows and its association with productive and reproductive performance. *Animal Feed Science and Technology*, *186*, 139–147. https://doi.org/10.1016/j.anifeedsci.2013.10.003

Moallem, U., Altmark, G., Lehrer, H., & Arieli, A. (2010). Performance of high-yielding dairy cows supplemented with fat or concentrate under hot and humid climates. *Journal of Dairy Science*, *93*, 3192–3202. https://doi.org/10.3168/jds.2009-2979

Moretti, R., Biffani, S., Chessa, S., & Bozzi, R. (2017). Heat stress effects on Holstein dairy cows' rumination. *Animal*, *11*, 2320–2325. https://doi.org/10.1017/S1751731117001173

Noro, M., Cid, P., Wagemann, C., Arnés, V., & Wittwer, F. (2013). Diagnostic assessment of liver enzymes in blood profiles from dairy cows. *Journal of the Faculty of Veterinary Medicine and Zootechny of University of Cordoba*, *18*, 3474–3479.

Olmos, G., Boyle, L., Hanlon, A., Patton, J., Murphy, J. J., & Mee, J. F. (2009). Hoof disorders, locomotion ability and lying times of cubicle-housed compared to pasture-based dairy cows. *Livestock Science*, *125*, 199–207. https://doi.org/10.1016/j.livsci.2009.04.009

Roca-Fernández, A. I., Ferris, C. P., & González-Rodríguez, A. (2013). Short communication. behavioural activities of two dairy cow genotypes (Holstein-Friesian vs. Jersey × Holstein-Friesian) in two milk production systems (grazing vs. confinement). *Spanish Journal of Agricultural Research*, *11*, 120–126. https://doi.org/10.5424/sjar/2013111-2682

Schären, M., Jostmeier, S., Ruesink, S., Hüther, L., Frahm, J., Bulang, M., … Dänicke, S. (2016). The effects of a ration change from a total mixed ration to pasture on health and production of dairy cows. *Journal of Dairy Science*, *99*, 1183–1200. https://doi.org/10.3168/jds.2015-9873

Schirmann, K., Chapinal, N., Weary, D. M., Heuwieser, W., & von Keyserlingk, M. A. G. (2012). Rumination and its relationship to feeding and lying behavior in Holstein dairy cows. *Journal of Dairy Science*, *95*, 3212–3217. https://doi.org/10.3168/jds.2011-4741

Schütz, K. E., Rogers, A. R., Poulouin, Y. A., Cox, N. R., & Tucker, C. B. (2010). The amount of shade influences the behavior and physiology of dairy cattle. *Journal of Dairy Science*, *93*, 125–133. https://doi.org/10.3168/jds.2009-2416

Soriano, F. D., Polan, C. E., & Miller, C. N. (2001). Supplementing pasture to lactating Holsteins fed a total mixed ration diet. *Journal of Dairy Science*, *84*, 2460–2468. https://doi.org/10.3168/jds.S0022-0302(01)74696-6

Tucker, C. B., Weary, D. M., & Fraser, D. (2004). Free-stall dimensions: Effects on preference and stall usage. *Journal of Dairy Science*, *87*, 1208–1216. https://doi.org/10.3168/jds.S0022-0302(04)73271-3

Van Soest, P. V., Robertson, J. B., & Lewis, B. A. (1991). Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *Journal of Dairy Science*, *74*, 3583–3597. https://doi.org/10.3168/jds.S0022-0302(91)78551-2

Vibart, R. E., Fellner, V., Burns, J. C., Huntington, G. B., & Green, J. T. (2008). Performance of lactating dairy cows fed varying levels of total mixed ration and pasture. *Journal of Dairy Research*, *75*, 471–480. https://doi.org/10.1017/S0022029908003361

Wales, W. J., Marret, L. C., Greenwood, J. S., Wright, M. M., Thornhill, J. B., Jacovs, J. L., … Auldist, M. J. (2013). Use of partial mixed ration in pasture-based dairying in temperate regions of Australia. *Animal Production Science*, *53*, 1167–1178. https://doi.org/10.1071/AN13207

Welch, J. G., & Smith, A. M. (1970). Forage quality and rumination time in cattle 1, 2. *Journal of Dairy Science*, *53*, 797–800. https://doi.org/10.3168/jds.S0022-0302(70)86293-2

White, S. L., Benson, G. A., Washburn, S. P., & Green, J. T. (2002). Reproduction, mastitis, and body condition of seasonally calved Holstein and Jersey cows in confinement or pasture systems. *Journal of Dairy Science*, *85*, 105–111. https://doi.org/10.3168/jds.S0022-0302(02)74058-7

White, S. L., Bertrand, J. A., Wade, M. R., Washburn, S. P., Green, J. T., & Jenkins, T. C. (2001). Comparison of fatty acid content of milk from Jersey and Holstein cows consuming pasture or a total mixed ration. *Journal of Dairy Science*, *84*, 2295–2301. https://doi.org/10.3168/jds.S0022-0302(01)74676-0

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Research Article

Milk Fatty Acid Profile of Holstein Cows When Changed from a Mixed System to a Confinement System or Mixed System with Overnight Grazing

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This study is aimed at comparing the milk fatty acid profile (FAP) of cows that changed from a mixed system (MS) of double grazing plus total mixed ration (TMR) to a total confinement system (TCS, 100% TMR) with cows that changed to another MS with one overnight grazing plus TMR and compare with cows that were kept unchanged in TCS. The diet change was made in the second month of lactation. The milk samples were collected at one (M1-spring) and three months of lactation (M3-summer). Three treatments are as follows (each $n = 10$): confined cows fed with TMR throughout the period (GTMR), cows that changed from MS with double grazing plus TMR in M1 to TCS in M3 (GCHD), and cows that changed from a MS with double grazing plus TMR in M1 to a MS with overnight grazing plus TMR in M3 (GTMR+P). Unlike GTMR+P, GCHD improved milk production after change (increased 14% from M1 to M3), but milk FAP was impaired. In M3, conjugated linoleic acid (C18 : 2-CLA) in GTMR and GCHD was lower than GTMR+P ($p < 0.05$), and linolenic (C18 : 3-n-3) was lower in GCHD than GTMR+P. Maintaining grazing in summer overnight sustained milk fat quality, evidenced by higher C18 : 3 (n-3); C18 : 2 (CLA); and n-6/n-3 ratio than cows that changed to TCS.

1. Introduction

Milk is the most complete liquid food of animal origin in nature, and milk and dairy products are among the most important human food [1–3]. Although for decades, ruminant's milk had a negative image due to its high content in saturated fatty acid (SFA) and the relationship of these with cardiovascular diseases [4–7], milk fatty acids are still important nutritional elements. Besides, it has also been reported that milk fat would have beneficial effects on health [8–10]. In this regard, it has been shown that oleic acid (OA; C18 : 1 cis 9), vaccenic fatty acid (VA; C18 : 1 trans 11), and conjugated linoleic acid (CLA) are considered beneficial for human health [11], due to anticarcinogenic, anti-inflammatory, and antiatherogenic effect [12–14]. The main isomer of CLA found in dairy products is rumenic acid (RA; C18 : 2 cis 9 trans 11) [15, 16] which together with the VA are exclusively from ruminants [11]. Furthermore, linolenic fatty acid (FA) (C18 : 3 (n-3)) and linoleic FA (C18 : 2 (n-6)) are the most abundant omega 3 (n-3) and omega 6 (n-6) FA in milk, respectively, both considered essential FA [17]. Moreover, the n-6/n-3 ratio (recommended to be below 4/1) is considered an indicator for nutritional impact of milk fat on human health [18–20]. Therefore, due to the great consumer demand for dairy products, milk composition modification in favor of healthy FA and against SFA and trans FA continues to be a challenge [13].

It is widely reported that animal nutrition is the factor that most influences milk fat composition [21–25]. Fresh pasture has high proportion of unsaturated FA (70–90%) with a large amount of C18 : 2 (n-6) and C18 : 3 (n-3), precursors of stearic acid (C18 : 0), VA, and CLA [26, 27], whereas oilseed contain a high proportion of C18 : 2 [28]. Therefore, the inclusion of fresh forage in mixed dairy systems (pasture plus TMR) improves milk FA profile in favor of those beneficial to human health [25, 27, 29, 30].

The dairy systems in Uruguay are characterized by being pasture-based systems (pasture 55%, supplementation as roughage 19% and concentrates 25%), with an average stocking rate of 1.15 milking cow per hectare, housed in open-sky facilities during the time cows are out of the pasture [31]. In this country, the average milk production in 2020 was 5245 Lt/cow/year [32], and according to Fariña and Chilibroste [31], the "mean productivity was 8831 Lt. per hectare of milking platform (total area of the farm potentially grazable by the milking herd)," data from 2013 to 2017. In such systems, cows are exposed to extreme weather conditions (such as heat stress); notwithstanding, ~63% of dairy farms have access to water near the milking parlor or the paddocks, and although 75% of the farms have natural shade [33], it is usually located on cow trails and not in the paddock or resting areas. In this sense, confinement systems are used to mitigate climatic conditions in summer and minimize its negative effects on milk yield [34, 35], provide independence from forage availability fluctuation [36, 37], and increase total dry matter (DMI) and energy intake per cow to achieve better productive levels [38, 39]. However, beyond the productive advantages provided by confinement systems, the lack of pasture in diet would negatively affect milk fat composition [25, 28, 40, 41], animal welfare [42], and consumer perception of dairy products [20, 43]. Regarding milk fat composition, when cows change from a pasture-based system (grass plus concentrate mix or grass plus total mixed ration: TMR) to 100% TMR, milk SFA increases in detriment of beneficial FA [44, 45]. Meanwhile, when cows switch from TMR to a pasture-based feeding system, the healthy FA increases [41, 44–46]. Hence, during summer, an alternative mixed system consisting of one grazing session at night would improve milk fat composition, while at the same time reducing heat stress negative effects. In addition, although the change from a mixed system to one in confinement in summer improves milk production and biochemical profile in blood, from behavioral point of view, cows fail to adapt in the short term to the lack of pasture, affecting their animal welfare [34]. Although the effect of dietary change on milk FAP when cows switch from pasture to TMR (and vice versa) has been evaluated, to our knowledge, the consequences of changing to a mixed system with one overnight grazing in summer on milk FAP have not been studied. Therefore, we hypothesize that the change from a mixed system with double grazing to overnight grazing during the summer could be an effective management strategy to achieve better milk fat composition than those cows that were changed from mixed to confinement systems or ever-confined cows. The objective of this study was to compare the milk FAP of cows that were changed from a system that combines TMR plus double grazing to a single confinement system (TMR) with cows that changed to a mixed system with only one night grazing and with cows that were kept unchanged in a confinement system (100% TMR).

2. Materials and Methods

2.1. Location, Animals, and Treatments. The experimental protocol was evaluated and approved by the Comisión Honoraria de Experimentación Animal (CHEA), Universidad de la República, Montevideo, Uruguay (N°149). The study was conducted at a commercial farm located in the Department of Paysandú, Uruguay.

Thirty cows with 2.1 ± 1.2 lactations and an average body weight of $660\,kg \pm 82.1\,kg$ were used. All cows were under the same management and feeding conditions throughout the 21 days before the expected calving date (prepartum diet). Cows were blocked by calving date, number of lactation, precalving body condition, and live weight and randomly assigned to one of the three following treatments immediately after calving: (1) cows confined and fed with TMR ad libitum (GTMR, $n = 10$) throughout both periods; (2) mixed system cows that changed their diet from double grazing plus 25% TMR of the GTMR (GCHD, $n = 10$) to confinement system (100% TMR); and (3) cows that kept mixed system from double grazing plus 25% TMR of the GTMR to overnight grazing with 35% TMR (GTMR+P, $n = 10$).

Diet change in GCHD and GTMR+P was carried out on November 16th, according to historical records of THI values in the region [34], which corresponds to 70 ± 14 days in milk (DIM). The TMR was offered from 11:00 to 15:00 H in open stalls for all treatments, and cows had free access to water. Feeders covered ($70\,m \times 3\,m$) with concrete floor and metal roof and an area with dirt floor without roof but with shade in each treatment ($50\,m \times 4\,m$). All treatments were in the same environment and confinement system but in different and adjacent pens, as described by Grille et al. [34]. The drinking troughs were plastic made ($3.76\,m \times 0.76\,m \times 0.44\,m$). Before diet change, GCHD and GTMR+P grazed in two sessions after each milking (08:00–11:00 H and 19:00–06:00 H) and were fed with TMR in one session (11:00–15:00 H, until milking in the afternoon) equivalent to 25% of that received by the GTMR. After diet change, GTMR+P cows grazed in one night session (19:00–06:00 H) and were fed with TMR (from 11:00 to 15:00 H, until milking in the afternoon) with an amount equivalent to 35% of that received by GTMR.

The pasture was composed of *Festuca arundinacea* and *Dactylis perseo*. Daily herbage allowance was 40 kg dry matter/cow. Herbage mass (kg DM/ha) to ground level was estimated weekly using the double sampling technique adapted from Haydock and Shaw [47]. The method was calibrated monthly using a 5-point scale with 3 replicates for each point. Herbage allowance was then determined adjusting daily strip area for grazing. On dry matter (DM) basis, the total mixed ration was composed of whole plant sorghum silage (33%), sorghum dry grain (12.5%), citrus pulp (10%), canola expeller (16.5%), sorghum distillery grain by-product (10%), and soybean husk (16%). In addition, a premix of minerals and vitamins (1.3%) and urea (0.2%) was added.

2.2. Data Collection, Measurements, and Estimates. Ration components were analyzed by near-infrared spectroscopy (methods 167.03, 42.05, and 984.13) [48]. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were measured sequentially [49], without sodium sulphite in the neutral detergent solution using an ANKOM200 Fibre Analyzer (ANKOM Technology Corp., Fairport, NY, USA). NDF was assayed without a heat-stable amylase. Both fiber contents were expressed inclusive of residual ash (Table 1). The TMR was formulated according to the National Research Council [50] for a body weight of 600 kg and 40 Lt/d milk production (4% milk fat).

All cows were milked twice a day at 6:30 and 18:30 H. Milk production was individually recorded with Waikato® meters. Milk samples were collected in two moments, one (M1: week 4, 35 ± 15 DIM) and three months of lactation (M3: week 13, 98 ± 14 DIM) for milk composition and FAP (composite sample and representative of both daily milkings). Total mixed ration and pasture samples were taken in the same moments of milk sampling (M1 and M3) (Table 1). Analyzes for the determination of milk fat were performed using LactoScope FT infrared (FTIR) (Delta Instruments, Drachten the Netherlands).

Milk fat was extracted according to Folch et al. [51], and FA methyl esters were prepared by the transmethylation procedure described by IUPAC 2.301 [52]. Fatty acid methyl esters were quantified using a gas chromatograph (Agilent Technologies 6890, Palo Alto, CA, USA) and a mass spectrometer (Agilent Technologies 5973) in electron ionization mode at 200°C with an electron current of 70 electron volts, acquiring spectra over the mass 35–600 daltons at a rate of 0.7 s/scan with an interscan delay of 0.5 ms. The column chromatography was a SP 2560 (Supelco), highly polar bis-cyanopropyl capillary column (100 m · 0.25 mm i.d. with 0.2-lm film thickness; Supelco, Bellefonte, PA, USA). Helium was used as the carrier gas with a flow rate of 0.5 mL/min. The injector temperature (split ratio of 1 : 50) was set to 250°C. The initial column temperature (120°C) increased at 10°C/min to 175°C and held for 5 min. Finally, column temperature was increased at a rate of 3°C/min to 240°C and held for 30 min [53]. Samples were run in duplicate, and FAME standard (Supelco 47885-U, Bellefonte; 37 FAME from C4 : 0 to C24 : 0) was analyzed at regular intervals for quality control purposes and to determine recovery and correction factors for individual FA. The intra- and interassay coefficients of variation for each analyte measured were on average 3% and 6%, respectively. Milk fat composition is expressed as grams of each individual FA per 100 g of total FA.

Table 1: Chemical composition and fatty acid profile of total mixed ration (TMR) and pasture in two moments (M1 and M3) of the experiment.

	M1		M3	
Component	TMR	Pasture	TMR	Pasture
DM (%)	66.8	26.7	57.8	21.3
CP (% DM)	16.8	13.6	16.6	10.4
NDF (% DM)	40.4	48.9	35.7	62.0
ADF (% DM)	22.3	24.0	20.5	32.2
Ash (% DM)	6.5	10.1	6.0	10.9
FAs (g/100 g)				
C10 : 0	0.06	nd	nd	nd
C12 : 0	0.09	0.22	nd	nd
C14 : 0	0.41	0.63	0.14	0.55
C15 : 0	0.07	nd	0.05	nd
C16 : 0	14.1	25.2	13.5	29.1
C16 : 1 cis	0.41	0.26	0.63	nd
C16 : 1 trans	0.06	1.98	0.1	1.92
C16 : 2	0.04	nd	nd	nd
C17 : 0	0.15	0.85	nd	0.65
C17 : 1 cis	0.07	nd	0.11	nd
C18 : 0	5.6	13.0	5.6	10.3
C18 : 1 cis	37.3	10.6	41.6	9.3
C18 : 1 trans	0.34	0.09	0.37	nd
C18 : 2 cis (n-6)	36.8	14.7	32.6	17.9
C18 : 2 trans	0.42	0.25	0.43	0.22
C18 : 2 (CLA)	0.05	nd	nd	nd
C18 : 3 (n-3)	2.3	25.0	2.2	23.8
C18 : 3 (n-6)	0.03	0.31	nd	0.34
C20 : 0	0.53	1.12	0.77	1.19
C20 : 1 cis	0.55	0.68	0.54	0.75
C20 : 1 trans	0.05	nd	nd	nd
C20 : 2 cis (n-6)	0.15	nd	0.15	nd
C21 : 0	0.04	0.18	0.05	0.18
C22 : 0	0.29	1.73	0.46	1.55
C23 : 0	0.14	0.22	0.1	0.47
C24 : 0	0.36	1.32	0.45	1.82
C25 : 0	0.07	0.27	0.09	0.11
C26 : 0	0.09	1.51	0.08	nd
C28 : 0	0.03	nd	nd	nd
n-3	2.27	25.0	2.17	23.8
n-6	37.0	15.0	37.1	18.2
Ether extract (g/100)	3.48	2.29	3.14	1.91

M1: one month after calving; M3: three months after calving; CP: crude protein; NDF: neutral detergent fiber; ADF: acid detergent fiber; nd: not detected.

2.3. Statistical Analyses. Milk yield, fat (% and kg/d), and FAP were analyzed with an ANOVA for repeated measures using the PROC MIXED of SAS (SAS Institute Inc., Cary, NC, USA (2004). The statistical model for milk yield, fat (content and yield), and FAP included the treatment (GTMR, GCHD, and GTMR+P), month (M1 and M3), and interaction between treatment and month (M1 and M3). The original model also included the parity, but as it was not significant, it was removed from the model. Cows in each treatment were considered as a random effect. In all variables, post-calving days were included as covariable. Post hoc comparisons were performed with the Tukey-Kramer test. Results were considered significant with $p \leq 0.05$ and tendency when the p value was between 0.05 and 0.10. Data are presented as mean ± SEM (standard error of the mean).

3. Results

3.1. Milk Yield and Composition. There was no difference between treatment and month in milk yield, but a significant interaction between treatment and month was observed (Table 2). In M3, milk yield in GTMR and GCHD was higher than in GTMR+P ($p = 0.04$), but there was no difference between treatments in M1. Milk yield increased from M1 to M3 ($p = 0.05$) in GCHD cows, while GTMR+P cows decreased milk yield between these months ($p = 0.004$).

Milk fat content was affected by treatment, month, and their interaction (Table 2). The cows of GTMR had lower milk fat content than GCHD and GTMR+P ($p < 0.05$), while GCHD and GTMR+P showed no difference between each other. Milk fat content decreased from M1 to M3 ($p = 0.04$). In M1, the GTMR cows had lower milk fat content than GCHD and GTMR+P ($p = 0.01$) and in M3 GTMR+P tended to be higher than GTMR ($p = 0.08$), but there were no differences between GCHD and the other treatments in M3. The cows of GCHD decreased from M1 to M3 ($p = 0.0005$), but the GTMR and GTMR+P showed no difference between these months.

Regarding milk fat yield (kg/d), there were no differences between treatments, but month effect and interaction between treatment and month were observed (Table 2). Fat yield decreased from M1 to M3 ($p = 0.01$). In M1, the GTMR cows had lower fat yield than GCHD and GTMR +P cows ($p = 0.006$), but there was no difference between treatments in M3. Fat yield decreased from M1 to M3 in GTMR+P cows ($p = 0.0001$), but there was no change between these months in GCHD and GTMR cows.

3.2. Milk Fatty Acid Profile. There was no treatment effect in SFA, MUFA, PUFA, n-6, n-3, trans, de novo FA, and preformed FA (Table 3).

No interaction between treatment and month was observed in SFA and MUFA (Table 3). An interaction between treatment and month was observed for PUFA (Table 3). There was no difference between treatments in M1 and M3. All treatments decreased from M1 to M3 ($p < 0.001$). The n-6 showed interaction between treatment and month (Table 3). In M1, the percentage of n-6 was higher in GTMR than in GCHD and GTMR+P ($p < 0.001$), but GCHD and GTMR+P had no difference. In M3, there was no difference between treatments.

There was interaction between treatment and month in n-3 (Table 3). No difference between treatments was observed during M1. In M3, the GCHD had lower n-3 than GTMR+P ($p = 0.02$, Table 3), while GTMR showed no difference between GCHD and GTMR+P. The n-3 decreased from M1 to M3 in GCHD ($p = 0.0001$), while in GTMR and GTMR+P did not change between months.

In the n-6/n-3 ratio, the treatment effect was observed (Table 3). The GTMR showed higher n-6/n-3 ratio than GCHD and GTMR+P ($p < 0.001$), and GCHD had higher n-6/n-3 than GTMR+P ($p < 0.001$). An interaction between treatment and month was observed for the n-6/n-3 ratio (Table 3). In M1, the GTMR had higher n-6/n-3 ratio than GCHD cows and GTMR+P ($p < 0.0001$), while GCHD and GTMR+P showed no difference. In M3, there was no difference between GTMR and GCHD, but both were higher than GTMR+P ($p < 0.0001$). The n6/n3 ratio of GTMR decreased from M1 to M3 ($p < 0.0001$, Table 3), while GCHD increased from M1 to M3 ($p < 0.0001$), and GTMR+P did not change between months.

There was interaction between treatment and month in de novo FA (Table 3). In each month, no difference between treatments was observed. The percentage of de novo FA increased from M1 to M3 in the GCHD and GTMR+P ($p < 0.0001$). In fact, in M1, capric (C10 : 0) percentages were higher in GTMR than in GCHD ($p = 0.05$), and lauric (C : 12 : 0) was higher in GTMR than in GCHD and GTMR +P ($p < 0.05$), but there were no differences between treatments in M3 (Table 4). These FA (capric and lauric) increased from M1 to M3 in GCHD ($p < 0.05$), and the lauric FA increased from M1 to M3 in GTMR+P ($p = 0.01$).

There was a trend in the interaction between treatment and month in preformed FA (Table 3). Of them, stearic (C18 : 0), oleic (C18 : 1 cis), linoleic (C18 : 2 cis), linoelaidic (C18 : 2 trans), CLA (C18 : 2), and linolenic (C18 : 3 (n-3)) showed no effect of treatment, but there was an interaction between treatment and month (Table 4). There was no difference in stearic FA (C18 : 0) between treatments.

In M1, GTMR had higher linoleic (C18 : 2 cis (n-6)) than GCHD and GTMR+P ($p < 0.01$), while GCHD and GTMR +P cows showed no difference.

The percentage of C18 : 2 trans was lower in GTMR than in GCHD and GTMR+P ($p < 0.05$). In GCHD, the percentage was lower than in GTMR+P ($p = 0.02$). In M3, there were no differences between treatments in both, C18 : 2 cis and trans (Table 4). The conjugated linoleic acid (C18 : 2) and linolenic (C18 : 3 (n-3)) showed no differences between treatments in M1, while in M3, the percentage of both FA in GCHD was lower than in GTMR+P ($p < 0.05$). Linolenic (C18 : 3 (n-3)) decreased from M1 to M3 in GCHD and GTMR+P cows ($p < 0.0001$). Stearic (C18 : 0), linoleic (C18 : 2 cis), and CLA (C18 : 2) decreased from M1 to M3 in the three treatments ($p < 0.01$). C18 : 1 cis and C18 : 2 trans decreased from M1 to M3 in GCHD and GTMR+P ($p < 0.01$), but in GTMR had no difference between months.

There was month effect in SFA, MUFA, PUFA, n-6, n-3, trans, de novo FA, and preformed FA (Table 3). Saturated FA, as well as de novo and mixed origin FA, increased from M1 to M3 ($p < 0.001$), while MUFA, PUFA, n-6, n-3, and preformed FA decreased from M1 to M3 ($p = 0.004$). The saturated FA increased from M1 to M3, mainly due to C11 : 0, C12 : 0, C13 : 0, C14 : 0, and C15 : 0 FA increase

Table 2: Effect of treatment (T), month (M), and interaction between treatment and month ($T * M$) on milk yield and fat (mean ± SEM) in month 1 (M1) and month 3 (M3) of lactation.

		GTMR		GCHD		GTMR+P		SEM		p value	
		M1	M3	M1	M3	M1	M3		T	M	$T * M$
Milk yield (kg/d)		33.89^{Aa}	32.34^{Aa}	30.88^{Aa}	35.30^{Ab}	32.48^{Aa}	25.99^{Bb}	3.5	ns	ns	0.005
Fat	(%)	2.09^{Aa}	2.47^{Aa}	4.12^{Ba}	2.8^{ABb}	3.53^{Ba}	3.28^{Ba}	0.2	0.04	0.04	0.003
	(kg/d)	0.76^{Aa}	0.8^{Aa}	1.08^{Ba}	0.96^{Aa}	1.09^{Ba}	0.78^{Ab}	0.1	ns	0.01	0.013

Treatments: GTMR: cows fed TMR; GCHD: cows diet change; GTMR+P: cows TMR+pasture. When there was significant interaction between treatment and month: differences between treatments in the same month (different capital letters) and difference between months within each treatment (different small letters) indicate $p < 0.05$ were shown. ns: no significance; SEM: standard error of the mean.

Table 3: Effect of treatment (T), month (M), and interaction between treatment and month ($T * M$) on fatty acid profile (FAP) in month 1 (M1) and month 3 (M3) of lactation.

	GTMR		GCHD		GTMR+P		SEM		p value	
	M1	M3	M1	M3	M1	M3		T	M	$T * M$
FA saturation (g/100 g of fat)										
SFA	64.84	71.57	61.01	70.70	63.01	70.40	1.67	ns	<0.001	ns
MUFA	30.01	25.82	35.18	26.83	32.90	26.71	1.54	ns	<0.001	ns
PUFA	5.10^{Aa}	2.60^{Ab}	3.80^{Aa}	2.46^{Ab}	4.07^{Aa}	2.87^{Ab}	0.35	ns	<0.001	<0.001
n-3	0.31^{Aa}	0.19^{ABa}	0.41^{Aa}	0.18^{Ab}	0.43^{Aa}	0.30^{Ba}	0.04	ns	<0.001	0.01
n-6	3.66^{Aa}	1.48^{Ab}	2.09^{Ba}	1.64^{Ab}	1.94^{Ba}	1.39^{Ab}	0.19	ns	<0.001	<0.001
n6/n3	12.30^{Aa}	8.52^{Ab}	5.02^{Ba}	8.95^{Ab}	4.47^{Ba}	4.72^{Ba}	0.37	<0.01	ns	<0.001
Trans	4.93	1.87	4.14	2.12	5.37	3.16	0.50	ns	<0.001	ns
FA origin (g/100 g of fat)										
De novo (C4 : 0-C15 : 1)	24.72^{Aa}	25.98^{Aa}	18.23^{Aa}	25.64^{Ab}	19.84^{Aa}	25.0^{Ab}	1.90	ns	<0.001	0.02
Mixed origin (C16 : 0+C16 : 1)	26.73	36.2	29.59	38.95	28.58	36.3	1.20	ns	<0.001	ns
Preformed (>C17 : 0)	48.07	37.45	52.31	35.58	51.43	38.49	2.60	ns	<0.001	0.08

Treatments: GTMR: cows fed TMR; GCHD: cows diet change; GTMR+P: cows TMR+pasture. When there was significant interaction between treatment and month: differences between treatments in the same month (different capital letters) and difference between months within each treatment (different small letters) indicate $p < 0.05$ were shown. ns: no significance; SFA: saturated; MUFA: monounsaturated; PUFA: polyunsaturated; SEM: standard error of the mean.

(Table 4). Meanwhile, MUFA decreased from M1 to M3, largely explained by C18 : 1 decrease ($p < 0.001$), and PUFA decreased as a result of C18 : 2 cis (n-6), C18 : 2 trans, C18 : 2 CLA, C18 : 3 (n-3), and n-6 which also decreased from M1 to M3 ($p \leq 0.04$) (Table 4).

4. Discussion

The change from a system that combines double grazing and total mixed ration to a single confinement system (GCHD) or to a mixed system with overnight grazing (GTMR+P) during summer affected milk yield, milk fat content and yield (% and kg/d), and FAP. In the first case (GCHD), milk yield was improved, but milk FAP quality was impaired, in agreement with Pastorini et al. [40], Salado et al. [54], and Mendoza et al. [55]. The higher milk yield in cows fed with TMR could be due in part to the higher total DM and energy intake achieved in a more nutrient-concentrated diet [54, 56, 57]. In relation to milk fat, although the content was diminished with diet change, fat yield (kg/d) did not change possibly by the improvement in milk production. In the second case (GTMR+P), cows showed lower milk yield from M1 to M3, while maintaining a healthier milk FAP for humans after the change (M3). The inability to maintain the productive level in summer (M3) could be due to the worst environmental condition (heat stress) [34], which could also decrease pasture quality [58, 59]. In addition, during summer (M3), pasture NDF and ADF content increased and CP content decreased in comparison with spring (M1). According to these results, it is suggested an advanced phenological stage in plants (decrease in green leaf in relation to stem) which could lead to a decrease in digestibility [60], lower pasture DMI, and lower milk production. Therefore, despite the change in management to reduce heat stress effects in summer (i.e., one overnight grazing and increase of TMR proportion in the diet), cows were not able to maintain their performance.

When comparing the treatments that changed their management strategy in summer (GCHD and GTMR+P), differences in milk FAP were observed, mainly in some FA

Table 4: Effect of treatment (T), month (M), and interaction between treatment and month ($T * M$) on individual milk fatty acid profile in dairy cows in month 1 (M1) and month 3 (M3) of lactation.

	GTMR		GCHD		GTMR+P		SEM	p value		
	M1	M3	M1	M3	M1	M3		T	M	$T * M$
FA (g/100 g of fat)										
C6 : 0	1.67	1.38	1.38	1.26	1.43	1.34	0.30	ns	0.04	ns
C8 : 0	1.48	1.24	1.04	1.07	1.08	1.16	0.20	ns	ns	ns
C10 : 0	3.81^{Aa}	3.34^{Aa}	2.31^{Ba}	2.95^{Ab}	2.52^{Aa}	3.0^{Aa}	0.40	ns	ns	0.03
C11 : 0	0.30^{Aa}	0.32^{Aa}	0.14^{Ba}	0.32^{Ab}	0.16^{Ba}	0.26^{Ab}	0.03	ns	<0.001	<0.001
C12 : 0	3.93^{Aa}	3.77^{Aa}	2.32^{Ba}	3.49^{Ab}	2.65^{Ba}	3.36^{Ab}	0.30	ns	<0.001	0.008
C12 : 1 cis	0.05	0.07	0.03	0.06	0.03	0.07	0.04	ns	<0.001	ns
C13 : 0	0.17^{A}	0.20^{A}	0.10^{B}	0.26^{A}	0.12^{AB}	0.18^{A}	0.02	ns	<0.001	<0.001
C14 : 0	11.16	12.6	9.12	12.7	9.83	12.68	0.80	ns	<0.001	0.09
C14 : 1 cis	0.51	0.84	0.39	0.89	0.43	0.82	0.10	ns	<0.001	ns
C15 : 0	1.63^{Aa}	2.22^{Ab}	1.38^{Aa}	2.60^{Ab}	1.57^{Aa}	2.16^{Ab}	0.10	ns	<0.001	<0.01
C16 : 0	26	34.87	28.04	37.17	27.24	34.8	1.20	ns	<0.001	ns
C16 : 1 cis	0.65	1.26	1.42	1.67	1.2	1.38	0.10	0.03	0.001	ns
C16 : 1 trans	0.08	0.07	0.13	0.11	0.13	0.11	0.01	0.07	<0.001	ns
C17 : 0	1.46	1.66	1.27	1.47	1.42	1.5	0.10	ns	<0.01	ns
C17 : 1 cis	0.11	0.16	0.22	0.23	0.18	0.19	0.02	0.04	ns	ns
C18 : 0	12.5^{Aa}	9.43^{Ab}	13.86^{Aa}	7.43^{Ab}	14.6^{Aa}	9.62^{Ab}	1.00	ns	<0.001	0.02
C18 : 1 cis	24.0^{Aa}	21.8^{Aa}	29.43^{Aa}	22.15^{Ab}	26.2^{Aa}	21.4^{Ab}	1.80	ns	<0.001	0.05
C18 : 1 trans	4.45	1.39	3.47	1.59	4.58	2.55	0.40	ns	<0.001	ns
C18 : 2 cis (n-6)	3.49^{Aa}	1.33^{Ab}	2.01^{Ba}	1.55^{Ab}	1.84^{Ba}	1.29^{Ab}	0.20	0.08	<0.001	<0.001
C18:2 trans	0.34^{Aa}	0.34^{Aa}	0.49^{Ba}	0.34^{Ab}	0.59^{Ca}	0.42^{Ab}	0.04	0.07	<0.001	<0.001
C18 : 2 CLA	0.78^{Aa}	0.57^{Ab}	0.80^{Aa}	0.29^{Ab}	1.10^{Aa}	0.76^{Bb}	0.10	0.05	<0.001	0.02
C18 : 3 (n-3)	0.23^{Aa}	0.15^{ABb}	0.37^{Aa}	0.13^{Bb}	0.37^{Aa}	0.25^{Ab}	0.04	ns	<0.001	<0.001
C20 : 0	0.1	0.09	0.11	0.09	0.12	0.11	0.02	ns	0.04	ns
C20 : 1 cis	0.05	0.04	0.05	0.04	0.05	0.04	0.01	ns	0.04	ns
C20 : 1 trans	0.05	0.07	0.05	0.07	0.06	0.08	0.01	ns	<0.001	ns
C20 : 3 cis	0.07^{Aa}	0.05^{Ab}	0.04^{Ba}	0.05^{Ab}	0.05^{Ba}	0.05^{Aa}	0.01	ns	ns	<0.001
C20 : 4 (n-6)	0.18	0.17	0.08	0.09	0.1	0.11	0.02	ns	ns	ns
C22 : 0	0.11	0.09	0.04	0.03	0.07	0.06	0.02	ns	0.02	ns

Treatments: GTMR: cows fed TMR; GCHD: cows diet change; GTMR+P: cows TMR+pasture. When there was significant interaction between treatment and month: differences between treatments in the same month (different capital letters) and difference between months within each treatment (different small letters) indicate $p < 0.05$ were shown. ns: no significance; SEM: standard error of the mean.

considered healthy for human consumption. The cows that accessed to overnight grazing during summer (GTMR+P) achieved higher content of healthy FA (e.g., n-3, C18 : 2 (CLA), and C18 : 3) compared to those that changed to a confinement system (GCHD). In addition, GTMR+P showed higher C18 : 2 (CLA) than GTMR in M3. On the other hand, GCHD cows decreased those healthy FA when they were changed to confinement systems. Therefore, cows in a mixed system, even during the summer and overnight grazing, had higher concentration of CLA and linolenic (n-3) in milk than cows in confinement. These results ratify that pasture inclusion in the diet (main source of C18 as linolenic (n-3) and linoleic FA) [46] could increase FA intermediates (VA and RA) and therefore CLA proportion in milk [26, 27]. Our results are also consistent with Morales-Almaraz et al. [61] and Barca et al. [41] who found higher amounts of n-3 in systems that include pasture compared to 100% TMR systems. Besides, in cows with grazing diet (mixed system), lower n-6/n-3 was observed in comparing to confinement system cows. Our results are consistent with studies previously conducted by Barca et al. [41] and Pastorini et al. [40]. In this sense, after change (M3), the GCHD increased the n-6/n-3 ratio to values considered inappropriate for human consumption (above 4/1), while GTMR+P maintained adequate values for human health, as suggested by Simopoulos [18]. Hence, this work highlights the importance of maintaining grazing at night in mixed systems during summer, as it leads to a fatty acid profile considered beneficial for human health, such as high n-3 and C18 : 2 (CLA) content, and low n-6/n-3 ratio [46, 62]. In addition, overnight grazing in a mixed system could be a good management tool as it allows cows to better express their normal behavior, while mitigating heat stress during daylight hours, enabling better welfare [42] and with lower feeding cost in comparison to confinement system [31, 63].

The higher de novo FA percentages in the GCHD and GTMR+P after change (M3) could be caused by lower synthesis inhibition due to lower long-chain FA [16, 64]. Long-chain FAs are potent inhibitors of mammary FA synthesis [24]. These long-chain FAs may reach the mammary gland through circulation from body fat mobilization [64] and diet FA (mainly pasture). In this sense, in both treatments (GCHD and GTMR+P), the preformed FA tended to be higher in M1 than M3, which suggests that there was greater mobilization of reserves in M1 [16, 65, 66]. However, only mixed system cows had greater C18 : 1 (cis) in M1 than in M3, while that GTMR had no difference between months. According to Rukkwamsuk et al. [67], stearic acid (C18 : 0) and oleic acid (C18 : 1cis) are predominant in adipocytes and are released during lipolysis, so we suggest that in our experiment, the cows in mixed system had higher body fat mobilization than confinement cows in M1, which coincides with the inhibition of the synthesis de novo FA. This is consistent with the results reported by Grille et al. [34], where it was observed that mixed system cows during the first month of lactation had greater negative energy balance compared to GTMR cows (evidenced by greater values of NEFA and lower body condition score). Thus, de novo FA synthesis inhibition in mixed systems during M1 could be due to the high proportion of long-chain FA from preformed FAs (lipomobilization) added to those that come directly from the diet (pastures). Moreover, the latter is also noted due to the high content of C18 : 3 in mixed systems than in confinement during M1.

5. Conclusion

Maintaining overnight grazing in summer improved milk fat composition, as was evidenced by higher n-3, CLA percentages, especially C18 : 3 (n-3), C18 : 2 (CLA), and n-6/n-3 ratio than those cows that changed from mixed system to confinement system. Therefore, a mixed system with confinement during the day and one overnight grazing could be a good management alternative during the summer, generating a healthier fatty acid profile in milk for consumers.

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare that there is no conflict of interest that could be perceived as harming the impartiality of the research reported.

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References

[1] K. Lambrini, F. Aikaterini, K. Konstantinos, I. Christos, P. V. Ioanna, and T. Areti, “Milk nutritional composition and its role in human health,” *The Journal of Pharmacy and Pharmacology*, vol. 9, no. 1, p. 8, 2021.

[2] I. T. Khan, M. Nadeem, M. Imran, R. Ullah, M. Ajmal, and M. H. Jaspal, “Antioxidant properties of milk and dairy products: a comprehensive review of the current knowledge,” *Lipids in Health and Disease*, vol. 18, no. 1, article 41, 2019.

[3] J. B. German and C. J. Dillard, “Saturated fats: a perspective from lactation and milk composition,” *Lipids*, vol. 45, no. 10, pp. 915–923, 2010.

[4] A. Astrup, F. Magkos, D. Bier et al., “Saturated fats and health: a reassessment and proposal for food-based recommendations,” *Journal of the American College of Cardiology*, vol. 76, no. 7, pp. 844–857, 2020.

[5] C. Bär, M. Sutter, C. Kopp et al., “Impact of herbage proportion, animal breed, lactation stage and season on the fatty acid and protein composition of milk,” *International Dairy Journal*, vol. 109, article 104785, 2020.

[6] M. E. Kleber, G. E. Delgado, S. Lorkowski, W. März, and C. Von Schacky, “Trans-fatty acids and mortality in patients referred for coronary angiography: the Ludwigshafen risk and cardiovascular health study,” *European Heart Journal*, vol. 37, no. 13, pp. 1072–1078, 2016.

[7] M. Y. Yakoob, P. Shi, W. C. Willet et al., “Circulating biomarkers of dairy fat and risk of incident diabetes mellitus among men and women in the United States in two large prospective cohorts,” *Circulation*, vol. 133, no. 17, pp. 1645–1654, 2016.

[8] M. L. Bainbridge, L. M. Cersosimo, A. D. G. Wright, and J. Kraft, “Content and composition of branched-chain fatty acids in bovine milk are affected by lactation stage and breed of dairy cow,” *PLoS One*, vol. 11, no. 3, article e0150386, 2016.

[9] P. Gómez-Cortés, M. Juárez, and M. A. de la Fuente, “Milk fatty acids and potential health benefits: an updated vision,” *Trends in Food Science and Technology*, vol. 81, pp. 1–9, 2018.

[10] J. P. Drouin-Chartier, J. A. Coté, M. E. Labonté et al., “Comprehensive review of the impact of dairy foods and dairy fat on cardiometabolic risk,” *Advances in Nutrition*, vol. 7, no. 6, pp. 1041–1051, 2016.

[11] A. Elgersma, S. Tamminga, and G. Ellen, “Modifying milk composition through forage,” *Animal Feed Science and Technology*, vol. 131, no. 3-4, pp. 207–225, 2006.

[12] E. Lopez-Huertas, “Health effects of oleic acid and long chain omega-3 fatty acids (EPA and DHA) enriched milks. A review of intervention studies,” *Pharmacological Research*, vol. 61, no. 3, pp. 200–207, 2010.

[13] A. Ferlay, L. Bernard, A. Meynadier, and C. Malpuech-Brugère, “Production of trans and conjugated fatty acids in dairy ruminants and their putative effects on human health: a review,” *Biochimie*, vol. 141, pp. 107–120, 2017.

[14] P. W. Parodi, “Conjugated linoleic acid and other anticarcinogenic agents of bovine milk fat,” *Journal of Dairy Science*, vol. 82, no. 6, pp. 1339–1349, 1999.

[15] J. A. Kelsey, B. A. Corl, R. J. Collier, and D. E. Bauman, "The effect of breed, parity, and stage of lactation on conjugated linoleic acid (CLA) in milk fat from dairy cows," *Journal of Dairy Science*, vol. 86, no. 8, pp. 2588–2597, 2003.

[16] A. L. Lock and D. E. Bauman, "Modifying milk fat composition of dairy cows to enhance fatty acids beneficial to human health," *Lipids*, vol. 39, no. 12, pp. 1197–1206, 2004.

[17] D. L. Palmquist, "Omega-3 fatty acids in metabolism, health, and nutrition and for modified animal product foods," *The Professional Animal Scientist*, vol. 25, no. 3, pp. 207–249, 2009.

[18] A. P. Simopoulos, "The importance of the Omega-6/Omega-3 fatty acid ratio in cardiovascular disease and other chronic diseases," *Experimental Biology and Medicine*, vol. 233, no. 6, pp. 674–688, 2008.

[19] M. Markiewicz-Keszycka, G. Czyzak-Runowska, P. Lipinska, and J. Wójtowski, "Fatty acid profile of milk-a review," *Bulletin of the Veterinary Institute in Pulawy*, vol. 57, no. 2, pp. 135–139, 2013.

[20] B. H. Schwendel, T. J. Wester, P. C. H. Morel et al., "Invited review: organic and conventionally produced milk—an evaluation of factors influencing milk composition," *Journal of Dairy Science*, vol. 98, no. 2, pp. 721–746, 2015.

[21] V. Artegoitia, A. Meikle, L. Olazabal et al., "Milk casein and fatty acid fractions in early lactation are affected by nutritional regulation of body condition score at the beginning of the transition period in primiparous and multiparous cows under grazing conditions," *Journal of Animal Physiology and Animal Nutrition*, vol. 97, p. 919, 2013.

[22] D. E. Bauman and J. M. Griinari, "Nutritional regulation of milk fat synthesis," *Annual Review of Nutrition*, vol. 23, no. 1, pp. 203–227, 2003.

[23] Y. Chilliard, A. Ferlay, J. Loor, J. Rouel, and B. Martin, "Trans and conjugated fatty acids in milk from cows and goats consuming pasture or receiving vegetable oils or seeds," *Italian Journal of Animal Science*, vol. 1, no. 4, pp. 243–254, 2002.

[24] Y. Chilliard, A. Ferlay, R. M. Mansbridge, and M. Doreau, "Ruminant milk fat plasticity: nutritional control of saturated, polyunsaturated,transand conjugated fatty acids," *Animal Research*, vol. 49, no. 3, pp. 181–205, 2000.

[25] R. J. Dewhurst, K. J. Shingfield, M. R. Lee, and N. D. Scollan, "Increasing the concentrations of beneficial polyunsaturated fatty acids in milk produced by dairy cows in high-forage systems," *Animal Feed Science and Technology*, vol. 131, no. 3-4, pp. 168–206, 2006.

[26] D. E. Bauman and J. M. Griinari, "Regulation and nutritional manipulation of milk fat: low-fat milk syndrome," *Livestock Production Science*, vol. 70, no. 1-2, pp. 15–29, 2001.

[27] Y. Chilliard, F. Glasser, A. Ferlay, L. Bernard, J. Rouel, and M. Doreau, "Diet, rumen biohydrogenation and nutritional quality of cow and goat milk fat," *European Journal of Lipid Science and Technology*, vol. 109, no. 8, pp. 828–855, 2007.

[28] G. F. Schroeder, G. A. Gagliostro, F. Bargo, J. E. Delahoy, and L. D. Muller, "Effects of fat supplementation on milk production and composition by dairy cows on pasture: a review," *Livestock Production Science*, vol. 86, no. 1-3, pp. 1–18, 2004.

[29] M. Alothman, S. A. Hogan, D. Hennessy et al., "The "grass-fed" milk story: understanding the impact of pasture feeding on the composition and quality of bovine milk," *Foods*, vol. 8, no. 8, p. 350, 2019.

[30] F. Bargo, J. E. Delahoy, G. F. Schroeder, L. H. Baumgard, and L. D. Muller, "Supplementing total mixed rations with pasture increase the content of conjugated linoleic acid in milk," *Animal Feed Science and Technology*, vol. 131, no. 3-4, pp. 226–240, 2006.

[31] S. R. Fariña and P. Chilibroste, "Opportunities and challenges for the growth of milk production from pasture: the case of farm systems in Uruguay," *Agricultural Systems*, vol. 176, article 102631, 2019.

[32] DIEA, 2021, https://descargas.mgap.gub.uy/DIEA/Anuarios/Anuario2021/LIBRO%20ANUARIO%202021%20Web.pdf.

[33] INALE, 2014, http://www.inale.org/estadisticas/encuesta-2014-produccion-de-leche.

[34] L. Grille, M. L. Adrien, M. Olmos, P. Chilibroste, and J. P. Damián, "Diet change from a system combining total mixed ration and pasture to confinement system (total mixed ration) on milk production and composition, blood biochemistry and behavior of dairy cows," *Animal Science Journal*, vol. 90, no. 11, pp. 1484–1494, 2019.

[35] K. E. Schütz, A. R. Rogers, Y. A. Poulouin, N. R. Cox, and C. B. Tucker, "The amount of shade influences the behavior and physiology of dairy cattle," *Journal of Dairy Science*, vol. 93, no. 1, pp. 125–133, 2010.

[36] J. L. Cajarville, C. Mendoza, A. Santana, and A. Repetto, "Section1: conference program," *preview*, vol. 2012, no. 156, pp. 35–48, 2012.

[37] P. Chilibroste, P. Soca, D. A. Mattiauda, O. Bentancur, and P. H. Robinson, "Short term fasting as a tool to design effective grazing strategies for lactating dairy cattle: a review," *Australian Journal of Experimental Agriculture*, vol. 47, no. 9, p. 1075, 2007.

[38] F. Bargo, L. D. Muller, E. S. Kolver, and J. E. Delahoy, "Invited review: production and digestion of supplemented dairy cows on pasture," *Journal of Dairy Science*, vol. 86, no. 1, pp. 1–42, 2003.

[39] M. Fajardo, D. A. Mattiauda, G. Motta et al., "Use of mixed rations with different access time to pastureland on productive responses of early lactation Holstein cows," *Livestock Science*, vol. 181, pp. 51–57, 2015.

[40] M. Pastorini, N. Pomiés, J. L. Repetto, A. Mendoza, and C. Cajarville, "Productive performance and digestive response of dairy cows fed different diets combining a total mixed ration and fresh forage," *Journal of Dairy Science*, vol. 102, no. 5, pp. 4118–4130, 2019.

[41] J. Barca, M. Carriquiry, L. Olazabal, M. Fajardo, P. Chilibroste, and A. Meikle, "Milk fatty acid profile from cows fed with mixed rations and different access time to pastureland during early lactation," *Journal of Animal Physiology and Animal Nutrition*, vol. 102, no. 3, pp. 620–629, 2018.

[42] G. Arnott, C. P. Ferris, and N. E. O'connell, "Review: welfare of dairy cows in continuously housed and pasture-based production systems," *Animal*, vol. 11, no. 2, pp. 261–273, 2017.

[43] G. L. Charlton and S. M. Rutter, "The behaviour of housed dairy cattle with and without pasture access: a review," *Applied Animal Behaviour Science*, vol. 192, pp. 2–9, 2017.

[44] R. C. Khanal, T. R. Dhiman, and R. L. Boman, "Changes in fatty acid composition of milk from lactating dairy cows during transition to and from pasture," *Livestock Science*, vol. 114, no. 2-3, pp. 164–175, 2004.

[45] O. A. Rego, A. R. Cabrita, H. J. Rosa et al., "Changes in milk production and milk fatty acid composition of cows switched from pasture to a total mixed ration diet and back to pasture,"

Italian Journal of Animal Science, vol. 15, no. 1, pp. 76–86, 2016.

[46] A. Elgersma, "Grazing increases the unsaturated fatty acid concentration of milk from grass fed cows: a review of the contributing factors, challenges and future perspectives," *European Journal of Lipid Science and Technology*, vol. 117, no. 9, pp. 1345–1369, 2015.

[47] K. P. Haydock and N. H. Shaw, "The comparative yield method for estimating dry matter yield of pasture," *Australian Journal of Experimental Agriculture*, vol. 15, no. 76, p. 663, 1975.

[48] *Official Methods of Analysis*, Association of Official Analytical Chemists, AOAC, Arlington, VA, 15th edition, 1990.

[49] P. J. Van Soest, J. B. Robertson, and B. A. Lewis, "Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition," *Journal of Dairy Science*, vol. 74, no. 10, pp. 3583–3597, 1991.

[50] National Research Council, *Nutrient Requirements of Dairy Cattle*, Natl. Acad Sci, Washington, DC, 2001.

[51] J. Floch, "A simple method for the isolation and purification of total lipides from animal tissues," *The Journal of Biological Chemistry*, vol. 226, no. 1, pp. 497–509, 1957.

[52] M. M. Mossoba, M. P. Yurawecz, J. A. G. Roach, R. E. McDonald, B. D. Flickinger, and E. G. Perkins, "Analysis of cyclic fatty acid monomer 2-alkenyl-4,4-dimethyloxazoline derivatives by gas chromatography–matrix isolation–fourier transform infrared spectroscopy," *Journal of Agricultural and Food Chemistry*, vol. 44, no. 10, pp. 3193–3196, 1996.

[53] P. A. Golay and J. Moulin, "Determination of labeled fatty acids content in milk products, infant formula, and adult/pediatric nutritional formula by capillary gas chromatography: Collaborative study, final action 2012.13," *Journal of AOAC International*, vol. 99, no. 1, pp. 210–222, 2016.

[54] E. E. Salado, G. Bretschneider, A. Cuatrin, A. M. Descalzo, and G. A. Gagliostro, "Productive response of dairy cows fed with different levels of totally mixed ration and pasture," *Agricultural Sciences*, vol. 9, no. 7, pp. 824–851, 2018.

[55] A. Mendoza, C. Cajarville, and J. L. Repetto, "Digestive response of dairy cows fed diets combining fresh forage with a total mixed ration," *Journal of Dairy Science*, vol. 99, no. 11, pp. 8779–8789, 2016.

[56] F. Bargo, L. D. Muller, J. E. Delahoy, and T. W. Cassidy, "Performance of high producing dairy cows with three different feeding systems combining pasture and total mixed rations," *Journal of Dairy Science*, vol. 85, no. 11, pp. 2948–2963, 2002.

[57] E. S. Kolver and L. D. Muller, "Performance and nutrient intake of high producing Holstein cows consuming pasture or a total mixed ration," *Journal of Dairy Science*, vol. 81, no. 5, pp. 1403–1411, 1998.

[58] W. J. Fulkerson and D. J. Donaghy, "Plant-soluble carbohydrate reserves and senescence-key criteria for developing an effective grazing management system for ryegrass-based pastures: a review," *Australian Journal of Experimental Agriculture*, vol. 41, no. 2, p. 261, 2001.

[59] E. Kennedy, M. O. Donovan, J. P. Murphy, L. Delaby, and F. P. O. Mara, "Effect of spring grazing date and stocking rate on sward characteristics and dairy cow production during midlactation," *Journal of Dairy Science*, vol. 90, no. 4, pp. 2035–2046, 2007.

[60] R. E. Vibart, V. Fellner, J. C. Burns, G. B. Huntington, and J. T. Green, "Performance of lactating dairy cows fed varying levels of total mixed ration and pasture," *The Journal of Dairy Research*, vol. 75, no. 4, pp. 471–480, 2008.

[61] E. Morales-Almaráz, A. Soldado, A. González et al., "Improving the fatty acid profile of dairy cow milk by combining grazing with feeding of total mixed ration," *The Journal of Dairy Research*, vol. 77, no. 2, pp. 225–230, 2010.

[62] C. H. S. Ruxton, S. C. Reed, M. J. A. Simpson, and K. J. Millington, "The health benefits of omega-3 polyunsaturated fatty acids: a review of the evidence," *Journal of Human Nutrition and Dietetics*, vol. 17, no. 5, pp. 449–459, 2004.

[63] S. L. White, G. A. Benson, S. P. Washburn, and J. T. Green, "Reproduction, mastitis, and body condition of seasonally calved holstein and jersey cows in confinement or pasture systems," *Journal of Dairy Science*, vol. 85, no. 1, pp. 105–111, 2002.

[64] J. Gross, H. A. Van Dorland, R. M. Bruckmaier, and F. J. Schwarz, "Milk fatty acid profile related to energy balance in dairy cows," *The Journal of Dairy Research*, vol. 78, no. 4, pp. 479–488, 2011.

[65] A. Meikle, M. de Lourdes Adrien, D. A. Mattiauda, and P. Chilibroste, "Effect of sward condition on metabolic endocrinology during the early postpartum period in primiparous grazing dairy cows and its association with productive and reproductive performance," *Animal Feed Science and Technology*, vol. 186, no. 3-4, pp. 139–147, 2013.

[66] M. L. Adrien, D. A. Mattiauda, V. Artegoitia et al., "Nutritional regulation of body condition score at the initiation of the transition period in primiparous and multiparous dairy cows under grazing conditions: milk production, resumption of post-partum ovarian cyclicity and metabolic parameters," *Animal*, vol. 6, no. 2, pp. 292–299, 2012.

[67] T. Rukkwamsuk, M. J. H. Geelen, T. A. M. Kruip, and T. Wensing, "Interrelation of fatty acid composition in adipose tissue, serum, and liver of dairy cows during the development of fatty liver postpartum," *Journal of Dairy Science*, vol. 83, no. 1, pp. 52–59, 2000.

animals

Article

Different Conditions during Confinement in Pasture-Based Systems and Feeding Systems Affect the Fatty Acid Profile in the Milk and Cheese of Holstein Dairy Cows

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Simple Summary: Pasture-based systems have advantages compared to confined systems, such as a higher proportion of beneficial milk fatty acids for their consumers. However, in mixed systems (grazing + total mixed ration), cows are more exposed to external climatic conditions. Due to climate change (high temperature or heavy rains), cows spend more time in confinement facilities where supplementation is offered, mainly in intensive mixed systems (high stocking rate). Therefore, the conditions of the facility during their confinement acquire great importance. To our knowledge, no studies have evaluated how the different facility conditions during confinement in a mixed system affect the fatty acid profiles in milk and cheese. The objective of this study was to compare the fatty acid profiles between mixed systems with different conditions during confinement (compost-bedded pack barns vs. outdoor soil-bedded pen) and mixed systems and confinement systems (100% total mixed ration) in a compost-bedded pack barns. In conclusion, the compost-bedded pack barns during confinement in a mixed system ensued a better milk quality (a higher percentage of Omega 3 (n-3) and C18:3 in the milk) compared to the outdoor soil-bedded pen. However, the fatty acid profiles in the milk, pooled milk (MilkP), and cheese were affected to a greater extent by the feeding management than by controlling the environment during the confinement.

Abstract: The diet of dairy cows influences the fatty acid (FA) profiles of their milk and cheese, but how these are affected by different conditions during confinement in a mixed system (MS:grazing + total mixed ration:TMR) is not known. The aim of this study was to compare the FAs of the milk and cheese from MS in a compost-bedded pack barns (CB-GRZ) versus an outdoor soil-bedded pen (OD-GRZ) during confinement, and with a confinement system (100%TMR) in a compost-bedded pack barns (CB-TMR). Individual milk samples (n = 12 cows/group), cheese, and pooled milk (MilkP) samples were collected. The saturated FA percentages in the milk and the omega 6/omega 3 ratio in the MilkP and cheese were greater for the CB-TMR ($p < 0.0001$), while the unsaturated and monounsaturated FA percentages in the milk were lower for the CB-TMR than the MS ($p < 0.001$). The milk n-3, C18:3, and conjugated linoleic acid percentages were lower for the CB-TMR than the MS ($p < 0.001$). The milk n-3 and C18:3 were higher for the CB-GRZ than the OD-GRZ ($p < 0.01$), but no differences were observed between the MS in the MilkP and cheese. In conclusion, CB-GRZ cows during confinement produced better quality milk compared to OD-GRZ cows. However, the FA profiles of the milk, MilkP, and cheese were affected to a greater extent by the feeding management than by the conditions during confinement.

Keywords: dairy products; lipid; bovine; grazing; total mixed ration; climatic condition

1. Introduction

Milk fat is one of the main components that determines the nutritional value of milk for humans, due to its energetic content and the bioactive properties of some of its fatty acids for human health [1,2]. The fatty acid profile (FAP) of milk is characterized by a higher proportion of saturated fatty acids (SFA) and a lower proportion of monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) [3]. The fat content and FAP are the most variable components of milk. One of the factors that most influences its variability is the nutritional management of the cow [4–7]. Pasture-based feeding systems or the inclusion of fresh pasture in the diet of confined dairy cows improves the FAP of milk, benefiting human health [8,9]. In this sense, in pasture-based systems, the milk's SFA content (C12:0, C14:0, and C16:0) and omega 6/omega 3 (n-6/n-3) are lower and some PUFAs are higher than those in TMR systems, such as 18:3 (n-3) and C18:2 (cis9-trans 11; conjugated linoleic acid: CLA), which is essential for humans [7,10–14]. The differences generated by changes in a cow's diet that have reported in milk FAPs are also reflected in cheeses. Higher concentrations of vaccenic acid (VA), CLA, and C18:3 (n-3) have been obtained from cheeses elaborated from pasture-based milk systems [15–18]. Therefore, pasture inclusion in a dairy cow's diet improves the nutritional profile of the FA in its milk and cheese, due to an increase in some of the FAs that are beneficial for human health, such as CLA, VA, and C18:3 (n-3), and a reduction in the n-6/n-3 ratio.

Cows are exposed to environmental and seasonal weather changes within pasture-based systems [19]. In countries with temperate climates, cows graze throughout the year, resulting in a better nutritional quality of their milk than that in confined systems (i.e., [9,20]). However, climate change has caused more extreme weather events, such as increased temperatures in summer, droughts, frosts in spring, and heavy rains, which affect the growth of the pastures and the time available for grazing. Therefore, these climatic phenomena become more important in temperate countries [21–23]. Extreme weather conditions can affect the physiology (an energy demand for thermoregulation, a decrease in dry matter intake, and a decrease in milk production) and behavior of dairy cows [24–27]. Cows can suffer from heat stress during spring–summer, which impacts their milk production and its quality. It has been reported that heat stress (a temperature humidity index (THI) above 68) negatively impacts their welfare [28], milk production, and its yield and fat content [29–32], as well as the milk's FAP [33]. Salamon et al. [34] showed higher percentages of C18:2, C18:3 (n-3), C18:1 (Oleic), and CLA in milk obtained during summer compared to those in milk from winter. In addition, lipid catabolism in thermally stressed dairy cows leads to an increased plasmatic FA content (as linolenic and oleic), and consequently, increased long-chain FAs and decreased short- and medium-chain fatty acids in the milk [33]. Furthermore, higher levels of MUFAs, PUFAs, and CLAs and lower n-6/n-3 ratios and palmitic acid concentrations (C16:0) in milk and cheese have been reported during spring compared to winter and autumn [35–37]. A possible explanation for this may be the proportion of conserved forages in the diet (mainly autumn), which have a poor fat quality compared to spring diets; thus, more fresh pasture is generally included in these diets [22,36]. In addition, in pasture-based systems, the season determines the rate of the grass growth, which affects the quality, digestibility, and consumption of the grass [38]. Therefore, in pasture-based systems, the season of the year could impact the FAP and cheese quality.

Uruguayan dairy systems are characterized by pasture-based systems (pasture 55%, supplementation with conserved forage 19%, and concentrates 25%) [22]. Unlike confined systems, pasture-based systems have the advantage of cows being able to display behaviors that are typical of their species, which therefore improves the animal welfare, but they are also more exposed to extreme environmental conditions, such as heavy rains and mud in winter or heat stress in summer, which could also affect their welfare [39,40]. Most dairy systems in Uruguay keep cows confined in open sky paddocks when they are not grazing, without shade over the resting and feeding areas [41]. Pasture-based system cows are supplemented in open areas without environmental protection (i.e., shade and a roof), and only 22% of dairy farms provide supplementation in specific feeding facilities [41]. However, over the last few years, confinement (places where cows are kept while they are not in grazing) has changed. There are many farms where confinements have fully covered sheds, drinkers, feeders, and different types of bedding. These confinements are characterized because the cows have little exposure to the external climate (a better control of the environment and better comfort). Some examples of these are the systems called "compost barns" or "compost-bedded pack barns" [42,43].

The milk and cheese quality from different feeding systems (confinement vs. pasture) and different seasons have been studied, but confounding effects between the environmental factors and feeding systems are evident [16,44,45]. To our knowledge, there are no studies that have evaluated how different facility conditions (compost-bedded pack barns vs. outdoor soil-bedded pen) during confinement in a mixed system (MS; under the same feeding condition: grazing + TMR) influence the milk fat quality and affect the dairy products' quality (i.e., cheeses). Therefore, firstly, we hypothesized that both MSs would achieve a healthier FAP in the milk and cheese from spring calving cows compared to the confined system (CB-TMR), although we predicted a lower milk and fat yield. Secondly, we hypothesized that different facility conditions during confinement in an MS (compost-bedded pack barns vs. outdoor soil-bedded pen) during summer could impact the FAP in the milk and cheese from spring calving cows. The aim of this study was to compare the milk and cheese FAPs from MSs with different facility conditions during confinement (compost-bedded pack barns vs. outdoor soil-bedded pen), and compare them with a confinement system (100% TMR) in spring calving cows from a compost-bedded pack barns.

2. Materials and Methods

2.1. Location, Animals, and Treatments

The experimental protocol was evaluated and approved by the Comisión Honoraria de Experimentación Animal (CHEA), Universidad de la República, Montevideo, Uruguay (ID 682; Exp: 020300-000602-18). The study was conducted at the Estación Experimental 'Dr. M. A. Cassinoni"-Facultad de Agronomía Paysandú, Uruguay (Location 32°22′49″ S, 58°03′04″ W).

In total, thirty-six spring calving Holstein cows with 2.7 ± 1.2 lactation and 620 ± 64 kg of body weight were used. The average calving date was 16 August 2019 ± 8.2 d. All the cows were under the same management and feeding conditions throughout the 21 d before the expected calving date. The cows were blocked by their calving date, number of lactations, pre-calving body conditions, and body weight, randomly assigned to one of the following treatments immediately after calving, and grouped to 4 cows/pen. This constituted three treatments: (1) cows that were fed with TMR ad libitum in compost-bedded pack barns (CB) facilities (CB-TMR, $n = 12$) (a low environment exposure); (2) mixed-system (MS) cows in CB facilities (a low environment exposure, same barn as previous) during confinement (CB-GRZ, $n = 12$); and (3) mixed-system cows in outdoor soil-bedded pens during confinement (a high environment exposure) (OD-GRZ, $n = 12$).

In the CB-TMR and CB-GRZ systems, the cows were confined within a fully roofed barn with CBs, fans, and sprinklers (activated when the temperature exceeded 25 °C) and protected from solar radiation, rain, mud, and wind (a low environment exposure). Each pen was 6 m wide by 13.5 m long (9 m of bed and 4.5 m of feeder space). In the OD-GRZ treatment, the cows were confined in an outdoor soil-bedded pen with shade. The outdoor soil-bedded pens (OD) consisted of 2 paddocks (which alternated according to the mud conditions), each with the following measurements: 5.2 m wide by 46 m long (shaded area: 4.8 m^2/cow). The water was disposed of ad libitum for all the treatments.

The total mixed rations were composed of whole plant sorghum or corn silage (CP: 5.9, 5.8%; NDF: 51.5, 41.7%; and ADF: 30.7, 22.5%; respectively), rye grass silage (CP: 10.9%; NDF: 49.6%; and ADF: 29.9%), fescue hay (CP: 9.7%; NDF: 68.3%; and ADF: 37.4%), and a commercial concentrate mix (CP: 25.5%; NDF: 34.5%; and ADF: 13.5%). Their chemical composition is presented in Table 1. The TMRs were formulated according to the National Research Council [46], for a body weight of 650 kg and 45 L/d of milk production (4% milk fat) for the CB-TMR treatment. In the MSs' (CB-GRZ and OD-GRZ), the supplementation with TMRs was adjusted to ensure that the cow requirements and productivity goals were met. In the CB-TMR treatment, the cows were fed ad libitum twice a day (5:30 am and 8:00 pm). In the mixed systems, TMRs were offered once a day at 8:00 am from November to April.

Table 1. Chemical compositions and fatty acid profiles in pasture and total mixed rations (TMR) during Section 1: individual milk samples in both moments of lactation (ML1 and ML2) and Section 2: MilkP and cheese manufacture.

	Individual Milk Samples				Cheese Manufacture	
	Section 1				Section 2	
	ML1		ML2			
	TMR	Pasture	TMR	Pasture	TMR	Pasture
CP (% DM)	21.6	12.2	20.1	14.2	16.4	21.25
NDF (% DM)	34	59	33	26	26	44
ADF (% DM)	19	30	19	19	15	20
FAs (g/100 g)						
C8:0	0.07	nd	0.11	nd	0.5	0.7
C10:0	0.04	1.56	0.02	nd	1.1	1.5
C12:0	0.35	0.05	0.24	0.55	1.4	1.9
C14:0	0.74	0.57	0.59	0.65	4.3	5.6
C14:1	0.08	0.7	0.08	0.11	0.3	0.5
C15:0	0.07	3.17	0.08	0.83	0.5	0.7
C16:0	31.95	20.74	28.27	18.09	21.6	25.1
C16:1	0.47	2.19	0.49	1.93	0.8	0.9
C17:0	0.15	0.37	0.12	0.24	0.4	0.6
C17:1	0.03	0.03	0.04	nd	0	0.1
C18:0	3.51	2.04	2.99	5.23	4.8	6.9
C18:1cis	29.71	5.7	34	4.17	32	14.9
C18:1trans	0.19	nd	0.22	nd	0.9	1.7
C18:2cis	24.34	12.63	24.3	17.25	23.9	12.1
C18:3 (n-3)	3.86	32.51	3.97	39.1	4.3	18.5
C18:3 (n-6)	0.03	0.18	0.06	0.13	.	.
C20:0	0.45	0.77	0.44	1.05	0.3	0.4
C22:0	0.15	2.56	0.42	1.78	0.3	0.3
C23:0	nd	nd	nd	nd	0.4	1.5
C24:0	0.35	0.89	0.37	2	0.2	0.2
Ether extract (g/100)	3.5	1.6	4.1	1.5	3.5	2.4

ML1: 80 ± 15 days in milk (DIM), ML2: 155 ± 15 DIM. CP: crude protein; NDF: neutral detergent fiber; and ADF: acid detergent fiber. nd: not detected.

The pasture was composed of tall fescue (*Festuca arundinacea*) (October–spring), soy (*Glycine max*) (January–summer), and lucerne (*Medicago sativa*) + orchard grass (*Dactylis glomerata*) (March–late summer). The herbage allowances (HA) were 28 kg DM/cow/day, 46 kg DM/cow/day, and 31 kg DM/cow/day, respectively. The herbage mass (kg DM/ha) at the ground level was estimated weekly using the double sampling technique adapted from Haydock and Shaw [47]. The method was calibrated fortnightly using a 5-point scale with 3 replicates for each point. The herbage allowance was then determined, adjusting the daily strip area for grazing. The grazing occurred in weekly occupation plots.

The THI values and precipitation from 10 d prior to the milk and cheese sampling are shown in Figure 1. The temperature humidity index was calculated according to Bernabucci et al. [29].

2.2. Experimental Design

Based on the times of the sample collection (spring and summer vs. late summer) and the type of the samples (individual milk vs. pooled milk and cheese), the study was divided into two sections. The first section was based on individual cow milk during spring and summer, and the second section was based on polled milk and cheese during late summer (Figure 2).

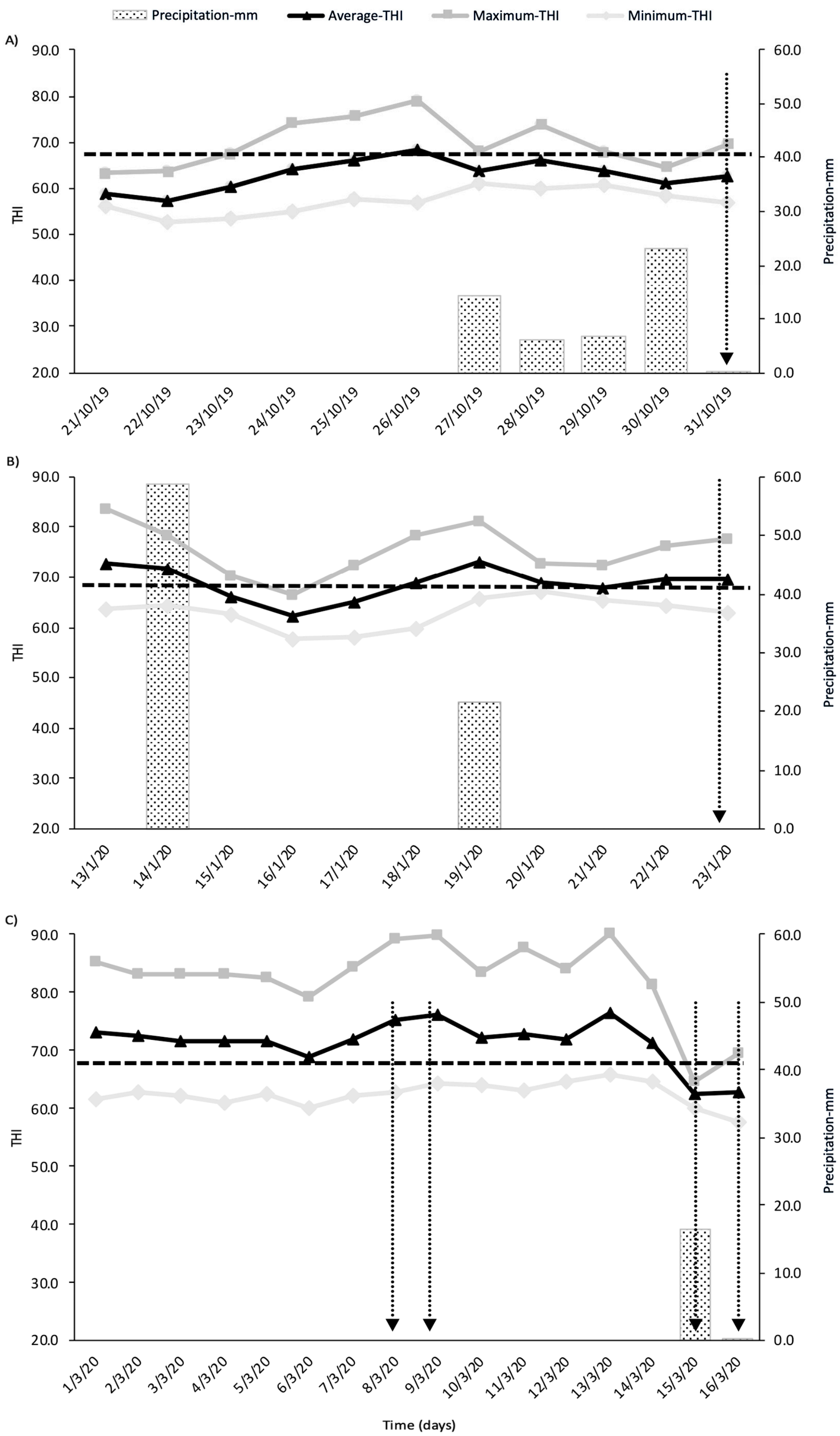

Figure 1. Temperature humidity index (THI) (average, maximum, and minimum) and precipitation (mm) (**A**) 10 days before milk sampling in spring (ML1); (**B**) 10 days before milk sampling in summer (ML2); and (**C**) late summer (MilkP sampling for cheese manufacture). Dotted arrow indicates sampling time.

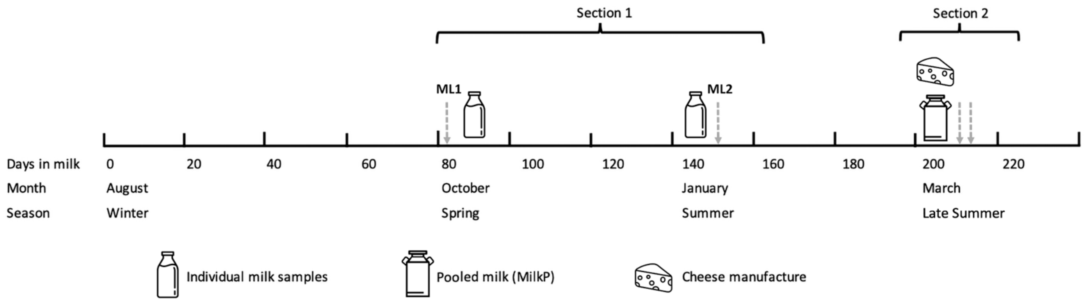

Figure 2. Experimental design. Section 1: Individual cow milk during spring and summer. Section 2: Pooled milk and cheese manufacture during late summer.

2.2.1. Individual Cow Milk during Spring and Summer

All the cows were milked twice a day (4:00 am and 5:00 pm) and the individual milk yields were individually recorded.

The fat-corrected milk (FCM) yield was standardized at 3.5% fat and calculated according to Pastorini et al. [48] and Mendoza et al. [49]

The total mixed rations, pasture samples (Table 1), and individual milk samples (the composite sample representative of both daily milkings) were collected at two moments of lactation: ML1: 80 ± 15 days in milk (DIM; October: spring; early lactation) and ML2: 155 ± 15 DIM (January: summer; mid-lactation)

2.2.2. Pooled Milk and Cheese Manufacture during Late Summer

For the cheese making, pooled milk (MilkP) was collected from both milkings (am and pm) in each pen into 60 L buckets. The trials were performed over two consecutive weeks during March (late summer: 210 ± 15 DIM). The pooled milk was obtained from 2 pens for each treatment in each week. This resulted in a total of 12 cheeses being manufactured in the experiment (each treatment: $n = 4$). This study followed a similar methodology to other studies, with the production system having effects on the characteristics of the cheese [16,44,45]. Unlike the aforementioned studies, ours used more experimental units. The pooled milk was stored and transported under refrigerated conditions (4 °C) to the Latitud–Fundación LATU pilot plant (Montevideo, Uruguay). The MilkP was standardized to 3.0% fat in a Westfalia Separator skimmer model LWA 205-1 (Oelde, Germany). Dambo-type cheeses were produced, as previously described by Escobar et al. [50]. Briefly, the cheese making process was carried out in a 50 L double O vat with a double jacket and a controlled mechanical agitation and cutting system. The milk was pasteurized at 72 °C for 15 s when the milk had cooled to 32 °C and $CaCl^2$ (Promilk®, Arras, France) and a mesophilic homofermentative starter culture (CHR HANSEN R 704, Christian Hansen, Denmark) were added. Thirty minutes later, the coagulant 100% chymosin (Maxiren®, Hørsholm, Denmark) was added, and the cultured milk was allowed to set for approximately 30 min. The curd was cut until a size of "corn grain" was obtained, which was cooked until 42 °C (stirring continuously). After this cooking, the whey was drained off, and the curd was distributed into a 1 kg polypropylene cylinder mold and pressed for 3 h (with intermediate rotations). The acidification of the cheese was measured after the pressing, and the pH was checked until it reached the final pH of 5.40 ± 0.1, where it was kept at 4 °C for 16 h. Afterward, the cheeses were salted in brine (19 °B) for 5 h. Then, they were kept in a chamber at 4 °C ± 2 for 24 h, after which, the cheeses were vacuum packed. The cheeses were kept in a ripening chamber at 12 °C for 30 d until the analysis.

The total mixed rations and pasture were taken on the same day of the milk collection (MilkP) for the cheese manufacture (Table 1). The MilkP samples for the FAP analysis were taken from the pilot plant (LATU) after the pasteurization (before cheese manufacture) and the cheese samples were taken after 30 d of ripening.

2.3. Samples Analysis

2.3.1. Fat Yield

The milk fat content (%) was determined using LactoScope FT infrared (FTIR) (Delta Instruments, Drachten, The Netherlands) at the COLAVECO in Colonia Suiza, Uruguay.

2.3.2. Fatty Acid Profile in Milk (Individual and Pooled) and Cheeses

The milk fat was extracted according to Hara and Radin [51] and the cheese fat was extracted according to Folch et al. [52]. FA methyl esters were prepared via the trans-methylation procedure described by Mossoba et al. [53]. These fatty acid methyl esters were quantified using a gas chromatograph (Agilent Technologies 6890, Palo Alto, CA, USA) and a mass spectrometer (Agilent Technologies 5973), as described by Grille et al. [7]. The samples were run in duplicate and the FAME standards (Supelco 47885-U, Bellefonte; 37 FAME from C4:0 to C24:0) were analyzed at regular intervals for quality control purposes and to determine the recovery and correction factors for the individual FAs. The intra- and inter- assay coefficients of variation for each measured analyte were, on average, 3% and 6%, respectively. The milk fat compositions were expressed in grams of each individual FA per 100 g of the total FAs. The de novo FAs were considered to be the sum of the C4:0 to C15:1 FAs, the mixed FAs to be the sum of C16 and C16:1, and the preformed FAs to be a sum of >C17:0. The Atherogenicity and Thrombogenicity indexes (AI and TI) in the milk were calculated as described by Ulbricht and Southgate [54]. The Atherogenicity index (AI) was calculated as (12:0 + 4 × 14:0 + 16:0/(MUFA + PUFA) and the Thrombogenicity index (TI) was (C14:0 + C16:0 + C18:0) (0.5 × MUFA + 0.5 × (n − 6) +3 ×(n − 3)/(n − 6). The FA analysis was performed at the Technological Laboratory of Uruguay (LATU) in Montevideo, Uruguay.

2.3.3. Pasture and TMR Chemical Composition and Fatty Acid Profile

The pasture and TMRs were weighed and oven dried at 55 °C for 48 h, in order to be later analyzed to determine their dry matter (DM), crude protein (CP), neutral detergent fiber (NDF), and acid detergent fiber (ADF) contents, according to AOAC (2000) [55]. The total nitrogen for the CP estimation was analyzed with the Kjeldahl method according to AOAC (1984), as were the digestion with sulfuric acid and the subsequent distillation and titration [56]. The neutral detergent fiber used α-amylase and both the NDF and ADF were measured using an ANKOM200 Fiber Analyzer (ANKOM Technology Corp., Fairport, NY, USA), according to Méndez et al. [23]. The lipids were extracted according to Folch et al. [52], and the FAP was performed as previously described for the milk and cheese samples.

2.4. Statistical Analysis

The milk yield (kg/d), fat yield (kg/d), fat content (%), and FAP (%) from Section 1 were analyzed with an ANOVA for repeated measures using the GLIMMIX of SAS (SAS Studio, Shinjuku, Tokyo). The Shapiro test was used to evaluate the normal distribution of the data. The goodness of fit for each model was checked by a visual inspection of the residuals. The election of the final models considered the Akaike's information criterion (AIC). The statistical model for the milk yield, fat (content and yield), and FAP included the treatments (CB-TMR, CB-GRZ, and OD-GRZ), moments of lactation (ML1 and ML2), and interactions between the treatments and moments of lactation as fixed effects, and the cow was considered as a random effect within each treatment. For the individual milk data (Section 1), the experimental unit was the cow, while for the MilkP and cheese (Section 2), the pen was considered as the experimental unit and the treatment was considered as a fixed effect. In Section 2, the week was considered as a random effect. In all the variables, the post-calving days were included as co-variables. Post hoc comparisons were performed with the Tukey–Kramer test. For a better visualization of the results, when the data sets from both moments of lactation (ML1 and ML2) in each treatment were analyzed, they were expressed as the average of the treatments (AT). In total, three treatments (CB-TMR, CB-GRZ, and OD-GRZ) were analyzed and the data sets from each moment of lactation were expressed as the average lactation moment (AML), either for the M1 (AML1) or M2 (AML2). The results were considered to be significant with an alpha of ≤0.05 and trends between 0.05 and 0.10. The data are presented as mean ± SEM (standard error of the mean).

3. Results

3.1. Milk Yield and Fat (% and kg/d)

There were treatment and moment of lactation effects on the milk and fat yields (kg/d), but the interactions between the factors were not significant (Table 2). The milk and fat yields were greater for the CB-TMR treatment than for the CB-GRZ and OD-GRZ treatments ($p < 0.001$), and there was no difference between both the MSs (CB-GRZ and OD-GRZ). The milk yield was higher for AM1 than AM2 ($p = 0.03$) and the fat yield tended to be higher for AML1 than AML2 ($p = 0.07$). There were no treatment and moment of lactation effects and there was no interaction between the treatments and moments of lactation in the milk fat content (%) (Table 2).

Table 2. Milk yield and fat (mean ± SEM) in confined (CB-TMR) and mixed systems with low (CB-GRZ) and high (OD-GRZ) environmental exposure in spring calving cows at two moments of lactation (ML1 and ML2).

	CB-TMR			CB-GRZ			OD-GRZ					*p* Value		
	ML1	ML2	AT	ML1	ML2	AT	ML1	ML2	AT	AML1	AML2	T	ML	T * ML
Milk yield (kg/d)	41.3 ± 1.0	39.4 ± 1.0	40.7 ± 0.7 ^{x}	29.9 ± 1.1	27.7 ± 1.1	28.3 ± 0.7 ^{y}	28.7 ± 1.0	26.5 ± 1.0	27.5 ± 0.7 ^{y}	32.9 ± 0.6 ^{A}	31.1 ± 0.6 ^{B}	<0.001	0.03	ns
3.5% FCM (kg/d)	39.5 ± 1.1	36.5 ± 1.1	38.5 ± 0.8 ^{x}	26.8 ± 1.2	25.9 ± 1.2	26.4 ± 0.8 ^{y}	27.4 ± 1.2	25.7 ± 1.2	26.6 ± 0.8 ^{y}	31.4 ± 0.7 ^{A}	29.5 ± 0.7 ^{B}	<0.001	0.04	ns
Fat														
(%)	3.4 ± 0.1	3.2 ± 0.1	3.4 ± 0.1	3.1 ± 0.2	3.2 ± 0.2	3.1 ± 0.1	3.3 ± 0.2	3.3 ± 0.2	3.3 ± 0.1	3.3 ± 0.1	3.2 ± 0.1	ns	ns	ns
(kg/d)	1.4 ± 0.05	1.2 ± 0.05	1.3 ± 0.04 ^{x}	0.9 ± 0.05	0.8 ± 0.05	0.9 ± 0.04 ^{y}	0.9 ± 0.05	0.8 ± 0.05	0.9 ± 0.04 ^{y}	1.05 ± 0.03	0.98 ± 0.03	<0.001	0.07	ns

T: treatment; ML: moments of lactation; and T * ML: interaction between treatments and moments of lactation. Treatments: CB-TMR, CB-GRZ, and OD-GRZ. ML1: 80 ± 15 days in milk (DIM), and ML2: 155 ± 15 days in milk (DIM). AT: average from both moments of lactation in each treatment; AML1: average from treatments (CB-TMR, CB-GRZ, and OD-GRZ) in ML1; and AML2: average from treatments (CB-TMR, CB-GRZ, and OD-GRZ in ML2). Fat-corrected milk standardized at 3.5% fat (3.5% FCM). Different letters indicate significant difference ($p < 0.05$): ^{x,y} indicate significant difference between AT; ^{A,B} indicate significant difference between AML. ns: no significance. 3.2. Milk fatty acid profile.

3.1.1. Individual Cow Milk during Spring and Summer

There was a treatment effect ($p < 0.05$) on the SFAs, UFAs, and MUFAs, n-3, n-6, and n-6/n-3, trans, mixed, and preformed FA content, and atherogenicity (AI) and thrombogenicity indexes (TI) of the milk (Table 3). The saturated FAs, n-6, n-6/n-3 ratio, AI, and TI were greater for the CB-TMR treatment than the CB-GRZ and OD-GRZ treatments ($p < 0.001$), and CB-TMR had lower UFAs, MUFAs, and trans FAs than CB-GRZ and OD-GRZ ($p < 0.001$). Additionally, CB-TMR had a lower n-3 than the milk from the CB-GRZ cows ($p = 0.0001$), but no difference was observed for OD-GRZ. Between the mixed systems, CB-GRZ had a greater n-3 than OD-GRZ ($p = 0.005$). There was moment of lactation effect on the SFAs, UFAs, MUFAs, n-6, novo FAs, mixed FAs, AI, and TI (Table 3). The saturated FAs, n-6, de novo, AI, and TI decreased from AML1 to AML2 ($p < 0.01$). The MUFAs, UFAs, and mixed FAs increased from AML1 to AML2 ($p < 0.001$). The polyunsaturated FAs tended to be greater at AML1 than AML2 ($p = 0.08$). There was an interaction of the n-3, n-6, and mixed FAs between the treatment and the moment of lactation (Table 3). In ML1, CB-GRZ had a greater n-3 than CB-TMR and OD-GRZ ($p = 0.02$; $p = 0.004$). For ML2, CB-GRZ had a greater n-3 than CB-TMR ($p = 0.04$), while OD-GRZ was intermediate. None of the three treatments showed differences in their n-3 values between ML1 and ML2 (Table 3). The milk of CB-TMR had a greater n-6 than that of CB-GRZ and OD-GRZ ($p < 0.001$) at ML1 and ML2, and no differences were observed between the mixed systems at both moments of lactation. The mixed FAs at ML1 were higher for CB-TMR than CB-GRZ and OD-GRZ ($p < 0.0001$), which were similar to each other. At ML2, no differences were found between the treatments for the mixed FAs (Table 3). The main changes in the SFAs were due to C4:0; C6:0; C8:0; C10:0, and C12:0, which were greater for CB-TMR than CB-GRZ and OD-GRZ, but no differences were observed between both MSs (Table 4). Regarding C14:0 and C16:0, there was an interaction between the treatment and moment of lactation (Table 4). At ML1, the C16:0 in the milk was greater for CB-TMR than the MS ($p < 0.001$), but the C14:0 was lower for CB-TMR and CB-GRZ than OD-GRZ ($p = 0.04$). At ML2, no differences were found between the treatments for any FAs. Regarding PUFAs, C18:2, CLA, and C18:3 had a treatment effect (Tables 3 and 4). Both FAs were greater for CB-GRZ and OD-GRZ than CB-TMR ($p < 0.0001$). Furthermore, the C18:3 was greater for CB-GRZ than OD-GRZ ($p = 0.001$) (Table 4).

Table 3. Fatty acid profile (FAP) (mean ± SEM) in confined (CB-TMR) and mixed systems with low (CB-GRZ) and high (OD-GRZ) environmental exposure in spring calving cows at two moments of lactation (ML1 and ML2).

	CB-TMR			CB-GRZ			OD-GRZ					*p* Value		
	ML1	**ML 2**	**AT**	**ML 1**	**ML 2**	**AT**	**ML 1**	**ML 2**	**AT**	**AML 1**	**A ML2**	**T**	**ML**	**T * ML**
FA saturation (g/100 g of fat)														
SFA	65.7 ± 0.5	62.8 ± 0.5	64.3 ± 0.4 ^{x}	62.4 ± 0.6	61.4 ± 0.6	61.9 ± 0.4 ^{y}	63.4 ± 0.6	61.6 ± 0.6	62.3 ± 0.4 ^{y}	63.7 ± 0.3 ^{A}	61.9 ± 0.3 ^{B}	<0.001	<0.001	ns
UFA	34.2 ± 0.5	37.1 ± 0.5	35.7 ± 0.4 ^{y}	37.5 ± 0.6	38.4 ± 0.6	37.9 ± 0.4 ^{x}	36.5 ± 0.6	38.2 ± 0.6	37.4 ± 0.4 ^{x}	36.1 ± 0.3 ^{B}	37.9 ± 0.3 ^{A}	<0.001	<0.001	ns
MUFA	29.1 ± 0.5	32.3 ± 0.5	30.7 ± 0.3 ^{y}	32.7 ± 0.5	33.7 ± 0.5	33.2 ± 0.4 ^{x}	31.8 ± 0.5	33.6 ± 0.5	32.7 ± 0.4 ^{x}	31.2 ± 0.3 ^{B}	33.2 ± 0.3 ^{A}	<0.001	<0.001	ns
PUFA	5.1 ± 0.1	4.8 ± 0.1	4.9 ± 0.1	4.8 ± 0.1	4.7 ± 0.1	4.7 ± 0.1	4.7 ± 0.1	4.6 ± 0.1	4.7 ± 0.1	4.9 ± 0.1	4.7 ± 0.1	ns	0.08	ns
n-3	0.48 ± 0.04 ^{bc}	0.45 ± 0.04 ^{c}	0.46 ± 0.02 ^{y}	0.67 ± 0.04 ^{a}	0.61 ± 0.04 ^{ab}	0.64 ± 0.03 ^{x}	0.43 ± 0.04 ^{c}	0.57 ± 0.04 ^{abc}	0.51 ± 0.03 ^{y}	0.53 ± 0.03	0.54 ± 0.03	<0.001	ns	0.03
n-6	3.4 ± 0.1 ^{a}	2.9 ± 0.1 ^{b}	3.1 ± 0.07 ^{x}	2.6 ± 0.1 ^{bc}	2.4 ± 0.1 ^{c}	2.5 ± 0.07 ^{y}	2.2 ± 0.1	2.3 ± 0.1 ^{c}	2.2 ± 0.07 ^{y}	2.7 ± 0.05 ^{A}	2.5 ± 0.06 ^{B}	<0.001	0.03	0.02
n6/n3	7.1 ± 0.3	6.4 ± 0.3	6.8 ± 0.1 ^{x}	3.4 ± 0.3	4.7 ± 0.3	3.8 ± 0.2 ^{y}	4.4 ± 0.3	3.9 ± 0.3	4.2 ± 0.2 ^{y}	4.9 ± 0.2	4.8 ± 0.1	<0.001	ns	0.08
Trans	4.1 ± 0.2	4.2 ± 0.2	4.2 ± 0.1 ^{y}	5.1 ± 0.2	5.2 ± 0.2	5.2 ± 0.1 ^{x}	5.5 ± 0.2	5.1 ± 0.2	5.3 ± 0.1 ^{x}	4.9 ± 0.1	4.8 ± 0.1	<0.001	ns	ns
FA origin (g/100 g of fat)														
De novo (C4:0-C15:1)	19.3 ± 0.5	17.2 ± 0.5	18.2 ± 0.3	19.4 ± 0.5	16.1 ± 0.5	17.7 ± 0.3	19.5 ± 0.5	15.9 ± 0.5	17.7 ± 0.3	19.4 ± 0.3 ^{A}	16.4 ± 0.3 ^{B}	ns	<0.001	ns
Mixed origin (C16:0 + C16:1)	37.6 ± 0.5 ^{a}	37.4 ± 0.5 ^{a}	37.5 ± 0.4 ^{x}	33.6 ± 0.5 ^{b}	36.6 ± 0.5 ^{a}	35.1 ± 0.4 ^{y}	33.2 ± 0.5 ^{b}	36.5 ± 0.5 ^{a}	34.8 ± 0.4 ^{y}	34.8 ± 0.3 ^{B}	36.8 ± 0.3 ^{A}	<0.001	<0.001	0.002
Preformed (>C17:0)	43.6 ± 0.7	45.2 ± 0.7	44.2 ± 0.5 ^{x}	46.9 ± 0.7	47.1 ± 0.7	47.0 ± 0.5 ^{y}	46.5 ± 0.7	47.4 ± 0.7	46.9 ± 0.5 ^{y}	45.5 ± 0.7	46.5 ± 0.7	<0.001	0.06	ns
SFA/UFAratio	1.9 ± 0.04	1.7 ± 0.04	1.8 ± 0.03 ^{x}	1.6 ± 0.04	1.5 ± 0.04	1.6 ± 0.03 ^{y}	1.7 ± 0.04	1.6 ± 0.04	1.6 ± 0.03 ^{y}	1.7 ± 0.02 ^{A}	1.6 ± 0.02 ^{B}	<0.001	<0.001	ns
Atherogenicity index (AI)	2.2 ± 0.05	1.9 ± 0.05	2.1 ± 0.04 ^{x}	1.9 ± 0.05	1.8 ± 0.05	1.9 ± 0.04 ^{y}	2.1 ± 0.06	1.8 ± 0.06	1.9 ± 0.04 ^{y}	2.1 ± 0.03 ^{A}	1.9 ± 0.03 ^{B}	<0.001	<0.001	ns
Thrombogenicity index (TI)	3.4 ± 0.05	3.0 ± 0.07	3.2 ± 0.05 ^{x}	2.9 ± 0.08	2.8 ± 0.07	2.8 ± 0.05 ^{y}	3.0 ± 0.07	2.9 ± 0.07	2.9 ± 0.05 ^{y}	3.1 ± 0.04 ^{A}	2.9 ± 0.04 ^{B}	<0.001	0.005	ns

T: treatment; ML: moments of lactation; and T * ML: treatment and moments of lactation interaction effect. Treatments: CB-TMR, CB-GRZ, and OD-GRZ. ML1: 80 ± 15 days in milk (DIM), and ML2: 155 ± 15 DIM. AT: average of both moments of lactation in each treatment; AML1: average of treatments in ML1; and AML2: average of treatments in ML2. Different letters indicate significant difference ($p < 0.05$): ^{x,y} indicate significant difference between AT; ^{A,B} indicate significant difference between AML; and ^{a,b,c} indicate significant difference in treatment by moment of lactation interaction. ns: not significant. SFA: saturated; MUFA: monounsaturated; PUFA: polyunsaturated; and UFA: unsaturated.

Table 4. Individual fatty acid profilse (FAP) (mean ± SEM) in confined (CB-TMR) and mixed systems with low (CB-GRZ) and high (OD-GRZ) environmental exposure in spring calving cows at two moments of lactation (ML1 and ML2).

	CB-TMR			CB-GRZ			OD-GRZ					*p* Value		
	ML1	**ML2**	**AT**	**ML1**	**ML2**	**AT**	**ML1**	**ML2**	**AT**	**AML1**	**AML2**	**T**	**ML**	**T * ML**
FA (g/100 g of fat)														
C4:0	0.84 ± 0.03	0.6 ± 0.03	0.72 ± 0.02 ^{x}	0.79 ± 0.03	0.52 ± 0.03	0.65 ± 0.02 ^{xy}	0.7 ± 0.03	0.52 ± 0.03	0.61 ± 0.02 ^{y}	0.77 ± 0.02 ^{A}	0.55 ± 0.02 ^{B}	0.003	<0.001	ns
C6:0	0.94 ± 0.03	0.73 ± 0.03	0.83 ± 0.02 ^{x}	0.85 ± 0.03	0.67 ± 0.03	0.76 ± 0.02 ^{y}	0.84 ± 0.03	0.61 ± 0.03	0.72 ± 0.02 ^{y}	0.88 ± 0.01 ^{A}	0.67 ± 0.01 ^{B}	0.001	<0.001	ns
C8:0	0.77 ± 0.02	0.6 ± 0.02	0.68 ± 0.02 ^{x}	0.70 ± 0.03	0.52 ± 0.03	0.68 ± 0.02 ^{y}	0.66 ± 0.03	0.47 ± 0.03	0.57 ± 0.002 ^{y}	0.71 ± 0.02 ^{A}	0.53 ± 0.01 ^{B}	<0.001	<0.001	ns
C10:0	2.2 ± 0.07	1.7 ± 0.02	1.2 ± 0.05 ^{x}	1.9 ± 0.08	1.4 ± 0.08	1.7 ± 0.06 ^{y}	1.7 ± 0.08	1.3 ± 0.08	1.5 ± 0.06 ^{y}	1.9 ± 0.04 ^{A}	1.5 ± 0.04 ^{B}	<0.001	<0.001	ns
C12:0	2.5 ± 0.08	2.2 ± 0.08	2.4 ± 0.05 ^{x}	2.3 ± 0.08	1.8 ± 0.08	2.1 ± 0.06 ^{y}	2 ± 0.08	1.8 ± 0.08	1.9 ± 0.06 ^{y}	2.3 ± 0.04 ^{A}	1.9 ± 0.04 ^{B}	<0.001	<0.001	ns
C14:0	9.7 ± 0.3 ^{b}	9.0 ± 0.2 ^{bc}	9.3 ± 0.25	9.7 ± 0.3 ^{b}	8.5 ± 0.2 ^{c}	9.1 ± 0.26	10.8 ± 0.3 ^{a}	8.4 ± 0.2 ^{c}	9.6 ± 0.3	10.3 ± 0.2 ^{A}	8.26 ± 0.2 ^{B}	ns	<0.001	0.005
C14:1 cis	0.63 ± 0.05 ^{b}	0.81 ± 0.05 ^{ab}	0.72 ± 0.03	0.88 ± 0.06 ^{a}	0.76 ± 0.06 ^{ab}	0.82 ± 0.04	0.64 ± 0.06 ^{ab}	0.76 ± 0.06 ^{ab}	0.72 ± 0.04	0.72 ± 0.03	0.78 ± 0.03	0.08	ns	0.03
C15:0	1.2 ± 0.04	1.2 ± 0.04	1.2 ± 0.03 ^{y}	1.9 ± 0.04	1.5 ± 0.04	1.7 ± 0.03 ^{x}	1.7 ± 0.04	1.6 ± 0.04	1.7 ± 0.03 ^{x}	1.6 ± 0.02 ^{A}	1.46 ± 0.02 ^{B}	<0.001	<0.001	ns
C16:0	35.7 ± 0.5 ^{a}	35.3 ± 0.5 ^{a}	35.5 ± 0.35 ^{x}	31.2 ± 0.5 ^{b}	34.3 ± 0.5 ^{a}	32.7 ± 0.38 ^{y}	31.3 ± 0.5 ^{b}	34.1 ± 0.5 ^{a}	32.7 ± 0.29 ^{y}	32.7 ± 0.3 ^{B}	34.6 ± 0.3 ^{A}	<0.001	<0.001	0.001
C16:1 cis	1.6 ± 0.08 ^{ab}	1.8 ± 0.08 ^{a}	1.7 ± 0.06	1.8 ± 0.09 ^{a}	1.8 ± 0.09 ^{a}	1.8 ± 0.06	1.3 ± 0.09 ^{a}	1.9 ± 0.09 ^{a}	1.6 ± 0.06	1.3 ± 0.05 ^{B}	1.9 ± 0.05 ^{A}	ns	<0.001	0.001
C16:1 trans	0.33 ± 0.03 ^{d}	0.35 ± 0.03 ^{cd}	0.35 ± 0.02 ^{z}	0.51 ± 0.03 ^{b}	0.44 ± 0.03 ^{bcd}	0.47 ± 0.02 ^{y}	0.75 ± 0.03 ^{a}	0.48 ± 0.03 ^{bc}	0.62 ± 0.02 ^{x}	0.53 ± 0.02 ^{A}	0.43 ± 0.02 ^{B}	<0.001	<0.001	<0.001
C18:0	10.6 ± 0.43	10.3 ± 0.43	10.5 ± 0.31	11.5 ± 0.45	10.6 ± 0.45	11 ± 0.32	11.5 ± 0.45	11.2 ± 0.45	11.4 ± 0.32	11.2 ± 0.2	10.7 ± 0.02	ns	ns	ns
C18:1 cis	22.8 ± 0.43 ^{b}	25.6 ± 0.43 ^{a}	24.2 ± 0.31	24.7 ± 0.45 ^{a}	25.7 ± 0.45 ^{a}	25.2 ± 0.32	24.6 ± 0.45 ^{a}	25.7 ± 0.45 ^{a}	25.2 ± 0.32	24.1 ± 0.3 ^{B}	25.7 ± 0.3 ^{A}	0.03	<0.001	0.07
C18:1 trans	3.3 ± 0.15	3.3 ± 0.15	3.3 ± 0.11 ^{y}	4.1 ± 0.16	4.4 ± 0.16	4.2 ± 0.11 ^{x}	4 ± 0.16	4 ± 0.16	4 ± 0.11 ^{x}	3.7 ± 0.09	3.9 ± 0.09	<0.001	ns	ns
C18:2 CLA	0.73 ± 0.06 ^{c}	0.82 ± 0.06 ^{bc}	0.77 ± 0.04 ^{y}	1.0 ± 0.06 ^{ab}	1.15 ± 0.06 ^{a}	1.1 ± 0.04 ^{x}	1.2 ± 0.06 ^{a}	1.1 ± 0.06 ^{ab}	1.12 ± 0.04 ^{x}	0.98 ± 0.03	1.0 ± 0.03	<0.001	ns	0.04
C18:3 (n-3)	0.26 ± 0.03	0.24 ± 0.03	0.25 ± 0.02 ^{z}	0.53 ± 0.03	0.46 ± 0.03	0.49 ± 0.02 ^{x}	0.39 ± 0.03	0.36 ± 0.03	0.38 ± 0.02 ^{y}	0.39 ± 0.02	0.35 ± 0.02	<0.001	ns	ns

T: treatment; ML: moment of lactation; and T * ML: treatments and moment of lactation interaction. Treatments: CB-TMR, CB-GRZ, and OD-GRZ. ML1: 80 ± 15 days in milk (DIM), and ML2: 155 ± 15 days in milk (DIM). AT: average of both moments of lactation in each treatment; AML1: average of treatments in ML1; and AML2: average of treatments in ML2. Different letters indicate significant difference ($p < 0.05$): ^{x,y,z} indicate significant difference between AT; ^{A,B} indicate significant difference between AML; and ^{a,b,c,d} indicate significant difference in treatment and moment of lactation interaction. ns: no significance.

3.1.2. Fatty Acid Profile in Pooled Milk and Cheese

A treatment effect was observed for the n-3, n-6, and n-6/n-3 ratios in the MilkP and cheese (Table 5). The MilkP and cheese from the CB-TMR treatment had a greater n-6 and n-6/n-3 ratio than the CB-GRZ and OD-GRZ treatments ($p < 0.01$). Regarding n-3, for the MilkP, CB-TMR was lower than that of CB-GRZ and OD-GRZ ($p < 0.0001$). In the cheese, the n-3 was lower for CB-TMR than CB-GRZ ($p = 0.03$), but no difference was found between CB-TMR and OD-GRZ. There was no difference in any of the FAs between the mixed systems for both the MilkP and cheese (Table 5).

Table 5. Fatty acid profiles (FAP) of milk and cheese (mean ± SEM) in confined (CB-TMR) and mixed systems with low (CB-GRZ) and high (OD-GRZ) environmental exposure in spring calving cows.

	MilkP				Cheese			
	CB-TMR	**CB-GRZ**	**OD-GRZ**	***p* Value T**	**CB-TMR**	**CB-GRZ**	**OD-GRZ**	***p* Value T**
FA saturation (g/100 g of fat)								
SFA	63.5 ± 0.9	64.5 ± 0.9	64.1 ± 0.9	ns	64.2 ± 0.5	64.4 ± 0.5	64.7 ± 0.5	ns
MUFA	32.3 ± 0.7	31.2 ± 0.7	31.6 ± 0.7	ns	31.8 ± 0.5	31.5 ± 0.5	31.3 ± 0.5	ns
PUFA	4.0 ± 0.2	3.0 ± 0.2	4.1 ± 0.2	ns	3.8 ± 0.1	3.8 ± 0.1	3.8 ± 0.1	ns
n-3	0.26 ± 0.03 x	0.51 ± 0.03 y	0.50 ± 0.03 y	<0.001	0.43 ± 0.03 x	0.55 ± 0.03 y	0.46 ± 0.03 xy	<0.001
n-6	2.5 ± 0.1 x	1.9 ± 0.1 y	1.85 ± 0.1 y	<0.001	2.9 ± 0.1 x	2.6 ± 0.1 y	2.3 ± 0.1 y	<0.001
n6/n3	9.5 ± 0.1 x	3.7 ± 0.1 y	3.7 ± 0.1 y	<0.001	7.1 ± 0.5 x	4.8 ± 0.5 y	5.2 ± 0.5 y	<0.001
Trans	3.5 ± 0.1	4.2 ± 0.1	2.0 ± 0.1	<0.001	3.6 ± 0.1	3.8 ± 0.1	3.9 ± 0.2	ns

MilkP: milk from pen. T: treatment. Treatments: (CB-TMR, CB-GRZ, and OD-GRZ). Different letters indicate significant difference ($p < 0.05$). x,y indicate significant difference between treatment (CB-TMR, CB-GRZ, and OD-GRZ). ns: no significance. SFA: saturated; MUFA: monounsaturated; and PUFA: polyunsaturated.

4. Discussion

In this study, it was evidenced that the main changes in the individual milk, MilkP, and cheese were observed between the confined system (CB-TMR) and mixed systems (CB-GRZ, and OD-GRZ). However, the differences in the facilities during the confinements in the mixed systems (compost-bedded pack barns vs. outdoor soil-bedded pen) affected some of the FAs in the milk which are beneficial to humans.

The CB-TMR system had a higher milk yield and fat yield (kg/d) compared to both the mixed systems, which is in accordance with the findings of Salado et al. [57] and Bargo et al. [58]. This difference in the fat yield (kg/d) was due to the higher milk yield in CB-TMR, since there were no differences in the fat contents (%) between the treatments. The milk from CB-TMR (Section 1), as well as the MilkP and cheese from this treatment (Section 2), showed clear differences in their fatty acid profiles compared to those from the mixed systems (CB-GRZ and OD-GRZ), as evidenced by a higher percentage of UFAs, MUFAs, C18:2 (CLA), and C18:3 (n-3), as well as a lower percentage of SFAs and a lower n-6/n-3 ratio. Given that low concentrations of UFAs, MUFAs, C18:2 (CLA), and C18:3 (n-3), a higher percentage of SFAs, and a higher n-6/n-3 ratio are associated with less healthy milk [59,60], our results show that the fatty acid profiles in the milk from CB-TMR are less healthy than those from the mixed systems. In addition, the mixed-system milk (at both moments of lactation) had better atherogenicity and thrombogenicity indexes compared to that from CB-TMR. For the MilkP and cheese, those from the CB-TMR treatment had a lower n-3, a higher n-6, and a higher n-6/n-3 ratio than those from the MSs. The fact that the MS cows had a better fatty acid profile in their milk, MilkP, and cheese compared to the CB-TMR cows (in favor of the beneficial FAs for human health) could be due to the greater contribution of the precursors to the milk FAs, such as C18:2 and C18:3, which are provided by the pasture [2,8]. These results confirm what has been reported in other studies, where the milk and dairy products from mixed systems (grazing + TMR) present higher proportions of MUFAs and PUFAs, lower atherogenic risks and SFAs, a lower n-6/n-3 ratio (the recommended value should be below 4/1; Simopoulos [61]), AI, and TI, and a higher content of healthy FAs (i.e., n-3), from the point of view of human health, compared to TMR-fed systems [7,9,20,35,48,62]. In addition, although the cows from the pasture-based systems had a better milk fat quality, this was to the detriment of the milk's fat yield. In this sense, the lower milk and fat yields in the mixed-system cows could be due to the lower dry matter, lower energy intake, and higher energy requirements during grazing, in comparison to the cows fed with TMRs ad libitum in confinement, which resulted in a lower productive performance [58,63,64]. Therefore, our results highlight the importance of pasture consumption for cows in mixed systems, presenting a better-quality fatty acid profile in their milk and cheese compared to those without pasture consumption (100% confined systems), despite its detriment to the productive variables.

Regarding the effect of environmental exposure (low and high) during confinement on the milk fatty acid profiles, differences between the mixed systems were only found at ML1 (spring, Section 1), but no differences were found between the mixed systems for the MilkP and cheese in late summer (Section 2). In spring (ML1), when the OD-GRZ cows were exposed to rain during confinement (Figure 1), a negative impact on their milk fatty acid profiles was evidenced by the lower content of n-3 in their milk, in contrast to the CB-GRZ cows. It has been reported that, when the facility conditions are not comfortable enough, cows modify their behavior, with less time lying and more time standing during confinement [65–68], which could later alter their grazing behavior in order to recover the required resting time for their welfare [69,70]. In fact, this is consistent with the results obtained by our team (in the same experiment), in which it was observed that, when extreme external conditions occurred (accumulated rain and mud), the OD-GRZ cows were lying less frequently during confinement, but more frequently during grazing than the CB-GRZ cows [65]. Meanwhile, it is possible to speculate that these changes in their ingestive behavior during grazing could have altered their dry matter intake, finally impairing the milk fat quality (a decrease in n-3). Therefore, a lower exposure to the environment during confinement (CB-GRZ) in a mixed system (confined in a fully roofed barn with CB, protected from climatic conditions outside) improved some of the parameters in the milk (C18:3 and n-3), thus making it healthier for human consumption. Contrary to our original hypothesis, we did not find any differences in the milk FAPs between both the mixed systems during the summer (ML2). Several studies have reported that heat stress in summer affects milk production, yield, and fat content, as well as milk fatty acid profiles [29–31,33,71,72]. Therefore, it was expected that, during summer, the OD-GRZ cows, being more exposed to the environment during confinement, would present a greater heat stress, resulting in a negative impact on their milk quality and FAs. However, such differences in the milk fat quality between the mixed systems were not found, which could be associated with the particular weather during that summer and the good conditions of the outdoor soil-bedded pen used in our study. Therefore, this lack of difference could be due to: (i) no high THI (as would be expected in the region during this season) or rainfall values during the days prior to sampling; or (ii) the OD-GRZ facilities having enough shade (according to the number of animals per pen) and water ad libitum, which could have contributed to mitigating the negative effects of the summer on the OD-GRZ cows, therefore preventing a negative effect on the milk fat quality. Román et al. [40] observed that heat stress mitigation strategies, such as a pen with shade (even with a THI below 72), improved the performance and behavior of dairy cows. Based on all of the above, under the conditions carried out in this study and the environmental particularities of summer and the outdoor soil-bedded pens, the OD-GRZ cows probably did not present negative repercussions due to heat stress; thus, there were no changes in their milk.

Regarding the MilkP and cheese, we anticipated that, if the OD-GRZ cows suffered more heat stress during the summer, this higher stress response would have had a greater negative impact not only on the milk, but also on the final product, cheese. However, in line with what was mentioned in Section 1 (with the individual milk during summer), the greater exposure to the environment during confinement in a mixed system did not affect the fatty acid profiles of the milk and cheese. Probably, as we commented before, the good conditions of the outdoor soil-bedded pens and the characteristics of the environment in the moments close to the sampling did not generate great enough changes in the animals to impact the quality of their milk and cheese, even with a THI above 72. In addition, the fact that the cheeses were made during late lactation could have influenced the lack of differences found in the fatty acid profiles between the mixed systems, given that heat stress in late lactation would have had a lower effect on the solids than that in early lactation [40]. Furthermore, it is also important to consider that, in Section 2, the experimental unit was smaller than that in Section 1; therefore, we are cautious in saying that there were no differences. Future studies using a larger number of experimental units are needed to confirm whether exposure to the environment during confinement in a mixed system affects the FAs in pooled milk and cheese. In any case, in Section 2, it is highlighted that the changes in the milk fatty acid profiles between the treatments were reflected in the fatty acid profiles of the cheese; therefore, the cheeses reflected and retained the fat quality of the milk.

5. Conclusions

The most important changes in the FAPs of the milk, MilkP, and cheese were due to the presence of pasture in the cows' diet, rather than a difference in the facilities during confinement in an MS (compost-bedded pack barns vs. outdoor soil-bedded pen), which was evidenced by the higher UFAs and MUFAs and lower SFAs and n-6/n-3 ratio in the milk, and the higher n-3 and lower n-6 and n-6/n-3 in the MilkP and cheese from the MSs than those from the CB-TMR treatment. Nevertheless, the compost-bedded pack barns conditions presented a better quality of milk fat (a higher percentage of n-3 and C18:3 in the milk) compared to that from the cows in the outdoor soil-bedded pen.

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References

1. German, J.B.; Dillard, C.J. Composition, Structure and Absorption of Milk Lipids: A Source of Energy, Fat-Soluble Nutrients and Bioactive Molecules. *Crit. Rev. Food Sci. Nutr.* **2006**, *46*, 57–92. [CrossRef] [PubMed]
2. Moscovici Joubran, A.; Pierce, K.M.; Garvey, N.; Shalloo, L.; O'Callaghan, T.F. Invited Review: A 2020 Perspective on Pasture-Based Dairy Systems and Products. *J. Dairy Sci.* **2021**, *104*, 7364–7382. [CrossRef] [PubMed]
3. Lock, A.L.; Bauman, D.E. Modifying Milk Fat Composition of Dairy Cows to Enhance Fatty Acids Beneficial to Human Health. *Lipids* **2004**, *39*, 1197–1206. [CrossRef]
4. Chilliard, Y.; Ferlay, A.; Mansbridge, R.M.; Doreau, M. Ruminant Milk Fat Plasticity: Nutritional Control of Saturated, Polyunsaturated, Trans and Conjugated Fatty Acids. *Ann. Zootech.* **2000**, *49*, 181–205. [CrossRef]

5. Dewhurst, R.J.; Shingfield, K.J.; Lee, M.R.F.; Scollan, N.D. Increasing the Concentrations of Beneficial Polyunsaturated Fatty Acids in Milk Produced by Dairy Cows in High-Forage Systems. *Anim. Feed. Sci. Technol.* **2006**, *131*, 168–206. [CrossRef]
6. Barca, J.; Carriquiry, M.; Olazabal, L.; Fajardo, M.; Chilibroste, P.; Meikle, A. Milk Fatty Acid Profile from Cows Fed with Mixed Rations and Different Access Time to Pastureland during Early Lactation. *J. Anim. Physiol. Anim. Nutr.* **2017**, *102*, 620–629. [CrossRef]
7. Grille, L.; Adrien, M.L.; Méndez, M.N.; Chilibroste, P.; Olazabal, L.; Damián, J.P. Milk Fatty Acid Profile of Holstein Cows When Changed from a Mixed System to a Confinement System or Mixed System with Overnight Grazing. *Int. J. Food Sci.* **2022**, *2022*, 5610079. [CrossRef]
8. Elgersma, A. Grazing Increases the Unsaturated Fatty Acid Concentration of Milk from Grass-Fed Cows: A Review of the Contributing Factors, Challenges and Future Perspectives. *Eur. J. Lipid Sci. Technol.* **2015**, *117*, 1345–1369. [CrossRef]
9. Alothman, M.; Hogan, S.A.; Hennessy, D.; Dillon, P.; Kilcawley, K.N.; O'donovan, M.; Tobin, J.; Fenelon, M.A.; O'callaghan, T.F. The "Grass-Fed" Milk Story: Understanding the Impact of Pasture Feeding on the Composition and Quality of Bovine Milk. *Foods* **2019**, *8*, 350. [CrossRef]
10. Bauman, D.E.; Griinari, J.M. Regulation and Nutritional Manipulation of Milk Fat: Low-Fat Milk Syndrome. *Livest. Prod. Sci.* **2001**, *70*, 15–29. [CrossRef]
11. Hu, F.B.; Manson, J.A.E.; Willett, W.C. Types of Dietary Fat and Risk of Coronary Heart Disease: A Critical Review. *J. Am. Coll. Nutr.* **2001**, *20*, 5–19. [CrossRef]
12. Chilliard, Y.; Glasser, F.; Ferlay, A.; Bernard, L.; Rouel, J.; Doreau, M. Diet, Rumen Biohydrogenation and Nutritional Quality of Cow and Goat Milk Fat. *Eur. J. Lipid Sci. Technol.* **2007**, *109*, 828–855. [CrossRef]
13. Moate, P.J.; Chalupa, W.; Boston, R.C.; Lean, I.J. Milk Fatty Acids. I. Variation in the Concentration of Individual Fatty Acids in Bovine Milk. *J. Dairy Sci.* **2007**, *90*, 4730–4739. [CrossRef] [PubMed]
14. Kucevic, D.; Trivunovic, S.; Bogdanovic, V.; Cobanovic, K.; Jankovic, D.; Stanojevic, D. Composition of Raw Milk from Conventional and Organic Dairy Farming. *Biotehnol. Stocarstvu* **2016**, *32*, 133–143. [CrossRef]
15. Lucas, A.; Agabriel, C.; Martin, B.; Ferlay, A.; Verdier-Metz, I.; Coulon, J.-B.; Rock, E. Relationships between the Conditions of Cow's Milk Production and the Contents of Components of Nutritional Interest in Raw Milk Farmhouse Cheese. *Le Lait* **2006**, *86*, 177–202. [CrossRef]
16. O'Callaghan, T.F.; Mannion, D.T.; Hennessy, D.; McAuliffe, S.; O'Sullivan, M.G.; Leeuwendaal, N.; Beresford, T.P.; Dillon, P.; Kilcawley, K.N.; Sheehan, J.J.; et al. Effect of Pasture versus Indoor Feeding Systems on Quality Characteristics, Nutritional Composition, and Sensory and Volatile Properties of Full-Fat Cheddar Cheese. *J. Dairy Sci.* **2017**, *100*, 6053–6073. [CrossRef] [PubMed]
17. Frétin, M.; Martin, B.; Buchin, S.; Desserre, B.; Lavigne, R.; Tixier, E.; Cirié, C.; Bord, C.; Montel, M.-C.; Delbès, C.; et al. Milk Fat Composition Modifies the Texture and Appearance of Cantal-Type Cheeses but Not Their Flavor. *J. Dairy Sci.* **2019**, *102*, 1131–1143. [CrossRef]
18. Lee, B.-H.; Corazzin, M.; Piasentier, E.; Huh, C.-S.; Sung, K.-I.; Park, S.-Y. Milk and Montasio-Type Cheese Fatty Acid Composition from Cows Grazing on Timothy and Reed Canarygrass Pasture or Fed Indoor. *Grassl. Sci.* **2019**, *65*, 226–232. [CrossRef]
19. Charlton, G.L.; Rutter, S.M. The Behaviour of Housed Dairy Cattle with and without Pasture Access: A Review. *Appl. Anim. Behav. Sci.* **2017**, *192*, 2–9. [CrossRef]
20. O'callaghan, T.F.; Hennessy, D.; McAuliffe, S.; Kilcawley, K.N.; O'donovan, M.; Dillon, P.; Ross, R.P.; Stanton, C. Effect of Pasture versus Indoor Feeding Systems on Raw Milk Composition and Quality over an Entire Lactation. *J. Dairy Sci.* **2016**, *99*, 9424–9440. [CrossRef]
21. Hennessy, D.; Delaby, L.; van den Pol-van Dasselaar, A.; Shalloo, L. Increasing Grazing in Dairy Cow Milk Production Systems in Europe. *Sustainability* **2020**, *12*, 2443. [CrossRef]
22. Fariña, S.R.; Chilibroste, P. Opportunities and Challenges for the Growth of Milk Production from Pasture: The Case of Farm Systems in Uruguay. *Agric. Syst.* **2019**, *176*, 102631. [CrossRef]
23. Méndez, M.N.; Grille, L.; Mendina, G.R.; Robinson, P.H.; Adrien, M.d.L.; Meikle, A.; Chilibroste, P. Performance of Autumn and Spring Calving Holstein Dairy Cows with Different Levels of Environmental Exposure and Feeding Strategies. *Animals* **2023**, *13*, 1211. [CrossRef] [PubMed]
24. Schütz, K.E.; Rogers, A.R.; Poulouin, Y.A.; Cox, N.R.; Tucker, C.B. The Amount of Shade Influences the Behavior and Physiology of Dairy Cattle. *J. Dairy Sci.* **2010**, *93*, 125–133. [CrossRef]
25. Van Laer, E.; Tuyttens, F.A.M.; Ampe, B.; Sonck, B.; Moons, C.P.H.; Vandaele, L. Effect of Summer Conditions and Shade on the Production and Metabolism of Holstein Dairy Cows on Pasture in Temperate Climate. *Animal* **2015**, *9*, 1547–1558. [CrossRef]
26. Kadzere, C.T.; Murphy, M.R.; Silanikove, N.; Maltz, E. Heat Stress in Lactating Dairy Cows: A Review. *Livest. Prod. Sci.* **2002**, *77*, 59–91. [CrossRef]
27. Moretti, R.; Biffani, S.; Chessa, S.; Bozzi, R. Heat Stress Effects on Holstein Dairy Cows' Rumination. *Animal* **2017**, *11*, 2320–2325. [CrossRef] [PubMed]
28. Napolitano, F.; Bragaglio, A.; Sabia, E.; Serrapica, F.; Braghieri, A.; De Rosa, G. The Human–Animal Relationship in Dairy Animals. *J. Dairy Res.* **2020**, *87*, 47–52. [CrossRef]
29. Bernabucci, U.; Basiricò, L.; Morera, P.; Dipasquale, D.; Vitali, A.; Cappelli, F.; Calamari, L. Effect of Summer Season on Milk Protein Fractions in Holstein Cows. *J. Dairy Sci.* **2015**, *98*, 1815–1827. [CrossRef]

30. Becker, C.A.; Collier, R.J.; Stone, A.E. Invited Review: Physiological and Behavioral Effects of Heat Stress in Dairy Cows. *J. Dairy Sci.* **2020**, *103*, 6751–6770. [CrossRef]
31. Morales-Piñeyrúa, J.T.; Damián, J.P.; Banchero, G.; Sant Anna, A.C. The Effects of Heat Stress on Milk Production and the Grazing Behavior of Dairy Holstein Cows Milked by an Automatic Milking System. *J. Anim. Sci.* **2022**, *100*, skac225. [CrossRef]
32. Maggiolino, A.; Dahl, G.E.; Bartolomeo, N.; Bernabucci, U.; Vitali, A.; Serio, G.; Cassandro, M.; Centoducati, G.; Santus, E.; De Palo, P. Estimation of Maximum Thermo-Hygrometric Index Thresholds Affecting Milk Production in Italian Brown Swiss Cattle. *J. Dairy Sci.* **2020**, *103*, 8541–8553. [CrossRef]
33. Kumar, G.; Devi, P.; Sharma, N.; Yallappa, I.; Somagond, M.; Somagond, Y.M. Impact of Thermal Stress on Milk Production, Composition and Fatty Acid Profile in Dairy Cows: A Review. *J. Entomol. Zool. Stud.* **2020**, *8*, 1278–1283.
34. Salamon, R.; Varga-Visi, É.; Sára, P.; Csapó-Kiss, Z.; Csapó, J. The Influence of the Season on the Fatty Acid Composition and Conjugated Linolic Acid Content of the Milk. *Krmiva Časopis O Hranidbi Zivotinj. Proizv. I Tehnol. Krme* **2006**, *48*, 193–200.
35. Maniaci, G.; Di Grigoli, A.; Bonanno, A.; Giosuè, C.; Ilardi, V.; Alabiso, M. Fatty Acids as Biomarkers of the Production Season of Caciocavallo Palermitano Cheese. *Animals* **2021**, *11*, 2675. [CrossRef]
36. Tzamaloukas, O.; Neofytou, M.C.; Simitzis, P.E.; Miltiadou, D. Effect of Farming System (Organic vs. Conventional) and Season on Composition and Fatty Acid Profile of Bovine, Caprine and Ovine Milk and Retail Halloumi Cheese Produced in Cyprus. *Foods* **2021**, *10*, 1016. [CrossRef]
37. Paszczyk, B.; Polak-Śliwińska, M.; Zielak-Steciwko, A.E. Chemical Composition, Fatty Acid Profile, and Lipid Quality Indices in Commercial Ripening of Cow Cheeses from Different Seasons. *Animals* **2022**, *12*, 198. [CrossRef] [PubMed]
38. Vibart, R.E.; Fellner, V.; Burns, J.C.; Huntington, G.B.; Green, J.T. Performance of Lactating Dairy Cows Fed Varying Levels of Total Mixed Ration and Pasture. *J. Dairy Res.* **2008**, *75*, 471–480. [CrossRef]
39. Chen, J.M.; Stull, C.L.; Ledgerwood, D.N.; Tucker, C.B. Muddy Conditions Reduce Hygiene and Lying Time in Dairy Cattle and Increase Time Spent on Concrete. *J. Dairy Sci.* **2017**, *100*, 2090–2103. [CrossRef]
40. Román, L.; Saravia, C.; Astigarraga, L.; Bentancur, O.; La Manna, A. Shade Access in Combination with Sprinkling and Ventilation Effects Performance of Holstein Cows in Early and Late Lactation. *Anim. Prod. Sci.* **2019**, *59*, 347. [CrossRef]
41. INALE. Available online: https://www.inale.org/estadisticas/encuesta-lechera-2019-resultados-definitivos/ (accessed on 26 January 2023).
42. Leso, L.; Barbari, M.; Lopes, M.A.; Damasceno, F.A.; Galama, P.; Taraba, J.L.; Kuipers, A. Invited Review: Compost-Bedded Pack Barns for Dairy Cows. *J. Dairy Sci.* **2020**, *103*, 1072–1099. [CrossRef] [PubMed]
43. Bewley, J.M.; Robertson, L.M.; Eckelkamp, E.A. A 100-Year Review: Lactating Dairy Cattle Housing Management. *J. Dairy Sci.* **2017**, *100*, 10418–10431. [CrossRef] [PubMed]
44. Esposito, G.; Masucci, F.; Napolitano, F.; Braghieri, A.; Romano, R.; Manzo, N.; Di Francia, A. Fatty Acid and Sensory Profiles of Caciocavallo Cheese as Affected by Management System. *J. Dairy Sci.* **2014**, *97*, 1918–1928. [CrossRef] [PubMed]
45. Bonanno, A.; Tornambè, G.; Bellina, V.; De Pasquale, C.; Mazza, F.; Maniaci, G.; Di Grigoli, A. Effect of Farming System and Cheesemaking Technology on the Physicochemical Characteristics, Fatty Acid Profile, and Sensory Properties of Caciocavallo Palermitano Cheese. *J. Dairy Sci.* **2013**, *96*, 710–724. [CrossRef]
46. National Research Council. *Nutrient Requirements of Dairy Cattle*, 7th ed.; National Academy of Sciences: Washington, DC, USA, 2001; Volume 519.
47. Haydock, K.P.; Shaw, N.H. The Comparative Yield Method for Estimating Dry Matter Yield of Pasture. *Aust. J. Exp. Agric.* **1975**, *15*, 663–670. [CrossRef]
48. Pastorini, M.; Pomiés, N.; Repetto, J.L.; Mendoza, A.; Cajarville, C. Productive Performance and Digestive Response of Dairy Cows Fed Different Diets Combining a Total Mixed Ration and Fresh Forage. *J. Dairy Sci.* **2019**, *102*, 4118–4130. [CrossRef]
49. Mendoza, A.; Cajarville, C.; Repetto, J.L. Short Communication: Intake, Milk Production, and Milk Fatty Acid Profile of Dairy Cows Fed Diets Combining Fresh Forage with a Total Mixed Ration. *J. Dairy Sci.* **2016**, *99*, 1938–1944. [CrossRef]
50. Escobar, D.; Pelaggio, R.; Grille, L.; Colzada, E.; Rampoldi, C.; Carro, S.; Delucci, I.; Viola, N.; Nolla, J.P.; Reinares, R.; et al. Effect of Caseins Profile, Somatic Cells Count and Milk Composition in Dambo Cheese Yield. *Innotec* **2014**, *9*, 31–42.
51. Hara, A.; Radin, N.S. Lipid Extraction of Tissues. *Anal. Biochem.* **1978**, *90*, 420–426. [CrossRef]
52. Folch, J.; Lees, M.; Sloane Stanley, G.H. A Simple Method for the Isolation and Purification of Total Lipids from Animal Tissues. *J. Biol. Chem.* **1957**, *226*, 497–509. [CrossRef]
53. Mossoba, M.M.; Yurawecz, M.P.; Roach, J.A.G.; McDonald, R.E.; Flickinger, B.D.; Perkins, E.G. Analysis of Cyclic Fatty Acid Monomer 2-Alkenyl-4,4-dimethyloxazoline Derivatives by Gas Chromatography−Matrix Isolation−Fourier Transform Infrared Spectroscopy. *J. Agric. Food Chem.* **1996**, *44*, 3193–3196. [CrossRef]
54. Ulbricht, T.L.V.; Southgate, D.A.T. Coronary Heart Disease: Seven Dietary Factors. *Lancet* **1991**, *338*, 985–992. [CrossRef] [PubMed]
55. AOAC. *Official Methods of Analysis*, 17th ed.; Association of Official Analytical Chemists: Washington, DC, USA, 2000.
56. AOAC. *Official Methods of Analysis*, 14th ed.; Association of Official Analytical Chemists: Washington, DC, USA, 1984.
57. Salado, E.E.; Bretschneider, G.; Cuatrin, A.; Descalzo, A.M.; Gagliostro, G.A. Productive Response of Dairy Cows Fed with Different Levels of Totally Mixed Ration and Pasture. *Agric. Sci.* **2018**, *09*, 824–851. [CrossRef]
58. Bargo, F.; Muller, L.D.; Varga, G.A.; Delahoy, J.E.; Cassidy, T.W. Ruminal Digestion and Fermentation of High-Producing Dairy Cows with Three Different Feeding Systems Combining Pasture and Total Mixed Rations. *J. Dairy Sci.* **2002**, *85*, 2964–2973. [CrossRef] [PubMed]

59. Mollica, M.P.; Trinchese, G.; Cimmino, F.; Penna, E.; Cavaliere, G.; Tudisco, R.; Musco, N.; Manca, C.; Catapano, A.; Monda, M.; et al. Milk Fatty Acid Profiles in Different Animal Species: Focus on the Potential Effect of Selected PUFAs on Metabolism and Brain Functions. *Nutrients* **2021**, *13*, 1111. [CrossRef]
60. Ferlay, A.; Bernard, L.; Meynadier, A.; Malpuech-Brugère, C. Production of Trans and Conjugated Fatty Acids in Dairy Ruminants and Their Putative Effects on Human Health: A Review. *Biochimie* **2017**, *141*, 107–120. [CrossRef]
61. Simopoulos, A.P. The Importance of the Omega-6/Omega-3 Fatty Acid Ratio in Cardiovascular Disease and Other Chronic Diseases. *Exp. Biol. Med.* **2008**, *233*, 674–688. [CrossRef]
62. Hirigoyen, D.; Santos, R.D.L.; Calvo, M.F.; González-Revello, A.; Constantin, M. Chemical Composition and Seasonal Changes in the Fatty Acid Profile of Uruguayan "Colonia" Cheeses. *Grasas y Aceites* **2018**, *69*, 254. [CrossRef]
63. Kolver, E.S.; Muller, L.D. Performance and Nutrient Intake of High Producing Holstein Cows Consuming Pasture or a Total Mixed Ration. *J. Dairy Sci.* **1998**, *81*, 1403–1411. [CrossRef]
64. Auldist, M.J.; Marett, L.C.; Greenwood, J.S.; Wright, M.M.; Hannah, M.; Jacobs, J.L.; Wales, W.J. Milk Production Responses to Different Strategies for Feeding Supplements to Grazing Dairy Cows. *J. Dairy Sci.* **2016**, *99*, 657–671. [CrossRef]
65. Pons, V.; Damián, J.P.; Mattiauda, D.A.; Meikle, A.; Adrien, M.L.; Chilibroste, P. ¿Como Afectan Las Caracteristicas Del Encierro El Comportamiento de Vacas Lecheras En Sistemas Mixtos? In Proceedings of the IV Congreso de Colegio Latinoamericano de Bienestar Animal y Medicina del Comportamiento, Montevideo, Uruguay, 5–7 November 2021.
66. Cook, N.B.; Bennett, T.B.; Nordlund, K.V. Monitoring Indices of Cow Comfort in Free-Stall-Housed Dairy Herds. *J. Dairy Sci.* **2005**, *88*, 3876–3885. [CrossRef] [PubMed]
67. Krawczel, P.; Grant, R. Effects of Cow Comfort on Milk Quality, Productivity and Behavior. In Proceedings of the NMC Annual Meeting Proceedings, Charlotte, NC, USA, 25–28 January 2009; pp. 15–24.
68. Grille, L.; Adrien, M.L.; Olmos, M.; Chilibroste, P.; Damián, J.P. Diet Change from a System Combining Total Mixed Ration and Pasture to Confinement System (Total Mixed Ration) on Milk Production and Composition, Blood Biochemistry and Behavior of Dairy Cows. *Anim. Sci. J.* **2019**, *90*, 1484–1494. [CrossRef] [PubMed]
69. Cooper, M.D.; Arney, D.R.; Phillips, C.J.C. Two- Or Four-Hour Lying Deprivation on the Behavior of Lactating Dairy Cows. *J. Dairy Sci.* **2007**, *90*, 1149–1158. [CrossRef] [PubMed]
70. Schütz, K.E.; Cave, V.M.; Cox, N.R.; Huddart, F.J.; Tucker, C.B. Effects of 3 Surface Types on Dairy Cattle Behavior, Preference, and Hygiene. *J. Dairy Sci.* **2019**, *102*, 1530–1541. [CrossRef] [PubMed]
71. Bernabucci, U.; Biffani, S.; Buggiotti, L.; Vitali, A.; Lacetera, N.; Nardone, A. The Effects of Heat Stress in Italian Holstein Dairy Cattle. *J. Dairy Sci.* **2014**, *97*, 471–486. [CrossRef] [PubMed]
72. Saravia, C.; Astigarraga, L.; Van Lier, E.; Bentancur, O. Impacto de Las Olas de Calor En Vacas Lecheras En Salto (Uruguay). *Agrociencia Urug.* **2011**, *15*, 93–102. [CrossRef]