UNIVERSIDAD DE LAS PALMAS DE GRAN CANARIA

Facultad Veterinaria
Universidad de Las Palmas de Gran Canaria

IUSA
Instituto Universitario de Sanidad Animal y Seguridad Alimentaria

Tesis Doctoral

Influencia de la dieta de los corderos sobre el sistema inmune de los mismos

Influence of diet on lamb immune system

Lorenzo Enrique Hernández Castellano

Las Palmas de Gran Canaria, octubre, 2013

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Anexo I

Dª. MARÍA SORAYA DÉNIZ SUÁREZ, SECRETARIA DEL INSTITUTO UNIVERSITARIO DE SANIDAD ANIMAL Y SEGURIDAD ALIMENTARIA DE LA UNIVERSIDAD DE LAS PALMAS DE GRAN CANARIA.

CERTIFICA

Que el Consejo de Doctores del Departamento en su sesión de fecha 25 de junio de 2013 tomó el acuerdo de dar el consentimiento para su tramitación, a la tesis doctoral europea titulada: **"INFLUENCIA DE LA DIETA DE LOS CORDEROS EN EL SISTEMA INMUNE DE LOS MISMOS."** presentada por el doctorando D. Lorenzo Enrique Hernández Castellano y dirigida por el Dr. D. Anastasio Argüello Henríquez.

Y para que así conste, y a efectos de lo previsto en el Artº 73.2 del reglamento de Estudios de Doctorado de esta Universidad, firmo la presente en Las Palmas de Gran Canaria, a veintiséis de junio de dos mil trece.

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Anexo II

UNIVERSIDAD DE LAS PALMAS DE GRAN CANARIA

Departamento: Instituto Universitario de Sanidad Animal y Seguridad Alimentaria

Programa de Doctorado: Sanidad Animal

Título de la Tesis

"INFLUENCIA DE LA DIETA DE LOS CORDEROS SOBRE EL SISTEMA INMUNE DE LOS MISMOS"

Tesis Doctoral Europea presentada por Don Lorenzo Enrique Hernández Castellano

Dirigida por el Dr. Anastasio Argüello Henríquez

ector,

nríquez

Las Palmas de Gran Canaria, a 21 de octubre de 2013

ANASTASIO ARGÜELLO HENRÍQUEZ, PROFESOR TITULAR DE UNIVERSIDAD EN EL DEPARTAMENTO DE PATOLOGÍA ANIMAL, PRODUCCIÓN ANIMAL, BROMATOLOGÍA Y TECNOLOGÍA DE LOS ALIMENTOS DE LA FACULTAD DE VETERINARIA DE LA UNIVERSIDAD DE LAS PALMAS DE GRAN CANARIA

INFORMA:

Que Don Lorenzo Enrique Hernández Castellano, Licenciado en Veterinaria, ha realizado bajo mi dirección y asesoramiento el presente trabajo titulado **"INFLUENCIA DE LA DIETA DE LOS CORDEROS SOBRE EL SISTEMA INMUNE DE LOS MISMOS"** considerando que reúne las condiciones y calidad científica para optar al título de Doctor por la Universidad de Las Palmas de Gran Canaria.

Las Palmas de Gran Canaria, a 21 de octubre de 2013

Fdo. Anastasio Argüello Henríquez

UNIVERSIDAD DE LAS PALMAS DE GRAN CANARIA

FACULTAD DE VETERINARIA

TESIS DOCTORAL

INFLUENCIA DE LA DIETA DE LOS CORDEROS SOBRE EL SISTEMA INMUNE DE LOS MISMOS

Lorenzo Enrique Hernández Castellano

Las Palmas de Gran Canaria, a 21 de octubre de 2013

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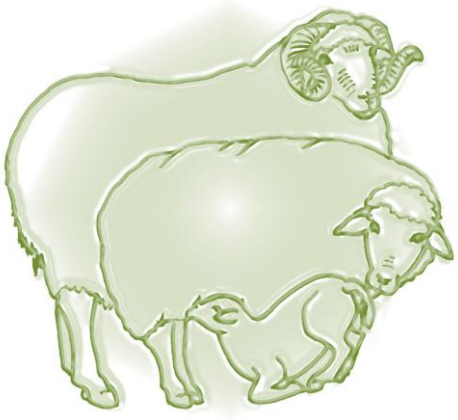
Introducción



1. Generalidades

Son varios los estudios que han demostrado la relación existente entre la supervivencia de los rumiantes neonatos y un correcto encalostrado (Argüello et al., 2004b; Castro et al., 2005b; Castro et al., 2009; Castro et al., 2011). De hecho, se ha descrito que el calostro se basa en una mezcla de diversos componentes tales como grasa, lactosa, vitaminas y minerales que presentan un alto valor nutricional (Ontsouka et al., 2003). Además, y a pesar de la clara importancia nutricional que posee el calostro, éste contiene una potente mezcla de proteínas que participan, de manera activa, en la protección del neonato contra diversos patógenos y otros cambios ambientales producidos tras el parto (Bendixen et al., 2011).

Por otro lado, la proteómica es campo de la ciencia que se encarga de estudiar los proteomas, entendiendo estos últimos como grandes y complejos conjuntos de proteínas expresadas procedentes de un tejido, órgano, organismo o fluido, entre otras. Pese a que el estudio de la proteómica es un campo relativamente nuevo, es uno de los que más rápido se han desarrollado en los últimos años, recibiendo un gran reconocimiento internacional y siendo una herramienta usada de forma amplia y diversa tanto en la investigación animal como en la humana (Penque, 2009; Eckersall et al., 2012). Dentro de esta área de trabajo se incluyen por ejemplo, la transformación de músculo a carne (Paredi et al., 2012) o su uso en la industria láctea (Roncada et al., 2012). En cuanto a los estudios proteómicos del calostro, existen diversos autores que han usado la proteómica para describir por ejemplo, los cambios que se producen en el perfil proteico durante la transición entre calostro y leche (Reinhardt and Lippolis, 2008; Stelwagen et al., 2009; Nissen et al., 2012) o los procesos proteolíticos que se producen en el estómago de los lechones después de la ingesta de calostro (Danielsen et al., 2011).

2. Definición y funciones del calostro en los rumiantes domésticos

El calostro se define como la primera secreción formada en la glándula mamaria de las hembras de los mamíferos unas semanas antes del parto. En cuanto a las funciones del calostro, cabe destacar que el calostro es la primera fuente de energía para

los rumiantes neonatos, estimula la eliminación de los meconios y por último y más importante, participa de forma activa en la transferencia de inmunidad pasiva desde la madre al neonato, protegiendo a éste frente a infecciones durante los primero días de vida (Kramer et al., 2001).

2.1. El calostro como fuente de energía

En cuanto a la importancia del calostro como fuente de energía, éste participa de forma activa en el incremento de la temperatura corporal de los neonatos. Así, Vermorel et al. (1983) describieron que la temperatura corporal de terneros mantenidos a 10°C aumentaba un 18 % y un 9 % en la primera y segunda hora tras la administración del calostro. Debido a lo anteriormente descrito y tras observar que una de las principales causas de mortalidad durante las primeras horas de vida en los corderos era la hipotermia, Eales et al. (1982) propusieron realizar un adecuado encalostrado como medida preventiva para evitar el descenso de temperatura de estos animales. En este mismo sentido, Hamadeh et al. (2000) afirmaron que la ingesta temprana de calostro es primordial para la inducción de la termogénesis en corderos neonatos.

2.2. Expulsión de los meconios

Si bien es cierto que esta función puede parecer menos importante que la anterior, el calostro juega un papel fundamental como estimulador del peristaltismo intestinal en los rumiantes neonatos debido a su alto contenido en sales de magnesio. Son varios autores (García de Jalón et al., 1990; Barza et al., 1993) los que describen que, esta función es de vital importancia para favorecer la expulsión del meconio, evitando así la colonización bacteriana de la mucosa intestinal.

2.3. Establecimiento del vínculo materno-filial

Los patrones de comportamiento que desarrollan los corderos neonatos durante los primeros momentos de vida tienen como objetivo fundamental el establecimiento del vínculo materno-filial, durante el periodo sensible de la madre, y a realizar lo antes posible el primer amamantamiento (Val-Laillet et al., 2004). El citado vínculo se instaura después del parto y tiene lugar en un periodo muy específico y breve de tiempo (Ramirez et al., 1997).

En corderos, el consumo de calostro produce una mayor actividad y vigorosidad sobre los mismos, viéndose favorecido el reconocimiento materno (Nowak et al., 1989). Por el contrario, Gonzalez and Goddard (1998) encontraron que el aporte de una dosis extra de calostro comercial no mejoraba el establecimiento del vínculo materno-filial. Estos autores describen como la existencia de un reflejo de saciedad por repleción estomacal podría reducir las posibilidades de que se produzcan mejoras en la citada relación entre la madre y el cordero.

2.4. El calostro como elemento esencial para la transferencia de inmunidad pasiva

Por otro lado, y tal y como ya ha sido descrito anteriormente, la complejidad de la placenta epiteliocorial (vacas y búfalos; Wildman et al., 2006; Padua et al., 2010) o sinepitelicorial (pequeños rumiantes; Wooding et al., 1986) hace que la transferencia de inmunoglobulinas a través de la placenta no sea posible, al contrario de lo que ocurre en otras especies con una placenta hemocorial (humanos y ratones; Chucri et al., 2010). Por esto mismo, los rumiantes neonatos son considerados agammaglobulinémicos (terneros y búfalos) o hipogammaglobulinémicos (corderos y cabritos) al nacimiento (Argüello et al., 2004b; Castro et al., 2005b; Castro et al., 2009; Moreno-Indias et al., 2012c), siendo extremadamente importante la ingesta de calostro en estas especies, ya que de ello depende la absorción de proteínas esenciales para conseguir una correcta transferencia de inmunidad pasiva, y por consiguiente disminuir los porcentajes de mortalidad perinatal (Stelwagen et al., 2009; Castro et al., 2011; Danielsen et al., 2011). En cuanto a la absorción de proteínas calostrales, las inmunoglobulinas son las proteínas que han sido estudiadas en profundidad. Sin embargo, cada vez se está teniendo en mayor consideración la importancia del resto de proteínas presentes en el calostro en el desarrollo de una correcta transferencia de inmunidad pasiva (Smith and Foster, 2007). No obstante, y a pesar de la funciones previamente descritas, el calostro es también importante desde el punto de vista nutricional, ya que provee al animal de una serie de elementos nutritivos tales como grasa, proteína, lactosa, minerales o vitaminas que son fundamentales en este período tan temprano de la vida (Ontsouka et al., 2003). Por otro lado, el calostro contiene otro tipo de sustancias tales como agentes antiinflamatorios y antimicrobianos (Stelwagen et al., 2009), factores de crecimiento que controlan el

desarrollo gastrointestinal temprano (Purup et al., 2007), citoquinas, enzimas y otros muchos péptidos (Koldovsky, 1980; Blum and Hammon, 2000), tal y como puede ser observado en la figura 1. Por último, es necesario remarcar que muchos de los péptidos bioactivos presentes en el suero y en la membrana de los glóbulos grasos, tales como las inmunoglobulinas, la lactoferrina o los factores de crecimiento, están presentes en una mayor concentración en calostro que en leche, reflejando una vez más las importancia del calostro en la salud y estado inmune de los rumiantes neonatos (Pakkanen and Aalto, 1997; Scammel, 2001).

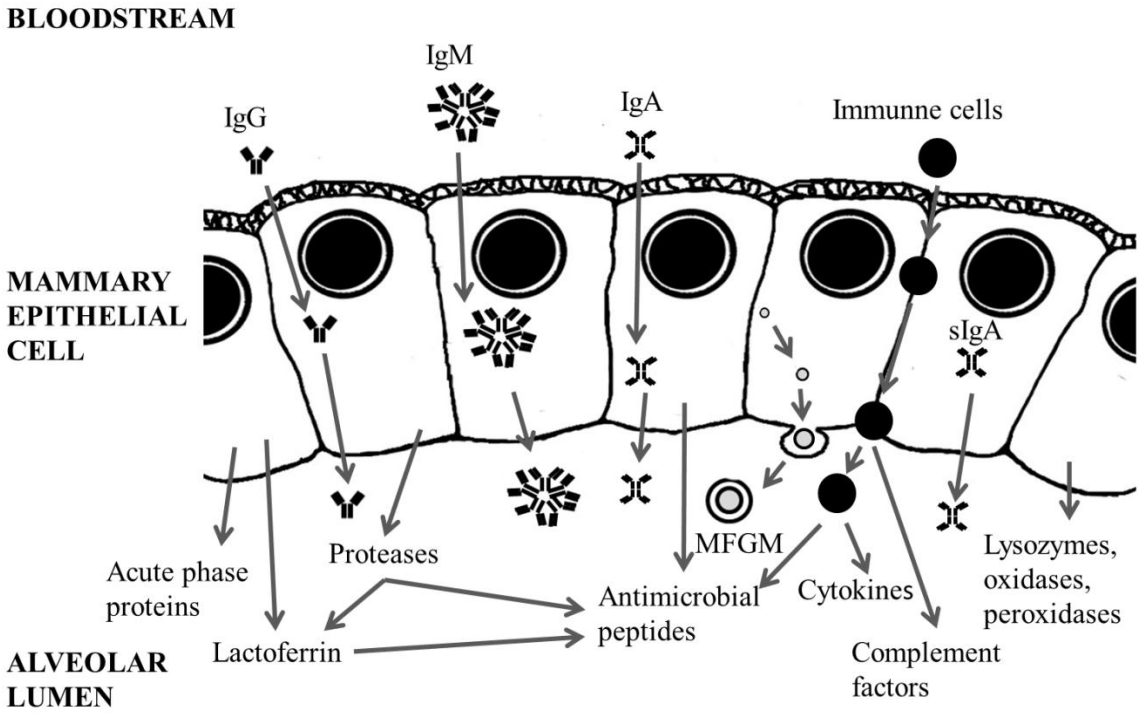


Figura 1. Visión esquemática de algunos de los principales componentes del calostro y de la leche que participan en la defensa del rumiante neonato.(Adaptado de Wheeler et al., 2007). MFGM: Membrana del glóbulo graso.

La correcta absorción de estos componentes, se lleva a cabo gracias a la acción combinada de la baja actividad proteolítica presente en el tracto gastrointestinal de los rumiantes neonatos (Guilloteau et al., 1983), la presencia de α1- antitripsina presente en el calostro (Jensen, 1978; Pallavicini et al., 1984; Ramos et al., 2010) y el gran porcentaje de enterocitos en estado de apoptosis (Castro-Alonso et al., 2008). Sin embargo, estas condiciones especiales para absorber las proteínas de forma intacta van desapareciendo durante las primeras 48 horas de vida, por lo que es de vital importancia alimentar a los rumiantes con calostro durante este periodo (Bush and Staley, 1980; Moore et al., 2005) con el objetivo de que estos animales adquieran unos adecuados niveles iniciales de inmunoglobulinas y otros componentes proteicos en el plasma sanguíneo (Quigley et al., 2000; Christley et al., 2003).

La falta de un correcto encalostrado y por lo tanto de una correcta transferencia de inmunidad pasiva, lleva consigo que los rumiantes neonatos sean considerablemente más susceptibles a enfermedades infecciosas, incrementándose los porcentajes de mortalidad a estas edades (Ahmad et al., 2000; da Nobrega et al., 2005; Nowak and Poindron, 2006). Por todo lo anteriormente descrito, la fase de encalostrado es considerada fundamental para mantener el bienestar de los rumiantes neonatos.

3.Componentes mayoritarios del calostro relacionados con el sistema inmune

3.1. Inmunoglobulinas

Las inmunoglobulinas son proteínas de un alto peso molecular, pertenecientes al grupo de las glicoproteínas. Estas pueden clasificarse de diversas formas en base a su movilidad electroforética, su peso molecular y su estructura antigénica.

Estas proteínas son producidas por las células plasmáticas y los linfocitos B, siendo responsables de la inmunidad humoral. Como principales funciones biológicas de las inmunoglobulinas figuran la activación de la vía clásica del sistema de complemento y la formación de los complejos antígeno-anticuerpo.

De los diferentes isotipos de inmunoglobulinas existentes en los mamíferos (IgG, IgM, IgA, IgD, IgE), la IgG es la que se encuentra en mayor concentración en el plasma sanguíneo, desempeñando el papel más importante en cuanto a los mecanismos de defensa mediados por anticuerpos se refiere (Tizard, 1992).

Esta inmunoglobulina presenta un peso molecular de 180 kDa y contiene cuatro cadenas polipeptídicas enlazadas y unidas mediante puentes disulfuro. Debido a este bajo peso molecular y a su estructura simple, constituye, junto con la IgD, el grupo de las inmunoglobulinas con menor peso molecular, lo que facilita la salida de estas inmunoglobulinas del torrente circulatorio con el fin de alcanzar rápidamente los espacios tisulares y las superficies corporales.

Figura 2. Oveja en el último estadío de la gestación.

Específicamente, dentro de la IgG se pueden distinguir dos subisotipos (IgG_1 e IgG_2), los cuales son diferenciables mediante el uso de métodos electroforéticos (Meckenzie, 1970; Butler et al., 1971; Kehoe, 1971). Además la presencia de estos dos isotipos en el calostro se encuentra regulada de forma diferente, ya que mientras que la IgG_1 presenta un tipo de transporte selectivo, la IgG_2 lo realiza por medio de la difusión pasiva (Lascelles, 1979). Sin embargo, la mayoría de la IgG presente en el calostro es IgG_1, la cual proviene del torrente sanguíneo. Así, Castro et al. (2006) encontraron que la concentración de IgG sanguínea, en cabras de raza Majorera, descendía un 38,70% durante los dos últimos meses de gestación.

En el caso de los rumiantes, la IgG es la que aparece con una mayor concentración en sangre y calostro, pudiendo llegar a representar entre el 80 y 90% del total de las inmunoglobulinas presentes en los mismos (Klaus et al., 1969; Neubauer and Schone, 1979; Lacetera et al., 1996).

En ganado bovino la IgG_1 supone el 50% de las inmunoglobulinas del suero sanguíneo, siendo la más predominante en leche (Tizard, 1992). En un estudio realizado por Ciupersescu (1977) en corderos, se estudió como la IgG_2 representa escasamente el 1% de las inmunoglobulinas presentes en la sangre durante las primeras etapas de la vida. Siguiendo esta misma línea, Lascelles (1979) encontró que la concentración de IgG_1 en el calostro de vaca y oveja era de 48,24 y 94,10 mg/ml, respectivamente; mientras que la concentración de IgG_2 en el calostro de estas mismas especies era de 3,98 y 2,50 mg/ml.

Por otro lado, el segundo subtipo de inmunoglobulina más importante en el torrente sanguíneo es la IgM, ya que tras la IgG, es la que más concentrada está en el suero sanguíneo de la mayoría de los mamíferos. La IgM es la inmunoglobulina de mayor peso molecular (900 kDa) y se encuentra constituida por la unión de 5 monómeros estabilizados mediante puentes disulfuro. Contiene además un pequeño péptido rico en cisteína, llamado cadena J, la cual, al igual que los monómeros, se encuentra unida mediante puentes disulfuro. Debido a su gran tamaño, estas inmunoglobulinas quedan retenidas en el torrente sanguíneo y no participan en la defensa extravascular. Sin embargo, pueden difundir a través de los epitelios gracias a la cadena J, ya que puede unirse a los receptores presentes en la superficie de las células. En el calostro, a diferencia de la IgG, la IgM se produce a nivel local (Lascelles, 1979) .

3.2. Chitotriosidasa

La Chitotriosidasa (ChT) es una enzima secretada por los macrófagos en su último estadío de diferenciación. La función de esta enzima es la de hidrolizar la quitina de la pared celular de hongos y nematodos. Han sido varios los autores que han descrito como la ChT juega un papel importante durante la respuesta inmune (Di Rosa et al., 2005; Malaguarnera et al., 2005) y los procesos inflamatorios (Di Rosa et al., 2006; Malaguarnera et al., 2006). Por otro lado, la actividad de esta enzima se encuentra muy elevada en el suero plasmático de pacientes que padecen enfermedades como la malaria, la enfermedad de Gaucher tipo 1 o en alteraciones hematológicas (Barone et al., 1999; Barone et al., 2003). En este sentido, Musumeci et al. (2005) observaron como la actividad de la ChT en el calostro y suero sanguíneo de mujeres africanas era significativamente mayor que en las mujeres caucásicas (1230 nmol/ml/hora y 293

nmol/ml/hora, mujeres africanas y caucásicas, respectivamente). En este estudio, no se encontró una correlación entre la actividad de la ChT del calostro y el suero sanguíneo en ninguno de los dos grupos estudiados, lo cual llevó a estos autores a sugerir que la actividad de la ChT es debida a una secreción local por la activación de los macrófagos.

Estudios similares fueron realizados en cabras de raza Majorera por Argüello et al. (2008b), encontrando que en el calostro de estos animales la actividad de esta enzima era de 3912 nmol/ml/hora en el momento del parto y de 465 nmol/ml/hora el día 4 después del parto. En otro estudio realizado con la misma raza, Moreno-Indias et al. (2012c) observaron valores de hasta 9431 nmol/ml/hora, justo después del parto, llegando a 3553 nmol/ml/hora a las 10 horas tras el parto. Si se realiza una comparación entre especies, se observa que la actividad de la ChT descrita en el calostro de cabra es mayor que la descrita en la especie humana debido probablemente a que las cabras presentan un mayor contacto con parásitos y hongos.

En cuanto a la actividad de la ChT descrita en cabritos (Argüello et al., 2008b), se han observado valores de 2664 nmol/ml/hora al nacimiento y de 9231 nmol/ml/hora a los 49 días de vida, concluyendo que la actividad de esta enzima en torrente sanguíneo aumenta con la edad. Esto puede ser explicado, tal y como se ha descrito anteriormente, por la progresiva activación de los macrófagos con la edad de los animales. En cambio, Moreno-Indias et al. (2012b) no encontraron diferencias en la actividad de la ChT en los primeros 35 días de vida de los animales. Asimismo, Rodríguez et al. (2009) no encontraron diferencias en la actividad de la citada enzima durante los 5 primeros días de vida de cabritos alimentados con distintas concentraciones de calostro caprino liofilizado. Esto refuerza la idea de que la ChT no es absorbida a nivel intestinal y puede jugar un rol importante en la defensa local en el lumen intestinal (Argüello et al., 2008b). Sin embargo, Wold and Adlerberth (2000) sugieren que la ChT debe ser destruida o inactivada antes de llegar al intestino, al igual que ocurre con otras proteínas de la leche materna.

3.3. Sistema de Complemento

El Sistema de Complemento es un componente central del sistema inmune innato, además de interceder en los mecanismos de respuesta mediante anticuerpos. Tiene tres actividades fisiológicas principales: defensa frente a infecciones bacterianas,

puente entre el sistema inmune innato y adquirido y de limpieza de los desechos de los complejos inmunes y daños de la inflamación (Walport, 2001).

Por otro lado, Korhonen et al. (2000), Rainard (2003) y Rainard and Riollet (2006) se han centrado en investigar la relación existente entre el Sistema de Complemento y la glándula mamaria y la leche, debido sobre todo a la importancia en enfermedades tan comunes como la mastitis.

Junto a los anticuerpos absorbidos del calostro, el Sistema de Complemento desempeña un papel crucial en la inmunización pasiva del ternero recién nacido (Butler et al., 1971; Staak, 1992). Además y tal y como ha sido demostrado en varios estudios (Reiter and Brock, 1975; Eckblad et al., 1981b; Korhonen et al., 1995), la actividad del Sistema de Complemento presente en la leche y el calostro bovino posee una gran actividad hemolítica y bactericida, confiriendo una mayor protección al rumiante neonato frente a agentes infecciosos.

4.Efecto del manejo en los rumiantes neonatos

Tal y como ha sido descrito por Castro et al. (2011), el manejo durante las últimas semanas de gestación es muy importante, ya que es en este periodo cuando el calostro es secretado en la sección alveolar de la ubre. Además, existen otros muchos factores tales como el tamaño de la camada o el número de lactaciones, que pueden afectar a la composición final del calostro.

4.1 Nutrición durante la gestación

Este aspecto ha sido ampliamente descrito por Mellor and Murray (1985), quienes observaron la relación entre la nutrición de la oveja durante la gestación y el desarrollo de la glándula mamaria, el volumen de calostro producido después del parto y la composición del mismo. Además, los componentes inmunes presentes en el calostro también pueden variar en función del manejo de la hembra en los últimos meses de gestación. De acuerdo con Awadeh et al. (1998b), las vacas suplementadas con selenio durante la fase final de gestación, incrementan sus niveles de IgG en el calostro. Por el

contrario, Swanson et al. (2008) no observaron ninguna variación en la concentración de IgG cuando suplementaron ovejas con selenio durante la última fase de gestación.

4.2 Tamaño de la camada y número de lactaciones

El número de crías nacidas es un factor con cierta controversia. Por un lado, Csapó et al. (1994) encontraron que la concentración de IgG en el calostro fue mayor en partos dobles que en partos simples de cabras de raza Hungarian White y ovejas de raza Hungarian Merino. Sin embargo, Argüello et al. (2006) no observaron diferencias entre la concentración de IgG de calostro en cabras de raza Majorera con diferentes tipo de partos (simple, doble y triple).

Con respecto al número de lactaciones, Oyeniyi and Hunter (1978) observaron que el número de lactaciones tenía una correlación positiva en vacas, incrementándose la concentración calostral de IgG. Sin embargo, no se ha probado este efecto en pequeños rumiantes, ya que mientras que Ha et al. (1986) encontraron que el número de lactaciones incrementó la concentración de IgG presente en el calostro de cabras de raza Korean Native, Santos (1994) y Argüello et al. (2006) no observaron ninguna diferencia debida al número de lactaciones, en la concentración de IgG presente en el calostro de cabras de raza Saanen y Majorera, respectivamente.

4.3 Primera ingesta de calostro por parte de los rumiantes recién nacidos

Una de las grandes condiciones excepcionales que poseen los rumiantes neonatos en sus primeras horas de vida, es la capacidad de absorber grandes moléculas a través del intestino. Sin embargo, en relación a la absorción de componentes del calostro, esta habilidad va desapareciendo progresivamente hasta las 36-48 horas tras el nacimiento, siendo imposible la absorción de dichas moléculas transcurrido este periodo de tiempo, lo que hace que la primera ingesta de calostro posea una gran relevancia a la hora de realizar una correcta transferencia de inmunidad pasiva. Por otro lado, existen algunos factores con respecto al calostro, que tienen una gran relevancia a la hora de determinar una correcta TIP, tales como la concentración de inmunoglobulinas o la cantidad de calostro ingerido. Así, Chigerwe et al. (2009) describieron cómo terneros de raza Holstein alimentados con 3 litros de calostro durante las primeras 4 horas tras el parto adquirieron una buena TIP. Sin embargo, estos autores observaron que los terneros que

recibían calostro entre las 4 y 12 horas, no presentaban un fallo en la TIP, siempre y cuando estos animales fueran alimentados con un calostro de alta calidad (concentración de IgG en calostro de > 100µg/µL). Del mismo modo, Rajala and Castrén (1995) correlacionaron el tiempo transcurrido entre el nacimiento de los terneros y la primera ingesta de calostro con los niveles de inmunoglobulinas en el suero sanguíneo, de forma que, cada 30 minutos de retraso en el consumo de calostro la concentración de inmunoglobulinas en el suero sanguíneo disminuyó en 2 mg/ml. Del mismo modo, Castro-Alonso et al. (2008) relacionaron la apoptosis de los enterocitos y el nivel de transferencia de inmunidad pasiva en cabritos de raza Majorera, observando que un mayor nivel de apoptosis, el cual desciende conforme aumentan las primeras 36-48 horas de vida, se corresponde con un mayor grado de absorción de IgG. Sin embargo, Orsel et al. (2000), trabajando con cabritos, no encontraron diferencias en los niveles de gammaglobulinas en el suero sanguíneo cuando el calostro era suministrado a los 30, 60 o 90 minutos de vida.

4.4. Peso al nacimiento y sexo

Respecto al peso al nacimiento, Bekele et al. (1992) no hallaron diferencias en la concentración de inmunoglobulinas en suero sanguíneo a las 48 horas de vida, entre corderos nacidos con un peso entre 1 y 1,5 kg (36,3 mg/ml) y animales cuyo peso al nacimiento superaba los 3 Kg (40,8 mg/ml). Por otro lado, Castro et al. (2009) usando cabritos de raza Majorera, describieron diferencias significativas en la concentración plasmática de IgG entre los distintos pesos al nacimiento, siendo los cabritos con pesos inferiores a 2,8 kg los que mostraron un menor valor de IgG en sangre.

En cuanto al efecto del sexo de los animales con su capacidad de absorción de componentes calostrales, Ciupersescu (1977) no observó diferencias, debidas al sexo, en la concentración plasmática de inmunoglobulinas de corderos tras la ingesta de calostro. Similares observaciones fueron realizadas por Chen et al. (1999) en un estudio realizado con cabritos.

5.La proteómica como herramienta para analizar e identificar las proteínas del calostro

El calostro es un complejo fluido corporal secretado por la glándula mamaria de las hembras mamíferas tras el parto. La composición de este fluido es regulada por hormonas (principalmente estradiol y progesterona) durante la formación del calostro, también denominado colostrogénesis (Castro et al., 2011). Dicha formación se define como la transferencia de componentes, principalmente inmunoglobulinas, desde el torrente sanguíneo materno hacia el calostro durante un periodo finito y discreto previo al parto (Barrington and Parish, 2001). Este proceso se detiene de forma drástica justo después del parto (Brandon et al., 1971). Existen multitud de factores que pueden afectar la calostrogénesis, tales como la especie, la raza, la edad, la nutrición, el tamaño de la camada, el periodo de secado y la salud del animal (Csapo et al., 1994; Awadeh et al., 1998a; Maunsell et al., 1998). Además, los componentes del calostro son secretados por diferentes mecanismos (Patton and Jensen, 1975), basándose principalmente en la producción de la glándula mamaria o la transferencia desde el torrente sanguíneo de esa proteína. Así por ejemplo, mientras que la IgG pasa directamente desde el torrente sanguíneo a la glándula mamaria, la IgA es secretada en la glándula mamaria por la acción de células plasmáticas que han migrado desde el torrente sanguíneo a la glándula mamaria (Wheeler et al., 2007) tal y como puede ser observado en la Figura 1.

La proteómica es una poderosa técnica que es capaz de analizar cientos de proteínas en complejas muestras, sin embargo debido al amplio rango de concentraciones de las proteínas y de las diferentes localizaciones de las mismas, no existe un protocolo general que permita una separación completa del proteoma del calostro. Es por esto mismo, que se hace necesario el análisis de los componentes proteicos de forma separada. Algunas de estas técnicas están basadas en el prefraccionamiento de la muestra de calostro, para poder reducir así la complejidad antes de realizar un estudio proteómico (Gagnaire et al., 2009). En general, estos pasos previos se basan en las diferentes propiedades de las proteínas, tales como la carga, el tamaño o la hidrofobicidad.

Durante décadas, los estudios proteómicos se han basado en los geles de electroforesis y aún hoy en día son ampliamente usados, siendo la electroforesis

bidemensional (2DE) el método comúnmente usado para realizar estudios cuantitativos (Wu et al., 2006). Sin embargo, cada vez se hace más evidente el interés por los métodos de separación basados en la cromatografía líquida (Danielsen et al., 2011), los cuales tienen varias ventajas si se comparan con los métodos de 2DE. Por otro lado, la Espectrometría de Masas (MS) ha sido el método de elección para el análisis de complejas muestras de proteínas, siendo por ejemplo el MALDI-TOF/TOF un método robusto, sensitivo, relativamente barato y que ha producido la mayoría de las identificaciones de proteínas que se pueden encontrar hoy en día en la bibliografía (Webster and Oxley, 2012). Sin embargo, hay otras técnicas, como el iTRAQ (isobaric tag for relative and absolute quantitation) que nos permite analizar el proteoma con una mayor profundidad, debido a que es muchísimo más sensible, si bien es verdad que el coste de ésta es mucho mayor.

Son muchos los estudios que han caracterizado los componentes del calostro en diferentes especies, principalmente vacas y humanos (Reinhardt and Lippolis, 2008; Stelwagen et al., 2009; Agarwal et al., 2011). A pesar de esto, poco se conoce sobre el proceso de absorción de componentes calostrales durante las primeras horas de vida del rumiante neonato, tales como degradación, protección frente a la acción proteolítica e internalización y retención por parte del intestino. Además, es necesario describir como esta fuente primaria de alimento pasa a través del tracto gastrointestinal del rumiante neonato y cómo los mencionados efectos pueden afectar a los diferentes componentes del calostro.

6. Fracciones del calostro y proteínas mayoritarias presentes en el mismo

6.1 Fracciones del calostro

Tal y como ha sido descrito en el apartado 3, no existe un protocolo para analizar completamente el calostro, por lo que es necesario el fraccionamiento del mismo mediante diferentes técnicas, para luego ser analizadas por separado. Las proteínas presentes en el calostro se encuentran divididas en tres grandes grupos, los

cuales son: las caseínas, las proteínas del suero y las proteínas de la membrana del glóbulo de grasa (MFGM), tal y como se recoge en la figura 2.

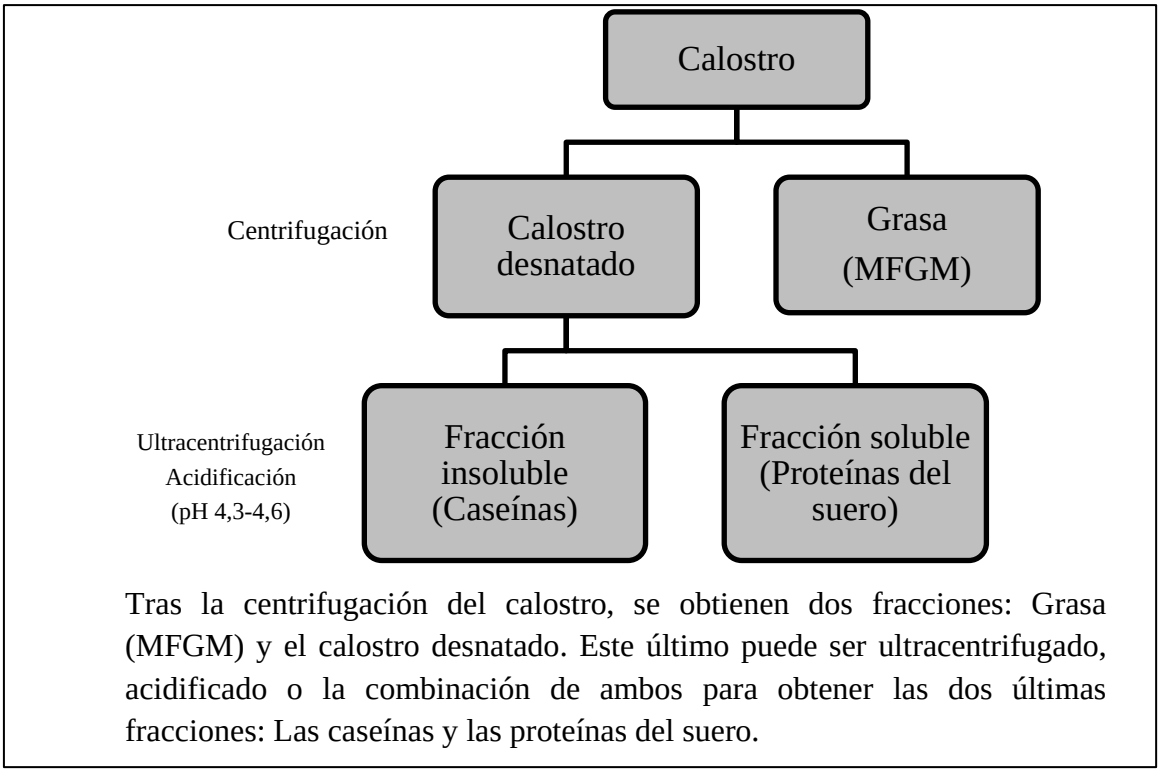


Figura 3. Procedimientos de separación de las tres fracciones del calostro.

Estas tres fracciones pueden ser separadas en dos pasos. El primero de ellos se basa en una centrifugación del calostro con el fin de separar por un lado la capa de grasa que va a contener las proteínas de la MFGM y por el otro la el calostro desnatado, que contendrá las otras dos fracciones (caseínas y proteínas del suero).

En la tabla 1 se recogen algunos de los protocolos de centrifugación observados en la bibliografía para leche y calostro, aunque algunos de ellos sólo han sido estudiados en leche.

Tabla 1. Procedimientos de centrifugación para separar las MFGM.

Especie	Centrifugación			Referencia
	Velocidad(*g*)	Tiempo (min.)	Temp. (ºC)	
Vaca	5000	15	4	(Zhang et al., 2011)
Vaca	15000	15	10	(Fong et al., 2008)
Vaca	10000	15	4	(Reinhardt and Lippolis, 2008)
Humano*	1500	20	25	(Patton and Huston, 1986)
Humano	4000	30	4	(Lonnerdal and Forsum, 1985)
Oveja*	1500	20	25	(Martini et al., 2013)

*Adición necesaria de 5 gr de Sacarosa/100 ml

Después de este proceso, el calostro desnatado debe de ser dividido en las dos últimas fracciones. En la bibliografía encontramos multitud de métodos para separar las caseínas del suero de calostro, sin embargo, todos ellos coinciden en el uso de la ultracentrifugación, la precipitación por acidificación del calostro o la combinación de ambos. Algunas de las técnicas más usadas se encuentran en la tabla 2.

Tabla 2. Procedimientos para la separación de las caseínas y del suero de calostro.

Especie	Centrifugación			Acidificación	Referencia
	Velocidad (*g*)	Tiempo (min.)	Temp. (ºC)		
Vaca	16500	30	4	$C_2H_4O_2$ (1N)	(Golinelli et al., 2011)
Vaca				$C_2H_4O_2$ (30% v/v, pH 4,6)	(Mier et al., 2008; Zhang et al., 2011)
Vaca	15000	15	10	HCl (1M, pH 4,6)	(Fong et al., 2008)
Vaca*	44000	30	4		(Boehmer et al., 2008)
Vaca	100000	60	4		(Le et al., 2010)
Humano*	13000	30	4	HCl (1M, pH 4,6) + 60 mmol $CaCl_2$	(Liao et al., 2011)
Humano*	189000	60	4	HCl (1M, pH 4,3) + 60 mmol $CaCl_2$	(Kunz and Lonnerdal, 1989)
Vaca	1500	10	5	$C_2H_4O_2$ (1N) + CH_3COONa (1N)	(Fox, 2003; Jensen et al., 2012)
Vaca	100000	60	4		(Jensen et al., 2012)
Humano	10000	15	20	HCl (1M, pH 4,6)	(Lonnerdal and Forsum, 1985)

*La centrifugación fue repetida dos veces

6.2. Proteínas mayoritarias presentes en el calostro

El primer grupo de proteínas que pueden ser aisladas del calostro o de la leche, son las localizadas en la MFGM, la cual envuelve al glóbulo de grasa (Lonnerdal and Forsum, 1985). Durante los últimos años, la mayoría de los estudios en la MFGM han sido realizados en vacas lecheras (Mondy and Keenan, 1993; Mather, 2000; Ye et al., 2002; Fong et al., 2007; Bianchi et al., 2009), sin embargo, existe un rápido crecimiento del interés por la MFGM de otras especies lecheras. Debido a este motivo, algunos de los estudios mas recientes sobre la MFGM han sido realizados en otras especies, tales como cabras (Cebo et al., 2010), ovejas (Addis et al., 2011; Pisanu et al., 2011) o búfalas (D'Ambrosio et al., 2008).

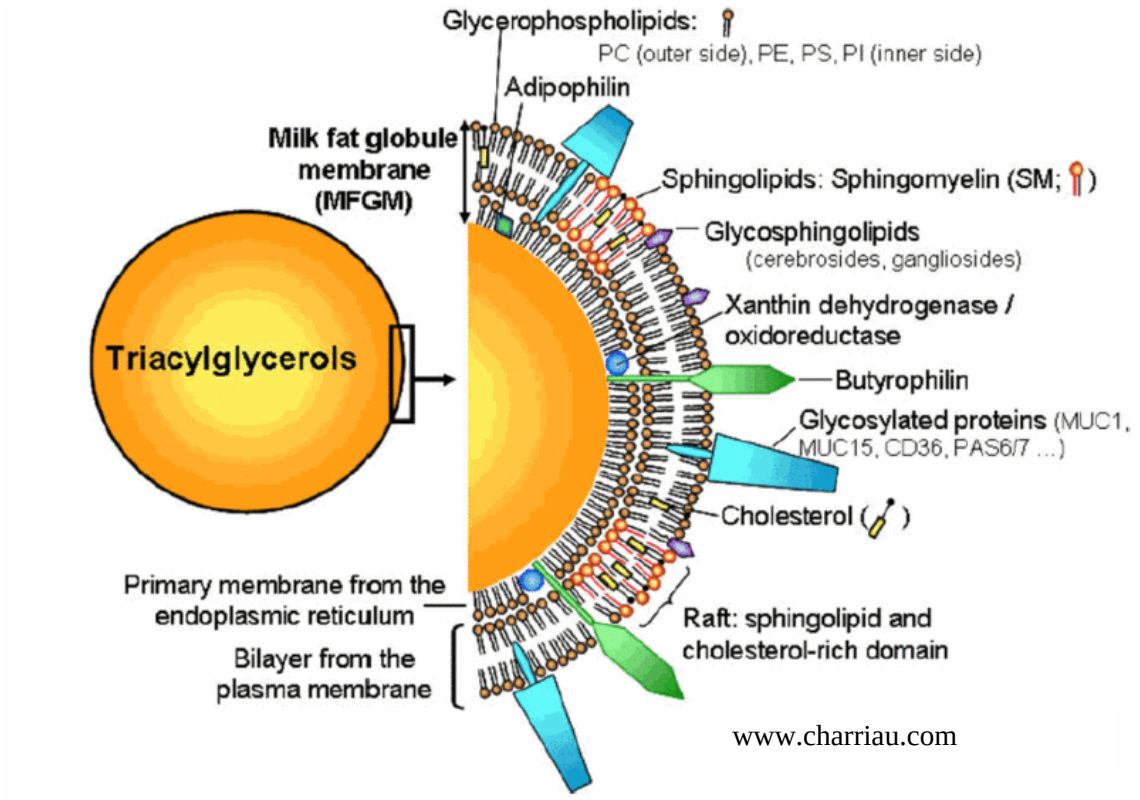


Figura 4. Principales componentes de la MFGM.

Tal y como ha sido descrito por Mather (2000) son 7 las principales proteínas mayoritarias presentes en la MFGM, siendo 2 de ellas las que más relación poseen con la inmunidad. Estas proteínas son la mucin-1 (MUC-1) y la xanthine dehydrogenase/oxidase (XDH/XO).

MUC-1 es una proteína glicosilada presente en la superficie apical de las células epiteliales de muchos órganos, tales como pulmones, estómago o intestino (Hollingsworth and Swanson, 2004). Esta proteína ha sido aislada en vacas (Patton et al., 1995), donde ésta se encuentra en una baja concentración (40 mg/l) si es comparada con la encontrada en la MFGM de humanos (729-805 mg/l) (Peterson et al., 1998) . Esta proteína también fue aislada en cabras (Campana et al., 1992) y pese a que no existe información en la bibliografía sobre su concentración en calostro, se ha descrito que la MUC-1 caprina presenta una reacción cruzada con la bovina, pero no con la humana. Esta proteína puede jugar un papel importante en la protección de superficies expuestas frente a daños físicos o a infecciones microbianas (Schroten et al., 1992; Patton et al., 1995; Peterson et al., 1998). Además, la MUC-1 podría participar en la reacción inmune de los neonatos, debido a la capacidad de esta proteína de unirse y secuestrar a los microorganismos patógenos en el tracto gastrointestinal (Schroten et al., 1992; Peterson et al., 1998).

Con respecto a la XDH/XO, ésta es principalmente sintetizada en la glándula mamaria (Bruder et al., 1983; Parks and Granger, 1986) y alcanza su máxima concentración durante el último periodo de la gestación e inmediatamente después del parto (Kurosaki et al., 1996). Esta proteína ha sido aislada de la MFGM de vacas, cabras y ovejas, aunque aún no han sido establecidas las concentraciones de esta proteína en dichas especies. Sin embargo, basándose en muestras de leche, se ha descrito que la XDH/XO ovina y bovina tienen una concentración similar (Mondy and Keenan, 1993; Ye et al., 2002; Pisanu et al., 2011). La función de esta proteína no está definida claramente, pero se ha sugerido que podría desempeñar un papel estructural y funcional en la formación de la MFGM (Ishii et al., 1995), además de ser uno de los componentes antibacterianos del calostro y de la leche, proporcionando una fuente de H_2O_2 para la lactoperoxidasa (Bjorck and Claesson, 1979). Por último, esta proteína podría actuar como inmunomodulador, produciendo una exacerbación de la respuesta inflamatoria o induciendo la expresión de genes que codifican para diferentes componentes tales como proteínas de adhesión, receptores celulares y otros componentes de la respuesta inmune (Mather, 2000).

El segundo grupo de proteínas que puede ser obtenido del calostro, son las denominadas proteínas del suero. Estableciendo el suero de calostro bovino como referencia, ha sido estudiado que este contiene más de 200 tipos de proteínas, de las cuales la β -lactoglobulina, α -lactalbumina, albúmina sérica bovina (BSA), inmunoglobulinas y la lactoferrina se encuentran en una mayor concentración (Sgarbieri, 2004; Korhonen and Pihlanto, 2007; Kawecka and Radko, 2011; Roncada et al., 2012). De este grupo de proteínas, las inmunoglobulinas juegan el papel más importante en cuanto a la función inmune se refiere, sin embargo existen otras proteínas como la α –lactalbumina y la lactoferrina que poseen otras funciones inmunes importantes (Korhonen and Pihlanto, 2007; Tang et al., 2011).

En cuanto a la α –lactalbumina, ésta ha sido asociada a la regulación de la producción de lactosa (Kleinberg et al., 1983), sin embargo ha sido descrito que esta proteína también juega un papel importante como inmunomodulador en el calostro y la leche humana y de vaca (Montagne et al., 2000; Korhonen and Pihlanto, 2007; Marnila and Korhonen, 2011). Además son varios los estudios que describen la presencia de esta proteína en calostro (Kawecka and Radko, 2011; Nissen et al., 2012).

.Ha sido observado que la concentración de esta proteína en calostro no difiere significativamente entre cabras, vacas y ovejas (2,77 mg/ml, 2 mg/ml y 2,3 mg/ml, respectivamente) (Perez et al., 1990; Levieux et al., 2002), mostrando que la concentración de esta proteína en calostro es 1,5 veces mayor que la encontrada en leche.

La lactoferrina, es otra de las proteínas mayoritarias del suero de calostro, siendo muy importante la acción de ésta durante las infecciones. Así, Konuspayeva et al. (2008) estudiaron las diferencias de concentración en calostro de varias especies tales como camella (5,1 mg/ml), vaca (0,84 mg/ml), cabra (3,09 mg/ml), oveja (1,56 mg/ml) o búfala (2,1 mg/ml). Resultados similares fueron observados por Nissen et al. (2012) en calostro de vaca usando 2DE-LC-MS/MS y ELISA. En cuanto a su función biológica primaria, la lactoferrina es una proteína transportadora de los iones hierro. Asimismo, la lactoferrina tiene una amplia variedad de funciones biológicas, muchas de las cuales no parecen estar relacionadas con su capacidad de transportar iones de hierro (Brock, 2002), así, la lactoferrina participa, por ejemplo, como un factor indiscutible en la

inmunidad innata, siendo sintetizada por el epitelio de la glándula mamaria (Sánchez et al., 1992; Adlerova et al., 2008), proveyendo actividad antimicrobiana (bactericida y fungicida) a la glándula mamaria y también al recién nacido (Bellamy et al., 1992). Por último, debido a que la concentración de esta proteína incrementa durante las reacciones inflamatorias e infecciones virales, ésta ha sido clasificada como una proteína inflamatoria de fase agua (Kanyshkova et al., 2001).

Con respecto a la última de las fracciones que podemos obtener del calostro, las caseínas pueden ser clasificadas en 4 subtipos (αs1, αs2, β and κ), siendo además responsables de algunas funciones biológicas importantes, tales como el transporte de iones (Ca, PO_4, Fe, Zn, Cu), el de actuar como inmunomoduladores y por último el ser precursores de péptidos bioactivos (Korhonen and Pihlanto, 2007). En cuanto a esta última característica de las caseínas, ha sido descrito que los fragmentos proteolíticos de las caseínas poseen funciones antimicrobianas (Lahov and Regelson, 1996), sugiriendo que las proteasas presentes en el calostro podrían participar en la propia inmunidad del individuo. Estos péptidos bioactivos están despertando el interés de los investigadores, ya que podrían ser usados como posibles fuentes naturales de alimento que reporten un beneficio directo a la salud de los seres humanos (Gauthier et al., 2006), debido probablemente a que estimulan el sistema inmune innato en la glándula mamaria y pueden prevenir las infecciones de esta durante el periodo de secado (Silanikove et al., 2005).

7.Proteínas minoritarias en calostro

Pese a la gran importancia de las proteínas mayoritarias del calostro anteriormente descritas, existe un creciente interés en aumentar el conocimiento general de las proteínas minoritarias presentes en el mismo, asi como su relación con los procesos relacionados con la salud de humanos y animales (Yamada et al., 2002; Reinhardt and Lippolis, 2008; Golinelli et al., 2011). Poniendo como ejemplo el proteoma del calostro humano, un total de 151 proteínas han sido identificadas, siendo 83 de ellas nunca descritas en leche o calostro humano (Palmer et al., 2006). Probablemente muchas de estas proteínas pueden estar relacionadas con importantes

funciones en el recién nacido, tales como la regulación del crecimiento, el transporte de nutrientes o lo que es más importante, la TIP. En los rumiantes, la identificación y el conocimiento de la función de las proteínas presentes en el suero de calostro y la MFGM están aún incompletos y por lo tanto muchas de las funciones de estas proteínas no ha sido aún descritas (Fong et al., 2008; Reinhardt and Lippolis, 2008; Rusu et al., 2009).

Las técnicas bioquímicas, principalmente los métodos basados en los geles 2DE, han sido las más usadas para la identificación de las proteínas minoritarias del calostro (Yamada et al., 2002; O'Donnell et al., 2004). Sin embargo, actualmente estas técnicas están empezando a ser reemplazadas por otras técnicas como el iTRAQ o el Label-free, ya que confieren varias ventajas en comparación con las técnicas basadas en los geles 2DE, siendo capaces de identificar un mayor número de proteínas, usando una menor cantidad de muestra y obteniendo unos resultados más reproducibles. Sin embargo, debido al elevado coste del equipamiento, de los instrumentos y los reactivos, estas técnicas no se encuentran disponibles en la mayoría de los laboratorios.

7.1. Técnicas comúnmente usadas para identificar proteínas minoritarias

Los procedimientos tales como inmunoabsorción (Murakami et al., 1998; Yamada et al., 2002; Palmer et al., 2006), isoelectro enfoque (IEF) (Zuo and Speicher, 2002), affinity tagging (Holland et al., 2006), y semi-coupled anion- and cation-exchange chromatography (Fong et al., 2008) han sido usados como herramientas para eliminar las proteínas mayoritarias e incrementar la abundancia relativa de éstas en análisis proteómicos de muestras complejas como el calostro. A pesar de las técnicas descritas en la tabla 2 para eliminar las caseínas de las muestras de suero de calostro, es necesario además depleccionar las proteínas mayoritarias (principalmente inmunoglobulinas), ya que estas enmascaran a las proteínas minoritarias en los análisis proteómicos (Yamada et al., 2002).

Este efecto adverso de las inmunoglobulinas en los estudios proteómicos de proteínas minoritarias se produce porque éstas están localizadas en la misma región del gel 2DE que muchas de las proteínas minoritarias, tales como la transferrina, plasmina, lipoproteína lipasa y fosfatasa alcalina, entre otras, que quedan enmascaradas por esta

proteína mayoritaria (Fong et al., 2008). Por este motivo es necesario eliminar las proteínas mayoritarias del calostro y así poder analizar los cambios en el perfil proteómico de las proteínas minoritarias. Actualmente existen varios kits capaces de eliminar o reducir la presencia de proteínas mayoritarias en el calostro. Así, Golinelli et al. (2011) usaron y compararon la eficacia del kit Albumin and IgG removal (GE Healthcare Life Sciences, UK) y del kit Vivaspin 500 ultrafiltration cartridge (GE Healthcare Life Sciences, UK) con una membrana de 100 kDa de peso molecular en la depleción de las proteínas mayoritarias del calostro. Estos autores observaron como el kit Albumin and IgG removal fue ineficaz a la hora de eliminar la IgG del suero de calostro bovino, probablemente porque estos kits están compuestos por una agarosa que contiene anticuerpos frente a la IgG de humano, lo que hace que debido a la falta de reacción cruzada entre la IgG humana y bovina, estas últimas no hayan sido captadas por los anticuerpos frente a las IgG de humano. Sin embargo, estos autores también describen que el tratamiento del suero de calostro con el kit Vivaspin 500 eliminó la mayoría de la IgG e IgA, mejorando la detección de proteínas minoritarias en las muestras.

Otro kit usado comúnmente para la depleción de las proteínas mayoritarias es el ProteoMiner (Bio-Rad, USA), el cual se basa en una exclusiva librería de hexapéptidos con la propiedad de unirse con la misma capacidad a proteínas de diferente abundancia (Bandhakavi et al., 2011). Por lo tanto, cuando una muestra que contiene una mezcla compleja de proteínas es expuesta a estos hexapéptidos, cada uno de éstos se unen a las proteínas mayoritarias y por lo tanto se saturan rápidamente, permaneciendo la gran mayoría de las proteínas mayoritarias en suspensión y sin unir a estos hexapéptidos. En el caso de las proteínas minoritarias, estas no llegan a saturar a sus hexapéptidos y por lo tanto todas las proteínas minoritarias son captadas por estos ligandos. Como resultado del tratamiento con este kit, tendremos que pese a que las proteínas mayoritarias siguen estando presentes, estas se encuentran en una proporción mucho más reducida, permitiendo así un incremento relativo del porcentaje de las proteínas minoritarias (Boschetti and Righetti, 2008; D'Amato et al., 2009). Cabe destacar, que pese a que este kit fue creado específicamente para muestras de plasma y suero, ha sido también comprobada su eficacia en otro tipo de muestras tales como orina (Castagna et al.,

2005), bilis (Guerrier et al., 2007), plaquetas, glóbulos rojos y clara de huevo (Guerrier et al., 2008).

Con respecto a las muestras de leche y calostro, Liao et al. (2011) usaron el kit Proteominer para depleciones en leche y calostro de humano, mostrando que la presencia de muchas proteínas minoritarias incrementó y que a su vez la presencia de proteínas mayoritarias decreció considerablemente. Sin embargo, estos autores describieron que este kit tiene un efecto muy limitado a la hora de eliminar las caseínas restantes en el suero de calostro y leche. De la misma forma, este kit fue usado para estudiar el proteoma de la leche de vaca, obteniendo un incremento considerable de las proteínas minoritarias (D'Amato et al., 2009). Es necesario remarcar que pese a que este kit es capaz de producir un incremento en la presencia de proteínas minoritarias, las proteínas mayoritarias presentes en el calostro y leche tales como la lactoferrina o la α-lactalbúmina, además de las caseínas, son aún detectadas tras el tratamiento con este kit.

Figura 5: Presentación comercial del kit Proteominer (Bio-Rad, USA) y del kit Albumin and IgG removal (GE Healthcare Life Sciences, UK).

Sin embargo, a pesar de que se ha demostrado la efectividad de este kit en la depleción de proteínas mayoritarias, es necesario resaltar el efecto que produce este kit en estudios proteómicos cuantitativos usando iTRAQ, en los cuales se mide la abundancia relativa de cada proteína (Bandhakavi et al., 2011). Tal y como describieron Mouton-Barbosa et al. (2010) después del tratamiento de las muestras con este kit, las proteínas mayoritarias no mantiene su verdadera cantidad relativa después del tratamiento, ya que saturan los ligandos de la librería de hexapéptidos, sin embargo las

proteínas minoritarias, al no saturar los ligandos, mantienen su concentración original en la muestra.

Por lo tanto, y tomando la información descrita anteriormente, el kit de depleción Proteominer es una excelente herramienta a la hora de descubrir las partes más profundas del proteoma de multitud de muestras compuestas por una gran mezcla de proteínas de diferente concentración. Sin embargo, algunas consideraciones deben de tenerse cuando este kit es usado en estudios proteómicos cuantitativos.

7.2.Función de algunas proteínas minoritarias y su relación con el sistema inmune

Es muy importante entender como las proteínas minoritarias del calostro pueden mejorar o modificar la respuesta inmune ya sea a nivel de la madre o a nivel del recién nacido, por lo que el estudio de estas proteínas por separado es de extrema importancia.

Una de estas proteínas minoritarias del calostro es la plasmina. Esta proteína es una enzima fibrinolítica que juega un papel muy importante en la disolución de los coágulos de fibrina, previniendo así procesos de trombosis (Ogiwara et al., 2010). A pesar de su función en el torrente sanguíneo, esta proteína ha sido identificada en calostro y leche (Dupont et al., 1998; Le et al., 2010). Tal y como fue descrito por Dupont et al. (1998), el calostro de vaca posee una concentración en plasmina 10 veces superior (0,49 µg/ml) a la que se encuentra en leche (0,04 µg/ml). Además Rebucci et al. (2005) encontraron que tanto la plasmina descrita en la leche de vaca, oveja y cabra es idéntica a la encontrada en sangre. Asimismo, otros autores han observado que la plasmina tiene una función inmune, ya que contribuye a la migración de los neutrófilos hasta el punto de infección (Renckens et al., 2006). Siguiendo esta línea, Theodorou et al. (2010) observaron como la concentración de plasmina se incrementaba en sangre y leche tras una mastitis aguda en ovejas lactantes.

Otra de las proteínas minoritarias presentes en el calostro es la serum amyloid A (SAA). Esta proteína se encuentra principalmente en complejos con lipoproteínas, existiendo varias isoformas que varían en concentración en función de la especie (Uhlar and Whitehead, 1999). La SAA es una de las proteínas más conservadas dentro de los

mamíferos, confirmando la premisa de que esta proteína tiene una función básica y esencial en el sistema inmune, formando parte de la fase aguda de la inflamación (Eckersall et al., 2006; Pyorala et al., 2011). Esta proteína ha sido también identificada en el calostro de varias especies tales como humana (Kumon et al., 2011) yegua, vaca y oveja (McDonald et al., 2001; Le et al., 2010). Así mismo, se ha descrito que la concentración de SAA circulante en plasma sanguíneo se incrementa 1000 veces entre las 24 y 48 horas tras la infección o inflamación, partiendo de niveles basales de 5-8 mg/ml (Faty et al., 2012), mostrando así la importancia de esta proteína en los procesos inflamatorios. Además, una variante de esta proteína de fase agua, la SAA-3, ha sido descrita su expresión en células de la glándula mamaria en respuesta a patógenos, estando presente en la leche durante las mastitis (McDonald et al., 2001) y en el calostro (McDonald et al., 2001; Larson et al., 2005), sugiriendo el importante papel de esta proteína en la reacción inmune. Así, McDonald et al. (2001) encontraron que la concentración de la SAA-3 es mucho mayor en calostro que en leche (267,45 µg/ml y 2,63 µg/ml, respectivamente). Estos autores además describieron que la concentración de esta proteína en calostro de ovejas es menor que la encontrada en vacas (62,83 µg/ml y 267,45 µg/ml, respectivamente).

De manera general, la proteína SAA desempeña un gran número de acciones proinflamatorias, tales como quimiotáctico de los neutrófilos, monocitos y linfocitos T, causando la infiltración de los leucocitos y promoviendo la adhesión de los neutrófilos a la células endoteliales (Badolato et al., 1994; Xu et al., 1995; Su et al., 1999), estimulando a la liberación de citoquinas (Furlaneto and Campa, 2000; He et al., 2003) y metaloproteínasas (Lee et al., 2005b) por parte de los neutrófilos y los monocitos. De acuerdo a lo descrito por He et al. (2009), estas características de la SAA sugieren que tiene una función clave no solo en el establecimiento, sino también en el mantenimiento de la inflamación.

Otra interesante proteína minoritaria presente en el calostro es el fibrinógeno, precursor de la fibrina, el componente mayoritario de los trombos sanguíneos. Sin embargo esta proteína tiene también una función defensiva, ya que ha sido demostrado que la concentración de fibrinógeno se incrementa durante los procesos de inflamación aguda (Tamzali et al., 2001; Ganheim et al., 2003). Además, Yamada et al. (2002)

estudiaron diferencias en proteínas minoritarias entre calostro y leche de vaca, destacando que había proteínas que se encontraban exclusivamente en el calostro, tales como el fibrinógeno.

De forma similar, varios autores describieron que el fibrinógeno tiene la capacidad para unirse a las integrinas, las cuales son normalmente expresadas por las células del sistema inmune, tales como CD11b/CD18 (Ugarova and Yakubenko, 2001; Ryu et al., 2009). El receptor formado por integrinas en las células CD11b/CD18 (αMβ2, Mac-1, complemento receptor 3) es un miembro de la familia de las integrinas β2, las cuales son expresadas por monocitos y macrófagos. Cuando el fibrinógeno se une a las integrinas de las células CD11b/CD18, se produce un proceso de señalización que produce la adhesión, migración, quimiotaxis y fagocitosis de las células B activadas (Akassoglou and Strickland, 2002). Por lo tanto, debido a las funciones descritas previamente, el fibrinógeno presente en el calostro podría ser considerado beneficioso para el sistema inmune de los recién nacidos.

El inhibidor de la tripsina (TI), también conocido como α1-antitripsina, es una proteína que reduce la tripsina biológicamente activa (Rawlings et al., 2004), disminuyendo los procesos de proteólisis. Esta proteína ha sido descrita en calostro de vaca usando diferentes técnicas proteómicas, tales como los geles de 2DE (Yamada et al., 2002) o LC-MS/MS (Nissen et al., 2012). Es de destacar que ha sido descrito que el calostro contiene 100 veces mayor concentración de esta proteína que la leche (Sandholm and Honkanenbuzalski, 1979; Honkanenbuzalski and Sandholm, 1981), probablemente para proteger a las proteínas calostrales de los procesos proteolíticos, permitiendo la absorción de los componentes inmunes del calostro de forma intacta por los rumiantes neonatos.

La lipopolysaccharide-binding protein (LBP) es una de las proteínas producidas durante las infecciones por las bacterias Gram negativas (Schumann et al., 1990). Además, la LPB parece participar también de forma activa durante las infecciones por bacterias Gram positivas (Weber et al., 2003). Esta proteína ha sido detectada en calostro de vaca usando 2D-LC-MS/MS (Nissen et al., 2012), cuantificándose a posteriori con ELISA (0,85 mg/ml, aproximadamente) por los mismos autores. No se ha encontrado ninguna referencia sobre la presencia de esta proteína en calostro de oveja o

de cabra. La principal función de esta proteína es unirse a los lipopolisacáridos de la pared celular, actuando como transportador y controlador de la respuesta de los monocitos (Stelwagen et al., 2009).

Uno de los componentes más interesantes del calostro, son los péptidos antimicrobianos (AMPs) que se caracterizan por su capacidad para inactivar agentes infecciosos (Zasloff, 2002). Muchos de los AMPs actúan desestabilizando la integridad de la membranas microbianas (Zasloff, 2002). Los dos grupos mayoritarios de AMPs son las defensinas (Roncada et al., 2012) y otro grupo de moléculas catiónicas denominadas catelicidinas (Lehrer and Ganz, 2002; Zaiou and Gallo, 2002).

Las defensinas son un grupo de pequeños péptidos antimicrobianos que interactúan con la respuesta inmune adaptativa (Yang et al., 2002a). Estos péptidos son producidos en respuesta a la presencia de productos de origen microbiano o de citoquinas proinflamatorias (Ganz and Lehrer, 1998; Lehrer and Ganz, 1999; Schroder, 1999). Concretamente se ha descrito que los miembros de la familia de las defensinas β han sido expresados por las células epiteliales de la glándula mamaria durante procesos de mastitis (Goldammer et al., 2004; Swanson et al., 2004). Aunque el mecanismo de estos péptidos no ha sido totalmente descrito, podrían estar envueltos en la desestabilización de la integridad de las membranas microbianas. Cabe destacar que este grupo de péptidos se presenta en una mayor concentración en calostro que en leche (Armogida et al., 2004).

La familia de las catelicidinas es un grupo de péptidos que desempeñan un papel fundamental en la respuesta inmune innata de los mamíferos contra infecciones bacterianas (Nizet et al., 2001). Pese a que su mecanismo de acción no está claro, parece que está mediado por el reconocimiento de ciertos patrones en la membrana celular de la bacteria. Este grupo de péptidos produce una disrupción de la membrana bacteriana basada en una interacción electrostática entre la parte catiónica del péptido y los componentes aniónicos de la membrana (Gennaro and Zanetti, 2000). La presencia de los componentes de esta familia de péptidos ha sido descrita en vacas (Stelwagen et al., 2009), ovejas (Mahoney et al., 1995; Shamova et al., 1999) y cabras (Shamova et al.,

1999), siendo además descrita la presencia de estas en calostro y leche (Park, 2009; Stelwagen et al., 2009).

8.Referencias bibliográficas

Addis, M. F., S. Pisanu, S. Ghisaura, D. Pagnozzi, G. Marogna, A. Tanca, G. Biosa, C. Cacciotto, A. Alberti, M. Pittau, T. Roggio, and S. Uzzau. 2011. Proteomics and Pathway Analyses of the Milk Fat Globule in Sheep Naturally Infected by Mycoplasma agalactiae Provide Indications of the In Vivo Response of the Mammary Epithelium to Bacterial Infection. Infection and immunity 79(9):3833-3845.

Adlerova, L., A. Bartoskova, and M. Faldyna. 2008. Lactoferrin: a review. Vet Med-Czech 53(9):457-468.

Agarwal, S., W. Karmaus, S. Davis, and V. Gangur. 2011. Immune Markers in Breast Milk and Fetal and Maternal Body Fluids: A Systematic Review of Perinatal Concentrations. J Hum Lact 27(2):171-186.

Ahmad, R., A. Khan, M. T. Javed, and I. Hussain. 2000. The level of immunoglobulins in relation to neonatal Lamb mortality in Pak-Karakul sheep. Vet Arhiv 70(3):129-139.

Akassoglou, K. and S. Strickland. 2002. Nervous system pathology: The fibrin perspective. Biol Chem 383(1):37-45.

Argüello, A., N. Castro, S. Álvarez, and J. Capote. 2006. Effects of the number of lactations and litter size on chemical composition and physical characteristics of goat colostrum. Small Ruminant Research 64(1–2):53-59.

Argüello, A., N. Castro, M. Batista, I. Moreno-Indias, A. Morales-delaNuez, D. Sanchez-Macias, E. Quesada, and J. Capote. 2008. Chitotriosidase activity in goat blood and colostrum. J Dairy Sci 91(5):2067-2070.

Argüello, A., N. Castro, J. Capote, J. W. Tyler, and N. M. Holloway. 2004. Effect of colostrum administration practices on serum IgG in goat kids. Livest Prod Sci 90(2-3):235-239.

Armogida, S. A., N. M. Yannaras, A. L. Melton, and M. D. Srivastava. 2004. Identification and quantification of innate immune system mediators in human

breast milk. Allergy and asthma proceedings : the official journal of regional and state allergy societies 25(5):297-304.

Awadeh, F. T., M. M. Abdelrahman, R. L. Kincaid, and J. W. Finley. 1998a. Effect of selenium supplements on the distribution of selenium among serum proteins in cattle. Journal of Dairy Science 81(4):1089-1094.

Awadeh, F. T., R. L. Kincaid, and K. A. Johnson. 1998b. Effect of level and source of dietary selenium on concentrations of thyroid hormones and immunoglobulins in beef cows and calves. Journal of animal science 76(4):1204-1215.

Badolato, R., J. M. Wang, W. J. Murphy, A. R. Lloyd, D. F. Michiel, L. L. Bausserman, D. J. Kelvin, and J. J. Oppenheim. 1994. Serum amyloid A is a chemoattractant: induction of migration, adhesion, and tissue infiltration of monocytes and polymorphonuclear leukocytes. The Journal of experimental medicine 180(1):203-209.

Bandhakavi, S., S. K. Van Riper, P. N. Tawfik, M. D. Stone, T. Haddad, N. L. Rhodus, J. V. Carlis, and T. J. Griffin. 2011. Hexapeptide Libraries for Enhanced Protein PTM Identification and Relative Abundance Profiling in Whole Human Saliva. J Proteome Res 10(3):1052-1061.

Barone, R., F. Di Gregorio, M. A. Romeo, G. Schiliro, and L. Pavone. 1999. Plasma chitotriosidase activity in patients with beta-thalassemia. Blood Cell Mol Dis 25(1):1-8.

Barone, R., J. Simpore, L. Malaguarnera, S. Pignatelli, and S. Musumeci. 2003. Plasma chitotriosidase activity in acute Plasmodium falciparum malaria. Clinica chimica acta; international journal of clinical chemistry 331(1-2):79-85.

Barrington, G. M. and S. M. Parish. 2001. Bovine neonatal immunology. The Veterinary clinics of North America. Food animal practice 17(3):463-476.

Barza, H., M. Marinescu, and L. Blaga. 1993. Disorders of foals 0-10 days of age. Part I. Revista Romana de Medicina Veterinaria 3:9-20.

Bekele, T., O. B. Kasali, and T. Woldeab. 1992. Causes of lamb morbidity and mortality in the Ethiopian highlands. Vet Res Commun 16(6):415-424.

Bellamy, W., M. Takase, H. Wakabayashi, K. Kawase, and M. Tomita. 1992. Antibacterial Spectrum of Lactoferricin-B, a Potent Bactericidal Peptide Derived from the N-Terminal Region of Bovine Lactoferrin. J Appl Bacteriol 73(6):472-479.

Bendixen, E., M. Danielsen, K. Hollung, E. Gianazza, and I. Miller. 2011. Farm animal proteomics - A review. Journal of proteomics 74(3):282-293.

Bianchi, L., M. Puglia, C. Landi, S. Matteoni, D. Perini, A. Armini, M. Verani, C. Trombetta, P. Soldani, P. Roncada, G. Greppi, V. Pallini, and L. Bini. 2009. Solubilization methods and reference 2-DE map of cow milk fat globules. Journal of proteomics 72(5):853-864.

Bjorck, L. and O. Claesson. 1979. Xanthine-Oxidase as a Source of Hydrogen-Peroxide for the Lactoperoxidase System in Milk. Journal of Dairy Science 62(8):1211-1215.

Blum, J. W. and H. Hammon. 2000. Colostrum effects on the gastrointestinal tract, and on nutritional, endocrine and metabolic parameters in neonatal calves. Livest Prod Sci 66(2):151-159.

Boehmer, J. L., D. D. Bannerman, K. Shefcheck, and J. L. Ward. 2008. Proteomic Analysis of Differentially Expressed Proteins in Bovine Milk During Experimentally Induced Escherichia coli Mastitis. Journal of Dairy Science 91(11):4206-4218.

Boschetti, E. and P. G. Righetti. 2008. The ProteoMiner in the proteomic arena: A non-depleting tool for discovering low-abundance species. Journal of proteomics 71(3):255-264.

Brandon, M. R., D. L. Watson, and Lascelle.Ak. 1971. Mechanism of Transfer of Immunoglobulin into Mammary Secretion of Cows. Aust J Exp Biol Med 49(Dec):613-&.

Brock, J. H. 2002. The physiology of lactoferrin. Biochem Cell Biol 80(1):1-6.

Bruder, G., H. W. Heid, E. D. Jarasch, and I. H. Mather. 1983. Immunological Identification and Determination of Xanthine-Oxidase in Cells and Tissues. Differentiation 23(3):218-225.

Bush, L. J. and T. E. Staley. 1980. Absorption of Colostral Immunoglobulins in Newborn Calves. Journal of Dairy Science 63(4):672-680.

Butler, J. E., A. J. Winter, and G. G. Wagner. 1971. Symposium: bovine immune system. J Dairy Sci 54(9):1309-1314.

Campana, W. M., R. V. Josephson, and S. Patton. 1992. Presence and Genetic-Polymorphism of an Epithelial Mucin in Milk of the Goat (Capra-Hircus). Comp Biochem Phys B 103(1):261-266.

Castagna, A., D. Cecconi, L. Sennels, J. Rappsilber, L. Guerrier, F. Fortis, E. Boschetti, L. Lomas, and P. G. Rigetti. 2005. Exploring the hidden human urinary proteome via ligand library beads. J Proteome Res 4(6):1917-1930.

Castro-Alonso, A., N. Castro, J. Capote, A. Morales-DelaNuez, I. Moreno-Indias, D. Sanchez-Macias, P. Herraez, and A. Argullo. 2008. Short communication: Apoptosis regulates passive immune transfer in newborn kids. Journal of Dairy Science 91(5):2086-2088.

Castro, N., J. Capote, S. Alvarez, and A. Arguello. 2005. Effects of lyophilized colostrum and different colostrum feeding regimens on passive transfer of immunoglobulin g in Majorera goat kids. J Dairy Sci 88(10):3650-3654.

Castro, N., J. Capote, R. M. Bruckmaier, and A. Arguello. 2011. Management effects on colostrogenesis in small ruminants: a review. Journal of Applied Animal Research 39(2):85-93.

Castro, N., J. Capote, D. Martin, and A. Arguello. 2006. The influence of dietary conjugated linoleic acid on blood serum and colostrum immunoglobulin G concentration in female goats before and after parturition. Journal of animal physiology and animal nutrition 90(9-10):429-431.

Castro, N., J. Capote, A. Morales-Delanuez, C. Rodriguez, and A. Arguello. 2009. Effects of newborn characteristics and length of colostrum feeding period on passive immune transfer in goat kids. J Dairy Sci 92(4):1616-1619.

Cebo, C., H. Caillat, F. Bouvier, and P. Martin. 2010. Major proteins of the goat milk fat globule membrane. Journal of Dairy Science 93(3):868-876.

Ciupersescu, D. D. 1977. Dynamics of serum immunoglobulin concentrations in sheep during pregnancy and lactation. Research in veterinary science 22(1):23-27.

Csapo, J., Z. Csapo-Kiss, T. G. Martin, J. Szentpeteri, and G. Wolf. 1994. Composition of Colostrum from Goats, Ewes and Cows Producing Twins. Int. Dairy J. 4(5):445-458.

Csapó, J., Z. Csapó-Kiss, T. G. Martin, J. Szentpeteri, and G. Wolf. 1994. Composition of colostrum from goats, ewes and cows producing twins. Int. Dairy J. 4(5):445-458.

Chen, J. C., C. J. Chang, H. C. Peh, and S. Y. Chen. 1999. Serum protein levels and neonatal growth rate of Nubian goal kids in Taiwan area. Small Ruminant Research 32(2):153-160.

Chigerwe, M., J. W. Tyler, M. K. Summers, J. R. Middleton, L. G. Schultz, and D. W. Nagy. 2009. Evaluation of factors affecting serum IgG concentrations in bottle-fed calves. Javma-J Am Vet Med A 234(6):785-789.

Christley, R. M., K. L. Morgan, T. D. H. Parkin, and N. P. French. 2003. Factors related to the risk of neonatal mortality, birth-weight and serum immunoglobulin concentration in lambs in the UK. Prev Vet Med 57(4):209-226.

Chucri, T. M., J. M. Monteiro, A. R. Lima, M. L. B. Salvadori, J. R. Kfoury, and M. A. Miglino. 2010. A review of immune transfer by the placenta. J Reprod Immunol 87(1-2):14-20.

D'Ambrosio, C., S. Arena, A. M. Salzano, G. Renzone, L. Ledda, and A. Scaloni. 2008. A proteomic characterization of water buffalo milk fractions describing PTM of major species and the identification of minor components involved in nutrient delivery and defense against pathogens. Proteomics 8(17):3657-3666.

D'Amato, A., A. Bachi, E. Fasoli, E. Boschetti, G. Peltre, H. n. Sénéchal, and P. G. Righetti. 2009. In-Depth Exploration of Cow's Whey Proteome via Combinatorial Peptide Ligand Libraries. J Proteome Res 8(8):3925-3936.

da Nobrega, J. E., F. Riet-Correa, R. S. Nobrega, J. M. de Medeiros, J. S. de Vasconcelos, S. V. D. Simoes, and I. M. Tabosa. 2005. Perinatal mortality of lambs in the semi-arid region of Paraiba, Brazil. Pesquisa Vet Brasil 25(3):171-178.

Danielsen, M., L. J. Pedersen, and E. Bendixen. 2011. An in vivo characterization of colostrum protein uptake in porcine gut during early lactation. Journal of proteomics 74(1):101-109.

Di Rosa, M., N. Dell'Ombra, A. M. Zambito, M. Malaguarnera, F. Nicoletti, and L. Malaguarnera. 2006. Chitotriosidase and inflammatory mediator levels in Alzheimer's disease and cerebrovascular dementia. The European journal of neuroscience 23(10):2648-2656.

Di Rosa, M., M. Musumeci, A. Scuto, S. Musumeci, and L. Malaguarnera. 2005. Effect of interferon-gamma, interleukin-10, lipopolysaccharide and tumor necrosis factor-alpha on chitotriosidase synthesis in human macrophages. Clin Chem Lab Med 43(5):499-502.

Dupont, D., B. Remond, and J. C. Collin. 1998. ELISA determination of plasmin and plasminogen in milk of individual cows managed without the dry period. Milchwissenschaft 53(2):66-69.

Eales, F. A., J. S. Gilmour, R. M. Barlow, and J. Small. 1982. Causes of hypothermia in 89 lambs. The Veterinary record 110(6):118-120.

Eckblad, W. P., K. M. Hendrix, and D. P. Olson. 1981. Total complement hemolytic activity of colostral whey and sera from dairy cows. The Cornell veterinarian 71(1):54-58.

Eckersall, P. D., A. M. de Almeida, and I. Miller. 2012. Proteomics, a new tool for farm animal science. Journal of proteomics 75(14):4187-4189.

Eckersall, P. D., F. J. Young, A. M. Nolan, C. H. Knight, C. McComb, M. M. Waterston, C. J. Hogarth, E. M. Scott, and J. L. Fitzpatrick. 2006. Acute phase proteins in bovine milk in an experimental model of Staphylococcus aureus subclinical mastitis. J Dairy Sci 89(5):1488-1501.

Faty, A., P. Ferre, and S. Commans. 2012. The acute phase protein Serum Amyloid A induces lipolysis and inflammation in human adipocytes through distinct pathways. PloS one 7(4):e34031.

Fong, B. Y., C. S. Norris, and A. K. H. MacGibbon. 2007. Protein and lipid composition of bovine milk-fat-globule membrane. Int. Dairy J. 17(4):275-288.

Fong, B. Y., C. S. Norris, and K. P. Palmano. 2008. Fractionation of bovine whey proteins and characterisation by proteomic techniques. Int. Dairy J. 18(1):23-46.

Fox, P. F. 2003. Milk proteins: general and historical aspects. Pages 1-41 in Advanced dairy Chemistry. Vol. 1. 3rd ed. P. F. P. McSweeney, ed. Kluwer Academic/Plenum Publishers, New York.

Furlaneto, C. J. and A. Campa. 2000. A novel function of serum amyloid A: a potent stimulus for the release of tumor necrosis factor-alpha, interleukin-1beta, and interleukin-8 by human blood neutrophil. Biochemical and biophysical research communications 268(2):405-408.

Gagnaire, V., J. Jardin, G. Jan, and S. Lortal. 2009. Invited review: Proteomics of milk and bacteria used in fermented dairy products: From qualitative to quantitative advances. Journal of Dairy Science 92(3):811-825.

Ganheim, C., C. Hulten, U. Carlsson, H. Kindahl, R. Niskanen, and K. P. Waller. 2003. The acute phase response in calves experimentally infected with bovine viral diarrhoea virus and/or Mannheimia haemolytica. Journal of veterinary medicine. B, Infectious diseases and veterinary public health 50(4):183-190.

Ganz, T. and R. I. Lehrer. 1998. Antimicrobial peptides of vertebrates. Curr Opin Immunol 10(1):41-44.

García de Jalón, J. A., M. De las Heras, L. M. Ferrer, and F. Sancho. 1990. Síndrome de la boca mojada. Medicina Veterinaria 7:505-509.

Gauthier, S. F., Y. Pouliot, and D. Saint-Sauveur. 2006. Immunomodulatory peptides obtained by the enzymatic hydrolysis of whey proteins. Int. Dairy J. 16(11):1315-1323.

Gennaro, R. and M. Zanetti. 2000. Structural features and biological activities of the cathelicidin-derived antimicrobial peptides. Biopolymers 55(1):31-49.

Goldammer, T., H. Zerbe, A. Molenaar, H. J. Schuberth, R. M. Brunner, S. R. Kata, and H. M. Seyfert. 2004. Mastitis increases mammary mRNA abundance of beta-defensin 5, toll-like-receptor 2 (TLR2), and TLR4 but not TLR9 in cattle. Clinical and diagnostic laboratory immunology 11(1):174-185.

Golinelli, L. P., C. A. Conte, V. M. F. Paschoalin, and J. T. Silva. 2011. Proteomic Analysis of Whey from Bovine Colostrum and Mature Milk. Braz Arch Biol Techn 54(4):761-768.

Gonzalez, S. G. and P. J. Goddard. 1998. The provision of supplementary colostrum to newborn lambs: effects on post-natal lamb and ewe behaviour. Applied Animal Behaviour Science 61(1):41-50.

Guerrier, L., S. Claverol, L. Finzi, F. Paye, F. Fortis, E. Boschetti, and C. Housset. 2007. Contribution of solid-phase hexapeptide ligand libraries to the repertoire of human bile proteins. J. Chromatogr. A 1176(1-2):192-205.

Guerrier, L., P. G. Righetti, and E. Boschetti. 2008. Reduction of dynamic protein concentration range of biological extracts for the discovery of low-abundance proteins by means of hexapeptide ligand library. Nat. Protocols 3(5):883-890.

Guilloteau, P., T. Corring, P. Garnot, P. Martin, R. Toullec, and G. Durand. 1983. Effects of Age and Weaning on Enzyme-Activities of Abomasum and Pancreas of the Lamb. Journal of Dairy Science 66(11):2373-2385.

Ha, W. K., J. W. Lim, and C. K. Choi. 1986. A study on the immunoglobulin G concentration in milk and blood serum of Korean native goats. I. Changes of IgG concentration on the lactation period of Korean native goats. Korean Journal of Animal Science 28:679-683.

Hamadeh, S. K., P. G. Hatfield, R. W. Kott, B. F. Sowell, B. L. Robinson, and N. J. Roth. 2000. Effects of breed, sex, birth type and colostrums intake on cold tolerance in newborn lambs. . Sheep and Goat Research Journal 16:46-51.

He, R., H. Sang, and R. D. Ye. 2003. Serum amyloid A induces IL-8 secretion through a G protein-coupled receptor, FPRL1/LXA4R. Blood 101(4):1572-1581.

He, R. L., J. Zhou, C. Z. Hanson, J. Chen, N. Cheng, and R. D. Ye. 2009. Serum amyloid A induces G-CSF expression and neutrophilia via Toll-like receptor 2. Blood 113(2):429-437.

Holland, J. W., H. C. Deeth, and P. F. Alewood. 2006. Resolution and characterisation of multiple isoforms of bovine kappa-casein by 2-DE following a reversible cysteine-tagging enrichment strategy. Proteomics 6(10):3087-3095.

Hollingsworth, M. A. and B. J. Swanson. 2004. Mucins in cancer: Protection and control of the cell surface. Nat Rev Cancer 4(1):45-60.

Honkanenbuzalski, T. and M. Sandholm. 1981. Trypsin-Inhibitors in Mastitic Milk and Colostrum - Correlation between Trypsin-Inhibitor Capacity, Bovine Serum-Albumin and Somatic-Cell Contents. J. Dairy Res. 48(2):213-223.

Ishii, T., N. Aoki, A. Noda, T. Adachi, R. Nakamura, and T. Matsuda. 1995. Carboxy-terminal cytoplasmic domain of mouse butyrophilin specifically associates with a 150-kDa protein of mammary epithelial cells and milk fat globule membrane. Bba-Gen Subjects 1245(3):285-292.

Jensen, H. B., N. A. Poulsen, H. S. Moller, A. Stensballe, and L. B. Larsen. 2012. Comparative proteomic analysis of casein and whey as prepared by chymosin-induced separation, isoelectric precipitation or ultracentrifugation. J. Dairy Res. 79(4):451-458.

Jensen, P. T. 1978. Trypsin-Inhibitor in Sow Colostrum and Its Function. Ann Rech Vet 9(2):225-228.

Kanyshkova, T. G., V. N. Buneva, and G. A. Nevinsky. 2001. Lactoferrin and its biological functions. Biochemistry-Moscow+ 66(1):1-7.

Kawecka, A. and A. Radko. 2011. Genetic polymorphism of beta-lactoglobulin in sheep raised for milk production. Journal of Applied Animal Research 39(1):68-71.

Kehoe, J. M. 1971. Subclasses of bovine IgG. J Dairy Sci 54(9):1317.

Klaus, G. G., A. Bennett, and E. W. Jones. 1969. A quantitative study of the transfer of colostral immunoglobulins to the newborn calf. Immunology 16(3):293-299.

Kleinberg, D. L., J. Todd, and G. Babitsky. 1983. Inhibition by Estradiol of the Lactogenic Effect of Prolactin in Primate Mammary Tissue - Reversal by Anti-Estrogens Ly-156758 and Tamoxifen. P Natl Acad Sci-Biol 80(13):4144-4148.

Koldovsky, O. 1980. Hormones in milk. Life sciences 26(22):1833-1836.

Konuspayeva, G., G. Loiseau, D. Levieux, and B. Faye. 2008. Lactoferrin and immunoglobulin content in camel milk from Bactrian, Dromedary and hybrids in Kazakhstan. 1:54-62.

Korhonen, H., P. Marnila, and H. S. Gill. 2000. Milk immunoglobulins and complement factors. The British journal of nutrition 84 Suppl 1:S75-80.

Korhonen, H. and A. Pihlanto. 2007. Technological options for the production of health-promoting proteins and peptides derived from milk and colostrum. Curr Pharm Design 13(8):829-843.

Korhonen, H., E. L. Syvaoja, H. Ahola-Luttila, S. Sivela, S. Kopola, J. Husu, and T. U. Kosunen. 1995. Bactericidal effect of bovine normal and immune serum, colostrum and milk against Helicobacter pylori. J Appl Bacteriol 78(6):655-662.

Kramer, M. S., B. Chalmers, E. D. Hodnett, Z. Sevkovskaya, I. Dzikovich, S. Shapiro, J. P. Collet, I. Vanilovich, I. Mezen, T. Ducruet, G. Shishko, V. Zubovich, D. Mknuik, E. Gluchanina, V. Dombrovskiy, A. Ustinovitch, T. Kot, N. Bogdanovich, L. Ovchinikova, E. Helsing, and P. S. Grp. 2001. Promotion of breastfeeding intervention trial (PROBIT) - A randomized trial in the Republic of Belarus. Jama-J Am Med Assoc 285(4):413-420.

Kumon, Y., Y. Yasuoka, S. Yamanaka, A. Wada, H. Takeuchi, and T. Sugiura. 2011. Acute-phase serum amyloid A is present in human colostrum and milk. Amyloid : the international journal of experimental and clinical investigation : the official journal of the International Society of Amyloidosis 18 Suppl 1:11-13.

Kunz, C. and B. Lonnerdal. 1989. Human-Milk Proteins - Separation of Whey Proteins and Their Analysis by Polyacrylamide-Gel Electrophoresis, Fast Protein Liquid-Chromatography (Fplc) Gel-Filtration, and Anion-Exchange Chromatography. American Journal of Clinical Nutrition 49(3):464-470.

Kurosaki, M., S. Zanotta, M. L. Calzi, E. Garattini, and M. Terao. 1996. Expression of xanthine oxidoreductase in mouse mammary epithelium during pregnancy and lactation: Regulation of gene expression by glucocorticoids and prolactin. Biochem J 319:801-810.

Lacetera, N., U. Bernabucci, B. Ronchi, and A. Nardone. 1996. Effects of selenium and vitamin E administration during a late stage of pregnancy on colostrum and milk production in dairy cows, and on passive immunity and growth of their offspring. Am J Vet Res 57(12):1776-1780.

Lahov, E. and W. Regelson. 1996. Antibacterial and immunostimulating casein-derived substances from milk: Casecidin, isracidin peptides. Food and Chemical Toxicology 34(1):131-145.

Larson, M. A., A. Weber, A. T. Weber, and T. L. McDonald. 2005. Differential expression and secretion of bovine serum amyloid A3 (SAA3) by mammary epithelial cells stimulated with prolactin or lipopolysaccharide. Vet Immunol Immunopathol 107(3-4):255-264.

Lascelles, A. K. 1979. Immune system of the ruminant mammary-gland and its role in the control of mastitis. Journal of Dairy Science 62(1):154-160.

Le, A., L. D. Barton, J. T. Sanders, and Q. Zhang. 2010. Exploration of Bovine Milk Proteome in Colostral and Mature Whey Using an Ion-Exchange Approach. J Proteome Res 10(2):692-704.

Lee, H. Y., M. K. Kim, K. S. Park, Y. H. Bae, J. Yun, J. I. Park, J. Y. Kwak, and Y. S. Bae. 2005. Serum amyloid A stimulates matrix-metalloproteinase-9 upregulation via formyl peptide receptor like-1-mediated signaling in human monocytic cells. Biochemical and biophysical research communications 330(3):989-998.

Lehrer, R. I. and T. Ganz. 1999. Antimicrobial peptides in mammalian and insect host defence. Curr Opin Immunol 11(1):23-27.

Lehrer, R. I. and T. Ganz. 2002. Defensins of vertebrate animals. Curr Opin Immunol 14(1):96-102.

Levieux, D., F. Morgan, N. Geneix, I. Masle, and F. Bouvier. 2002. Caprine immunoglobulin G, beta-lactoglobulin, alpha-lactalbumin and serum albumin in colostrum and milk during the early post partum period. J. Dairy Res. 69(3):391-399.

Liao, Y., R. Alvarado, B. Phinney, and B. Lönnerdal. 2011. Proteomic Characterization of Human Milk Whey Proteins during a Twelve-Month Lactation Period. J Proteome Res 10(4):1746-1754.

Lonnerdal, B. and E. Forsum. 1985. Casein Content of Human-Milk. American Journal of Clinical Nutrition 41(1):113-120.

Mahoney, M. M., A. Y. Lee, D. J. BrezinskiCaliguri, and K. M. Huttner. 1995. Molecular analysis of the sheep cathelin family reveals a novel antimicrobial peptide. Febs Lett 377(3):519-522.

Malaguarnera, L., M. Di Rosa, A. M. Zambito, N. dell'Ombra, R. Di Marco, and M. Malaguarnera. 2006. Potential role of chitotriosidase gene in nonalcoholic fatty liver disease evolution. The American journal of gastroenterology 101(9):2060-2069.

Malaguarnera, L., M. Musumeci, M. Di Rosa, A. Scuto, and S. Musumeci. 2005. Interferon-gamma, tumor necrosis factor-alpha, and lipopolysaccharide promote chitotriosidase gene expression in human macrophages. J Clin Lab Anal 19(3):128-132.

Marnila, P. and H. Korhonen. 2011. Milk | Colostrum. Pages 591-597 in Encyclopedia of Dairy Sciences (Second Edition). W. F. Editor-in-Chief: John, ed. Academic Press, San Diego.

Martini, M., I. Altomonte, R. Pesi, M. G. Tozzi, and F. Salari. 2013. Fat globule membranes in ewes' milk: The main enzyme activities during lactation. Int. Dairy J. 28(1):36-39.

Mather, I. H. 2000. A review and proposed nomenclature for major proteins of the milk-fat globule membrane. Journal of Dairy Science 83(2):203-247.

Maunsell, F. P., D. E. Morin, P. D. Constable, W. L. Hurley, G. C. McCoy, I. Kakoma, and R. E. Isaacson. 1998. Effects of mastitis on the volume and composition of colostrum produced by Holstein cows. Journal of Dairy Science 81(5):1291-1299.

McDonald, T. L., M. A. Larson, D. R. Mack, and A. Weber. 2001. Elevated extrahepatic expression and secretion of mammary-associated serum amyloid A 3 (M-SAA3) into colostrum. Vet Immunol Immunop 83(3–4):203-211.

Meckenzie, H. A. 1970. Milk protein. Vol. I. New York, USA, .

Mellor, D. J. and L. Murray. 1985. Effects of Maternal Nutrition on Udder Development during Late Pregnancy and on Colostrum Production in Scottish Blackface Ewes with Twin Lambs. Research in veterinary science 39(2):230-234.

Mier, M. P., R. Ibanez, and I. Ortiz. 2008. Influence of process variables on the production of bovine milk casein by electrodialysis with bipolar membranes. Biochem. Eng. J. 40(2):304-311.

Mondy, B. L. and T. W. Keenan. 1993. Butyrophilin and Xanthine-Oxidase Occur in Constant Molar Proportions in Milk Lipid Globule-Membrane but Vary in Amount with Breed and Stage of Lactation. Protoplasma 177(1-2):32-36.

Montagne, P. M., M. L. Cuillière, C. M. Molé, M. C. Béné, and G. C. Faure. 2000. Dynamics of the Main Immunologically and Nutritionally Available Proteins of

Human Milk during Lactation. Journal of Food Composition and Analysis 13(2):127-137.

Moore, M., J. W. Tyler, M. Chigerwe, M. E. Dawes, and J. R. Middleton. 2005. Effect of delayed colostrum collection on colostral IgG concentration in dairy cows. Javma-J Am Vet Med A 226(8):1375-1377.

Moreno-Indias, I., A. Morales-delaNuez, L. E. Hernandez-Castellano, D. Sanchez-Macias, J. Capote, N. Castro, and A. Arguello. 2012a. Docosahexaenoic acid in the goat kid diet: Effects on immune system and meat quality. Journal of animal science 90(11):3729-3738.

Moreno-Indias, I., D. Sanchez-Macias, N. Castro, A. Morales-delaNuez, L. E. Hernandez-Castellano, J. Capote, and A. Arguello. 2012b. Chemical composition and immune status of dairy goat colostrum fractions during the first 10 h after partum. Small Ruminant Research 103(2-3):220-224.

Mouton-Barbosa, E., F. Roux-Dalvai, D. Bouyssie, F. Berger, E. Schmidt, P. G. Righetti, L. Guerrier, E. Boschetti, O. Burlet-Schiltz, B. Monsarrat, and A. G. de Peredo. 2010. In-depth Exploration of Cerebrospinal Fluid by Combining Peptide Ligand Library Treatment and Label-free Protein Quantification. Mol Cell Proteomics 9(5):1006-1021.

Murakami, K., M. Lagarde, and Y. Yuki. 1998. Identification of minor proteins of human colostrum and mature milk by two-dimensional electrophoresis. Electrophoresis 19(14):2521-2527.

Musumeci, M., L. Malaguarnera, J. Simpore, R. Barone, M. Whalen, and S. Musumeci. 2005. Chitotriosidase activity in colostrum from African and Caucasian women. Clin Chem Lab Med 43(2):198-201.

Neubauer, H. P. and H. H. Schone. 1979. Transfer of insulin-binding antibodies from nanny goats to kids. Am J Vet Res 40(7):962-965.

Nissen, A., E. Bendixen, K. L. Ingvartsen, and C. M. Rontved. 2012. In-depth analysis of low abundant proteins in bovine colostrum using different fractionation techniques. Proteomics 12(18):2866-2878.

Nizet, V., T. Ohtake, X. Lauth, J. Trowbridge, J. Rudisill, R. A. Dorschner, V. Pestonjamasp, J. Piraino, K. Huttner, and R. L. Gallo. 2001. Innate antimicrobial peptide protects the skin from invasive bacterial infection. Nature 414(6862):454-457.

Nowak, R. and P. Poindron. 2006. From birth to colostrum: early steps leading to lamb survival. Reprod Nutr Dev 46(4):431-446.

Nowak, R., P. Poindron, and I. G. Putu. 1989. Development of mother discrimination by single and multiple newborn lambs. Developmental psychobiology 22(8):833-845.

O'Donnell, R., J. W. Holland, H. C. Deeth, and P. Alewood. 2004. Milk proteomics. Int. Dairy J. 14(12):1013-1023.

Ogiwara, K., K. Nogami, K. Nishiya, and M. Shima. 2010. Plasmin-induced procoagulant effects in the blood coagulation: a crucial role of coagulation factors V and VIII. Blood Coagul Fibrin 21(6):568-576.

Ontsouka, C. E., R. M. Bruckmaier, and J. W. Blum. 2003. Fractionized milk composition during removal of colostrum and mature milk. J Dairy Sci 86(6):2005-2011.

Orsel, K., J. J. Amerongen, R. N. Zadoks, D. C. K. Doorn, T. Wensing, J. J. van Amerongen, and D. C. K. van Doorn. 2000. Serum gamma-globulin concentration in goat kids after colostrum administration: effect of time of administration, and amount and type of colostrum. Tijdschrift voor Diergeneeskunde 125:709-712.

Oyeniyi, O. O. and A. G. Hunter. 1978. Colostral constituents including immunoglobulins in 1st 3 milkings postpartum. Journal of Dairy Science 61(1):44-48.

Padua, M. B., A. A. Kowalski, M. Y. Canas, and P. J. Hansen. 2010. The molecular phylogeny of uterine serpins and its relationship to evolution of placentation. Faseb J 24(2):526-537.

Pakkanen, R. and J. Aalto. 1997. Growth factors and antimicrobial factors of bovine colostrum. Int. Dairy J. 7(5):285-297.

Palmer, D. J., V. C. Kelly, A. M. Smit, S. Kuy, C. G. Knight, and G. J. Cooper. 2006. Human colostrum: Identification of minor proteins in the aqueous phase by proteomics. Proteomics 6(7):2208-2216.

Pallavicini, C., B. Ciruzzi, and V. Laudadio. 1984. Isolation and Partial Characterization of Ovine Colostrum Trypsin-Inhibitor. Latte 9(4):309-312.

Paredi, G., S. Raboni, E. Bendixen, A. M. de Almeida, and A. Mozzarelli. 2012. "Muscle to meat" molecular events and technological transformations: The proteomics insight. Journal of proteomics 75(14):4275-4289.

Park, Y. W. 2009. Overview of Bioactive Components in Milk and Dairy Products. Pages 1-12 in Bioactive Components in Milk and Dairy Products. Wiley-Blackwell.

Parks, D. A. and D. N. Granger. 1986. Xanthine-Oxidase - Biochemistry, Distribution and Physiology. Acta physiologica Scandinavica 126:87-99.

Patton, S., S. J. Gendler, and A. P. Spicer. 1995. The epithelial mucin, MUC1, of milk, mammary gland and other tissues. Bba-Rev Biomembranes 1241(3):407-423.

Patton, S. and G. E. Huston. 1986. A Method for Isolation of Milk-Fat Globules. Lipids 21(2):170-174.

Patton, S. and R. G. Jensen. 1975. Lipid metabolism and membrane functions of the mammary gland. Progress in the chemistry of fats and other lipids 14(4):163-277.

Penque, D. 2009. Two-dimensional gel electrophoresis and mass spectrometry for biomarker discovery. Proteom Clin Appl 3(2):155-172.

Perez, M. D., L. Sanchez, P. Aranda, J. M. Ena, R. Oria, and M. Calvo. 1990. Synthesis and Evolution of Concentration of Beta-Lactoglobulin and Alpha-Lactalbumin from Cow and Sheep Colostrum and Milk Throughout Early Lactation. Cell Mol Biol 36(2):205-212.

Peterson, J. A., S. Patton, and M. Hamosh. 1998. Glycoproteins of the human milk fat globule in the protection of the breast-fed infant against infections. Biology of the neonate 74(2):143-162.

Pisanu, S., S. Ghisaura, D. Pagnozzi, G. Biosa, A. Tanca, T. Roggio, S. Uzzau, and M. F. Addis. 2011. The sheep milk fat globule membrane proteome. Journal of proteomics 74(3):350-358.

Purup, S., M. Vestergaard, O. P. L, and K. Sejrsen. 2007. Biological activity of bovine milk on proliferation of human intestinal cells. The Journal of dairy research 74(1):58-65.

Pyorala, S., M. Hovinen, H. Simojoki, J. Fitzpatrick, P. D. Eckersall, and T. Orro. 2011. Acute phase proteins in milk in naturally acquired bovine mastitis caused by different pathogens. The Veterinary record 168(20):535.

Quigley, J. D., P. French, and R. E. James. 2000. Short communication: Effect of pH on absorption of immunoglobulin G in neonatal calves. Journal of Dairy Science 83(8):1853-1855.

Rainard, P. 2003. The complement in milk and defense of the bovine mammary gland against infections. Veterinary research 34(5):647-670.

Rainard, P. and C. Riollet. 2006. Innate immunity of the bovine mammary gland. Veterinary research 37(3):369-400.

Rajala, P. and H. Castrén. 1995. Serum Immunoglobulin Concentrations and Health of Dairy Calves in Two Management Systems from Birth to 12 Weeks of Age. Journal of Dairy Science 78(12):2737-2744.

Ramirez, A., A. Quiles, M. L. Hevia, F. Sotillo, and M. D. Ramirez. 1997. Influence of forced contact on the maternal-filial bond in the domestic goat after different periods of post-partum separation. Small Ruminant Research 23(2-3):75-81.

Ramos, J. J., A. Loste, L. M. Ferrer, A. Fernandez, N. Castro, A. Ortin, M. T. Verde, A. Arguello, and L. Figueras. 2010. Effect of addition of soybean trypsin inhibitor to colostrum on immunological status in goat kids. Journal of animal physiology and animal nutrition 94(1):93-98.

Rawlings, N. D., D. P. Tolle, and A. J. Barrett. 2004. Evolutionary families of peptidase inhibitors. Biochem J 378:705-716.

Rebucci, R., E. Fusi, C. Pecorini, L. Pinotti, F. Cheli, and A. Baldi. 2005. Evaluation of the biological activation of plasmin plasminogen system in sheep and goat milk. Ital J Anim Sci 4:330-332.

Reinhardt, T. A. and J. D. Lippolis. 2008. Developmental changes in the milk fat globule membrane proteome during the transition from colostrum to milk. Journal of Dairy Science 91(6):2307-2318.

Reiter, B. and J. H. Brock. 1975. Inhibition of Escherichia coli by bovine colostrum and post-colostral milk. I. Complement-mediated bactericidal activity of antibodies to a serum susceptible strain of E. coli of the serotype O 111. Immunology 28(1):71-82.

Renckens, R., J. J. T. H. Roelofs, S. Florquin, and T. van der Poll. 2006. Urokinase-type plasminogen activator receptor plays a role in neutrophil migration during lipopolysaccharide-induced peritoneal inflammation but not during Escherichia coli-induced peritonitis. Journal of Infectious Diseases 193(4):522-530.

Rodríguez, C., N. Castro, J. Capote, A. Morales-delaNuez, I. Moreno-Indias, D. Sanchez-Macias, and A. Arguello. 2009. Effect of colostrum immunoglobulin concentration on immunity in Majorera goat kids. Journal of Dairy Science 92(4):1696-1701.

Roncada, P., C. Piras, A. Soggiu, R. Turk, A. Urbani, and L. Bonizzi. 2012. Farm animal milk proteomics. Journal of proteomics 75(14):4259-4274.

Rusu, D., R. Drouin, Y. Pouliot, S. Gauthier, and P. E. Poubelle. 2009. A Bovine Whey Protein Extract Can Enhance Innate Immunity by Priming Normal Human Blood Neutrophils. Journal of Nutrition 139(2):386-393.

Ryu, J. K., D. Davalos, and K. Akassoglou. 2009. Fibrinogen signal transduction in the nervous system. J Thromb Haemost 7:151-154.

Sánchez, L., L. Luján, R. Oria, H. Castillo, D. Pérez, J. M. Ena, and M. Calvo. 1992. Synthesis of Lactoferrin and Transport of Transferrin in the Lactating Mammary-Gland of Sheep. Journal of Dairy Science 75(5):1257-1262.

Sandholm, M. and T. Honkanenbuzalski. 1979. Colostral Trypsin-Inhibitor Capacity in Different Animal Species. Acta Vet Scand 20(4):469-476.

Santos, D. 1994. Variabilidade em imunoglobina G (IgG) no colostro de cabra de primeira ordenha e absorcao intestinal de IgG pelos cabritos recém-nascidos [Variability of immunoglobulins (IgG) in the colostrum of goats at first milking and intestinal absorption IgG by neonatal kids]. Braz Arch Biol Techn 37:285.

Scammel, A. W. 2001. Production and uses of colostrum. Aust J Dairy Technol 56.

Schroder, J. M. 1999. Epithelial antimicrobial peptides: innate local host response elements. Cellular and Molecular Life Sciences 56(1-2):32-46.

Schroten, H., F. G. Hanisch, R. Plogmann, J. Hacker, G. Uhlenbruck, R. Nobisbosch, and V. Wahn. 1992. Inhibition of Adhesion of S-Fimbriated Escherichia-Coli to Buccal Epithelial-Cells by Human-Milk Fat Globule-Membrane Components - a Novel Aspect of the Protective Function of Mucins in the Nonimmunoglobulin Fraction. Infection and immunity 60(7):2893-2899.

Schumann, R. R., S. R. Leong, G. W. Flaggs, P. W. Gray, S. D. Wright, J. C. Mathison, P. S. Tobias, and R. J. Ulevitch. 1990. Structure and Function of Lipopolysaccharide Binding-Protein. Science 249(4975):1429-1431.

Sgarbieri, V. C. 2004. Propriedades fisiológicas-funcionais das proteínas do soro de leite. Revista de Nutrição 17:397-409.

Shamova, O., K. A. Brogden, C. Q. Zhao, T. Nguyen, V. N. Kokryakov, and R. I. Lehrer. 1999. Purification and properties of proline-rich antimicrobial peptides from sheep and goat leukocytes. Infection and immunity 67(8):4106-4111.

Silanikove, N., F. Shapiro, A. Shamay, and G. Leitner. 2005. Role of xanthine oxidase, lactoperoxidase, and NO in the innate immune system of mammary secretion during active involution in dairy cows: manipulation with casein hydrolyzates. Free Radical Bio Med 38(9):1139-1151.

Smith, G. W. and D. M. Foster. 2007. Short Communication: Absorption of Protein and Immunoglobulin G in Calves Fed a Colostrum Replacer. Journal of Dairy Science 90(6):2905-2908.

Staak, C. 1992. [Bovine colostrum and protection of young animals]. Berl Munch Tierarztl Wochenschr 105(7):219-224.

Stelwagen, K., E. Carpenter, B. Haigh, A. Hodgkinson, and T. T. Wheeler. 2009. Immune components of bovine colostrum and milk. Journal of animal science 87(13 Suppl):3-9.

Su, S. B., W. Gong, J. L. Gao, W. Shen, P. M. Murphy, J. J. Oppenheim, and J. M. Wang. 1999. A seven-transmembrane, G protein-coupled receptor, FPRL1, mediates the chemotactic activity of serum amyloid A for human phagocytic cells. The Journal of experimental medicine 189(2):395-402.

Swanson, K., S. Gorodetsky, L. Good, S. Davis, D. Musgrave, K. Stelwagen, V. Farr, and A. Molenaar. 2004. Expression of a beta-defensin mRNA, lingual antimicrobial peptide, in bovine mammary epithelial tissue is induced by mastitis. Infection and immunity 72(12):7311-7314.

Swanson, T. J., C. J. Hammer, J. S. Luther, D. B. Carlson, J. B. Taylor, D. A. Redmer, T. L. Neville, J. J. Reed, L. P. Reynolds, J. S. Caton, and K. A. Vonnahme. 2008. Effects of gestational plane of nutrition and selenium supplementation on mammary development and colostrum quality in pregnant ewe lambs. Journal of animal science 86(9):2415-2423.

Tamzali, Y., J. F. Guelfi, and J. P. Braun. 2001. Plasma fibrinogen measurement in the horse: comparison of Millar's technique with a chronometric technique and the QBC-Vet Autoreader. Research in veterinary science 71(3):213-217.

Tang, Z. R., Z. H. Sun, X. S. Tang, Z. M. Feng, D. Zhou, D. F. Xiao, B. Zhang, and L. L. Li. 2011. Dietary bovine lactoferricin improved gut microflora, benefited intestinal mucosal morphology and inhibited circular cytokines delivery in 21-day weaned piglets. Journal of Applied Animal Research 39(2):153-157.

Theodorou, G., M. Daskalopoulou, R. Chronopoulou, A. Baldi, V. Dell'Orto, and I. Politis. 2010. Acute mastitis induces upregulation of expression of plasminogen activator-related genes by blood monocytes and neutrophils in dairy ewes. Vet Immunol Immunop 138(1-2):124-128.

Tizard, I. 1992. Inmunidad en el feto y en el neonato. Page 558 pp. in Inmunología Veterinaria. I. McGraw-Hill, ed, México D.F.

Ugarova, T. P. and V. P. Yakubenko. 2001. Recognition of fibrinogen by leukocyte integrins. Ann Ny Acad Sci 936:368-385.

Uhlar, C. M. and A. S. Whitehead. 1999. Serum amyloid A, the major vertebrate acute-phase reactant. European Journal of Biochemistry 265(2):501-523.

Val-Laillet, D., M. Simon, and R. Nowak. 2004. A full belly and colostrum: two major determinants of filial love. Developmental psychobiology 45(3):163-173.

Vermorel, M., C. Dardillat, J. Vernet, Saido, and C. Demigne. 1983. Energy metabolism and thermoregulation in the newborn calf. Ann Rech Vet 14(4):382-389.

Walport, M. J. 2001. Complement. First of two parts. The New England journal of medicine 344(14):1058-1066.

Weber, J. R., P. Moreillon, and E. I. Tuomanen. 2003. Innate sensors for Gram-positive bacteria. Curr Opin Immunol 15(4):408-415.

Webster, J. and D. Oxley. 2012. Protein Identification by MALDI-TOF Mass Spectrometry. Pages 227-240 in Chemical Genomics and Proteomics. Vol. 800. E. D. Zanders, ed. Humana Press.

Wheeler, T. T., A. J. Hodgkinson, C. G. Prosser, and S. R. Davis. 2007. Immune components of colostrum and milk--a historical perspective. Journal of mammary gland biology and neoplasia 12(4):237-247.

Wildman, D. E., C. Y. Chen, O. Erez, L. I. Grossman, M. Goodman, and R. Romero. 2006. Evolution of the mammalian placenta revealed by phylogenetic analysis. Proceedings of the National Academy of Sciences of the United States of America 103(9):3203-3208.

Wold, A. E. and I. Adlerberth. 2000. Breast feeding and the intestinal microflora of the infant - Implications for protection against infectious diseases. Pages 77-93 in Short and Long Term Effects of Breast Feeding on Child Health. Vol. 478. B. Koletzko, K. F. Michaelsen, and O. Hernell, ed. Kluwer Academic / Plenum Publ, New York.

Wooding, F. B., A. P. Flint, R. B. Heap, G. Morgan, H. L. Buttle, and I. R. Young. 1986. Control of binucleate cell migration in the placenta of sheep and goats. Journal of reproduction and fertility 76(2):499-512.

Wu, W. W., G. H. Wang, S. J. Baek, and R. F. Shen. 2006. Comparative study of three proteomic quantitative methods, DIGE, cICAT, and iTRAQ, using 2D gel- or LC-MALDI TOF/TOF. J Proteome Res 5(3):651-658.

Xu, L., R. Badolato, W. J. Murphy, D. L. Longo, M. Anver, S. Hale, J. J. Oppenheim, and J. M. Wang. 1995. A novel biologic function of serum amyloid A. Induction of T lymphocyte migration and adhesion. J Immunol 155(3):1184-1190.

Yamada, M., K. Murakami, J. C. Wallingford, and Y. Yuki. 2002. Identification of low-abundance proteins of bovine colostral and mature milk using two-dimensional electrophoresis followed by microsequencing and mass spectrometry. Electrophoresis 23(7-8):1153-1160.

Yang, D., A. Biragyn, L. W. Kwak, and J. J. Oppenheim. 2002. Mammalian defensins in immunity: more than just microbicidal. Trends Immunol 23(6):291-296.

Ye, A., H. Singh, M. W. Taylor, and S. Anema. 2002. Characterization of protein components of natural and heat-treated milk fat globule membranes. Int. Dairy J. 12(4):393-402.

Zaiou, M. and R. Gallo. 2002. Cathelicidins, essential gene-encoded mammalian antibiotics. J Mol Med 80(9):549-561.

Zasloff, M. 2002. Antimicrobial peptides of multicellular organisms. Nature 415(6870):389-395.

Zhang, L. Y., J. Q. Wang, Y. X. Yang, D. P. Bu, S. S. Li, and L. Y. Zhou. 2011. Comparative Proteomic Analysis of Changes in the Bovine Whey Proteome during the Transition from Colostrum to Milk. Asian Austral J Anim 24(2):272-278.

Zuo, X. and D. W. Speicher. 2002. Comprehensive analysis of complex proteomes using microscale solution isoelectrofocusing prior to narrow pH range two-dimensional electrophoresis. Proteomics 2(1):58-68

Objetivos

Capítulo 1

Revisar el importante papel que juega el calostro en la transferencia de inmunidad pasiva, especialmente las proteínas mayoritarias presentes en el mismo. Además, también se detallará como las proteínas minoritarias presentes en éste pueden intervenir en procesos inmunes, dado que estos procesos tiene una gran relevancia no solo en la supervivencia del recién nacido, sino en el incremento de la protección frente a agentes infecciosos.

Capítulo 2

Determinar la evolución del peso vivo, así como la concentración de IgG e IgM y la actividad del Sistema de Complemento y la Chitotriosidasa durante los primeros 5 días de vida de los corderos criados debidos al sistema de lactancia (natural *vs.* artificial), la fuente de calostro (cabra *vs.* oveja) y el tiempo transcurrido hasta la primera toma de calostro (2 *vs.* 14 h después del nacimiento).

Capítulo 3

Analizar el efecto de la dieta de los corderos (leche de oveja, lactorremplazante y leche en polvo entera para consumo humano) sobre la evolución del peso vivo, la concentración de IgG e IgM y la actividad del Sistema de Complemento y la Chitotriosidasa durante la lactancia y el destete.

Capítulo 4

Investigar el peso vivo y el estado inmune de corderos criados con un sistema de lactancia natural y corderos criados en un sistema de lactancia artificial y encalostrados con dos cantidades de IgG diferentes (4 g de IgG/ kg de peso vivo y 8 g de IgG/ kg de peso vivo).

Capítulo 5

Analizar las proteínas minoritarias presentes en el plasma sanguíneo de corderos debido a la ingesta de calostro durante las primeras 14 horas de vida, contribuyendo así a incrementar el conocimiento de la importancia del calostro en la transferencia de inmunidad pasiva y el desarrollo del sistema inmune de los corderos.

Capítulo 6

Describir el proteoma del calostro ovino y el plasma de cordero, así como hacer una cuantificación relativa de cómo la ingesta de calostro puede modificar el proteoma del plasma de los corderos recién nacidos.

Objectives

Chapter 1

To describe the important role of colostrum in the passive immune transfer (PIT), particularly the high abundant proteins (HAP) present in colostrum from different ruminant species. Moreover, it is also described in detail the low abundant proteins (LAP) that could be involved in immunological processes. These processes have a high relevance to animal survival as they increase the protection against infections in newborn ruminants.

Chapter 2

To determine the BW evolution and the immune parameters, such as IgG and IgM concentration and ChT and Complement System activity during the first 5 d after birth in relation to rearing system (natural *vs.* artificial), colostrum source (goat *vs.* sheep) and time of the first colostrum intake (2 *vs.* 14 h after birth).

Chapter 3

To analyze the effect of diet (sheep milk-NR, milk replacer-MR and whole powdered cow milk-CM) on the BW evolution, the IgG and IgM blood concentration and the ChT and Complement System activity during milk feeding period and weaning.

Chapter 4

To investigate the BW and immune status of lambs reared under natural conditions and lambs reared under artificial conditions fed with two different colostrum amounts (4 g IgG/ kg of BW and 8 g IgG/ kg of BW).

Chapter 5

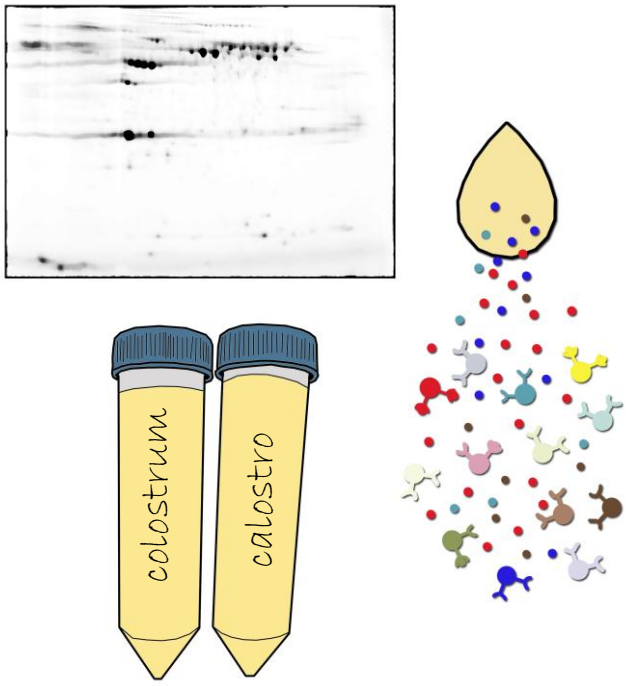
To analyze blood plasma low abundance proteins changes in newborn lambs due to the colostrum intake during the first 14 hours after birth, in order to identify such protein changes. Results showed in this study can contribute to understand the importance of colostrum on the passive immune transfer and the lamb immune system development.

Chapter 6

To describe the sheep colostrum and lamb plasma proteome, using an SDS-LC-MS/MS approach, and to provide relative quantification of whether and how neonate plasma protein concentrations change as an effect of early colostrum intake, using an iTRAQ-based proteomics approach.

Capítulo 1

Chapter 1



The colostrum proteome, ruminant nutrition and immunity: a review

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Short summary

In this review authors address colostrum proteins implications in different domestic ruminant species. The colostrogenesis process and how different factors, such as litter size or nutrition during gestation can alter the different components concentration in colostrum are also reviewed. The different colostrum fractions will be described, focusing on high and low abundant proteins. This review describes the major function of such proteins and their role on the passive immune transfer and nutrition in the newborn animal. It will be also performed a comprehensive review on different techniques and commercial kits available for high abundant protein depletion in colostrum. We will finally focus on how proteomics has been used to address this issue and how it can contribute to the major questions about colostrum associated immunology.

Keywords: Colostrum, Depletion, Immune System, Low Abundant Proteins, Proteomics.

1. INTRODUCTION

The relation between colostrum and newborn ruminant survival has been deeply characterized (Argüello et al., 2004b; Castro et al., 2005b; Castro et al., 2009; Castro et al., 2011). In fact, it has been described that colostrum is a mixture of diverse components, such as fat, lactose, vitamins and minerals that have a high nutritional importance (Ontsouka et al., 2003). However, in spite of the nutritional function,

colostrum contains a complex mixture of proteins that actively participate in the protection of the neonate against pathogens and other post-partum environmental challenges (Bendixen et al., 2011).

Proteomics is the field of science that, on a large scale, studies the proteome, i.e. the set of proteins being expressed in a given tissue, organ, organism or fluid. Proteomics is a relatively novel field and one of the fastest-growing areas in biological research, receiving international recognition and, additionally, it's applications in animal science are wide and diverse (Penque, 2009; Eckersall et al., 2012), including for instance, the transformation of muscle to meat (Paredi et al., 2012), or dairy production (Roncada et al., 2012). Concerning colostrum, proteomics has been used for instance to characterize protein changes in the transition from colostrum to milk in cattle (Reinhardt and Lippolis, 2008; Stelwagen et al., 2009; Nissen et al., 2012) and also to determine proteolytic processes in stomach and gut of piglets upon colostrum intake (Danielsen et al., 2011).

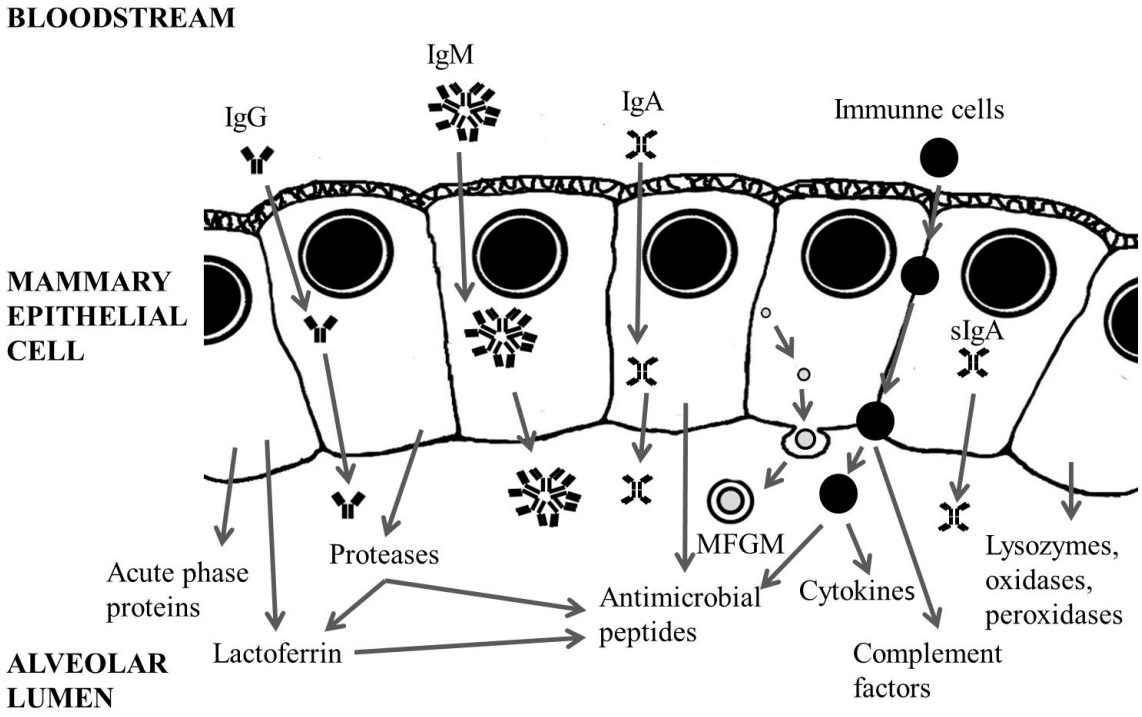
This review describes the important role of colostrum in the passive immune transfer (PIT), particularly the high abundant proteins (HAP) present in colostrum from different ruminant species. Moreover, it is also described in detail the low abundant proteins (LAP) that could be involved in immunological processes. These processes have a high relevance to animal survival as they increase the protection against infections in newborn ruminants.

2. DEFINITION, TIME OF SECRETION AND IMPORTANCE OF COLOSTRUM IN DOMESTIC RUMINANT SPECIES

Colostrum is the first source of nutrition in neonate ruminants, having a fundamental biological function in these animals, promoting immunoglobulin transfer from the dam to the newborn and providing protection against infections in the newborn (Kramer et al., 2001). As long described, the complexity of the epitheliochorial (cows and water buffaloes; (Wildman et al., 2006; Padua et al., 2010)) or synepitheliochorial (small ruminants; (Wooding et al., 1986)) placentas do not allow an adequate transfer of immunoglobulins (Ig's) from the dam to the foetus, as in other species such as humans or mice (hemochorial placenta; Chucri et al., 2010). For this reason, newborn ruminants are considered agammaglobulinemic (calves) or hypo-gammaglobulinemic (lambs and kids) at birth (Argüello et al., 2004b; Castro et al., 2005b; Castro et al., 2009; Moreno-Indias et al., 2012c). As a consequence,

colostrum intake and the absorption of colostrum proteins play an essential role in PIT and ultimately on newborn survival rates (Stelwagen et al., 2009; Castro et al., 2011; Danielsen et al., 2011). Such protein absorption is mainly based on immunoglobulins (IgG, IgM and IgA, mainly), however it has been also described the importance of non-immunoglobulin proteins absorption from colostrum to the newborn ruminants blood in order to ensure a correct PIT (Smith and Foster, 2007). In spite of this primary role, colostrum is also important to the newborn animal as the first source of nutrition, supplying essential nutrients such as fat, proteins, lactose and minerals at this early stage of life (Ontsouka et al., 2003). Finally, colostrum also contains other substances such as antimicrobial and anti-inflammatory agents (Stelwagen et al., 2009), as well as growth factors that control early gastrointestinal development (Purup et al., 2007), cytokines, enzymes and numerous other peptides (Koldovsky, 1980; Blum and Hammon, 2000) as shown in figure 1. Many of the bioactive whey and milk fat globule membrane (MFGM) proteins, notably immunoglobulins, lactoferrin and growth factors, are present in colostrum in higher concentration than in milk, reflecting the importance of colostrum in the health of newborn ruminants (Pakkanen and Aalto, 1997; Scammel, 2001).

Figure 1. Schematic representation of the major known proteins involved in the host defence system in milk and colostrum. (Adapted from Wheeler et al. (Wheeler et al., 2007)). MFGM: Milk Fat Globule Membrane.



The absorption of these colostrum components is favoured by a low proteolytic activity in the gastrointestinal tract of newborn animals (Guilloteau et al., 1983) and also by trypsin inhibitors present in colostrum (Jensen, 1978; Pallavicini et al., 1984; Ramos et al., 2010). However, these special conditions for intact colostrum proteins absorption decrease along the first 48 hours after birth, so it is crucial to feed newborn ruminants with colostrum during this period (Bush and Staley, 1980; Moore et al., 2005), in order for them to acquire an adequate initial concentration of serum Ig's (Quigley et al., 2000; Christley et al., 2003). In addition, it has been described that newborn ruminants that are not fed with colostrum during the first hours of life are more susceptible to diseases, considerably increasing mortality rates (Ahmad et al., 2000; da Nobrega et al., 2005; Nowak and Poindron, 2006). For all these reasons, colostrum intake is very important to ensure the survival of newborn ruminants.

3. PROTEOMICS AS A TOOL TO ANALYZE AND IDENTIFY PROTEINS FROM COLOSTRUM

Colostrum is a complex body fluid produced by the mammary gland of pregnant mammals. Colostrum composition is regulated by hormones (estradiol and progresterone) during colostrogenesis (Castro et al., 2011) that may be defined as the prepartum transfer of components, mainly immunoglobulins, from maternal bloodstream into mammary secretions during a short period (Barrington and Parish, 2001). This process ceases abruptly immediately before parturition (Brandon et al., 1971). There are several factors that affect colostrogenesis, such as species, breed, age, nutrition, litter size, length of dry period and health status (Csapo et al., 1994; Awadeh et al., 1998a; Maunsell et al., 1998). Moreover, colostrum components are secreted by different mechanisms (Patton and Jensen, 1975), so there are proteins directly produced in the mammary gland or transferred from the bloodstream (or both). For example, while IgG is transported to the mammary gland from the blood stream, part of the IgA is synthesized within the mammary gland by plasma cells which had migrated into the gland (Wheeler et al., 2007), as shown in figure 1 .

Proteomics is a powerful tool that can simultaneously analyse several hundred proteins in complex mixtures; however, because of the wide range of protein concentrations and subcellular locations, there is no general protocol for the separation of the complete colostrum proteome. Accordingly, and in order to increase the coverage of the colostrum proteome, it is necessary to study the different components separately. Some of these techniques are based in the pre-fractionation of the colostrum sample in order to

reduce complexity before proteomics analysis(Gagnaire et al., 2009). These previous steps are in accordance with the different properties of the proteins, namely charge, size or hydrophobicity.

The gel-based approach has been the most used method for decades, and is still widely used, being the two-dimensional gel electrophoresis (2DE) method commonly employed in quantitative proteomics (Wu et al., 2006). However, it has been also described a clear interest on liquid chromatography separations (Danielsen et al., 2011), which have several distinct advantages in comparison with the 2DE studies. Finally, mass spectrometry (MS) has increasingly become the method of choice for analysis of complex protein samples. MALDI TOF/TOF MS, for instance is a robust, sensitive and relatively inexpensive. Moreover, this method has produced much of the protein identifications data reported in the literature(Webster and Oxley, 2012). However, there are other techniques, such as isobaric tag for relative and absolute quantitation (iTRAQ) that allows studying deeply the proteome of complex samples with higher accuracy.

Several proteome studies have characterized the components of colostrum in different species such as bovine and human (Reinhardt and Lippolis, 2008; Stelwagen et al., 2009; Agarwal et al., 2011). Nevertheless, little is known about colostrum and the process that takes place in the newborn, namely degradation, proteolytic protection, internalization and retention in the intestinal tissue. Moreover, it is necessary to study how this primary food source passes through the gastrointestinal tract of the suckling neonate and how the above-mentioned factors affect the different colostrum components.

4. MANAGEMENT EFFECTS ON COLOSTRUM AND NEWBORN RUMINANTS

As described by Castro et al. (Castro et al., 2011) the management during the last weeks of pregnancy is extraordinarily important, because it is in this period that colostrum is secreted. There are other factors such as litter size and number of lactation that affect final colostrum composition. Moreover, colostrum management in the first hours of life is crucial for the survival of the newborn ruminant. However, in spite of the importance of these factors in the final composition and on colostrum protein absorption by newborn ruminants, no proteomics studies have been published on these effects.

4.1 Nutrition during gestation

The relation between maternal nutrition during late pregnancy and mammary gland development, colostrum composition and volume produced after partum has long been described (Mellor and Murray, 1985). In fact, dairy animals that receive a balanced feed produce more colostrum than underfed animals. Regarding immune components present in colostrum, nutrition management may vary the final concentration of some immune components. Accordingly, Awadeh et al. (Awadeh et al., 1998b) described how cows supplemented during late pregnancy with selenium increased their IgG concentration in colostrum. On contrast, Swanson et al. (Swanson et al., 2008) did not observe any effect in sheep supplemented during the late pregnancy neither in the colostrum yield nor in IgG concentration.

4.2 Litter size and number of lactations

Litter size is a very controversial effect. Csapó et al. (Csapó et al., 1994) found that colostrum IgG concentration was higher in twin Hungarian White goats and Hungarian Merino sheep than in singles. However, Argüello et al. (Argüello et al., 2006) did not find differences in colostrum IgG concentration between Majorera goats with different litter sizes.

There has been some controversy on the issue of the number of lactations. While it has been shown to increase IgG concentration in bovine (Oyeniyi and Hunter, 1978) and caprine colostrum (Ha et al., 1986), Dos Santos et al. (Santos, 1994) and Argüello et al. (Argüello et al., 2006) did not observe differences in the colostrum IgG concentration due to the number of lactations in Saanen and Majorera goats, respectively .

4.3 First colostrum intake by newborn ruminants

As described in section 2, newborn ruminants can absorb large molecules. Nevertheless and considering colostrum, this special ability decreases along the first 48 hours after birth. It is important to notice that the first colostrum ingestion is very important to acquire a correct PIT, although other aspects such as colostrum IgG concentration or volume of ingested colostrum also take high relevance in PIT. As reported (Chigerwe et al., 2009) Holstein calves fed with 3 litres of colostrum between the first 4 hours after birth acquire an adequate PIT. However, the same author observed that calves that took their first

colostrum intake between 4 and 12 hours after birth also had an adequate PIT, although the latter animals were fed with a high quality colostrum (IgG concentration > 100µg/µL).

5. COLOSTRUM FRACTIONS AND HIGH ABUNDANT PROTEINS

5.1 Colostrum fractions

As described in Section 3, there is no adequate protocol for the separation of whole colostrum. It is therefore necessary to separate different fractions. Colostrum proteins are divided into three main groups according to the fraction where they are found: caseins, whey proteins and proteins from the MFGM, as summarized in figure 2.

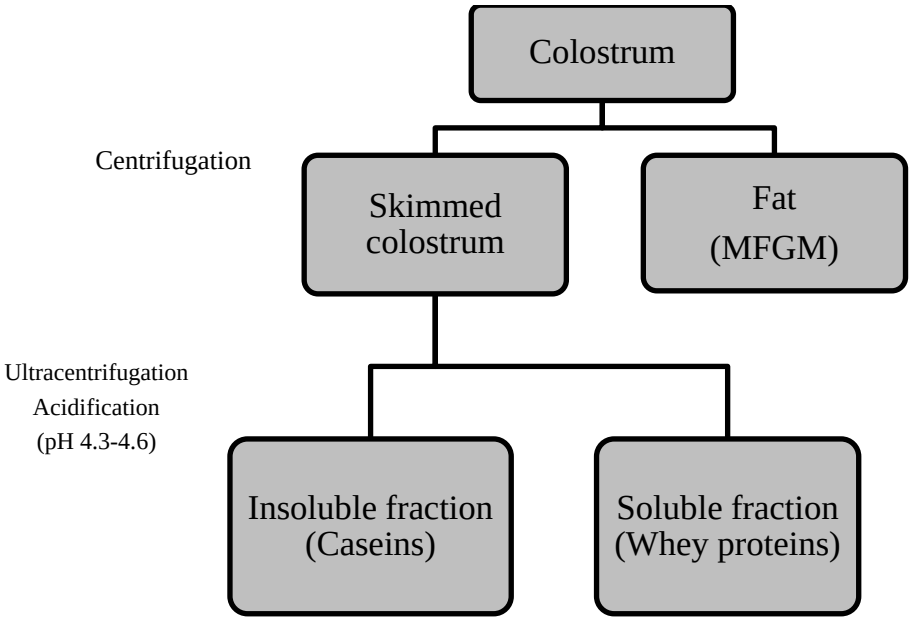


Figure 2. Schematic procedure for the separation of the three main groups of colostrums proteins. Two fractions can be obtained upon centrifugation: Milk Fat Globule Membrane (MFGM) and skimmed colostrum. The skimmed colostrum can be treated with ultracentrifugation or acidification (or both) to obtain the last last fractions: Caseins and Whey proteins.

These three fractions can be isolated in two steps. The first step is based in the centrifugation of the colostrum in order to separate the fat layer (MFGM) and the skimmed colostrum that includes caseins and whey proteins. Several centrifugation protocols may be found in the literature as summarized in table 1, although some of them have only been tested in milk samples.

Table 1. Centrifugation procedures for isolation of MFGM.

Specie	Centrifugation			Reference
	Speed (*g*)	Time (min.)	Temp. (ºC)	
Cow	5,000	15	4	(Zhang et al., 2011)
Cow	15,000	15	10	(Fong et al., 2008)
Cow	10,000	15	4	(Reinhardt and Lippolis, 2008)
Human*	1,500	20	25	(Patton and Huston, 1986)
Human	4,000	30	4	(Lonnerdal and Forsum, 1985)
Ewe*	1,500	20	25	(Martini et al., 2013)

*With addition of 5g of Sucrose/100mL of milk

After this process, the resulting skimmed colostrum needs to be divided in the two last fractions (whey proteins and caseins). In the literature, several methods may be found to isolate caseins from the skimmed colostrum or milk sample; however, all of them have in common the use of ultracentrifugation, acid precipitation or the combination of both methods. Some of the most used techniques are shown in table 2.

Table 2. Procedures to isolate caseins and whey proteins from milk and colostrum.

Species	Centrifugation			Acid precipitation	Reference
	Speed (*g*)	Time (min.)	Temp. (ºC)		
Cow	16,500	30	4	$C_2H_4O_2$ (1N)	(Golinelli et al., 2011)
Cow				$C_2H_4O_2$ (30% v/v, pH 4.6)	(Mier et al., 2008; Zhang et al., 2011)
Cow	15,000	15	10	HCl (1M, pH 4.6)	(Fong et al., 2008)
Cow*	44,000	30	4		(Boehmer et al., 2008)
Cow	100,000	60	4		(Le et al., 2010)
Human*	13,000	30	4	HCl (1M, pH 4.6) + 60 mmol $CaCl_2$	(Liao et al., 2011)
Human*	189,000	60	4	HCl (1M, pH 4.3) + 60 mmol $CaCl_2$	(Kunz and Lonnerdal, 1989)
Cow	1,500	10	5	$C_2H_4O_2$ (1N) + CH_3COONa (1N)	(Fox, 2003; Jensen et al., 2012)
Cow	100,000	60	4		(Jensen et al., 2012)
Human	10,000	15	20	HCl (1M, pH 4.6)	(Lonnerdal and Forsum, 1985)

*Centrifugation repeated twice

5.2. High abundant proteins

As described above, one of the three groups of proteins that can be found in colostrum and milk, are located in a complex membrane, called milk fat globule membrane (MFGM) that surrounds triacylglycerols droplets (Lonnerdal and Forsum, 1985).Traditionally, most of the research on MFGM has been focused in dairy cattle (Mondy and Keenan, 1993; Mather, 2000; Ye et al., 2002; Fong et al., 2007; Bianchi et al., 2009), however the interest in different aspects of non-bovine milk is fast growing. For this reason, some recent studies on MFGM of other species, such as goats (Cebo et al., 2010), sheep (Addis et al., 2011; Pisanu et al., 2011) or water buffaloes (D'Ambrosio et al., 2008) may be found in the literature.

As described (Mather, 2000), there are 7 HAP in the MFGM. Two of the most important proteins related to immunity are mucin-1 (MUC-1) and xanthine dehydrogenase/oxidase (XDH/XO).

MUC-1 is a glicycoprotein that is present in the apical surface of epithelial cells from different organs, such as lungs, stomach, intestine and others (Hollingsworth and Swanson, 2004). This protein has been isolated in cattle (Patton et al., 1995) where it's concentration is lower (40 mg/L) by comparison to human MFGM (729-805 mg/L) (Peterson et al., 1998). This protein was isolated in goats (Campana et al., 1992), albeit no information seems to be available on its concentration levels. Nevertheless, it has been demonstrated that it has cross-reactivity with cow MUC-1 and no cross-reactivity with human MUC-1. Regarding its biological function, this protein may play an important role in the protection of exposed surfaces from physical damage and invasive pathogenic microorganisms (Schroten et al., 1992; Patton et al., 1995; Peterson et al., 1998). In addition, MUC-1 may participate in the immune reaction in the suckling neonate because of its capacity of binding and sequestering pathogenic microorganisms within the gut lumen (Schroten et al., 1992; Peterson et al., 1998).

Concerning XDH/XO, it is essentially synthesized in the mammary gland (Bruder et al., 1983; Parks and Granger, 1986) and it reaches maximum values during late pregnancy and immediately after parturition (Kurosaki et al., 1996). This protein has been isolated from MFGM in cows, goats and sheep, although differences between them have so far not been analysed in colostrum. However, using milk samples, it has been described that XDH/XO from sheep and cow MFGM have similar concentrations (Mondy and Keenan, 1993; Ye et al., 2002; Pisanu et al., 2011). The function of this protein is not completely defined, but it has been suggested that it may play structural and functional roles in the formation of the MFGM

(Ishii et al., 1995) and also as having antibacterial properties in colostrum and milk, providing a source of H_2O_2 for lactoperoxidase (Bjorck and Claesson, 1979). Finally, this protein could act as an immunomodulator either causing tissue damage and exacerbation of the inflammatory response or inducing expression of genes encoding, for example, adhesive proteins, cell receptors, and components of the immune system (Mather, 2000).

Another group of proteins that can be found in colostrum are whey proteins. Setting bovine milk as reference, whey contains more than 200 different proteins, with β -lactoglobulin, α -lactalbumin, bovine serum albumin (BSA), immunoglobulins and lactoferrin being the major constituents (Sgarbieri, 2004; Korhonen and Pihlanto, 2007; Kawecka and Radko, 2011; Roncada et al., 2012). From this group of proteins, immunoglobulins play the most important role in the innate immune transfer, however there are other proteins with important immune functions, particularly α –lactalbumin and lactoferrin (Korhonen and Pihlanto, 2007; Tang et al., 2011).

Conventionally, α –lactalbumin has been associated to the regulation of lactose production (Kleinberg et al., 1983), however it has been recently described that this protein plays also a role as an immunomodulator in human and bovine colostrum and milk (Montagne et al., 2000; Korhonen and Pihlanto, 2007; Marnila and Korhonen, 2011). The concentration of this protein in colostrum does not differ significantly between goats, cows and sheep (2.77 mg/mL, 2 mg/mL and 2.3 mg/mL, respectively) (Perez et al., 1990; Levieux et al., 2002), showing that the concentration of this protein is approximately 1.5 times higher than those found in milk.

The other important HAP in the immune response against infections is lactoferrin. Konuspayeva et al. (Konuspayeva et al., 2008) studied the differences of this protein concentration in colostrum from several mammals such as camels (5.1 mg/mL), cows (0.84 mg/mL), goats (3.09 mg/mL), sheep (1.56 mg/mL) or water buffaloes (2.1 mg/mL). Similar results were found by Nissen et al. (Nissen et al., 2012) in bovine colostrum, using 2DE-LC-MS/MS and quantitative ELISA. The main function of lactoferrin is the binding and transport of iron ions. Additionally, lactoferrin has a wide variety of biological functions, many of which do not appear to be connected with its iron binding ability (Brock, 2002). Lactoferrin works as an innate immune factor synthesized by the mammary epithelium (Sánchez et al., 1992; Adlerova et al., 2008), that provides antimicrobial activity (bactericide and fungicide) to the mammary

gland and also to the newborn (Bellamy et al., 1992). Due to the increase in its concentration during most inflammatory reactions and some viral infections, it has been classified as an acute-phase protein (Kanyshkova et al., 2001).

With reference to casein, there are four main types (αs1, αs2, β and κ), that are responsible for important biological functions such as ion carriers (Calcium, Fosfate, Iron, Zinc, Cupper), bioactive peptide precursors and immunomodulators (Korhonen and Pihlanto, 2007). It was also demonstrated that the casein proteolytic fragments have an antimicrobial activity (Lahov and Regelson, 1996), suggesting that proteases may also play a role in the host defence. In addition, peptides derived from caseins are receiving much attention as possible sources of natural bioactivity with health benefits for humans (Gauthier et al., 2006), probably because they stimulate the innate immune system within the mammary gland and prevent udder infections during the dry phase (Silanikove et al., 2005).

6. LOW ABUNDANT PROTEINS FROM COLOSTRUM

In addition to the high importance of HAP in colostrum, there is a growing interest in LAP in order to increase the general knowledge about their biological role in animal and human health (Yamada et al., 2002; Reinhardt and Lippolis, 2008; Golinelli et al., 2011). In humans for example, a total of 151 proteins were recently identified, albeit the fact that 83 of them had never been previously described in human colostrum or milk (Palmer et al., 2006). Probably, many of this newly identified proteins could be involved in important colostrum roles such as regulation of growth, nutrient transport and what it is more important, PIT. In ruminants, knowledge on the identity and function of the LAP constituents of whey and MFGM is still incomplete, although many of these proteins may have uncharacterized biological properties (Fong et al., 2008; Reinhardt and Lippolis, 2008; Rusu et al., 2009).

Biochemical techniques, namely 2DE gel methods have been one of the most common techniques used to identify LAP in both milk and colostrum (Yamada et al., 2002; O'Donnell et al., 2004). However, these techniques are starting to be replaced by other such as iTRAQ or label-free methodologies that confer several advantages in comparison to 2DE gel methods. These new techniques are able to identify a higher number of proteins using less amount of sample and producing more reproducible results. On the other hand, the main disadvantage of these techniques is the expensive cost of the equipment, instruments and reagents, not frequently available in most laboratories.

6.1. Most common techniques used to identify low abundant proteins

Approaches such as immunoabsorption (Murakami et al., 1998; Yamada et al., 2002; Palmer et al., 2006), isoelectric focusing (IEF) (Zuo and Speicher, 2002), affinity tagging (Holland et al., 2006) , and semi-coupled anion- and cation-exchange chromatography (Fong et al., 2008) have been used as a tool to remove HAP and increase the relative abundant of LAP in proteomic analysis of complex samples such as colostrum. Despite of the removal of caseins by procedures such as those described in table 2, the large amount of immunoglobulins and high abundant proteins in, for example, ruminant whey is particularly difficult for LAP proteomic analysis (Yamada et al., 2002).

As a consequence of their high abundant in colostrum, similar isoelectric point and molecular weight, immunoglobulins are located in the same region of the 2DE-gel where most of the LAP, such as transferrin, plasmin, lipoprotein lipase, alkaline phosphatase, and others yet to be identified are also found (Fong et al., 2008). For this reason, it is necessary to remove HAP (namely immunoglobulins) from colostrum as they can mask low abundant proteins. Presently, several commercial kits are available to remove immunoglobulins. Golinalli et al. (Golinelli et al., 2011) used and compared the removal efficacy of whey HAP proteins from colostrum using the albumin and IgG removal kit (GE Healthcare Life Sciences, UK) and the Vivaspin 500 ultrafiltration cartridge (GE Healthcare Life Sciences, UK) with a membrane of a molecular-mass cut-off of 100 kDa. These authors observed that the Albumin and IgG removal kit was ineffective in removing IgG from the bovine colostrum whey. It is known that the albumin and IgG removal kit contains agarose-immobilized anti-IgG against human proteins. The failure of these antibodies to capture the IgG present in bovine colostrum whey could be ascribed to a lack of cross-reactivity of the antibodies to the bovine proteins. However, these authors described that the treatment of colostrum whey with Vivaspin 500 removed most of the IgG and IgA, rendering possible the detection of LAP.

Another common depletion technique used to deplete high abundant proteins is the ProteoMiner kit (Bio-Rad, USA) that consists of a unique library of hexapeptides, which bind proteins of different abundance with same capacity (Bandhakavi et al., 2011). Consequently, when a complex protein mixture is exposed to a ligand library, each bead with affinity to an abundant protein will rapidly become saturated and therefore the vast majority of the same protein will remain unbound. In contrast, low abundant proteins

will not saturate the corresponding partner beads, so all of them are captured, producing a progressively increase in the amount of those proteins. After elution of the captured proteins, all proteins will be present, but in a reduced dynamic range dependent on each single protein concentration before depletion (Boschetti and Righetti, 2008; D'Amato et al., 2009). In addition, ProteoMiner kit is a very extended method that was created for plasma and serum samples, but that has successfully been tested in other samples types including urine (Castagna et al., 2005), bile (Guerrier et al., 2007), platelets, red blood cell extract and egg white extract (Guerrier et al., 2008).

With reference to colostrum and milk, Liao et al. (Liao et al., 2011) treated human colostrum and milk samples with the ProteoMiner kit, showing that the abundance of LAP was increased while some of the high abundant proteins were decreased. However, these authors described that the ProteoMiner kit beads had little effect on remaining casein residues in the human colostrum and milk whey. Similarly, the ProteoMiner kit was used to study the whey proteome of cow's milk, obtaining an increase of the low abundant proteins (D'Amato et al., 2009). It should be noticed that although this kit is able to produce an enrichment of LAP, whey HAP proteins (e.g., lactoferrin and α-lactalbumin) and caseins, are still detected. For these reasons, and according to the authors, the ProteoMiner kit is a valid approach to increase the detection of low abundant proteins.

However, despite of the demonstrated effectiveness of the ProteoMiner kit in increasing the number of protein identifications in a complex protein mixture, it is necessary to remark its effect on quantitative proteomic studies, measuring relative protein abundance levels between samples via stable isotope labelling (Bandhakavi et al., 2011). Accordingly, Mouton-Barbosa et al. (Mouton-Barbosa et al., 2010) showed that high abundant proteins did not retain their quantitative accuracy after the treatment with a hexapeptide library (e.g. ProteoMiner kit), probably because they saturated their peptide binding partners, resulting in a loss of the unbound portion of the protein. On the other hand, low abundant proteins retained their quantitative accuracy after a hexapeptide library treatment, since they did not saturate their peptide binding partners and were fully recovered.

As a result, and taking the information described before, Proteominer kit seems to be an adequate tool to discover the deep proteome of several samples compound by a potent and a high mixture of proteins. Nevertheless, some considerations must be taken if this kit is to be used in quantitative studies.

6.2. Function of selected low abundant proteins and their relation to the immune system

It is very important to understand how LAP from colostrum can improve or modify the immune response either at the level of the mother or at the level of the newborn, for this reason, the function of some important LAP will be described separately.

One of these LAP in colostrum is plasmin. This protein is a fribrinolytic enzyme that plays an important role in the dissolution of fibrin blood clots in order to prevent thrombosis (Ogiwara et al., 2010). Nevertheless, this protein has been identified not only in blood, but also in colostrum and milk (Dupont et al., 1998; Le et al., 2010). As described by Dupont et al. (Dupont et al., 1998), bovine colostrum contents 10 times more plasmin (0.49 µg/mL) than milk (0.04 µg/mL). In bovine, sheep and goat milk, plasmin is essentially identical to those found in blood (Rebucci et al., 2005). Additionally, other authors have observed that plasmin has immune activity, as it contributes to the neutrophil migration to the site of infection (Renckens et al., 2006). In agreement with these findings, an increase in plasmin concentration in blood and milk during acute mastitis in lactating dairy ewes has been described (Theodorou et al., 2010).

Another interesting protein is serum amyloid A (SAA). This protein is normally found in complexes with lipoproteins and different isoforms, varying in concentration according to species (Uhlar and Whitehead, 1999). SAA represents one of the most conserved proteins among mammals supporting the premise that it has a basic and essential role in the innate immune system, taking part of the acute phase of inflammation (Eckersall et al., 2006; Pyorala et al., 2011). This protein has been also identified in colostrum from several species, namely human (Kumon et al., 2011) horse, cattle and sheep (McDonald et al., 2001; Le et al., 2010). It has been described that the circulating concentration of SAA is increased by 1000-fold within 24 to 48 h following infection/inflammation from a basal level of 5–8 mg/mL (Faty et al., 2012), showing the importance of this protein in inflammatory processes. Moreover, a variant of this acute phase protein, serum amyloid A3 (SAA-3), was found to be expressed in mammary cells in response to pathogens or pathogen-derived lipoteichoic acid and to be present in milk during mastitis (McDonald et al., 2001) and colostrum (McDonald et al., 2001; Larson et al., 2005) suggesting a role for this protein in host defence. Accordingly, McDonald et al. (McDonald et al., 2001) found that SAA-3 concentration in

cow colostrum is much higher than in milk (267.45 µg/mL and 2.63 µg/mL, respectively). These authors also described the concentration of this protein in sheep colostrum, being lower than in cow colostrum (62.83 µg/mL and 267.45 µg/mL, respectively).

In general, the SAA protein has numerous pro-inflammatory actions: chemoattractant to neutrophils, monocytes, and T lymphocytes, causing leukocyte infiltration and promoting neutrophil adhesion to endothelial cells (Badolato et al., 1994; Xu et al., 1995; Su et al., 1999) and it stimulates neutrophils and monocytes to release not only cytokines (Furlaneto and Campa, 2000; He et al., 2003), but also matrix metalloproteinases (Lee et al., 2005b). According to He et al. (He et al., 2009), these findings suggest a key function for SAA not only in the establishment, but also in the maintenance of inflammation.

Fibrinogen is the precursor of fibrin, the most abundant component in blood clots. However, this protein has also a defensive function, as it has been demonstrated that fibrinogen concentration increases during acute-phase reactions (Tamzali et al., 2001; Ganheim et al., 2003). Moreover, Yamada et al. (Yamada et al., 2002) studied differences in low abundant proteins between cow colostrum and milk, showing that some of them were only present in colostrum, such as fibrinogen. Additionally, several authors have described that fibrinogen can bind integrins, that are normally expressed by cells of the immune system, such as CD11b/CD18 (Ugarova and Yakubenko, 2001; Ryu et al., 2009). The CD11b/CD18 integrin receptor (αMβ2, Mac-1, complement receptor 3) is a member of the β2 integrin family, which is expressed on monocytes and macrophages. When fibrinogen binds to CD11b/CD18, integrin causes an extensive array of cell signalling responses, namely the activation of the nuclear factor kappa-light-chain-enhancer of activated B cells and mitogen-activated protein kinase/phosphatidylinositol 3-kinase, that mediate adhesion, migration, chemotaxis and phagocytosis (Akassoglou and Strickland, 2002). All these reasons, confirm that fibrinogen and fibrin from colostrum may result beneficial to the newborn immune system efficiency.

Trypsin inhibitor (TI), also known as α1-antitrypsin, is a protein that reduces biologically active trypsin (Rawlings et al., 2004), decreasing proteolysis. This protein has been described in bovine colostrum using different proteomics techniques, such as 2DE gel electrophoresis (Yamada et al., 2002) or LC-MS/MS

(Nissen et al., 2012). It has been studied that colostrum contains 100 times more TI than milk (Sandholm and Honkanenbuzalski, 1979; Honkanenbuzalski and Sandholm, 1981), probably to protect against the proteolytic cleavage, allowing the absorption of the intact immune components by the newborn ruminant.

The lipopolysaccharide-binding protein (LBP) is one of the a produced during infections with Gram-negative bacteria(Schumann et al., 1990). Additionally, LPB seems to actively participate during gram-positive bacterial infections (Weber et al., 2003). This protein has been detected in cow colostrum using 2D-LC-MS/MS (Nissen et al., 2012) and then quantified using ELISA (0.85 mg/mL, approximately) by the same authors. No references about the presence of this protein has been found neither goat nor sheep colostrum. The main function of this protein is to bind bacterial lipopolysaccharides (LPS) expressed on the outer cell wall of bacteria, acting as a carrier for LPS and to help control LPS-dependent monocyte responses (Stelwagen et al., 2009).

Antimicrobial peptides (AMPs) are defined as such for their capacity to rapidly inactivate infectious agents (Zasloff, 2002). Most AMPs act by disrupting the integrity of the microbial membranes (Zasloff, 2002). The two major AMP families in mammals are the defensins [10] and another group of cationic molecules, classified as cathelicidin peptides (Lehrer and Ganz, 2002; Zaiou and Gallo, 2002).

Defensins are a group of small antimicrobial peptides that interact with the adaptive immune response (Yang et al., 2002a). These peptides are produced constitutively and/or in response to microbial products or pro-inflammatory cytokines (Ganz and Lehrer, 1998; Lehrer and Ganz, 1999; Schroder, 1999). Specifically, members of the β-defensin family were found to be expressed by mammary epithelial cells during mastitis (Goldammer et al., 2004; Swanson et al., 2004). Although the mechanism of these peptides has not been fully known, they may be involved in the disruption of the microbial membrane. Moreover, these peptides have been described in milk and at elevated levels in human colostrum (Armogida et al., 2004).

Cathelicidin family is a group of peptides that serves a critical role in mammalian innate immune defence against invasive bacterial infection (Nizet et al., 2001). Its mechanism appears to be mediated by

recognition of molecular patterns at the microbial surface. This step is largely based on electrostatic interactions between the cationic peptide and anionic bacterial components and subsequent insertion into the lipid moiety, affecting the integrity of the microbial membranes (Gennaro and Zanetti, 2000). Cathelicidin family components have since been found in cattle (Stelwagen et al., 2009), sheep (Mahoney et al., 1995; Shamova et al., 1999), goats (Shamova et al., 1999) , and the presence of these peptides has been described in both colostrum and milk (Park, 2009; Stelwagen et al., 2009).

7. CONCLUSION

In summary, colostrum plays an essential role in several important processes in the newborn, such as PIT and immune system development, decreasing ruminant neonate mortality rates and increasing farmers economic benefits. However, colostrum is not only important for its Ig´s content but also for the non-immunoglobulin proteins that play a fundamental role in the activation and attraction of immune cells and low gastric secretion, among others. It has been also observed the relation of different proteins from the three colostrum fractions (caseins, whey and MFGM) with the newborn immune protection.

In this field, proteomics is a powerful tool of interest to increase the general knowledge of proteins present in colostrum and their relation with PIT and the immune system development. Moreover, further studies will be necessary in order to understand the biological process of colostrum proteins uptake trough the gut in newborn ruminants, particularly in less studied domestic ruminants such as sheep, goats and water buffalo

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Conflict of interest

The authors have no conflict of interests regarding commercial products, or any other aspects, referred to in this manuscript.

8. REFERENCES

[1]Argüello, A.; Castro, N.; Capote, J.; Tyler, J. W.; Holloway, N. M., Effect of colostrum administration practices on serum IgG in goat kids. *Livest Prod Sci* **2004,** *90* (2-3), 235-239.
[2]Castro, N.; Capote, J.; Alvarez, S.; Arguello, A., Effects of lyophilized colostrum and different colostrum feeding regimens on passive transfer of immunoglobulin g in Majorera goat kids. *J Dairy Sci* **2005,** *88* (10), 3650-4.
[3]Castro, N.; Capote, J.; Bruckmaier, R. M.; Arguello, A., Management effects on colostrogenesis in small ruminants: a review. *Journal of Applied Animal Research* **2011,** *39* (2), 85-93.
[4]Castro, N.; Capote, J.; Morales-Delanuez, A.; Rodriguez, C.; Arguello, A., Effects of newborn characteristics and length of colostrum feeding period on passive immune transfer in goat kids. *J Dairy Sci* **2009,** *92* (4), 1616-9.
[5]Ontsouka, C. E.; Bruckmaier, R. M.; Blum, J. W., Fractionized milk composition during removal of colostrum and mature milk. *J Dairy Sci* **2003,** *86* (6), 2005-11.
[6]Bendixen, E.; Danielsen, M.; Hollung, K.; Gianazza, E.; Miller, I., Farm animal proteomics - A review. *Journal of proteomics* **2011,** *74* (3), 282-293.
[7]Eckersall, P. D.; de Almeida, A. M.; Miller, I., Proteomics, a new tool for farm animal science. *Journal of proteomics* **2012,** *75* (14), 4187-4189.
[8]Penque, D., Two-dimensional gel electrophoresis and mass spectrometry for biomarker discovery. *Proteom Clin Appl* **2009,** *3* (2), 155-172.
[9]Paredi, G.; Raboni, S.; Bendixen, E.; de Almeida, A. M.; Mozzarelli, A., "Muscle to meat" molecular events and technological transformations: The proteomics insight. *Journal of proteomics* **2012,** *75* (14), 4275-4289.
[10]Roncada, P.; Piras, C.; Soggiu, A.; Turk, R.; Urbani, A.; Bonizzi, L., Farm animal milk proteomics. *Journal of proteomics* **2012,** *75* (14), 4259-4274.
[11]Reinhardt, T. A.; Lippolis, J. D., Developmental changes in the milk fat globule membrane proteome during the transition from colostrum to milk. *Journal of Dairy Science* **2008,** *91* (6), 2307-2318.
[12]Nissen, A.; Bendixen, E.; Ingvartsen, K. L.; Rontved, C. M., In-depth analysis of low abundant proteins in bovine colostrum using different fractionation techniques. *Proteomics* **2012,** *12* (18), 2866-2878.
[13]Stelwagen, K.; Carpenter, E.; Haigh, B.; Hodgkinson, A.; Wheeler, T. T., Immune components of bovine colostrum and milk. *Journal of animal science* **2009,** *87* (13 Suppl), 3-9.
[14]Danielsen, M.; Pedersen, L. J.; Bendixen, E., An in vivo characterization of colostrum protein uptake in porcine gut during early lactation. *Journal of proteomics* **2011,** *74* (1), 101-109.
[15]Kramer, M. S.; Chalmers, B.; Hodnett, E. D.; Sevkovskaya, Z.; Dzikovich, I.; Shapiro, S.; Collet, J. P.; Vanilovich, I.; Mezen, I.; Ducruet, T.; Shishko, G.; Zubovich, V.; Mknuik, D.; Gluchanina, E.; Dombrovskiy, V.; Ustinovitch, A.; Kot, T.; Bogdanovich, N.; Ovchinikova, L.; Helsing, E.; Grp, P. S., Promotion of breastfeeding intervention trial (PROBIT) - A randomized trial in the Republic of Belarus. *Jama-J Am Med Assoc* **2001,** *285* (4), 413-420.

[16]Wildman, D. E.; Chen, C. Y.; Erez, O.; Grossman, L. I.; Goodman, M.; Romero, R., Evolution of the mammalian placenta revealed by phylogenetic analysis. *Proceedings of the National Academy of Sciences of the United States of America* **2006,** *103* (9), 3203-3208.
[17]Padua, M. B.; Kowalski, A. A.; Canas, M. Y.; Hansen, P. J., The molecular phylogeny of uterine serpins and its relationship to evolution of placentation. *Faseb J* **2010,** *24* (2), 526-537.
[18]Wooding, F. B.; Flint, A. P.; Heap, R. B.; Morgan, G.; Buttle, H. L.; Young, I. R., Control of binucleate cell migration in the placenta of sheep and goats. *Journal of reproduction and fertility* **1986,** *76* (2), 499-512.
[19]Chucri, T. M.; Monteiro, J. M.; Lima, A. R.; Salvadori, M. L. B.; Kfoury, J. R.; Miglino, M. A., A review of immune transfer by the placenta. *J Reprod Immunol* **2010,** *87* (1-2), 14-20.
[20]Moreno-Indias, I.; Sanchez-Macias, D.; Castro, N.; Morales-delaNuez, A.; Hernandez-Castellano, L. E.; Capote, J.; Arguello, A., Chemical composition and immune status of dairy goat colostrum fractions during the first 10 h after partum. *Small Ruminant Research* **2012,** *103* (2-3), 220-224.
[21]Smith, G. W.; Foster, D. M., Short Communication: Absorption of Protein and Immunoglobulin G in Calves Fed a Colostrum Replacer. *Journal of Dairy Science* **2007,** *90* (6), 2905-2908.
[22]Purup, S.; Vestergaard, M.; L, O. P.; Sejrsen, K., Biological activity of bovine milk on proliferation of human intestinal cells. *The Journal of dairy research* **2007,** *74* (1), 58-65.
[23]Koldovsky, O., Hormones in milk. *Life sciences* **1980,** *26* (22), 1833-6.
[24]Blum, J. W.; Hammon, H., Colostrum effects on the gastrointestinal tract, and on nutritional, endocrine and metabolic parameters in neonatal calves. *Livest Prod Sci* **2000,** *66* (2), 151-159.
[25]Scammel, A. W., Production and uses of colostrum. *Aust J Dairy Technol* **2001,** *56*.
[26]Pakkanen, R.; Aalto, J., Growth factors and antimicrobial factors of bovine colostrum. *Int. Dairy J.* **1997,** *7* (5), 285-297.
[27]Wheeler, T. T.; Hodgkinson, A. J.; Prosser, C. G.; Davis, S. R., Immune components of colostrum and milk--a historical perspective. *Journal of mammary gland biology and neoplasia* **2007,** *12* (4), 237-47.
[28]Guilloteau, P.; Corring, T.; Garnot, P.; Martin, P.; Toullec, R.; Durand, G., Effects of Age and Weaning on Enzyme-Activities of Abomasum and Pancreas of the Lamb. *Journal of Dairy Science* **1983,** *66* (11), 2373-2385.
[29]Jensen, P. T., Trypsin-Inhibitor in Sow Colostrum and Its Function. *Ann Rech Vet* **1978,** *9* (2), 225-228.
[30]Pallavicini, C.; Ciruzzi, B.; Laudadio, V., Isolation and Partial Characterization of Ovine Colostrum Trypsin-Inhibitor. *Latte* **1984,** *9* (4), 309-312.
[31]Ramos, J. J.; Loste, A.; Ferrer, L. M.; Fernandez, A.; Castro, N.; Ortin, A.; Verde, M. T.; Arguello, A.; Figueras, L., Effect of addition of soybean trypsin inhibitor to colostrum on immunological status in goat kids. *Journal of animal physiology and animal nutrition* **2010,** *94* (1), 93-98.
[32]Bush, L. J.; Staley, T. E., Absorption of Colostral Immunoglobulins in Newborn Calves. *Journal of Dairy Science* **1980,** *63* (4), 672-680.
[33]Moore, M.; Tyler, J. W.; Chigerwe, M.; Dawes, M. E.; Middleton, J. R., Effect of delayed colostrum collection on colostral IgG concentration in dairy cows. *Javma-J Am Vet Med A* **2005,** *226* (8), 1375-1377.
[34]Quigley, J. D.; French, P.; James, R. E., Short communication: Effect of pH on absorption of immunoglobulin G in neonatal calves. *Journal of Dairy Science* **2000,** *83* (8), 1853-1855.
[35]Christley, R. M.; Morgan, K. L.; Parkin, T. D. H.; French, N. P., Factors related to the risk of neonatal mortality, birth-weight and serum immunoglobulin concentration in lambs in the UK. *Prev Vet Med* **2003,** *57* (4), 209-226.

[36]da Nobrega, J. E.; Riet-Correa, F.; Nobrega, R. S.; de Medeiros, J. M.; de Vasconcelos, J. S.; Simoes, S. V. D.; Tabosa, I. M., Perinatal mortality of lambs in the semi-arid region of Paraiba, Brazil. *Pesquisa Vet Brasil* **2005,** *25* (3), 171-178.
[37]Ahmad, R.; Khan, A.; Javed, M. T.; Hussain, I., The level of immunoglobulins in relation to neonatal Lamb mortality in Pak-Karakul sheep. *Vet Arhiv* **2000,** *70* (3), 129-139.
[38]Nowak, R.; Poindron, P., From birth to colostrum: early steps leading to lamb survival. *Reprod Nutr Dev* **2006,** *46* (4), 431-446.
[39]Barrington, G. M.; Parish, S. M., Bovine neonatal immunology. *The Veterinary clinics of North America. Food animal practice* **2001,** *17* (3), 463-76.
[40]Brandon, M. R.; Watson, D. L.; Lascelle.Ak, Mechanism of Transfer of Immunoglobulin into Mammary Secretion of Cows. *Aust J Exp Biol Med* **1971,** *49* (Dec), 613-&.
[41]Awadeh, F. T.; Abdelrahman, M. M.; Kincaid, R. L.; Finley, J. W., Effect of selenium supplements on the distribution of selenium among serum proteins in cattle. *Journal of Dairy Science* **1998,** *81* (4), 1089-1094.
[42]Csapo, J.; Csapo-Kiss, Z.; Martin, T. G.; Szentpeteri, J.; Wolf, G., Composition of Colostrum from Goats, Ewes and Cows Producing Twins. *Int. Dairy J.* **1994,** *4* (5), 445-458.
[43]Maunsell, F. P.; Morin, D. E.; Constable, P. D.; Hurley, W. L.; McCoy, G. C.; Kakoma, I.; Isaacson, R. E., Effects of mastitis on the volume and composition of colostrum produced by Holstein cows. *Journal of Dairy Science* **1998,** *81* (5), 1291-1299.
[44]Patton, S.; Jensen, R. G., Lipid metabolism and membrane functions of the mammary gland. *Progress in the chemistry of fats and other lipids* **1975,** *14* (4), 163-277.
[45]Gagnaire, V.; Jardin, J.; Jan, G.; Lortal, S., Invited review: Proteomics of milk and bacteria used in fermented dairy products: From qualitative to quantitative advances. *Journal of Dairy Science* **2009,** *92* (3), 811-825.
[46]Wu, W. W.; Wang, G. H.; Baek, S. J.; Shen, R. F., Comparative study of three proteomic quantitative methods, DIGE, cICAT, and iTRAQ, using 2D gel- or LC-MALDI TOF/TOF. *J Proteome Res* **2006,** *5* (3), 651-658.
[47]Webster, J.; Oxley, D., Protein Identification by MALDI-TOF Mass Spectrometry. In *Chemical Genomics and Proteomics*, Zanders, E. D., Ed. Humana Press: 2012; Vol. 800, pp 227-240.
[48]Agarwal, S.; Karmaus, W.; Davis, S.; Gangur, V., Immune Markers in Breast Milk and Fetal and Maternal Body Fluids: A Systematic Review of Perinatal Concentrations. *J Hum Lact* **2011,** *27* (2), 171-186.
[49]Mellor, D. J.; Murray, L., Effects of Maternal Nutrition on Udder Development during Late Pregnancy and on Colostrum Production in Scottish Blackface Ewes with Twin Lambs. *Research in veterinary science* **1985,** *39* (2), 230-234.
[50]Awadeh, F. T.; Kincaid, R. L.; Johnson, K. A., Effect of level and source of dietary selenium on concentrations of thyroid hormones and immunoglobulins in beef cows and calves. *Journal of animal science* **1998,** *76* (4), 1204-1215.
[51]Swanson, T. J.; Hammer, C. J.; Luther, J. S.; Carlson, D. B.; Taylor, J. B.; Redmer, D. A.; Neville, T. L.; Reed, J. J.; Reynolds, L. P.; Caton, J. S.; Vonnahme, K. A., Effects of gestational plane of nutrition and selenium supplementation on mammary development and colostrum quality in pregnant ewe lambs. *Journal of animal science* **2008,** *86* (9), 2415-2423.
[52]Csapó, J.; Csapó-Kiss, Z.; Martin, T. G.; Szentpeteri, J.; Wolf, G., Composition of colostrum from goats, ewes and cows producing twins. *Int. Dairy J.* **1994,** *4* (5), 445-458.
[53]Argüello, A.; Castro, N.; Álvarez, S.; Capote, J., Effects of the number of lactations and litter size on chemical composition and physical characteristics of goat colostrum. *Small Ruminant Research* **2006,** *64* (1–2), 53-59.
[54]Oyeniyi, O. O.; Hunter, A. G., Colostral constituents including immunoglobulins in 1st 3 milkings postpartum. *Journal of Dairy Science* **1978,** *61* (1), 44-48.

[55]Ha, W. K.; Lim, J. W.; Choi, C. K., A study on the immunoglobulin G concentration in milk and blood serum of Korean native goats. I. Changes of IgG concentration on the lactation period of Korean native goats. *Korean Journal of Animal Science* **1986,** *28*, 679-683.
[56]Santos, D., Variabilidade em imunoglobina G (IgG) no colostro de cabra de primeira ordenha e absorcao intestinal de IgG pelos cabritos recém-nascidos [Variability of immunoglobulins (IgG) in the colostrum of goats at first milking and intestinal absorption IgG by neonatal kids]. *Braz Arch Biol Techn* **1994,** *37*, 285.
[57]Chigerwe, M.; Tyler, J. W.; Summers, M. K.; Middleton, J. R.; Schultz, L. G.; Nagy, D. W., Evaluation of factors affecting serum IgG concentrations in bottle-fed calves. *Javma-J Am Vet Med A* **2009,** *234* (6), 785-789.
[58]Zhang, L. Y.; Wang, J. Q.; Yang, Y. X.; Bu, D. P.; Li, S. S.; Zhou, L. Y., Comparative Proteomic Analysis of Changes in the Bovine Whey Proteome during the Transition from Colostrum to Milk. *Asian Austral J Anim* **2011,** *24* (2), 272-278.
[59]Fong, B. Y.; Norris, C. S.; Palmano, K. P., Fractionation of bovine whey proteins and characterisation by proteomic techniques. *Int. Dairy J.* **2008,** *18* (1), 23-46.
[60]Patton, S.; Huston, G. E., A Method for Isolation of Milk-Fat Globules. *Lipids* **1986,** *21* (2), 170-174.
[61]Lonnerdal, B.; Forsum, E., Casein Content of Human-Milk. *American Journal of Clinical Nutrition* **1985,** *41* (1), 113-120.
[62]Martini, M.; Altomonte, I.; Pesi, R.; Tozzi, M. G.; Salari, F., Fat globule membranes in ewes' milk: The main enzyme activities during lactation. *Int. Dairy J.* **2013,** *28* (1), 36-39.
[63]Golinelli, L. P.; Conte, C. A.; Paschoalin, V. M. F.; Silva, J. T., Proteomic Analysis of Whey from Bovine Colostrum and Mature Milk. *Braz Arch Biol Techn* **2011,** *54* (4), 761-768.
[64]Mier, M. P.; Ibanez, R.; Ortiz, I., Influence of process variables on the production of bovine milk casein by electrodialysis with bipolar membranes. *Biochem. Eng. J.* **2008,** *40* (2), 304-311.
[65]Boehmer, J. L.; Bannerman, D. D.; Shefcheck, K.; Ward, J. L., Proteomic Analysis of Differentially Expressed Proteins in Bovine Milk During Experimentally Induced Escherichia coli Mastitis. *Journal of Dairy Science* **2008,** *91* (11), 4206-4218.
[66]Le, A.; Barton, L. D.; Sanders, J. T.; Zhang, Q., Exploration of Bovine Milk Proteome in Colostral and Mature Whey Using an Ion-Exchange Approach. *J Proteome Res* **2010,** *10* (2), 692-704.
[67]Liao, Y.; Alvarado, R.; Phinney, B.; Lönnerdal, B., Proteomic Characterization of Human Milk Whey Proteins during a Twelve-Month Lactation Period. *J Proteome Res* **2011,** *10* (4), 1746-1754.
[68]Kunz, C.; Lonnerdal, B., Human-Milk Proteins - Separation of Whey Proteins and Their Analysis by Polyacrylamide-Gel Electrophoresis, Fast Protein Liquid-Chromatography (Fplc) Gel-Filtration, and Anion-Exchange Chromatography. *American Journal of Clinical Nutrition* **1989,** *49* (3), 464-470.
[69]Jensen, H. B.; Poulsen, N. A.; Moller, H. S.; Stensballe, A.; Larsen, L. B., Comparative proteomic analysis of casein and whey as prepared by chymosin-induced separation, isoelectric precipitation or ultracentrifugation. *J. Dairy Res.* **2012,** *79* (4), 451-458.
[70]Fox, P. F., Milk proteins: general and historical aspects. In *Advanced dairy Chemistry*, 3rd ed.; McSweeney, P. F. P., Ed. Kluwer Academic/Plenum Publishers: New York, 2003; Vol. 1, pp 1-41.
[71]Fong, B. Y.; Norris, C. S.; MacGibbon, A. K. H., Protein and lipid composition of bovine milk-fat-globule membrane. *Int. Dairy J.* **2007,** *17* (4), 275-288.
[72]Mather, I. H., A review and proposed nomenclature for major proteins of the milk-fat globule membrane. *Journal of Dairy Science* **2000,** *83* (2), 203-247.

[73]Bianchi, L.; Puglia, M.; Landi, C.; Matteoni, S.; Perini, D.; Armini, A.; Verani, M.; Trombetta, C.; Soldani, P.; Roncada, P.; Greppi, G.; Pallini, V.; Bini, L., Solubilization methods and reference 2-DE map of cow milk fat globules. *Journal of proteomics* **2009,** *72* (5), 853-864.
[74]Mondy, B. L.; Keenan, T. W., Butyrophilin and Xanthine-Oxidase Occur in Constant Molar Proportions in Milk Lipid Globule-Membrane but Vary in Amount with Breed and Stage of Lactation. *Protoplasma* **1993,** *177* (1-2), 32-36.
[75]Ye, A.; Singh, H.; Taylor, M. W.; Anema, S., Characterization of protein components of natural and heat-treated milk fat globule membranes. *Int. Dairy J.* **2002,** *12* (4), 393-402.
[76]Cebo, C.; Caillat, H.; Bouvier, F.; Martin, P., Major proteins of the goat milk fat globule membrane. *Journal of Dairy Science* **2010,** *93* (3), 868-876.
[77]Addis, M. F.; Pisanu, S.; Ghisaura, S.; Pagnozzi, D.; Marogna, G.; Tanca, A.; Biosa, G.; Cacciotto, C.; Alberti, A.; Pittau, M.; Roggio, T.; Uzzau, S., Proteomics and Pathway Analyses of the Milk Fat Globule in Sheep Naturally Infected by Mycoplasma agalactiae Provide Indications of the In Vivo Response of the Mammary Epithelium to Bacterial Infection. *Infection and immunity* **2011,** *79* (9), 3833-3845.
[78]Pisanu, S.; Ghisaura, S.; Pagnozzi, D.; Biosa, G.; Tanca, A.; Roggio, T.; Uzzau, S.; Addis, M. F., The sheep milk fat globule membrane proteome. *Journal of proteomics* **2011,** *74* (3), 350-358.
[79]D'Ambrosio, C.; Arena, S.; Salzano, A. M.; Renzone, G.; Ledda, L.; Scaloni, A., A proteomic characterization of water buffalo milk fractions describing PTM of major species and the identification of minor components involved in nutrient delivery and defense against pathogens. *Proteomics* **2008,** *8* (17), 3657-3666.
[80]Hollingsworth, M. A.; Swanson, B. J., Mucins in cancer: Protection and control of the cell surface. *Nat Rev Cancer* **2004,** *4* (1), 45-60.
[81]Patton, S.; Gendler, S. J.; Spicer, A. P., The epithelial mucin, MUC1, of milk, mammary gland and other tissues. *Bba-Rev Biomembranes* **1995,** *1241* (3), 407-423.
[82]Peterson, J. A.; Patton, S.; Hamosh, M., Glycoproteins of the human milk fat globule in the protection of the breast-fed infant against infections. *Biology of the neonate* **1998,** *74* (2), 143-162.
[83]Campana, W. M.; Josephson, R. V.; Patton, S., Presence and Genetic-Polymorphism of an Epithelial Mucin in Milk of the Goat (Capra-Hircus). *Comp Biochem Phys B* **1992,** *103* (1), 261-266.
[84]Schroten, H.; Hanisch, F. G.; Plogmann, R.; Hacker, J.; Uhlenbruck, G.; Nobisbosch, R.; Wahn, V., Inhibition of Adhesion of S-Fimbriated Escherichia-Coli to Buccal Epithelial-Cells by Human-Milk Fat Globule-Membrane Components - a Novel Aspect of the Protective Function of Mucins in the Nonimmunoglobulin Fraction. *Infection and immunity* **1992,** *60* (7), 2893-2899.
[85]Bruder, G.; Heid, H. W.; Jarasch, E. D.; Mather, I. H., Immunological Identification and Determination of Xanthine-Oxidase in Cells and Tissues. *Differentiation* **1983,** *23* (3), 218-225.
[86]Parks, D. A.; Granger, D. N., Xanthine-Oxidase - Biochemistry, Distribution and Physiology. *Acta physiologica Scandinavica* **1986,** *126*, 87-99.
[87]Kurosaki, M.; Zanotta, S.; Calzi, M. L.; Garattini, E.; Terao, M., Expression of xanthine oxidoreductase in mouse mammary epithelium during pregnancy and lactation: Regulation of gene expression by glucocorticoids and prolactin. *Biochem J* **1996,** *319*, 801-810.
[88]Ishii, T.; Aoki, N.; Noda, A.; Adachi, T.; Nakamura, R.; Matsuda, T., Carboxy-terminal cytoplasmic domain of mouse butyrophilin specifically associates with a 150-kDa protein of mammary epithelial cells and milk fat globule membrane. *Bba-Gen Subjects* **1995,** *1245* (3), 285-292.
[89]Bjorck, L.; Claesson, O., Xanthine-Oxidase as a Source of Hydrogen-Peroxide for the Lactoperoxidase System in Milk. *Journal of Dairy Science* **1979,** *62* (8), 1211-1215.

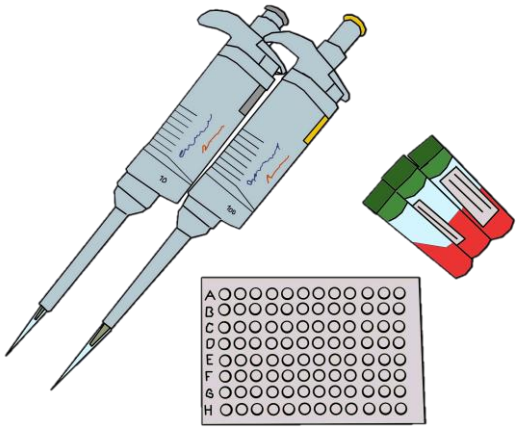
[90]Sgarbieri, V. C., Propriedades fisiológicas-funcionais das proteínas do soro de leite. *Revista de Nutrição* **2004,** *17*, 397-409.
[91]Korhonen, H.; Pihlanto, A., Technological options for the production of health-promoting proteins and peptides derived from milk and colostrum. *Curr Pharm Design* **2007,** *13* (8), 829-843.
[92]Kawecka, A.; Radko, A., Genetic polymorphism of beta-lactoglobulin in sheep raised for milk production. *Journal of Applied Animal Research* **2011,** *39* (1), 68-71.
[93]Tang, Z. R.; Sun, Z. H.; Tang, X. S.; Feng, Z. M.; Zhou, D.; Xiao, D. F.; Zhang, B.; Li, L. L., Dietary bovine lactoferricin improved gut microflora, benefited intestinal mucosal morphology and inhibited circular cytokines delivery in 21-day weaned piglets. *Journal of Applied Animal Research* **2011,** *39* (2), 153-157.
[94]Kleinberg, D. L.; Todd, J.; Babitsky, G., Inhibition by Estradiol of the Lactogenic Effect of Prolactin in Primate Mammary Tissue - Reversal by Anti-Estrogens Ly-156758 and Tamoxifen. *P Natl Acad Sci-Biol* **1983,** *80* (13), 4144-4148.
[95]Montagne, P. M.; Cuillière, M. L.; Molé, C. M.; Béné, M. C.; Faure, G. C., Dynamics of the Main Immunologically and Nutritionally Available Proteins of Human Milk during Lactation. *Journal of Food Composition and Analysis* **2000,** *13* (2), 127-137.
[96]Marnila, P.; Korhonen, H., Milk | Colostrum. In *Encyclopedia of Dairy Sciences (Second Edition)*, Editor-in-Chief: John, W. F., Ed. Academic Press: San Diego, 2011; pp 591-597.
[97]Levieux, D.; Morgan, F.; Geneix, N.; Masle, I.; Bouvier, F., Caprine immunoglobulin G, beta-lactoglobulin, alpha-lactalbumin and serum albumin in colostrum and milk during the early post partum period. *J. Dairy Res.* **2002,** *69* (3), 391-399.
[98]Perez, M. D.; Sanchez, L.; Aranda, P.; Ena, J. M.; Oria, R.; Calvo, M., Synthesis and Evolution of Concentration of Beta-Lactoglobulin and Alpha-Lactalbumin from Cow and Sheep Colostrum and Milk Throughout Early Lactation. *Cell Mol Biol* **1990,** *36* (2), 205-212.
[99]Konuspayeva, G.; Loiseau, G.; Levieux, D.; Faye, B., Lactoferrin and immunoglobulin content in camel milk from Bactrian, Dromedary and hybrids in Kazakhstan. **2008,** *1*, 54-62.
[100]Brock, J. H., The physiology of lactoferrin. *Biochem Cell Biol* **2002,** *80* (1), 1-6.
[101]Sánchez, L.; Luján, L.; Oria, R.; Castillo, H.; Pérez, D.; Ena, J. M.; Calvo, M., Synthesis of Lactoferrin and Transport of Transferrin in the Lactating Mammary-Gland of Sheep. *Journal of Dairy Science* **1992,** *75* (5), 1257-1262.
[102]Adlerova, L.; Bartoskova, A.; Faldyna, M., Lactoferrin: a review. *Vet Med-Czech* **2008,** *53* (9), 457-468.
[103]Bellamy, W.; Takase, M.; Wakabayashi, H.; Kawase, K.; Tomita, M., Antibacterial Spectrum of Lactoferricin-B, a Potent Bactericidal Peptide Derived from the N-Terminal Region of Bovine Lactoferrin. *J Appl Bacteriol* **1992,** *73* (6), 472-479.
[104]Kanyshkova, T. G.; Buneva, V. N.; Nevinsky, G. A., Lactoferrin and its biological functions. *Biochemistry-Moscow+* **2001,** *66* (1), 1-7.
[105]Lahov, E.; Regelson, W., Antibacterial and immunostimulating casein-derived substances from milk: Casecidin, isracidin peptides. *Food and Chemical Toxicology* **1996,** *34* (1), 131-145.
[106]Gauthier, S. F.; Pouliot, Y.; Saint-Sauveur, D., Immunomodulatory peptides obtained by the enzymatic hydrolysis of whey proteins. *Int. Dairy J.* **2006,** *16* (11), 1315-1323.
[107]Silanikove, N.; Shapiro, F.; Shamay, A.; Leitner, G., Role of xanthine oxidase, lactoperoxidase, and NO in the innate immune system of mammary secretion during active involution in dairy cows: manipulation with casein hydrolyzates. *Free Radical Bio Med* **2005,** *38* (9), 1139-1151.
[108]Yamada, M.; Murakami, K.; Wallingford, J. C.; Yuki, Y., Identification of low-abundance proteins of bovine colostral and mature milk using two-dimensional electrophoresis followed by microsequencing and mass spectrometry. *Electrophoresis* **2002,** *23* (7-8), 1153-1160.

[109]Palmer, D. J.; Kelly, V. C.; Smit, A. M.; Kuy, S.; Knight, C. G.; Cooper, G. J., Human colostrum: Identification of minor proteins in the aqueous phase by proteomics. *Proteomics* **2006,** *6* (7), 2208-2216.
[110]Rusu, D.; Drouin, R.; Pouliot, Y.; Gauthier, S.; Poubelle, P. E., A Bovine Whey Protein Extract Can Enhance Innate Immunity by Priming Normal Human Blood Neutrophils. *Journal of Nutrition* **2009,** *139* (2), 386-393.
[111]O'Donnell, R.; Holland, J. W.; Deeth, H. C.; Alewood, P., Milk proteomics. *Int. Dairy J.* **2004,** *14* (12), 1013-1023.
[112]Murakami, K.; Lagarde, M.; Yuki, Y., Identification of minor proteins of human colostrum and mature milk by two-dimensional electrophoresis. *Electrophoresis* **1998,** *19* (14), 2521-2527.
[113]Zuo, X.; Speicher, D. W., Comprehensive analysis of complex proteomes using microscale solution isoelectrofocusing prior to narrow pH range two-dimensional electrophoresis. *Proteomics* **2002,** *2* (1), 58-68.
[114]Holland, J. W.; Deeth, H. C.; Alewood, P. F., Resolution and characterisation of multiple isoforms of bovine kappa-casein by 2-DE following a reversible cysteine-tagging enrichment strategy. *Proteomics* **2006,** *6* (10), 3087-3095.
[115]Bandhakavi, S.; Van Riper, S. K.; Tawfik, P. N.; Stone, M. D.; Haddad, T.; Rhodus, N. L.; Carlis, J. V.; Griffin, T. J., Hexapeptide Libraries for Enhanced Protein PTM Identification and Relative Abundance Profiling in Whole Human Saliva. *J Proteome Res* **2011,** *10* (3), 1052-1061.
[116]Boschetti, E.; Righetti, P. G., The ProteoMiner in the proteomic arena: A non-depleting tool for discovering low-abundance species. *Journal of proteomics* **2008,** *71* (3), 255-264.
[117]D'Amato, A.; Bachi, A.; Fasoli, E.; Boschetti, E.; Peltre, G.; Sénéchal, H. n.; Righetti, P. G., In-Depth Exploration of Cow's Whey Proteome via Combinatorial Peptide Ligand Libraries. *J Proteome Res* **2009,** *8* (8), 3925-3936.
[118]Castagna, A.; Cecconi, D.; Sennels, L.; Rappsilber, J.; Guerrier, L.; Fortis, F.; Boschetti, E.; Lomas, L.; Rigetti, P. G., Exploring the hidden human urinary proteome via ligand library beads. *J Proteome Res* **2005,** *4* (6), 1917-1930.
[119]Guerrier, L.; Claverol, S.; Finzi, L.; Paye, F.; Fortis, F.; Boschetti, E.; Housset, C., Contribution of solid-phase hexapeptide ligand libraries to the repertoire of human bile proteins. *J. Chromatogr. A* **2007,** *1176* (1-2), 192-205.
[120]Guerrier, L.; Righetti, P. G.; Boschetti, E., Reduction of dynamic protein concentration range of biological extracts for the discovery of low-abundance proteins by means of hexapeptide ligand library. *Nat. Protocols* **2008,** *3* (5), 883-890.
[121]Mouton-Barbosa, E.; Roux-Dalvai, F.; Bouyssie, D.; Berger, F.; Schmidt, E.; Righetti, P. G.; Guerrier, L.; Boschetti, E.; Burlet-Schiltz, O.; Monsarrat, B.; de Peredo, A. G., In-depth Exploration of Cerebrospinal Fluid by Combining Peptide Ligand Library Treatment and Label-free Protein Quantification. *Mol Cell Proteomics* **2010,** *9* (5), 1006-1021.
[122]Ogiwara, K.; Nogami, K.; Nishiya, K.; Shima, M., Plasmin-induced procoagulant effects in the blood coagulation: a crucial role of coagulation factors V and VIII. *Blood Coagul Fibrin* **2010,** *21* (6), 568-576.
[123]Dupont, D.; Remond, B.; Collin, J. C., ELISA determination of plasmin and plasminogen in milk of individual cows managed without the dry period. *Milchwissenschaft* **1998,** *53* (2), 66-69.
[124]Rebucci, R.; Fusi, E.; Pecorini, C.; Pinotti, L.; Cheli, F.; Baldi, A., Evaluation of the biological activation of plasmin plasminogen system in sheep and goat milk. *Ital J Anim Sci* **2005,** *4*, 330-332.
[125]Renckens, R.; Roelofs, J. J. T. H.; Florquin, S.; van der Poll, T., Urokinase-type plasminogen activator receptor plays a role in neutrophil migration during lipopolysaccharide-induced

peritoneal inflammation but not during Escherichia coli-induced peritonitis. *Journal of Infectious Diseases* **2006,** *193* (4), 522-530.
[126]Theodorou, G.; Daskalopoulou, M.; Chronopoulou, R.; Baldi, A.; Dell'Orto, V.; Politis, I., Acute mastitis induces upregulation of expression of plasminogen activator-related genes by blood monocytes and neutrophils in dairy ewes. *Vet Immunol Immunop* **2010,** *138* (1-2), 124-128.
[127]Uhlar, C. M.; Whitehead, A. S., Serum amyloid A, the major vertebrate acute-phase reactant. *European Journal of Biochemistry* **1999,** *265* (2), 501-523.
[128]Eckersall, P. D.; Young, F. J.; Nolan, A. M.; Knight, C. H.; McComb, C.; Waterston, M. M.; Hogarth, C. J.; Scott, E. M.; Fitzpatrick, J. L., Acute phase proteins in bovine milk in an experimental model of Staphylococcus aureus subclinical mastitis. *J Dairy Sci* **2006,** *89* (5), 1488-501.
[129]Pyorala, S.; Hovinen, M.; Simojoki, H.; Fitzpatrick, J.; Eckersall, P. D.; Orro, T., Acute phase proteins in milk in naturally acquired bovine mastitis caused by different pathogens. *The Veterinary record* **2011,** *168* (20), 535.
[130]Kumon, Y.; Yasuoka, Y.; Yamanaka, S.; Wada, A.; Takeuchi, H.; Sugiura, T., Acute-phase serum amyloid A is present in human colostrum and milk. *Amyloid : the international journal of experimental and clinical investigation : the official journal of the International Society of Amyloidosis* **2011,** *18 Suppl 1*, 11-3.
[131]McDonald, T. L.; Larson, M. A.; Mack, D. R.; Weber, A., Elevated extrahepatic expression and secretion of mammary-associated serum amyloid A 3 (M-SAA3) into colostrum. *Vet Immunol Immunop* **2001,** *83* (3–4), 203-211.

Capítulo 2

Chapter 2



Interpretative summary

Immune parameters and body live weight evolution in lambs raised under different rearing systems, colostrum sources and first time of colostrum feedings. By Hernández-Castellano

There are several factors that can affect the final lamb body condition and immune status, such as the rearing system (natural *vs*. artificial), colostrum source (goat *vs*. sheep) and time of first colostrum feeding (2 h *vs*. 14 h after birth). These factors may produce a negative impact on the economic benefits of farmers and breeders and severe animal welfare consequences. The present manuscript details that in general there were no differences between colostrum sources and time of first colostrum feeding in an artificial rearing system; however, lambs reared under a natural system usually presented higher values during the first days after birth.

25 **Running head: REARING MANAGEMENT SYSTEM AND IMMUNE STATUS**

26

27 **TITLE**

28 **The effect of rearing system (natural *vs.* artificial), colostrum source (goat *vs.***
29 **sheep) and time at first colostrum intake (2 h *vs.* 14 h after birth) on body live**
30 **weight and immune status of lambs during 5 d after birth.**

31

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ABSTRACT

There are several factors that can affect the immune parameters and BW before weaning in lambs, but some of the most important factors are the rearing system, the colostrum source and the time of the first colostrum intake. The aim of this study was to evaluate the effect of the rearing system (natural *vs.* artificial), colostrum source (goat and sheep) and the time of the first colostrum intake (2 h and 14 h after birth) on the lamb BW and immune parameters during the first 5 d after birth. In this study 60 lambs were randomly assigned to 5 treatments. Twenty lambs remained with their dams. Forty lambs were removed from their dams at birth and were fed at 2 or 14 h after birth with goat or lamb colostrum respectively. Blood plasma samples and BW recording were taken before feeding. Blood plasma was used to measure the immunoglobulin concentration (IgG and IgM), the Chitotriosidase activity and the Complement system activity (Total and Alternative pathways). Results showed that lambs from the natural rearing group presented higher BW, IgG, IgM concentration and Complement System activity (total and alternative pathways) than animals reared under artificial systems during the first days after birth. No differences in Chitotriosidase activity between groups were observed during this period. Although these findings may improve the management in lamb farms that feed animals under artificial conditions, because it will be not necessary to feed with colostrum immediately after birth and also goat colostrum could be used to bottle fed newborn lambs, further studies will be necessary in order to achieve a similar initial IgG concentration in the artificial rearing groups in reference to the natural ones.

Keywords: Chitotriosidase, Immune, lamb, goat colostrum, weaning

INTRODUCTION

Newborn ruminants have three critical periods related to their immune system during the first two months of life; colostrum intake, milk feeding and weaning. The management in these periods affects the final animal performances (Marsico et al., 1993; Massimini et al., 2007; Mastellone et al., 2011).

Today there are an increasing number of high production dairy sheep farms, where lambs are reared under an artificial feeding system in order to increase marketable sheep milk. Under this system, lambs are separated from dams at early age, and then they are fed with colostrum and milk replacer in order to increase the amount of milk available for processing like cheese or yogurt (Demiroren et al., 1995; Napolitano et al., 2008) and simplify the management (Emsen et al., 2004). However, animals growing under an artificial rearing system need to be fed with an adequate amount of colostrum in order to increase their performance.

Colostrum is essential to ensure the survival of the litter because ruminants are born hypo gammaglobulinemic, due to the complexity of the synepitheliochorial ruminant placenta which does not allow sufficient transfer of Immunoglobulins from the dam to the foetus (Wooding et al., 1986; Castro et al., 2009). As a consequence, the consumption of colostrum by the progeny of these species (cow, sheep and goat) has a fundamental role in passive immunity transfer and on the newborn survival rate (Campbell et al., 1977; Lascelles, 1979; Stelwagen et al., 2009).

Moreover, it has been described that lambs not fed with colostrum in the first hours of life are more susceptible to diseases and mortality (Ahmad et al., 2000; da Nobrega et

al., 2005; Nowak and Poindron, 2006). Therefore, it is important to provide an optimal colostrum source, and as a consequence, several studies have been performed using bovine colostrum as an alternative source of colostrum to feed lambs at this early stage of life (Quigley et al., 2002a; Moretti et al., 2010). However, these studies report that when lambs are fed with cow colostrum they suffer from anemia (Winter and Clarkson, 1992; Winter, 2011; Ruby et al., 2012), so it is necessary to study other colostrum sources, such as goat colostrum.

Another important factor that affects the immune parameters in lambs is the time of the first colostrum intake, because for ruminants the period between 12 h and 36 h after birth is extremely critical for absorption of IgG from colostrum (Chen et al., 1999; Nowak and Poindron, 2006; Castro-Alonso et al., 2008) to acquire an adequate initial immunoglobulin concentration on blood (O'Doherty and Crosby, 1997; Quigley et al., 2000; Christley et al., 2003). Nevertheless, it is necessary to study how a delay in the first colostrum intake could affect the final immune status on the lamb bloodstream.

One of the most important immune parameters is the immunoglobulin concentration (IgG and IgM, mainly); however there are other immune parameters that can be studied in blood, such as the Chitotriosidase (ChT) activity and the Complement System activity (Total and Alternative pathways). Chitotriosidase is a functional chitinase with high homology to chitinases that belong to family 18 of glycosyl hydrolases, and although research has been undertaken in humans (Musumeci et al., 2005) and goats (Argüello et al., 2008a; Moreno-Indias et al., 2012c), this enzyme has never been described in sheep or lambs. The enzyme is predominantly a secretory protein that is

only expressed at the late stage of monocyte differentiation. Chitotriosidase is capable of hydrolyzing chitin in the cell wall of fungi and nematodes (Barone et al., 1999).

The Complement System activity, Total (TCA) and Alternative (ACA) pathways play an important role in host defense mechanisms against infectious microbes, as they are involved in specific and nonspecific immunity (Rodríguez et al., 2009). The Complement System in mammals has been well described, particularly in human and mice, and it has been also examined in ruminants, such as cows and goats (Castro et al., 2008; Mayilyan et al., 2008; Rodríguez et al., 2009; Moreno-Indias et al., 2012b). However, there are few studies about the Complement System activity in sheep and lambs, with studies based on an old determination technique.

The aim of this study was to determine the BW evolution and the immune parameters, such as IgG and IgM concentration and ChT and Complement System activity during the first 5 d after birth in relation to rearing system (natural *vs.* artificial), colostrum source (goat *vs.* sheep) and time of the first colostrum intake (2 *vs.* 14 h after birth).

MATERIAL AND METHODS

The present study was performed in the Department of Animal Science of the Universidad de Las Palmas de Gran Canaria in Canary Island (Spain) on 60 lambs (30 males and 30 females) from Canary dairy breed. Animal procedures were approved by the Ethical Committee of the University.

Colostrum feeding period

At birth, animals were randomly divided in five different groups. The natural rearing (NR) group was composed by 20 lambs who fed colostrum directly from their dams. The other 40 lambs were removed from their dams, dried, weighed and ear tagged and then divided into 4 blocks (10 lambs each) in accordance with the method of colostrum feeding and without contact with the dam. Lambs blocks were allocated into rooms equipped with central heating at a room temperature of 20ºC and providing at least 0.3 m^2 floor space per lamb. Goat Colostrum 2 h (GC2) and Goat Colostrum 14 h (GC14) groups received a goat colostrum pool that was previously pasteurized at 63ºC for 30 minutes according to Trujillo et al. (2007). Sheep Colostrum 2 h (SC2) and Sheep Colostrum 14 h (SC14) groups received a sheep colostrum pool that was pasteurized by the procedure previously described. Lambs from GC2 and SC2 were bottle-fed colostrum at 2 h, 14 h and 24 h after birth and GC14 and SC14 lambs were bottle-fed colostrum at 14 h and 24 h after birth. Because there are no references concerning the amount/concentration of colostrum that should be given to lambs in order to achieve a correct PIT, by the end of the colostrum feeding period all the artificial rearing groups (GC2, GC14, SC2 and SC14) received a total colostrum amount equivalent to 4 g of IgG/kg of BW, which is the amount recommended by Castro et al. (2005b) for goat kids.

The goat and sheep colostrum IgG concentration (41.32 and 64.37 mg/mL, respectively) was determined using a commercial ELISA kit (Bethyl laboratories, Montgomery, TX, USA).

The NR group was raised with dams and had free access to dam colostrum/milk until end of the experiment. After colostrum period, artificial rearing groups (GC2, GC14, SC2 and SC14) received a commercial milk replacer at 16% (w/w; MR group, Bacilactol Corderos y Cabritos, Saprogal, La Coruña, Spain; 95.5% DM, 23.6% CP and 22.7% ether extract, air-dry powder basis). These groups were fed *ad libitum* (37ºC), using nipple buckets twice a day (8:00 am and 05:00 pm).

BW recording and sample collection

All experimental animals were weighed before each blood extraction and data was expressed in kg (MOBBA, Barcelona, Spain; accuracy, 5g). Blood samples were taken before morning feeding from the jugular vein in 2.5 ml K-EDTA tubes. Subsequently, blood was centrifuge at 2190 x g during 5 minutes at 4º C (Hettich-Zentrifugen, Universal 32 R, Germany), storing the obtained plasma at -80° C until analysis. In the colostrum and milk feeding period, blood samples were taken at 2 h after birth (labeled as sample 0) and then at 1, 2, 3, 4, 5 d after birth.

To determine plasma IgG and IgM concentration commercial ELISA kits (Bethyl Laboratories, Montgomery, TX, USA) were used, setting a purified sheep IgG and IgM as a standard reference. Results were expressed as mg of immunoglobulin/mL of plasma.

Chitotriosidase activity was measured following the procedure described previously by Argüello et al. (2008a) in goat blood plasma. In this procedure, 1 µL of undiluted blood plasma with 100 µL of a solution containing 22 m*M* artificial substrate (4-methylumbelliferyl-d-N, N′, N″ triacetylchitotriose) in 0.5 *M* citrate phosphate buffer (pH 5.2) was incubated for 15 min at 37°C. The reaction was stopped with 5 mL of 0.5

198 *M* Na_2CO_3-$NaHCO_3$ buffer (pH 10.7). Fluorescence was measured using a fluorimeter
199 (Perkin Elmer, Norwalk, CT) at 365 nm of excitation and 450 nm of emission. The ChT
200 activity was expressed as nanomoles of substrate hydrolyzed/mL/h.
201
202 Complement System activity (TCA and ACA) was measured by the hemolytic rate
203 according to a novel technique described by Moreno-Indias et al. (2012b) in goat kid
204 blood plasma. In this technique a $DGHB^{++}$ buffer [Hepes Gelatin Veronal Buffer with
205 Ca^{++} and Mg^{++}: 5 m*M* HEPES, 71 m*M* NaCl, 0.15 m*M* $CaCl_2$, 0.5 m*M* $MgCl_2$, 2.5%
206 (w/v) glucose, 0.1% (w/v) gelatin, pH 7.4] was used to measure total Complement
207 System activity, and DGHB-Mg-EGTA buffer [4.2 m*M* Hepes, 59 m*M* NaCl, 7.0 m*M*
208 $MgCl_2$, 2.08% (w/v) glucose, 0.08% (w/v) gelatin, 10 m*M* EGTA, pH 7.4] to measure
209 the alternative pathway. For total Complement System activity, rabbit red blood cells
210 and lamb plasma were diluted to 5% in $DGHB^{++}$; 100 µL of each was then mixed in a
211 microtiter plate and incubated at 37°C for 1 h. Cells were removed by centrifugation
212 (2500 × *g*, 5 min, 4ºC), and supernatant absorbance was measured at 405 nm using a
213 micro-plate reader. Complete hemolysis was achieved by mixing the cells with distilled
214 water (100 µL), and spontaneous lysis was produced by mixing the diluted rabbit red
215 blood cells with $DGHB^{++}$. Complement-induced hemolysis of rabbit red blood cells by
216 the test sera was calculated using the formula: [(A405 sample − A405 spontaneous
217 lysis) / (A405 complete hemolysis − A405 spontaneous lysis)] × 100. The same protocol
218 was performed with DGHB-Mg-EGTA buffer to measure the alternative pathway.
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222 ***Statistical analyses***

223 Statistical analyses were performed using SAS, Version 9.00 (SAS Institute Inc., Cary,
224 NC). The SAS PROC MIXED procedure for repeated measurements was used to
225 evaluate the effect of rearing system (natural *vs.* artificial) colostrum source (goat *vs.*
226 sheep) and timing of the first colostrum intake (2 *vs.* 14 h after birth) on IgG and IgM
227 concentration, ChT and Complement System activity and BW of lambs from birth to 5 d
228 after birth. A Bonferroni's test was used to evaluate differences between groups.

229

230 **RESULTS AND DISCUSSION**

231 Results relating to BW, IgG and IgM concentration, ChT activity, TCA and ACA
232 during the first 5 d after birth are shown in Table 1. NR was the only group that
233 increased the BW after birth; however weights for groups reared under artificial
234 conditions decreased from partum to 4 d after birth, with a trend of increasing weight at
235 5 d. It was observed that there were no BW differences between the animals at birth (0
236 d), although at 4 d and 5 d the NR group was heavier than the others (GC2, GC14, SC2
237 and SC14), with no BW differences being found between these four artificial rearing
238 groups. According to Lanza et al. (2006), factors associated with an increased suckling
239 frequency could be related to the differences between NR and the other groups. As
240 observed by Rodríguez et al. (2008), lambs that are reared under restricted conditions
241 (twice ad libitum daily) presented a lower BW gain rate (253 g/d) than animals that
242 were raised with their dams (307 g/d). In addition, Argüello et al. (2004a) observed that
243 goat kids reared under a natural rearing system had a higher weight than those from an
244 artificial rearing system. In contrast of these findings, Napolitano et al. (2002) did not
245 find BW gain rate differences between lambs reared under natural or artificial
246 conditions (180 g/d and 170 g/d, respectively). It is important to remark that not only

neither the colostrum source (goat *vs.* sheep), nor the delay of the colostrum intake (2 *vs.*14 h after birth) showed effects on lamb growth during the colostrum period.

Focusing on the IgG concentration, at birth (0 d) all the animals had a basal IgG concentration in their blood plasma, probably from maternal origin through the placenta, as it has been referenced by Castro et al. (2011). These values increased during the first 24 h after birth, although the highest concentration was obtained in the NR group (16.79 mg/mL), probably because the colostrum intake were not restricted in these animals and a higher amount of IgG could be absorbed. However, IgG concentration in the artificial rearing groups (GC2, GC14, SC2 and SC14) remained constant in the following days (2, 3, 4 and 5 d after birth). No differences were observed between artificial rearing groups in this period, probably because all these animals received the same amount of IgG in relation to their BW at birth. Several authors have observed how an increase of the total amount of IgG present in colostrum intake increase the IgG present in newborn ruminant blood not only in lambs (Halliday and Williams, 1979) but also in calves (Muller and Ellinger, 1981; Stott and Fellah, 1983) and goat kids (Castro et al., 2005b; Rodríguez et al., 2009). In addition Firat et al. (2003) reported differences in the milk feeding period between lambs raised under natural and artificial conditions, describing that lambs raised under artificial rearing system had generally lower plasma IgG levels than lambs raised under natural conditions. Results observed in this study show that no differences in IgG concentration were found to be due to the colostrum source (goat *vs.* sheep) or the colostrum intake delay (2 *vs.* 14 h).

No animals from any of the groups presented detectable IgM in the blood at birth (0 d). Groups studied showed an increase in IgM concentration during the first days after birth; however this concentration tended to gradually decrease in the following days in groups NR, SC2 and SC14. When groups were compared, the largest differences were perceived between the NR group and the artificial rearing groups (GC2, GC14, SC2 and GC14), showing that NR group presented the highest IgM concentration from 1 d to 5 d. These differences between natural and artificial rearing groups during this period could be produced because the artificial rearing groups received a restricted amount of colostrum in comparison with those who were reared under natural conditions. The same effect was observed in newborn goat kids by Rodríguez et al. (2009) who observed that animals fed with a higher IgM amount in colostrum presented a higher IgM concentration on blood. Moreover, Stott and Fellah (1983) observed a quadratic relationship between the amount of IgM in colostrum and plasma IgM concentration in calves. According to the results of this study, no differences were observed due to the colostrum source (goat *vs.* sheep) and colostrum intake time (2 h *vs.* 14 h after birth) when artificial rearing groups were analyzed in this period.

The ChT activity was found to be similar during the first 4 d after birth, increasing in GC14 and SC14 at the end of this period, although no differences were observed between groups in the whole period. The evolution of this enzyme activity has not been previously described in sheep, although Argüello et al. (2008a) described higher values in goat kids at birth (2664 nmol/mL/h) and if this is compared with results of the present study (1017.22, 804.68, 897.46, 946.33 and 1019.69 nmol/mL/h in NR, GC2, GC14, SC2 and SC14, respectively), it demonstrates an increase of this enzyme activity in goat kids blood at 21 days of life (6000 nmol/mL/h). In accordance with these findings,

Rodríguez et al. (2009) did not find any differences in blood ChT activity of goat kids that were fed with colostrum at different IgG concentrations. As tends to happen with other proteins from breast milk, ChT may be inactivated or destroyed before arriving in the intestine (Wold and Adlerberth, 2000), so the primary role of ChT from colostrum must be to protect the intestinal lumen of the newborn, increasing the activity of this enzyme when the animal becomes older by the progressively macrophages activation (Argüello et al., 2008a). The present results suggest that neither rearing method (natural *vs.* artificial) nor colostrum source (goat *vs.* sheep) nor colostrum intake time (2 h *vs.*14 h after birth) seemed to affect ChT activity in newborn lambs.

Complement System activity (TCA and ACA) was not detectable at birth (0 d) in any of the studied groups, becoming detectable from 1 d after birth and reaching the maximum value at the end of this period (5 d). NR group obtained the highest Complement System activity (TCA and ACA) from 2 d (20.56% and 15.05%, TCA and ACA, respectively) to 5 d (42.43% and 36.26% of TCA and ACA, respectively). In general, no differences in Complement System activity were found between artificial rearing groups. In accordance with these findings, Eckblad et al. (1981a) suggested that Complement System components in colostrum may play an important role in the development of the Complement System activity in newborn animals through gut absorption, so a higher amount of colostrum intake may produce an earlier development of the Complement System activity, as happened in the NR group. Similarly, Castro et al. (2008) showed the importance of dam milk in the earlier Complement System activation compared with milk replacer feeding. Results of the current study indicate that neither the colostrum source (goat *vs.* sheep) nor the colostrum intake delay (2 h *vs.* 14 h after birth) affect the Complement System development.

CONCLUSION

In this study, lambs from the natural rearing system showed in general, higher immune condition and BW than animals reared under the different artificial system during the first days after birth. However, further studies will be necessary in order to achieve a similar initial IgG concentration in the artificial rearing groups compared to the natural ones.

These findings may improve the management systems in place in lamb farms as it shows that it is not necessary to colostrum feed immediately after birth and, also, that goat colostrum could be used to bottle fed newborn lambs.

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REFERENCES

Ahmad, R., A. Khan, M. T. Javed, and I. Hussain. 2000. The level of immunoglobulins in relation to neonatal Lamb mortality in Pak-Karakul sheep. Vet Arhiv 70(3):129-139.

Argüello, A., N. Castro, M. Batista, I. Moreno-Indias, A. Morales-DelaNuez, D. Sanchez-Macias, E. Quesada, and J. Capote. 2008. Chitotriosidase activity in goat blood and colostrum. Journal of Dairy Science 91(5):2067-2070.

Argüello, A., N. Castro, and J. Capote. 2004. Growth of milk replacer kids fed under three different managements. Journal of Applied Animal Research 25(1):37-40.

Barone, R., F. Di Gregorio, M. A. Romeo, G. Schiliro, and L. Pavone. 1999. Plasma chitotriosidase activity in patients with beta-thalassemia. Blood Cell Mol Dis 25(1):1-8.

Campbell, S. G., M. J. Siegel, and B. J. Knowlton. 1977. Sheep inmmunoglobulind and their transmission to neonatal lamb. New Zealand veterinary journal 25(12):361-365.

350 Castro-Alonso, A., N. Castro, J. Capote, A. Morales-DelaNuez, I. Moreno-Indias, D. Sanchez-
351 Macias, P. Herraez, and A. Argullo. 2008. Short communication: Apoptosis regulates passive
352 immune transfer in newborn kids. Journal of Dairy Science 91(5):2086-2088.
353 Castro, N., F. Acosta, T. Nino, J. Vivas, E. Quesada, J. Capote, and A. Arguello. 2008. The effects
354 of diet and age on serum complement system activity in goat kids. Livest Sci 119(1-3):102-106.
355 Castro, N., J. Capote, S. Alvarez, and A. Arguello. 2005. Effects of lyophilized colostrum and
356 different colostrum feeding regimens on passive transfer of immunoglobulin g in Majorera
357 goat kids. J Dairy Sci 88(10):3650-3654.
358 Castro, N., J. Capote, R. M. Bruckmaier, and A. Arguello. 2011. Management effects on
359 colostrogenesis in small ruminants: a review. Journal of Applied Animal Research 39(2):85-93.
360 Castro, N., J. Capote, A. Morales-Delanuez, C. Rodriguez, and A. Arguello. 2009. Effects of
361 newborn characteristics and length of colostrum feeding period on passive immune transfer in
362 goat kids. J Dairy Sci 92(4):1616-1619.
363 Chen, J. C., C. J. Chang, H. C. Peh, and S. Y. Chen. 1999. Serum protein levels and neonatal
364 growth rate of Nubian goal kids in Taiwan area. Small Ruminant Research 32(2):153-160.
365 Christley, R. M., K. L. Morgan, T. D. H. Parkin, and N. P. French. 2003. Factors related to the risk
366 of neonatal mortality, birth-weight and serum immunoglobulin concentration in lambs in the
367 UK. Prev Vet Med 57(4):209-226.
368 da Nobrega, J. E., F. Riet-Correa, R. S. Nobrega, J. M. de Medeiros, J. S. de Vasconcelos, S. V. D.
369 Simoes, and I. M. Tabosa. 2005. Perinatal mortality of lambs in the semi-arid region of Paraiba,
370 Brazil. Pesquisa Vet Brasil 25(3):171-178.
371 Demiroren, E., J. N. B. Shrestha, and W. J. Boylan. 1995. Breed and Environmental-Effects on
372 Components of Ewe Productivity in Terms of Multiple Births, Artificial Rearing and 8-Month
373 Breeding Cycles. Small Ruminant Research 16(3):239-249.
374 Eckblad, W. P., K. M. Hendrix, and D. P. Olson. 1981. Total complement hemolytic-activity of
375 colostral whey and sera from dairy-cows. Cornell Veterinarian 71(1):54-58.
376 Emsen, E., M. Yaprak, O. C. Bilgin, B. Emsen, and H. W. Ockerman. 2004. Growth performance
377 of Awassi lambs fed calf milk replacer. Small Ruminant Research 53(1-2):99-102.
378 Firat, A., A. Ozpinar, and M. H. Erhard. 2003. Serum Immunoglobulin G Levels in Lambs Fed
379 Colostrum and Dam Milk or Cow Milk After Birth. Pages 24-25 in Proc. The 11th International
380 Simposyum of the World Association of Veterinary Laboratory Diagnosticians and OIE Seminars
381 on Biotechnology, Bangkok, Thailand.
382 Halliday, R. and M. R. Williams. 1979. Absorption of inmunoglobulin from colostrum by bottle-
383 fed lambs. Ann Rech Vet 10(4):549-556.
384 Lascelles, A. K. 1979. Immune system of the ruminant mammary-gland and its role in the
385 control of mastitis. Journal of Dairy Science 62(1):154-160.
386 Marsico, G., A. Vicenti, P. Centoducati, and A. Braghieri. 1993. Influence of Weaning age on
387 Productive performance of kids slaughtered at 107 days of age. Small Ruminant Research
388 12(3):321-328.
389 Massimini, G., V. Mastellone, D. Britti, P. Lombardi, and L. Avallone. 2007. Effect of passive
390 transfer status on preweaning growth performance in dairy goat kids. Javma-J Am Vet Med A
391 231(12):1873-1877.
392 Mastellone, V., G. Massimini, M. E. Pero, L. Cortese, D. Piantedosi, P. Lombardi, D. Britti, and L.
393 Avallone. 2011. Effects of Passive Transfer Status on Growth Performance in Buffalo Calves.
394 Asian Austral J Anim 24(7):952-956.
395 Mayilyan, K. R., Y. H. Kang, A. W. Dodds, and R. B. Sim. 2008. The Complement System in
396 Innate Immunity. Pages 219-236 in Innate Immunity of Plants, Animals, and Humans. Vol. 21.
397 H. Heine, ed. Springer Berlin Heidelberg.

398 Moreno-Indias, I., A. Morales-delaNuez, L. E. Hernandez-Castellano, D. Sanchez-Macias, J.
399 Capote, N. Castro, and A. Arguello. 2012a. Docosahexaenoic acid in the goat kid diet: Effects on
400 immune system and meat quality. Journal of animal science 90(11):3729-3738.
401 Moreno-Indias, I., D. Sanchez-Macias, N. Castro, A. Morales-delaNuez, L. E. Hernandez-
402 Castellano, J. Capote, and A. Arguello. 2012b. Chemical composition and immune status of
403 dairy goat colostrum fractions during the first 10 h after partum. Small Ruminant Research
404 103(2-3):220-224.
405 Moretti, D. B., L. Kindlein, P. Pauletti, and R. Machado-Neto. 2010. IgG absorption by Santa
406 Ines lambs fed Holstein bovine colostrum or Santa Ines ovine colostrum. Animal : an
407 international journal of animal bioscience 4(6):933-937.
408 Muller, L. D. and D. K. Ellinger. 1981. Colostral immunoglobulin concentrations among breeds
409 of dairy-cattle. Journal of Dairy Science 64(8):1727-1730.
410 Musumeci, M., L. Malaguarnera, J. Simpore, R. Barone, M. Whalen, and S. Musumeci. 2005.
411 Chitotriosidase activity in colostrum from African and Caucasian women. Clin Chem Lab Med
412 43(2):198-201.
413 Napolitano, F., G. F. Cifuni, C. Pacelli, A. M. Riviezzi, and A. Girolami. 2002. Effect of artificial
414 rearing on lamb welfare and meat quality. Meat Science 60(3):307-315.
415 Napolitano, F., C. Pacelli, A. Girolami, and A. Braghieri. 2008. Effect of information about
416 animal welfare on consumer willingness to pay for yogurt. Journal of Dairy Science 91(3):910-
417 917.
418 Nowak, R. and P. Poindron. 2006. From birth to colostrum: early steps leading to lamb survival.
419 Reprod Nutr Dev 46(4):431-446.
420 O'Doherty, J. V. and T. F. Crosby. 1997. The effect of diet in late pregnancy on colostrum
421 production and immunoglobulin absorption in sheep. Anim Sci 64(01):87-96.
422 Quigley, J. D., 3rd, A. F. Carson, and J. Polo. 2002. Immunoglobulin derived from bovine plasma
423 as a replacement for colostrum in newborn lambs. Veterinary therapeutics : research in
424 applied veterinary medicine 3(3):262-269.
425 Quigley, J. D., P. French, and R. E. James. 2000. Short communication: Effect of pH on
426 absorption of immunoglobulin G in neonatal calves. Journal of Dairy Science 83(8):1853-1855.
427 Rodríguez, A. B., R. Landa, R. Bodas, N. Prieto, A. R. Mantecon, and F. J. Giraldez. 2008. Carcass
428 and meat quality of Assaf milk fed lambs: Effect of rearing system and sex. Meat Science
429 80(2):225-230.
430 Rodríguez, C., N. Castro, J. Capote, A. Morales-delaNuez, I. Moreno-Indias, D. Sanchez-Macias,
431 and A. Arguello. 2009. Effect of colostrum immunoglobulin concentration on immunity in
432 Majorera goat kids. Journal of Dairy Science 92(4):1696-1701.
433 Ruby, R. E., C. C. Balcomb, S. A. Hunter, and K. E. Lawrence. 2012. Bovine colostrum-induced
434 anaemia in a 2-week-old lamb. New Zealand veterinary journal 60(1):82-83.
435 Stelwagen, K., E. Carpenter, B. Haigh, A. Hodgkinson, and T. T. Wheeler. 2009. Immune
436 components of bovine colostrum and milk. Journal of animal science 87(13 Suppl):3-9.
437 Stott, G. H. and A. Fellah. 1983. Colostral immunoglobulin absorption linearly related to
438 concentration for calves. Journal of Dairy Science 66(6):1319-1328.
439 Trujillo, A. J., N. Castro, J. M. Quevedo, A. Arguello, J. Capote, and B. Guamis. 2007. Effect of
440 heat and high-pressure treatments on microbiological quality and immunoglobulin G stability
441 of caprine colostrum. Journal of Dairy Science 90(2):833-839.
442 Winter, A. 2011. Bovine neonatal pancytopenia, and anaemia in lambs caused by feeding cow
443 colostrum. Veterinary Record 168(3):84-84.
444 Winter, A. C. and M. J. Clarkson. 1992. Farm investigations of anemia in labms caused by
445 feeding cow colostrum. Veterinary Record 131(10):213-216.
446 Wold, A. E. and I. Adlerberth. 2000. Breast feeding and the intestinal microflora of the infant -
447 Implications for protection against infectious diseases. Pages 77-93 in Short and Long Term

448 Effects of Breast Feeding on Child Health. Vol. 478. B. Koletzko, K. F. Michaelsen, and O.
449 Hernell, ed. Kluwer Academic / Plenum Publ, New York.
450 Wooding, F. B., A. P. Flint, R. B. Heap, G. Morgan, H. L. Buttle, and I. R. Young. 1986. Control of
451 binucleate cell migration in the placenta of sheep and goats. Journal of reproduction and
452 fertility 76(2):499-512.

453

454 Table 1. BW, ChT activity, TCA, ACA and IgG and IgM concentration evolution in
455 NR, GC2, GC14, SC2 and SC14 during the first 5 d after birth.

	Group	Days						SEM
		0	1	2	3	4	5	
BW (kg)	NR	4.15^{a}	4.42^{b}	4.69^{c}	4.95^{d,z}	5.19^{e,z}	5.43$^{e.z}$	0.09
	GC2	4.17^{ab}	4.04^{b}	3.91^{c}	4.00^{c,y}	4.11^{ab,y}	4.22^{a,y}	0.15
	GC14	4.26^{a}	4.09^{b}	3.99^{b}	3.98^{b,y}	4.04^{b,y}	4.16^{ab,y}	0.10
	SC2	4.49^{a}	4.23^{b}	4.20^{b}	4.22^{b,zy}	4.25^{b,y}	4.35^{ab,y}	0.18
	SC14	4.55^{a}	4.34^{b}	4.25^{b}	4.21^{b,zy}	4.18^{b,y}	4.26^{b,y}	0.12
IgG (mg/mL)	NR	0.22^{a}	16.79^{b,z}	13.84^{c,z}	12.75^{cd,z}	12.07^{cd,z}	11.43^{d,z}	0.76
	GC2	0.18^{a}	6.17^{b,y}	8.73^{c,y}	8.56^{c,y}	6.80^{b,y}	6.96^{bc,y}	1.70
	GC14	0.15^{a}	5.83^{b,y}	7.40^{b,y}	6.90^{b,y}	6.49^{b,y}	6.07^{b,y}	0.95
	SC2	0.15^{a}	8.84^{b,y}	9.52^{b,y}	6.26^{b,y}	7.17^{b,y}	6.24^{b,y}	0.77
	SC14	0.20^{a}	5.40^{b,y}	7.80^{b,y}	6.26^{b,y}	5.57^{b,y}	5.77^{b,y}	0.78
IgM (mg/mL)	NR	-	1.88^{a,z}	1.44^{b,z}	1.32^{bc,z}	1.15^{c,z}	0.98^{c,z}	0.10
	GC2	-	0.69^{yx}	0.67^{y}	0.69^{y}	0.60^{y}	0.44^{y}	0.06
	GC14	-	0.57^{x}	0.43^{y}	0.42^{y}	0.54^{y}	0.40^{y}	0.05
	SC2	-	0.98^{a,y}	0.72^{a,y}	0.58^{ab,y}	0.46^{b,y}	0.50^{ab,y}	0.11
	SC14	-	0.60^{a,yx}	1.10^{b,zy}	0.69^{ab,y}	0.42^{a,y}	0.50^{a,y}	0.09
ChT activity (nmol/mL/hr)	NR	1017.22	1060.58	926.52	875.57	1057.22	1033.95	50.07
	GC2	804.68	847.03	791.44	821.63	866.74	868.43	23.62
	GC14	897.46^{a}	944.70^{a}	787.50^{a}	717.67^{a}	860.51^{a}	1128.23^{b}	58.00
	SC2	946.33	996.13	815.72	784.38	885.25	986.77	99.770
	SC14	1019.69^{a}	907.80^{a}	770.64^{a}	845.58^{a}	913.39^{a}	1195.50^{b}	166.35
TCA (%)	NR	-	9.05^{a}	20.56^{b,z}	31.36^{c,z}	36.58^{c,z}	42.43^{d,z}	2.10
	GC2	-	2.46^{a}	4.34^{ab,y}	7.05^{ab,y}	13.47^{b,y}	15.42^{b,y}	1.42
	GC14	-	0.23^{a}	4.08^{ab,y}	6.39^{ab,y}	8.27^{ab,y}	9.70^{b,y}	0.92
	SC2	-	1.11^{a}	4.66^{ab,y}	13.56^{b,y}	15.58^{b,y}	16.06^{b,y}	1.96
	SC14	-	0.86^{a}	8.70^{b,y}	13.74^{bc,y}	18.12^{c,y}	23.03^{c,y}	2.81
ACA (%)	NR	-	7.67^{a,z}	15.05^{b,z}	24.48^{c,z}	27.29^{d,z}	36.26^{e,z}	1.69
	GC2	-	0.71^{a,y}	0.77^{a,y}	3.16^{ab,x}	9.42^{b,y}	11.54^{b,y}	1.39
	GC14	-	0.30^{a,y}	2.45^{a,y}	6.30^{ab,yx}	8.68^{b,y}	12.32^{b,y}	1.15
	SC2	-	0.23^{a,y}	3.89^{b,y}	6.99^{ab,yx}	10.08^{b,y}	14.90^{b,y}	1.40
	SC14	-	0.40^{a,y}	3.99^{b,y}	8.69^{b,y}	10.18^{b,y}	16.85^{b,y}	1.99

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457 $^{a–e}$ Means within a row with different superscript letters differ significantly ($P < 0.05$).
458 $^{x–z}$ Means within a column for a specific item with different superscript letters differ
459 significantly ($P < 0.05$).
460 Data with - means non-detectable.
461 ChT: Chitotriosidase; TCA: Total Complement System activity; ACA: Alternative
462 Complement System activity.
463

Capítulo 3

Chapter 3

Interpretative Summary

Influence of rearing feed system (sheep milk *vs.* milk replacer *vs.* whole powdered cow milk) on final lamb weight and immune status. By Hernández-Castellano

The rearing feed system (sheep milk *vs.* milk replacer *vs.* whole powdered cow milk) may affect the immune status and therefore the final lamb performance. These factors may produce a negative impact on the economic benefits of farmers and breeders with severe consequences on the animal welfare. This manuscript details BW and immune differences due to the rearing system. In addition, it is also described that there was neither weight nor immune differences between non sheep milk groups due to the milk source (milk replacer *vs.* whole powdered cow milk).

25 **Running head: EFFECT OF MILK SOURCE LAMB STATUS**

26

27 **TITLE**

28 **The effect of rearing system on BW and immune status of lambs from birth to**

29 **weaning.**

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ABSTRACT

There are several factors that can affect the lamb BW and immune parameters before weaning, such as rearing system or milk source. For this reason the aim of this experiment was to evaluate the effect of diet (sheep milk-NR *vs.* milk replacer-MR *vs.* whole powdered cow milk-CM) on the BW and immune parameters during milk feeding period and weaning. In this study 60 lambs were randomly divided according to treatment (NR, MR and CM). Blood plasma was used to measure the immunoglobulin concentration (IgG and IgM), Chitotriosidase activity and Complement System activity (Total and Alternative pathways, TCA and ACA, respectively). Results showed that lambs reared with NR presented higher BW, IgG, IgM, TCA and ACA than animals reared with MR or CM at 3 d and 5 d. However, during the weaning, these differences disappeared. At the end of this period, animals reared with MR and CM showed higher BW than lambs reared with NR (15.28, 16.89 and 17.66 kg in NR, MR and CM groups, respectively, $P<0.05$). Moreover, MR and CM groups showed higher IgM concentrations at the end of this period than NR group (1.05, 1.90 and 1.60 mg/mL in NR, MR and CM, respectively, $P<0.05$). The findings showed in this study, may improve the management in sheep farms, reducing the expenses of the artificial rearing systems and, consequently, increasing the economic benefits of the sheep producers.

Keywords: Chitotriosidase, Immunity, lamb, whole powdered cow milk, weaning

INTRODUCTION

Newborn ruminants have three critical periods related to their immune system in the first month of life: colostrum intake, milk feeding, and weaning. The management in these periods affects the final animal performances (Marsico et al., 1993; Massimini et al., 2007; Mastellone et al., 2011).

Nowadays, there is an increasing number of high production dairy farms, in which artificial rearing is chosen in order to increase the amount of sheep milk available for processing (cheese, yogurt) (Demiroren et al., 1995; Napolitano et al., 2008) and simplifying management (Emsen et al., 2004). In this system, lambs are separated from dams at early age (0–2 d) and then, they are fed with a milk replacer. The milk feeding period is an important stage in the newborn lamb because milk is the only energy source for these animals. Furthermore, in order to achieve an optimum performance, it has been recommended to feed lambs with milk replacers formulated specifically for them (Frederiksen, 1980), which are mainly based on cow milk, cereals and vegetable fats (Bañón et al., 2006). However, milk replacer for lambs is usually considerably more expensive than high quality calf milk replacer or even whole powdered cow milk for human consumption (in some regions).

Weaning is a critical phase in domestic ruminant production, mainly because any change produced in feeding strategies (frequency and composition) can be perceived as a stressor in preweaning ruminants. Weaning is well known to increase susceptibility to a variety of infectious diseases in ruminants due to the attenuation of the immune system under high stress conditions, such as diet changes (Sowinska et al., 2001; Hickery, 2003). For this reason, there is an increasing interest in finding effective

dietary stress reducers and immune enhancers that may improve the disease resistance in weaning ruminants (Kwon et al., 2011).

The immunoglobulin plasma concentration (IgG and IgM, mainly) is probably the most important humoral immune parameter, being deeply studied in lambs (Klobasa and Werhahn, 1989; Mukkur et al., 1998; Hashemi et al., 2008); however, there are other immune components, such as the Chitotriosidase (ChT) activity and the Complement System activity, that play a fundamental role in the innate immune response, acting as a part of the host defense in newborn ruminants. Chitotriosidase is predominantly a secretory protein that is able to hydrolyze chitin in the cell wall of fungi and nematodes (Barone et al., 1999). This enzyme is a functional chitinase with high homology to chitinases that belong to family of 18 glycosyl hydrolases. Chitotriosidase has an important relation with the host defense and for this reason it has been investigated in humans (Musumeci et al., 2005) and goats (Argüello et al., 2008a; Hernández-Castellano et al., 2011; Moreno-Indias et al., 2012c), being this enzyme has never been described in sheep or lambs .

The Complement System activity (Total (TCA) and Alternative (ACA) pathways) is involved in specific and nonspecific immunity, playing an important role in defense mechanisms against infectious microorganisms (Rodríguez et al., 2009), being one of the first immune barriers to pathogens (Petrova and Mehta, 2007). The Complement System in mammals has been well described, particularly in humans and mice, but it has been also examined in ruminants, such as cows and goats (Mayilyan et al., 2008; Moreno-Indias et al., 2012a; Moreno-Indias et al., 2012c). However, there are few studies about the Complement System activity in sheep and lambs.

The aim of the present study was to analyze the effect of diet (sheep milk-NR, milk replacer-MR and whole powdered cow milk-CM) on the BW evolution, the IgG and IgM blood concentration and the ChT and Complement System activity during milk feeding period and weaning.

MATERIAL AND METHODS

The present study was performed in the Department of Animal Science of the Universidad de Las Palmas de Gran Canaria in Canary Islands (Spain) on 60 lambs (30 males and 30 females) of Canary breed. Animal procedures were approved by the Ethical Committee of the University.

Colostrum Period

At birth, animals were randomly divided in three different groups according to diets (NR, MR and CM). The NR group was composed of 20 lambs that were fed colostrum directly from their dams. The other 40 lambs were artificial reared without dam contact. The MR and CM animals received a pool of fresh sheep colostrum that was previously pasteurized at 63ºC for 30 minutes according to Trujillo et al. (2007). Because no recommendation about the requirements of colostrum IgG/BW in artificially reared lambs was found in the literature, lambs received 4g of IgG/Kg of BW during the colostrum period (0-2 d after birth), according to recommend concentration for goat kids (Castro et al., 2005a). Sheep colostrum IgG concentration was determined using a commercial ELISA kit (Bethyl laboratories, Montgomery, TX, USA), using purified sheep IgG (Bethyl laboratories, Montgomery, TX, USA) for the standard curve.

148 ***Milk Feeding Period***

149 The NR group was raised with unlimited access to their dams until weaning period.
150 Ewes were not milked during this period. After colostrum period, the other 40 lambs
151 were randomly allotted into two artificial rearing groups: one of them received a
152 commercial milk replacer at 16% (w/w; MR group, Bacilactol Corderos y Cabritos,
153 Saprogal, La Coruña, Spain; 95.5% DM, 23.6% CP and 22.7% ether extract, air-dry
154 powder basis), and the other one received whole powdered cow milk for human
155 consumption at 16% (w/w; CM group, whole powdered milk, Arla foods, Denmark;
156 97% DM, 27% CP, 28% ether extract, air-dry powder basis). Both groups were fed *ad*
157 *libitum* (37ºC), using nipple buckets twice a day (8:00 a.m. and 05:00 p.m.).
158
159 ***Weaning Period***
160 Animals started the weaning period when they reached 10 Kg of BW. During the
161 weaning period (30 d) the three studied groups had free access to starter feed (18% CP
162 and 3.4% ether extract), alfalfa hay and water. The NR lambs were removed from dams
163 and placed in a pen. Ewes were milked during the whole weaning period once daily
164 (09:00 a.m.). During the first wk, lambs had access to ewes twice daily (10:00 a.m. after
165 milking, and 05:00 p.m.), reducing this access to once a day (05:00 p.m.) during the
166 second wk, and thereafter, lambs had no more access to ewes. The MR and CM groups
167 were fed with half a liter of the selected diet twice a day during the first wk (10:00 a.m.
168 and 05:00 p.m.), reducing to once daily at the second wk (05:00 p.m.). During the rest
169 of the period animals received neither cow milk nor milk replacer.
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BW and sample collection

During the milk feeding period, blood samples were taken at 3 d, 5 d and 20 d after birth. Another sample was collected when animals reached 10 Kg of BW. During the weaning period, samples were obtained every 5 days until the end of the experiment. Animals were weighed before blood extraction (MOBBA, Barcelona, Spain; accuracy, 5g). Blood samples were taken before feeding from the jugular vein in 2.5 mL tubes with EDTA. After that, blood was centrifuged at 2190 *g* for 5 minutes at 4ºC (Hettich-Zentrifugen, Universal 32 R, Germany), storing the plasma at -80° C until analysis.

To determine plasma IgG and IgM concentrations, a commercial ELISA kit (Bethyl Laboratories, Montgomery, TX, USA) was used, setting a purified sheep IgG and IgM as a standard reference. Results were expressed in mg of immunoglogulin/mL of plasma.

Chitotriosidase activity was measured following the procedure described by Argüello et al. (2008a). In this procedure, 1 µL of undiluted blood plasma with 100 µL of a solution containing 22 m*M* artificial substrate (4-methylumbelliferyl-d-N, N′, N″ triacetylchitotriose) in 0.5 *M* citrate phosphate buffer (pH 5.2) was incubated for 15 min at 37°C. The reaction was stopped with 5 mL of 0.5 *M* Na_2CO_3-$NaHCO_3$ buffer (pH 10.7). Fluorescence was measured using a fluorimeter (Perkin Elmer, Norwalk, CT) set to an exictation at 365 nm and emission at 450 nm. The ChT activity was expressed as nanomoles of substrate hydrolyzed per milliliter per hour.

Complement System activity (TCA and ACA) was measured using a novel technique described by Moreno-Indias et al. (2012b). A $DGHB^{++}$ buffer [Hepes Gelatin Veronal

198 Buffer with Ca^{++} and Mg^{++}: 5 m*M* HEPES, 71 m*M* NaCl, 0.15 m*M* $CaCl_2$, 0.5 m*M*
199 $MgCl_2$, 2.5% (w/v) glucose, 0.1% (w/v) gelatin, pH 7.4] was used to measure the total
200 Complement System activity, and DGHB-Mg-EGTA buffer [4.2 m*M* Hepes, 59 m*M*
201 NaCl, 7.0 m*M* $MgCl_2$, 2.08% (w/v) glucose, 0.08% (w/v) gelatin, 10 m*M* EGTA, pH
202 7.4] was used to measure the alternative pathway. For total Complement System
203 activity, rabbit red blood cells and lamb plasma were both diluted to 5% in $DGHB^{++}$;
204 100 µL of each was then mixed in a microtiter plate and incubated at 37°C for 1 h. Cells
205 were removed by centrifugation (2500 × *g*, 5 min, 4ºC), and supernatant absorbance was
206 measured at 405 nm using a microplate reader. Complete hemolysis was achieved by
207 mixing the cells with distilled water (100 µL), and spontaneous lysis was produced by
208 mixing the diluted rabbit red blood cells with $DGHB^{++}$. Complement-induced hemolysis
209 of rabbit red blood cells by the test sera was calculated using the formula: [(A405
210 sample − A405 spontaneous lysis) / (A405 complete hemolysis − A405 spontaneous
211 lysis)] × 100. The same protocol was performed with DGHB-Mg-EGTA buffer to
212 measure the alternative pathway.
213
214 ***Statistical analyses***
215 Statistical analyses were performed using SAS, Version 9.00 (SAS Institute Inc., Cary,
216 NC). The SAS PROC MIXED procedure for repeated measures was used to evaluate
217 the effect of the milk source (NR, MR, CM) on BW evolution, IgG and IgM
218 concentration, ChT and Complement System activity during milk feeding and weaning
219 period. A Tukey's test was used to evaluate differences between groups.
220
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RESULTS AND DISCUSSION

Table 1 shows the results of BW, IgG and IgM concentration, ChT activity, TCA and ACA during the milk feeding period. In reference to BW evolution, all the studied groups increased their initial BW at 3 d after birth; although differences between groups where observed. Sheep milk group showed the highest BW at 3 d, 5 d and 20 d after birth (4.95, 5.43, 8.96 kg, respectively). Due to the similar BW at birth between groups (4.15, 4.35 and 4.46 kg in NR, MR and CM groups, respectively), differences between NR and the other two groups could be related to factors associated with the increase of suckling frequency in animals reared in the NR group (Lanza et al., 2006). As it has been studied by Rodríguez et al. (2008), lambs that are reared under restricted conditions presented lower ADG (253 g/d) than animals that were raised with their dams (307 g/d). Baumrucker and Blum (1993) found that dam's milk has a growth promoter that is not present in milk replacers, which could explain the higher BW in NR lambs. In agreement with these findings, Argüello et al. (2004a) obtain heavier goat kids in a natural rearing system than goat kids fed with milk replacer. However, when MR and CM groups were compared, no differences due to the diet were observed during this period.

Focusing on the IgG concentrations, all animals decreased the IgG concentrations in blood from 3 d until 5 d after birth. In the NR group, IgG concentrations also decreased at 20 d (6.47 mg/mL) and at the end of the period (5.22 mg/mL). On the other hand, IgG concentrations in MR and CM groups remained similar at 20 d (3.15 and 4.44 mg/mL in MR and CM groups, respectively), but decreased at the end of the milk feeding period (2.78 and 2.88 g/mL in MR and CM groups, respectively). The highest IgG concentrations were obtained by the NR lambs throughout the studied period, probably

248 because of the unrestricted amount of colostrum ingested. In agreement with these
249 results, Altiner et al. (2005) described that lambs reared with their dams obtained higher
250 IgG concentrations in blood than lambs reared with milk replacer. Nevertheless, Firat et
251 al. (2003) found no differences in the milk feeding period, between lambs that received
252 a similar colostrum intake and lambs raised under natural and artificial conditions,
253 obtaining higher IgG concentrations than the results showed in this study. On the other
254 hand, comparing both artificial rearing groups (MR and CM), show that no differences
255 were observed in IgG concentrations during the whole studied period. To the best of our
256 knowledge, no references about the immune evolution due to the different milk sources
257 used in artificial rearing has been found in lambs, moreover, Moreno-Indias et al.
258 (2012b) did not find any difference in IgG concentration between goat kids artificial
259 reared with fresh goat milk or cow milk. These results support the idea that there were
260 no differences between lambs reared with MR or CM on blood IgG concentrations,
261 although higher IgG values were obtained in the NR group, probably produced by the
262 higher colostrum intake of this last group.
263
264 The NR group obtained the highest IgM concentration at 3 d after birth (1.32 mg/mL),
265 decreasing at 5 d and 20 d and remaining constant until the end of the milk feeding
266 period (0.98, 0.71 and 0.78 mg/mL, respectively). A different evolution was observed in
267 MR and CM groups, whereas the IgM concentration decreased at 5 d (0.41 mg/mL in
268 both MR and CM) and kept constant at 20 d (0.50 and 0.39 mg/mL, respectively),
269 increasing and obtaining the highest value at the end of the milk feeding period (0.82
270 mg/mL and 0.65 mg/mL in MR and CM, respectively). According to findings observed
271 by Nonnecke et al. (2012) in colostrum-fed and colostrum-deprived calves, animals fed
272 with colostrum showed a rapid increase of serum IgG and IgM concentrations after

colostrum intake, decreasing these concentrations with age. The colostrum-deprived calves, in contrast, had very low or undetectable serum IgG and IgM concentrations, followed by an age-related increase of IgG and IgM concentrations, suggesting endogenous production of these immunoglobulins. Although in the present study this evolution was only observed in IgM concentrations, it has been described that IgM is the first immunoglobulin produced by the organism (Ehrenstein and Notley, 2010). The NR group registered higher IgM concentrations at 5 d (0.98 mg/mL) and at 20 d (0.71 mg/mL) than the MR and CM groups at the same time. As it was described before, the unrestricted access of the NR group to colostrum could produce a higher IgM concentration in blood. At the end of this period, no differences were observed between groups. No differences between MR and CM were detected during the whole milk feeding period. Moreno-Indias et al. (2012b) did not observed differences, due to the milk source (Goat milk and cow milk) in goat kids reared under an artificial rearing system from partum to 35 d of life. In this study, no differences in blood IgM concentrations were observed between NR, MR and CM diets at the end of this period, although NR group showed higher IgM concentrations at 5 d than the other two groups.

Chitotriosidase activity remained constant from 3 d after birth to the end of this period in the three studied groups. Moreover, even a punctual exception was found at 20 d; no differences were found between different groups in this period. The ChT activity is related to the immune status of animals; however there are no studies that have described this enzyme in sheep. In goat kids similar values to these results were reported by Rodríguez et al. (2009), who similarly demonstrated that ChT activity in goat kids does not vary with age during the first 5 d of life. A similar evolution was also described by Moreno-Indias et al. (2012b), although higher values than present results

were described, concluding that the diet and the time after birth affected plasma ChT activity. In this study, milk source (NR *vs.* MR *vs.* CM) did not affect the plasma ChT activity in the milk feeding period.

Complement System activity (TCA and ACA) increased until the end of this period in the three studied groups, following a similar evolution previously observed by Oswald et al. (1990) in lambs and by Castro et al. (2008) in goat kids. As described by these last authors, during this period ACA seems to be the most important pathway in the three studied groups.

Complement System activity (TCA and ACA) was higher in NR than in MR and CM at 3 d, 5 d and 20 d after birth. Conversely, at the end of the milk feeding period, differences between groups were only observed for ACA, NR being higher than MR or CM groups. Oswald et al. (1990) described the evolution of the Complement System activity (TCA and ACA) in lambs, showing that the great increase of the Complement System activity after birth may be explained by the passive transfer of Complement factors from colostrum. As the NR group received an unrestricted amount of colostrum and milk, lambs from this group could absorb a higher amount of complement factors than lambs from MR and CM groups, as it has been observed in newborn goat kids (Castro et al., 2008). In agreement with this study, no differences in TCA and ACA were observed by Moreno-Indias et al. (2012b) in goat kids fed under artificial rearing system with goat milk and CM. Results in the present study showed no differences in the Complement System activity (TCA *vs.* ACA) between MR and CM groups. However, NR used to display higher TCA and ACA percentages than MR and CM groups in this period.

323 Table 2 shows the values of BW, IgG and IgM concentrations, ChT activity, TCA and
324 ACA during the weaning period. Increases in BW during this period were observed in
325 the three experimental groups. However, at the end of the weaning period (30 d),
326 animals from MR and CM groups were heavier (16.89 and 17.66 kg in MR and CM
327 group, respectively) than the NR group (15.28 kg). It has been described by Napolitano
328 et al. (2002) that lambs raised with dams suffer a higher stress in the weaning period
329 than animals from artificial rearing, resulting in a decrease of the growth rate. Present
330 results showed that there were no differences in the final BW obtained at the end of the
331 weaning period in MR and CM groups, both of them being higher than the NR group.
332
333 During this period there were no important variations in IgG levels in the three groups
334 (NR, MR and CM), however all groups experienced a slightly higher blood IgG
335 concentration at the end of this period. When differences between groups were
336 established, it was found that the NR group presented higher IgG concentrations in
337 blood (5.22 mg/mL) than the MR and CM group (2.78 and 2.88 mg/mL, respectively) at
338 the beginning of this period (0 d). No IgG concentration differences between MR and
339 CM groups were observed at the same time. However, during the rest of the period,
340 differences between groups were not found, probably due to the combination of food
341 restriction and dam separation stress that was present in the NR group. It has been
342 demonstrated that stress produces a decrease of IgG and IgM concentrations in blood
343 (Matos-Gomes et al., 2010). Additionally, the increase of IgG shown in the MR and CM
344 groups, could suggest the beginning of the endogenous immunoglobulin production by
345 these animals. This stress likely reduced the rate of IgG blood concentration increase in
346 NR group as compared to the other two studied groups. In contrast of these findings,
347 Rhind et al. (1998) found that weaning at 4 months of age determined increased plasma

348 cortisol concentrations, but did not affect the immune response of lambs. Results in the
349 present study showed that milk source (NR *vs.* MR *vs.*CM) seems to have no
350 remarkable effects on the evolution of IgG concentration.
351
352 In reference to the blood IgM concentration, the NR group expressed an increase of this
353 immunoglobulin from the beginning of this period (0.78mg/mL) until 15 d (1.02
354 mg/mL) when the maximum value was obtained, remaining at this level until the end of
355 weaning. A similar evolution was followed by MR and CM groups, but their
356 concentrations increased from the beginning (0.82 mg/mL and 0.60 mg/mL in MR and
357 CM groups, respectively) to the end of the weaning period (1.90 mg/mL and 1.60
358 mg/mL in MR and CM groups, respectively). When IgM concentrations between groups
359 (NR, MR, and CM) were compared, no differences were detected at the beginning of
360 the weaning period (0.78 mg/mL, 0.82 mg/mL and 0.60 mg/mL, respectively), however
361 in spite of the increase of IgM concentration observed in all groups along this time, the
362 increasing rate was different and for this reason at the end of this period, animals from
363 MR and CM groups obtained a higher IgM concentration (1.90 mg/mL and 1.60
364 mg/mL, respectively) than the NR group (0.95 mg/mL). As it has been described before,
365 the higher stress level suffered by lambs from the NR group could possibly affect the
366 IgM differences between groups at the end of the weaning period. These results showed
367 that animals from the NR group had a lower IgM concentration than the the MR and
368 CM groups. Moreover, there were no differences in IgM concentrations between
369 animals reared with MR or CM.
370
371 The ChT activity increased in the NR group at 15 d (1511.25 nmol/mL/hr). However,
372 this activity abruptly decreased at 20 d (1194.85 nmol/mL/hr), remaining at this level

until the end of weaning period. On the other hand, MR and CM showed an increase of the ChT activity in this period. The observed reduction by the NR group was probably because it was at this time when animals stop the milk feeding, that also means the stop of dam contact in case of NR group. As it has been described before (Napolitano et al., 2002; Matos-Gomes et al., 2010), the combination of both stressors could produce a decrease of immune components, such as ChT activity. On the other hand, when experimental groups were compared, no differences were observed during the weaning period. These results reveal that, in general, ChT activity was not affected by the milk source (NR *vs.*MR *vs.* CM) during the weaning period.

In general, the Complement System activity (TCA and ACA) increased in the three studied groups during the weaning period. As previously observed in the milk feeding period, ACA seems to be the most important Complement System pathway at this time. Moreover, no differences were observed between groups during the whole period. There are few references about the Complement System activity during weaning not only in lambs, but also in ruminants (cow, sheep and goat). Nevertheless, Oswald et al. (1990) described the Complement System activity (TCA and ACA) in lambs during a similar studied period, showing a similar evolution to present results, although different values were described, probably due to the differences between analytical techniques used. According to the results showed in the present study, Complement System activity (TCA and ACA) was not affected by any of the diets used in this study (NR *vs.* MR *vs.* CM).

CONCLUSIONS

In the present study lambs reared under a natural rearing system showed, in general, higher BW and immune parameters than lambs reared under artificial systems with different milk sources during the milk feeding period. However, it is interesting to highlight that during the weaning period, animals from both artificial rearing groups compensated for these differences. For this reason, at the end of the studied period MR and CM groups showed a higher BW than NR group.

These findings may improve the dairy sheep management, reducing the expenses of the artificial rearing system and, consequently, increasing the economic benefit for sheep farmers.

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REFERENCES

Altiner, A., A. Ozpinar, and M. Erhard. 2005. Serum immunoglobulin G levels in lambs fed colostrum and dam milk or cow milk and milk replacer after birth. Med Weter 61(10):1135-1137.

Argüello, A., N. Castro, M. Batista, I. Moreno-Indias, A. Morales-DelaNuez, D. Sanchez-Macias, E. Quesada, and J. Capote. 2008. Chitotriosidase activity in goat blood and colostrum. Journal of Dairy Science 91(5):2067-2070.

Argüello, A., N. Castro, and J. Capote. 2004. Growth of milk replacer kids fed under three different managements. Journal of Applied Animal Research 25(1):37-40.

425 Bañón, S., R. Vila, A. Price, E. Ferrandini, and M. D. Garrido. 2006. Effects of goat milk or milk
426 replacer diet on meat quality and fat composition of suckling goat kids. Meat Science
427 72(2):216-221.
428 Barone, R., F. Di Gregorio, M. A. Romeo, G. Schiliro, and L. Pavone. 1999. Plasma
429 chitotriosidase activity in patients with beta-thalassemia. Blood Cell Mol Dis 25(1):1-8.
430 Baumrucker, C. R. and J. R. Blum. 1993. Secretion of Insulin-Like Growth-Factors in Milk and
431 Their Effect on the Neonate. Livest Prod Sci 35(1-2):49-72.
432 Castro, N., F. Acosta, T. Nino, J. Vivas, E. Quesada, J. Capote, and A. Arguello. 2008. The effects
433 of diet and age on serum complement system activity in goat kids. Livest Sci 119(1-3):102-106.
434 Castro, N., J. Capote, S. Alvarez, and A. Arguello. 2005. Effects of lyophilized colostrum and
435 different colostrum feeding regimens on passive transfer of immunoglobulin G in Majorera
436 goat kids. Journal of Dairy Science 88(10):3650-3654.
437 Demiroren, E., J. N. B. Shrestha, and W. J. Boylan. 1995. Breed and Environmental-Effects on
438 Components of Ewe Productivity in Terms of Multiple Births, Artificial Rearing and 8-Month
439 Breeding Cycles. Small Ruminant Research 16(3):239-249.
440 Ehrenstein, M. R. and C. A. Notley. 2010. The importance of natural IgM: scavenger, protector
441 and regulator. Nat Rev Immunol 10(11):778-786.
442 Emsen, E., M. Yaprak, O. C. Bilgin, B. Emsen, and H. W. Ockerman. 2004. Growth performance
443 of Awassi lambs fed calf milk replacer. Small Ruminant Research 53(1-2):99-102.
444 Firat, A., A. Ozpinar, and M. H. Erhard. 2003. Serum Immunoglobulin G Levels in Lambs Fed
445 Colostrum and Dam Milk or Cow Milk After Birth. Pages 24-25 in Proc. The 11th International
446 Simposyum of the World Association of Veterinary Laboratory Diagnosticians and OIE Seminars
447 on Biotechnology, Bangkok, Thailand.
448 Frederiksen, K. R. 1980. Rearing lambs on milk replacer diets. Dept. of Agriculture, Science and
449 Education Administration : for sale by the Supt. of Docs., U.S. Govt. Print. Off., [Washington] :.
450 Hashemi, M., M. J. Zamiri, and M. Safdarian. 2008. Effects of nutritional level during late
451 pregnancy on colostral production and blood immunoglobulin levels of Karakul ewes and their
452 lambs. Small Ruminant Research 75(2-3):204-209.
453 Hernández-Castellano, L. E., A. Torres, A. Alavoine, M. D. Ruiz-Diaz, A. Arguello, J. Capote, and
454 N. Castro. 2011. Effect of milking frequency on milk immunoglobulin concentration (IgG, IgM
455 and IgA) and chitotriosidase activity in Majorera goats. Small Ruminant Research 98(1-3):70-
456 72.
457 Hickery, J. 2003. Just the berries Prostate-specific antigen testing. Should we recommend it?
458 (vol 49, pg 303, 2003). Can Fam Physician 49:568-568.
459 Klobasa, F. and E. Werhahn. 1989. Variations in the levels of immunoglobulin-IgG1,
460 Immunoglobulin-IgG2, Inmunoglobulin-IgM and Immunoglobulin-IgA in sheep.2. Changes of
461 blood-levels in pure-bred and cross-bred lambs during the course of rearing. Berliner
462 Munchener Tierarztl. Wochenschr. 102(10):331-337.
463 Kwon, I. H., M. H. Kim, C. H. Yun, J. Y. Go, C. H. Lee, H. J. Lee, W. Phipek, and J. K. Ha. 2011.
464 Effects of Fermented Soybean Meal on Immune Response of Weaned Calves with
465 Experimentally Induced Lipopolysaccharide Challenge. Asian Austral J Anim 24(7):957-964.
466 Lanza, M., M. Bella, A. Priolo, D. Barbagallo, V. Galofaro, C. Landi, and P. Pennisi. 2006. Lamb
467 meat quality as affected by a natural or artificial milk feeding regime. Meat Science 73(2):313-
468 318.
469 Marsico, G., A. Vicenti, P. Centoducati, and A. Braghieri. 1993. Influence of Weaning age on
470 Productive performance of kids slaughtered at 107 days of age. Small Ruminant Research
471 12(3):321-328.
472 Massimini, G., V. Mastellone, D. Britti, P. Lombardi, and L. Avallone. 2007. Effect of passive
473 transfer status on preweaning growth performance in dairy goat kids. Javma-J Am Vet Med A
474 231(12):1873-1877.

475 Mastellone, V., G. Massimini, M. E. Pero, L. Cortese, D. Piantedosi, P. Lombardi, D. Britti, and L.
476 Avallone. 2011. Effects of Passive Transfer Status on Growth Performance in Buffalo Calves.
477 Asian Austral J Anim 24(7):952-956.
478 Matos-Gomes, N., M. Katsurayama, F. H. Makimoto, L. L. O. Santana, E. Paredes-Garcia, M. A.
479 D. Becker, and M. C. Dos-Santos. 2010. Psychological Stress and Its Influence on Salivary Flow
480 Rate, Total Protein Concentration and IgA, IgG and IgM Titers. Neuroimmunomodulation
481 17(6):396-404.
482 Mayilyan, K. R., Y. H. Kang, A. W. Dodds, and R. B. Sim. 2008. The Complement System in
483 Innate Immunity. Pages 219-236 in Innate Immunity of Plants, Animals, and Humans. Vol. 21.
484 H. Heine, ed. Springer Berlin Heidelberg.
485 Moreno-Indias, I., A. W. Dodds, A. Arguello, N. Castro, and R. B. Sim. 2012a. The complement
486 system of the goat: Haemolytic assays and isolation of major proteins. Bmc Vet Res 8.
487 Moreno-Indias, I., A. Morales-delaNuez, L. E. Hernandez-Castellano, D. Sanchez-Macias, J.
488 Capote, N. Castro, and A. Arguello. 2012b. Docosahexaenoic acid in the goat kid diet: Effects on
489 immune system and meat quality. Journal of animal science 90(11):3729-3738.
490 Moreno-Indias, I., D. Sanchez-Macias, N. Castro, A. Morales-delaNuez, L. E. Hernandez-
491 Castellano, J. Capote, and A. Arguello. 2012c. Chemical composition and immune status of
492 dairy goat colostrum fractions during the first 10 h after partum. Small Ruminant Research
493 103(2-3):220-224.
494 Mukkur, T. K. S., K. H. Walker, and G. H. McDowell. 1998. Passive immunisation of neonatal
495 lambs via colostrum and milk of ewes previously immunised with live attenuated Salmonella
496 typhimurium protects neonatal lambs from experimental salmonellosis. Comp. Immunol.
497 Microbiol. Infect. Dis. 21(4):327-336.
498 Musumeci, M., L. Malaguarnera, J. Simpore, R. Barone, M. Whalen, and S. Musumeci. 2005.
499 Chitotriosidase activity in colostrum from African and Caucasian women. Clin Chem Lab Med
500 43(2):198-201.
501 Napolitano, F., G. F. Cifuni, C. Pacelli, A. M. Riviezzi, and A. Girolami. 2002. Effect of artificial
502 rearing on lamb welfare and meat quality. Meat Science 60(3):307-315.
503 Napolitano, F., C. Pacelli, A. Girolami, and A. Braghieri. 2008. Effect of information about
504 animal welfare on consumer willingness to pay for yogurt. Journal of Dairy Science 91(3):910-
505 917.
506 Nonnecke, B. J., W. R. Waters, J. P. Goff, and M. R. Foote. 2012. Adaptive immunity in the
507 colostrum-deprived calf: Response to early vaccination with Mycobacterium bovis strain bacille
508 Calmette Guerin and ovalbumin. Journal of Dairy Science 95(1):221-239.
509 Oswald, I. P., F. Lantier, and G. Bourgy. 1990. Classical and Alternative Pathway Hemolytic
510 Activities of Ovine Complement - Variations with Age and Sex. Vet Immunol Immunop
511 24(3):259-266.
512 Petrova, A. and R. Mehta. 2007. Dysfunction of innate immunity and associated pathology in
513 neonates. Indian J Pediatr 74(2):185-191.
514 Rhind, S. M., H. W. Reid, S. R. McMillen, and G. Palmarini. 1998. The role of cortisol and beta-
515 endorphin in the response of the immune system to weaning in lambs. Anim Sci 66:397-402.
516 Rodríguez, A. B., R. Landa, R. Bodas, N. Prieto, A. R. Mantecon, and F. J. Giraldez. 2008. Carcass
517 and meat quality of Assaf milk fed lambs: Effect of rearing system and sex. Meat Science
518 80(2):225-230.
519 Rodríguez, C., N. Castro, J. Capote, A. Morales-delaNuez, I. Moreno-Indias, D. Sanchez-Macias,
520 and A. Arguello. 2009. Effect of colostrum immunoglobulin concentration on immunity in
521 Majorera goat kids. Journal of Dairy Science 92(4):1696-1701.
522 Sowinska, J., H. Brzostowski, Z. Tanski, and K. Czaja. 2001. The weaning stress response in
523 lambs of different age. Czech J Anim Sci 46(11):465-468.

524 Trujillo, A. J., N. Castro, J. M. Quevedo, A. Arguello, J. Capote, and B. Guamis. 2007. Effect of
525 heat and high-pressure treatments on microbiological quality and immunoglobulin G stability
526 of caprine colostrum. Journal of Dairy Science 90(2):833-839.

527

Table 1. BW, IgG and IgM concentrations, ChT activity, TCA and ACA evolution in NR, MR and CM groups during feeding period.

	Group	3 d	5 d	20 d	End	SEM
BW (kg)	NR	$4.95^{a,z}$	$5.43^{b,z}$	$8.96^{c,z}$	10.35^{d}	0.35
	MR	$4.22^{a,y}$	$4.40^{b,y}$	$7.17^{c,y}$	10.10^{d}	0.48
	CM	$4.05^{a,y}$	$4.06^{a,y}$	$6.36^{b,y}$	10.10^{c}	0.55
IgG (mg/mL)	NR	$12.75^{a,z}$	$11.43^{b,z}$	$6.47^{c,z}$	$5.22^{d,z}$	0.69
	MR	$6.51^{a,y}$	$5.72^{b,y}$	$3.15^{b,y}$	$2.78^{c,y}$	0.44
	CM	$8.79^{a,y}$	$6.30^{b,y}$	$4.44^{b,y}$	$2.88^{c,y}$	0.57
IgM (mg/mL)	NR	$1.32^{a,z}$	$0.98^{b,z}$	$0.71^{c,z}$	0.78^{c}	0.07
	MR	$0.64^{a,y}$	$0.41^{b,y}$	$0.50^{b,yz}$	0.82^{a}	0.06
	CM	$0.67^{a,y}$	$0.41^{b,y}$	$0.39^{b,y}$	0.65^{a}	0.04
ChT activity (nmol/mL/hr)	NR	875.57	1033.95	1416.90^{z}	1351.64	79.56
	MR	741.70	1377.80	1223.48^{yz}	1315.25	142.81
	CM	582.76	795.21	906.31^{y}	1045.50	68.65
TCA (%)	NR	$31.36^{a,z}$	$42.43^{b,z}$	$50.75^{bc,z}$	53.17^{c}	2.63
	MR	$12.90^{a,y}$	$15.26^{a,y}$	$34.76^{b,y}$	49.31^{c}	3.92
	CM	$11.63^{a,y}$	$16.15^{a,y}$	$37.88^{b,zy}$	44.16^{b}	3.53
ACA (%)	NR	$24.48^{a,z}$	$36.26^{b,z}$	$40.56^{bc,z}$	$46.80^{c,z}$	2.39
	MR	$9.94^{a,y}$	$14.32^{a,y}$	$28.27^{b,y}$	$33.70^{b,y}$	3.16
	CM	$6.40^{a,y}$	$10.95^{a,y}$	$29.37^{b,y}$	$33.45^{b,y}$	3.19

$^{a-d}$ Means within a row with different superscript letters differ significantly ($P < 0.05$).
$^{y-z}$ Means within a column for a specific item with different superscript letters differ significantly ($P < 0.05$).
TCA: Total Complement System activity; ACA: Alternative Complement System activity; ChT: Chitotriosidase; NR: Sheep milk; MR: Milk replacer; CM: Whole powdered cow milk.

Table 2. BW, IgG and IgM concentrations, ChT activity, TCA and ACA evolution in NR, MR and CM during weaning period.

	Group	0 d	5 d	10 d	15 d	20 d	25 d	30 d	SEM
BW (kg)	NR	10.35^{a}	11.41^{b}	12.02^{c}	12.95^{d}	13.89^{e}	$14.62^{f,z}$	$15.28^{g,z}$	0.24
	MR	10.10^{a}	10.83^{b}	11.56^{c}	13.07^{d}	14.46^{e}	$15.72^{f,yz}$	$16.89^{g,y}$	0.35
	CM	10.10^{a}	10.86^{b}	12.062^{c}	13.53^{d}	15.02^{e}	$16.33^{f,y}$	$17.66^{g,y}$	0.37
IgG (mg/mL)	NR	$5.22^{a,z}$	4.39^{a}	4.55^{a}	4.11^{a}	4.69^{a}	5.06^{a}	7.11^{b}	0.27
	MR	$2.78^{a,y}$	3.59^{a}	4.02^{ab}	4.44^{ab}	5.46^{ab}	4.03^{ab}	5.72^{b}	0.29
	CM	$2.88^{a,y}$	4.08^{ab}	3.92^{ab}	4.20^{b}	4.63^{b}	3.75^{ab}	5.51^{b}	0.23
IgM (mg/mL)	NR	0.78^{a}	0.84^{ab}	$0.88^{ab,z}$	1.02^{b}	$0.90^{ab,z}$	$0.96^{ab,z}$	$1.05^{b,z}$	0.05
	MR	0.82^{a}	1.10^{a}	$1.52^{b,y}$	1.36^{ab}	$1.38^{ab,yz}$	$1.49^{b,y}$	$1.90^{c,y}$	0.08
	CM	0.60^{a}	0.84^{ab}	$0.94^{b,z}$	1.27^{c}	$1.13^{bc,y}$	$1.09^{bc,yz}$	$1.60^{d,y}$	0.08
ChT activity (nmol/mL/hr)	NR	1351.60^{ab}	1375.59^{ab}	1223.52^{b}	1511.25^{a}	1194.85^{b}	1274.34^{b}	1212.54^{b}	51.15
	MR	1315.25^{a}	1237.29^{ab}	1202.20^{b}	1253.40^{ab}	1467.61^{ac}	1478.08^{ac}	1562.44^{c}	46.62
	CM	955.84^{a}	1074.52^{a}	1231.41^{ab}	1373.97^{b}	1282.79^{ab}	1362.56^{b}	1326.79^{b}	49.11
TCA (%)	NR	53.17^{a}	51.32^{a}	49.86^{a}	61.01^{ab}	55.39^{a}	65.20^{b}	65.06^{b}	1.85
	MR	49.31^{a}	54.58^{ab}	51.79^{ab}	50.35^{ab}	51.93^{ab}	50.91^{b}	60.05^{b}	2.48
	CM	44.16^{a}	49.69^{a}	49.32^{a}	51.77^{a}	61.64^{ab}	60.59^{ab}	67.78^{b}	2.29
ACA (%)	NR	46.80^{a}	46.99^{ab}	44.42^{a}	53.44^{ab}	43.21^{a}	55.52^{b}	55.40^{b}	1.92
	MR	33.70^{a}	37.69^{a}	47.41^{ab}	42.35^{ab}	39.465^{a}	41.15^{ab}	50.77^{b}	2.18
	CM	33.45^{a}	37.41^{ab}	40.87^{a}	40.49^{ab}	45.49^{b}	48.39^{b}	47.54^{b}	1.82

$^{a-g}$ Means within a row with different superscript letters differ significantly ($P < 0.05$).

$^{y-z}$ Means within a column for a specific item with different superscript letters differ significantly ($P < 0.05$).

TCA: Total Complement System activity; ACA: Alternative Complement System activity; ChT: Chitotriosidase; NR: Sheep milk; MR: Milk replacer; CM: Whole powdered cow milk.

Capítulo 4

Chapter 4

Running head: Colostrum importance on lamb immune status

The importance of colostrum period management on the weight and immune parameters in lambs: from birth to weaning.

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ABSTRACT

The aim of this study was to investigate the BW and immune status of lambs reared under natural conditions and lambs reared under artificial conditions fed with two different colostrum amounts (4 g/kg of BW and 8 g/kg of BW of IgG). In this study 60 lambs were randomly divided according to treatment. Twenty lambs remained with their dams (**NR** group). Forty lambs were removed from their lambs at birth and were bottle fed with 4 g/kg of BW (**C4** group) or 8 g/kg of BW of IgG (**C8** group). Blood plasma samples and BW recording were done before feeding. Blood plasma was used to determine the immunoglobulin concentration (IgG and IgM) and the Complement system activity (Total and Alternative pathways). Lambs from NR group showed higher BW than C4 and C8 groups during milk feeding period, while those groups had higher BW than NR group at the end of weaning period (15.28, 16.89 and 16.95 kg in NR, C4 and C8 groups, respectively, $P<0.05$). With reference to immune parameters, C8 and NR groups had higher plasma IgG and IgM concentration than C4 group during milk feeding period ($P<0.05$). Addtionally, C4 and C8 groups showed a similar IgG concentration and higher IgM concentration than NR group at the end of weaning period ($P<0.05$). Complement system activity was higher in NR group than in C4 and C8 groups during the first 3 days after birth ($P<0.05$). As a conclusion, lambs fed with the amount of colostrum equivalent to 8g of IgG/ kg of BW showed similar immune parameters than lambs from NR group getting a better BW at the end of the weaning period.

Keywords: Immune, lamb, colostrum, weaning, artificial rearing.

INTRODUCTION

The relation between colostrum and the survival of newborn ruminants has been long characterized (Argüello et al., 2004b; Castro et al., 2005b; Castro et al., 2009; Castro et al., 2011). In fact, colostrum contains a complex mixture of proteins that actively participate in the protection of the dam and the neonate (Passive Inmune Transfer; PIT) against pathogens and other post-partum environmental challenges (Bendixen et al., 2011). Moreover, colostrum contains diverse components, such as fat, lactose, vitamins or minerals that have a high nutritional importance (Ontsouka et al., 2003).

In particular, colostrum plays an important role in newborn lambs, because they are born hypo gammaglobulinemic, due to the complexity of the synepitheliochorial ruminant placenta which does not allow a sufficient transfer of immunoglobulins from the dam to the foetus (Castro et al., 2009). Additionally, it has been described that lambs which are not fed with colostrum in the first hours of life are more susceptible to diseases and mortality (Ahmad et al., 2000; da Nobrega et al., 2005; Nowak and Poindron, 2006).

Nowadays, there are an increasing number of high production dairy farms, where lambs are reared under an artificial feeding system. In those cases, lambs are bottle-fed with colostrum and milk replacer in order to increase the amount of milk available for processing (cheese, yogurt) (Demiroren et al., 1995; Napolitano et al., 2008), simplifying the animal management (Emsen et al., 2004).

It has been described by several authors (Castro et al., 2005b; Morales-delaNuez et al., 2009; Moreno-Indias et al., 2012b) that goat kids need to be fed with an amount of colostrum equivalent to 4 gr of IgG/kg of BW, divided into 3 meals before 48 h after

birth, in order to make an appropriate PIT. However, no references about the amount of colostrum required by newborn lambs reared under artificial conditions have been found. This knowledge has a high relevance on the survival of the lambs during these first days of life, because it is essential to reach an adequate initial immunoglobulin concentration on blood (O'Doherty and Crosby, 1997; Quigley et al., 2000; Christley et al., 2003).

The aim of this study was to investigate the BW and immune status of lambs reared under natural conditions and lambs reared under artificial conditions fed with two different colostrum amounts (4 g IgG/ kg of BW and 8 g IgG/ kg of BW).

MATERIAL AND METHODS

The present study was performed in the Department of Animal Science of the Universidad de Las Palmas de Gran Canaria in the Canary Islands (Spain) on 60 lambs (30 males and 30 females) from Canary breed. Animal procedures were approved by the Ethical Committee of the University.

Colostrum and Feeding Period

At birth, animals were randomly divided into three different groups. The natural rearing (NR) group was composed of 20 lambs which sucked colostrum directly from their dams. The other 40 lambs were removed from their dams and then divided in 2 blocks (20 lambs each) according to colostrum feeding treatment (4 g of IgG/ kg of BW and 8 g of IgG/kg of BW) without dam contact. Each room had central heating conferring a room temperature of 20ºC (approximately) and providing at least 0.3 m^2 floor space per lamb. Both artificial rearing groups (C4 and C8) received a sheep colostrum pool that

was previously pasteurized at 63ºC for 30 minutes according to Trujillo et al. (2007)and frozen. Lambs from C4 and C8 groups were bottle fed with a frozen pool of colostrum at 2, 14 and 24 hours after birth. At the end of the colostrum period lambs from C4 received 4 gr of IgG/kg of BW, according to recommended concentration for goat kids by Castro et al. (2005b) and C8 group received 8 gr of IgG/kg of BW.

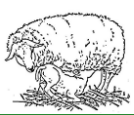
Colostrum IgG concentration (64.37 mg/mL) was determined using a commercial ELISA kit (Bethyl laboratories, Montgomery, TX, USA), using a purified sheep IgG as the standard curve.

The NR group was raised with dams and had free access to dam milk until weaning period. After the colostrum period, both artificial rearing lambs (C4 and C8) received a commercial milk replacer at 16% (w/w; MR group, Bacilactol Corderos y Cabritos, Saprogal, La Coruña, Spain; 95.5% DM, 23.6% CP and 22.7% ether extract, air-dry powder basis). These groups were fed *ad libitum* (37ºC), using nipple buckets twice a day (8:00 am and 17:00 pm).

Weaning Period

Animals from the 3 studied groups were weaned when they reached 10 kg of BW. During the weaning period, animals had free access to starter feed (18% CP and 3.4% ether extract), alfalfa hay and water. Lambs from the natural rearing group were removed from dams and placed in a pen for 4 weeks. Ewes were milked once a day (09:00 a.m.). During the first week of weaning, lambs had access to ewes twice daily (10:00 a.m. after milking and 5:00 p.m.), reducing this access to once a day (5:00 p.m.) during the second week, and thereafter, lambs had no more access to ewes. Artificial

115 rearing groups were fed with half a liter of milk replacer twice a day during the first
116 week (10:00 a.m. and 5:00 p.m.), reducing to once daily in the second week (5:00 p.m.).
117 During third and fourth weeks, animals did not receive milk replacer.
118
119 ***Weight and sample collection***
120 All experimental animals were weighed before each blood extraction and the results
121 were expressed in kg (MOBBA, Barcelona, Spain; accuracy, 5g). Blood samples were
122 taken before morning feeding from the jugular vein in 2.5 mL tubes with K3EDTA.
123 Subsequently, blood was centrifuged at 2190 x g during 5 minutes at 4º C (Hettich-
124 Zentrifugen, Universal 32 R, Germany), storing the plasma at -80° C until analysis. In
125 the colostrum and feeding period, blood samples were taken at 2 hours after birth
126 labeling it as sample 0, and then blood samples were collected at day 1, 2, 3, 4, 5 and 20
127 after birth. Another sample was taken when each animal reached 10 kg BW. During the
128 weaning period samples were obtained every 5 days until the end of the experimental
129 period.
130
131 To determine blood plasma IgG and IgM concentration, commercial ELISA kits (Bethyl
132 Laboratories, Montgomery, TX, USA) were used, using a purified sheep IgG and IgM
133 as the standard curve. Results were expressed in mg of immunoglobulin/mL of plasma.
134
135 Complement System activity (TCA and ACA) was measured by the hemolytic rate
136 according to a novel technique described by (Moreno-Indias et al., 2012b) in goat kid
137 blood plasma. In this technique, a $DGHB^{++}$ buffer [Hepes Gelatin Veronal Buffer with
138 Ca^{++} and Mg^{++}: 5 m*M* HEPES, 71 m*M* NaCl, 0.15 m*M* $CaCl_2$, 0.5 m*M* $MgCl_2$, 2.5%
139 (w/v) glucose, 0.1% (w/v) gelatin, pH 7.4] was used to measure total Complement



System activity, and DGHB-Mg-EGTA buffer [4.2 m*M* Hepes, 59 m*M* NaCl, 7.0 m*M* $MgCl_2$, 2.08% (w/v) glucose, 0.08% (w/v) gelatin, 10 m*M* EGTA, pH 7.4] to measure the alternative pathway. For total Complement System activity, rabbit red blood cells and lamb plasma were both diluted to 5% in $DGHB^{++}$; 100 µL of each was then mixed in a microtiter plate and incubated at 37°C for 1 h. Cells were removed by centrifugation (2,500 × *g*, 5 min, 4ºC), and supernatant absorbance was measured at 405 nm using a microplate reader. Complete hemolysis was achieved by mixing the cells with distilled water (100 µL), and spontaneous lysis was produced by mixing the diluted rabbit red blood cells with $DGHB^{++}$. Complement-induced hemolysis of rabbit red blood cells by the test sera was calculated using the formula: [(A405 sample − A405 spontaneous lysis) / (A405 complete hemolysis − A405 spontaneous lysis)] × 100. The same protocol was performed with DGHB-Mg-EGTA buffer to measure the alternative pathway.

Statistical analyses

Statistical analyses were performed using SAS, Version 9.00 (SAS Institute Inc., Cary, NC). The SAS PROC MIXED procedure for repeated measurements was used to evaluate the effect of colostrum IgG intake (NR, C4 and C8) on plasma IgG and IgM concentration, Complement System activity and BW evolution of lambs from birth to weaning. A Tukey's test was used to evaluate differences between groups.

RESULTS AND DISCUSSION

Colostrum and Milk Feeding Period

Table 1 compiles data about BW, IgG and IgM concentration and Complement System

activity in NR, C4 and C8 groups during the milk feeding period. Focusing on BW evolution, NR group values increased during the whole period, however BW in C4 and C8 remained constant during the first 5 days after birth, increasing at day 20 and at the end of this period. When NR, C4 and C8 were compared, no differences were observed in any group at birth; however NR showed higher BW than C4 and C8 during the rest of the period until they reached 10 kg (end of milk intake period) and started the weaning period. Differences observed between groups could be related to the amount of colostrum and amount and quality of milk consumed. As has been described in lambs by several authors (Murphy et al., 1994; Napolitano et al., 2002), food restriction could reduce the average daily gain of animals from C4 and C8, resulting in a lower BW.

With reference to IgG concentration, all groups (NR, C4 and C8) had a very low IgG concentration in blood plasma at birth, probably from maternal origin through the placenta, as has been referenced by Castro et al. (2011). This concentration rapidly increased in NR, C4 and C8 groups after colostrum intake, obtaining the highest concentration in blood plasma at 1-2 days after birth (16.80, 6.45 and 17.73 mg of IgG/mL, respectively, $P<0.05$). After these days, IgG concentration continuously decreased until the end of the milk feeding period (5.22, 2.18 and 5.62 mg of IgG/mL, respectively, $P<0.05$). No differences were observed between groups at birth; however, during the rest of the period, IgG concentration was lower in lambs from C4 group than those from NR and C8 groups. Moreover, no differences were observed in IgG concentration between NR and C8 group during this period. In contrast to these findings, Firat et al. (2003) appreciated differences in the milk feeding period between lambs raised under natural and artificial conditions, describing that lambs raised under artificial rearing system had generally lower plasma IgG levels than lambs raised under

natural conditions. However, these authors used a commercial colostrum supplement to feed lambs under artificial rearing system, this fact being, as the authors suggested, the main reason that could affect the immune concentration in blood. Additionally, when only artificial rearing groups (C4 and C8) were compared, it was noted that plasma IgG concentration did not linearly increase according to colostrum IgG concentration, showing that the C8 group had approximately 3 times higher plasma values than C4 group. A similar non-linear absorption has been observed in lambs (Halliday and Williams, 1979), goat kids (Castro et al., 2005b; Rodríguez et al., 2009) and calves (Muller and Ellinger, 1981; Quigley et al., 2002b). In contrast to these findings, Stott and Fellah (1983) have observed how an increase of the total amount of IgG present in colostrum intake, linearly increased the plasma IgG concentration in calves. However, both views agree that an increase in the amount of IgG intake through colostrum produces an increase of plasma IgG in newborn ruminants. This fact could explain the higher IgG concentration showed by lambs from C8 compared with lambs from C4.

A similar evolution was observed in the case of IgM concentration in blood, although this immunoglobulin was not detectable at birth in any of the studied groups. A rapid increase of IgM in blood plasma was produced in NR, C4 and C8 at 1-2 days after birth (1.63, 0.35 and 2.84 mg of IgM/mL, respectively, $P<0.05$) because of the colostrum intake. Although, C8 group was fed with a double amount of colostrum than C4 group, IgM concentration was proportionally much higher in C8 group than C4 group during these days. After that, IgM concentration in blood plasma decreased until the end of the milk feeding period in NR and C8 groups. However, C4 group increased IgM concentration at 20 days and at the end of the milk feeding period. According to findings, Nonnecke et al. (2012) working with colostrum-fed and colostrum-deprived

calves, observed that animals fed with colostrum showed a rapid increase of plasma IgG and IgM concentrations after colostrum intake, decreasing these concentrations with age. The colostrum-deprived calves, in contrast, had very low or undetectable plasma IgG and IgM concentration, followed by an age-related increase of IgG and IgM concentration, suggesting endogenous production of these immunoglobulins. In the present study, although this evolution was only observed in IgM concentration, it has been described that IgM is the first immunoglobulin produced by the organism (Ehrenstein and Notley, 2010). When differences between groups were analyzed, it was noticed that C8 group reached a higher IgM concentration at 1-3 days (2.84, 2.58 and 1.83 mg of IgM/mL, respectively, $P<0.05$), than NR group (1.63, 1.24 and 1.19 mg of IgM/mL, respectively, $P<0.05$) and finally the lowest IgM concentrations were obtained by C4 group (0.35, 0.47 and 0.45 mg of IgM/mL, respectively, $P<0.05$). No references have been found about the IgM absorption rate in lambs; however it could be suggested that, as in the case of IgG, IgM does not follow a linear absorption in relation with the amount of IgM given to lambs. Additionally, no differences between groups were observed at 20 days and at the end of the milk feeding period. As was described above, the endogenous production of IgM by the C4 group related with the deficient colostrum intake, could produce the increase of IgM concentration in this group, and in consequence, obtain similar results to NR and C8 groups at that time.

Even the evolution of Complement System activity showed that all groups did not show any detectable activity at birth, it did, however increase in NR, C4 and C8 during the whole studied period, although a different rate was observed. In the case of NR group, lambs showed an earlier Complement System activation, being higher than C4 and C8 at days 1-3.

240 Despite that colostrum freezing for storage does not affect immunoglobulin
241 concentration in newborn ruminants (Holloway et al., 2002; Argüello et al., 2003), other
242 colostrum components could be affected by the freezing process (Ramírez-Santana et
243 al., 2012) . As was described before, C4 and C8 groups were fed with colostrum from a
244 frozen pool that may produce a reduction in some Complement components present in
245 colostrum. Moreover, dam milk contains some Complement System components that
246 are not present in milk. In a similar way, Castro et al. (2008) showed the importance of
247 dam milk in the earlier Complement System activation compared to milk replacer
248 feeding. Nevertheless, no differences were observed between C8 and NR groups at day
249 4, showing that C8 group had an earlier Complement System activation than C4,
250 perhaps because of the higher amount of colostrum received in C8 group. This fact may
251 produce an earlier activation because it has been described that colostrum contains high
252 amounts of Complement System components (Wheeler et al., 2007). However, even
253 lambs from C4 group showed lower TCA and ACA during most of this period, no
254 differences between groups were observed at the end of the milk feeding period.
255 Additionally, ACA seems to be the most important pathway in the three groups, as was
256 described by Castro et al. (2008) in newborn goat kids.
257
258 ***Weaning period***
259 Table 2 shows the BW, IgG and IgM concentration and Complement System activity in
260 NR, C4 and C8 groups during the weaning period. With reference to BW, NR, C4 and
261 C8 groups increased their BW during this period. No differences were observed
262 between groups, however lambs from C4 and C8 showed a higher BW than lambs from
263 NR group at day 25 (15.72, 15.63 and 14.62 kg, respectively, $P<0.05$) and day 30
264 (16.89, 16.95 and 15.28 kg, respectively, $P<0.05$). It has been described by Napolitano

et al. (2002) that lambs raised with dams suffer a higher stress by separation during the weaning period than animals from artificial rearing system, resulting in a decrease of the growth rate.

Regarding IgG concentration, NR and C8 showed slight decreases in this immunoglobulin concentration in plasma in some specific moments of weaning. However, both groups showed an increase of these values at the end of this period (30 days). In contrast, the values from C4 group never decreased, reaching the maximum value at 30 days. Regarding to differences between groups, NR and C8 group exhibited higher values than animals from the C4 group at the beginning of weaning period (5.22, 2.78, 5.62 mg/mL in NR, C4 and C8, respectively, $P<0.05$). These differences may probably be due to the lower colostrum amount given to lambs from C4 group, those consequences persisting until that time. However, no differences between groups were observed during the rest of the weaning. As was described above, the reduction of colostrum intake in C4 could produce an earlier IgG synthesis than in NR and C8 as was described by Nonnecke et al. (2012). This fact could explain the increase of IgG in lambs from N4 group during this period.

Conversely to the evolution observed in IgG concentration, IgM constantly increased in NR, C4 and C8 groups during the whole period. At the beginning of weaning (0 and 5 days), no differences were perceived between groups, probably because at that time animals were able to produce the earliest immunoglobulin synthesized by the young immune system (Ehrenstein and Notley, 2010). Additionally and in spite of no differences being observed between groups at 15 days, C4 and C8 showed higher values than NR group during the rest of the period. It has been demonstrated that stress

produces a decrease of IgM concentration in blood (Matos-Gomes et al., 2010). The combination of food restriction and dam separation stress that was present in the NR group could probably produce these differences between groups during the last part of the weaning period.

Relating to the evolution of Complement System activity (TCA and ACA) observed in NR, C4 and C8 groups during the weaning period, all groups increased both Complement System pathways during this period, although different increase rates were observed in the studied groups. In relation to differences between groups, C8 used to show higher Complement System activity (TCA and ACA) than NR and C4 during the whole period. However, at the end of the weaning period, all the studied groups showed a similar TCA (65.06, 60.05 and 78.10 % of hemolysis in NR, C4 and C8, respectively, $P<0.05$) and ACA (55.40, 50.77 and 64.65 % of hemolysis in NR, C4 and C8, respectively, $P<0.05$). Unfortunately, the Complement System activity during weaning has not been described only in lambs, but also in ruminants (cow, sheep and goat). Nevertheless, Oswald et al. (1990) described the Complement System activity (TCA and ACA) in lambs, showing a similar evolution to present results, although different values were described, probably due to the differences between analytical techniques used.

CONCLUSION

In this study, lambs that received 8 g of IgG/ kg of BW (C8 group) were able to reach similar IgG and higher IgM concentration in blood than lambs reared under natural conditions (NR group) during the first days after birth. However, NR group obtained higher Complement System activity than any of the other groups (C4 and C8) in the

same period. During weaning, both artificial rearing groups (C4 and C8) showed a higher BW and IgM and a similar IgG concentration and Complement system activity than lambs reared under natural conditions.

This study reveals important information about the amount of colostrum that needs to be given to newborn lambs reared under artificial conditions in order to reach similar immune values to those reared under natural conditions.

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REFERENCES

Ahmad, R., A. Khan, M. T. Javed, and I. Hussain. 2000. The level of immunoglobulins in relation to neonatal Lamb mortality in Pak-Karakul sheep. Vet Arhiv 70(3):129-139.

Argüello, A., N. Castro, J. Capote, R. Gines, F. Acosta, and J. L. Lopez. 2003. Effects of refrigeration, freezing-thawing and pasteurization on IgG goat colostrum preservation. Small Ruminant Research 48(2):135-139.

Argüello, A., N. Castro, J. Capote, J. W. Tyler, and N. M. Holloway. 2004. Effect of colostrum administration practices on serum IgG in goat kids. Livest Prod Sci 90(2-3):235-239.

Bendixen, E., M. Danielsen, K. Hollung, E. Gianazza, and I. Miller. 2011. Farm animal proteomics - A review. Journal of proteomics 74(3):282-293.

Castro, N., F. Acosta, T. Nino, J. Vivas, E. Quesada, J. Capote, and A. Arguello. 2008. The effects of diet and age on serum complement system activity in goat kids. Livest Sci 119(1-3):102-106.

Castro, N., J. Capote, S. Alvarez, and A. Arguello. 2005. Effects of lyophilized colostrum and different colostrum feeding regimens on passive transfer of immunoglobulin g in Majorera goat kids. J Dairy Sci 88(10):3650-3654.

Castro, N., J. Capote, R. M. Bruckmaier, and A. Arguello. 2011. Management effects on colostrogenesis in small ruminants: a review. Journal of Applied Animal Research 39(2):85-93.

Castro, N., J. Capote, A. Morales-Delanuez, C. Rodriguez, and A. Arguello. 2009. Effects of newborn characteristics and length of colostrum feeding period on passive immune transfer in goat kids. J Dairy Sci 92(4):1616-1619.

Christley, R. M., K. L. Morgan, T. D. H. Parkin, and N. P. French. 2003. Factors related to the risk of neonatal mortality, birth-weight and serum immunoglobulin concentration in lambs in the UK. Prev Vet Med 57(4):209-226.

da Nobrega, J. E., F. Riet-Correa, R. S. Nobrega, J. M. de Medeiros, J. S. de Vasconcelos, S. V. D. Simoes, and I. M. Tabosa. 2005. Perinatal mortality of lambs in the semi-arid region of Paraiba, Brazil. Pesquisa Vet Brasil 25(3):171-178.

Demiroren, E., J. N. B. Shrestha, and W. J. Boylan. 1995. Breed and Environmental-Effects on Components of Ewe Productivity in Terms of Multiple Births, Artificial Rearing and 8-Month Breeding Cycles. Small Ruminant Research 16(3):239-249.

Ehrenstein, M. R. and C. A. Notley. 2010. The importance of natural IgM: scavenger, protector and regulator. Nat Rev Immunol 10(11):778-786.

Emsen, E., M. Yaprak, O. C. Bilgin, B. Emsen, and H. W. Ockerman. 2004. Growth performance of Awassi lambs fed calf milk replacer. Small Ruminant Research 53(1-2):99-102.

Firat, A., A. Ozpinar, and M. H. Erhard. 2003. Serum Immunoglobulin G Levels in Lambs Fed Colostrum and Dam Milk or Cow Milk After Birth. Pages 24-25 in Proc. The 11th International Simposyum of the World Association of Veterinary Laboratory Diagnosticians and OIE Seminars on Biotechnology, Bangkok, Thailand.

Halliday, R. and M. R. Williams. 1979. Absorption of inmunoglobulin from colostrum by bottle-fed lambs. Ann Rech Vet 10(4):549-556.

Holloway, N. M., J. W. Tyler, J. Lakritz, S. L. Carlson, R. K. Tessman, and J. Holle. 2002. Serum immunoglobulin G concentrations in calves fed fresh colostrum or a colostrum supplement. Journal of veterinary internal medicine / American College of Veterinary Internal Medicine 16(2):187-191.

Matos-Gomes, N., M. Katsurayama, F. H. Makimoto, L. L. O. Santana, E. Paredes-Garcia, M. A. D. Becker, and M. C. Dos-Santos. 2010. Psychological Stress and Its Influence on Salivary Flow Rate, Total Protein Concentration and IgA, IgG and IgM Titers. Neuroimmunomodulation 17(6):396-404.

Morales-delaNuez, A., N. Castro, I. Moreno-Indias, M. C. Juste, D. Sánchez-Macías, H. Briggs, J. Capote, and A. Argüello. 2009. Effects of a reputed immunostimulant on the innate immune system of goat kids. Small Ruminant Research 85(1):23-26.

Moreno-Indias, I., A. Morales-delaNuez, L. E. Hernandez-Castellano, D. Sanchez-Macias, J. Capote, N. Castro, and A. Arguello. 2012a. Docosahexaenoic acid in the goat kid diet: Effects on immune system and meat quality. Journal of animal science 90(11):3729-3738.

Moreno-Indias, I., A. Morales-delaNuez, L. E. Hernandez-Castellano, D. Sanchez-Macias, J. Capote, N. Castro, and A. Arguello. 2012b. Docosahexaenoic acid in the goat kid diet: effects on immune system and meat quality. Journal of animal science 90(11):3729-3738.

Muller, L. D. and D. K. Ellinger. 1981. Colostral immunoglobulin concentrations among breeds of dairy-cattle. Journal of Dairy Science 64(8):1727-1730.

Murphy, T. A., S. C. Loerch, K. E. McClure, and M. B. Solomon. 1994. Effects of restricted feeding on growth performance and carcass composition of lambs. Journal of animal science 72(12):3131-3137.

Napolitano, F., G. F. Cifuni, C. Pacelli, A. M. Riviezzi, and A. Girolami. 2002. Effect of artificial rearing on lamb welfare and meat quality. Meat Science 60(3):307-315.

Napolitano, F., C. Pacelli, A. Girolami, and A. Braghieri. 2008. Effect of information about animal welfare on consumer willingness to pay for yogurt. Journal of Dairy Science 91(3):910-917.

Nonnecke, B. J., W. R. Waters, J. P. Goff, and M. R. Foote. 2012. Adaptive immunity in the colostrum-deprived calf: Response to early vaccination with Mycobacterium bovis strain bacille Calmette Guerin and ovalbumin. Journal of Dairy Science 95(1):221-239.

Nowak, R. and P. Poindron. 2006. From birth to colostrum: early steps leading to lamb survival. Reprod Nutr Dev 46(4):431-446.
O'Doherty, J. V. and T. F. Crosby. 1997. The effect of diet in late pregnancy on colostrum production and immunoglobulin absorption in sheep. Anim Sci 64(01):87-96.
Ontsouka, C. E., R. M. Bruckmaier, and J. W. Blum. 2003. Fractionized milk composition during removal of colostrum and mature milk. J Dairy Sci 86(6):2005-2011.
Oswald, I. P., F. Lantier, and G. Bourgy. 1990. Classical and Alternative Pathway Hemolytic Activities of Ovine Complement - Variations with Age and Sex. Vet Immunol Immunop 24(3):259-266.
Quigley, J. D., 3rd, C. J. Kost, and T. M. Wolfe. 2002. Absorption of protein and IgG in calves fed a colostrum supplement or replacer. J Dairy Sci 85(5):1243-1248.
Quigley, J. D., P. French, and R. E. James. 2000. Short communication: Effect of pH on absorption of immunoglobulin G in neonatal calves. Journal of Dairy Science 83(8):1853-1855.
Ramírez-Santana, C., F. J. Pérez-Cano, C. Audí, M. Castell, M. G. Moretones, M. C. López-Sabater, C. Castellote, and A. Franch. 2012. Effects of cooling and freezing storage on the stability of bioactive factors in human colostrum. Journal of Dairy Science 95(5):2319-2325.
Rodríguez, C., N. Castro, J. Capote, A. Morales-delaNuez, I. Moreno-Indias, D. Sanchez-Macias, and A. Arguello. 2009. Effect of colostrum immunoglobulin concentration on immunity in Majorera goat kids. Journal of Dairy Science 92(4):1696-1701.
Stott, G. H. and A. Fellah. 1983. Colostral immunoglobulin absorption linearly related to concentration for calves. Journal of Dairy Science 66(6):1319-1328.
Trujillo, A. J., N. Castro, J. M. Quevedo, A. Arguello, J. Capote, and B. Guamis. 2007. Effect of heat and high-pressure treatments on microbiological quality and immunoglobulin G stability of caprine colostrum. Journal of Dairy Science 90(2):833-839.
Wheeler, T. T., A. J. Hodgkinson, C. G. Prosser, and S. R. Davis. 2007. Immune components of colostrum and milk--a historical perspective. Journal of mammary gland biology and neoplasia 12(4):237-247.

Table 1. BW, TCA, ACA and IgG and IgM concentration evolution in NR, C4 and C8 groups during colostrum and milk feeding period.

		Days								SEM
	Group	0	1	2	3	4	5	20	End	
BW (kg)	NR	4.15^{a}	4.42^{a,z}	4.69^{a,z}	4.95^{ab,z}	5.20^{b,z}	5.43^{b,z}	8.96^{c,z}	10.35^{d}	0.216
	C4	4.25^{a}	4.05^{a,y}	3.95^{a,y}	3.96^{a,y}	4.00^{a,y}	4.06^{a,y}	6.36^{b,y}	10.10^{c}	0.274
	C8	3.94^{a}	3.77^{a,y}	3.58^{a,y}	3.65^{a,y}	3.72^{a,y}	3.79^{a,y}	6.07^{b,y}	9.61^{c}	0.332
IgG (mg/mL)	NR	0.20^{a}	16.79^{b,z}	13.84^{bc,z}	12.75^{c,z}	12.07^{c,z}	11.43^{c,z}	6.47^{d,zy}	5.22^{e,z}	0.628
	C4	0.17^{a}	5.81^{b,y}	6.45^{b,y}	5.83^{b,y}	5.09^{b,y}	6.30^{b,y}	4.44^{c,y}	2.18^{d,y}	0.452
	C8	0.23^{a}	17.74^{b,z}	17.44^{b,z}	15.86^{bc,z}	15.91^{bc,x}	14.66^{c,z}	9.67^{d,z}	5.62^{e,z}	1.004
IgM (mg/mL)	NR	-	1.63^{a,z}	1.24^{ab,z}	1.19^{b,z}	1.01^{b,z}	0.90^{c,z}	0.70^{c}	0.78^{c}	0.069
	C4	-	0.35^{a,y}	0.47^{a,y}	0.45^{a,y}	0.41^{a,y}	0.41^{a,y}	0.54^{b}	0.78^{c}	0.039
	C8	-	2.84^{a,x}	2.58^{a,x}	1.83^{b,x}	1.51^{b,z}	1.29^{c,z}	0.78^{d}	0.71^{d}	0.164
TCA (%)	NR	-	9.05^{a,z}	20.56^{b,z}	31.36^{c,z}	36.58^{cd,z}	42.43^{d,z}	50.75^{e,z}	53.17^{e}	1.810
	C4	-	1.04^{a,y}	2.84^{a,y}	8.33^{b,y}	16.15^{c,y}	17.43^{c,y}	31.13^{d,y}	55.26^{e}	1.809
	C8	-	1.43^{a,y}	2.85^{ab,y}	8.16^{b,y}	28.08^{c,zy}	29.67^{c,y}	30.61^{c,y}	60.13^{e}	4.502
ACA (%)	NR	-	7.67^{a,z}	15.05^{b,z}	24.48^{c,z}	27.29^{c,z}	36.26^{d,z}	40.56^{d,z}	46.80^{e}	4.849
	C4	-	0.53^{a,y}	4.86^{b,y}	7.09^{c,y}	12.59^{d,y}	13.02^{d,y}	27.27^{e,y}	40.38^{f}	3.869
	C8	-	0.53^{a,y}	2.06^{b,y}	7.11^{c,y}	26.45^{d,z}	24.81^{d,x}	26.40^{d,y}	41.40^{e}	2.519

$^{a–f}$ Means within a row with different superscript letters differ significantly ($P < 0.05$).
$^{x–z}$ Means within a column for a specific item with different superscript letters differ significantly ($P < 0.05$).
Data with - means non-detectable.
TCA: Total Complement System activity; ACA: Alternative Complement System activity.

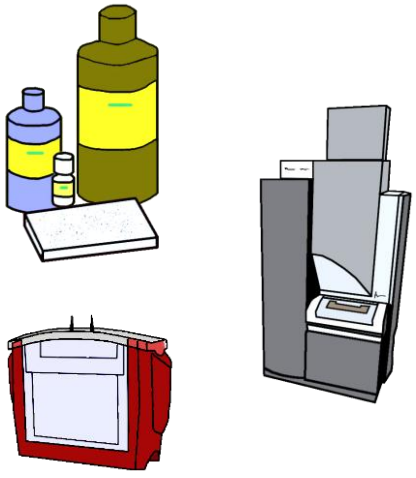
Table 2. BW, TCA, ACA and IgG and IgM concentration evolution in NR, C4 and C8 groups during weaning period.

	Group	Days							SEM
		0	5	10	15	20	25	30	
BW (kg)	NR	10.35^{a}	11.41^{b}	12.02^{c}	12.95^{d}	13.89^{e}	$14.62^{f,z}$	$15.28^{g,z}$	0.236
	C4	10.10^{a}	10.83^{b}	11.56^{c}	13.07^{d}	14.46^{e}	$15.72^{f,y}$	$16.89^{g,y}$	0.373
	C8	10.05^{a}	10.74^{b}	11.74^{c}	13.35^{d}	14.26^{e}	15.63^{y}	$16.95^{g,y}$	0.403
IgG (mg/mL)	NR	$5.22^{a,z}$	4.39^{ab}	4.55^{a}	4.11^{b}	4.69^{a}	5.06^{a}	7.11^{b}	0.266
	C4	$2.78^{a,y}$	3.59^{a}	4.02^{ab}	4.44^{ab}	5.46^{ab}	4.03^{ab}	5.72^{b}	0.230
	C8	$5.62^{a,z}$	4.62^{ab}	4.45^{ab}	4.26^{b}	4.2^{6b}	4.19^{b}	5.62^{a}	0.254
IgM (mg/mL)	NR	0.78^{a}	0.84^{ab}	$0.88^{ab,z}$	1.02^{b}	0.90^{ab}	$0.96^{b,z}$	$1.05^{b,z}$	0.046
	C4	0.82^{a}	1.10^{a}	$1.52^{b,y}$	1.36^{ab}	1.38^{ab}	$1.49^{b,y}$	$1.90^{c,y}$	0.080
	C8	0.71^{a}	0.77^{a}	0.86^{ab}	1.22^{b}	1.39^{b}	1.39^{b}	$1.77^{c,y}$	0.051
TCA (%)	NR	$53.17^{a,zy}$	$51.32^{a,z}$	$49.86^{a,z}$	$61.01^{ab,zy}$	$55.39^{a,z}$	$65.20^{b,zy}$	65.06^{b}	1.846
	C4	$49.31^{a,y}$	$54.58^{ab,z}$	$51.79^{ab,y}$	$50.35^{ab,y}$	$51.93^{ab,z}$	$50.91^{ab,y}$	60.05^{b}	2.573
	C8	$62.46^{a,z}$	$64.92^{a,y}$	$72.74^{a,y}$	$73.95^{ab,z}$	$75.82^{ab,y}$	$78.08^{b,z}$	78.10^{b}	4.406
ACA (%)	NR	$46.80^{a,zy}$	$46.99^{ab,zy}$	$44.42^{a,z}$	$53.44^{ab,zy}$	$43.21^{a,z}$	$55.52^{b,zy}$	55.40^{b}	1.900
	C4	$33.70^{a,y}$	$37.69^{a,y}$	$47.41^{ab,z}$	$42.35^{ab,y}$	$39.465^{a,z}$	$41.15^{ab,y}$	50.77^{b}	2.167
	C8	$52.33^{a,z}$	$54.47^{a,z}$	$67.76^{b,y}$	$68.39^{b,z}$	$67.50^{b,y}$	$63.38^{b,z}$	64.65^{b}	2.530

$^{a–g}$ Means within a row with different superscript letters differ significantly ($P < 0.05$).
$^{x–z}$ Means within a column for a specific item with different superscript letters differ significantly ($P < 0.05$).
Data with - means non-detectable.
TCA: Total Complement System activity; ACA: Alternative Complement System activity.

Capítulo 5

Chapter 5



The effect of colostrum intake on blood plasma proteome profile in newborn lambs: low abundant proteins

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Abstract

Colostrum intake by newborn lambs plays a fundamental role in the perinatal period, ensuring lamb survival. In this study, blood plasma samples from two groups of newborn lambs (Colostrum group and Delayed Colostrum group) at 2 and 14 hours after birth were depleted of high abundant proteins and analyzed using Two-Dimensional Differential in Gel Electrophoresis and MALDI MS/MS for protein identification in order to investigate the expression level of proteins with immune function in newborn lambs. The results showed that the expression of four proteins was increased in the blood plasma of lambs due to colostrum intake. These proteins have not been previously described as increased in blood plasma of newborn ruminants by colostrum intake. Moreover, these proteins have been described to have an immune function in other species; some of them were previously identified in colostrum and milk. In conclusion, the colostrum intake modified the blood plasma low abundant proteome profile of newborn lambs, increasing the expression of apolipoprotein A-IV, plasminogen, serum amyloid A and fibrinogen, demonstrating that colostrum is essential, not only for the immunoglobulins, but also for the supply of several low abundant proteins with novel roles herein described in sheep.

Keywords: lamb, plasma proteome, immune, colostrum, DIGE

INTRODUCTION

Colostrum is the first source of nutrition in neonatal ruminants, supplying not only nutrients, but having also a fundamental biological function, promoting immunoglobulin transfer from the dam to the newborn. As a consequence, colostrum intake and colostrum protein absorption play an essential role in passive immune transfer and ultimately in the newborn survival rate (Stelwagen et al., 2009; Danielsen et al., 2011). However, the conditions to absorb intact colostrum proteins decrease along the first 48 hours after birth, being crucial to feed newborn ruminants with colostrum during this period [1]. On the other hand, it has been described how feeding newborn ruminants with colostrum with an inadequate Ig´s concentration results in a negative impact on the economic benefits of farmers and breeders with severe consequences to animal welfare.

In what concerns colostrum, proteomics has been used to characterize protein changes in the transition from colostrum to milk in cattle [2,3]. Additionally, the study of low abundant proteins from different body fluids such as blood plasma, colostrum or milk is becoming more relevant.

Presently, a wide variety of colostrum and milk bioactive peptides and proteins have not only been linked to the passive immune transfer, but also promoting gastrointestinal growth and development of the newborn [4].

In spite of the previous proteomic studies in colostrum and milk, it is still not fully known which proteins are absorbed or increased in lamb blood plasma due to the colostrum intake. It is hypothesized that early colostrum fed in newborn lambs modify

the blood plasma proteome. For this reason, the aim of this study is to analyze blood plasma low abundance proteins changes in newborn lambs due to the colostrum intake during the first 14 hours after birth, in order to identify such protein changes. Results showed in this study can contribute to understand the importance of colostrum on the passive immune transfer and the lamb immune system development.

MATERIAL AND METHODS

The experiment was approved by the ethics committee of the Faculty of Veterinary of the *Universidad de Las Palmas de Gran Canaria.*

Samples Collection

The Canarian dairy sheep breed was used for this experiment. This breed is a high milk yield breed (1.8 L/d) with a lactation period of 180-200 d [5]. Ewes were fed with corn, soy 44 (crude protein 44 per cent), dehydrated lucerne, dehydrated beetroot, lucerne hay and a vitamin-mineral supplement in accordance with the guidelines issued by L´Institut Nationale de la Recherche Agronomique [6]. Two groups of 6 newborn male lambs each from single births (Canarian dairy breed) were used in this experiment. The experiment took place at the experimental farm of the Veterinary Faculty of the Universidad de Las Palmas de Gran Canaria (Canary Islands, 28º 8' 20.66" N, 15º 30' 24.97" W, Spain) in spring. Animals were fed with sheep colostrum at different time points. One group (named Colostrum group; C group) received three colostrum meals, at 2, 14 and 26 hours after birth. The other group (named Delayed Colostrum group; DC group) was not fed with colostrum at 2 hours after birth but received two colostrum

meals at 14 and 26 hours after birth in order to ensure the survival of these animals. At the end of the colostrum period (26 hours after birth) each animal (from both groups) was fed with a total volume of colostrum equivalent to 4g of IgG/kg of BW as it has been described in previous colostrum immune studies [7,8] . The colostrum source was from a frozen pool with 64.74 mg of Immunoglobulin G (IgG)/mL. Blood samples were collected before feeding at 2 and 14 hours after birth using K-EDTA tubes, and the obtained plasma was frozen at -80ºC until further analysis. During this experiment, lambs were accommodated in artificial rearing rooms, providing at least 0.3 m^2 floor space per lamb. Each room had central heating conferring a room temperature of approximately 20ºC.

Blood Plasma IgG and IgM was measured as to determine the presence of colostrum proteins in lamb plasma from both groups (C group and DC group) at the two studied times (2 and 14 hours after birth). To determine plasma IgG and IgM concentration a commercial ELISA kit (Bethyl Laboratories, Montgomery, TX, USA) was used, setting a purified sheep IgG and IgM as a standard reference.

Statistical analyses of IgG and IgM were performed using SAS, Version 9.00 (SAS Institute Inc., Cary, NC). The SAS PROC MIXED procedure for repeated measurements was used to evaluate the effect of colostrum intake (C group *vs.* DC group) at 2 and 14 hours after birth. A Bonferroni's test was used to evaluate differences between groups.

Samples Treatment For Analysis

The proteomic assays were carried out at the *Instituto de Tecnología Química e Biológica* (Oeiras, Portugal). In order to reduce the high-abundance proteins present in blood plasma (albumin and immunoblogulins), plasma samples (200µL) were depleted with a Protein Enrichment Kit (ProteoMiner® Bio-Rad, Hercules, CA, USA) following the manufacturer's instructions. Samples were subsequently desalted with 2D-Clean-up® kit (GE Healthcare, Piscataway, NJ, USA) and quantified with 2D-Quant® kit (GE Healthcare, Piscataway, NJ, USA) following manufacturer's instructions.

Two-Dimensional Differential in Gel Electrophoresis (DIGE)

For each DIGE gel, 50 µg of each treated sample was labeled with Cy3 or Cy5 cyanine dyes (GE Healthcare, Piscataway, NJ, USA), whilst the internal standard pool, created from an equal amount of protein from each studied sample, was labeled with Cy2 dye. After labeling, samples were mixed with 7M urea, 2M thiourea, 4% (w/v) CHAPS, 2% (w/v) DTT , 2% (v/v) ampholytes and 0.04% bromophenol blue solution (1%) up to a final volume of 150 µL. Immobiline DryStrips with pH 3-10 and 24 cm length (GE Healthcare, Piscataway, NJ, USA) were passively rehydrated with 450 µL of rehydration buffer (7M urea, 2M thiourea, 4% (w/v) CHAPS and 0.04% bromophenol blue solution (1%)) for 6 hours at room temperature. Isoelectric focusing was performed with an Ettan IPGphor 3 Isoelectric Focusing System coupled to a Manifold strip holding system (GE Healthcare, Piscataway, NJ, USA) following the program: 150V for 3 h, 300V for 3 h, a gradient of 1000V for 6 h, a gradient of 10000V for 1 h and 10000V for 3 h. Subsequently, strips were equilibrated with 50mM Tris-HCl pH 8.8, 6M urea, 30% (v/v) glycerol, 2% (w/v) SDS and 0.02% bromophenol blue solution (1%), in two steps of 15 min with 1% (w/v) DTT and 2.5% (w/v) iodoacetamide, respectively.

After equilibration, second dimension was conducted using 12.5% polyacrylamide gels on an Ettan Dalt Six electrophoresis system (GE Healthcare, Piscataway, NJ, USA) using the running conditions recommended by the manufacturer (1 W/gel for 1 h and 2 W/gel for 14-16 h at 12ºC) and using low-florescence glass plates.

Each DIGE gel was scanned with a Fluorescent Image Analyzer (Fujifilm FLA-5100, Fujifim, Tokyo, Japan), using preferred excitation/emission wavelengths for Cy2, Cy3, and Cy5 of 488/520, 532/580, and 633/670 nm, respectively, generating images that were used in gel analysis

Image Analysis

In order to detect differentially expressed proteins, gels were analyzed using Progenesis SameSpots software (Nonlinear Dynamics, Newcastle upon Tyne, UK) following manufacturer's instructions for DIGE gels. Spots with $p<0.05$ and an intensity of at least 1.3 fold higher were considered to have significantly different expression levels.

Visible Gel Staining, Spot Excision and Digestion

In order to excise the selected spots, DIGE gels were stained with Coomassie Brillant Blue G-250 as previously described by Almeida et al. [9]. Spots were then manually excised for individual in-gel digestion using trypsin as described by Almeida et al. [9]. Briefly, spots were washed with 30µL of water for 30 minutes, washed in acetonitrile (50%), reduced with 10mM DTT at 56ºC for 45 minutes, alkylated with 55mM iodoacetamide for 30 minutes, washed in acetonitrile (100%) and vacuum dried

(SpeedVac®, Thermo Fisher Scientific, Waltham, MA, USA). Gel pieces were rehydrated with a digestion buffer (50 mM NH_4HCO_3 buffer) containing trypsin (Promega, Madison, WI, USA) and incubated overnight at 37°C. The digestion buffer containing peptides was acidified with formic acid, desalted and concentrated using C8 microcolumns (POROS R2®, Applied Biosystems, Foster City, CA, USA), as described by Almeida et al.(2010).

Protein Identification

Protein identification was conducted as described by Marcelino et al. [10]. Briefly protein identification was conducted using a MALDI-TOF–TOF data acquired with an Applied Biosystem 4800 Proteomics Analyzer (Applied Biosystems, Foster City, CA, USA) in both MS and MS/MS mode. Positively charged ions were analyzed in the reflectron mode over the m/z range of 800–3500 Da. Each MS spectrum was obtained in a result independent acquisition mode with a total of 800 laser shots per spectra and a fixed laser intensity of 3500 V, being externally calibrated using des-Arg-Bradykinin (904.468 Da), angiotensin 1 (1296.685 Da), Glu-Fibrinopeptide B (1570.677 Da), ACTH (1–17) (2093.087 Da), and ACTH (18–39) (2465.199) (Calibration Mix from Applied Biosystems). Fifteen best precursors from each MS spectrum were selected for MS/MS analysis. MS/MS analyses were performed using CID (Collision Induced Dissociation) assisted with air, using a collision energy of 1 kV and a gas pressure of 1 × 106 Torr. Two thousand laser shots were collected for each MS/MS spectrum using a fixed laser intensity of 4500 V. The S/N ratio was set at 20 as recommended by manufacturer. Raw data were generated by the 4000 Series Explorer Software v3.0 RC1 (Applied Biosystems, Foster City, CA, USA) and all contaminant m/z peaks originating from human keratin, trypsin autodigestion, or matrix were

included in the exclusion list used to generate the peptide mass list used in the database search.

The generated mass spectra were used to search the NCBI predicted protein database, setting a taxonomical restriction (mammals database). Searches were conducted using Mowse from MASCOT-demon 2.1.0 Software (Matrix-Science) algorithm. Protein identifications were accepted if protein score was above a threshold of 95% ($p < 0.05$). The interpretation of the combined MS + MS/MS data was carried out using the GPS Explorer Software (Version 3.5, Applied Biosystems, Foster City, CA, USA), using the following parameters: missed-cleavage, one; peptide tolerance, 50 ppm; fragment mass tolerance, 0.25 Da; fixed modification, carbamidomethylation of cysteine; and variable modification, methionine oxidation. From the predicted protein database, the theoretical molecular mass and pI of the identified proteins was obtained using the Expasy Mw/pI Tool (http://www.expasy.org/tools/pi_tool.html). The identified proteins were only considered if a MASCOT protein scores above 61 ($p < 0.05$) was obtained.

RESULTS AND DISCUSSION

Because the newborn lamb immune system is not fully developed at birth, colostrum plays a highly relevant role in animal survival. Colostrum intake is necessary for the onset of a correct passive immune transfer; mainly as it transfers a high amount of Ig´s. However, there is no information about the relation of the role played on the passive immune transfer of non-immunoglobulin proteins, i.e. the minor or less abundant proteins and colostrum intake. In this experiment we have used an approach based on the analysis of the proteome of depleted plasma from newborn lambs using the

Proteominer® technology that would allow the removal of the higher-abundance proteins in the plasma, particularly albumin, IgG and IgM, followed by a 2-DE DIGE analysis and protein identification using mass spectrometry. With reference to this topic, many methodologies can be found in the market, however several authors have observed better results using ProteoMiner® (Bio-Rad, Hercules, CA, USA) not only in the removal of high abundance proteins but also in the high concentration and intensity of low abundance proteins [11,12].

We have nevertheless evaluated the levels of IgG and IgM in non-depleted plasma samples in order to determine the presence or absence of colostrum proteins in both groups (C group and DC group) at the two studied times (2 and 14 hours after birth). Results are shown in table 1 where the concentration of the two Ig´s, expressed in mg/mL of blood plasma in the two studied groups (C and DC groups) and at 2 and 14 hours after birth is presented. Considering the IgG concentration, at birth (2 hours) animals from both groups had no detectable (ND) IgG concentration in blood. However, when both groups were compared at 14 hours, C group had a higher IgG concentration than DC group (7.406 mg/mL *vs.* ND in C and DC group, respectively). A similar evolution was observed when IgM concentrations were analyzed, showing no differences in blood plasma concentration of this immunoglobulin at 2 hours after birth and increasing in the C group at 14 hours after birth. Several authors have observed a similar evolution in Ig´s level, depending on the total amount of Ig´s present in colostrum, in newborn blood from lambs [13] calves [14,15] and goat kids [7,16]. As expected, these results confirm that the presence of colostrum IgG and IgM in the C group in blood at 14 hours after birth is due to colostrum intake.

As can be observed in figure 1, a total of 11 spots showing over-expression in lambs at 14 hours after birth were detected only for the C group. Of the eleven spots, we were able to identify a total of 7 spots, as presented in table 2. Spots showed in table 2 had a similar value between C and DC group at 2 hours after birth and did not show any increase between DC group at 2 and 14 hours after birth. The 7 spots were identified as apolipoprotein A-IV (spots 563,565 and 572), plasminogen (spot 201), serum amyloid A (spot 726) and fibrinogen gamma chain (spots 475 and 490). These proteins may play an important role either in the immune-system development or in the immune protection or even both at the early stages of life.

Apolipoprotein A-IV (Apo A-IV)

The metabolic function of apolipoprotein A-IV (Apo A-IV) has not been fully established yet, however it has been suggested that Apo A-IV plays an important role at this early stage of life, modulating the enterocyte lipid transport efficiency in fatty foods such as colostrum [17]. For this reason, the intestinal synthesis and secretion of Apo A-IV increases during fat absorption in accordance to Simon et al. [18]. Additionally, Apo A-IV has antioxidant properties, acts as a postprandial satiety signal, and reduces gastric acid secretion [17]. An increase in the expression of this protein could be therefore related to the preservation of the immunoglobulin molecule structure, reducing the gastric acid secretion in the stomach of the newborn lamb and increasing the total amount of intact Ig´s absorbed at the intestine level.

Finally, Apo A-IV has been described to have an immunomodulatory effect against external agents in mice [19], consequently an increase in the blood plasma levels

of Apo A-IV could also contribute to protect the newborn animal from infections at this early stage of life.

Plasminogen (PLG)

This glycoprotein is the precursor of plasmin, a fribrinolytic enzyme that plays an important role in the dissolution of fibrin blood clots in order to prevent thrombosis [20,21]. Nevertheless, this protein has been identified not only in blood, but also in colostrum and milk [22]. In bovine, sheep and goat milk, plasmin and plasminogen forms are also essentially identical to those found in blood [23].

In spite of its main role in the dissolution of fibrin blood clots, it has been also found that plasminogen is structurally similar to Apo A-IV [24]. This structural homology endows Apo A-IV with the capacity to bind Fibrin and proteins of endothelial cells and monocytes, and thereby to inhibit plasminogen binding and plasmin generation [24], so the presence of this protein in colostrum and the increase of this protein in blood plasma could thwart the Apo A-IV to bind fibrin in newborn lambs.

Additionally, other authors have observed that plasminogen has immune activity, as it contributes to the neutrophil migrating to the site of infection [25]. In agreement with these findings, Theodorou et al. [26] found an increase of plasminogen concentration in blood and milk during acute mastitis in lactating dairy ewes. As it has been described before, an increase of this protein promotes the neutrophil migration in blood, contributing to the immune response against potential infections in the newborn

lamb; therefore the colostrum intake seems to be an important factor that increases plasminogen concentration on blood at this stage of life.

Finally, it has been also established that plasminogen participates in the regulation of cellular apoptosis [27], specifically the apoptosis of adherent cells induced by disruption of integrin-mediated cell-matrix interactions. This has been described under physiological conditions, such as the involution of the mammary gland after lactation and the renewal of intestinal epithelial cells. The apoptotic processes in the latter tissue is of important relevance in the absorption of Ig´s by newborn ruminants during the first hours after birth as described by Castro-Alonso et al. [28]. Therefore it could be assumed that the increase of this protein in blood could delay the decrease of the apoptosis rate of intestinal epithelial cells during the early stage of life, increasing the available time for colostrum components absorption, including both Ig´s and other proteins.

Serum Amyloid A (SAA)

Serum Amyloid A (SAA) is normally found in different isoforms and complexes with lipoproteins, varying its concentration according to species [29]. SAA represents one of the most conserved proteins among mammals supporting the premise that it has a basic and essential role in the innate immune system. Serum Amyloid A is an apolipoprotein that takes part of the acute phase of inflammation [30-32], although it has been also identified in colostrum from several species, such as human [33], horse, cattle and sheep [34,35]. The importance of this protein in inflammatory processes has been fully monitored, showing that the circulating concentration of SAA protein is

316 increased by 1000-fold within 24 to 48 h following infection/inflammation from a basal
317 level of 5–8 mg/mL [36].
318
319 The SAA protein has numerous pro-inflammatory actions: it works as a
320 chemoattractant to neutrophils, monocytes, and T lymphocytes, causing leukocyte
321 infiltration and promoting neutrophil adhesion to endothelial cells [37-39] and it
322 stimulates neutrophils and monocytes to release not only cytokines [40,41], but also
323 matrix metalloproteinases [42]. According to He et al. [43], these findings suggest a key
324 function for SAA not only in the establishment, but also in the maintenance of
325 inflammation, meaning that newborn lambs fed with colostrum at this early stage of life
326 reports a clear advantage, increasing this protein level on blood, and consequently
327 producing a more efficient immune status.
328
329 *Fibrinogen gamma chain (FGG)*
330 The fibrinogen gamma chain (FGG) is one of the three components of
331 fibrinogen that is the precursor of fibrin, the most abundant component in blood clots.
332 However, this protein also has a defensive function, as it has been demonstrated that
333 fibrinogen concentration increases during acute-phase reactions [44,45]. Moreover,
334 Yamada et al. [46] studied differences in low abundance proteins between cow
335 colostrum and milk, showing that some of them were only present in colostrum, such as
336 fibrinogen, which could explain the plasma increase of this protein in animals that were
337 fed with it. Additionally, several authors have described that fibrinogen can bind to
338 integrins, that are normally expressed on cells of the immune system, such as
339 $CD11b^{+}/CD18^{+}$ monocytes [47,48]. The $CD11b^{+}/CD18^{+}$ integrin receptor (αMβ2, Mac-
340 1, complement receptor 3) is a member of the β2 integrin family, which is expressed on

monocytes and macrophages. When fibrinogen binds to $CD11b^{+}/CD18^{+}$, integrin causes an extensive array of cell signaling responses, namely the activation of the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and mitogen-activated protein kinase (MAPK) / phosphatidylinositol 3-kinase (PI3K). These molecules mediate adhesion, migration, chemotaxis and phagocytosis [49]. All of these factors explain the importance of fibrinogen on the immune reaction. Therefore, colostrum intake produces an increase of fibrinogen in blood plasma resulting in benefit for the newborn immune system efficiency.

In spite of the great potential of these novel results, this manuscript showed initial results about the relation between non-immunoglobulin proteins and colostrum and the passive immune transfer in newborn lambs. Because of the limitations of this study, further proteomic studies will be necessary in order to increase the general knowledge about the new roles of colostrum in the passive immune transfer.

CONCLUSIONS

In conclusion, early colostrum intake produced an overexpression of non-immunoglobulin proteins, such as apoliprotein A-IV, plasminogen, serum amyloid A and fibrinogen. In addition, all of these overexpressed proteins have been previously described as having immune functions, demonstrating that colostrum is essential not only by its immunoglobulin content but also by the non-immunoglobulin proteins that play a fundamental role in the activation and attraction of immune cells, the apoptosis rate of the enterocytes and the low gastric secretion, among others. The results of this work contribute information about proteins with immune function that are increased after colostrum intake, that can be used to decrease the lamb mortality and for instance

366 to increase the economic benefit for farmers. In future, further proteomic studies will be
367 necessary in order to increase the general knowledge about the role of colostrum in the
368 passive immune transfer.

369

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381

382 **REFERENCES**

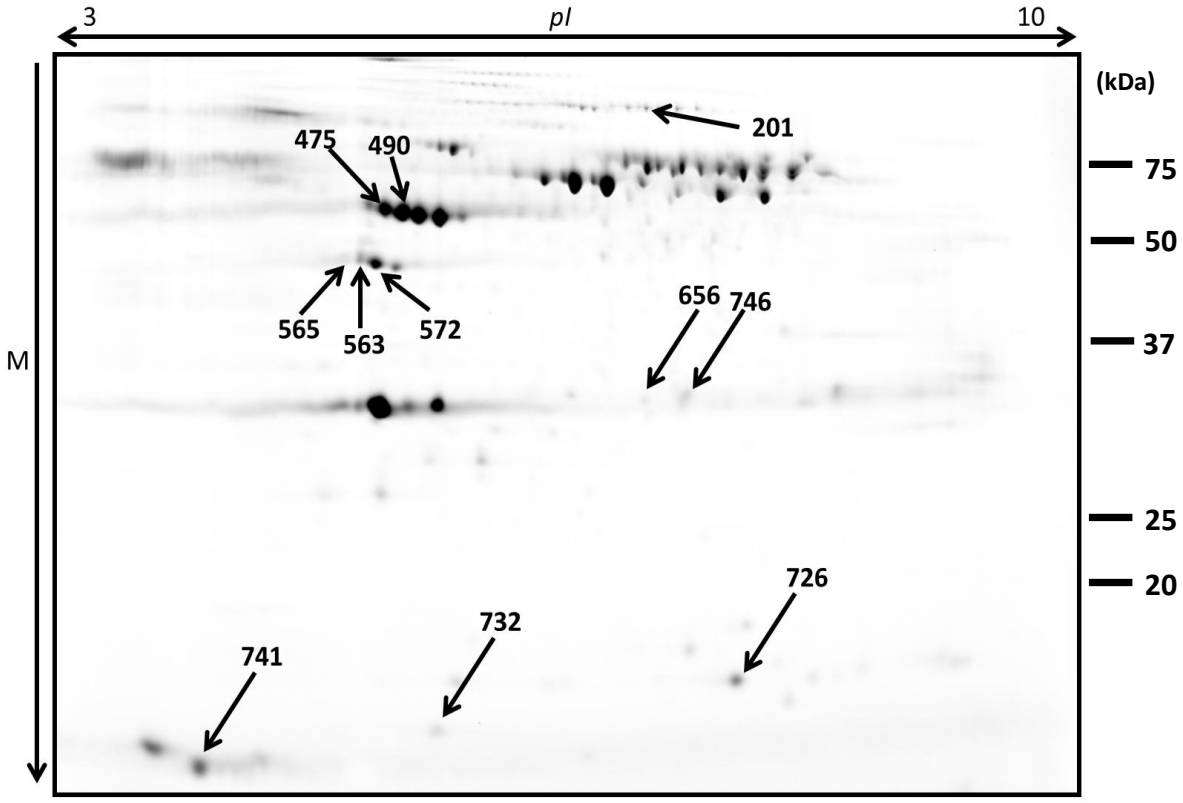
383 1. Moore M, Tyler JW, Chigerwe M, Dawes ME, Middleton JR (2005) Effect of delayed
384 colostrum collection on colostral IgG concentration in dairy cows. J Am Vet Med Assoc
385 226: 1375-1377.
386 2. Reinhardt TA, Lippolis JD (2008) Developmental changes in the milk fat globule membrane
387 proteome during the transition from colostrum to milk. J Dairy Sci 91: 2307-2318.
388 3. Nissen A, Bendixen E, Ingvartsen KL, Rontved CM (2012) In-depth analysis of low abundant
389 proteins in bovine colostrum using different fractionation techniques. Proteomics 12:
390 2866-2878.
391 4. Bendixen E, Danielsen M, Hollung K, Gianazza E, Miller I (2011) Farm animal proteomics - A
392 review. J Proteomics 74: 282-293.
393 5. Piñan DFO (2010) Guia de Campo de las Razas Autóctonas Españolas. 766 p.
394 6. Jarrige R (1988) Alimentation Des Bovins, Ovins & Caprins: Inra-Quae.

7. Rodríguez C, Castro N, Capote J, Morales-delaNuez A, Moreno-Indias I, et al. (2009) Effect of colostrum immunoglobulin concentration on immunity in Majorera goat kids. J Dairy Sci 92: 1696-1701.
8. Castro N, Capote J, Morales L, Quesada E, Briggs H, et al. (2007) Short communication: Addition of milk replacer to colostrum whey: effect on immunoglobulin G passive transfer in Majorera kids. J Dairy Sci 90: 2347-2349.
9. Almeida AM, Campos A, Francisco R, van Harten S, Cardoso LA, et al. (2010) Proteomic investigation of the effects of weight loss in the gastrocnemius muscle of wild and NZW rabbits via 2D-electrophoresis and MALDI-TOF MS. Anim Genet 41: 260-272.
10. Marcelino I, de Almeida AM, Ventosa M, Pruneau L, Meyer DF, et al. (2012) Tick-borne diseases in cattle: Applications of proteomics to develop new generation vaccines. J Proteomics 75: 4232-4250.
11. Boschetti E, Righetti PG (2008) The ProteoMiner in the proteomic arena: A non-depleting tool for discovering low-abundance species. J Proteomics 71: 255-264.
12. Marco-Ramell A, Bassols A (2010) Enrichment of low-abundance proteins from bovine and porcine serum samples for proteomic studies. Res Vet Sci 89: 340-343.
13. Halliday R, Williams MR (1979) Absorption of inmunoglobulin from colostrum by bottle-fed lambs. Ann Rech Vet 10: 549-556.
14. Muller LD, Ellinger DK (1981) Colostral immunoglobulin concentrations among breeds of dairy-cattle. J Dairy Sci 64: 1727-1730.
15. Stott GH, Fellah A (1983) Colostral immunoglobulin absorption linearly related to concentration for calves. J Dairy Sci 66: 1319-1328.
16. Castro N, Capote J, Alvarez S, Arguello A (2005) Effects of lyophilized colostrum and different colostrum feeding regimens on passive transfer of immunoglobulin g in Majorera goat kids. J Dairy Sci 88: 3650-3654.
17. Stan S, Delvin E, Lambert M, Seidman E, Levy E (2003) Apo A-IV: an update on regulation and physiologic functions. Biochim Biophys Acta 1631: 177-187.
18. Simon T, Cook VR, Rao A, Weinberg RB (2011) Impact of murine intestinal apolipoprotein A-IV expression on regional lipid absorption, gene expression, and growth. J Lipid Res 52: 1984-1994.
19. Vowinkel T, Mori M, Krieglstein CF, Russell J, Saijo F, et al. (2004) Apolipoprotein A-IV inhibits experimental colitis. J Clin Invest 114: 260-269.
20. Ogiwara K, Nogami K, Nishiya K, Shima M (2010) Plasmin-induced procoagulant effects in the blood coagulation: a crucial role of coagulation factors V and VIII. Blood Coagul Fibrinolysis 21: 568-576.
21. Booth NA, Bachmann F (2006) Plasminogen-plasmin system. In: Colman RW, editor. Hemostasis and Thrombosis. Philadelphia, PA: Lippincott Williams & Wilkins. pp. 335-361.
22. Dupont D, Remond B, Collin JC (1998) ELISA determination of plasmin and plasminogen in milk of individual cows managed without the dry period. Milchwissenschaft 53: 66-69.
23. Rebucci R, Fusi E, Pecorini C, Pinotti L, Cheli F, et al. (2005) Evaluation of the biological activation of plasmin plasminogen system in sheep and goat milk. Ital J Anim Sci 4: 330-332.
24. Feric NT, Boffa MB, Johnston SM, Koschinsky ML (2008) Apolipoprotein(a) inhibits the conversion of Glu-plasminogen to Lys-plasminogen: a novel mechanism for lipoprotein(a)-mediated inhibition of plasminogen activation. J Thromb Haemost 6: 2113-2120.
25. Renckens R, Roelofs JJTH, Florquin S, van der Poll T (2006) Urokinase-type plasminogen activator receptor plays a role in neutrophil migration during lipopolysaccharide-

induced peritoneal inflammation but not during Escherichia coli-induced peritonitis. J Infect Dis 193: 522-530.
26. Theodorou G, Daskalopoulou M, Chronopoulou R, Baldi A, Dell'Orto V, et al. (2010) Acute mastitis induces upregulation of expression of plasminogen activator-related genes by blood monocytes and neutrophils in dairy ewes. Vet Immunol Immunopathol 138: 124-128.
27. O'Mullane MJ, Baker MS (1999) Elevated plasminogen receptor expression occurs as a degradative phase event in cellular apoptosis. Immunol Cell Biol 77: 249-255.
28. Castro-Alonso A, Castro N, Capote J, Morales-DelaNuez A, Moreno-Indias I, et al. (2008) Short communication: Apoptosis regulates passive immune transfer in newborn kids. J Dairy Sci 91: 2086-2088.
29. Uhlar CM, Whitehead AS (1999) Serum amyloid A, the major vertebrate acute-phase reactant. Eur J Biochem 265: 501-523.
30. Eckersall PD, Young FJ, Nolan AM, Knight CH, McComb C, et al. (2006) Acute phase proteins in bovine milk in an experimental model of Staphylococcus aureus subclinical mastitis. J Dairy Sci 89: 1488-1501.
31. Pyorala S, Hovinen M, Simojoki H, Fitzpatrick J, Eckersall PD, et al. (2011) Acute phase proteins in milk in naturally acquired bovine mastitis caused by different pathogens. Vet Rec 168: 535.
32. Soler L, Molenaar A, Merola N, Eckersall PD, Gutierrez A, et al. (2013) Why working with porcine circulating serum amyloid A is a pig of a job. J Theor Biol 317: 119-125.
33. Kumon Y, Yasuoka Y, Yamanaka S, Wada A, Takeuchi H, et al. (2011) Acute-phase serum amyloid A is present in human colostrum and milk. Amyloid 18 Suppl 1: 11-13.
34. McDonald TL, Larson MA, Mack DR, Weber A (2001) Elevated extrahepatic expression and secretion of mammary-associated serum amyloid A 3 (M-SAA3) into colostrum. Vet Immunol Immunopathol 83: 203-211.
35. Le A, Barton LD, Sanders JT, Zhang Q (2010) Exploration of Bovine Milk Proteome in Colostral and Mature Whey Using an Ion-Exchange Approach. J Proteome Res 10: 692-704.
36. Faty A, Ferre P, Commans S (2012) The acute phase protein Serum Amyloid A induces lipolysis and inflammation in human adipocytes through distinct pathways. PLoS One 7: e34031.
37. Badolato R, Wang JM, Murphy WJ, Lloyd AR, Michiel DF, et al. (1994) Serum amyloid A is a chemoattractant: induction of migration, adhesion, and tissue infiltration of monocytes and polymorphonuclear leukocytes. J Exp Med 180: 203-209.
38. Xu L, Badolato R, Murphy WJ, Longo DL, Anver M, et al. (1995) A novel biologic function of serum amyloid A. Induction of T lymphocyte migration and adhesion. J Immunol 155: 1184-1190.
39. Su SB, Gong W, Gao JL, Shen W, Murphy PM, et al. (1999) A seven-transmembrane, G protein-coupled receptor, FPRL1, mediates the chemotactic activity of serum amyloid A for human phagocytic cells. J Exp Med 189: 395-402.
40. He R, Sang H, Ye RD (2003) Serum amyloid A induces IL-8 secretion through a G protein-coupled receptor, FPRL1/LXA4R. Blood 101: 1572-1581.
41. Furlaneto CJ, Campa A (2000) A novel function of serum amyloid A: a potent stimulus for the release of tumor necrosis factor-alpha, interleukin-1beta, and interleukin-8 by human blood neutrophil. Biochem Biophys Res Commun 268: 405-408.
42. Lee HY, Kim M-K, Park KS, Bae YH, Yun J, et al. (2005) Serum amyloid A stimulates matrix-metalloproteinase-9 upregulation via formyl peptide receptor like-1-mediated signaling in human monocytic cells. Biochem Biophys Res Commun 330: 989-998.

493 43. He RL, Zhou J, Hanson CZ, Chen J, Cheng N, et al. (2009) Serum amyloid A induces G-CSF
494 expression and neutrophilia via Toll-like receptor 2. Blood 113: 429-437.
495 44. Tamzali Y, Guelfi JF, Braun JP (2001) Plasma fibrinogen measurement in the horse:
496 comparison of Millar's technique with a chronometric technique and the QBC-Vet
497 Autoreader. Res Vet Sci 71: 213-217.
498 45. Ganheim C, Hulten C, Carlsson U, Kindahl H, Niskanen R, et al. (2003) The acute phase
499 response in calves experimentally infected with bovine viral diarrhoea virus and/or
500 Mannheimia haemolytica. J Vet Med B Infect Dis Vet Public Health 50: 183-190.
501 46. Yamada M, Murakami K, Wallingford JC, Yuki Y (2002) Identification of low-abundance
502 proteins of bovine colostral and mature milk using two-dimensional electrophoresis
503 followed by microsequencing and mass spectrometry. Electrophoresis 23: 1153-1160.
504 47. Ugarova TP, Yakubenko VP (2001) Recognition of fibrinogen by leukocyte integrins.
505 Fibrinogen 936: 368-385.
506 48. Ryu JK, Davalos D, Akassoglou K (2009) Fibrinogen signal transduction in the nervous
507 system. J Thromb Haemost 7: 151-154.
508 49. Akassoglou K, Strickland S (2002) Nervous system pathology: The fibrin perspective. Biol
509 Chem 383: 37-45.
510

Figure 1. Image of the lamb plasma pool labeled with Cy2 used as a reference gel in gel analysis. Protein extracts were depleted using PROTEOMINER® commercial kit and used in DIGE, where a total of 50µg were loaded per CyDye. Spots showing differential expression are highlighted with arrows. pI – Isoelectric point and M – Molecular Mass.



516 Table 1. IgG and IgM evolution in Colostrum group (C) and Delayed Colostrum group
517 (DC) at 2 and 14 hours after birth.

518

	Groups	Time after birth (h)		SEM
		2	14	
519 IgG	C	ND	7.406	0.837
520	DC	ND	ND	
521 IgM	C	ND	0.443	0.100
522	DC	ND	ND	

523 ND means no detectable.

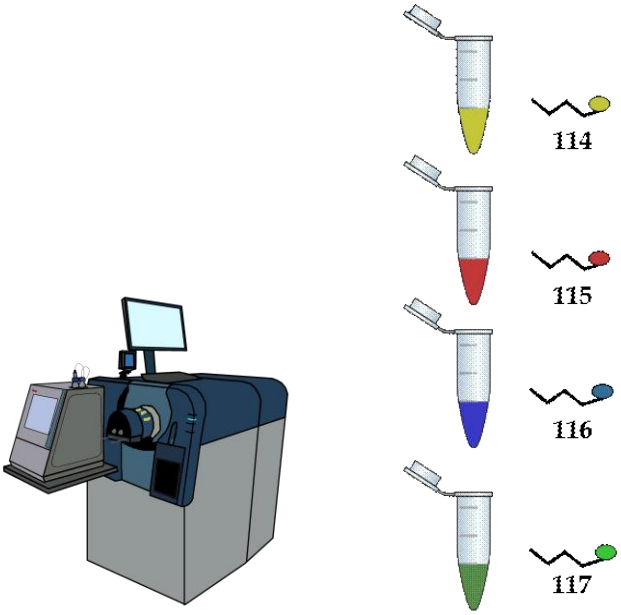
Table 2. Differential expression analysis between Colostrum group at 2 (C 2h) and 14 hours (C 14h) after birth and Mass Spectrometry Identification of differentially expressed proteins from lamb plasma.

Spot reference	P-value	Fold	Average Normalized Volumes		Spot Image		Protein name	Accession number	Theoretical molecular mass (kDa)	Theoretical PI	Matched Peptides[b]		Sequence coverage (%)[c]	Protein Score[d]
			C 2h	C 14h	C 2h[a]	C 14h					MS	MS/MS		
490	<0.001	2.1	0.835	1.770			Fibrinogen gamma-B chain	FIBG_BOVIN	50.8	5.5	7	5	16	361
201	<0.001	2.1	0.843	1.810			Plasminogen (Fragment)	PLMN_SHEEP	38.6	7.5	7	-	23	86
565	0.002	1.3	0.835	1.122			Apolipoprotein A-IV	APOA4_BOVIN	42.9	5.3	12	1	26	183
475	0.005	1.3	1.187	1.930			Fibrinogen gamma-B chain	FIBG_BOVIN	50.8	5.5	8	4	15	332
572	0.007	3.0	0.832	2.531			Apolipoprotein A-IV	APOA4_BOVIN	42.9	5.3	17	8	35	738
726	0.007	4.0	0.442	1.779			Serum amyloid A	SAA_BOVIN	14.5	7.8	4	1	30	62
563	0.008	1.3	0.891	1.116			Apolipoprotein A-IV	APOA4_BOVIN	42.9	5.3	15	1	35	432

[a] Spots showed in this table had a similar value between C and DC group at 2 hours after birth and did not show any increase between DC group at 2 and 14 hours after birth. [b] Number of peptides, matching the identified protein, whose sequence differs in at least one amino acid residue; [c] Percentage of the identified protein sequence covered by the matched peptides; [d] Identification Score obtained with the Mowse algorithm. A result is considered to be significant when a score above 61 is attained.

Capítulo 6

Chapter 6



Colostrum protein uptake in neonatal lambs studied by descriptive and quantitative LC-MS/MS.

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Abstract

Colostrum intake is a key factor for newborn mammals survival, particularly ruminants. As ruminant placenta impedes the transfer of immune components, newborn ruminants entirely depend on Passive Immunity Transfer from the mother to the neonate, through early suckling of colostrum. Understanding the important role of colostrum proteins has gained much attention. However, proteomics studies of sheep colostrum proteomes and their uptake in neonate lamb has not yet been presented.

The aim of this study was hence to describe the sheep colostrum and lamb plasma proteome, using a top-down mapping approach based on SDS-PAGE separation and LC-MS/MS based protein identification, and to provide relative quantification of whether and how neonate plasma protein concentrations change as an effect of early colostrum intake, using an iTRAQ-based proteomics approach.

The results of this study described the presence of 90 proteins in the sheep colostrum proteome. Moreover, the colostrum intake produced an increase of eight proteins with immune function in the lamb blood plasma. Further proteomic studies will be necessary, particularly using the SRM approach, in order to increase the general knowledge about the role of colostrum in the PIT.

Biological Significance

This manuscript reveals novel information to Journal of Proteomics readers since, many sheep colostrum and lamb plasma proteins were mapped using an SDS-LC-MS/MS approach. Furthermore, quantitative protein changes in lamb blood plasma, because of the colostrum intake, were deeply described, using an iTRAQ approach. This technique

provided the most comprehensive description of sheep colostrum and lamb plasma proteome, since no information about them has been fully described yet. This manuscript contributes information about proteins with immune function that are increased after colostrum intake, that can be used to decrease lamb mortality rates and consequently to increase the economic benefit for sheep producers.

Keywords: Colostrum, lamb, plasma, immune, iTRAQ, LC-MS/MS

1. INTRODUCTION

It is well known that early access to colostrum is a key factor for neonatal survival in mammals. But for ruminants, this is particularly critical, because the synepitheliochorial placenta of ruminants implies that the placental membrane is attached rather than invasive to the maternal endometrial tissues, hence the transfer of immunoglobulins from the mother to the foetus is very limited. [1-4]. Hence, ruminants are hypo gammaglobulinemic at birth, and entirely depend on Passive Immunity Transfer (PIT) from the mother to the neonate, through early suckling of colostrum [5, 6]. Colostrum contains essential nutrients like fat, lactose, vitamins and minerals [7], but is also rich in unique proteins that play active roles in regulating growth and development of the gut tissue, and for protecting the neonate against pathogens and post-partum environmental challenges [8].

Although transfer of immunoglobulins is a key factor in host defense to pathogens, also a wider range of colostrum components have been suggested to contribute to the early

protection of the neonate [9]. These include many unique proteins with specific functional roles, including proteins secreted by neutrophils and macrophages, proteins taking part in the blood complement system, acute-phase proteins, as well as specific proteins and peptides that possess direct antimicrobial activity [10-12]. Besides mediating host defense, this wide range of bioactive colostrum proteins also play important roles as key regulators of gastrointestinal growth and development in early life [8].

Colostrum proteins are protected by the trypsin inhibitors present in colostrum [13], as well as by the low proteolytic activity in the gastrointestinal tract of newborn animals [14]. Moreover, the uptake of entire proteins through the gut epithelium is facilitated by a unique feature of an regulated apoptosis process of the neonatal ruminant enterocytes that allow unrestricted passage of large molecules, such as immunoglobulins, into the gut [5, 15]. It is crucial to feed newborn ruminants during the first 48 hours after birth, because the mechanisms that facilitates PIT and colostrum uptake are only operational in the first neonatal days [16]. Neither the cellular mechanisms of colostrum protein transfer to blood, nor their bioactive roles in the neonate ruminants are understood in much detail. While bovine milk and colostrum proteomes have attracted much attention, including reports of more than 257, 138, and 403 proteins characterized from whey [17], milk fat globule membrane (MFGF) [18] and colostrum [19] samples, respectively, very limited knowledge exists about sheep colostrum proteins. Even some ELISA based studies have reported the concentration levels of some immune proteins such as Immunoglobulin G in sheep colostrum (48.1-60.9 mg/mL [20-22]) and newborn lamb blood plasma (13.7-26.6 mg/mL [23, 24]), proteomics studies of sheep colostrum proteomes and their uptake in neonate lamb has not yet been presented.

The aim of this study was hence to describe the sheep colostrum and lamb plasma proteome, using an SDS-LC-MS/MS approach, and to provide relative quantification of whether and how neonate plasma protein concentrations change as an effect of early colostrum intake, using an iTRAQ-based proteomics approach.

2. MATERIAL AND METHODS

This experiment was approved by the ethics committee of the Faculty of Veterinary of the *Universidad de Las Palmas de Gran Canaria* (Spain).

2.1.Sample Collection

Two groups of 4 newborn lambs each (Canarian dairy breed) were used in this experiment. The experiment took place at the experimental farm of the Veterinary Faculty of the *Universidad de Las Palmas de Gran Canaria* (Canary Islands, 28º 8' 20.66" N, 15º 30' 24.97" W, Spain) in spring. The two groups of animals were fed from a common pool of sheep colostrum that contained 64.74 mg/mL of Immunoglobulin G. The two experimental groups were fed according to 2 different time schemes. The colostrum-fed group (C group) received early colostrum feeding, at 2, 14 and 26 hours after birth. The delayed-colostrum group (DC group) was not fed until 14 and 26 hours. At the end of the experimental period (26 hours after birth) all animals took the same amount of fresh colostrum. Blood samples were collected before feeding from the jugular vein in 2.5 mL tubes with K-EDTA at 2 and 14 hours after birth. Blood was centrifuged at 2190 *g* for 5 minutes at 4ºC (Hettich-Zentrifugen, Universal 32 R,

Germany) and the obtained plasma was frozen at -80ºC until analysis. All lambs were accommodated in rearing rooms with at least 0.3 m^2 floor-space per lamb. Each room had central heating conferring a room temperature of approximately 20ºC.

2.2 Sample Prepraration

Plasma samples were analysed at Aarhus University (Denmark). 200 mL from each sample were homogenized with 1 mL of TES buffer (10 mM Tris-HClpH 7.6, 1 mM EDTA, 0.25 M sucrose) using ULTRAturrax at 12.000 rpm. Homogenates were centrifuged at 10,000 x *g* for 30 min at 4°C to remove insoluble components. Protein concentration of the supernatant was determined with the Quick Start™ Bradford Protein Assay (Bio-Rad, Hercules, CA, USA), using BSA as standard reference [25].

Aliquots of 100 µg protein from each sample was recovered after precipitated with 6 volumes of ice-cold acetone (-20ºC), centrifuged at 15,000 x *g* for 10 minutes at 4ºC.

For the descriptive SDS-PAGE-LC-MS/MS analyses of plasma and colostrum proteomes, aliquots of 60 µg protein from the C group and DC group at 14 hours (2 biological replicates from each group), as well as the pooled colostrum used for the feeding experiment ,were prepared (2 technical replicates). Aliquots were resuspended in 20 µL TES buffer. The samples were boiled for 5 min in SDS sample buffer (7.5 mL) containing 500 mM DTT, separated by SDS-PAGE using 10% (w/v) gels [26] and stained using Coomassie blue (RAPIDstain, G-Biosciences). Each lane was cut into 9 equal sized slices, each of these were washed 3 times in milli-Q water and incubated for two times 15 min in 130 µL 50% acetonitrile, dehydrated in 130 µL acetonitrile for 15 min and equilibrated in 150 µL 0.1 M NH_4HCO_3 for 5 min before 150 µL acetonitrile

was added. After 15 min the supernatants were removed and the gel pieces were lyophilized for 20 min. In-gel digestions were performed by incubating the gel pieces with sequencing grade modified trypsin (Promega) in 50 mM NH_4HCO_3 at 37 °C for 16 h, using 400 ng trypsin for heavy loaded gel slices, and 200 ng trypsin for the more faint gel slices. The resulting peptides were desalted using C_{18} StageTips (Thermo Scientific) and stored at -20 °C before LC−MS/MS analysis as described below.

2.3. LC−MS/MS Analysis

LC−MS/MS analyses of gel slices were performed on an EASY-nLC II system (Thermo Scientific) connected to a TripleTOF 5600 mass spectrometer (AB SCIEX) equipped with a NanoSpray III source (AB Sciex) and operated under Analyst TF 1.6 control. Tryptic peptides were dissolved in 0.1% formic acid, injected, trapped and desalted isocratically on a ReproSil-Pur C18-AQ column (5 µm, 2 cm × 100 µm I.D;Thermo Scientific), eluted from the trap column and separated on a ReproSil-Pur C18-AQ 3 µm capillary column (16 cm × 75 µm I.D) connected in-line to the mass spectrometer at 250 nL/min using a 50 min gradient from 5% to 35% of buffer B (0.1% formic acid and 90% acetonitrile), followed by a 10 minute re-equilibration time in buffer A. The TripleTOF 5600 was run in positive ion mode using 2500 V for ion spray, curtain gas at 30 V, ion source gas at 5 and an interface heater temperature of 150°C. The automated IDA method acquired up to 50 MS/MS spectra per cycle using 2.3 s cycle times and an exclusion window of 6 s.

2.4. iTRAQ based quantitative LC-MS/MS

Six sets of iTRAQ experiments were designed in order to study the differential protein abundances found in plasma collected from C versus DC groups. A total of 16 plasma samples were compared, including 4 biological replicates and 2 times points (2 and 14 h after birth) from both treatment conditions (C versus DC). Consistently, the iTRAQ reagent 114 was used to label an internal control sample made as a pool of 100 ug plasma protein aliquots taken from each of the 16 individual plasma samples. The remaining iTRAQ reagents (115, 116 and 117) were used to label the 16 individual plasma samples.

Cysteine residues were reduced with 2.5 mM tris (2-carboxyethyl) phosphine hydrochloride at 60°C for 1 hour and then blocked with 10mM methylmethanethiosulfate solution at room temperature for 10 minutes. Proteins were then digested with trypsin (1:10 w/w) at 37°C overnight. The tryptic peptides were labeled with iTRAQ reagents (Applied Biosystems, Forster City, CA, USA) at room temperature for 1 hour as previously described by the manufacturer. Finally, samples were combined to create 6 different 4-plexed samples.

2.5. SCX fractionation and LC-MS/MS analysis

A total of 50 µg protein from each of the iTRAQ 4-plexed samples were injected into an Agilent 1100 Series capillary HPLC equipped with a Zorbax Bio-SCX Series II, and peptides were eluted with a gradient of increasing NaCl solution. Fractions were collected every minute for 65 minutes and combined according to their peptide loads into 9-10 pooled samples.

LC–MS/MS analyses were performed on an Easy-nLC II system (Thermo Scientific) connected to a QSTAR Elite mass spectrometer (AB SCIEX) equipped with a NanoSpray source (AB Sciex) and operated under Analyst QS 2.0 control. The trypsin digested samples were dissolved in 0.1% formic acid, injected, trapped and desalted isocratically on a ReproSil-Pur C18-AQ column (5 μm, 2 cm × 100 μm I.D; Thermo Scientific), after which the peptides were eluted from the trap column and separated on an analytical ReproSil-Pur C18-AQ capillary column (3 μm, 10 cm × 75 μm I.D; Thermo Scientific) connected in-line to the mass spectrometer at 250 nL/min using a 63 min gradient from 5% to 38% phase B (0.1% formic acid and 90% acetonitrile). The automated IDA method was set to acquire up to 3 MS/MS spectra per cycle using 2.0 s cycle times and an exclusion window of 80 s.

2.6. Protein Identification and Quantitation

The collected MS files were converted to Mascot generic format (MGF) using the AB SCIEX MS Data Converter beta 1.3 (AB SCIEX) and the "proteinpilot MGF" parameter. The peak lists were used to interrogate a combined sheep and goat database consisting of sequences from TrEMBL, Swiss-Prot and NCBInr (Updated November 2012, 32.444 sequences) using Mascot 2.3.02 [Matrix Science; 27]. For in-gel digests, the search parameters were set to allow one missed trypsin cleavage site and propionamide as a fixed modification. The mass accuracy of the precursor and product ions were 15 ppm and 0.2 Da and the instrument settings was specified as ESI-QUAD-TOF. For the iTRAQ samples, the search parameters were set to allow one missed trypsin cleavage site and methylthio (MMTS) (C) as a fixed modification with oxidation

of methionine as variable modification. iTRAQ 4-plex was selected as quantitation method. The mass accuracy of the precursor and product ions were 0.2 Da and the instrument setting was specified as ESI-QUAD-TOF. The significance threshold (p) was set at 0.01 with an ion score cutoff at 31 for all samples.

Mascot results were parsed using MS Data Miner v.1.1 [MDM; 28]. A final report was generated using MDM, comparing all identified and quantified proteins from the 6 sets of iTRAQ data.

2.7. Statistical Analysis

Statistical analyses from iTRAQ approach were performed using SAS, Version 9.00 (SAS Institute Inc., Cary, NC). The SAS PROC MIXED procedure for repeated measurements was used to evaluate the effect of colostrum intake (C group *vs.* DC group) at 2 and 14 hours after birth. A Bonferroni's test was used to evaluate differences between groups ($P<0.05$).

3. RESULTS AND DISCUSSION

In order to investigate which proteins can be detected in colostrum and plasma using shotgun proteomics approaches, we used a top-down mapping approach based on SDS-PAGE separation and LC-MS/MS based protein identification. Only proteins observed in both of the replicated analyses from each sample type were regarded as significantly and uniquely observed. Analyses of the pooled colostrum which was used in our feeding experiment, allowed the identification of 88 proteins, while 65 and 127 proteins could be observed in plasma from respectively early and delayed colostrum-fed lambs. The aim of this initial and descriptive study was to collect detailed information about the

protein subsets detectable in these different and complex proteome samples, and to provide information about the overlap and uniqueness of the protein subsets across these body fluids. The venn diagram in figure 1 presents an overview of the distribution of proteins across these body fluids. It is clear that the majority (30 proteins) of the observed proteins could be observed in all 3 body fluids. Moreover, comparing these proteome profiles clearly demonstrated that a wide range of immunoglobulins originating from colostrum are transferred to plasma, while a large majority of colostrum specific proteins cannot be detected in plasma 12 hours after early suckling, indicating that transport of proteins from colostrum, over the gut epithelial layer and into plasma is a controlled and selective process, although mechanisms responsible for selective transfer are not yet well understood [29].

Another interesting observation provided by our direct comparison of body fluid proteomes, is, that a much larger range of the well-known plasma proteins and also intracellular protein (e.g. Fructose –biphosphate aldolase and carbonic anhydrase) are detectable in plasma from animals that lacked access to colostrum. This is likely due to the fact that peptide selection in shotgun-based LC-MS/MS analyses is greatly biased by the relative abundance of the protein and peptide components in complex biological samples. This is indeed the case in plasma collected after colostrum suckling, where massive amounts of immunoglobulins are observed in plasma, and can therefore outnumber and overshadow detection of the plasma-protein background.

In order to achieve a more direct comparison and also a relative quantification of the effect of early colostrum feeding, an iTRAQ based tagging approach was used to compare the plasma proteomes of eight individually sampled animals. This

investigation also aimed to study how early colostrum affects the expression levels of plasma-proteins related to growth and development of the neonatal lamb.

In the iTRAQ tagged analyses, we observed a total of 148 proteins. Compared to similar shotgun analyses of cow plasma [30] and colostrum [18, 19], the number of proteins we could identify and quantify in colostrum and plasma samples of sheep was much lower. This is partly due to the dynamic range, as discussed above [31, 32], but is mainly a direct effect of the current lack of well-covered sheep genome and protein databases. Particularly, the detailed annotation of sheep proteins is very limited when compared to bovine and goat databases.

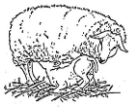
A depletion-step aimed to remove high abundant plasma proteins from colostrum and plasma samples before MS/MS analysis may improve proteome coverage of body fluid shotgun analyses of body fluids, but because the main objective of this study was to compare changes in plasma proteins as an effect of early suckling, this approach is not applicable in this study.

The recent technological progress in targeted and absolute quantitation methods [33] may allow more accurate analyses in future experiments. Particularly, the development of selected reaction monitoring (SRM) based monitoring of candidate target proteins would greatly benefit further studies of the absolute absorption of specific colostrum proteins in plasma. However, these methods require that the detection of specific proteotypic signature peptides are described and validated for each protein to be analyzed across tissues [33]. Such SRM methods are not yet available for the specific ovine proteins we have analyzed. However, this study clearly points to interesting candidate proteins that could be of immediate interest for SRM method development.

3.1. Protein identification

Table 1 compiles information about non-redundant proteins identified in colostrum and plasma samples from C and DC group at 14 hours after birth. The first group of proteins listed in table 1 was identified in all samples (from colostrum as well as plasma). The second group represents the proteins that were uniquely found in colostrum and plasma from colostrum-fed animals. This list of proteins is of specific interest because they represent specific transfer from colostrum, and in our data these consist mainly of immunoglobulins. The third set of proteins in table 1 shows proteins that were exclusively identified in colostrum. Most of these are well known from bovine studies to be highly abundant in colostrum, including immunoglobulins and caseins, but also proteins which from similar studies of cow colostrum are known to be of lower abundance (such as serum amyloid A-3, 267.45 µg/mL [34] or plasmin, 0.49 µg/mL [35]) could be observed. Finally, the fourth group presents proteins that could only detected in animals that lacked access to colostrum (the DC group). This list reflect proteins that are expressed as a response to physiological stress caused by lack of normal colostrum intake.

Regarding the subset of 30 proteins that were identified in all 6 samples in the descriptive study, it should be noted that certain immunoglobulin isoforms could also be detected in plasma of animals who had not received colostrum. Despite that several authors have described that ruminant placenta entirely impedes the transfer of immunoglobulins from the dam to the foetus [36, 37], other authors [1-3, 38] agree with these findings, suggesting that small but detectable amounts of immunoglogulins can be transferred via placenta. Likewise, different apolipoprotein isoforms, fibrinogen, plasminogen, serotransferrin, ceruloplasmin and trypsin inhibitor were also present in



all the studied samples. Despite that these proteins are mainly known as components of blood, their presence in cow colostrum has been confirmed, including apolipoproteins A-I, A-IV, E [18, 39], plasminogen [35, 39], trypsin inhibitor [39, 40], ceruloplasmin [41], fibrinogen and serotransferrin [39], and in some cases reported to be more abundant in colostrum than in plasma. Colostrum contributes with several proteins that provides immunomodulatory effects in the newborn, as reviewed by Bendixen et al. [8], hence an iTRAQ labeling approach allows analyses of the relative changes of the immunomodulatory colostrum proteins as an effect of early and delayed access to colostrum in neonate lambs.

The second group of proteins was identified in colostrum and plasma from colostrum-fed lambs. As can been observed, immunoglobulins were the main colostrum proteins absorbed by colostrum-fed lambs. These findings are in agreement with Hurley et al. [42] and Moreno-Indias et al. [43] who described that immunoglobulins are among the most important proteins in colostrum in order to acquire a correct passive immune transfer.

The third group of proteins, showed in table 1, was only identified in colostrum and therefore was not absorbed by the colostrum-fed newborn lambs. As can be appreciated, colostrum used in this experiment contains a high amount of immunoglobulins. As was showed above, even some immunoglobulin fragments were only identified in colostrum, all immunoglobulins seem to be efficiently absorbed, as these colostrum proteins dominate the plasma proteome of the colostrum-fed animals. Additionally to immunoglobulin content of the colostrum used in this experiment, other high abundance proteins such as caseins or alpha-lactoalbumin were observed. It has been noted that caseins are responsible for some important biological functions such as Ion carriers (Ca,

PO4, Fe, Zn, Cu), bioactive peptide precursors and immunomodulators [44]. It was also demonstrated that the casein proteolytic fragments have antimicrobial activity [45], suggesting that the proteases may also play a role in the host defence. In addition, peptides derived from caseins are receiving much attention as possible sources of natural bioactivity with health benefits for humans [46], probably because they stimulate the innate immune system within the mammary gland and prevent udder infections during the dry phase [47]. Moreover, it has been recently shown how alpha-lactoalbumin, plays a role as an immunomodulator in human and cow colostrum and milk [44, 48, 49]. Despite that neither caseins nor alpha-lactoalbumin seems to be absorbed in colostrum fed newborn lambs, they may have a protective function in the intestine of that animals.

Moreover, a wide range of proteins components from colostrum were not detectable in colostrum-fed animals, including proteins like neutrophil gelatinase-associated lipocalin isoform 2, peptidoglycan recognition protein 1 or junction plakoglobin which all provide protection against bacterial infections. The first acts as an iron-trafficking protein [50], the second one interfering with peptidoglycan biosynthesis [51, 52] and finally junction plakiglobin in conjunction with MUC-1 prevents pathogens from reaching the cell surface [53, 54]. Other proteins like calcium binding protein, matrix gla protein and nucleobindin-1, that takes part in the Ca2+ transport, or adipocyte fatty acid-binding protein, which participates in the lipid transport were also observed in colostrum, but could not be detected in plasma after suckling.

Finally, the last group of proteins, which were observed only in lambs that lacked access to colostrum until 14 h after birth are presented. As it has been described by several authors [55-58], lambs are dependent on the intestinal transmission of immunoglobulins

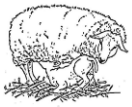
and other immune modulating factors present in colostrum, which may affect the development of the immune system of newborn lambs. Moreover, present findings described how delayed-colostrum lambs showed a wide range of plasma proteins related to stress, inflammation, coagulation activity, catabolic metabolism and cells destruction. The effect of this starvation is reflected by the presence of proteins such as, haptoglobin, chymotripsinogen b, hepatocyte growth factor activator, or different complement factors, indicate stress in this groups of lambs, probably derived from the colostrum deprivation, and therefore, the lack of energy intake. Also, the presence of several proteins that take part during the catabolic metabolism (adiponectin, flavin reductase (NADPH), Insulin-like growth factor-binding protein 2, L-lactacte dehydrogenase B chain, n-acetylmuranoyl-l-alanine amidase, phospholipid transfer protein, plasma serine protease inhibitor, among others) were observed, together with proteins that are involved in the blood coagulation processes, including coagulation factor a1, X and XIII, alpha-2 antiplasmin, kininogen-1 or serpin A3-2, A3-5 and D1. This observation may be related to the well-known fact that the coagulation system appears to be activated and upregulated during periods of acute stress, as is indeed the case during lack of feed intake [59-61]. Finally, the deprivation of colostrum produced the activation of some homeostatic proteins such as Angiotensin-1 and Carbonic anhydrase 2, trying to balance the lack of water and minerals given through colostrum. For this reason, these proteins produce vasoconstriction and promote Na^{2+} retention in the kidney, which also drives blood pressure up [62-64].

3.2.Protein quantification using an iTRAQ approach

Only approximately 148 proteins could be observed and characterized from the shotgun proteome data. From this result, a total of 31 proteins were identified, which followed a normal distribution in at least 3 out of the 4 biological replicates in each of the 4 studied groups. A statistical analysis was performed on the selected proteins, as described above, and a total of eight proteins were found increased in the C group at 14 hours after birth.

Table 2 shows significant ($P<0.05$) proteins ratio changes between C and DC group because of the colostrum feeding. In reference to apolipoproteins, three different classes (apolipoprotein A-IV, B-100 and E) were increased in the C group at 14 h after birth due to the colostrum feeding. The primary function of apolipoproteins in the efficacy of fat absorption by the intestine has been deeply described [65-68]. In this way, fat absorption plays an essential role in newborn lambs, because colostrum is not only necessary for the immune protection but also as the only energy source at this early period after birth. In spite of these findings, their relations to immune response have also been described. In particular, apolipoprotein B-100 and apolipoprotein-E have been demonstrated to be an innate barrier against infections [69, 70]. Moreover, it has been observed that apolipoprotein A-IV, not only exhibits a protective effect against external agents [71], but also produces a decrease of gastric secretions [72]. This last effect, added to the increase of trypsin inhibitor produced in C group, promotes the absorption of proteins with their native structure.

Lambs that were colostrum fed also increased their plasma levels of several acute-phase proteins, such as fibrinogen alpha chain, tetranectin or ceruloplasmin. The first of them



participates in coagulation process [73], but also during inflammation, stimulating the adhesion, migration, chemotaxis and phagocytosis of monocytes and macrophages to the point of infection [74, 75]. As happen with the previous described protein, tetranectin produced the activation of plasmin [76], stimulating the neutrophil migration to the infection [77]. Finally, in spite of the main function of ceruloplasmin as cupper and iron transporter, it has been described that this protein may contribute to defense responses via its ferroxidase activity, which may drive iron homeostasis in a direction unfavorable to invasive organisms [78].

As can be observed in table 2 early colostrum intake produced a relative increase of eight plasma proteins in the C group. This demonstrates that colostrum is essential not only for its immunoglobulin content but also for the non-immunoglobulin proteins that play a fundamental role in the activation and attraction of immune cells, the low gastric secretion, among others.

4. CONCLUSION

The results of this work described the presence of 90 proteins in the sheep colostrum proteome. Moreover, eight proteins with immune function were increased in the lamb blood plasma after colostrum intake. Understanding uptake and effect of colostrum is important for future reduction of lamb mortality rates and will also contribute to increase the economic benefit of sheep producers. Further proteomic studies will be necessary, particularly using the SRM approach, in order to increase the general knowledge about the role of colostrum in the PIT.

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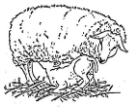
6. REFERENCES

[1] Argüello A, Castro N, Capote J, Tyler JW, Holloway NM. Effect of colostrum administration practices on serum IgG in goat kids. Livest Prod Sci. 2004;90:235-9.
[2] Castro N, Capote J, Alvarez S, Arguello A. Effects of lyophilized colostrum and different colostrum feeding regimens on passive transfer of immunoglobulin g in Majorera goat kids. J Dairy Sci. 2005;88:3650-4.
[3] Castro N, Capote J, Bruckmaier RM, Arguello A. Management effects on colostrogenesis in small ruminants: a review. Journal of Applied Animal Research. 2011;39:85-93.
[4] Castro N, Capote J, Morales-Delanuez A, Rodriguez C, Arguello A. Effects of newborn characteristics and length of colostrum feeding period on passive immune transfer in goat kids. J Dairy Sci. 2009;92:1616-9.
[5] Stelwagen K, Carpenter E, Haigh B, Hodgkinson A, Wheeler TT. Immune components of bovine colostrum and milk. Journal of animal science. 2009;87:3-9.
[6] Danielsen M, Pedersen LJ, Bendixen E. An in vivo characterization of colostrum protein uptake in porcine gut during early lactation. Journal of proteomics. 2011;74:101-9.
[7] Ontsouka CE, Bruckmaier RM, Blum JW. Fractionized milk composition during removal of colostrum and mature milk. J Dairy Sci. 2003;86:2005-11.
[8] Bendixen E, Danielsen M, Hollung K, Gianazza E, Miller I. Farm animal proteomics - A review. Journal of proteomics. 2011;74:282-93.
[9] Smith GW, Foster DM. Short Communication: Absorption of Protein and Immunoglobulin G in Calves Fed a Colostrum Replacer. Journal of Dairy Science. 2007;90:2905-8.
[10] Rainard P, Riollet C. Innate immunity of the bovine mammary gland. Veterinary research. 2006;37:369-400.

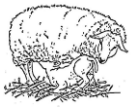
[11] Oviedo-Boyso J, Valdez-Alarcon JJ, Cajero-Juarez M, Ochoa-Zarzosa A, Lopez-Meza JE, Bravo-Patino A, et al. Innate immune response of bovine mammary gland to pathogenic bacteria responsible for mastitis. J Infect. 2007;54:399-409.
[12] Leitner G, Krifucks O, Jacoby S, Lavi Y, Silanikove N. Concentrations of ganglioside type M1 and immunoglobulin G in colostrum are inversely related to bacterial infection at early lactation in cows. Journal of Dairy Science. 2008;91:3337-42.
[13] Ramos JJ, Loste A, Ferrer LM, Fernandez A, Castro N, Ortin A, et al. Effect of addition of soybean trypsin inhibitor to colostrum on immunological status in goat kids. Journal of animal physiology and animal nutrition. 2010;94:93-8.
[14] Guilloteau P, Corring T, Garnot P, Martin P, Toullec R, Durand G. Effects of Age and Weaning on Enzyme-Activities of Abomasum and Pancreas of the Lamb. Journal of Dairy Science. 1983;66:2373-85.
[15] Castro-Alonso A, Castro N, Capote J, Morales-DelaNuez A, Moreno-Indias I, Sanchez-Macias D, et al. Short communication: Apoptosis regulates passive immune transfer in newborn kids. Journal of Dairy Science. 2008;91:2086-8.
[16] Moore M, Tyler JW, Chigerwe M, Dawes ME, Middleton JR. Effect of delayed colostrum collection on colostral IgG concentration in dairy cows. Javma-J Am Vet Med A. 2005;226:1375-7.
[17] Le A, Barton LD, Sanders JT, Zhang Q. Exploration of Bovine Milk Proteome in Colostral and Mature Whey Using an Ion-Exchange Approach. J Proteome Res. 2010;10:692-704.
[18] Reinhardt TA, Lippolis JD. Developmental changes in the milk fat globule membrane proteome during the transition from colostrum to milk. Journal of Dairy Science. 2008;91:2307-18.
[19] Nissen A, Bendixen E, Ingvartsen KL, Rontved CM. In-depth analysis of low abundant proteins in bovine colostrum using different fractionation techniques. Proteomics. 2012;12:2866-78.
[20] Higaki S, Nagano M, Katagiri S, Takahashi Y. Effects of parity and litter size on the energy contents and immunoglobulin G concentrations of Awassi ewe colostrum. Turk J Vet Anim Sci. 2013;37:109-12.
[21] Moretti DB, Pauletti P, Kindlein L, Machado-Neto R. Enteric cell proliferation in newborn lambs fed bovine and ovine colostrum. Livest Sci. 2010;127:262-6.
[22] Moretti DB, Kindlein L, Pauletti P, Machado-Neto R. IgG absorption by Santa Ines lambs fed Holstein bovine colostrum or Santa Ines ovine colostrum. Animal : an international journal of animal bioscience. 2010;4:933-7.
[23] Rodinova H, Kroupova V, Travnicek J, Stankova M, Pisek L. Dynamics of IgG in the blood serum of sheep with different selenium intake. Vet Med-Czech. 2008;53:260-5.
[24] Quigley JD, 3rd, Carson AF, Polo J. Immunoglobulin derived from bovine plasma as a replacement for colostrum in newborn lambs. Veterinary therapeutics : research in applied veterinary medicine. 2002;3:262-9.
[25] Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem. 1976;72:248-54.
[26] Bury AF. Analysis of protein and peptide mixtures : Evaluation of three sodium dodecyl sulphate-polyacrylamide gel electrophoresis buffer systems. J Chromatogr A. 1981;213:491-500.
[27] Perkins DN, Pappin DJC, Creasy DM, Cottrell JS. Probability-based protein identification by searching sequence databases using mass spectrometry data. Electrophoresis. 1999;20:3551-67.
[28] Dyrlund TF, Poulsen ET, Scavenius C, Sanggaard KW, Enghild JJ. MS Data Miner: A web-based software tool to analyze, compare, and share mass spectrometry protein identifications. Proteomics. 2012;12:2792-6.

[29] Johnson JL, Godden SM, Molitor T, Ames T, Hagman D. Effects of feeding heat-treated colostrum on passive transfer of immune and nutritional parameters in neonatal dairy calves. J Dairy Sci. 2007;90:5189-98.
[30] Faulkner S, Elia G, Mullen MP, O'Boyle P, Dunn MJ, Morris D. A comparison of the bovine uterine and plasma proteome using iTRAQ proteomics. Proteomics. 2012;12:2014-23.
[31] Klobasa F, Werhahn E, Butler JE. Composition of sow milk during lactation. Journal of animal science. 1987;64:1458-66.
[32] Hortin GL, Sviridov D. The dynamic range problem in the analysis of the plasma proteome. Journal of proteomics. 2010;73:629-36.
[33] Picotti P, Bodenmiller B, Mueller LN, Domon B, Aebersold R. Full dynamic range proteome analysis of S. cerevisiae by targeted proteomics. Cell. 2009;138:795-806.
[34] McDonald TL, Larson MA, Mack DR, Weber A. Elevated extrahepatic expression and secretion of mammary-associated serum amyloid A 3 (M-SAA3) into colostrum. Vet Immunol Immunop. 2001;83:203-11.
[35] Dupont D, Remond B, Collin JC. ELISA determination of plasmin and plasminogen in milk of individual cows managed without the dry period. Milchwissenschaft. 1998;53:66-9.
[36] Constant SB, LeBlanc MM, Klapstein EF, Beebe DE, Leneau HM, Nunier CJ. Serum immunoglobulin G concentration in goat kids fed colostrum or a colostrum substitute. Journal of the American Veterinary Medical Association. 1994;205:1759-62.
[37] Argüello A, Castro N, Zamorano MJ, Castroalonso A, Capote J. Passive transfer of immunity in kid goats fed refrigerated and frozen goat colostrum and commercial sheep colostrum. Small Ruminant Research. 2004;54:237-41.
[38] Gabriël S, Geldhof P, Phiri IK, Cornillie P, Goddeeris BM, Vercruysse J. Placental transfer of immunoglobulins in cattle infected with Schistosoma mattheei. Vet Immunol Immunop. 2005;104:265-72.
[39] Yamada M, Murakami K, Wallingford JC, Yuki Y. Identification of low-abundance proteins of bovine colostral and mature milk using two-dimensional electrophoresis followed by microsequencing and mass spectrometry. Electrophoresis. 2002;23:1153-60.
[40] Honkanenbuzalski T, Sandholm M. Trypsin-Inhibitors in Mastitic Milk and Colostrum - Correlation between Trypsin-Inhibitor Capacity, Bovine Serum-Albumin and Somatic-Cell Contents. J Dairy Res. 1981;48:213-23.
[41] Albera E, Kankofer M. Antioxidants in colostrum and milk of sows and cows. Reproduction in domestic animals = Zuchthygiene. 2009;44:606-11.
[42] Hurley WL, Theil PK. Perspectives on Immunoglobulins in Colostrum and Milk. Nutrients. 2011;3:442-74.
[43] Moreno-Indias I, Sanchez-Macias D, Castro N, Morales-delaNuez A, Hernandez-Castellano LE, Capote J, et al. Chemical composition and immune status of dairy goat colostrum fractions during the first 10 h after partum. Small Ruminant Research. 2012;103:220-4.
[44] Korhonen H, Pihlanto A. Technological options for the production of health-promoting proteins and peptides derived from milk and colostrum. Curr Pharm Design. 2007;13:829-43.
[45] Lahov E, Regelson W. Antibacterial and immunostimulating casein-derived substances from milk: Casecidin, isracidin peptides. Food and Chemical Toxicology. 1996;34:131-45.
[46] Gauthier SF, Pouliot Y, Saint-Sauveur D. Immunomodulatory peptides obtained by the enzymatic hydrolysis of whey proteins. Int Dairy J. 2006;16:1315-23.
[47] Silanikove N, Shapiro F, Shamay A, Leitner G. Role of xanthine oxidase, lactoperoxidase, and NO in the innate immune system of mammary secretion during active involution in dairy cows: manipulation with casein hydrolyzates. Free Radical Bio Med. 2005;38:1139-51.
[48] Montagne PM, Cuillière ML, Molé CM, Béné MC, Faure GC. Dynamics of the Main Immunologically and Nutritionally Available Proteins of Human Milk during Lactation. Journal of Food Composition and Analysis. 2000;13:127-37.



546 [49] Marnila P, Korhonen H. Milk | Colostrum. In: Editor-in-Chief: John WF, editor.
547 Encyclopedia of Dairy Sciences (Second Edition). San Diego: Academic Press; 2011. p. 591-7.
548 [50] Yang J, Goetz D, Li JY, Wang W, Mori K, Setlik D, et al. An iron delivery pathway mediated
549 by a lipocalin. Molecular cell. 2002;10:1045-56.
550 [51] Liu C, Xu Z, Gupta D, Dziarski R. Peptidoglycan recognition proteins: a novel family of four
551 human innate immunity pattern recognition molecules. The Journal of biological chemistry.
552 2001;276:34686-94.
553 [52] Lu X, Wang M, Qi J, Wang H, Li X, Gupta D, et al. Peptidoglycan recognition proteins are a
554 new class of human bactericidal proteins. The Journal of biological chemistry. 2006;281:5895-
555 907.
556 [53] Li Y, Yu WH, Ren J, Chen W, Huang L, Kharbanda S, et al. Heregulin targets gamma-catenin
557 to the nucleolus by a mechanism dependent on the DF3/MUC1 oncoprotein. Molecular cancer
558 research : MCR. 2003;1:765-75.
559 [54] Moncada DM, Kammanadiminti SJ, Chadee K. Mucin and Toll-like receptors in host
560 defense against intestinal parasites. Trends Parasitol. 2003;19:305-11.
561 [55] Schlimme E, Martin D, Meisel H. Nucleosides and nucleotides: natural bioactive
562 substances in milk and colostrum. The British journal of nutrition. 2000;84 Suppl 1:S59-68.
563 [56] Butler JE. Immunologic aspects of breast feeding, antiinfectious activity of breast milk.
564 Seminars in perinatology. 1979;3:255-70.
565 [57] Boutinaud M, Jammes H. Potential uses of milk epithelial cells: a review. Reprod Nutr Dev.
566 2002;42:133-47.
567 [58] Blum JW, Baumrucker CR. Colostral and milk insulin-like growth factors and related
568 substances: mammary gland and neonatal (intestinal and systemic) targets. Domestic animal
569 endocrinology. 2002;23:101-10.
570 [59] Wirtz PH, Redwine LS, Baertschi C, Spillmann M, Ehlert U, von Kanel R. Coagulation activity
571 before and after acute psychosocial stress increases with age. Psychosom Med. 2008;70:476-
572 81.
573 [60] Austin AW, Wirtz PH, Patterson SM, Stutz M, von Kanel R. Stress-induced alterations in
574 coagulation: assessment of a new hemoconcentration correction technique. Psychosom Med.
575 2012;74:288-95.
576 [61] Binev R, Slavova P, Laleva S. Effects of fasting on blood cells from labms of various breeds.
577 Trakia Journal of Sciences. 2006;4.
578 [62] Wu H, Chen L, Zhou Q, Zhang X, Berger S, Bi J, et al. Aqp2-expressing cells give rise to renal
579 intercalated cells. J Am Soc Nephrol. 2013;24:243-52.
580 [63] Kanaide H, Ichiki T, Nishimura J, Hirano K. Cellular mechanism of vasoconstriction induced
581 by angiotensin II: it remains to be determined. Circ Res. 2003;93:1015-7.
582 [64] Harrison-Bernard LM. The renal renin-angiotensin system. Advances in physiology
583 education. 2009;33:270-4.
584 [65] Simon T, Cook VR, Rao A, Weinberg RB. Impact of murine intestinal apolipoprotein A-IV
585 expression on regional lipid absorption, gene expression, and growth. Journal of lipid research.
586 2011;52:1984-94.
587 [66] Stan S, Delvin E, Lambert M, Seidman E, Levy E. Apo A-IV: an update on regulation and
588 physiologic functions. Bba-Mol Cell Biol L. 2003;1631:177-87.
589 [67] Kendrick JS, Chan L, Higgins JA. Superior role of apolipoprotein B48 over apolipoprotein
590 B100 in chylomicron assembly and fat absorption: an investigation of apobec-1 knock-out and
591 wild-type mice. Biochem J. 2001;356:821-7.
592 [68] Mahley RW, Rall SC. Apolipoprotein E: Far more than a lipid transport protein. Annu Rev
593 Genom Hum G. 2000;1:507-37.

[69] Peterson MM, Mack JL, Hall PR, Alsup AA, Alexander SM, Sully EK, et al. Apolipoprotein B Is an Innate Barrier against Invasive Staphylococcus aureus Infection. Cell Host Microbe. 2008;4:555-66.
[70] Maezawa I, Maeda N, Montine TJ, Montine KS. Apolipoprotein E-specific innate immune response in astrocytes from targeted replacement mice. J Neuroinflamm. 2006;3.
[71] Vowinkel T, Mori M, Krieglstein CF, Russell J, Saijo F, Bharwani S, et al. Apolipoprotein A-IV inhibits experimental colitis. The Journal of clinical investigation. 2004;114:260-9.
[72] Okumura T, Fukagawa K, Tso P, Taylor IL, Pappas TN. Intracisternal injection of apolipoprotein A-IV inhibits gastric secretion in pylorus-ligated conscious rats. Gastroenterology. 1994;107:1861-4.
[73] Tamzali Y, Guelfi JF, Braun JP. Plasma fibrinogen measurement in the horse: comparison of Millar's technique with a chronometric technique and the QBC-Vet Autoreader. Research in veterinary science. 2001;71:213-7.
[74] Ganheim C, Hulten C, Carlsson U, Kindahl H, Niskanen R, Waller KP. The acute phase response in calves experimentally infected with bovine viral diarrhoea virus and/or Mannheimia haemolytica. Journal of veterinary medicine B, Infectious diseases and veterinary public health. 2003;50:183-90.
[75] Akassoglou K, Strickland S. Nervous system pathology: The fibrin perspective. Biol Chem. 2002;383:37-45.
[76] Holtet TL, Graversen JH, Clemmensen I, Thogersen HC, Etzerodt M. Tetranectin, a trimeric plasminogen-binding C-type lectin. Protein Science. 1997;6:1511-5.
[77] Renckens R, Roelofs JJTH, Florquin S, van der Poll T. Urokinase-type plasminogen activator receptor plays a role in neutrophil migration during lipopolysaccharide-induced peritoneal inflammation but not during Escherichia coli-induced peritonitis. Journal of Infectious Diseases. 2006;193:522-30.
[78] Mazumder B, Mukhopadhyay CK, Prok A, Cathcart MK, Fox PL. Induction of ceruloplasmin synthesis by IFN-gamma in human monocytic cells. J Immunol. 1997;159:1938-44.



621 Table 1. Colostrum proteins identified in sheep colostrum and lamb plasma from

622 colostrum and delayed colostrum lambs.

Protein name	Accession No.
Proteins identified in all samples	
alpha globin chain [Ovis aries]	gi|1787
alpha-2-macroglobulin precursor (Blast2Go, Similarity: 88.35%)	E1BJW0
Apolipoprotein A-I	P15497
RecName: Full=Apolipoprotein E; Short=Apo-E; Flags: Precursor	gi|41016830
Apolipoprotein A-IV	F1N3Q7
Apolipoprotein D	F1MS32
beta-lactoglobulin [Ovis sp.]	gi|165839
Complement C3	Q2UVX4
RecName: Full=Ceruloplasmin; AltName: Full=Ferroxidase; Flags: Precursor	gi|75075054
Fibrinogen beta chain	F1MAV0
gamma fibrinogen, partial [Ovis dalli]	gi|1916272
fibronectin isoform 1 (Blast2Go, Similarity: 97.0%)	G5E5A9
gelsolin isoform a precursor (Blast2Go, Similarity: 99.05%)	F1MJH1
Ig lambda chain C region - sheep (fragment)	gi|109030
immunoglobulin lambda light chain constant region segment 1 [Ovis aries]	gi|52366986
immunoglobulin mu chain, partial [Ovis aries]	gi|165945
immunoglobulin V lambda chain [Ovis aries]	gi|2766644
RecName: Full=Alpha-1-antiproteinase; AltName: Full=Alpha-1-antitrypsin; AltName: Full=Alpha-1-proteinase inhibitor; Flags: Precursor	gi|112890
trypsin inhibitor (contrapsin) – goat	gi|1363062
inter-alpha-trypsin inhibitor heavy chain h2 (Blast2Go, Similarity: 93.4%)	F1MNW4
keratin 1 (Blast2Go, Similarity: 95.45%)	G3N0V2
Keratin 10 (Epidermolytic hyperkeratosis; keratosis palmaris et plantaris)	A6QNZ7
keratin 14 (Blast2Go, Similarity: 98.0%)	F1MC11
keratin 18 (Blast2Go, Similarity: 94.7%)	F6S1Q0
RecName: Full=Plasminogen	gi|3914364
Plasminogen	E1B726
pregnancy-zone protein (Blast2Go, Similarity: 80.65%)	F1MI18
Serotransferrin	G3X6N3
RecName: Full=Serum albumin; Flags: Precursor	gi|113582
type ii cytoskeletal 2 epidermal (Blast2Go, Similarity: 90.3%)	E1B991
Colostrum specific proteins which could also be observed in plasma 14 hours after early access to colostrum	
apolipoprotein b-100 (Blast2Go, Similarity: 85.6%)	E1BNR0
Ig heavy chain C region - sheep (fragment)	gi|109029
Ig kappa chain – sheep	gi|423311
immunoglobulin alpha heavy chain [Ovis aries]	gi|2582411
Immunoglobulin J chain	Q3SYR8
immunoglobulin lambda-6c light chain variable region [Ovis aries]	gi|2746699
immunoglobulin light chain variable region (Blast2Go, Similarity: 92.2%)	G3N2D7
Inter-alpha-trypsin inhibitor heavy chain H1	Q0VCM5

Colostrum proteins which could not be observed in plasma after 14 hours after early access to colostrum

45 kda calcium-binding protein (Blast2Go, Similarity: 95.0%)	F1MKI5
adipocyte fatty acid-binding protein [Capra hircus]	gi|118582237
RecName: Full=Alpha-lactalbumin; AltName: Full=Lactose synthase B protein; Flags: Precursor	gi|125998
RecName: Full=Alpha-S2-casein; Flags: Precursor	gi|115658
alpha-S1-casein precursor [Ovis aries]	gi|57526469
alpha-s2-casein [Ovis aries]	gi|284025656
ATP synthase subunit alpha	F1MLB8
ATP synthase subunit beta, mitochondrial	P00829
RecName: Full=Beta-casein; Flags: Precursor	gi|416752
cohesin subunit sa-1 (Blast2Go, Similarity: 98.95%)	F1MC39
desmoplakin isoform i (Blast2Go, Similarity: 96.35%)	E1BKT9
Elongation factor 1-alpha	E1B9F6
Fatty acid-binding protein, heart	P10790
Fc-gamma-RII-D	A8DC37
Ig mu chain – sheep	gi|478694
Ig mu heavy chain V region precursor - sheep (fragment)	gi|1083124
Ig mu heavy chain V region precursor - sheep (fragment)	gi|1083106
immunoglobulin gamma 2 heavy chain constant region [Capra hircus]	gi|147744654
immunoglobulin heavy chain precursor [Ovis aries]	gi|5815188
immunoglobulin heavy chain precursor [Ovis aries]	gi|5815218
immunoglobulin heavy chain variable region [Ovis aries]	gi|23304497
immunoglobulin heavy chain variable region precursor, partial [Capra hircus]	gi|164664986
immunoglobulin lambda-2b light chain variable region [Ovis aries]	gi|2746691
immunoglobulin light chain variable region [Ovis aries]	gi|5802430
immunoglobulin V lambda chain 4.1.3 [Ovis aries]	gi|26245483
junction plakoglobin (Blast2Go, Similarity: 98.35%)	F1N3P9
Keratin, type I cytoskeletal 17	A1L595
Keratin, type II cytoskeletal 75	Q08D91
KRT5 protein	A5D7M6
Malate dehydrogenase (Fragment)	G1K257
Matrix Gla protein	P07507
milk fat globule EGF factor 8 protein, partial [Ovis aries]	gi|347803268
neutrophil gelatinase-associated lipocalin isoform 2 (Blast2Go, Similarity: 86.45%)	E1B6Z6
Nucleobindin-1	Q0P569
pancreatic secretory granule membrane major glycoprotein gp2 (Blast2Go, Similarity: 86.0%)	F1N726
Peptidoglycan recognition protein 1	Q8SPP7
Peptidyl-prolyl cis-trans isomerase	Q2NKS8
Peptidyl-prolyl cis-trans isomerase B	P80311
Peroxiredoxin-1	Q5E947
Pigment epithelium-derived factor	Q95121
polymeric immunoglobulin receptor (Blast2Go, Similarity: 81.7%)	F1MR22
RecName: Full=Glycosylation-dependent cell adhesion molecule 1; Short=GlyCAM-1; AltName: Full=28 kDa milk glycoprotein PP3; AltName: Full=Lactophorin; AltName: Full=Proteose-peptone component 3; Short=PP3; Flags: Precursor	gi|22096365
secreted form immunoglobulin epsilon heavy chain constant partial (Blast2Go, Similarity: 74.3%)	G3N342
type i cytoskeletal 42-like (Blast2Go, Similarity: 95.15%)	G3N2P6
type ii cytoskeletal 3 (Blast2Go, Similarity: 95.8%)	G3MXL3

Proteins identified only in plasma from DC group at 14 h after birth

Adiponectin	Q3Y5Z3
Alpha-2-antiplasmin	P28800
antithrombin-III precursor [Ovis aries]	gi|57164383
Apolipoprotein A-II	P81644
apolipoprotein m (Blast2Go, Similarity: 93.7%)	F1MYX2
Beta-2-glycoprotein 1	P17690
bone marrow stromal cell antigen 1 (Blast2Go, Similarity: 86.65%)	F1MLP3
C1QC protein (Fragment)	Q1RMH5
C4b-binding protein alpha chain	Q28065
Carboxypeptidase N catalytic chain	Q2KJ83
Chain D, Crystal Structure Determination Of Sheep Methemoglobin At 2.7 Angstrom Resolution	gi|195927260
chymotrypsinogen b (Blast2Go, Similarity: 79.85%)	E1BDT3
coagulation factor a1 polypeptide (Blast2Go, Similarity: 94.3%)	F1MW44
Coagulation factor X	P00743
Coagulation factor XIII, B polypeptide	Q2TBQ1
collagen alpha-1 chain isoform 2 (Blast2Go, Similarity: 98.15%)	F1MSR8
Complement C1s subcomponent	Q0VCX1
Complement component 1, r subcomponent	A5D9E9
complement component 4a (rodgers blood group) (Blast2Go, Similarity: 90.3%)	F1N2Q0
complement component C4 [Ovis aries]	gi|1235
complement factor B [Ovis aries]	gi|148645283
CPN2 protein	A6QP30
C-reactive protein	C4T8B4
Dermatopontin	P19427
eph receptor a7 (Blast2Go, Similarity: 97.75%)	F1N780
extracellular matrix protein 1 (Blast2Go, Similarity: 81.65%)	F1MWT6
Fetuin-B	Q58D62
fibulin 1 (Blast2Go, Similarity: 93.9%)	F1MYN5
Flavin reductase (NADPH)	P52556
forkhead box protein k2 (Blast2Go, Similarity: 90.1%)	F1MW83
Fructose-bisphosphate aldolase	A6QLL8
Glutathione peroxidase	G3X8D7
glutathione s-transferase mu 3 (Blast2Go, Similarity: 87.9%)	E1B7U2
RecName: Full=Haptoglobin; AltName: Full=Zonulin; Contains: RecName: Full=Haptoglobin alpha chain; Contains: RecName: Full=Haptoglobin beta chain; Flags: Precursor	gi|226709030
haptoglobin [Rupicapra rupicapra]	gi|307742657
hemoglobin gamma	gi|229222
hepatocyte growth factor activator (Blast2Go, Similarity: 88.85%)	E1BCW0
Histidine-rich glycoprotein	F1MKS5
histone h2b type 1-like (Blast2Go, Similarity: 96.15%)	E1B7N8
Insulin-like growth factor-binding protein 2	F1N2P8
Kininogen-1	P01044
Leucine-rich alpha-2-glycoprotein 1	Q2KIF2
L-lactate dehydrogenase B chain	Q5E9B1
Lumican	Q05443
lysine-specific demethylase 5c isoform 1 (Blast2Go, Similarity: 97.45%)	F1MYV2
n-acetylmuramoyl-l-alanine amidase (Blast2Go, Similarity: 81.25%)	E1BH94
Phospholipid transfer protein	Q58DL9
pigment epithelium-derived factor precursor [Ovis aries]	gi|213021132
Plasma serine protease inhibitor	Q9N2I2
Proteasome subunit beta type-6	Q3MHN0
Protein HP-20 homolog	Q2KIT0
prothrombin precursor [Ovis aries]	gi|261244968
RecName: Full=Angiotensinogen; AltName: Full=Serpin A8; Contains: RecName:	gi|1703309

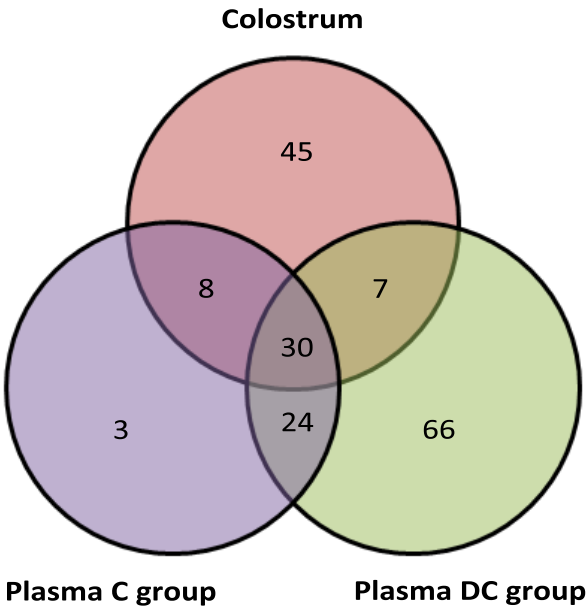
Full=Angiotensin-1; AltName: Full=Angiotensin I; Short=Ang I; Contains: RecName: Full=Angiotensin-2; AltName: Full=Angiotensin II; Short=Ang II; Contains: RecName: Full=Angio	
RecName: Full=Carbonic anhydrase 2; AltName: Full=Carbonate dehydratase II; AltName: Full=Carbonic anhydrase II; Short=CA-II	gi|118582300
Serpin A3-2	A2I7M9
Serpin A3-5	A2I7N1
SERPIND1 protein	A6QPP2
Serum albumin	P02769
Serum amyloid A-4 protein	Q32L76
SHBG protein	A5PKC2
Superoxide dismutase [Cu-Zn]	A3KLR9
transferrin receptor protein 1 (Blast2Go, Similarity: 87.9%)	E1BIG6
transferrin receptor, partial [Ovis aries]	gi|369726201
Triosephosphate isomerase	Q5E956
Vitamin K-dependent protein C (Fragment)	P00745
Vitamin K-dependent protein S	P07224

623 Table 2. Significantly different protein abundances (P<0.05) between animals receiving

624 colostrum early (2 hours) (C group) versus late (14 hours after birth) (DC group).

		C group		DC group		P-value		
Protein	Accession	2h	14h	2h	14h	C2 vs DC2	C2 vs C14	C14 vs DC14
Apolipoprotein A-IV	F1N3Q7	0.87	1.45	0.74	1.04	0.608	0.018	0.018
Apolipoprotein B-100	E1BNR0	0.58	1.17	0.82	0.90	0.355	0.041	0.028
Apolipoprotein E	Q03247	0.65	1.58	0.90	0.96	0.499	0.009	0.006
Ceruloplasmin precursor	F1N076	0.62	1.00	0.75	0.83	0.482	0.035	0.039
Fibrinogen Alpha Chain	A5PJE3	0.69	1.04	0.77	1.02	0.484	0.048	0.042
Tetranectin	Q2KIS7	0.69	1.07	0.85	1.02	0.737	0.013	0.004
Immunoglobulin mu chain partial	gi|165945	0.82	1.02	0.94	1.06	0.104	0.025	0.043
Trypsin Inhibitor	gi|1363062	0.77	0.96	0.98	1.00	0.250	0.013	0.041

625 Figure 1. Venn diagram of colostrum and lamb plasma from colostrum and delayed-
626 colostrum groups.



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Conclusiones

Capítulo 1

El calostro juega un papel fundamental en procesos tan importantes para el recién nacido como la transferencia de inmunidad pasiva y el desarrollo del sistema inmune, disminuyendo los porcentajes de mortalidad neonatal y por lo tanto, aumentando los beneficios económicos de los ganaderos. Sin embargo, el calostro no solo es importante por su contenido de Ig´s, sino también porque contiene otras proteínas que juegan un papel fundamental en la activación y atracción de células con función inmune, la disminución de las secreciones gástricas, entre otras. Además, ha sido descrito la relación existente entre las proteínas procedentes de las diferentes fracciones del calostro (caseínas, proteínas del suero y proteínas de la membrana del glóbulo graso).

En este campo, la proteómica es una herramienta fundamental para incrementar el conocimiento general de las proteínas presentes en el calostro y su relación con la transferencia de inmunidad pasiva y el desarrollo del sistema inmune. Sucesivos estudios serán necesarios para entender los procesos biológicos que ocurren durante la absorción de las proteínas a través del intestino de los rumiantes neonatos.

Capítulo 2

Los corderos criados bajo un sistema de lactancia natural mostraron, en general, pesos vivos y mayores valores de parámetros inmunes que los corderos alimentados en un sistema de lactancia artificial criados con diferentes dietas (lactorremplazante o leche en polvo entera para consumo humano) durante el periodo de lactancia. Sin embargo, es necesario resaltar que durante el destete estos grupos de lactancia artificial compensaron estas diferencias, mostrando, incluso al final de este periodo, mayores pesos vivos que los corderos de lactancia natural.

Los resultados obtenidos pueden ayudar a mejorar el manejo de las granjas ovinas, reduciendo los gastos que conlleva la lactancia artificial y por tanto, incrementando los beneficios económicos de los ganaderos.

Capítulo 3

Los corderos criados bajo un sistema de lactancia natural mostraron, en general, mayores valores en los parámetros inmunes y mayor peso vivo que los corderos criados bajo los diferentes sistemas de lactancia artificial usados durante los primeros días de vida. Sin embargo, será necesario realizar otros estudios basado en cambios en el manejo de la lactancia artificial, con el fin de conseguir que los corderos criados en este sistema presenten valores inmunes similares a los obtenidos en lactancia natural.

Los resultados de este estudio demuestran además, que cuando la lactancia artificial es usada, los corderos pueden ser encalostrados con calostro caprino, siendo además no necesario encalostrar a los animales inmediatamente después del parto.

Capítulo 4

Los corderos que recibieron 8 gramos de IgG/ kg de peso vivo obtuvieron similares concentraciones plasmáticas de IgG y mayores de IgM, que los corderos criados en lactancia natural (grupo NR) durante los primeros días de vida. Sin embargo, durante este periodo el grupo NR presentó una mejor actividad del sistema de Complemento que los otros dos grupos de estudio (grupo C4 y C8). Durante el destete, los dos grupos de lactancia artificial estudiados, presentaron un mayor peso vivo, una mayor

concentración de IgM, asi como similares concentraciones plasmáticas de IgG y actividad del Complemento que los corderos criados en lactancia natural.

Los hallazgos de este estudio muestran que cuando los corderos reciben la cantidad de calostro equivalente a 8g de IgG/kg de peso vivo, estos obtienen valores inmunes similares a los obtenidos en lactancia natural.

Capítulo 5

El consumo temprano de calostro produjo un incremento de la concentración de apolipoproteina-IV, plasminógeno, amiloide sérico A y fibrinógeno, lo cual es de gran interés, ya que presentan funciones relacionadas con el sistema inmune, demostrando que el calostro no solo es fundamental por el aporte de inmunoglobulinas, sino porque también contiene proteínas que desempeñan un papel fundamental en la respuesta inmune del neonato, tales como son la activación y atracción de las células inmunes, la regulación de la apoptosis en los enterocitos o la disminución de las secreciones gástricas.

Los resultados de este trabajo muestran información sobre las proteínas con función inmune que se incrementan en el plasma sanguíneo de los corderos recién nacidos tras la ingesta de calostro, los cuales pueden ser usados para disminuir la mortalidad neonatal y así aumentar el beneficio económico de los ganaderos. En el futuro será necesario realizar otros estudios proteómicos para así aumentar el conocimiento sobre el papel del calostro en la transferencia de inmunidad pasiva.

Capítulo 6

Los resultados de este trabajo describieron la presencia de 90 proteínas en el proteoma del calostro ovino. Además, en cuanto a los cambios en el perfil proteómico del plasma sanguíneo de cordero, la ingesta temprana de calostro produjo un incremento de 8 proteínas con función inmune. Estos datos pueden ser usados para disminuir los porcentajes de mortalidad y por ende contribuir al incremento del beneficio económico de los ganaderos. En el futuro, estudios proteómicos usando SRM, podrían aumentar el conocimiento general del calostro y su relación en la transferencia de inmunidad pasiva.

Conclusions

Chapter 1

Colostrum plays an essential role in several important processes in the newborn, such as PIT and immune system development, decreasing ruminant neonate mortality rates and increasing the economic benefit of farmers. However, colostrum is not only important for its Ig´s content but also for the non-immunoglobulin proteins that play a fundamental role in the activation and attraction of immune cells, the low gastric secretion, among others. It has been also observed the relation of different proteins from the three colostrum fractions (caseins, whey and MFGM) with the newborn immune protection.

In this field, proteomics is a powerful tool that needs to be used in the future to increase the general knowledge of proteins present in colostrum and their relation with the PIT and the immune system development. Moreover, further studies will be necessary in order to understand the biological process of colostrum proteins uptake trough the gut in newborn ruminants.

Chapter 2

Lambs reared under a natural rearing system showed, in general, higher BW and immune parameters than lambs reared under artificial systems with different milk sources during the milk feeding period. However, it is interesting to highlight that during the weaning period, animals from both artificial rearing groups compensated for these differences. For this reason, at the end of the studied period MR and CM groups showed a higher BW than NR group.

These findings may improve the dairy sheep management, reducing the expenses of the artificial rearing system and, consequently, increasing the economic benefit for sheep farmers.

Chapter 3

Lambs from the natural rearing system showed in general, higher immune condition and BW than animals reared under the different artificial system during the first days after birth. However, further studies will be necessary in order to achieve a similar initial IgG concentration in the artificial rearing groups compared to the natural ones.

These findings may improve the management systems in place in lamb farms as it shows that it is not necessary to colostrum feed immediately after birth and, also, that goat colostrum could be used to bottle fed newborn lambs.

Chapter 4

Lambs that received 8 g of IgG/ kg of BW (C8 group) were able to reach similar IgG and higher IgM concentration in blood than lambs reared under natural conditions (NR group) during the first days after birth. However, NR group obtained higher Complement System activity than any of the other groups (C4 and C8) in the same period. During weaning, both artificial rearing groups (C4 and C8) showed a higher BW and IgM and a similar IgG concentration and Complement system activity than lambs reared under natural conditions.

This study reveals important information about the amount of colostrum that needs to be given to newborn lambs reared under artificial conditions in order to reach similar immune values to those reared under natural conditions.

Chapter 5

Early colostrum intake produced an overexpression of non-immunoglobulin proteins, such as apoliprotein A-IV, plasminogen, serum amyloid A and fibrinogen. In addition, all of these overexpressed proteins have been previously described as having immune functions, demonstrating that colostrum is essential not only by its immunoglobulin content but also by the non-immunoglobulin proteins that play a fundamental role in the activation and attraction of immune cells, the apoptosis rate of the enterocytes and the low gastric secretion, among others. The results of this work contribute information about proteins with immune function that are increased after colostrum intake, that can be used to decrease the lamb mortality and for instance to increase the economic benefit for farmers. In future, further proteomic studies will be necessary in order to increase the general knowledge about the role of colostrum in the passive immune transfer.

Chapter 6

The results of this work described the presence of 90 proteins in the sheep colostrum proteome. Moreover, 8 proteins with immune function were increased in the lamb blood plasma after colostrum intake. Understanding uptake and effect of colostrum is important for future reduction of lamb mortality rates and will also contribute to increase the economic benefit of sheep producers. Further proteomic studies will be

necessary, particularly using the SRM approach, in order to increase the general knowledge about the role of colostrum in the PIT.