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A proteomics study of colostrum and milk from the two major small ruminant dairy breeds from the Canary Islands: a bovine milk comparison perspective

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Colostrum and milk feeding are key factors for the newborn ruminant survival, affecting the future performance of the animal. Nowadays, there is an increasing interest in the potential of feeding newborn ruminants (mainly goat kids and lambs) with colostrum and milk from other more productive ruminant species (mainly cows). Although some studies regarding differences between colostrum and milk from these three species have been performed, herein we conduct for the first time a comparison using a proteomics 2-Dimensional Electrophoresis gel-based approach between these three ruminant species. In this study colostrum and milk samples from six Holstein cows, six Canarian sheep and six Majorera goats were used to determine the chemical composition, immunoglobulin G (**IgG**) and M (**IgM**) concentrations and proteomics profiles. Results showed that in general sheep colostrum and milk contained higher fat, protein and lactose percentages compared to bovine and goat samples. Additionally, no differences in the IgG or IgM concentrations were found among any of the three studied species, with the exception of sheep colostrum that showed the highest IgM concentration. With reference to the proteomics-based approach, some high abundant proteins such as serum albumin precursor, beta-caseins or different immunoglobulins components were found in colostrum, milk or even both. Nevertheless, differences in other proteins with immune function such as serotransferrin or lactoperoxidase were detected. This study shows that despite the similar immunoglobulin concentrations in colostrum and milk from the three studied species, differences in several immune components can be detected when these samples are studied using a proteomics approach. Finally, this study also provides a base for future investigation in colostrum and milk proteomics and metabolomics.

Keywords: proteomics, colostrum, milk, small ruminants, immune.

Colostrum is the first secretion from the mammary gland after parturition and it starts changing after birth, becoming mature milk (Hernández-Castellano et al. 2014a; Lérias et al. 2014). Colostrum feeding in mammals is very important to provide protection (passive immune transfer, **PIT**) against infections in newborn mammals (Czesnikiewicz-Guzik et al. 2007a; Castro et al. 2011; Hernández-Castellano et al. 2015a). The interest for heterologous PIT such as immunoglobulins **(Ig)** obtained from one species and utilised for passive immunity in other species is

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increasing worldwide (Hurley & Theil, 2011; Hernández-Castellano et al. 2015b). This tendency is extended in orphan ruminants, because the homology between cattle, goats and sheep Ig ensures a biological activity of these macromolecules in the different species (Curtain & Fudenber, 1973). Additionally, this method can also be used in those born from dams with inadequate colostrum, or those not allowed to suckle their dams (Morales-delaNuez et al. 2011), where an alternative source of colostrum or commercially available colostrum substitute is used (Tsiligianni et al. 2012). Despite the importance of colostrum in newborn survival, differences in milk composition can also have severe consequences in newborn ruminants. In dairy intensive systems, such as those found throughout the Canary Islands, newborn ruminants are separated from dams and fed with a milk replacer or another milk source (Hernández-Castellano et al. 2015c). As described above, milk composition is an important aspect as a non-balanced feeding source may affect animal performance in young ruminants (Hernández-Castellano *et al.*, 2013a, b) with severe consequence for animal welfare. Therefore, further research focus on differences within colostrums and milks from different ruminant species is required. We hypothesise that even though some differences between colostrum and milk from different ruminant species has already been described, the use of proteomics, based on 2D-gel electrophoresis, complemented with traditional methods based on gross chemical composition (fat, protein, lactose, dry matter percentage and immunoglobulin concentration) will provide further information about these two fluids from the two most relevant ruminant species dairy breeds in the Canary Islands, the Canarian sheep and the Majorera goat (Lérias et al. 2013; Hernández-Castellano et al. 2014a). In order to obtain a comparison term, we have also contrasted the results for each fluid to those of colostrum and milk of the most widely found dairy ruminant species and breed across the globe: the Holstein cow.

Material and methods

Spanish and European Union guidelines and legislation on care, use and handling of experimental farm animals were followed. All samples (colostrum and milk) used in this study were obtained at the milking parlour after animals were completely milked. This study did not involve any capture of live animals or animal experimentation, thus no specific ethical approval was necessary. During the experimental period, animals were under veterinary supervision. Animal health status was monitored (for diarrhoea, mastitis or fever) and they were found to be healthy throughout the experimental period.

Sample collection

Six Holstein cows, six Canarian sheep and six Majorera goats in their second lactation were used in this experiment. Animals were fed following recommendations of the *Institut National de la Recherche Agronomique* (INRA, 2007). The experiment took place at the experimental farm of the Veterinary Faculty of the Universidad de Las Palmas de Gran Canaria (Canary Islands, 28°8′20·66″N, 15°30′ 24·97″W, Spain) during spring. Colostrum samples (20 ml) from the whole available colostrum were individually taken immediately after parturition in the three studied species. Similarly, milk samples (20 ml) were individually collected 20 d after parturition. Both colostrum and milk samples were frozen (−80 °C) until further analysis.

Sample treatment for analysis

Colostrum and milk samples (10 ml each) were centrifuged following the procedure described by Boehmer et al. (2008). In this procedure samples were centrifuged at 44 000 × ***g*** at 4 °C for 30 min, and the fat layer was removed with a spatula. The skimmed milk was decanted into a clean tube, centrifuged at 44 000 × ***g*** at 4 °C for 30 min and the translucent supernatant (whey fraction) was collected and stored at −80 °C. Caseins have been the principle target of high-abundance protein removal in sample preparation of milk from healthy cows.

Chemical composition and Ig concentration

Fat, protein, lactose and total solids contents of different colostrum and milk samples (as well as their respective whey fractions) were determined by routine laboratory procedures using an automated infrared method with a DMA2001 Milk Analyser (Miris Inc., Uppsala, Sweden). Quantification of Ig in colostrum and milk (and their respective whey fractions) were performed using IgG and IgM ELISA kits (Bethyl Laboratories, Montgomery, TX, USA).

Differences in chemical composition and Ig concentration within colostrum, milk, colostrum whey and milk whey from goat, sheep and cow were analysed using the ANOVA procedure of SAS (Version 9.0, Institute Inc., Cary, NC, USA). Results were expressed as mean ± SD. Significant differences required $P < 0{\cdot}05$ for all measured parameters.

Two-dimensional gel electrophoresis (2DE)

Samples from colostrum and milk whey fractions were desalted with ReadyPrep 2D-Clean-up kit (Biorad, Hercules, CA, USA). The protein concentration of these samples was subsequently determined using the Quick Start Protein Assay kit (Biorad, Hercules, CA, USA), setting bovine gammaglobulin as a standard (Biorad, Hercules, CA, USA). Then, every whey protein sample (400 µg) was diluted in rehydration buffer (8 M urea, 2% (w/v) 3-[(3-Cholamidopropyl) dimethylammonio]-1-propanesulfonate (CHAPS), 50 mM dithiothreitol, 0·2% (w/v) BioLyte 3/10 ampholyte, 0·002% (w/v) bromophenol Blue)) to a final sample volume of 300 µl. After dilution in rehydration buffer, samples were applied to

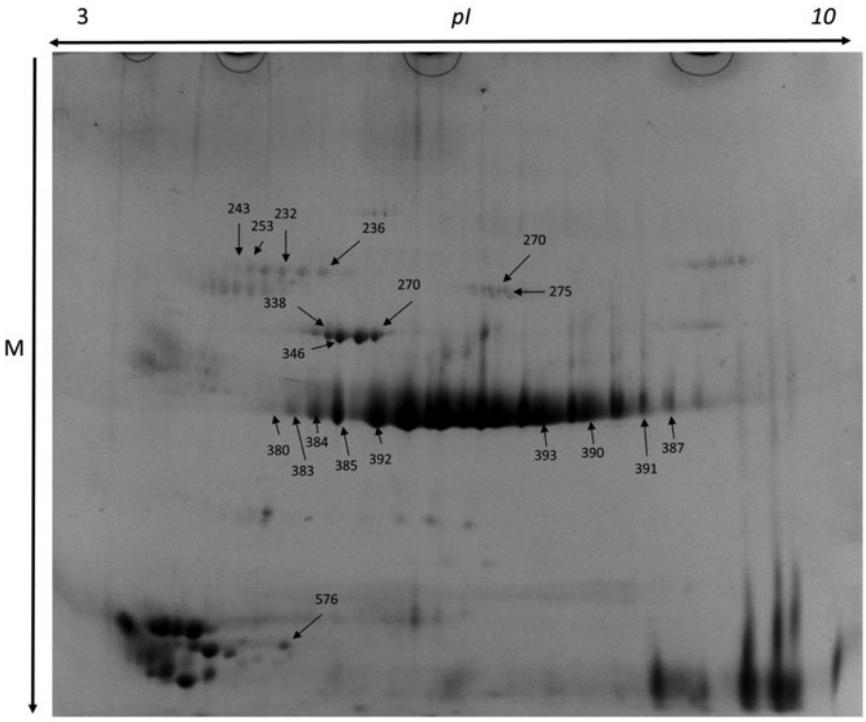

Fig. 1. Colostrum reference gel. Spots showing differential expression are highlighted with arrows. pI, Isoelectric point and M, Molecular Mass.

17-cm pH 3-10 nonlinear immobilised pH gradient (IPG) strips (Biorad, Hercules, CA, USA) and focused in a Bio-Rad Protean IEF Cell for 20 h using the following voltage intervals: 500 V for 1 h, 1000 V for 1 h, 2000 V for 2 h, 4000 V for 4 h, and 8000 V for 12 h, as described by Boehmer et al. (2008).

Subsequently, strips were equilibrated with equilibration buffer (6 M urea, 2% (w/v) SDS, 50 mM Tris-HCl pH 8·8, 30% (v/v) glycerol and 0·02% bromophenol blue solution (1%)), in two steps of 15 min with 1% (w/v) dithiothreitol and 2·5% (w/v) iodoacetamide, respectively as previously described by Almeida et al. (2010).

Second dimension was conducted after equilibration using 12·5% polyacrylamide gels on a Protean II xi Cell electrophoresis system (Biorad, Hercules, CA, USA) using the running conditions as recommended by the manufacturer (1 W/gel for 1 h and 2 W/gel for 14–16 h at 12 °C). Each gel was stained using Coomassie Brillant Blue G-250 as previously described by Almeida et al. (2010) and scanned with a Gel Doc XR system (Biorad, Hercules, CA, USA).

Image analysis

In order to detect differentially expressed proteins, gels were analysed using Progenesis SameSpots software (Nonlinear Dynamics, Newcastle upon Tyne, UK). Spots with $P < 0{\cdot}05$ and a fold intensity higher than 1·5 were considered to have significantly different expression levels.

Spot excision and digestion

Protein spots of interest (Figures 1 and 2) were excised from the gel with a sterile 1000 µl pipette tip or a sterile stainless scalpel blade for individual in-gel digestion using trypsin as described by Almeida et al. (2010). Briefly, spots were washed with 30 µl of water for 30 min, washed in acetonitrile (50%), reduced with 10 mM dithiothreitol at 56 °C for 45 min, alkylated with 55 mM iodoacetamide for 30 min, washed in acetonitrile (100%) and vacuum dried (SpeedVac®, Thermo Fisher Scientific, Waltham, MA, USA). Gel pieces were rehydrated with a digestion buffer (50 mM NH4HCO3 buffer) containing 50 µl of trypsin (6·7 ng/µl; Promega, Madison, WI, USA) and incubated overnight at 37 °C. The digestion buffer containing the 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 Printz et al. (2013). Briefly, peptide mass determinations were carried out using the 5800 Proteomics Analyser (ABsciex) in reflectron mode for both peptide mass fingerprint and MS/MS. Calibration was performed with the peptide mass calibration kit for 4700 (ABsciex). Protein identification

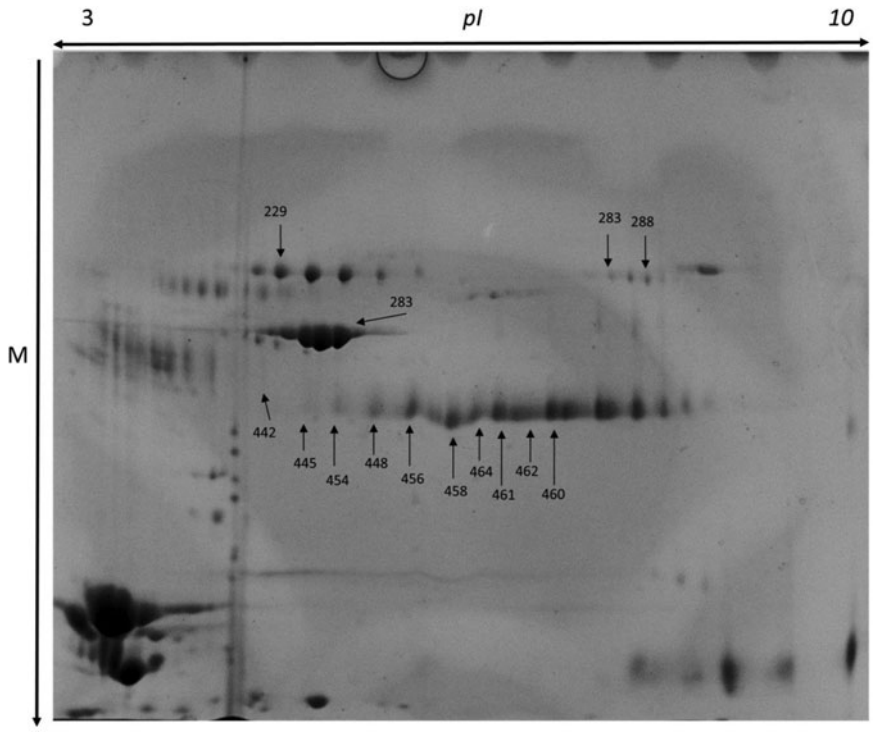

Fig. 2. Milk reference gel. Spots showing differential expression are highlighted with arrows. pI, Isoelectric point and M, Molecular Mass.

was done by searching the MS and MS/MS data against NCBI database in the Other Mammalia taxonomy (434 586 sequences), using an in house MASCOT 2·3 server (www.matrixscience.com). Two trypsin missed cleavages, four dynamic modifications (methionine and tryptophan oxidation, tryptophan dioxydation and tryptophan to kynurenin), and carbamidomethylation of cysteine as fixed modification were allowed. Mass accuracy was set to 100 ppm for parent ions and 0·5 Da for MS/MS fragments. Homology identification was retained with probability set at 95%. All identifications were confirmed manually.

Results and discussion

Chemical composition

Table 1 shows the chemical composition and Ig concentration of goat, sheep and cow colostrum and milk. As expected, fat percentage was lower in cow colostrum (6·66%) than those measured in goats and sheep (9·06 and 9·94%, respectively). Similarly, the fat percentage in cow milk was lower (3·26%) compared to goat or sheep milk (4·47 and 6·39%, respectively). No differences were detected when sheep and goat colostrum were compared, although sheep showed higher milk fat percentage. Results described above are in accordance with previous studies in these three different species. Abd El-Fattah et al. (2012) described similar milk fat percentages in Holstein cows to those presented in this study (4·00 and 3·26%, respectively), although these authors observed higher fat percentage in colostrum (8·04%). Similarly, Moreno-Indias et al. (2012) and Banchero et al. (2004) showed similar fat percentages in Majorera goat colostrum (8·70%) and Corriedale sheep colostrum (10·60%), respectively. Additionally, Álvarez et al. (2007) and James et al. (2001) found similar fat percentages to those reported in this study in Majorera goat milk (4·39%) and Lacaune sheep milk (6·86%), respectively.

Some differences in colostrum and milk protein percentages were also detected among the studied species. Sheep colostrum showed higher protein percentage than goat colostrum (13·94 and 10·16%, respectively). No differences were observed when goat or sheep colostrum was compared to bovine colostrum. Conversely, when these differences were studied in milk, sheep obtained the highest protein percentage (6·60%). This study shows lower values in colostrum protein percentages compared to other studies in Holstein cows (Abd El-Fattah et al. 2012), Majorera goats (Moreno-Indias et al. 2012) and Corriedale sheep (Banchero et al. 2004), although milk protein percentages were similar to those described in other studies (Park et al. 2007; Abd El-Fattah et al. 2012; Torres et al. 2013).

No differences in the lactose percentage in colostrum between studied species were detected. Nevertheless, sheep milk had a higher lactose percentage (5·75%) than cow or goat milk (4·35 and 4·17%, respectively). Similar values were observed by Abd El-Fattah et al. (2012) in Holstein cow colostrum and milk. Additionally, Moreno-Indias et al. (2012) and Banchero et al. (2006) found

Table 1. Chemical composition and immunoglobulins concentration (mean ± SD) of colostrum and milk from goat, sheep and cow

	Whole colostrum			Whole milk			Colostrum whey			Milk whey		
	Goat	Sheep	Cow	Goat	Sheep	Cow	Goat	Sheep	Cow	Goat	Sheep	Cow
Fat (%)	9·06 ± 3·30^{a}	9·94 ± 1·52^{a}	6·66 ± 0·76^{b}	4·47 ± 1·47^{a}	6·39 ± 0·44^{c}	3·26 ± 0·95^{b}	nd	nd	nd	nd	nd	nd
Protein (%)	10·16 ± 1·56^{a}	13·94 ± 2·03^{b}	11·65 ± 1·91^{ab}	3·81 ± 0·26^{a}	6·60 ± 0·56^{b}	3·71 ± 0·56^{a}	9·02 ± 0·91*	9·98 ± 0·29*	9·96 ± 1·39*	1·45 ± 0·33*	1·23 ± 0·43*	1·87 ± 1·22*
Lactose (%)	1·99 ± 0·75	2·33 ± 0·83	1·86 ± 0·17	4·17 ± 0·61^{a}	5·75 ± 0·19^{b}	4·35 ± 0·64^{a}	2·02 ± 0·20	2·23 ± 0·05	1·71 ± 0·10	5·09 ± 0·18	5·59 ± 0·10	4·13 ± 1·20
TS (%)	21·65 ± 2·70^{a}	26·76 ± 2·43^{b}	16·07 ± 1·66^{c}	13·65 ± 0·96^{a}	16·32 ± 0·78^{b}	11·20 ± 0·92^{c}	10·78 ± 0·76^{a*}	12·93 ± 0·26^{b*}	12·23 ± 1·55^{b*}	6·75 ± 0·35*	7·83 ± 0·58*	7·01 ± 0·29*
IgG (mg/ml)	41·13 ± 5·65	45·38 ± 9·81	40·28 ± 5·60	0·73 ± 0·21	0·91 ± 0·22	0·91 ± 0·61	35·94 ± 2·48^{ab*}	41·23 ± 7·05^{b*}	30·72 ± 6·98^{a*}	0·83 ± 0·43^{a}	0·94 ± 2·00^{b}	1·05 ± 0·55^{a}
IgM (mg/ml)	0·72 ± 0·28^{a}	5·23 ± 2·79^{b}	2·13 ± 1·20^{c}	0·41 ± 0·05	0·38 ± 0·07	0·58 ± 0·10	1·17 ± 0·56^{a}	4·65 ± 1·16^{b}	1·96 ± 0·64^{a}	0·11 ± 0·07^{a*}	0·24 ± 0·03^{b}	0·12 ± 0·03^{a*}

TS, Total Solids.
$^{a-c}$Means within a row for a specific item (whole colostrum, whole milk, colostrum whey and milk whey) with different superscript letters differ significantly ($P < 0·05$).
*Means within a row for a specific species (goat, sheep and cow) differ significantly between the whole and whey sample ($P < 0·05$).

similar values to those shown in this study, in Majorera goat and Corriedale sheep colostrum, respectively.

As can be observed in Table 1, sheep colostrum and milk usually contained higher percentages of protein, fat and lactose than those from goat and cow. As a result, the total solid percentage observed in colostrum and milk from sheep was also higher than those from goat and cow.

Ig concentration

No differences in the IgG concentration were observed in colostrum or milk from the three studied species. In accordance to these findings, Stelwagen et al. (2009); Moreno-Indias et al. (2012) and Tabatabaei et al. (2013) found similar IgG concentrations in colostrum from Holstein cows (47·60 mg/ml), Majorera goats (41·20 mg/ml) and Lori Bakhtiyari sheep (52·62 mg/ml), respectively. Similarly, no differences were detected regarding IgM concentration in milk from the different studied species, although sheep colostrum showed higher values (5·23 mg/ml) than cows and goats, being the goat colostrum the one that obtained the lowest IgM concentration in this study (0·72 mg/ml). Similar IgM concentration was described by Hernández-Castellano et al. (2015d) in colostrum from the same breed of sheep (5·61 mg/ml).

Colostrum and milk proteomics

As described above, there is a considerable interest in the potential for heterologous PIT, such as Ig obtained from one species and utilised for passive immunity in another species (Hurley & Theil, 2011). Traditional methods based on gross chemical composition (fat, protein, lactose and dry matter percentage) or even on Ig concentration are frequently used for colostrum or milk characterisation. However, they are not thorough enough to fully describe differences between colostrum and milk from different ruminant species. Because of the wide range of protein concentrations and subcellular locations present in colostrum and milk, samples need to be processed before running any type of proteomics approach (Hernández-Castellano et al. 2014b; Zhang et al. 2015). According to the results shown in Table 1, protein and total solids percentages, as well as IgG and IgM concentration were slightly affected by the centrifugation process. In contrast, lactose percentage was not affected by the centrifugation process.

The differentially expressed proteins identified in colostrum whey from the three studied ruminant species are shown in Table 2. In contrast to the results showed in Table 1 about IgG and IgM concentration in colostrum whey, most of the differences between species were based on the several immune components, such as the Ig heavy chain C region, immunoglobulin gamma 2 heavy chain constant region, Ig gamma-1 chain, polymeric immunoglobulin receptor isoform 1 and the immunoglobulin mu heavy chain constant region. The differences between the proteomics results and the ELISA-based results could be

Table 2. Spots showing differential expression between goat, sheep and cow colostrum whey samples ($P < 0{\cdot}05$ and fold change >1·5)

Spot	*P*-value	Fold	Average normalised volumes (E^{+06})			Protein name	Accession number	Theoretical molecular mass (kDa)	Theoretical PI	Matched peptides†		Sequence coverage (%)‡	Protein score§
			Goat	Sheep	Cow					MS	MS/MS		
232	0·020	1·9	6·01	11·50	9·05	Ig heavy chain C region	gi|109029	34 327	6·07	15	5	36	274
236	0·003	1·9	4·00	7·11	3·74	Albumin precursor	gi|193085052	68 266	5·58	17	6	21	428
243	0·017	2·6	6·71	9·93	17·50	Albumin precursor	gi|193085052	68 266	5·58	24	2	33	132
253	0·011	2·9	6·28	1·11	18·10	Immunoglobulin gamma 2 heavy chain constant region	gi|147744654	21 648	6·33	16	3	50	293
270	0·008	1·9	1·36	1·29	24·40	Ig heavy chain C region	gi|109029	34 327	6·07	17	5	40	453
275	0·002	2·8	0·13	0·80	2·22	Serum albumin precursor	gi|57164373	71 139	5·8	18	5	23	376
338	<0·001	3·3	10·10	13·70	4·20	Immunoglobulin gamma 2 heavy chain constant region	gi|147744654	21 648	6·33	12	3	43	239
346	<0·001	3·4	7·45	13·00	3·86	Ig heavy chain C region	gi|109029	34 327	6·07	16	4	40	285
351	<0·001	2·8	14·80	35·30	12·40	Serotransferrin	gi|2501351	79 870	6·75	25	3	39	291
380	<0·001	3·2	8·25	13·40	26·60	Ig heavy chain C region	gi|109029	34 327	6·07	15	5	36	491
383	<0·001	2·6	10·80	15·40	27·80	Ig gamma-1 chain	gi|346578	52 218	5·65	15	4	23	230
384	0·002	2·0	16·00	19·60	31·40	Polymeric immunoglobulin receptor isoform 1	gi|426239425	83 669	5·79	29	6	31	599
385	0·003	1·7	25·40	26·80	43·10	Serotransferrin precursor	gi|296490958	79 783	7·13	40	7	50	767
387	<0·001	3·7	10·90	8·35	30·50	Immunoglobulin mu heavy chain constant region	gi|162424563	52 881	5·15	26	8	35	658
390	<0·001	2·0	51·30	30·00	59·50	Ig heavy chain C region	gi|109029	34 327	6·07	19	5	45	490
391	<0·001	2·7	16·10	12·80	34·30	Immunoglobulin mu heavy chain constant region	gi|162424563	52 881	5·15	24	6	37	603
392	0·012	1·6	37·20	36·50	56·90	Polymeric immunoglobulin receptor isoform 1	gi|426239425	83 669	5·79	31	7	31	682
393	0·021	1·5	3·39	22·10	33·20	Immunoglobulin gamma 1 heavy chain constant region	gi|91982959	36 562	6·49	7	2	15	197
576	0·036	1·9	4·01	2·11	3·27	Beta-casein	gi|49781319	11 051	6·71	7	2	57	92

†Number of peptides, matching the identified protein, whose sequence differs in at least one amino acid residue.
‡Percentage of the identified protein sequence covered by the matched peptides.
§Identification score obtained with the Mowse algorithm. A result is considered to be significant when a score above 92 is attained.

Table 3. Spots showing differential expression between goat, sheep and cow milk whey samples ($P < 0{\cdot}05$ and fold change >1·5)

Spot	*P*-value	Fold	Average normalised volumes (E^{+06})			Protein name	Accession number	Theoretical molecular mass (kDa)	Theoretical PI	Matched peptides†		Sequence coverage (%)‡	Protein score§
			Goat	Sheep	Cow					MS	MS/MS		
229	<0·001	2·6	3·39	4·32	1·66	Polymeric immunoglobulin receptor isoform 1	gi|426239425	83 669	5·79	20	4	25	449
283	<0·001	3·9	1·31	1·40	5·06	Chain A, crystal structure of lactoperoxidase at 2·4a resolution	gi|158430634	68 285	7·53	36	8	45	668
288	0·002	3·1	0·55	0·54	1·67	Chain A, crystal structure of lactoperoxidase at 2·4a resolution	gi|158430634	68 285	7·53	41	9	46	958
359	<0·001	2·4	10·20	15·60	6·58	Albumin precursor	gi|193085052	68 266	5·58	23	7	25	756
442	0·049	2·2	2·74	6·11	3·32	Ig heavy chain C region	gi|109029	34 327	6·07	14	2	36	238
445	0·002	2·6	1·12	2·92	1·36	Ig heavy chain C region	gi|109029	34 327	6·07	12	3	36	222
448	0·002	2·2	8·42	18·80	9·84	Ig heavy chain C region	gi|109029	34 327	6·07	16	5	36	448
454	0·01	2·1	5·01	10·30	5·42	Ig heavy chain C region	gi|109029	34 327	6·07	17	5	40	526
456	0·001	1·9	6·41	11·90	6·32	Ig heavy chain C region	gi|109029	34 327	6·07	13	4	36	340
460	0·007	2·6	7·06	9·22	3·51	Ig heavy chain C region	gi|109029	34 327	6·07	13	4	36	462
461	<0·001	3·1	10·20	15·10	4·92	Ig heavy chain C region	gi|109029	34 327	6·07	15	4	36	460
462	<0·001	3·4	10·10	15·30	4·57	Ig heavy chain C region	gi|109029	34 327	6·07	12	3	36	337
464	0·001	2·4	7·03	11·50	4·70	Ig heavy chain C region	gi|109029	34 327	6·07	17	5	40	435
468	0·008	2·3	9·51	14·70	6·28	Ig heavy chain C region	gi|109029	34 327	6·07	18	5	40	577

†Number of peptides, matching the identified protein, whose sequence differs in at least one amino acid residue.
‡Percentage of the identified protein sequence covered by the matched peptides.
§Identification score obtained with the Mowse algorithm. A result is considered to be significant when a score above 92 is attained.

due to structural differences in the non-binding sites of the immunoglobulins, which would not be detected by ELISA-based methods, but it would influence the mass of the immunoglobulin fragment and therefore affect the results in the proteomics approach. Furthermore, the proteomics analysis leads to a much more refined analysis than one allowed by the ELISA analysis. The absence of differences from other non-immune proteins in colostrum could be due to the high concentration of Ig that could mask the presence of other less abundant proteins. Presently, several depletion kits for high abundance proteins removal are available, although their use has generated some controversy (Hernández-Castellano et al. 2014b). In agreement with this statement, Golinelli et al. (2011) observed that the treatment with the Albumin and IgG removal kit was ineffective in removing IgG and BSA from the bovine colostrum whey. It is known that the albumin and IgG removal kit contains agarose-immobilised 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.

In addition to the proteins described above, serotransferrin was also detected in the three studied species, although cow colostrum whey showed the highest value (2·8 and 1·9 fold change in spots 275 and 270, respectively). In agreement with these results, this protein has been also identified in cow (Yamada et al. 2002), sheep (Hernández-Castellano et al. 2015a) and goat colostrum (Fernandez et al. 2006). As described, the presence of this protein in colostrum is of high relevance to newborn ruminants as it plays a fundamental role in iron transport (Czesnikiewicz-Guzik et al. 2007b), explaining its immune activity against bacteria, fungi and viruses (Laporta et al. 2014). As described by Sanchez et al. (1988) this protein is found to be four times greater in cow colostrum than in mature or mastitic milk, suggesting a specific transport of this protein from blood into colostrum. Finally, serum albumin was less expressed in goat colostrum whey than sheep or cow colostrum whey. Even though this protein has no direct effect on the immune system, Wall et al. (2015) described that it can be used as a marker for the permeability of the blood-milk barrier and the transfer of other immune components such as lactate dehydrogenase.

Table 3 shows the differentially identified proteins in milk whey from the three studied ruminant species (goat, sheep and cow). As occurred with colostrum whey, the main differences between the three milk whey types are based on different immunoglobulins composition. Several differences were observed in the distribution of the IgG heavy chain C region, however, most of them showed higher intensity in sheep milk whey than the milk whey from the other two species. Another protein that was identified in milk whey was lactoperoxidase. The presence of this enzyme was higher in cow milk whey compared to sheep or goats. This protein is secreted by the mammary gland, participating in the oxidation of several products that have potent bactericidal activities.

The polymeric immunoglobulin receptor isoform 1 was found to be higher in sheep and goat whey compared to cow whey. This receptor binds polymeric IgA and IgM at the basolateral surface of epithelial cells. The complex is then transported across the cell to be secreted at the apical surface. During this process a cleavage occurs that separates the extracellular from the transmembrane segment. This protein has been described in cow milk by Smolenski et al. (2014) and in sheep milk by Ha et al. (2015), however, this is the first time that the presence of this protein has been observed in goat milk whey.

Conclusions

In conclusion, the differences observed in the chemical composition among different colostrums and milks could affect the final animal performance as a consequence of an unbalanced diet. Differences in the IgG and IgM content were observed between colostrum and milk from the three ruminant species studies, and several other differences were observed when the different proteomes were compared. Therefore, it seems that heterologous PIT could affect the final immune status of the animal or even lead to PIT failure, if the immune components of the selected colostrum or milk do not reach the requirements for the selected species. Further studies are necessary using more powerful proteomics and metabolomics techniques and other colostrum and milk fractions in other to have a broader overview of the differences between these two fluids among ruminant species.

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AA, AMA, NC and LEHC conceived and designed the experiments. LEHC performed the experiments. LEHC, AMA and JR analysed the data. AA, AMA, NC and JR contributed reagents/materials/analysis tools and LEHC, AMA, NC and AA wrote the paper.

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