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TRANSCRIPTOMIC CHANGES IN RUMINAL TISSUE INDUCED BY THE PERIPARTURIENT TRANSITION IN DAIRY COWS

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ABSTRACT

To understand how the capacity for fat metabolism (uptake, synthesis, modification) changes in rumen epithelia immediately before and after onset of lactation in dairy cows, rumen fluid Short Chain Fatty Acid (SCFA) concentrations and mRNA expression profiles of rumen epithelia was determined in twelve Holstein dairy cows at three weeks prior to calving (wk -3, n = 12), one week post calving (week +1, n = 12) and six weeks (week +6, n = 12) after calving. The diet was modified from a dry cow formulation to a lactating cow formulation immediately following parturition and raised the non-fiber carbohydrate level from 34 to 43%. All data was analyzed using the mixed procedure of SAS, with cows blocked by anticipated calving date and week of sampling as the repeated measure. Propionate, butyrate, isovalerate and valerate levels rose significantly following the diet change ($p \leq 0.001$), although acetate and isobutyrate levels were unchanged ($p > 0.05$). Mean rumen pH also changed during the transition period (6.38 Vs 5.81 and 5.85±0.08; -3 Vs +1 and +6; $p < 0.001$) as did mean BW (716.00 Vs 635.82 kg and 615.45 kg ±16.20; -3 Vs +1 and +6; $p \leq 0.002$). Microarray analysis of total RNA from rumen epithelial biopsies revealed 1476 differentially expressed genes at a false discovery rate of 10%. These results were filtered for genes that were directly related to both the immune system and fat metabolism/homeostasis. Consequently, the expression of the resulting 28 genes was analyzed by quantitative PCR (qRT-PCR) to compare their expression at period -3 versus +6 periods. qRT-PCR analysis revealed that 13 genes were upregulated ($p \leq 0.01$), 2 were downregulated ($p \leq 0.01$) and 13 were unchanged during the transition period. Pathway and context analysis yielded a unique interactome pathway map which revealed a set of genomic interactions that indicate a link between selected genes from the immune system and those involved in the preparation for lactation.

Keywords: Immune System, Parturition, Transition Period, Fat Metabolism, Microarray, Biological Interactome

1. INTRODUCTION

The maintenance of health and the preparation for oncoming parturition is of paramount importance for modern dairy cows during the transition period. As a result, considerable attention has been given to this area of research so that through nutritional interventions and adequate homeostasis of fatty acids, protein and carbohydrate metabolism can be maintained or even enhanced. A comprehensive review of the role of disease in the feeding and management of transition cows can be found in several sources (Mulligan and Doherty, 2008; Mulligan *et al*., 2006; Overton *et al*., 2004; Sordillo and Raphael, 2013). Indeed, the literature is replete with information about the role of the liver and adipose tissue in metabolic alterations during parturition (Loor, 2010). Yet despite its importance to ruminant physiology, genomic adaptations in the rumen underlying these processes have largely been ignored (Loor, 2010).

During the immediate few weeks following parturition, genomic changes take place which promote

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the production of milk at the expense of normal body maintenance (Ingvartsen, 2006). These changes are hallmarked by clear reductions in body condition score and dry matter intake in the modern dairy cow, so it is clear that a massive mobilization of nutrients must be taking place which is orchestrated by an as yet unknown series of mechanisms (Adrien *et al*., 2012). However, excellent information continues to be reported on tissues known to play important roles in these processes (liver and adipose). As a result of low feed intake, circulating insulin concentrations are low and result in drastic reductions in lipogenesis through alterations in the control pathways that regulate them (Ji *et al*., 2012; Leroy *et al*., 2008), enabling more nutrients available for milk production. These changes can be effectively tracked by studying genomic changes in key metabolic pathways and so, relative changes in gene expression can be used as a tool to track energy status in the periparturient dairy cow.

The advent of high throughput screening technologies such as microarray and quantitative PCR in the past few years has allowed researchers to amass a large database of information on global gene expression from a wide variety of conditions such as disease or drug treatment (Cao *et al*., 2006; Galindo *et al*., 2010). Using such technologies has allowed for the delineation of many disease processes and has enhanced our knowledge of the biochemistry of metabolic adaptations. Because most data concerning nutrient partitioning in the peri-parturient dairy cow has come from studies of liver and adipose tissue, there is a scarcity of genomic data concerning changes in the rumen during this time period. In dairy cattle, the rumen is the principle site of SCFA generation and although *de novo* fatty acid synthesis takes place primarily in adipose tissue in these animals, it is the rumen which is the primary control point for the entry of nutrients into the body (Laliotis *et al*., 2010). Indeed, our group has previously reported genomic changes related to cholesterol homeostasis in the rumen of dairy cattle (Steele *et al*., 2011b). Moreover, we recently reported that major structural changes in the rumen are facilitated by components of the immune system (Dionissopoulos *et al*., 2013). In order to help better understand the physiological changes that take place during the peripartal period, changes in gene expression of key nutrient metabolic pathways should be elucidated. The objective of the current study was to determine the magnitude and identification of genomic changes in the rumen related to nutrient (fat) homeostasis and energy availability.

2. MATERIALS AND METHODS

2.1. Animals, Treatments and Diet

Twelve dairy primiparous (mean wt. 644±13 kg) and multiparous (mean wt. 760±10 kg) Holstein dairy cows fitted with rumen cannulae (Duffield, 1999) were used in this study and were housed in a tie-stall facility at the Elora Dairy Research Station, University of Guelph. The animal utilization protocol (10R105) was approved for use by the University of Guelph Animal Care Committee in accordance with guidelines set forth by the Canadian Council on Animal Care. The cows were placed within the study three weeks prior to calving (-3) and were placed on a lactating cow diet immediately following parturition through the 6th week thereafter. The cows were fed a Total Mixed Ration (TMR) twice daily at 08:00 and 14:00, where the amount of feed allocated to the cows was monitored to allow for a maximum of 5 kg day^{-1} of orts on an as-fed basis. The diet formulations and their chemical composition are presented in **Table 1**.

2.2. Rumen SCFA and pH

Rumen fluid was collected at the same time each day (approximately 3 h after the last feeding; 17:00) from the ventral sac of the rumen and squeezed through 4 layers of cheesecloth into 50 mL capped, conical centrifuge tubes and frozen for subsequent analysis of SCFA via gas chromatography (Dionissopoulos *et al*., 2013). Rumen pH was spot measured on the last two days of each experimental week (week -3, +1 and +6), (AlZahal *et al*., 2007). Mean daily pH for each experimental week was calculated and tabulated in **Table 2**.

2.3. Rumen Papillae Biopsies, Microarray and qRT-PCR

Samples of rumen papillae (n = 12) were harvested from the ventral sac of the rumen at the end of each experimental week according to the methodology described in Steele *et al*. (2012b). Rumen contents were partially evacuated to allow access to the ventral sac. Approximately 150 mg of papillae was cut from this area and washed 20 times in ice-cold PBS and subsequently placed in RNAlater™ (Qiagen, Hilden, GmbH) until such time that the RNA could be isolated. Total tissue RNA was isolated as previously described by our group (Steele *et al*., 2012b) using the RNeasy midi kit (Qiagen, Mississauga, Ontario, Canada) and its concentration was determined by Nano-Drop (ND-1000, NanoDrop Technologies, Wilmington, Delaware). After isolation, the RNA was treated with DNAse (Invitrogen, Inc., Burlington, Ontario, Canada) and its purity was assessed

using a bio-analyzer (Agilent 2100, Agilent Technologies Inc., Palo Alto, California).

2.4. Microarray

The isolated RNA was subsequently prepared for microarray analysis to determine expression differences (n = 12) between weeks -3 and +6. Microarray hybridization and data acquisition was performed at the University of Kentucky Advanced Genomic Technologies Center using the 24K Affymetrix GeneChip Bovine array (Affymetrix, Santa Clara, CA) as was done previously (Steele *et al*., 2011b). The effect of week of transition on gene expression was assessed at the University of Guelph Genomics Facility using GeneSpring GX 7.3.1 (Agilent Technologies, Santa Clara, CA). Using the partial least squares methodology in SAS (2004), comparison differences were determined between the two weeks, employing a false discovery rate of 0.1 according to the methods of Benjamini as described by Reiner *et al*. (2003). Therefore, differences expressed between treatment weeks at an adjusted P value <0.10 were considered to be significant.

2.5. qRT-PCR

In order to confirm results on candidate genes involved in fat homeostasis and the immune system, quantitative PCR was performed on samples from the -3 (n = 12) and +6 (n = 12) periods. Five µg samples were reverse transcribed using iTaq SYBR Green (Bio-Rad Laboratories) performed in triplicate using an ABI Prism 7000 instrument (Applied Biosystems). Where permissible, PCR primers were designed to span exon-exon junctions using the NCBI/PrimerBLAST tool (http://www.ncbi.nlm.nih.gov/tools/primer-blast) and were verified to be specific for target genes using GenBank (NCBI, Bethesda, Maryland). Primer efficiency was determined through a 5-point standard curve method, where these dissociation curves were used to determine the amplification of a single gene product. Target gene expression was normalized to GAPDH mRNA expression which was used as the housekeeping gene (Steele *et al*., 2011a), where GAPDH stability was confirmed by low variance Ct. Differences in the expression of genes assessed by qRT-PCR were determined using the Pfaffl *et al*. (2004). mRNA content was expressed relative to the -3 week observation as described previously (Xue *et al*., 2010). Expression differences were assessed via the comparison of individual +6 week group samples to the -3 pooled value using a Student's t-test. All differentially expressed genes were analyzed for interaction pathways using the Ingenuity Pathway Analysis software tool (Ingenuity Inc., Redwood City, California) in conjunction with the University of Kentucky (Lexington, Kentucky). Values deemed significant were subjected to a screen of 95% confidence and a false discovery rate of 10% according to the methods of Benjamini (Reiner *et al*., 2003).

2.6. Statistical Analysis

SCFA concentration, BW, BCS and pH were analyzed using the Mixed procedure in SAS (2004). This model included cow parity (primi-or multiparous) and week (-3, +1, +6). The term "week" was used as the repeated measurement using "cow" as the subject term and the data was subjected to multiple covariance structures. The structure that yielded the smallest Bayesian information criterion was used for reporting of results.

3. RESULTS

3.1. Diet and Physiological Parameters

Table 1 indicates the formulation and chemical composition (analysis) of the diet in this study. As can be seen in the table, the analysis confirmed that the design actually matched the composition for both of the diets.

Table 1. Formulation and chemical composition of pre-and post-parturient TMR diets expressed on an a dry matter basis)

	% DM	
Component	Dry-cow	Lactating-cow
Corn silage	45.00	26.00
Alfalfa silage	11.00	26.00
Straw	27.00	6.00
High-moisture corn	-	20.00
Protein supplement[1]	18.00	21.00
Chemical composition		
DM, %	44.00	45.00
CP (N×6.25)	13.00	16.00
Soluble Protein	5.00	6.00
ADIN	3.00	4.00
ADF	31.00	23.00
NDF	46.00	34.00
NFC	34.00	43.00
Starch	18.00	22.00
Ether extract	3.00	3.00
Ash	8.00	7.00
NE_L, Mcal/kg	1.37	1.68

[1]Dry-cow protein supplement contained 48%-soybean meal, 10% canola meal, 10% wheat bran, 19% vitamin and mineral mix, 4% soybean hulls, 2% molasses, 3% beef tallow. Lactating cow supplement contained 9% high-protein corn gluten meal, 48% soybean meal, 7% Tri-Pro Gold, 14% canola meal, 10% beat pulp, 3% herring meal, 4% dry-corn distillers grain, 12% mineral mix, 5% soybean hulls, 2% molasses, 3% beef tallow

There was no effect of transition period (-3 vs +1 vs +6) on rumen acetate and isobutyrate concentrations or on total SCFA concentration (**Table 2**). However, propionate was significant between weeks -3 and +1 (17.57 vs 22.94), between weeks -3 and +6 (17.57 Vs 22.26), but not between weeks +1 and +6 (22.94 vs 22.26±1.25 mM). For weeks -3 Vs +1 and for weeks -3 vs +6, Butyrate (6.97 Vs 9.85±0.52 mM), isovalerate (1.09 Vs 1.29±0.07 mM) and valerate (1.74 Vs. 2.57 ± 0.15 mM) concentrations were affected by diet transition (**Table 2**; $p \leq 0.004$). No statistical differences were observed between weeks +3 vs +6.

Similarly, mean rumen pH changed significantly between weeks -3 and +1 (6.38 vs 5.81±0.08; $p<0.001$) and between weeks -3 and +6 (6.38 Vs 5.85±0.08; $p<0.001$). As was the case with the SCFA, no changes were observed in the comparisons between weeks +1 and +6.

Body weight and BCS (data not shown) also significantly changed between week -3 and +1 (716.00 vs. 635.82±16.20; P = 0.002) and between week -3 and +6 (716.00 vs. 615.45±16.20; $p<0.001$). BW and BCS did not change between weeks +1 and +6.

3.2. Microarray Screen, qRT-PCR and Pathway Analysis

Microarray analysis of total rumen papillae RNA revealed that 1476 genes were differentially expressed ($p<0.0001$; data not shown). Subsequently, the 1476 differentially expressed genes (10% FDR) were subjected to a stringent screening regimen in which only those genes related to nutrient homeostasis and the immune system were chosen. In addition, only those differentially expressed genes in which a direct interactive relationship was demonstrated in the literature and were found in the GIT were subjected to verification by qRT-PCR. The 28 genes found to correspond to these criteria which demonstrated microarray-identified differential expression, were confirmed by qRT-PCR. Of those, 13 were upregulated (ACSL1, ATF3, CLU, DUSP1, EPAS1, F2RL1, GSN, MAP2K1, MAPK3, PTEN, SMAD4, SREBF1 and TP63), 2 were downregulated (HMOX1 and LGALS1) and 13 were unchanged (BCL2L11, CAV1, HIF1A, HTR2A, KSR1, MIF, PPARA, PRKCD, SERPINE1, STAT4, TGFB1, TIMP1 and VDR). Gene expression results along with fold changes and significance are presented in **Table 3**. In addition, an interpretation is offered which places meaning on these results within a contextual interactome framework of the genes which intersect those involved in fat metabolism and those commonly associated with the immune system. Using these results and the IPA tool, a concrete graphical interactome was constructed and can be seen in **Fig. 1**.

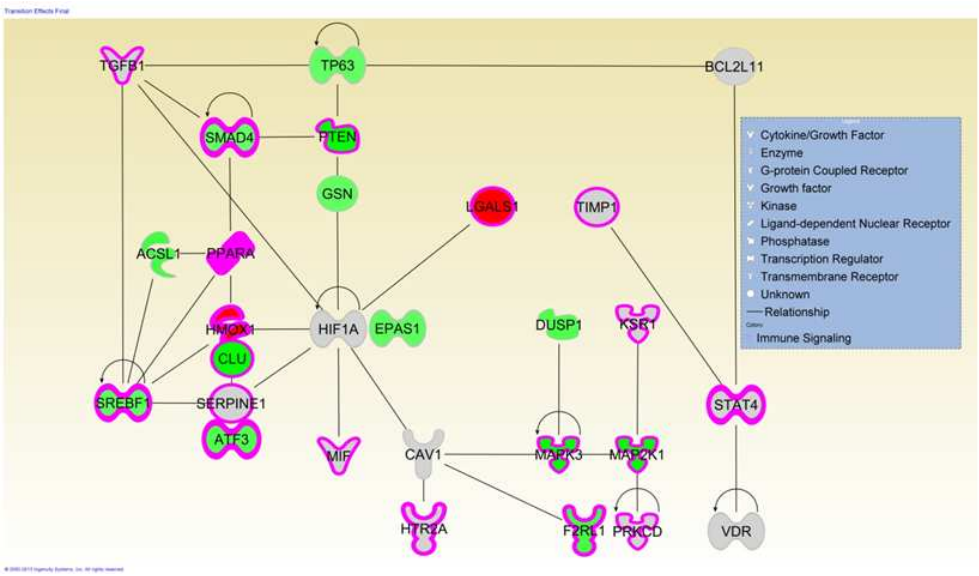

Fig. 1. Biological interactome pathway delineated by genomic analysis of immune-related and fat metabolism genes in the bovine rumen using Ingenuity Pathway Analysis. Findings were analysed using ANOVA and mixed models statistics and biological network analysis stemming from microarray and qRT-PCR analysis of 1476 differentially expressed genes (false discovery rate 10%). These results were further filtered to include only those genes that were confirmed to be direct interactions and related to both the immune system and fat metabolism in the gastrointestinal tract of mammals from **Table 3** Green = upregulated gene; Red = downregulated gene; Gray = unaffected. Genes outlined in magenta indicate concomitant involvement in immune system signaling

Table 2. Rumen SCFA concentration (mM), mean rumen pH and mean Body Weight (BW; kg) expressed through the transition period (-3, +1 and +6 weeks). Values are expressed as means ± SEM; n = 12 per treatment week.

					P-value		
	Day -3	Day +1	Day +6				
	A	B	C	SEM	AB	AC	BC
Total SCFA	70.61	73.48	81.90	4.43	0.645	0.0870	0.195
Acetate	40.75	40.53	43.20	1.32	0.911	0.2390	0.200
Propionate	17.57	22.94	22.26	1.25	0.001	0.0040	0.643
Isobutyrate	2.48	2.68	2.95	0.17	0.394	0.0640	0.282
Butyrate	6.97	9.85	9.40	0.52	<0.001	0.0020	0.510
Isovalerate	1.09	1.29	1.43	0.07	0.005	<0.0001	0.039
Valerate	1.74	2.57	2.47	0.15	<0.001	<0.0010	0.592
pH	6.38	5.81	5.85	0.08	<0.001	<0.0010	0.730
BW	716.00	635.82	615.45	16.20	0.002	<0.0010	0.381

Table 3. Gene expression results from qRT-PCR analysis and biological context analysis from rumen tissue of cows from the transition period between weeks -3 and +6. All pathway results were confirmed via the INGENUITY™ KEGG knowledge database

Symbol	Entrez Gene Name	Fold Change	SE	P-value	Function	Ref.
ACSL1	acyl-CoA synthetase long -chain family member 1	1.54	0.53	0.01	Promotes uptake of rumen SCFA	(Yang and Barouch, 2007)
ATF3	activating transcription factor 3	3.13	0.57	0.00	Promotes assimilation of fatty acids	(Zmuda *et al*., 2010)
BCL2L11	BCL2-like 11 (apoptosis facilitator)	Unchanged	NA	NS	NA	
CAV1	caveolin 1, caveolae protein, 22kDa	Unchanged	NA	NS	NA	
CLU	clusterin	1.33	0.10	0.01	Promotes fat mobilization	(Seo *et al*., 2013)
DUSP1	dual specificity phosphatase 1	2.09	0.27	0.00	Promotes fat mobilization	(Guenard *et al*., 2013)
EPAS1	endothelial PAS domain protein 1	1.72	0.21	0.00	Improves insulin sensitivity and fat mobilization	(Shimba *et al*., 2004)
F2RL1	coagulation factor II (thrombin) receptor-like 1	2.10	0.20	0.00	Promotes fat mobilization	(Badeanlou *et al*., 2011)
GSN	gelsolin	1.65	0.19	0.00	Promotes mammary gland Development	(Crowley *et al*., 2000)
HIF1A	hypoxia inducible factor 1, alpha subunit (basic helix -loop- helix transcription factor)	Unchanged	NA	NS	NA	
HMOX1	heme oxygenase (decycling) 1	0.75	0.06	0.00	Promotes efflux of hepatic triglycerides	(Czech *et al*., 2013)
HTR2A	5-hydroxytryptamine (serotonin) receptor 2A, G protein-coupled	Unchanged	NA	NS	NA	
KSR1	kinase suppressor of ras 1	Unchanged	NA	NS	NA	
LGALS1	lectin, galactoside-binding, soluble, 1	0.72	0.08	0.00	Promotes glucose homeostasis	(Camby *et al*., 2006)
MAP2K1	mitogen-activated protein kinase kinase 1	1.36	0.05	0.00	Maintains fat homeostasis	(Bost *et al*., 2005)
MAPK3	mitogen-activated protein kinase 3	1.40	0.14	0.01	Promotes adipogenesis	(Bost *et al*., 2005)
MIF	macrophage migration Inhibitory factor (glycosylation-inhibiting factor)	Unchanged	NA	NS	NA	
PPARA	peroxisome proliferator -activated receptor alpha	Unchanged	NA	NS	NA	
PRKCD	protein kinase C, delta	Unchanged	NA	NS	NA	
PTEN	phosphatase and tensin homolog	1.44	0.11	0.00	Promotes fat mobilization	(Sanchez-Gurmaches *et al*., 2012)
SERPINE1	serpin peptidase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 1	Unchanged	NA	NS	NA	
SMAD4	SMAD family member 4	1.65	0.14	0.00	Maintains fat homeostasis	(Wrana, 2009)
SREBF1	sterol regulatory element binding transcription factor 1	2.29	0.27	0.00	Promotes de novo fat synthesis and mobilization	(Eberle *et al*., 2004)
STAT4	signal transducer and activator of transcription 4	Unchanged	NA	NS	NA	
TGFB1	transforming growth factor, beta 1	Unchanged	NA	NS	NA	
TIMP1	TIMP metallopeptidase inhibitor 1	Unchanged	NA	NS	NA	
TP63	tumor protein p63	1.73	0.14	0.00	Promotes fatty acid synthesis	(Sabbisetti *et al*., 2009)
VDR	vitamin D (1,25-dihydroxyvitamin D3) receptor	Unchanged	NA	NS	NA	

4. DISCUSSION

The transition period for a high-producing dairy cow is metabolically stressful and it is during this time that most production diseases occur (Drackley, 1999; Ji *et al*., 2012; Osorio *et al*., 2013), most likely as a result of transient but sustained immunosuppression (Springer and Sciences, 2008). Many metabolomic and genomic studies have been performed to determine the role of macronutrients on gene expression of key metabolic pathways (Loor, 2010;

Mulligan and Doherty, 2008; Mulligan *et al*., 2006). However, these studies have mainly focused on liver and adipose tissue, largely ignoring the rumen epithelium. As the rumen is the source for most metabolic SCFA and nutrient transport and since immune activity seems to be omnipresent, we elected to elucidate changes in transition cow rumen epithelial tissue by studying local genomic alterations to determine what role, if any, is ascribed to the immune system.

4.1. Physiological Parameters

To achieve optimal health status during the transition period, the diet of the dairy cow must be modified to balance production goals while maintaining adequate health (NRC, 2001). As the cow progresses through the transition period, its diet changes from one typical of a dry cow formulation to one which is formulated for lactating cows (Grummer, 1995). The dietary formulations in this study follow this treatment paradigm, changing in such key parameters as protein level, ADF, energy density and most drastically, NFC (**Table 1**). In this study, the NFC content rose from 34% to 43%, a diet typically used for lactating dairy cows, but which is known to cause Subacute Ruminal Acidosis (SARA) (Dionissopoulos *et al*., 2012; Steele *et al*., 2012a). The rise in NFC over the transition period reflects the change in mean pH over time; where it was seen to drop from 6.38 to approximately 5.81 following a dietary switch post-calving. Although still below the threshold level for a diagnosis of SARA (Plaizier *et al*., 2008), the changes in pH likely reflect dietary alterations and hence changes in SCFA homeostasis rather than a physiological adaptation during the transition period. In addition to BW losses from calf, placenta and fluid expulsion, as expected, BW decreased significantly during the transition period, falling significantly from 716 kg to 635 kg. Negative changes in BW and body condition score have been associated with the transition period and have been documented for some time (Hutjens and Aalseth, 2005). It is important to note that the ensuing inflammation that occurs following calving can also be a contributing factor to reductions in DMI. Chronic immune system stimulation during this period has been known to result in anorexia, leading to feed depressions and reductions in body weight (Dionissopoulos *et al*., 2006; Esposito *et al*., 2013).

Although the total rumen SCFA did not change, the ruminal SCFA propionate, butyrate, isovalerate and valerate showed significant increases that coincided with the timing of the dietary change post calving. It is known that ruminal SCFA concentration is largely affected by dietary formulation and nutrient ecology (Aschenbach *et al*., 2011; Gabel and Sehested, 1997; Kristensen *et al*., 1998; Penner *et al*., 2011) and not as an adaptive mechanism during the transition period. The SCFA concentrations agree with previously published reports from our group using diets of similar composition (Dionissopoulos *et al*., 2013; Steele *et al*., 2012b).

4.2. Gene Expression and Biological Interactome Analysis

The changes accompanying the rumen epithelium in the transition cow have been largely ignored, due to the importance of metabolically active tissues such as liver and adipose (Loor, 2010). Yet this amazing tissue is the site of a great variety and quantity of nutrient absorption and so likely serves a significant role during the transition period in the high-producing dairy cow. Our group therefore, sought to determine genomic alterations in ruminal tissue during this period. To achieve this goal, a microarray screen followed by qRT-PCR analysis was performed on genes differentially expressed and associated with fat metabolism and the immune response. Furthermore, since we have previously shown a role for the immune system in facilitating rumen adaptation (Dionissopoulos *et al*., 2013), it was deemed prudent to also determine which immune-related genes were undergoing change. In this study, the genes significantly changed and verified by qRT-PCR were related to the immune system and fat metabolism (mobilization, synthesis).

Receptor signaling in adipose and liver tissues is known to enhance insulin sensitivity and fatty acid homeostasis during the postpartum period (Loor, 2010). In addition, the principle means by which metabolic alterations take place are through changes in a multitude of pathways involved in fatty acid homeostasis, with glucose and amino acid homeostasis following close behind (Dann *et al*., 2006; Drackley *et al*., 2006; Loor *et al*., 2006). Hence, since adipose tissue contains the key metabolic fuel for mobilization in the periparturient dairy cow, its signaling pathways must be of high concern as they are likely to affect fat metabolism in distant tissues (Vernon, 2005).

ACSL1 and SREBF1, which were upregulated during the transition period, are both involved in enhancing the uptake and assimilation of rumen SCFA (Yang *et al*., 2007) and in *de novo* fatty acid synthesis and mobilization. These results agree with those published earlier this year (Weber *et al*., 2013) which showed similar patterns of expression during the transition

period. ATF3 is an inducible transcription factor whose expression is enhanced by high levels of circulating fatty acids and glucose (Zmuda *et al*., 2010). ATF3 expression was enhanced in this study, which corresponds to its role in promoting the mobilization of fatty acids. CLU is a gene expressed in various tissues, but most notably its expression is reduced during lactation and is partially responsible for mammary gland involution following the cessation in lactation during the dry period (Piantoni *et al*., 2010). CLU was upregulated in our study, owing to the fact that comparisons were made among the interval between -3 and +6 weeks post calving, which presented the greatest differences. DUSP1 along with MAP2K1 and MAPK3 are well known genes involved in nutrient homeostasis. DUSP1 has been reported to be one of a series of upstream regulators of the MAP series of kinase activity (Liu *et al*., 2008) and has been shown to be downregulated during a shift from negative to positive energy balance (Moyes *et al*., 2011), likely affecting downstream transcription of MAP2K1 and MAPK3. These results agree with those presented here in that during transition, cows in general are in a negative energy balance state. Similarly, EPAS1 has been shown to improve insulin sensitivity and fat homeostasis (Shimba *et al*., 2004) and in this study, EPAS1 was upregulated during the transition period, likely as a response to the negative energy balance described above. F2RL1 and PPAR2 are central signaling genes and originate from the signal clotting cascade in a wide variety of tissues (Reinhardt *et al*., 2012). In the present study, F2RL1 expression increased likely as a result of MAPK signaling in negative energy balance. In an experimental model of obesity, Badeanlou *et al*. (2011) found that mice lacking the F2RL1 gene had better fat homeostasis and insulin sensitivity than their wild type counterparts. GSN is a cytoskeletal protein involved in actin signaling in order to strengthen and prepare the bovine mammary interstitium for lactation (Crowley *et al*., 2000). In the rumen, its upregulation may also be a response to a negative energy balanced state in order to mobilize body reserves for a successful lactation (Kuhla *et al*., 2011). Moreover, PTEN (upregulated in our study) is also a key gene in the pathway mediating fat liberation during this period (Sanchez-Gurmaches *et al*., 2012) and in response to increased levels of SMAD4 and control inputs from TGF-β and TP63 signaling (Wrana, 2009), acts through PPARA and back to ACSL1 to mediate the liberation of fats from metabolic stores and to promote the redistribution of energy to on-demand tissues.

To better understand the interconnectedness and adaptability of metabolism, a biological interactome was derived (**Fig. 1**) using data obtained from this study as well as from the Ingenuity Systems^{TM} KEGG database (Tanabe and Kanehisa, 2012; Zhou, 2013). As can be seen in **Fig. 1**, these genes act in a concerted fashion to regulate fat metabolism and hence energy availability. A central role for this pathway in the provision of energy during the transition period can clearly be seen.

5. CONCLUSION

To our knowledge, this is the first analysis of metabolic and genomic markers of ruminal tissue of dairy cows during the transition period. Although the transition period places severe metabolic and physical stress on the dairy cow, through changes in the expression of genes involved in fat metabolism, the immune system and energy homeostasis, the dairy cow can have a productive and healthy life. We have demonstrated altered expression of a subset of genes that seem to constitute an interactome representing associated changes in the expression of genes responsible for fatty acid, triglyceride and immune system capacity, which collectively prepare the dairy cow for the metabolic rigors associated with the transition period.

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