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Expression of selected genes related to energy mobilisation and insulin resistance in dairy cows

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Abstract. The physiological and metabolic adaptation characterising the transition period in the dairy cows is developed by a complex modulation of different metabolic pathways as well as the expression of selected tissue-specific gene. The aim of this study was to evaluate the age effect on expression of selected genes in adipose, hepatic and muscle tissues in dairy cows during their transition period using the quantitative real-time PCR. Twenty-two pluriparous dairy cows were divided into three groups in relation to age: Group A (38 ± 2 months); Group B (52 ± 2 months) and Group C (80 ± 8 months). Lower levels of peroxisome proliferator-activated receptor gamma and higher levels of adiponectin were found in adipose tissue in Group C than Groups A and B ($P < 0.05$). Higher levels of solute carrier family 2/facilitated glucose transporter member 4 were found in muscle in Group C than Group A ($P < 0.001$) and Group B ($P < 0.05$). The present study showed in dairy cows that the expression of selected genes associated with mobilisation of energy and with insulin resistance are influenced by age demonstrating and highlighting the importance of a genomics approach to assess the metabolic status of dairy cows during the transition period.

Additional keywords: adipose tissue, age, gene expression, hepatic tissue, muscle tissue.

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Introduction

The transition period of dairy cattle usually refers to the interval between 3 weeks pre-partum and 3 weeks post-partum and it is characterised by dramatic changes in metabolism and host defence mechanisms that are associated with an increased risk of disease (Fiore *et al.* 2014).

During this period an imbalance between intake and demand of energy and nutrients is commonly reported (Moyes *et al.* 2010; Fiore *et al.* 2014). The metabolic stress in association with the negative energy balance characterising the late period of gestation in high-yielding dairy cows, may develop into several diseases including ketosis, fatty liver, displaced abomasum, retained placenta, metritis, dystocia, lameness or milk fever (Ingvartsen *et al.* 2003; Sander *et al.* 2011). In response to this energy deficit period, hormone expression and tissue responsiveness are modified to increase lipolysis and decrease lipogenesis. In particular, energy is mobilised from body tissues while tissue sensitivity and responsiveness are decreased and tissue expression of the insulin-responsive glucose transporter-4 (SLC2A4) is decreased (Moyes *et al.* 2010; Saremi *et al.* 2014). The physiological and metabolic adaptation characterising the transition period in the dairy cows is developed by a complex modulation of different metabolic pathways: the expression of selected tissue-specific gene including glucose transporters, adiponectin, retinol-binding protein, prostaglandin-endoperoxide synthases, peroxisome proliferator-activated receptors and insulin-induced gene. The functional activity of peroxisome proliferator-activated receptors during this physiological state provides an example of the multi-tiered pathway, which links the biological molecules with the cellular responses in different tissues (Bionaz *et al.* 2013).

Therefore, a normal transition period is an important target in order to optimise performance and the overall welfare of dairy cows (Renquist *et al.* 2006). Advanced gene expression analysis techniques may give the rapid identification of multiple biomarkers that reflect the metabolic and immunological status of the animals. Although there are many methods available for the quantification of nucleic acids, the quantitative real-time PCR has been recognised as the most accurate and sensitive

method for quantifying mRNA transcript (Bustin 2002). This method was previously normalised in bovine tissues (Janovick-Guretzky *et al.* 2007; Lisowski *et al.* 2008).

Studies carried out on dairy cows demonstrated a relationship between peripartum diseases, nutrition, gene expression and the farm management conditions (Al-Trad *et al.* 2010; Khan *et al.* 2013). These researchers have helped to integrate data on gene expression, enzymatic rates, metabolism, and production to better understand the cellular adaptations mechanisms and biological responses in the cow during the transition period (Khan *et al.* 2013).

It has been reported that age of dairy cows influences body condition and production parameters, pregnancy rate and calving interval (Renquist *et al.* 2006). Some farmers decide breeding time on the basis of an animal's age as milk yield has been reported to be related to age at calving (Pirlo *et al.* 2000) in dairy cows. In addition, age at parturition is negatively related to the rate of genetic progress, as the generation interval decreases and the progeny test of sampling bulls is carried out earlier (Pirlo *et al.* 2000). However, the effects of age of dairy cows at calving on longevity as well as on animal physiology and the other underlying mechanisms are still unclear during the transition period. Many studies carried out on non-ruminant species have shown that aging is associated with perturbation of many metabolic pathways involved in insulin resistance and energy metabolism (Ye *et al.* 2006; Su *et al.* 2015). In contrast to non-ruminants, the age influence on gene expression in bovine species is not yet fully understood, particularly with regards to dairy cows.

The aim of this study was to compare the expression of selected genes involved in the physiological and metabolic adaptation during the transition period in adipose, hepatic and muscle tissues of pluriparous high-yielding dairy cows of different ages.

Material and methods

Animals

Twenty-two multiparous high-yielding dairy cows from the same dairy farm located in North-east Italy, were enrolled in the study. The farm makes a dry period of 60 days and a steaming-up of 15 days before the calving. The farm had a milk production of ~10 000 (kg) for year and milk quality was characterised by 3.7% milk-fat and 3.4% milk protein.

All animals were clinically healthy and free from internal and external parasites. Before and during the entire experimental period the animals' health status was monitored with the routinely clinical examination (rectal temperature, heart rate, respiratory profile, appetite, faecal consistency), and with the hematologic profile. Each animal was kept under natural winter photoperiod (sunrise 0746 hours, sunset 1630 hours) and environmental temperature (minimum temperature 0°C; maximum temperature 6.8°C). Body condition score (BCS, 0–5 scale) was evaluated for each subject.

Dairy cows were divided in three groups in relation to age: Group A included seven cows aged 38 ± 2 months and at second calving; Group B included eight cows aged 52 ± 2 months and at third calving; Group C included seven cows aged 80 ± 8 months and at fifth and sixth calvings.

A sample of visceral adipose, hepatic and muscle tissue was collected from each animal at 15 days before calving.

All treatments, housing and animal care were carried out in accordance with the standards recommended by the EU Directive 2010/63/EU for animal experiments.

Tissue collection

Animals were immobilised in a capture system for the animal and operator safety. Samples were collected by ultrasound-guided biopsy from visceral adipose, hepatic and muscle tissue according to procedures recommended by Herd (2012).

The 10th intercostal space was identified to insert the needle to collect visceral fat, hepatic and muscle tissues. At the site of insertion of the biopsy instrument 5 mL of 2% lidocaine solution was infiltrated under the skin and into the intercostal muscles. Shaving and disinfection scrubs of the skin were performed before the incision with a scalpel.

A Tru-Cut biopsy needle (UK Medical, Sheffield, United Kingdom) was used for the collection of the bioptic samples.

The MyLaOneVET ultrasound system with a convex probe SC3421 (ESAOTE S.p.A., Milan, Italy) was used to focus the area of the biopsy. The biopsy samples consisted of 5 mg of tissue.

The visceral adipose, hepatic and muscle tissues were stored in cryopreservation tubes with 1 mL of TRIzol (Life Technologies, Monza, Italy) and frozen at −80°C until RNA extraction.

RNA extraction and primer design

Total RNA was extracted from ~5 mg of visceral adipose, hepatic and muscle tissue using a PureLink RNA Mini Kit (Ambion, Life Technologies), following the manufacturer's instructions. The concentration of the extracted total RNA was quantified using a spectrophotometer (NanoDrop 1000 spectrophotometer, ThermoScientific, Wilmington, DE, USA) at 260 nm and an absorbance ratios of 260 : 280 nm and 260 : 230 nm were measured to check the good quality (1.8–1.9). The RNA integrity was evaluated through the observation of 18S and 28S ribosomal bands after electrophoresis on 0.4% agarose gel, in the presence of gel red (Biotium, Aurogene, Rome, Italy). TATA box-binding protein expression was used as an internal control, confirming thorough integrity of the RNA.

Primer3 Input software was used to design the primer sequences encoding for: tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein zeta (YWHAZ), actin β (ACTB), TATA box-binding protein (TBP), 18S subunit rRNA (18S rRNA), solute carrier family 2/facilitated glucose transporter member 1 (SLC2A1), solute carrier family 2/facilitated glucose transporter member 4 (SLC2A4), solute carrier family 2/facilitated glucose transporter member 8 (SLC2A8), adiponectin (ADIPOQ), insulin-induced gene 1 (INSIG1), insulin-induced gene 2 (INSIG2), resistin (RTN), interleukin 2 (IL2), tumour necrosis factor (TNF), prostaglandin-endoperoxide synthase 1/prostaglandin G/H synthase and cyclooxygenase (PTGS1), prostaglandin-endoperoxide synthase 2/prostaglandin G/H synthase and cyclooxygenase (PTGS2), peroxisome proliferator-activated receptor gamma (PPARG) and retinol-binding protein 4

(RBP4). According to the HUGO Gene Nomenclature Committee, primers and product lengths for each gene are detailed in Table 1.

Reverse transcription

Reverse transcriptions were performed with 400 ng of total RNA from visceral adipose and hepatic tissues and with 150 ng of total RNA from muscle tissue using Improm-II Reverse Transcriptase system (Promega, Milan, Italy).

Total RNA samples with 1-μL random hexamers (0.5 μg/μL, MBI Fermentas, Milan, Italy) and nuclease-free water to a final volume of 20 μL were incubated at 70°C for 5 min in a PTC-100 thermocycler (MJ Research Inc., Waltham, MA, USA). Then, a mix was prepared with 4 μL of Improm-II Reverse Transcriptase buffer (5X Promega), 2.4 μL $MgCl_2$ (50 mM), 1 μL of Improm-II Reverse Transcriptase and 1 μL of dNTP (10 mM) was added to the reaction and incubated at 37°C for 90 min and finally at 94°C for 5 min.

The final cDNA concentration of liver and adipose tissue was assumed as 20 ng/μL. Final concentration of muscle cDNA was 7.5 ng/μL.

Quantitative real-time PCR

For each gene, an aliquot of each cDNA samples was pooled and standard curves with serial dilution of pool were used to optimise PCR conditions and to calculate the efficiency, fluorescence baseline and threshold. The expression of target genes was normalised using geometric mean of four genes: 18S rRNA, ACTB, YWHAZ, TBP, which are known to be constitutively expressed (Bougarn *et al.* 2011).

The quantitative real-time PCR were performed in triplicate form using Platinum SYBR Green qPCR SuperMix-UDG (Invitrogen, Milan, Italy). For these reactions, a master mix with the following components was prepared to the indicated end concentration: 0.5 μL of cDNA, forward and reverse primers, 6.25 μL of 2X Platinum SYBR Green quantitative PCR SuperMix-UDG and water to a total volume of 12.5 μL. Complementary DNA concentrations and primers molarities were different for each gene and determined with standard curves analyses performed before real-time PCR reactions. Complementary DNA and primers concentrations are shown in Table 1. PCR amplifications were conducted applying 40 cycles (10 s at 95°C, 30 s at the specific annealing temperature, 30 s at 72°C) in a 96-well spectrofluorometric thermal cycler (CFX-96, BioRAD, Hercules, CA, USA). The melting curve analysis of amplification products was performed at the end of each PCR reaction to confirm that a single PCR product was detected. The quantitative real-time PCR data were normalised using the ΔCt method with geometric mean of four housekeeping genes: 18S rRNA, ACTB, YWHAZ, TBP (Bougarn *et al.* 2011). Ct (cycle threshold) represents the cycle number at which the signal reaches the threshold of detection. Each Ct used for these calculations is the mean of three replicates of the same reaction.

Statistical analyses

One-way ANOVA was applied to evaluate the possible effect of age on BCS, gene expression in adipose, hepatic and muscle tissues.

All statistical analyses were performed using STATISTICA 7.0 (Stat Soft Inc., Tulsa, OK, USA) software package. All data were expressed as Mean ΔCt ± standard deviation (s.d.).

Results

In this study no significant effect of age on BCS values ($P > 0.05$) was observed (Table 2).

Tables 3–5 show mean ΔCt ± s.d. of gene expressed in adipose, hepatic and muscle tissues, respectively, in high-yielding dairy cows with the relative significances.

Expression of PPARG was decreased ($P < 0.05$) and ADIPOQ was increased ($P < 0.05$) in the adipose tissue of dairy cows from Group C, whereas any difference for the other tested genes was not found in adipose tissue. No ADIPOQ expression was found in the hepatic tissue. No significant effect of age on the expression of tested genes was detected in hepatic tissue. Gene expression of SLC2A4 in muscle tissue showed significant changes in relation to age. Significantly higher levels of SLC2A4 were found in Group C than Group A ($P < 0.001$) and Group B ($P < 0.05$).

Discussion

In the present study, no significant changes in BCS values were observed among cow groups. The best BCS was recorded in Group A with a mean value of 3.25, followed by Groups C and B with a mean value of 3.27 and 3.47, respectively. It is well recognised that the optimum calving BCS is between 3.0 and 3.25 (5-point scale); cows with a BCS below 3.0 produce less milk, are less likely to get pregnant, and are more likely to present themselves in an animal welfare-risk category. Cows with a BCS over 3.25 have a reduced dry matter intake, produce less milk, and are more likely to succumb to periparturient metabolic disorders (Roche *et al.* 2009).

A different expression in adipose and muscle tissues of some genes associated to mobilisation of energetic substrate and insulin resistance was found in Group C than Groups A and B.

Lower expression of PPARG in adipose tissue was found in Group C than Groups A and B.

The PPARG isotypes are demonstrated to be ideal targets for the prevention and cure of the metabolic syndrome in pregnant women (Bionaz *et al.* 2013). In human medicine, the administration of PPARG agonists is a clinical approach currently used in practice to treat insulin resistance, which is one of the main problems related with the metabolic syndrome (Olefsky and Saltiel 2000).

Similarly, it was proposed that PPARG isotypes play an important role in the physiological adaptation in dairy cattle during the transition period (Bionaz *et al.* 2008).

The sensitivity of various tissues to PPARG isotype-specific agonists is closely related with the abundance of the specific isotype and other essential factors such as the abundance of coactivators or corepressors and hormones (Bionaz *et al.* 2013). Besides tissue-specific distribution, other factors may control the presence of PPARG isotypes in tissues. Among factors controlling PPARG isotypes expression in ruminants, several lipid molecules, such as retinoids and propionate, can

Table 1. Oligonucleotide primer sequences and reaction conditions for SybrGreen qRT-PCR

Gene	GenBank number	Sequence (5′→3′)	Length (bp)	Tissue	cDNA (ng)	Primer (nM)
YWHAZ	BC102382.1	F:TGTTGTAGGAGCCCGTAGGT	95	Adipose	5	200
		R:ATTCTCGAGCCATCTGCTGT		Liver	5	200
				Muscle	3.75	200
TBP	BC113308.1	F:ACAACAGCCTCCCACCCTAT	91	Adipose	20	200
		R:CCGTAAGGCATCATTGGACT		Liver	20	200
				Muscle	20	200
18S rRNA	NR_036642.1	F:AAACGGCTACCACATCCAAG	90	Adipose	0.5	25
		R:TCCTGTATTGTTATTTTTCGTCAC		Liver	0.5	50
				Muscle	0.94	200
ACTB	BC142413.1	F:GGCATCCTGACCCTCAAGTA	81	Adipose	1	200
		R:GGTGTGGTGCCAGATCTTCT		Liver	1	200
				Muscle	1.87	100
SLC2A1	NM_174602	F:GCCGAAGCGGGATCCACAGA	99	Adipose	20	200
		R:GTCAGCTTCTTGCTGGTGGGC		Liver	10	200
				Muscle	7.5	400
SLC2A4	NM_174604	F:ACCTTATGGCCACTCCTCCT	180	Adipose	20	400
		R:CTCAGCCAACACCTCAGACA		Liver	20	400
				Muscle	3.75	200
SLC2A8	NM_201528	F:CGCCGATACCAACGTGGGGC	72	Adipose	20	600
		R:ACAGCGAAGCCGGCGATGAA		Liver	20	600
				Muscle	7.5	600
ADIPOQ	AF269230.1	F:GTGGCTCTGATTCCACACCT	208	Adipose	10	400
		R:TCTCCAGGAGTGCCATCTCT		Liver	–	–
				Muscle	3.75	200
INSIG1	NM_001077909	F:CTGCCCTGCATCTTCTTCTC	154	Adipose	20	400
		R:CCAGTATCGCGGACTTTCTC		Liver	5	200
				Muscle	7.5	600
INSIG2	XM_002685428	F:AGTGTGATGCGGTGTGTAGC	159	Adipose	20	400
		R:TACTCCAAGGCCAAAACCAC		Liver	20	400
				Muscle	7.5	400
RETN	NM_183362.1	F:AGTCCACAGAGAGGCACCTG	133	Adipose	20	300
		R:TGGTGACCTCCTGGATCTTC		Liver	20	300
				Muscle	7.5	300
IL2	EU276068.1	F:ACGGGGAACACAATGAAAGA	99	Adipose	20	600
		R:CATCCTGGAGAGCTTGAGGT		Liver	20	600
				Muscle	7.5	600
TNF	AF348421.1	F:AGAAGGGAGATCGCCTCAGT	89	Adipose	20	600
		R:GGCGATGATCCCAAAGTAGA		Liver	20	600
				Muscle	7.5	600
PTGS1	AF004943.1	F:ATGCGGAGTTTCTGAGTCGT	98	Adipose	20	200
		R:GAAGTGTTGGGCAAAGAAGG		Liver	20	200
				Muscle	7.5	400
PTGS2	AF031699	F:CGGGAACACAACAGAGTGTG	88	Adipose	20	500
		R:GGATTAGCCTGCTTGTCTGG		Liver	20	800
				Muscle	7.5	500
PPARG	NM_181024	F:CATCTTCCAGGGGTGTCAGT	156	Adipose	20	200
		R:GAGGCCAGCATCGTGTAAAAT		Liver	20	200
				Muscle	7.5	200
RBP4	NM_001040475	F:CTTTTCCCCGGAAGTGCAGA	143	Adipose	10	200
		R:AAGGACACCTTCCATGTTGCT		Liver	5	200
				Muscle	7.5	200

affect the expression of PPARG isotypes, with a different sensitivity based on tissue type. The expression of ruminant PPARG isotypes is also affected by physiological status, level of energy in the diet, oxygen and peroxide levels, hormones and other growth factors and age (Ye *et al.* 2006; Bionaz *et al.* 2013).

Our findings suggest that the PPARG expression in dairy cows may be related with the different age. In literature, it is reported that aging may be associated with decreased PPARG expression affecting insulin resistance in the aged rats (Ye *et al.* 2006). The PPARG gene is recognised to be an activator of adipocyte differentiation and insulin sensitivity (Medina-Gomez *et al.*

Table 2. Mean ± standard deviation (M ± s.d.), minimum and maximum values (Min./Max.) of body condition score (BCS) obtained in cow groups

Groups	BCS	
	M ± s.d.	Min./Max.
A (n = 7)	3.25 ± 0.20	3.00–3.50
B (n = 8)	3.47 ± 0.25	3.25–4.00
C (n = 7)	3.27 ± 0.11	3.00–3.75

Table 3. Mean ΔCt (cycle threshold values) ± standard deviation (s.d.) of gene expressed in adipose tissue of high-yielding dairy cows

Gene	Group A Mean ΔCt ± s.d.	Group B Mean ΔCt ± s.d.	Group C Mean ΔCt ± s.d.
SLC2A1	12.21 ± 1.81	12.12 ± 2.10	12.51 ± 1.05
SLC2A4	8.63 ± 1.47	8.97 ± 1.61	9.04 ± 1.36
SLC2A8	10.27 ± 0.74	10.99 ± 0.90	10.50 ± 0.24
INSIG1	9.90 ± 1.64	9.57 ± 1.85	8.87 ± 1.52
INSIG2	8.10 ± 0.96	7.60 ± 1.89	6.90 ± 0.78
PPARG	7.09 ± 2.12	6.73 ± 1.65	4.02 ± 1.61
RTN	9.41 ± 1.50	8.50 ± 4.29	8.63 ± 3.07
RBP4	3.37 ± 3.34	4.24 ± 2.25	4.85 ± 0.91
ADIPOQ	8.75 ± 0.96	9.17 ± 1.22	10.92 ± 1.26
PTGS1	8.67 ± 2.12	9.37 ± 1.58	10.62 ± 1.01
PTGS2	5.82 ± 2.06	8.13 ± 2.76	7.77 ± 0.59
TNFα	11.96 ± 0.59	11.42 ± 1.81	10.28 ± 1.48
IL2	6.48 ± 1.27	7.23 ± 1.46	8.07 ± 1.98

Table 4. Mean ΔCt (cycle threshold values) ± standard deviation (s.d.) of gene expressed in hepatic tissue of high-yielding dairy cows

Gene	Group A Mean ΔCt ± s.d.	Group B Mean ΔCt ± s.d.	Group C Mean ΔCt ± s.d.
SLC2A1	3.20 ± 1.47	2.91 ± 1.14	3.00 ± 0.79
SLC2A4	4.01 ± 1.38	3.60 ± 1.64	3.68 ± 0.86
SLC2A8	3.56 ± 1.45	4.38 ± 1.07	4.11 ± 0.61
INSIG1	–0.82 ± 0.72	–0.83 ± 0.63	–0.76 ± 0.54
INSIG2	–1.78 ± 0.86	–1.89 ± 0.97	–2.26 ± 0.61
PPARG	4.95 ± 2.09	4.66 ± 1.26	4.70 ± 0.79
RTN	5.20 ± 1.13	4.98 ± 1.39	5.42 ± 0.63
RBP4	–6.74 ± 0.87	–5.51 ± 2.63	–6.77 ± 0.59
PTGS1	2.47 ± 1.82	2.89 ± 2.02	2.16 ± 0.81
PTGS2	3.47 ± 2.08	4.21 ± 1.58	4.43 ± 0.59
TNFα	3.57 ± 2.21	3.82 ± 2.01	3.57 ± 0.81
IL2	4.47 ± 2.46	4.06 ± 1.62	5.02 ± 0.65

2007), inducing the transcription of many adipocyte genes encoding proteins and enzymes involved in the development and the maintenance of the adipocyte phenotype (Gregoire *et al.* 1998). Although PPARG expression peaks during adipocyte differentiation, it is also expressed in mature adipocytes, at lower levels (Scollan *et al.* 2006). Harper and Pethick (2004) reported that the expression of PPARG decreases substantially as growth proceeds. Therefore, although the aged cows of Group C may have a greater number of mature adipocytes than cows in Groups A and B, at the cellular level, their expression of the PPARG gene was decreased.

Table 5. Mean ΔCt (cycle threshold values) ± standard deviation (s.d.) of gene expressed in muscle tissue of high-yielding dairy cows

Gene	Group A Mean ΔCt ± s.d.	Group B Mean ΔCt ± s.d.	Group C Mean ΔCt ± s.d.
SLC2A1	5.97 ± 2.42	4.51 ± 1.13	4.42 ± 1.44
SLC2A4	2.10 ± 0.58	2.50 ± 0.84	4.44 ± 1.54
SLC2A8	7.82 ± 1.38	8.02 ± 2.03	7.03 ± 0.94
INSIG1	4.16 ± 2.09	3.02 ± 1.54	3.16 ± 1.90
INSIG2	5.00 ± 1.58	5.05 ± 3.16	4.26 ± 1.20
PPARG	4.30 ± 1.60	3.24 ± 1.08	3.05 ± 1.27
RTN	4.15 ± 1.21	4.19 ± 2.61	4.46 ± 0.93
RBP4	–1.64 ± 0.79	–1.56 ± 0.66	–1.53 ± 0.96
ADIPOQ	4.22 ± 5.76	4.21 ± 5.12	5.28 ± 3.31
PTGS1	4.75 ± 2.17	3.22 ± 2.84	3.55 ± 1.17
PTGS2	5.69 ± 2.10	4.22 ± 2.34	4.47 ± 0.82
TNFα	9.93 ± 2.06	8.07 ± 2.78	7.59 ± 0.62
IL2	4.66 ± 1.79	3.45 ± 1.90	2.91 ± 1.21

The PPARG expression could also be related to the higher ADIPOQ expression in adipose tissue found in Group C than Groups A and B. Adiponectin mRNA expression in adipose tissue is downregulated by the expression of PPARG (Khan *et al.* 2013).

In addition, the upregulation of ADIPOQ could represent a response in coordinating the balance of lipogenesis and lipolysis (Khan *et al.* 2013). Adiponectin is an adipocytokine that has been demonstrated to have anti-atherogenic and anti-inflammatory roles as well a positive role in glucose metabolism (Lehnert *et al.* 2007). Adiponectin modulates energy metabolism in the maternal–fetal interface exchanges during pregnancy (Corona *et al.* 2007) and insulin sensitivity (Aldai *et al.* 2007). Moreover, adiponectin is involved in the regulation of energy homeostasis and endocrine feedback between adipose tissue and peripheral tissues including skeleton muscle (Widmann *et al.* 2011). In particular, this adipocytokine reduces gluconeogenesis and decreases hepatic glucose release. Beta-oxidation of fatty acids is increased by ADIPOQ in liver and skeletal muscle where glucose uptake is improved (Kadowaki and Yamauchi 2005; Kadowaki *et al.* 2006). This mechanism could explain the higher expression of SLC2A4 gene in muscle tissue in Group C compared with Groups A and B. Effectively, as shown in the KEGG (2016) adipocytokine signalling pathway, adiponectin signals increased glucose uptake via the SLC2A transporters (Diez and Iglesias 2003; Staiger *et al.* 2003; Nedvídková *et al.* 2005) and SLC2A4. SLC2A4 has been widely studied for its role as the main insulin-sensitive transporter and for its role in glucose metabolism. In bovine, SLC2A4 may contribute to the impaired insulin stimulation of glucose transporter (Abe *et al.* 1997) and its expression is highest in the insulin-sensitive tissues (Zhao and Keating 2007). Ogunyemi *et al.* (2013) reported that insulin-dependent glucose influx in skeletal muscle relies largely on SLC2A4 and it has been shown that SLC2A4 is downregulated in both adipose and skeletal muscle of woman with gestational diabetes (Colomiere *et al.* 2010).

Conclusion

Our findings suggest that the expression of some selected genes associated with the mobilisation of energy and with insulin resistance is associated with cow age.

This preliminary study demonstrates the importance of the genomics approach to assess the metabolic status of dairy cows during the transition period. Further studies are necessary to evaluate the application of other diagnostic and experimental tools (e.g. gene silencing), bioinformatic methods (e.g. transcription factor-binding site identification) and nutritional management focussed on genomics research to achieve the health status and production in dairy cows during the transition period.

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