

**Effects of parturition, feed energy and fibre contents to the expression
of lipogenic and lipolytic genes in dairy cows**

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<p>Metabolic disorders are known to predispose dairy cows to periparturient diseases. Main components of metabolic disorder are insulin resistance and severe negative energy balance which are responsible for the reduced fertility in addition of increased risk of disease in dairy cows. The mobilization of adipose tissue in response to energy deficiency is associated with metabolic and endocrine changes during early lactation. Reduced insulin sensitivity in peripheral tissues could potentially change the relative rates of lipolysis and lipogenesis. This study focused on expression of lipogenesis and lipolysis associated genes around parturition.</p> <p>Samples and data sets for this study were obtained from the feeding experiments conducted from September 2010 to April 2011 in Viikki Experimental Farm at the University of Helsinki. Sixteen multiparous Ayrshire cows were divided into two groups based on energy level and fibre content of their feed: (1) grass silage group (control) and (2) silage-roughage mixture group (experiment). During experimental period average energy intake (MJ/day) in silage group was 35% higher than in silage-roughage mixture group. Subcutaneous adipose tissue samples were collected a week before, one day and a week after parturition from cows. Total RNA was extracted from tissue samples quality and quantity of total RNA was analysed using electrophoresis and spectrophotometer. Complementary DNA (cDNA) was prepared from the total RNA for quantitative PCR (qPCR). QPCR was conducted to quantitate expression of the following genes: adiponectin (<i>ADIPOQ</i>), leptin (<i>LEP</i>), peroksisome proliferator activated receptor gamma (<i>PPAR-γ</i>), adiponectin receptor-1 (<i>ARI</i>), adiponectin receptor-2 (<i>AR2</i>), lipoprotein lipase (<i>LPL</i>), stearoyl-CoA desaturase (<i>SCD</i>) and hormone-sensitive lipase (<i>HSL</i>).</p> <p>Two genes, <i>AR2</i> and <i>LEP</i>, were downregulated in group 1. Reduced expression of <i>AR2</i> in group 1 may relate to an increased insulin resistance. The glucose metabolism was reduced further leading to reduced insulin sensitivity. Lower expression of <i>LEP</i> after parturition indicates usage of energy for milk production. The upregulation of <i>SCD</i> in group 1 before and after calving as well as after calving in group 2 may be a result of the uptake of fatty acids by the mammary tissues. The expression of <i>ADIPOQ</i>, <i>ARI</i>, <i>LPL</i>, <i>PPAR</i>, and <i>HSL</i> did not show any significant changes.</p>			
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LIST OF ABBREVIATIONS

<i>ADIPOQ</i>	adiponectin gene
ADIPOQ	protein coded by adiponectin gene
<i>ARI</i>	adiponectin receptor 1 gene
AR1	protein coded by adiponectin receptor 1 gene
<i>AR2</i>	adiponectin receptor 2 gene
AR2	protein coded by adiponectin receptor 2 gene
<i>HSL</i>	hormone-sensitive lipase gene
HSL	protein coded by hormone-sensitive lipase
<i>LEP</i>	leptin gene
LEP	protein coded by leptin gene
<i>LPL</i>	lipoprotein lipase
LPL	protein coded by lipoprotein lipase
NEFA	non-esterified fatty acids
<i>PPAR</i>	peroksisome proliferator activated receptor gamma gene
PPAR	protein coded by peroksisome proliferator activated receptor gamma gene
SNEB	severe negative energy balance
<i>SCD</i>	stearoyl-CoA desaturase gene
SCD	protein coded by stearoyl-CoA desaturase
TAG	triacylglycerol
-12	12 days before calving
+1	1 day after calving
+7	a week after calving
g	g-force, relative centrifugal force
rpm	revolutions per minute

1 INTRODUCTION

The selection of high yielding dairy cows is based on their predicted genetic merit. In most dairy cow populations the genetic merit is estimated for milk production traits (Snijders et al. 2001). The capability to produce large quantities of milk is partially dependent on capability to mobilize fat during early lactation. Milk production and development of foetus increase nutrient requirements during the periparturient period. Especially during early lactation period the cow fails to meet the energy demand of high milk production. Therefore, cows enter a state of negative energy balance. The period of SNEB can vary depending on the body condition, feed and the yield of milk (Beever et al. 2006). This period also accompanies an increase in the incidence of health problems, e.g. resulting in diseases like fatty liver and ketosis (Morris et al. 2009). The increased need for nutrients during the postpartum period is partially met by the increase in insulin resistance (Butler et al. 2003).

Adipose tissue has been considered a fat depot but recent studies have shown that it has important roles in various autocrine, paracrine and endocrine functions (Pittas et al. 2004). A decrease in the lipogenesis in dairy cows during the postpartum stage is associated with the negative energy balance (Chilliard et al. 2000). The insulin secretion affects the regulation of lipogenic enzymes (Sumner et al. 2011). Lipogenesis is linked with changes in the plasma concentration of insulin and substrates inducing formation of fatty acids (Pittas et al. 2004). The increase in the non-esterified fatty acids (NEFA) in the plasma might alter the expression of genes or activity of enzymes involved in lipogenesis.

The lipolysis in the adipose tissue results in the release of NEFA in to the plasma. The increase in the fat mobilization due to re-esterification and lipolysis in the early lactation counts for the negative energy balance (Sadri et al. 2010). The progress of the lactation period is marked by an increase in the esterification of fatty acids and a steady rate of lipolysis (Sumner & McNamara 2007).

Various studies of adipose tissue of periparturient dairy cows have led to the deeper understanding about metabolic adaptations to lactation (Namara et al. 1986, Sumner & McNamara 2007, Sadri et al. 2010, Sadri et al. 2011). The main objective of this study

was to analyze how feed energy and fibre contents affect the expression of lipogenic and lipolytic genes in high yielding cows.

2 LITERATURE REVIEW

2.1 Effects of SNEB on dairy cows

Severe negative energy balance (SNEB) is affecting high yielding dairy cows after parturition. It is known to predispose cows to periparturient diseases (Lemor et al. 2009). The high yielding cows encounter SNEB during the postpartum period, when the energy needed for maintenance and lactogenesis is more than the energy intake through diet (Carthy et al. 2010). The SNEB can last for few weeks and during that period, fatty acid catabolism can lead to accumulation of triglycerides in liver and affect its function. This can augment the concentration of lipid metabolites in blood to cause oxidative stress (Morris et al. 2009).

The increase in the rate of metabolism and weight results in changes in liver during the early postpartum period. In extreme cases it can lead to hepatic lipidosis and ketosis (Kokkonen et al. 2004). The dry period corresponds to the growth of the foetus where the requirement for the nutrients is at its peak. The demand for glucose, fatty acids and proteins further increase after calving for the production of milk for lactation. NEFA from the breakdown of lipids are used by the liver where they are oxidized to produce energy (Wathes et al. 2007). The increasing metabolic process can in turn use body reserves such as fat and protein. This might cause mitochondrial fatty acid oxidation in tissues like liver. This may be linked to weak immune system and make the animal more susceptible to infections (Morris et al. 2009).

The metabolic changes in the body can affect the mechanism of uterine repair, resulting in the reduced fertility (Wathes et al. 2009). The activity of ovary regains its form during the SNEB but it has severe effects on the conception rates (Fenwick et al. 2008). The changes in the negative energy balance can alter the expression of genes and cause inhibitory impact on the cell growth and repair. They decrease the DNA replication and also abnormally affect the cell cycle (McCarthy et al. 2010).

2.2 Feed intake and SNEB

The improvement in genetic selection, nutrition and management has increased the production of milk yield of dairy cows. One important factor here is the feed intake especially during the early lactation when the cows suffer from severe negative energy balance (Veerkamp et al. 2000). Inadequate feed intake has caused numerous problems in the postpartum cow. It has led to metabolic disorders, reduced fertility and adverse effects on the production of milk (Holtenius et al. 2003). Hence, nutritional strategies should be developed that increase the feed intake which will enhance BCS, the mobilization of lipid and reduce its storage in liver (Douglas et al. 2006).

An easy method to increase the feed intake is to increase the energy density of feed either by fat and carbohydrates or decreasing the forage (Andersen et al. 2002). It has been noticed that overfeeding during the postpartum period has led to high risks such as appetite depression and reduced milk synthesis. As mentioned above SNEB is responsible for various metabolic disorders like, ketosis, lipidosis, ruminal acidosis and decrease in the reproduction rate, hence, various studies have been done in order to cope up with this metabolic disorder by increasing intake of feed in the postpartum period (Reist et al. 2003). The ability of liver to oxidize long chain fatty acids can be helpful in reducing hepatic lipidosis. A study shows that fatty acid metabolism is related to feed and hormones (Andersen et al. 2002).

One strategy is a protein supplement in feed which can increase the milk production. This includes grass silage, a crude protein content which can affect on the performance of animal but still produces good quality milk (Beever et al. 2006). Another method to enhance BCS is to increase the energy density by adding concentrates out of cereal and low calorie supplements to a high forage meal. This will reduce the accumulation of TG in liver as compared to cows fed with lower or higher energy diets (Douglas et al. 2006). In line to this strategy to improve SNEB is to reduce the calorie needed for milk production in dairy cows. Intake of milk-fat depressing diets can prevent the accumulation of fat in the body. Addition of glucose rich feed, lower milk fat yield, reducing the energy needed to produce milk would enhance the energy partitioning to other tissues. Therefore, the SNEB will be improved with lower rates of fat mobilization (Knegsel et al. 2005). Thus high energy diet can yield positive results during SNEB and will have reduced risk of fatty acid infiltration in liver (Andersen et al. 2002).

2.3 SNEB and insulin resistance

A number of changes take place in the dairy cows around parturition. One component of SNEB is insulin resistance which means a failure of target organs to respond normally to the action of insulin (Pittas et al. 2004). The reduced fertility of dairy cows has been related to negative energy balance and insulin resistance (Wathes et al. 2009). The increase in need for glucose, fatty acids and amino acids is required for the production of milk during the period of lactation. The liver takes up the fatty acids and oxidizes them either to carbon dioxide for energy demand or to ketones for use by the body. Most of the cows undergo the stage of ketosis during the initial months of lactation. This is important in balancing the blood glucose level (Wathes et al. 2007).

During calving the amount of urea in the blood is not stable. It is marked by the increase in amino acid catabolism in muscles and various tissue proteins which also meets the demand for glucose (Wathes et al. 2007). Thus parturition and lactation initiates adaptations to increase the energy by decreasing lipogenesis, increasing lipolysis and the levels of insulin in serum (McNamara et al. 1986). Insulin regulates the function of adipose tissues such as TG synthesis, glucose metabolism and inhibiting lipolysis. These actions are triggered when the receptor binds to insulin and produces signaling pathway (Arner 2003).

The major adaptations of the ruminants during early lactations accounts for the increased reduced responsiveness of the adipose tissues towards insulin (Block et al. 2001). The demand for energy is fulfilled by insulin resistance in adipose tissues which decreases the uptake of peripheral glucose and increases the continuous flow of nutrients from mother to the placenta (Chagas et al. 2009). The further increase in demand for energy after calving decreases the amount of insulin during early lactation (Ingvarlsen et al. 2006). Insulin resistance impairs the functioning of adipocyte which is mediated by the increase in the fatty acids in adipose tissues (Arner 2003).

The amount of fat and the adipose tissue distribution affects the insulin resistance. Adipose tissue, the fat depot of the body produces proteins called as adipocytokines that might alter insulin metabolism through autonomic pathways. The differences in the breeds to produce milk are partially dependent on the response of tissues to insulin and also on the feed intake rich in glucose and fatty acids by the mammary tissues during the lactation (Chagas et al. 2009).

2.4 Role of adipose tissues in lipolysis and lipogenesis

Adipose tissue regulates energy metabolism, insulin sensitivity, endocrine, reproductive functions, inflammation and immunity (Ahima & Flirt 2000). It helps in overcoming the energy deficiency in tissues during the early lactation. Negative energy balance increases the amount of non-esterified fatty acid concentration in plasma by breaking down fat molecules in adipose tissues (Wathes et al. 2007). The mobilization of adipose tissue in response to energy deficient conditions is associated with metabolic and endocrine changes in dairy cows during early lactation (Sadri et al. 2010). It is involved in a variety of functions such as, glucose homeostasis, energy balance and metabolism of lipid mostly regulated by insulin (Arner 2003).

Adipose tissue metabolizes energy in two ways by first storing it during pregnancy and then releasing it for development and maintenance of the body. The hormonal changes increase the energy need in the animal and hence, the lipid synthesis (lipogenesis) decreases and the breaking down of fat molecules (lipolysis) increases (Namara et al. 1986). This metabolic regulation supports a process called as homeorhesis common amongst ruminants. This adaptation in dairy cows shows the basic principle of energy metabolism and its regulation (Chilliard et al. 2000). In adipose tissues during early lactation NEFA are released in response to synthesis of milk and energy metabolism. This process leads to the storage of glucose and amino acids to be used by the mammary gland (Chilliard et al. 2000).

The increase in NEFA in adipose tissue due to SNEB orients the tissue towards catabolism and reduces the insulin sensitivity (Sadri et al. 2010). During late gestation and early lactation, dairy cows undergo a moderate degree of peripheral tissue insulin resistance to support the uptake of glucose by mammary tissues (Lemor et al. 2009). Adipose tissue can be classified into 2 types- subcutaneous and visceral. Lipolysis is more in visceral as compared to subcutaneous as insulin plays an important role as an anti-lipolytic agent (Arner 2003). The cow copes up with the SNEB with decreased adipose tissue lipogenesis and increased lipolysis. The response varies in the animal from excess needed for the maintenance of body and milk production to increase in feed intake with less fat in the body (Sumner & McNamara 2007).

Reduced insulin sensitivity in peripheral tissue could change the relative rates of lipolysis and lipogenesis. However, little is known about the changes in mRNA

expression of key lipogenic and lipolytic enzymes in adipose tissue around parturition (Sadri et al. 2010).

2.5 Quantitative PCR in gene expression studies

Quantitative Polymerase Chain Reaction (qPCR) has been a popular method to quantify gene expression (Peirson et al. 2003). The quantification produced can either be exact number of copies (absolute) or relative amount (relative) in reference to the DNA input. The presence of one or more specific sequences in the sample can lead to detection as well as quantification of DNA. The absolute quantification relies on copy number of the transcript by forming standard curve in respect to PCR signal. The relative quantification explains the change in gene expression by taking a reference group under consideration (Peirson et al. 2003).

The quantification of gene expression relatively requires the use of equations, assumptions and testing to completely understand the data. One of the methods includes the delta CT method which is used to calculate gene expression from qPCR experiments (Livak et al. 2001). The use of this application is to determine the effect of treatment on the expression of internal control gene. The main steps to this approach include the selection of an internal control gene. The PCR should be performed by using cDNA including the experimental gene and control gene with same efficiency. The final data should be converted in to delta CT by using raw CT values statistically (Livak et al. 2001).

During the last few years, the gene expression analysis has been used to characterize the physiological processes in farm animals. It also provided a deeper insight into the metabolic process and functional states of the organism. Several studies were conducted in order to know the alterations in gene expression by negative energy balance (McCarthy et al. 2010, Wathes et al. 2009 & Morris et al. 2009). The data obtained from the gene expression data can give us an idea about the ways to reduce the risk of metabolic disorders in dairy cows and improve their reproductive performance.

2.6 Genes in this study

Genes in this study include adiponectin (*ADIPOQ*), secreted from adipose tissue. It is important for lipid metabolism, maintaining blood glucose level and insulin sensitivity (Lemor et al. 2009). Leptin (*LEP*) is a hormone secreted by adipose tissue and plays an important role in increasing lipolysis and decreasing liver lipogenesis (Sadri et al. 2010). The peroxisome proliferator activated receptor gamma (*PPAR- γ*) acts as a transcriptional regulator for fat specific genes (Rosen et al. 1999). Adiponectin receptor-1 (*AR1*) and receptor-2 (*AR2*) are liver specific hormones that are involved in fatty acid oxidation (Loor et al. 2010). Lipoprotein lipase (*LPL*) is expressed in large amounts by adipose tissue to facilitate the uptake of NEFA in peripheral tissues by hydrolyzing triglyceride rich lipoproteins (Ranganathan et al. 2006). Stearoyl-CoA desaturase (*SCD*) is a regulatory enzyme which helps in the synthesis of unsaturated fatty acids in liver (Ntambi et al. 1995). Hormone-sensitive lipase (*HSL*) is a major enzyme contributing to the breakdown of triacylglycerol in adipose tissue (Schweiger et al. 2006).

3 OBJECTIVES

The objectives of this master's thesis study:

- 1) To quantitate the expression of lipolysis and lipogenesis associated genes during late pregnancy and early lactation.
- 2) To analyse if feed energy and fibre contents have effect to the expression of lipolysis and lipogenesis associated genes in dairy cows.

Understanding the changes in expression levels of genes before and after calving based on feed intake and energy would increase our understanding of the regulation of metabolic pathways and help to improve the health and reproductive performance of the dairy cows.

4 MATERIALS AND METHODS

4.1 Animals and feeding experiment

Samples and data sets for this study were obtained from the feeding experiment conducted from September 2010 to April 2011 in Viikki Experimental Farm at the University of Helsinki. Sixteen multiparous Ayrshire cows were divided into two groups: (1) grass silage fed group (control) and (2) silage-roughage mixture fed group (experiment). Feeding experiment started 8 weeks before expected parturition. In the experiment, the treatments included feeding of grass silage (1) and a mixture of grass silage, wheat straw and rapeseed meal (55%: 40%: 5%) (2). During the dry period, the metabolizable energy was 35% higher in the group 1 than in the group 2.

These two groups control and experiment were formed based on the pairs of animals. Prior to the treatments, cows were divided into pairs according their expected calving date, parity and body condition score (BCS). After calving, all cows were offered wilted grass silage with high amounts of commercial concentrate. Subcutaneous adipose tissue samples were collected 12 days before, one day and 7 days after parturition from cows under local anesthesia at 1600-1700. Tissue samples were snap frozen using liquid nitrogen and then stored at -80 C. The final number of analysed animals was 14 because two animals were excluded from the experiment during experimental period (Table 1).

Table 1. List of animals and the schedule for collection of tissue samples.

Animal Id	Name of sample	-12	+1	+7
440	TUC	18-10-2010	11-11-2010	18-11-2010
449	Tenkapoo	01-11-2010	18-11-2010	24-11-2010
560	Assamix	8-12-2010	26-12-2010	2-1-2011
544	Yncell	11-11-2010	30-11-2010	7-12-2010
510	Virkatati	12-12-2010	21-12-2010	27-12-2010
403	Sibylla	1-2-2011	20-2-2011	27-2-2011
459	Utopia	27-12-2010	3-1-2011	10-1-2011

447	Ticoria	2-3-2011	23-3-2011	30-3-2011
455	Unetta	25-10-2011	8-11-2010	15-11-2010
460	Ukulele	7-2-2011	20-2-2011	27-2-2011
436	Tox tox	26-1-2011	7-2-2011	15-2-2011
525	Fantastico	15-2-2011	14-3-2011	9-3-2011
540	Yam yam	10-1-2011	25-1-2011	1-2-2011
439	Tavernassa	21-12-2010	31-12-2010	7-1-2011

4.2 Extraction of total RNA

Total RNA was extracted using RNeasy Lipid Tissue Kit (Qiagen GmbH, Hilden, Germany). A slice of adipose tissue (< 100 mg) was disrupted and homogenised in 1 ml of QIAZOL lysis reagent using the homogenizator (TissueRuptor, Qiagen GmbH, Hilden, Germany) and incubated at room temperature for 5 min. Then 200 µl of chloroform was added, followed by vigorous shaking for 15 sec and incubation at room temperature for 2-3 min.

A centrifuge was set to 4°C and the sample was centrifuged at 12,000g for 15 min. The upper aqueous phase was transferred to a new tube and mixed with equal amount of 70% ethanol and vortexed. The sample was transferred to a column placed in a 2 ml tube and centrifuged at 10,000 rpm for 15 sec. After this, 700µl of RW1 buffer was added to the column and centrifuged at 10,000 rpm for 15 sec. The same step was followed by addition of 500µl of RPE buffer to the column and it was centrifuged at 10,000 rpm for 15 sec. This step was repeated at the same rpm for 2 min.

The column was placed in a new tube and centrifuged at full speed for 1 min. The column was again placed in 1.5ml tube and total RNA was eluted adding 50 µl of RNase free water to the column and then it was centrifuged at 10,000 rpm for 1 min. The extracted sample was divided into two parts (1.5µl for RNA quality check and 48.5 µl for further analyses) and stored at - 80°C.

4.3 RNA quality analyses

The quality of total RNA was analyzed using Agilent Bioanalyzer 2100 chip electrophoresis system (Agilent Technologies, Santa Clara, CA, USA). The RNA 6000

Nano dye concentrate was allowed to equilibrate to room temperature for 30 min, followed by a vortex of 10 min and then 1 μ l of dye concentrate was added to 65 μ l of filtered gel. The solution was vortexed well and then centrifuged at 13,000g at room temperature for 10 min.

A new RNA 6000 chip was placed into the priming station and 9 μ l of the gel-dye mix was pipetted out into the well marked G. The plunger was positioned at 1 ml and the priming station was closed. The plunger was pressed until it was held by the chip. After 30 sec the tip was released. The plunger was pulled back after 5 sec to its previous position. The priming station was opened and 9 μ l of gel-dye mix was pipetted out in the remaining wells. 2.5 μ l of the RNA 6000 Nano marker was added to all of the 12 wells including the well for the ladder. 1 μ l of prepared ladder was also added to the well. In all the 12 sample wells, 1 μ l of samples were added and in the unused wells the marker was added.

The chip was vortexed for 1 min at 2400 rpm and placed in the bioanalyzer within 5 min. The quality and concentration of total RNA were also measured by the spectrophotometer by placing 1 μ l of the sample on the NanoDrop 1000 (Thermo Fischer Scientific, Wilmington, USA) and measuring the absorbance at wavelengths 260 nm and 280 nm.

4.4 cDNA preparation

cDNA was prepared by using Transcriptor First Strand cDNA Synthesis Kit (Roche Diagnostics GmbH, Mannheim, Germany).). All the reagents were thawed and centrifuged before starting the procedure. In a sterile, thin walled PCR tube, the primer template mixture was prepared for 20 μ l reaction. The reaction components, their volumes and concentrations are shown in Table 2.

Table 2. Reaction components for the first phase.

Component	Volume (μ l)	Final concentration
Total RNA	12	1 μ g total RNA
Anchored oligo (dT) ₁₈		
primer, 50pmol/ μ l	1	2.5 μ M
Total volume	13	

The template mixture was denatured by heating the tube at 65°C for 10 min in a thermal block cycler with a heated lid. This step ensured the denaturation of secondary structures of RNA. This step was followed by immediate cooling on ice. The remaining components of cDNA mixture were added to the tube in the order shown in Table 3.

Table 3. Reaction components for the second phase.

Component	Volume (μ l)	Final concentration
Transcriptor Reverse transcriptase reaction buffer, 5 \times conc.	4	1 \times (8mM Mgcl ₂)
Protector RNase Inhibitor, 40 U/ μ l	0.5	20U
Deoxynucleotide Mix, 10mM each	2	1mM each
Transcriptor Reverse Transcriptase, 20 U/ μ l	0.5	10U
Final volume	20	

The reagents were centrifuged and the tubes were placed on the thermal block cycler at temperature of 25°C for 10 min followed by 55°C for 30 min. The cDNA preparation was based on the primer used and the length of the target mRNA. The Transcriptor reverse transcriptase was inactivated by heating at 85°C for 5 min. The reaction was stopped by keeping it on ice and later stored at -25°C for longer durations.

4.5 Quantitative PCR

Quantitative PCR (qPCR) was done by using Light Cycler 480 SYBR Green I Master kit (Roche Diagnostics GmbH, Mannheim, Germany). The first three plates were made for genes adiponectin (ADIPOQ), adiponectin receptor-2 (AR2), leptin (LEP), lipoprotein lipase (LPL), stearoyl-CoA desaturase (SCD) and the last three plates for the remaining genes tumor necrosis factor alfa (TNF- α), carnitine palmitoyltransferase 1 (CPT-1), peroksisome proliferator activated receptor gamma (PPAR- γ), adiponectin receptor-1 (AR1), hormone-sensitive lipase (HSL). The internal control was eukaryotic

translation initiation factor 3 subunit K (EIF3K). Primer sequences and lengths for PCR products are given in Table 4.

Table 4. List of the studied genes, lengths of PCR products and primer sequences of genes.

Gene	PCR product (bp)	Forward primer	Reverse primer	Source
ADIPOQ	70	GATGGCACCCCTGGTGAGAA	CACCAGTGTCACCCTTAGGACCA	Kari Elo, unpublished
AR1	118	GGCTCTACTACTCCTTCTAC	ACACCCCTGCTCTTGTCTG	Lemor et al. 2009
AR2	200	GGCAACATCTGGACACATC	CTGGAGACCCCTTCTGAG	Lemor et al. 2009
EIF3K	125	CCAGGCCACCAAGAAGAA	TTATACCTTCCAGGAGGTCCATG T	Kadegowda et al. 2009
HSL	102	GAGTTTGAGCGGATCATTCA	TGAGGCCATGTTTGCTAGAG	Sumner & McNamara 2007
LEP	55	CTGTGCCCATCCGCAAGGT	CCAGTGACCCTCTGTTTGGAGGA	Kari Elo, unpublished
LPL	101	ACACAGCTGAGGACACTTGC C	GCCATGGATCACCACAAAGG	Bionaz & Loor 2008
PPAR γ	171	CTTGTGAAGGATGCAAGGGT TTCTT	CCAAACCTGATGGCATTATGAG ACA	Kari Elo, unpublished
SCD	101	TCCTGTTGTTGTGCTTCATCC	GGCATAACGGAATAAGGTGGC	Bionaz & Loor 2008

The reaction volume in PCR was 10 μ l (7.5 μ l Master Mix and 2.5 μ l of cDNA) and PCRs were pipette on the 384-well plates using epMotion 5075 pipetting robot (Eppendorf AG, Hamburg, Germany). The total volume of cDNA solution was 80 μ l (diluted 1:8, 10 μ l of cDNA and 70 μ l ddH₂O). The cDNA was manually pipetted into 96-well plate in the following order, A1, A2, B1, B2 and so on. The primer solution was prepared by diluting 6 μ l of forward and reverse primer with 108 μ l of ddH₂O to get a concentration of 5pmol/ μ l. The master mix was prepared for six genes by adding 128 μ l ddH₂O, 85 μ l of primer solution and 425 μ l of Roche's 2X master mix. Following the previous step, 79 μ l of the master mix was manually pipetted into the 96-well plate and correctly positioned on the epMotion 5075 worktable.

All the pipette racks were placed on B2 and B3 positions, 96-well cDNA plate to position C1, optical 384-well plate to C2 and 96-well master mix plate to C3 positions on the epMotion 5075 worktable. Reactions were performed in a Light Cycler 480 Real Time PCR instrument (Roche Diagnostics GmbH, Mannheim, Germany).

4.6 Statistical analyses

The threshold cycle value (Ct) is the starting point for exponential amplification in the PCR reaction. Typical amplification curves with different starting points for exponential amplification are shown in Figures 1-3.

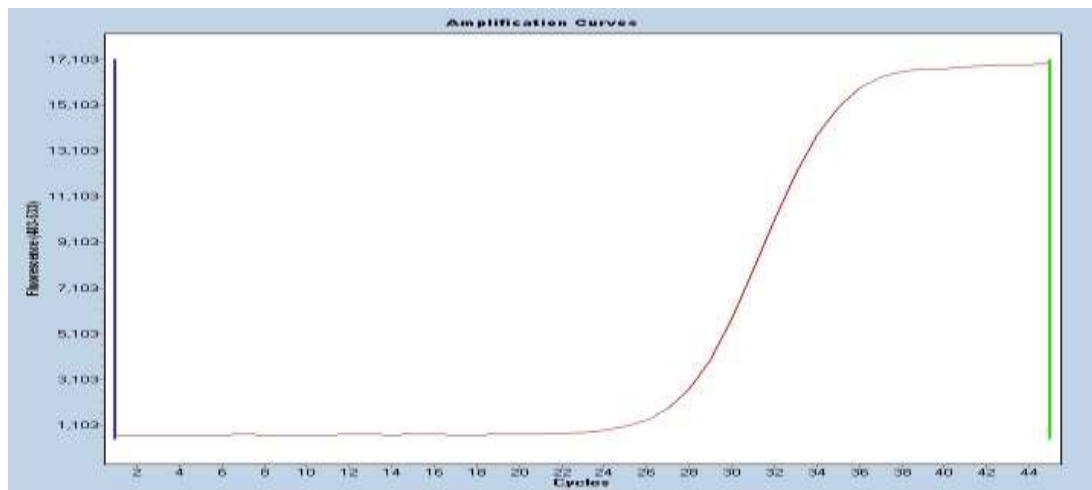


Figure 1. Amplification curve with Ct=27 for *EIF3K* (internal control gene).

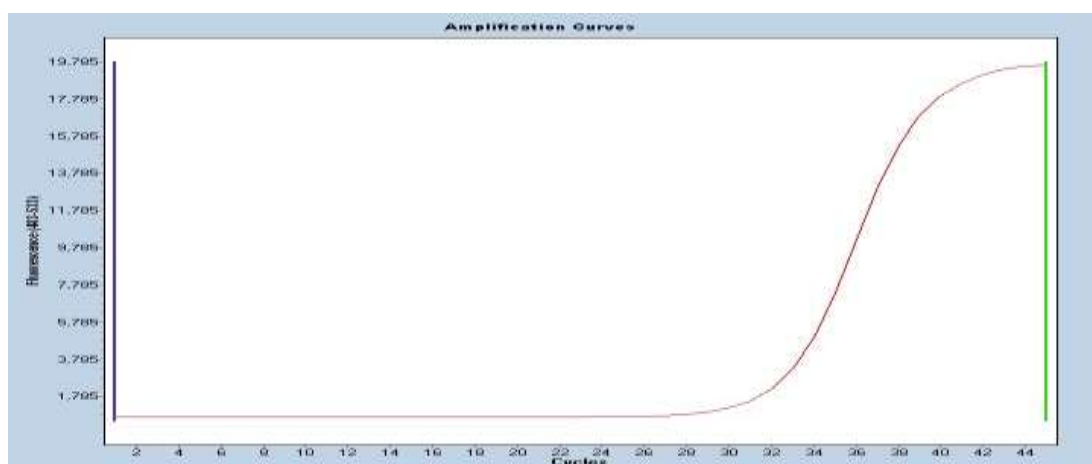


Figure 2. Amplification curve with Ct=31 for *AR2* gene.

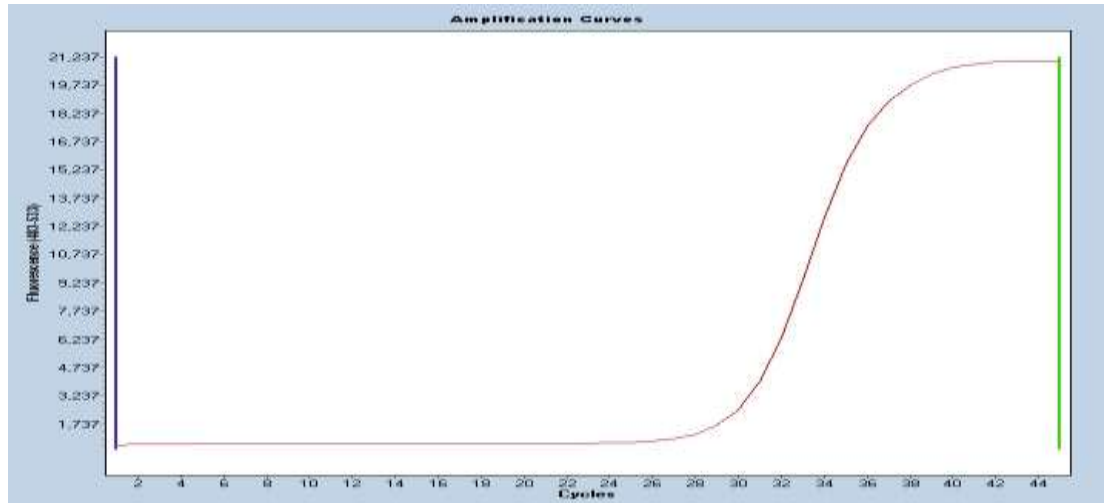


Figure 3. Amplification curve with Ct=29 for *HSL* gene.

The concentration of the target cDNA influences the value of threshold cycle. Using qPCR the differences in expression levels of control and the experimental genes were evaluated (EIF3K, ADIPOQ, AR1, LEP, LPL, SCD, PPAR, AR2 and HSL) using 4 replicates. Transcriptional levels of these genes were compared using the method of Livak & Schmittgen (2001). ΔCt values were calculated as follows:

$$\Delta Ct = (\text{average Ct of replicates})_{\text{studied gene}} - (\text{average Ct of replicates})_{\text{internal control gene}}$$

The ΔCt values were subtracted from 10 ($10 - \Delta Ct$) to get positive values before analyses. Most of the statistical analyses were performed using $10 - \Delta Ct$ as variable.

All data analyses were conducted using R statistical software package (<http://www.r-project.org/>). Distributions of the data sets were analysed using the Shapiro Wilk normality test. The gene expression differences between groups (control and experiment) and among time points (-12, +1 and +7) were studied using non-parametric methods (Kruskal Wallis Rank Sum test and Wilcoxon Rank Sum test). Differences among time points include repeated measurements from same animals, and thus data consists of dependent measurements. Differences between two time points were analysed using Wilcoxon Rank Sum test for dependent measurements. All differences were considered statistically significant at risk levels $P \leq 0.05$.

5 RESULTS

5.1 Quality and quantity of RNA

The quality and quantity of RNA were measured by automated capillary gel electrophoresis on a Agilent Bioanalyzer 2100. The samples had 28S/18S ratios (rRNA ratio) ranging from 0.5 to 1.5 and RNA Integrity Number (RIN) between 3.0 to 8.0 which indicated the acceptable quality of RNA (Table 5). Another method to detect the purity and quantity of the RNA was spectrophotometrical. Total RNA samples were measured by Nanodrop spectrophotometer giving for most samples absorption values close to 2.0 implying the acceptable quality of samples (Table 5).

Table 5. RIN values, rRNA ratio and spectrophotometer absorption at 260/280.

Animal Id	Time period	Agilent Bioanalyzer 2100		NanoDrop	
		rRNA ratio	RIN	260/280	ng/ μ l
440	-12	1.1	7.6	3.87	0.7
440	+1	0.9	7.2	1.98	21.2
440	+7	1.1	7.6	1.96	85.2
449	+1	1	7.5	1.97	27
449	+7	0	N/A	1.4	5.3
560	-12	0	4.3	1.43	4.7
560	+1	1.2	6.1	2.17	23.2
560	+7	0	N/A	-1.24	-0.9
544	-12	0.9	5.4	1.72	7.5
544	+7	0.9	5.9	1.62	44.2
510	-12	0	N/A	3.27	-4.1
510	+1	0	3.8	1.97	28.3
510	+7	1	6.3	1.82	6.1
403	-12	1	N/A	2.32	22.9
403	+1	1	7.7	1.94	18.7
403	+7	1	7.1	1.91	14.5
459	-12	1	7	1.92	6.2
459	+1	0.8	6.7	1.98	23.9

447	-12	1.1	7.6	2.01	23.5
447	+1	0	5.1	1.96	30.5
447	+7	1	7.1	1.84	16.4
455	-12	2	N/A	2.02	11.6
455	+1	0.6	6.2	1.7	19.9
455	+7	0	1.8	1.63	14.7
460	-12	0	1.7	1.93	19.9
460	+1	0.6	6.4	1.87	10.8
460	+7	1.2	7.1	1.93	35.1
436	+1	1	7.4	1.68	6.6
436	+7	1.3	6.9	2.06	43.6
525	-12	0	3.3	2.42	-1.9
525	+1	0	3.7	2.01	14.6
525	+7	0	N/A	1.99	8.2
540	-12	0.7	5.9	1.99	41.6
540	+1	0.5	5.5	1.5	3.7
540	+7	0.6	6	2.02	25
439	-12	0	N/A	2	38.2
439	+7	0	N/A	1.85	22

5.2 Statistical analyses

The null hypothesis for the Shapiro Wilk normality test is that the data is normally distributed. If the P value listed in the output is less than 0.05 then the data is not normally distributed. If the value of P is greater than 0.05 then the null hypothesis is not rejected and the data is normally distributed. Our data set is not completely normally distributed (Table 5). Based on this observation nonparametric tests were used in further analyses.

Table 5. Results of Shapiro Wilk normality test.

Values	ADIPOQ	AR2	LEP	LPL	SCD	PPAR	AR1	HSL
W	0.8971	0.9469	0.979	0.9779	0.9223	0.8421	0.8607	0.9892
p	0.001366*	0.07689	0.6966	0.5979	0.00807*	0.000*	0.0001*	0.9597

* $P < 0.05$ = no normal distribution of data

The null hypothesis states that there is no difference between control and treatment. When gene expressions levels were compared between grass silage fed group (control) and silage-roughage mixture fed group (experiment) using $10^{-\Delta Ct}$ as a variable, the expression of *AR2* ($P=0.036$) did not follow the null hypothesis (Table 6). There is potential difference ($P=0.064$) in gene expression between groups in *LEP* gene as well (Table 6).

Table 6. Statistical analyses by Kruskal Wallis Rank Sum test (degree of freedom=1)

Values	ADIPOQ	AR2	LEP	LPL	SCD	PPAR	AR1	HSL
Chi-squared	0.6751	4.3961	3.4358	1.4395	0	0.7409	0.1992	0.7864
P	0.4113	0.0360*	0.0638	0.2302	0.9792	0.3894	0.6553	0.3752

*Treatment was significantly different ($P < 0.05$) from control.

Differences between time points in the mRNA expression levels of all genes were analysed using Wilcoxon Rank Sum test. Statistically significant differences were observed in *LEP* (+1 vs +7 days, $P=0.055$) and in *SCD* (-12 vs. +1 days, $P=0.017$ and -12 vs. +7 days, $P=0.017$) (Table 7).

Table 7. Statistical analyses by Wilcoxon Rank Sum test

LOG VALUE	ADIPOQ	AR2	LEP	LPL	SCD	PPAR	AR1	HSL
dCt12&d V	57	65	59	41	15	56	48	41
ct7 P	0.8077	0.4631	0.7148	0.5016	0.0166*	0.8552	0.8077	0.5016
dCt7&dCV	48	21	14	62	55	64	60	59
t1 P	0.8077	0.1698	0.05461*	0.583	0.9032	0.5016	0.6698	0.7148
dCt12&d V	56	54	38	44	15	73	65	61
Ct1 P	0.8552	0.9515	0.391	0.6257	0.0166*	0.2166	0.4631	0.6257

*mRNA expression of genes are significantly different at different time points ($P < 0.05$)

Whether genes were down- or upregulated in different groups of animals can be concluded from averages of $10^{-\Delta Ct}$ (Tables 8-10). The gene expression of *AR2* and *LEP* was smaller in the control group than in the experiment group (Tables 8-10).

It is evident that *LEP* is downregulated in the control group from -12 to +7 days from calving because $10^{-\Delta Ct}$ values are decreasing from 9.819 to 7.803 (AVG1 in Tables 8-10). In the experiment group, there is both down- (-12 vs. +1) and upregulation (+1 vs. +7) (Fig. 4). Similar pattern was seen in the expression of *SCD*. The gene is downregulated from -12 to +7 in the control group but both down- (-12 vs. +1) and upregulated (+1 vs. +7) in the experiment group (Tables 8-10).

Table 8. Mean of mRNA expression on lipogenic and lipolysis related genes in adipose tissue 12 days before calving.

Gene Name	AVG1	AVG2	SD1	SD2
ADIPOQ	13.13893	11.31542	1.862484	1.978331
AR2	9.763571	10.01202	1.610167	0.813276
LEP	9.819405	10.42655	1.856294	1.626801
LPL	11.24786	11.23702	1.512752	1.086468
SCD	13.61143	12.67333	2.577535	2.411493
PPAR	11.00964	11.60321	2.016041	1.023636
AR1	5.171667	5.516786	1.317601	1.077812
HSL	7.669643	8.503333	2.632312	1.725175

AVG1&2 = average of control and experiment groups, SD1&2= Standard deviation of control and experiment groups.

Table 9. Mean of mRNA expression on lipogenic and lipolysis related genes in adipose tissue one day after calving.

Gene Name	AVG1	AVG2	SD1	SD2
ADIPOQ	12.32571	13.61	2.772565	1.008344
AR2	9.112083	10.06042	1.657691	1.748904
LEP	8.0125	8.7525	0.994279	1.699306
LPL	10.68393	10.80042	1.902427	1.572661
SCD	10.69429	9.5675	2.974829	2.032585
PPAR	11.54905	11.64958	1.238916	0.86585
AR1	5.515357	5.57625	1.758742	0.987131
HSL	8.731429	7.5475	2.829273	2.407774

Table 10. Mean of mRNA expression on lipogenic and lipolysis related genes in adipose tissue one week after calving.

Gene Name	AVG1	AVG2	SD1	SD2
ADIPOQ	11.55012	13.91298	2.647986	2.634173
AR2	8.735167	11.08722	1.128083	1.83393
LEP	7.8025	10.46708	1.589297	1.679485
LPL	9.719524	11.80798	2.767039	1.882248
SCD	9.614762	11.32869	2.438923	2.216924
PPAR	11.18964	11.82393	1.309604	0.936286
AR1	5.673571	6.24	0.473068	2.907868
HSL	7.089286	8.391071	1.454178	1.826168

There were no significant differences in the gene expression of ADIPOQ, LPL, PPAR, AR1 and HSL either between control and experiment or among time points (Tables 6-10).

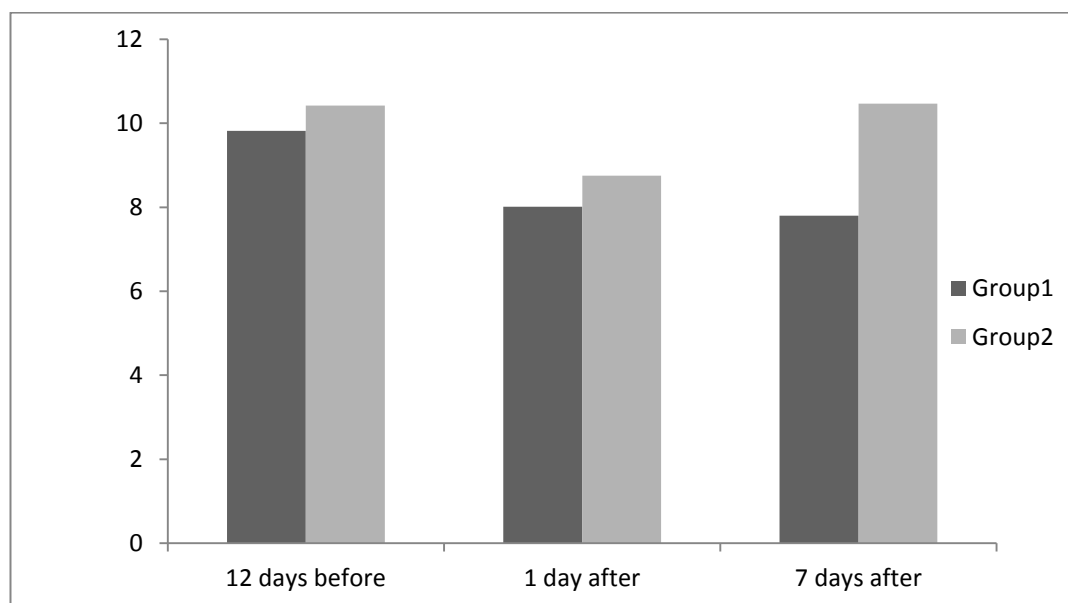


Figure 4. Pattern of *LEP* gene expression during weeks relative to parturition.

6 DISCUSSIONS

6.1 Background to gene expression analyses

Adipose tissue is marked by lipolysis and reduced fatty acid synthesis during the early lactation (Vernon et al. 2005). Insulin, responsible for glucose metabolism acts against lipolysis (Sadri et al. 2010). During the late pregnancy and early lactation the insulin resistance increases in adipose tissues of dairy cows so that the need for nutrients during

the postpartum period is met (Butler et al. 2003). This can interfere with the increasing amount of NEFA. High levels of plasma NEFA can reduce insulin sensitivity and further lead to diseases like ketosis and fatty acid accumulation (Vernon et al. 2005). In contrast to the current study, the increased intake of feed during the prepartum period increased the plasma insulin concentration (Holtenius et al. 2003). However, the energy intake did not show any changes in the level of NEFA before and after calving (Kokkonen et al., unpublished). This difference in the lipid mobilization could be a result of the minute difference in the weight gains between the treatment groups of animal.

In previous studies, it has been observed that large differences in the weight gains during the dry period increase the plasma NEFA concentration after calving (Holtenius et al. 2003 and Douglas et al. 2006). Adipose tissue controls the mobility of fatty acids according to the requirement of the body. High energy intake by group1 in this study affected the plasma NEFA levels during the experiment but the differences were not statistically significant (Kokkonen et al., unpublished). The cows in this group needed more insulin to lower down the rate of lipolysis. A decrease in the lipolysis of fatty acids shows an improvement in the glucose metabolism and insulin sensitivity (Schweiger et al. 2006).

6.2 mRNA abundance level of lipogenesis and lipolysis related genes

ADIPOQ is a cytokine produced in the adipose tissue which plays an important role in lipid metabolism and maintaining energy levels in the body (Sadri et al. 2010). Hepatic expression of AR2 in this study was down regulated in control ($P < 0.05$ and $P < 0.10$) as compared to experiment group. Three biopsy time points were used in this study with 48 animals in total. In concordance with the study by (Lemor et al. 2009) significant time effect was reported in *AR1* and *AR2* expression in subcutaneous adipose tissue postpartum compared with prepartum cows. This might lead to reduced insulin sensitivity in the adipose tissue further leading to a decrease in the glucose metabolism and increased rate of lipolysis. The study involved 2 biopsy time points with a varied time interval. This was in contrast to the study by (Sadri et al. 2011) which observed no significant time effect in the expression of adiponectin or its receptors. The study had three biopsy time points from 27 animals which varied in time difference as compared

to our samples. The body adaptations to the metabolic changes during the transition period allows for these changes in the expression of genes. However no significant differences were found in the expression of *ADIPOQ* and *ARI* in the current study in subcutaneous fat.

LEP is another regulatory cytokine produced by adipose tissues. It has multifunctional roles in decreasing the lipogenesis in adipose tissues or liver and increasing the lipolysis in adipose tissues (Havel et al. 2004). The *LEP* gene expression was found to be more in experiment group during the relative days of calving (-12, +1, +7) as compared to control group (Figure 4). The decrease in the expression of *LEP* was due to the negative energy balance produced by the reduced levels in plasma insulin. In accordance to the study by (Sadri et al. 2010) insulin controls the secretion of *LEP* in lactating cows. This state of the dairy cow may be related to the conservation of energy for development of body and foetus growth (Chilliard et al. 2005). This was found to be in contrast to the studies by (Chilliard et al. 2001) where the LEP plasma concentration was lower in moderately fed group as compared to high energy fed group. The decrease in expression can be correlated to the fatness in dairy cows which in turn correlated with loss of appetite and reduction in energy efficiency (Chilliard et al. 2000). Similar studies by (Block et al. 2001) showed that *LEP* mRNA levels were high at 1 and 4 weeks after calving as compared to first weeks of lactation.

SCD is an enzyme involved in the synthesis of fatty acids. The mRNA abundance of gene in the current study was upregulated in group 1 before calving and one day after calving. However, one week after calving, the mRNA abundance of *SCD* in the subcutaneous fat was more in group2 as compared to group1. This is in contrast to the studies by (Kinsella et al. 1972) where the regulation of SCD increased more than 40% after calving. The studies by (Bionaz et al. 2008) accounted for 23% of the relative mRNA abundance of *SCD* after calving. Thus the fatty acid determination in milk by *SCD* depends on selective uptake of TAG by mammary tissues and its utilization during lactation. Another similar study by (Loor et al. 2007) was contrasting to the current study where feed restriction and ketosis are associated with down regulation of desaturation of fatty acid. Ruminants with hepatic lipidosis have reduced amount of circulating lipoproteins as a result of down regulation of SCD.

The lipolysis of TAG by HSL is the rate limiting step in the process of lipolysis (Sadri et al. 2010). In relation to studies made by (Sumner & McNamara 2007) the expression of *HSL* increased in the prepartum cows as compared to postpartum. The rate of lipolysis was more on the day of after parturition. This proposed that the current study had lower rate of lipolysis as the recent study shows no significant changes in the mRNA abundance of *HSL* in the plasma which is in accordance with the studies made by (Sadri et al. 2010). The fatty acids are accumulated in the adipose tissue by *de novo* synthesis and uptake from the circulation.

LPL plays a major role in the uptake of fatty acids from the circulation. Its regulation is dependent on the nutrition and metabolic function of the body (Vernon et al. 2005). There were no significant differences in the expression of *LPL* in the recent study which shows that cows are still affected by the negative energy balance to incorporate lipogenesis (Sadri et al. 2010). Recent studies (Harvatine et al. 2009) showed an upregulation in the *SCD* and *LPL* genes related to fatty acid synthesis in adipose tissues. The experimental setup for this study included biopsy samples on 14 day after calving as compared to our biopsy time points for the study. The nuclear hormone *PPAR* is responsible for lipid metabolism, energy homeostasis and differentiation of adipose tissues (Lemberger et al. 1996).

In our study no specific significance was found in the expression of *PPAR*. In a recent study by (Bionaz et al. 2008) showed that the changes in the adipocytes might be related to stages of lactation and this could report for a down regulation of the expression of the gene. Here, the mRNA abundance was found to be less than 1% which was similar to our observation indicated that the mammary tissue of the lactating cow has low amounts of subcutaneous fat.

7 CONCLUSIONS

The feed had an effect on the expression of adipose related genes in this experiment. The regulation of lipolysis and lipogenic genes showed variations ranging from the period of prepartum to postpartum in dairy cows. The genes involved in the synthesis of fatty acids were down regulated only after 7 days of calving in control group with an increase in the expression in the experiment group.

The rate of lipolysis was also low due to the difference in the level NEFA in the plasma as well as the restricted feeding strategy during the prepartum period. This proves that the adipose tissue was unable to cover the energy needed for maintenance and lactation. Understanding the role of adipose tissue in lipid metabolism, feed intake and lactation would help reducing the metabolic disorders and its consequences in ruminants.

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10 APPENDICES

Appendix 2. The Ct values of EIF3K, ADIPOQ, AR2, LEP, LPL and SCD

Animal	Time	Gene	EIF3K	EIF3K	EIF3K	EIF3K	SD	Coverage	Sample1	Sample2	Sample3	Sample4	SD	Saverage	SD_deltaCt	deltaCt	deltadeltaCt (Ct-colCt)
440	12	ADIPOQ	20.74	20.95	21.45	20.6	0.372245	20.935	17.63	16.93	16.86	17.44	0.3786379	17.215	0.530973948	-3.72	0
440	1	ADIPOQ	20.38	19.59	20.41	20.44	0.410731	20.205	18.51	17.8	17.93	18.48	0.3677862	18.18	0.551331721	-2.025	1.695
440	7	ADIPOQ	20.48	20.37	20.37	20.8	0.203388	20.505	20.86	20.39	20.31	19.91	0.3897328	20.3675	0.439611571	-0.1375	3.5825
440	12	AR2	20.74	20.95	21.45	20.6	0.372245	20.935	21.46	21.66	21.73	21.92	0.1899781	21.6925	0.417921444	0.7575	0
440	1	AR2	20.38	19.59	20.41	20.44	0.410731	20.205	22.4	21.94	22.51	22.71	0.3262923	22.39	0.52456331	2.185	1.4275
440	7	AR2	20.48	20.37	20.37	20.8	0.203388	20.505	22.66	23.48	23.72	23.54	0.4711688	23.35	0.513192621	2.845	2.0875
440	12	LEP	20.74	20.95	21.45	20.6	0.372245	20.935	23.71	23.42	24.21	24.33	0.426722	23.9175	0.566267016	2.9825	0
440	1	LEP	20.38	19.59	20.41	20.44	0.410731	20.205	23.43	24.37	24.28	24.64	0.5228767	24.18	0.664906008	3.975	0.9925
440	7	LEP	20.48	20.37	20.37	20.8	0.203388	20.505	25.6	24.86	24.62	24.5	0.4932545	24.895	0.533541626	4.39	1.4075
440	12	LPL	20.74	20.95	21.45	20.6	0.372245	20.935	20.38	20.41	20.34	19.77	0.3046856	20.225	0.481040539	-0.71	0
440	1	LPL	20.38	19.59	20.41	20.44	0.410731	20.205	22.27	20.61	21.63	21.61	0.6856627	21.53	0.799270501	1.325	2.035
440	7	LPL	20.48	20.37	20.37	20.8	0.203388	20.505	23.73	23.62	24.35	24.47	0.4295249	24.0425	0.475245551	3.5375	4.2475
440	12	SCD	23.42	23.34	23.34	22.87	0.251181	23.2425	22.43	22.54	22.71	22.52	0.1169045	22.55	0.277052943	-0.6925	0
440	1	SCD	22.62	22.6	22.6	22.7	0.04761	22.63	26.43	26.31	26.39	25.85	0.2680174	26.245	0.272213152	3.615	4.3075

440	7	SCD	30.67	30.45	29.83	30.46	0.362802	30.3525	31.59	30.32	30.82	31.53	0.6074263	31.065	0.707525029	0.7125	1.405
560	12	ADIPOQ	27.96	28.59	28.82	28.35	0.367423	28.43	29.31	28.76	28.31	27.78	0.6511016	28.54	0.747618441	0.11	0
560	1	ADIPOQ	23.72	22.38	22.81	23.99	0.756417	23.225	21.81	21.44	21.32	20.82	0.40885	21.3475	0.859840101	-1.8775	-1.9875
560	7	ADIPOQ	27.81	27.44	27.44		0.21362	27.56333	24.48	24.28	24.4	24.3	0.0929157	24.365	0.232952069	3.198333333	3.308333333
560	12	AR2	27.96	28.59	28.82	28.35	0.367423	28.43		29.64	27.78	28.58	0.9330238	28.66667	1.00276285	0.23666667	0
560	1	AR2	23.72	22.38	22.81	23.99	0.756417	23.225	24.66	24.99	25.46	24.71	0.3666515	24.955	0.840595027	1.73	1.493333333
560	7	AR2	27.81	27.44	27.44		0.21362	27.56333	26.91	27.52	26.61	26.75	0.4008637	26.9475	0.454230118	0.615833333	-0.8525
560	12	LEP	27.96	28.59	28.82	28.35	0.367423	28.43		27.28	27.47	27.81	0.2685144	27.52	0.45508241	-0.91	0
560	1	LEP	23.72	22.38	22.81	23.99	0.756417	23.225	25.72	24.55	25.68	25.72	0.5786406	25.4175	0.952361101	2.1925	3.1025
560	7	LEP	27.81	27.44	27.44		0.21362	27.56333		24.29	26.73	27.37	1.6253	26.13	1.639278296	1.433333333	0.523333333

Animal	Time	Gene	EIF3K	EIF3K	EIF3K	EIF3K	SD	Coverage	Sample1	Sample2	Sample3	Sample4	SD	Saverage	SD_deltaCt	deltaCt	deltadeltaCt (Ct-colCt)
560	12	LPL	27.96	28.59	28.82	28.35	0.367423	28.43	27.34	27.49	27.53	27.31	0.1087428	27.4175	0.383177505	-1.0125	0
560	1	LPL	23.72	22.38	22.81	23.99	0.756417	23.225	22.97	23.49	23.53	23.33	0.2550817	23.33	0.798268961	0.105	1.1175
560	7	LPL	27.81	27.44	27.44		0.21362	27.56333	26.94	27.65	27.58	27.45	0.3208842	27.405	0.385486705	-0.158333	0.854166667

560	12	SCD	27.96	28.59	28.82	28.35	0.367423	28.43	23.54	22.98	23.52	22.85	0.3591077	23.2225	0.513768755	-5.2075	0
560	1	SCD	23.72	22.38	22.81	23.99	0.756417	23.225	23.8	23.67	24.34	23.8	0.2980352	23.9025	0.81301394	0.6775	5.885
560	7	SCD	27.81	27.44	27.44		0.21362	27.56333	27.46	27.53	27.94	27.78	0.2224672	27.6775	0.30842341	0.11416667	5.321666667
544	12	ADIPOQ	28.79	28.69	26.84	27.56	0.937337	27.97	24.37	24.49	24.77	24.33	0.1986622	24.49	0.958157955	-3.48	0
544	1	ADIPOQ			27.83	27.95	0.084853	27.89	23.35	22.85	22.91	22.58	0.3191003	22.9225	0.33018934	-4.9675	-1.4875
544	7	ADIPOQ	28.43	27.76	25.37	25.45	1.574471	26.7525	19.44	18.86	18.9	18.52	0.3803507	18.93	1.619760785	-7.8225	-4.3425
544	12	AR2	28.79	28.69	26.84	27.56	0.937337	27.97	26.6	27.55	27.53	27.61	0.4828647	27.3225	1.054399513	-0.6475	0
544	1	AR2			27.83	27.95	0.084853	27.89	24.58	24.36	24.69	24.72	0.1631717	24.5875	0.183915742	-3.3025	-2.655
544	7	AR2	28.43	27.76	25.37	25.45	1.574471	26.7525	21.65	21.74	22.38	22.28	0.3707088	22.0125	1.617523828	-4.74	-4.0925
544	12	LEP	28.79	28.69	26.84	27.56	0.937337	27.97		27.59	28.41	27.72	0.4407191	27.90667	1.035776681	-	0
544	1	LEP			27.83	27.95	0.084853	27.89	25.7	26.71	26.6	25.95	0.4920027	26.24	0.499266128	-1.65	1.586666667
544	7	LEP	28.43	27.76	25.37	25.45	1.574471	26.7525	23.5	23.33	24.88	23.7	0.7014925	23.8525	1.723673403	-2.9	2.836666667
544	12	LPL	28.79	28.69	26.84	27.56	0.937337	27.97	25.61	25.75	25.78	25.54	0.1140175	25.67	0.944245731	-2.3	0
544	1	LPL			27.83	27.95	0.084853	27.89	24.45	24.55	24.39	24.49	0.06733	24.47	0.108320512	-3.42	-1.12
544	7	LPL	28.43	27.76	25.37	25.45	1.574471	26.7525	21.8	21.82	22.36	22.38	0.3235223	22.09	1.607365858	-4.6625	-2.3625
544	12	SCD	28.79	28.69	26.84	27.56	0.937337	27.97	23.64	23.63	23.98	23.52	0.1992277	23.6925	0.958275361	-4.2775	0
544	1	SCD			27.83	27.95	0.084853	27.89	24.77	25.96	25.59	25.54	0.4997666	25.465	0.506918797	-2.425	1.8525
544	7	SCD	28.43	27.76	25.37	25.45	1.574471	26.7525	23.95	24.5	24.38	23.72	0.365	24.1375	1.616225026	-2.615	1.6625

449	12	ADIPOQ	28.57	29.52	28.91		0.481352	29	24.52	24.62	24.5	24.66	0.0772442	24.575	0.487510684	-4.425	0
449	1	ADIPOQ	24.71	24.61	24.9	24.8	0.123962	24.755	21.66	20.77	20.6	20.48	0.5350623	20.8775	0.549234316	-3.8775	0.5475
449	7	ADIPOQ		23.64	23.33	22.38	0.656531	23.11667	19.68	19.34	18.85	18.8	0.4196328	19.1675	0.779182264	3.94916667	0.475833333
449	12	AR2	28.57	29.52	28.91		0.481352	29	27.38	26.75	26.8	26.95	0.28624	26.97	0.560029761	-2.03	0
449	1	AR2	24.71	24.61	24.9	24.8	0.123962	24.755	24.76	24.63	24.86	25.72	0.4940563	24.9925	0.509370527	0.2375	2.2675
449	7	AR2		23.64	23.33	22.38	0.656531	23.11667	23.66	23.68	24.33	24.5	0.4357656	24.0425	0.787987944	0.92583333	2.955833333
Animal	Time	Gene	EIF3K	EIF3K	EIF3K	EIF3K	SD	Coverage	Sample1	Sample2	Sample3	Sample4	SD	Saverage	SD_deltaCt	deltaCt	deltadeltaCt (Ct-colCt)
449	12	LEP	28.57	29.52	28.91		0.481352	29	26.66	27.49	26.92	26.69	0.384621	26.94	0.616143923	-2.06	0
449	1	LEP	24.71	24.61	24.9	24.8	0.123962	24.755	26.77		26.51	26.88	0.19	26.72	0.22686266	1.965	4.025
449	7	LEP	24.66	23.64	23.33	22.38	0.939588	23.5025	24.79	25.67	25.72	26.73	0.793153	25.7275	1.229600206	2.225	4.285
449	12	LPL	28.57	29.52	28.91		0.481352	29	25.65	25.5	25.98	25.54	0.2177728	25.6675	0.528322818	-3.3325	0
449	1	LPL	24.71	24.61	24.9	24.8	0.123962	24.755	23.54	22.98	23.39	23.37	0.2390258	23.32	0.26925824	-1.435	1.8975
449	7	LPL		23.64	23.33	22.38	0.656531	23.11667	23.41	23.38	23.61	23.9	0.2395134	23.575	0.698856208	0.45833333	3.790833333
449	12	SCD	28.57	29.52	28.91		0.481352	29	24.84	25.52	24.78	25.5	0.4049691	25.16	0.629046898	-3.84	0
449	1	SCD	24.71	24.61	24.9	24.8	0.123962	24.755	22.75	22.76	23.28	22.77	0.2601282	22.89	0.288155051	-1.865	1.975
449	7	SCD		23.64	23.33	22.38	0.656531	23.11667	21.4	21.9	21.69	21.65	0.2051016	21.66	0.687822652	1.45666667	2.383333333
510	12	ADIPOQ													0	0	0
510	1	ADIPOQ	25.66	25.59	25.51	25.49	0.078049	25.5625	27.49	27.54	27.58	27.63	0.0594418	27.56	0.098107084	1.9975	1.9975

510	7	ADIPOQ	23.7	23.61	22.9	21.96	0.805497	23.0425	19.74	19.62	19.71	19.56	0.0826136	19.6575	0.809722175	-3.385	-3.385
510	12	AR2													0	0	0
510	1	AR2	25.66	25.59	25.51	25.49	0.078049	25.5625	28.76	29.64	27.66	30.32	1.1502319	29.095	1.152876836	3.5325	3.5325
510	7	AR2	23.7	23.61	22.9	21.96	0.805497	23.0425	23.99	24.75	25.53	25.5	0.7303595	24.9425	1.087313202	1.9	1.9
510	12	LEP													0	0	0
510	1	LEP	25.66	25.59	25.51	25.49	0.078049	25.5625	26.44	27.57			0.7990307	27.005	0.802833524	1.4425	1.4425
510	7	LEP	23.7	23.61	22.9	21.96	0.805497	23.0425	26.61	24.93	26.64		0.9787237	26.06	1.267566566	3.0175	3.0175
510	12	LPL													0	0	0
510	1	LPL	25.66	25.59	25.51	25.49	0.078049	25.5625	27.79	27.61	27.48	27.86	0.1725302	27.685	0.189362967	2.1225	2.1225
510	7	LPL	23.7	23.61	22.9	21.96	0.805497	23.0425	25.8	25.91	25.93	26.31	0.2224672	25.9875	0.835653437	2.945	2.945
510	12	SCD													0	0	0
510	1	SCD	25.66	25.59	25.51	25.49	0.078049	25.5625	30.35	28.98	28.53	28.42	0.8870551	29.07	0.890482079	3.5075	3.5075
510	7	SCD	23.7	23.61	22.9	21.96	0.805497	23.0425	24.8	25.59	25.93	24.73	0.5916854	25.2625	0.999458187	2.22	2.22
403	12	ADIPOQ	25.44	25.92	25.84	25.93	0.231858	25.7825	21.7	21.49	21.8	22.22	0.3068523	21.8025	0.384599359	-3.98	0
403	1	ADIPOQ	24.74	24.3	24.55	24.54	0.180254	24.5325	20.31	20.5	20.43	20.44	0.0795822	20.42	0.197040605	-4.1125	-0.1325
403	7	ADIPOQ	24.53	24.56	24.64	24.7	0.07719	24.6075	19.84	20.23	19.93	19.95	0.1685972	19.9875	0.185427434	-4.62	-0.64
403	12	AR2	25.44	25.92	25.84	25.93	0.231858	25.7825	26.4	26.56	26.54	26.22	0.1570563	26.43	0.280044639	0.6475	0
Animal	Time	Gene	EIF3K	EIF3K	EIF3K	EIF3K	SD	Coverage	Sample1	Sample2	Sample3	Sample4	SD	Saverage	SD_deltaCt	deltaCt	deltadeltaCt (Ct-colCt)
403	1	AR2	24.74	24.3	24.55	24.54	0.180254	24.5325	24	24.4	24.76	24.57	0.3236639	24.4325	0.370472671	-0.1	-0.7475

403	7	AR2	24.53	24.56	24.64	24.7	0.07719	24.6075	24.54	24.38	24.52	24.71	0.1352467	24.5375	0.155724115	-0.07	-0.7175
403	12	LEP	25.44	25.92	25.84	25.93	0.231858	25.7825	25.65	25.43	25.69	25.78	0.1486327	25.6375	0.275408787	-0.145	0
403	1	LEP	24.74	24.3	24.55	24.54	0.180254	24.5325	25.82	26.62	26.26	25.98	0.3508086	26.17	0.39440884	1.6375	1.7825
403	7	LEP	24.53	24.56	24.64	24.7	0.07719	24.6075	25.62	24.96	25.45	24.97	0.336353	25.25	0.345096605	0.6425	0.7875
403	12	LPL	25.44	25.92	25.84	25.93	0.231858	25.7825	23.5	23.42	23.51	23.79	0.1617611	23.555	0.282710099	-2.2275	0
403	1	LPL	24.74	24.3	24.55	24.54	0.180254	24.5325	23.5	23.35	23.38	23.51	0.0818535	23.435	0.197968853	-1.0975	1.13
403	7	LPL	24.53	24.56	24.64	24.7	0.07719	24.6075	24.52	23.94	24.63	23.96	0.3637192	24.2625	0.371819849	-0.345	1.8825
403	12	SCD	25.44	25.92	25.84	25.93	0.231858	25.7825	19.89	20.39	20.56	20.48	0.301441	20.33	0.380295937	-5.4525	0
403	1	SCD	24.74	24.3	24.55	24.54	0.180254	24.5325	20.55	21.75	21.52	21.42	0.5251666	21.31	0.555240188	-3.2225	2.23
403	7	SCD	24.53	24.56	24.64	24.7	0.07719	24.6075	26.47	26.95	26.49	26.5	0.2320022	26.6025	0.244506305	1.995	7.4475
459	12	ADIPOQ	26.25	26.53	25.59	26	0.398863	26.0925	21.5	21.67	21.39	21.6	0.1219289	21.54	0.417083125	-4.5525	0
459	1	ADIPOQ	24.69	24.83	25.53	24.93	0.37	24.995	21.4	21.58	21.37	21.43	0.0932738	21.445	0.381575681	-3.55	1.0025
459	7	ADIPOQ	27.79	26.85	27.23	26.93	0.425989	27.2	24.93	25.43	24.89	24.9	0.2622181	25.0375	0.500224949	-2.1625	2.39
459	12	AR2	26.25	26.53	25.59	26	0.398863	26.0925	23.91	24.57	24.63	24.77	0.3826225	24.47	0.552713006	-1.6225	0
459	1	AR2	24.69	24.83	25.53	24.93	0.37	24.995	24.77	23.57	23.81	23.93	0.5219195	24.02	0.639765582	-0.975	0.6475
459	7	AR2	27.79	26.85	27.23	26.93	0.425989	27.2	27.53		28.37	27.87	0.4225321	27.92333	0.6	0.72333333	2.345833333
459	12	LEP	26.25	26.53	25.59	26	0.398863	26.0925	23.87	24.4	23.98	24.59	0.3410767	24.21	0.524809489	-1.8825	0
459	1	LEP	24.69	24.83	25.53	24.93	0.37	24.995	26.72	26.47	25.98	26.57	0.3202603	26.435	0.489353315	1.44	3.3225
459	7	LEP	27.79	26.85	27.23	26.93	0.425989	27.2	27.66	28.71	28.47	26.81	0.8614087	27.9125	0.960984738	0.7125	2.595
459	12	LPL	26.25	26.53	25.59	26	0.398863	26.0925	23.27	22.98	23.55	23.33	0.2348581	23.2825	0.462871472	-2.81	0

459	1	LPL	24.69	24.83	25.53	24.93	0.37	24.995	24.83	24.74	24.54	24.66	0.1231192	24.6925	0.389946578	-0.3025	2.5075
459	7	LPL	27.79	26.85	27.23	26.93	0.425989	27.2	27.49	26.39	26.88	27.32	0.492409	27.02	0.651101631	-0.18	2.63
459	12	SCD	26.25	26.53	25.59	26	0.398863	26.0925	18.83	18.67	19.44	17.67	0.7342286	18.6525	0.835573655	-7.44	0
459	1	SCD	24.69	24.83	25.53	24.93	0.37	24.995	23.34	23.63	23.74	23.56	0.1687947	23.5675	0.406683743	-1.4275	6.0125
459	7	SCD	27.79	26.85	27.23	26.93	0.425989	27.2	29.47	29.58	27.86	28.54	0.8150409	28.8625	0.919651202	1.6625	9.1025
447	12	ADIPOQ	25.31	25.73	25.93	25.56	0.262853	25.6325	21.4	21.36	21.44	20.89	0.257083	21.2725	0.367672862	-4.36	0
447	1	ADIPOQ	25.85	25.55	25.31	25.59	0.221133	25.575	21.82	21.65	21.66	21.46	0.1472809	21.6475	0.265690923	-3.9275	0.4325

Animal	Time	Gene	EIF3K	EIF3K	EIF3K	EIF3K	SD	Coverage	Sample1	Sample2	Sample3	Sample4	SD	Saverage	SD_deltaCt	deltaCt	deltadeltaCt (Ct-colCt)
447	7	ADIPOQ	24.84	25.53	25.82	25.47	0.412674	25.415	20.43	19.79	19.87	19.95	0.2875181	20.01	0.502957917	-5.405	-1.045
447	12	AR2	25.31	25.73	25.93	25.56	0.262853	25.6325	24.96	24.83	24.42	24.58	0.2430878	24.6975	0.358027001	-0.935	0
447	1	AR2	25.85	25.55	25.31	25.59	0.221133	25.575	25.76	25.39	25.95	26.42	0.4285635	25.88	0.482251663	0.305	1.24
447	7	AR2	24.84	25.53	25.82	25.47	0.412674	25.415	24.64	23.86	24.42	25.55	0.7030588	24.6175	0.815224918	-0.7975	0.1375
447	12	LEP	25.31	25.73	25.93	25.56	0.262853	25.6325	24.37	24.65	24.6	24.43	0.1337597	24.5125	0.29492937	-1.12	0
447	1	LEP	25.85	25.55	25.31	25.59	0.221133	25.575	27.8	27.32	27.48	26.96	0.349285	27.39	0.413400532	1.815	2.935
447	7	LEP	24.84	25.53	25.82	25.47	0.412674	25.415	25.88	25.47	25.54	24.91	0.4020779	25.45	0.576165485	0.035	1.155
447	12	LPL	25.31	25.73	25.93	25.56	0.262853	25.6325	23.33	23.39	23.44	22.92	0.2376272	23.27	0.354342113	-2.3625	0
447	1	LPL	25.85	25.55	25.31	25.59	0.221133	25.575	23.75	23.9	23.96	24.43	0.2935984	24.01	0.367559519	-1.565	0.7975
447	7	LPL	24.84	25.53	25.82	25.47	0.412674	25.415	23.49	23.36	24.44	22.97	0.6237788	23.565	0.747930478	-1.85	0.5125
447	12	SCD	25.31	25.73	25.93	25.56	0.262853	25.6325	19.79	20.2	21.3	19.85	0.7004046	20.285	0.748103157	-5.3475	0

447	1	SCD	25.85	25.55	25.31	25.59	0.221133	25.575	24.38	24.37	24.61	24.25	0.1504161	24.4025	0.267441582	-1.1725	4.175
447	7	SCD	24.84	25.53	25.82	25.47	0.412674	25.415	23.91	23.95	24.38	24.53	0.3096638	24.1925	0.515937658	-1.2225	4.125
455	12	ADIPOQ	25.88	26.31	25.98	26.38	0.244728	26.1375	21.43	21.58	21.48	21.44	0.0684957	21.4825	0.254132511	-4.655	0
455	1	ADIPOQ	25.34	25.23	25.79	25.51	0.243909	25.4675	21.81	21.73	21.77	21.35	0.2125245	21.665	0.323509402	-3.8025	0.8525
455	7	ADIPOQ	25.93	25.55	25.77	25.51	0.196638	25.69	21.51	21.66	21.54	21.56	0.065	21.5675	0.207103034	-4.1225	0.5325
455	12	AR2	25.88	26.31	25.98	26.38	0.244728	26.1375	25.68	25.66	25.93	25.53	0.1671327	25.7	0.29635283	-0.4375	0
455	1	AR2	25.34	25.23	25.79	25.51	0.243909	25.4675	25.61	24.73	24.88	24.74	0.418967	24.99	0.484793771	-0.4775	-0.04
455	7	AR2	25.93	25.55	25.77	25.51	0.196638	25.69	25.42	24.82	25.46	25.32	0.2959167	25.255	0.355293306	-0.435	0.0025
455	12	LEP	25.88	26.31	25.98	26.38	0.244728	26.1375	25.69	26.31	25.84	25.29	0.4213767	25.7825	0.487288416	-0.355	0
455	1	LEP	25.34	25.23	25.79	25.51	0.243909	25.4675	25.91	25.66	25.78	24.71	0.5462905	25.515	0.598268334	0.0475	0.4025
455	7	LEP	25.93	25.55	25.77	25.51	0.196638	25.69	26.47	25.78	25.27	25.6	0.506162	25.78	0.543016267	0.09	0.445
455	12	LPL	25.88	26.31	25.98	26.38	0.244728	26.1375	23.88	23.83	24.44	23.86	0.2923896	24.0025	0.381291664	-2.135	0
455	1	LPL	25.34	25.23	25.79	25.51	0.243909	25.4675	24.55	24.48	23.97	24.44	0.2639444	24.36	0.359386051	-1.1075	1.0275
455	7	LPL	25.93	25.55	25.77	25.51	0.196638	25.69	24.71	24.51	24.55	24.62	0.0877021	24.5975	0.215309854	-1.0925	1.0425
455	12	SCD	25.88	26.31	25.98	26.38	0.244728	26.1375	23.33	23.69	24.44	23.72	0.4650806	23.795	0.525539405	-2.3425	0
455	1	SCD	25.34	25.23	25.79	25.51	0.243909	25.4675	27.39	27.54	27.5	27.4	0.0741058	27.4575	0.254918288	1.99	4.3325
455	7	SCD	25.93	25.55	25.77	25.51	0.196638	25.69	26.45	26.57	26.95	26.85	0.2340228	26.705	0.305668666	1.015	3.3575

Animal	Time	Gene	EIF3K	EIF3K	EIF3K	EIF3K	SD	Coverage	Sample1	Sample2	Sample3	Sample4	SD	Saverage	SD_deltaCt	deltaCt	deltadeltaCt (Ct-colCt)
460	12	ADIPOQ	26.36		26.51	26.31	0.104083	26.39333	23.28	23.22	22.87	23.52	0.2683747	23.2225	0.287851235	-	0

3.17083333

460	1	ADIPOQ	25.51	24.95	24.83	24.94	0.306526	25.0575	21.67	21.87	21.75	21.63	0.1058301	21.73	0.324281257	-3.3275	-	0.156666667
460	7	ADIPOQ	25.89	26.77	25.99	26.61	0.439811	26.315	22.32	22.45	22.24	22.33	0.0866025	22.335	0.448255879	-3.98	-	-0.809166666
460	12	AR2	26.36		26.51	26.31	0.104083	26.39333	27.81	27.28	27.55	27.69	0.2279437	27.5825	0.250582654	1.18916667	-	0
460	1	AR2	25.51	24.95	24.83	24.94	0.306526	25.0575	25.4	25.46	25.62	25.46	0.0943398	25.485	0.320715346	0.4275	-	0.761666667
460	7	AR2	25.89	26.77	25.99	26.61	0.439811	26.315	26.78	26.84	26.49	26.65	0.1551344	26.69	0.466368953	0.375	-	0.814166667
460	12	LEP	26.36		26.51	26.31	0.104083	26.39333	28.51	27.68		28.48	0.4707795	28.22333	0.482147972	1.83	-	0
460	1	LEP	25.51	24.95	24.83	24.94	0.306526	25.0575	27	27.56	27.42	27.8	0.3356089	27.445	0.45452356	2.3875	-	0.5575
460	7	LEP	25.89	26.77	25.99	26.61	0.439811	26.315	28.97		28.41	27.89	0.5401234	28.42333	0.696539063	2.10833333	-	0.278333333
460	12	LPL	26.36		26.51	26.31	0.104083	26.39333	26.5		25.99	25.83	0.3499047	26.10667	0.365057073	0.28666667	-	0
460	1	LPL	25.51	24.95	24.83	24.94	0.306526	25.0575	25.55	25.51	24.84	25.7	0.3821867	25.4	0.489923463	0.3425	-	0.629166667
460	7	LPL	25.89	26.77	25.99	26.61	0.439811	26.315	26.83	26.57	25.89	25.88	0.4823812	26.2925	0.652782506	-0.0225	-	0.264166667
460	12	SCD	26.36		26.51	26.31	0.104083	26.39333	26.89	26.93	26.54	26.7	0.1804624	26.765	0.208326667	0.37166667	-	0
460	1	SCD	25.51	24.95	24.83	24.94	0.306526	25.0575	24.46	25.45	26.43	25.36	0.8054191	25.425	0.861776266	0.3675	-	0.004166667
460	7	SCD	25.89	26.77	25.99	26.61	0.439811	26.315				27.33	#DIV/0!	27.33	#DIV/0!	1.015	-	0.643333333
436	12	ADIPOQ	29.69	29.73	29.82	29.75	0.054391	29.7475	25.98	25.44	25.21	24.97	0.4316634	25.4	0.435076622	-4.3475	-	0
436	1	ADIPOQ	29.96	29.36	29.27	28.58	0.565177	29.2925	23.83	24.28	23.55	23.36	0.3997082	23.755	0.692236713	-5.5375	-	-1.19

436	7	ADIPOQ	30.56	30.72	30.49	30.34	0.157771	30.5275		31.66	32.53	32.66	0.5437217	32.28333	0.566149274	1.75583333	6.103333333
436	12	AR2	29.69	29.73	29.82	29.75	0.054391	29.7475	31.57	32.95	32.78	31.66	0.7259477	32.24	0.727982372	2.4925	0
436	1	AR2	29.96	29.36	29.27	28.58	0.565177	29.2925	29.97	29.59	29.84	29.56	0.1981582	29.74	0.59890873	0.4475	-2.045
436	7	AR2	30.56	30.72	30.49	30.34	0.157771	30.5275					#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
436	12	LEP	29.69	29.73	29.82	29.75	0.054391	29.7475	31.83	31.77	31.7		0.0650641	31.76667	0.084803695	2.01916667	0
436	1	LEP	29.96	29.36	29.27	28.58	0.565177	29.2925	29.54	31.48	30.53	31.48	0.9270158	30.7575	1.085717888	1.465	0.554166667
436	7	LEP	30.56	30.72	30.49	30.34	0.157771	30.5275					#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
436	12	LPL	29.69	29.73	29.82	29.75	0.054391	29.7475	28.96	28.97	30.46	30.38	0.8406892	29.6925	0.842446833	-0.055	0
436	1	LPL	29.96	29.36	29.27	28.58	0.565177	29.2925	26.82	26.93	27.76	27.38	0.4325409	27.2225	0.711699843	-2.07	-2.015
Animal	Time	Gene	EIF3K	EIF3K	EIF3K	EIF3K	SD	Coverage	Sample1	Sample2	Sample3	Sample4	SD	Saverage	SD_deltaCt	deltaCt	deltadeltaCt (Ct-colCt)
436	7	LPL	30.56	30.72	30.49	30.34	0.157771	30.5275	25.68	25.57	25.51	25.48	0.0883176	25.56	0.18080837	-4.9675	-4.9125
436	12	SCD	29.69	29.73	29.82	29.75	0.054391	29.7475	25.75	25.34	26.5	25.68	0.48897	25.8175	0.491985772	-3.93	0
436	1	SCD	29.96	29.36	29.27	28.58	0.565177	29.2925	26.58	26.9	27.52	26.66	0.4256368	26.915	0.707525029	-2.3775	1.5525
436	7	SCD	30.56	30.72	30.49	30.34	0.157771	30.5275	25.88	25.9	26.65	26.33	0.3703152	26.19	0.402523291	-4.3375	-0.4075
525	12	ADIPOQ	28.63	28.46	28.34	28.51	0.120139	28.485	27.72	27.77	27	27.66	0.361144	27.5375	0.380602592	-0.9475	0
525	1	ADIPOQ		31.41			#DIV/0!	31.41		32.55	31.92		0.4454773	32.235	#DIV/0!	0.825	1.7725
525	7	ADIPOQ	29.73	30.44	29.56	29.76	0.388448	29.8725	32.37	31.55	31.62	30.54	0.7513987	31.52	0.845867405	1.6475	2.595
525	12	AR2	28.63	28.46	28.34	28.51	0.120139	28.485	29.39	29.68	29.88	30.63	0.5296854	29.895	0.543139025	1.41	0

525	1	AR2		31.41			#DIV/0!	31.41				#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
525	7	AR2	29.73	30.44	29.56	29.76	0.388448	29.8725				#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
525	12	LEP	28.63	28.46	28.34	28.51	0.120139	28.485	28.86	28.81		0.0353553	28.835	0.125233116	0.35	0	
525	1	LEP		31.41			#DIV/0!	31.41				#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
525	7	LEP	29.73	30.44	29.56	29.76	0.388448	29.8725				#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
525	12	LPL	28.63	28.46	28.34	28.51	0.120139	28.485	28.74	28.9	29.42	28.48	0.3964425	28.885	0.414246304	0.4	0
525	1	LPL		31.41			#DIV/0!	31.41	28.48	27.96	27.92	27.96	0.2673325	28.08	#DIV/0!	-3.33	-3.73
525	7	LPL	29.73	30.44	29.56	29.76	0.388448	29.8725	30.72	29.7	30.2	30.93	0.551566	30.3875	0.674623352	0.515	0.115
525	12	SCD	28.63	28.46	28.34	28.51	0.120139	28.485	24.45	24.61	24.69	24.49	0.1101514	24.56	0.162992842	-3.925	0
525	1	SCD		31.41			#DIV/0!	31.41	28.49	28.38	27.93	28.48	0.2647011	28.32	#DIV/0!	-3.09	0.835
525	7	SCD	29.73	30.44	29.56	29.76	0.388448	29.8725		32.51	31.51	31.3	0.646555	31.77333	0.754271171	1.90083333	5.825833333
540	12	ADIPOQ	25.26	24.8	24.83	24.62	0.271339	24.8775	22.45	21.68	21.55	21.42	0.4623491	21.775	0.536089234	-3.1025	0
540	1	ADIPOQ	23.5	23.74	23.35	22.35	0.611474	23.235	20.31	18.98	19.63	18.99	0.6328441	19.4775	0.879995265	-3.7575	-0.655
540	7	ADIPOQ	25.79	25.58	25.75	24.77	0.477101	25.4725	22.37	21.43	21.38	21.5	0.4692547	21.67	0.66919728	-3.8025	-0.7
540	12	AR2	25.26	24.8	24.83	24.62	0.271339	24.8775	24.36	24.35	24.67	24.49	0.1493039	24.4675	0.30970416	-0.41	0
540	1	AR2	23.5	23.74	23.35	22.35	0.611474	23.235	23.77	23.8	24.7	24.49	0.4756049	24.19	0.774661216	0.955	1.365
540	7	AR2	25.79	25.58	25.75	24.77	0.477101	25.4725	24.83	25.45	24.75	25.62	0.4369115	25.1625	0.646928641	-0.31	0.1
540	12	LEP	25.26	24.8	24.83	24.62	0.271339	24.8775	26	25.75	25.66	25.75	0.1462874	25.79	0.308261253	0.9125	0
540	1	LEP	23.5	23.74	23.35	22.35	0.611474	23.235	26.47	26.51	25.46	25.27	0.6543381	25.9275	0.895577095	2.6925	1.78
540	7	LEP	25.79	25.58	25.75	24.77	0.477101	25.4725	26.58	24.22	23.55	24.73	1.2998205	24.77	1.384614868	-0.7025	-1.615

Animal	Time	Gene	EIF3K	EIF3K	EIF3K	EIF3K	SD	Coverage	Sample1	Sample2	Sample3	Sample4	SD	Saverage	SD_deltaCt	deltaCt	deltadeltaCt (Ct-colCt)
540	12	LPL	25.26	24.8	24.83	24.62	0.271339	24.8775	25.39	24.82	25.62	25.52	0.3576194	25.3375	0.448906078	0.46	0
540	1	LPL	23.5	23.74	23.35	22.35	0.611474	23.235	23.61	23.9	24.36	24.44	0.3921203	24.0775	0.726400945	0.8425	0.3825
540	7	LPL	25.79	25.58	25.75	24.77	0.477101	25.4725	24.75	24.74	24.68	24.85	0.0704746	24.755	0.482277583	-0.7175	-1.1775
540	12	SCD	25.26	24.8	24.83	24.62	0.271339	24.8775	24.95	25.43	25.3	25.27	0.2038586	25.2375	0.339386702	0.36	0
540	1	SCD	23.5	23.74	23.35	22.35	0.611474	23.235	26.39	25.94	26.88	26.36	0.3844802	26.3925	0.722305337	3.1575	2.7975
540	7	SCD	25.79	25.58	25.75	24.77	0.477101	25.4725	20.3	20.54	20.85	20.51	0.2267157	20.55	0.52822817	-4.9225	-5.2825
439	12	ADIPOQ	31.25	30.37	30.58	29.76	0.614546	30.49	31.39	30.54	30.83	29.74	0.6874348	30.625	0.92208098	0.135	0
439	7	ADIPOQ			32.49		#DIV/0!	32.49				33.43	#DIV/0!	33.43	#DIV/0!	0.94	0.805
439	12	AR2	31.25	30.37	30.58	29.76	0.614546	30.49				31.41	#DIV/0!	31.41	#DIV/0!	0.92	0
439	7	AR2			32.49		#DIV/0!	32.49					#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
439	12	LEP	31.25	30.37	30.58	29.76	0.614546	30.49				27.21	#DIV/0!	27.21	#DIV/0!	-3.28	0
439	7	LEP			32.49		#DIV/0!	32.49					#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
439	12	LPL	31.25	30.37	30.58	29.76	0.614546	30.49	29.49	29.45	29.5	29.43	0.0330404	29.4675	0.615433452	-1.0225	0
439	7	LPL			32.49		#DIV/0!	32.49	28.46	28.37	28.53	27.99	0.2407454	28.3375	#DIV/0!	-4.1525	-3.13
439	12	SCD	31.25	30.37	30.58	29.76	0.614546	30.49		28.36	28.53	27.77	0.3988734	28.22	0.732643615	-2.27	0
439	7	SCD			32.49		#DIV/0!	32.49	29.42	30.32	29.85	29.63	0.3856164	29.805	#DIV/0!	-2.685	-0.415

Appendix 2. The Ct values of EIF3K, PPAR, AR1 and HSL

Animal	Time	Gene	EIF3K	EIF3K	EIF3K	EIF3K	SD	Coverage	Sample1	Sample2	Sample3	Sample4	SD	Saverage	SD_deltaCt	deltaCt	deltadeltaCt (Ct-colCt)
440	12	PPAR- gamma	24.47	24.49	23.95	23.82	0.3476948	24.1825	22.41	21.95	22.42	21.96	0.2656439	22.185	0.4375595	-1.9975	0
440	1	PPAR- gamma	27.41	27.40	27.30	26.57	0.4030715	27.17	24.36	24.53	24.73	24.51	0.1519594	24.5325	0.4307648	-2.6375	-0.64
440	7	PPAR- gamma	26.75	26.71	26.72	26.47	0.129454	26.6625	24.68	24.68	24.40	24.38	0.1676305	24.535	0.2117979	-2.1275	-0.13
440	12	AR1	24.47	24.49	23.95	23.82	0.3476948	24.1825	28.74	28.64	28.82	28.93	0.1228481	28.7825	0.3687592	4.6	0
440	1	AR1	27.41	27.40	27.30	26.57	0.4030715	27.17	31.84	32.39	32.38	32.89	0.4288356	32.375	0.5885292	5.205	0.605
440	7	AR1	26.75	26.71	26.72	26.47	0.129454	26.6625	31.36	31.61	31.76	31.64	0.168003	31.5925	0.2120927	4.93	0.33
440	12	HSL	24.47	24.49	23.95	23.82	0.3476948	24.1825	26.94	26.84	26.78	26.93	0.0763217	26.8725	0.3559728	2.69	0
Animal	Time	Gene	EIF3K	EIF3K	EIF3K	EIF3K	SD	Coverage	Sample1	Sample2	Sample3	Sample4	SD	Saverage	SD_deltaCt	deltaCt	deltadeltaCt (Ct-colCt)
440	1	HSL	27.41	27.40	27.30	26.57	0.4030715	27.17	26.44	26.79	26.74	26.37	0.2107922	26.585	0.4548626	-0.585	-3.275
440	7	HSL	26.75	26.71	26.72	26.47	0.129454	26.6625	30.57	30.88	30.80	31.51	0.4020779	30.94	0.4224038	4.2775	1.5875
560	12	PPAR- gamma	29.76	29.96	30.42	29.71	0.3235609	29.9625	29.31	30.46	29.76	29.82	0.4733128	29.8375	0.5733382	-0.125	0
560	1	PPAR- gamma	27.85	26.79	27.55	26.73	0.5568962	27.23	24.81	24.62	25.34	25.58	0.4479118	25.0875	0.7146736	-2.1425	-2.0175

560	7	PPAR-gamma	27.63	26.80	26.72	26.43	0.5151375	26.895	23.83	23.98	24.53	23.98	0.3082207	24.08	0.6003055	-2.815	-2.69
560	12	AR1	29.76	29.96	30.42	29.71	0.3235609	29.9625	35.34	35.70	35.90	35.47	0.2479751	35.6025	0.4076559	5.64	0
560	1	AR1	27.85	26.79	27.55	26.73	0.5568962	27.23	33.63	33.76	32.93	33.56	0.369414	33.47	0.6682814	6.24	0.6
560	7	AR1	27.63	26.80	26.72	26.43	0.5151375	26.895	32.00	32.83	32.87	33.52	0.6229232	32.805	0.8083316	5.91	0.27
560	12	HSL	29.76	29.96	30.42	29.71	0.3235609	29.9625		32.75			#DIV/0!	32.75	#DIV/0!	2.7875	0
560	1	HSL	27.85	26.79	27.55	26.73	0.5568962	27.23	32.52	33.69			0.8273149	33.105	0.997288	5.875	3.0875
560	7	HSL	27.63	26.80	26.72	26.43	0.5151375	26.895	31.87	31.92	32.86	31.70	0.5235376	32.0875	0.7344783	5.1925	2.405
544	12	PPAR-gamma	31.62	31.69	31.50	32.53	0.4699291	31.835	30.87	30.86	30.69	30.56	0.1484363	30.745	0.492815	-1.09	0
544	1	PPAR-gamma	28.45	27.82	27.90	27.52	0.3878466	27.9225	25.53	25.58	25.50	25.77	0.1212436	25.595	0.4063558	-2.3275	-1.2375
544	7	PPAR-gamma	28.96	29.26	29.28	28.76	0.2505328	29.065	28.99	28.57	28.51	28.56	0.2232151	28.6575	0.3355468	-0.4075	0.6825
544	12	AR1	31.62	31.69	31.50	32.53	0.4699291	31.835	36.93	35.92	38.82	39.63	1.7007939	37.825	1.7645207	5.99	0
544	1	AR1	28.45	27.82	27.90	27.52	0.3878466	27.9225	31.35	31.73	31.82	31.60	0.2043689	31.625	0.4383967	3.7025	-2.2875
544	7	AR1	28.96	29.26	29.28	28.76	0.2505328	29.065	35.99	34.96	36.97	36.72	0.9015912	36.16	0.9357528	7.095	1.105

544	12	HSL	31.62	31.69	31.50	32.53	0.4699291	31.835	33.98		32.32	32.42	0.9308777	32.906667	1.0427688	1.0716667	0
544	1	HSL	28.45	27.82	27.90	27.52	0.3878466	27.9225	31.77	30.93	31.59	31.64	0.3760652	31.4825	0.5402314	3.56	2.4883333
544	7	HSL	28.96	29.26	29.28	28.76	0.2505328	29.065	30.89	31.71	31.45	30.75	0.4550458	31.2	0.5194548	2.135	1.0633333
449	12	PPAR- gamma	29.51	29.62	29.74	29.36	0.1617354	29.5575	27.77	27.91	28.35	27.51	0.3515205	27.885	0.3869431	-1.6725	0
449	1	PPAR- gamma	27.82	27.30	27.86	26.98	0.425049	27.49	24.96	24.92	24.96	25.44	0.2473863	25.07	0.4917994	-2.42	-0.7475
Animal	Time	Gene	EIF3K	EIF3K	EIF3K	EIF3K	SD	Coverage	Sample1	Sample2	Sample3	Sample4	SD	Saverage	SD_deltaCt	deltaCt	deltadeltaCt (Ct-colCt)
449	7	PPAR- gamma	24.51	24.85	24.84	24.35	0.2483781	24.6375	22.63	22.67	22.69	22.56	0.057373	22.6375	0.2549183	-2	-0.3275
449	12	AR1	29.51	29.62	29.74	29.36	0.1617354	29.5575	37.49	36.75	36.55	35.41	0.8608523	36.55	0.8759138	6.9925	0
449	1	AR1	27.82	27.30	27.86	26.98	0.425049	27.49	32.77	32.78	32.73	32.94	0.0925563	32.805	0.4350096	5.315	-1.6775
449	7	AR1	24.51	24.85	24.84	24.35	0.2483781	24.6375	28.42	28.53	28.43	28.60	0.0858293	28.495	0.2627895	3.8575	-3.135
449	12	HSL	29.51	29.62	29.74	29.36	0.1617354	29.5575	31.77	31.61	32.32	32.40	0.3936581	32.025	0.4255878	2.4675	0
449	1	HSL	27.82	27.30	27.86	26.98	0.425049	27.49	29.49	29.50	29.80	30.33	0.3938697	29.78	0.5794825	2.29	-0.1775
449	7	HSL	24.51	24.85	24.84	24.35	0.2483781	24.6375	25.41	24.94	25.51	24.96	0.2973774	25.205	0.3874597	0.5675	-1.9

510	12	PPAR-gamma			34.87		#DIV/0!	34.87		37.87	38.79	0.6505382	38.33	#DIV/0!	3.46	0	
510	1	PPAR-gamma	28.68	28.75	28.54	28.63	0.0883176	28.65	28.52	28.98	29.62	29.49	0.5040751	29.1525	0.5117535	0.5025	-2.9575
510	7	PPAR-gamma	26.36	25.89	25.89	25.74	0.2694439	25.97	23.78	24.32	23.44	23.85	0.3623419	23.8475	0.4515436	-2.1225	-5.5825
510	12	AR1			34.87		#DIV/0!	34.87					#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
510	1	AR1	28.68	28.75	28.54	28.63	0.0883176	28.65	34.70	35.60	36.31	35.91	0.6847384	35.63	0.6904105	6.98	#DIV/0!
510	7	AR1	26.36	25.89	25.89	25.74	0.2694439	25.97	29.72	29.57	28.84	30.57	0.7097652	29.675	0.7591882	3.705	#DIV/0!
510	12	HSL			34.87		#DIV/0!	34.87	39.00				#DIV/0!	39	#DIV/0!	4.13	0
510	1	HSL	28.68	28.75	28.54	28.63	0.0883176	28.65		33.38	32.65	34.35	0.8528189	33.46	0.8573797	4.81	0.68
510	7	HSL	26.36	25.89	25.89	25.74	0.2694439	25.97	29.32	29.84	29.92	29.50	0.2830194	29.645	0.3907685	3.675	-0.455
403	12	PPAR-gamma	25.33	24.95	24.81	24.64	0.2937544	24.9325	24.82	23.51	23.64	23.99	0.5892934	23.99	0.6584515	-0.9425	0
403	1	PPAR-gamma	23.64	24.47	24.52	23.95	0.423989	24.145	23.98	24.28	24.44	23.59	0.3739318	24.0725	0.5653244	-0.0725	0.87
403	7	PPAR-gamma	25.36	25.65	25.36	24.81	0.3510461	25.295	23.45	23.38	23.91	22.99	0.3772157	23.4325	0.5152912	-1.8625	-0.92
403	12	AR1	25.33	24.95	24.81	24.64	0.2937544	24.9325	30.43	30.49	30.6	30.61	0.0873212	30.5325	0.3064583	5.6	0

403	1	AR1	23.64	24.47	24.52	23.95	0.423989	24.145	29.58	29.93	29.89	29.74	0.1592692	29.785	0.4529165	5.64	0.04
403	7	AR1	25.36	25.65	25.36	24.81	0.3510461	25.295	29.75	29.47	29.72	30.27	0.3359936	29.8025	0.4859269	4.5075	-1.0925
403	12	HSL	25.33	24.95	24.81	24.64	0.2937544	24.9325	30.84	30.5	30.51	32.58	0.9942962	31.1075	1.0367819	6.175	0
Animal	Time	Gene	EIF3K	EIF3K	EIF3K	EIF3K	SD	Coverage	Sample1	Sample2	Sample3	Sample4	SD	Saverage	SD_deltaCt	deltaCt	deltadeltaCt (Ct-colCt)
403	1	HSL	23.64	24.47	24.52	23.95	0.423989	24.145	28.58	27.95	28.94	28.45	0.409634	28.48	0.5895478	4.335	-1.84
403	7	HSL	25.36	25.65	25.36	24.81	0.3510461	25.295	28.73	29	28.73	28.57	0.1783956	28.7575	0.3937745	3.4625	-2.7125
459	12	PPAR- gamma	25.4	26.33	25.96	25.99	0.3851407	25.92	23.89	23.88	24.58	23.76	0.3730393	24.0275	0.5361825	-1.8925	0
459	1	PPAR- gamma	23.81	24.48	23.99	23.76	0.3285321	24.01	22.34		21.91	21.92	0.2454248	22.056667	0.4100813	1.9533333	-0.0608333
459	7	PPAR- gamma	24.85	25.43	25.55	25.69	0.3689625	25.38	25.39	25.44	25.41	25.41	0.0206155	25.4125	0.369538	0.0325	1.925
459	12	AR1	25.4	26.33	25.96	25.99	0.3851407	25.92	30.7	30.27	30.53	30.75	0.2165448	30.5625	0.4418427	4.6425	0
459	1	AR1	23.81	24.48	23.99	23.76	0.3285321	24.01	27.51	27.34	27.38	27.43	0.0732575	27.415	0.3366007	3.405	-1.2375
459	7	AR1	24.85	25.43	25.55	25.69	0.3689625	25.38	29.87	29.86	30.83	30.48	0.4779819	30.26	0.6038212	4.88	0.2375
459	12	HSL	25.4	26.33	25.96	25.99	0.3851407	25.92	28.92	28.44	29.65	28.43	0.5741661	28.86	0.6913754	2.94	0
459	1	HSL	23.81	24.48	23.99	23.76	0.3285321	24.01	24.52	24.52	24.66	24.81	0.1384136	24.6275	0.3564992	0.6175	-2.3225
459	7	HSL	24.85	25.43	25.55	25.69	0.3689625	25.38	29.33	28.79	28.77	29.4	0.3390551	29.0725	0.5010905	3.6925	0.7525
447	12	PPAR- gamma	23.64	23.64	23.52	23.59	0.0567891	23.5975	21.72	21.71	21.83	21.6	0.0939858	21.715	0.1098104	-1.8825	0
447	1	PPAR-	23.81	24.38	24.28	24.28	0.2560436	24.1875	22.99	22.94	23.33	22.72	0.2522565	22.995	0.3594324	-1.1925	0.69

Animal	Time	Gene	EIF3K	EIF3K	EIF3K	EIF3K	SD	Coverage	Sample1	Sample2	Sample3	Sample4	SD	Saverage	SD_deltaCt	deltaCt	deltadeltaCt (Ct-colCt)
		gamma															
		PPAR-gamma															
447	7	PPAR-gamma	24.53	25.33	24.93	24.88	0.3275541	24.9175	22.43	22.64	22.56	22.59	0.0896289	22.555	0.3395953	-2.3625	-0.48
447	12	AR1	23.64	23.64	23.52	23.59	0.0567891	23.5975	27.58	27.59	27.93	27.78	0.1675311	27.72	0.1768945	4.1225	0
447	1	AR1	23.81	24.38	24.28	24.28	0.2560436	24.1875	28.36	28.8	28.76	28.73	0.203695	28.6625	0.327185	4.475	0.3525
447	7	AR1	24.53	25.33	24.93	24.88	0.3275541	24.9175	28.39	28.59	29.32	28.66	0.4032369	28.74	0.519511	3.8225	-0.3
447	12	HSL	23.64	23.64	23.52	23.59	0.0567891	23.5975	26.67	26.81	27.39	26.98	0.3119161	26.9625	0.3170436	3.365	0
447	1	HSL	23.81	24.38	24.28	24.28	0.2560436	24.1875	27.92	27.71	28.35	27.84	0.2771883	27.955	0.3773482	3.7675	0.4025
447	7	HSL	24.53	25.33	24.93	24.88	0.3275541	24.9175	26.74	26.74	27.59	27.43	0.4493328	27.125	0.5560501	2.2075	-1.1575
455	12	PPAR-gamma	23.84	24.36	23.96	23.51	0.3510342	23.9175	20.96	20.95	21.33	20.91	0.1961929	21.0375	0.4021401	-2.88	0
455	1	PPAR-gamma	23.4	23.48	23.57	23.6	0.0906918	23.5125	22.46	21.98	22.51	21.94	0.304234	22.2225	0.3174639	-1.29	1.59
455	7	PPAR-gamma	23.31	23.32	23.29	22.7	0.3035896	23.155	21.77	21.72	21.94	21.65	0.1235584	21.77	0.3277702	-1.385	1.495
455	12	AR1	23.84	24.36	23.96	23.51	0.3510342	23.9175	26.74	26.75	26.38	26.68	0.1744276	26.6375	0.3919821	2.72	0
455	1	AR1	23.4	23.48	23.57	23.6	0.0906918	23.5125	27.29	26.83	26.84	26.88	0.2210581	26.96	0.2389386	3.4475	0.7275
455	7	AR1	23.31	23.32	23.29	22.7	0.3035896	23.155	26.66	26.73	26.95	26.78	0.1235584	26.78	0.3277702	3.625	0.905
455	12	HSL	23.84	24.36	23.96	23.51	0.3510342	23.9175	21.72	21.8	22.45	21.82	0.3377746	21.9475	0.4871516	-1.97	0
455	1	HSL	23.4	23.48	23.57	23.6	0.0906918	23.5125	22.7	23.36	23.42	22.83	0.3655475	23.0775	0.3766298	-0.435	1.535

455	7	HSL	23.31	23.32	23.29	22.7	0.3035896	23.155	22.88	22.93	22.8	22.89	0.0544671	22.875	0.3084369	-0.28	1.69
460	12	PPAR- gamma	24.2	24.67	24.32	24.43	0.2000833	24.405	23.87	23.72	23.69	23.88	0.0989949	23.79	0.2232338	-0.615	0
460	1	PPAR- gamma	23.67	23.9	23.7	23.65	0.115181	23.73	23.33	23.37	23.44	23.49	0.0713559	23.4075	0.1354929	-0.3225	0.2925
460	7	PPAR- gamma	22.54	22.63	22.7	22.92	0.1621471	22.6975	21.74	21.87	21.55	21.63	0.1388944	21.6975	0.2135025	-1	-0.385
460	12	AR1	24.2	24.67	24.32	24.43	0.2000833	24.405	28.94	28.74	28.92	28.75	0.1071992	28.8375	0.2269912	4.4325	0
460	1	AR1	23.67	23.9	23.7	23.65	0.115181	23.73	27.97	28.34	28.47	28.31	0.2132878	28.2725	0.2424012	4.5425	0.11
460	7	AR1	22.54	22.63	22.7	22.92	0.1621471	22.6975	26.52	26.53	26.63	26.57	0.0499166	26.5625	0.1696565	3.865	-0.5675
460	12	HSL	24.2	24.67	24.32	24.43	0.2000833	24.405	26.52	26.69	26.71	26.52	0.1042433	26.61	0.2256103	2.205	0
460	1	HSL	23.67	23.9	23.7	23.65	0.115181	23.73	25.39	25.73	25.69	25.59	0.1518771	25.6	0.190613	1.87	-0.335
460	7	HSL	22.54	22.63	22.7	22.92	0.1621471	22.6975	23.36	22.82	23.44	23.36	0.2858321	23.245	0.3286209	0.5475	-1.6575
436	12	PPAR- gamma	23.42	23.34	23.34	22.87	0.2511805	23.2425	21.39	20.71	20.72	20.83	0.3229422	20.9125	0.4091251	-2.33	0
436	1	PPAR- gamma	24.38	23.93	23.88	24.32	0.2588919	24.1275	21.81	22.39	21.65	21.6	0.3628935	21.8625	0.4457765	-2.265	0.065
436	7	PPAR- gamma	22.62	22.6	22.6	22.7	0.0476095	22.63	24.32	23.83	23.73	23.56	0.3262923	23.86	0.3297474	1.23	3.56
436	12	AR1	23.42	23.34	23.34	22.87	0.2511805	23.2425	26.28	26.54	26.54	26.84	0.2289105	26.55	0.3398406	3.3075	0
436	1	AR1	24.38	23.93	23.88	24.32	0.2588919	24.1275	26.66	26.97	26.73	26.86	0.1377195	26.805	0.2932434	2.6775	-0.63
436	7	AR1	22.62	22.6	22.6	22.7	0.0476095	22.63	26.8	26.7	26.74	26.95	0.1096586	26.7975	0.1195478	4.1675	0.86

436	12	HSL	23.42	23.34	23.34	22.87	0.2511805	23.2425	22.43	22.54	22.71	22.52	0.1169045	22.55	0.2770529	-0.6925	0
436	1	HSL	24.38	23.93	23.88	24.32	0.2588919	24.1275	20.78	20.93	20.89	20.71	0.1007886	20.8275	0.2778189	-3.3	-2.6075
436	7	HSL	22.62	22.6	22.6	22.7	0.0476095	22.63	26.43	26.31	26.39	25.85	0.2680174	26.245	0.2722132	3.615	4.3075
525	12	PPAR- gamma	25.96	26.28	26.41	25.91	0.2434475	26.14	24.35	24.45	24.49	24.5	0.0684957	24.4475	0.2528998	-1.6925	0
525	1	PPAR- gamma	30.67	30.45	29.83	30.46	0.3628016	30.3525	28.58	28.54	28.3	28	0.2670206	28.355	0.450472	-1.9975	-0.305
525	7	PPAR- gamma	28.8	28.53	28.4	28.51	0.1699019	28.56	27.27	26.94	27.29	26.83	0.2325762	27.0825	0.2880249	-1.4775	0.215
525	12	AR1	25.96	26.28	26.41	25.91	0.2434475	26.14	29.65	29.97	30.27	29.98	0.253295	29.9675	0.3513189	3.8275	0
525	1	AR1	30.67	30.45	29.83	30.46	0.3628016	30.3525	31.84	32.38	33.56	32.31	0.7320462	32.5225	0.8170169	2.17	-1.6575
Animal	Time	Gene	EIF3K	EIF3K	EIF3K	EIF3K	SD	Coverage	Sample1	Sample2	Sample3	Sample4	SD	Saverage	SD_deltaCt	deltaCt	deltadeltaCt (Ct-colCt)
525	7	AR1	28.8	28.53	28.4	28.51	0.1699019	28.56	32.42	33.31	32.56	32.9	0.3966842	32.7975	0.4315379	4.2375	0.41
525	12	HSL	25.96	26.28	26.41	25.91	0.2434475	26.14	24.84	24.77	24.72	24.64	0.084212	24.7425	0.2576011	-1.3975	0
525	1	HSL	30.67	30.45	29.83	30.46	0.3628016	30.3525	31.59	30.32	30.82	31.53	0.6074263	31.065	0.707525	0.7125	2.11
525	7	HSL	28.8	28.53	28.4	28.51	0.1699019	28.56	29.71	30.31	28.82	29.74	0.6153861	29.645	0.6384095	1.085	2.4825
540	12	PPAR- gamma	24.77	24.93	24.9	24.94	0.0785281	24.885	22.47	21.92	22.4	22.43	0.2582634	22.305	0.2699383	-2.58	0
540	1	PPAR- gamma	26.61	26.57	26.42	26.49	0.0846069	26.5225	23.97	23.8	23.89	23.94	0.0743864	23.9	0.1126573	-2.6225	-0.0425
540	7	PPAR- gamma	24.9	25.42	25.44	25.39	0.2591492	25.2875	23.56	23.37	23.24	23.28	0.1424488	23.3625	0.2957195	-1.925	0.655

540	12	AR1	24.77	24.93	24.9	24.94	0.0785281	24.885	28.89	28.95	29.41	29.3	0.2563039	29.1375	0.268064	4.2525	0
540	1	AR1	26.61	26.57	26.42	26.49	0.0846069	26.5225	30.48	30.6	30.68	30.87	0.1637834	30.6575	0.1843457	4.135	-0.1175
540	7	AR1	24.9	25.42	25.44	25.39	0.2591492	25.2875	29.32	29.47	29.63	29.3	0.1534058	29.43	0.3011506	4.1425	-0.11
540	12	HSL	24.77	24.93	24.9	24.94	0.0785281	24.885	26.32	25.96	26.75	26.53	0.3361547	26.39	0.3452053	1.505	0
540	1	HSL	26.61	26.57	26.42	26.49	0.0846069	26.5225	26.58	26.43	26.73	26.66	0.128841	26.6	0.1541374	0.0775	-1.4275
540	7	HSL	24.9	25.42	25.44	25.39	0.2591492	25.2875	26.37	26.36	26.36	26.51	0.0734847	26.4	0.2693665	1.1125	-0.3925
439	12	PPAR- gamma	27.34	27.69	27.33	27.27	0.1908533	27.4075	25.32	25.33	25.35	25.43	0.0499166	25.3575	0.1972731	-2.05	0
439	7	PPAR- gamma	33.56	34.64	32.33	35.2	1.2667379	33.9325	30.62	30.83	31.38	31.41	0.3964005	31.06	1.3273124	-2.8725	-0.8225
439	12	AR1	27.34	27.69	27.33	27.27	0.1908533	27.4075	30.92	32.42	31.35	31.84	0.6456715	31.6325	0.673288	4.225	0
439	7	AR1	33.56	34.64	32.33	35.2	1.2667379	33.9325	31.71	31.44	32.46	31.56	0.458503	31.7925	1.3471637	-2.14	-6.365
439	12	HSL	27.34	27.69	27.33	27.27	0.1908533	27.4075	28.53	29.33	29.27	28.55	0.4395452	28.92	0.479192	1.5125	0
439	7	HSL	33.56	34.64	32.33	35.2	1.2667379	33.9325	34.28				#DIV/0!	34.28	#DIV/0!	0.3475	-1.165
