



## Differential Expression of *PPAR $\gamma$* , *FASN*, and *ACADM* Genes in Various Adipose Tissues and *Longissimus dorsi* Muscle from Yanbian Yellow Cattle and Yan Yellow Cattle

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**ABSTRACT:** The objective of this study was to investigate the correlation between cattle breeds and deposit of adipose tissues in different positions and the gene expressions of peroxisome proliferator-activated receptor gamma (*PPAR $\gamma$* ), fatty acid synthase (*FASN*), and Acyl-CoA dehydrogenase (*ACADM*), which are associated with lipid metabolism and are valuable for understanding the physiology in fat depot and meat quality. Yanbian yellow cattle and Yan yellow cattle reared under the same conditions display different fat proportions in the carcass. To understand this difference, the expression of *PPAR $\gamma$* , *FASN*, and *ACADM* in different adipose tissues and *longissimus dorsi* muscle (LD) in these two breeds were analyzed using the Real-time quantitative polymerase chain reaction method (qRT-PCR). The result showed that *PPAR $\gamma$*  gene expression was significantly higher in adipose tissue than in LD in both breeds. *PPAR $\gamma$*  expression was also higher in abdominal fat, in perirenal fat than in the subcutaneous fat ( $p < 0.05$ ) in Yanbian yellow cattle, and was significantly higher in subcutaneous fat in Yan yellow cattle than that in Yanbian yellow cattle. On the other hand, *FASN* mRNA expression levels in subcutaneous fat and abdominal fat in Yan yellow cattle were significantly higher than that in Yanbian yellow cattle. Interestingly, *ACADM* gene shows greater fold changes in LD than in adipose tissues in Yan yellow cattle. Furthermore, the expressions of these three genes in lung, colon, kidney, liver and heart of Yanbian yellow cattle and Yan yellow cattle were also investigated. The results showed that the highest expression levels of *PPAR $\gamma$*  and *FASN* genes were detected in the lung in both breeds. The expression of *ACADM* gene in kidney and liver were higher than that in other organs in Yanbian yellow cattle, the comparison was not statistically significant in Yan yellow cattle. (**Key Words:** *PPAR $\gamma$* , *FASN*, *ACADM*, Fat Deposition, Cattle, qRT-PCR)

### INTRODUCTION

*PPAR $\gamma$*  is a subtype belonging to the PPARS receptor family which play central roles in carbohydrate and lipid metabolism in many different tissues (Aeberhard et al., 2001; Chawla et al., 2001; Escher et al., 2001). *PPAR $\gamma$*  has been located to chromosome 22 in cattle (Zimin et al., 2009). The *PPAR $\gamma$*  isoform expression is especially high in fat body (Rosen et al., 2000). It has been shown that

abnormal expression of *PPAR $\gamma$*  in fibroblasts induces the expression of a battery of genes including adipokines, leptin, resistin and the accumulation of triglyceride droplets (Tontonoz and Spiegelman, 2008). Activation of *PPAR $\gamma$*  target gene could influence the production of multiple signaling molecules in adipocytes (Barak et al., 1999). Thus, *PPAR $\gamma$*  is indeed a key transcription factor in the development and function of the adipose tissues.

*PPAR $\gamma$*  activation in adipose tissue also increases the capacity of fatty acids synthesis (Tontonoz and Spiegelman, 2008). The *FASN* gene is located on chromosome 17 in a region associated with body fat in Pima Indians (Norman et al., 1998). Furthermore, *FASN* encodes fatty acid synthase, which forms a complex homodimeric enzyme and plays an important role in biosynthesis of long chain fatty acids from

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acetyl coenzyme A (CoA) and malonyl-CoA (Semenkovich et al., 1995; Chakravarty et al., 2004). This enzyme has important roles in lipid metabolism, the regulation of body fat as well as the meat quality, which is determined by the fatty acid composition especially monounsaturated fatty acids (Laborde et al., 2001).

ACADM, a member of the acyl-CoA dehydrogenase (ACAD) family that comprises 9 known proteins, is involved in the oxidation of medium-chain fatty acids and amino acids (Kim Miura, 2004). Much effort has been devoted to research on ACADM deficiency as it is the most frequent fatty acid oxidation disorder, leading to disease or death (Smith et al., 2010). Greco et al. (2008) determined that *ACADM* mRNA level is decreased in humans with high liver fat content (Greco et al., 2008).

Yanbian yellow cattle are a native Chinese yellow breed with a slow growth rate. To improve production parameters, this breed is crossed with Limousin (25%) to form a new breed named Yan yellow cattle. The Yan yellow cattle breed has been developed for 27 years and is promoted across the country by the Ministry of Agriculture in China. The beef performance index (BPI) is 5.66 to 6.76 kg/cm for adult bulls and 4.06 to 4.59 kg/cm for adult cows over Yanbian yellow cattle. Fat percentages in carcass are different between different cattle breeds and fat depots in the bovine body are developed according different order (Lee et al., 2011).

The aim of this study was to compare the expression levels of *PPAR $\gamma$* , *FASN*, and *ACADM* mRNA in various adipose tissues (subcutaneous fat, abdominal fat, and perirenal fat), LD, lung, colon, kidney, liver, and heart between Yanbian yellow cattle and Yan yellow cattle. Based on the functions of *PPAR $\gamma$* , *FASN*, and *ACADM* genes, the present study was aimed to evaluate the possibility of using these candidate genes for determining fat deposition and meat quality in the two breeds as well as for marker directed selection.

## MATERIALS AND METHODS

### Animal tissues

Fat samples, LD and other tissues were obtained at

slaughter from nine Chinese (Yanbian yellow cattle and Yan yellow cattle) steers when they were 24 months old in BenFu cattle farm at Longjing city. The sample collection procedure was approved by the animal care committee of Husbandry Bureau of Yanji City. Samples were immediately frozen in liquid nitrogen and then stored at -80°C.

### RNA isolation

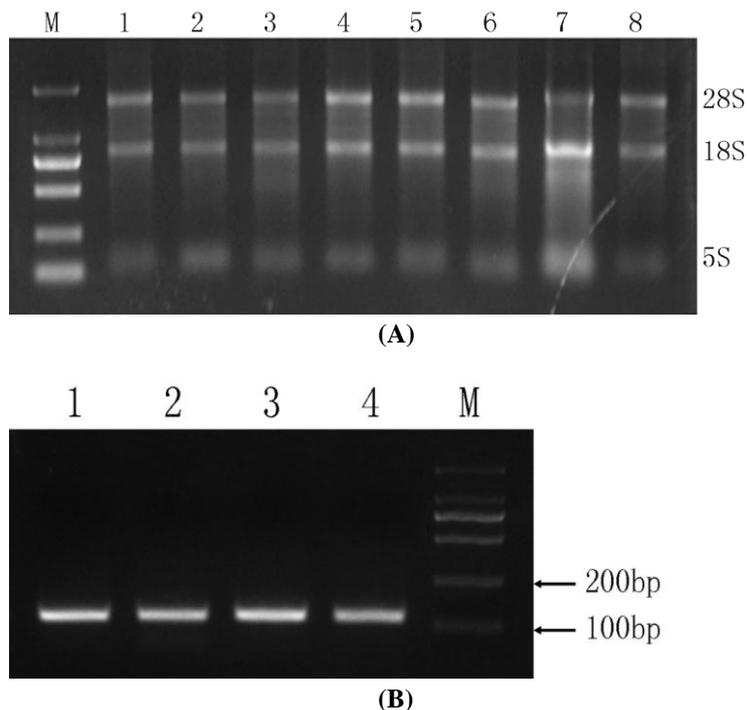
Total RNA was isolated from the adipose tissues, LD, heart, liver, kidney, colon and lung using Trizol Reagent (Invitrogen, Life Carlsbad, CA, USA). Due to the low numbers of cells in the adipose tissues, a maximum of 100 mg had to be processed to obtain a sufficient RNA yield. Also, the standard protocol for Trizol reagent had to be modified to suit adipose tissues. The adipose tissue was disrupted in liquid nitrogen and homogenized to achieve effective disruption. A centrifuge step was performed after incubating the homogenized sample to get rid of neutral lipids. The RNA concentration and purity were measured with a Nanodrop 2000/2000C (Thermo Scientific, Waltham, MA, USA). The integrity of the total RNA was checked by agarose gel electrophoresis. This modified protocol yielded pure and integral total RNA from fat tissues that was suitable for downstream RT-PCR. Total RNA (500 ng) was reverse-transcribed into cDNA using the ReverTra Ace qPCR RT Master Mix with a gDNA Remover kit (Toyobo, Co, Osaka, Japan) as described in the product information sheet.

### Quantitative real time polymerase chain reaction

Primers were designed for *PPAR $\gamma$* , *FASN*, *ACADM*, and glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) genes using Primer Express 3.0 software (PE Applied Biosystems, FosterCity, CA, USA) as shown in Table 1. The cDNA was amplified by PCR with gene-specific primer pairs using SYBR Green Realtime PCR Master Mix (Toyobo: QPK-201). Amplification was performed with a Mastercycler® ep (ependorf) under the following conditions: 95°C for 1 min followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. The PCR products were analyzed by Relative Quantification Soft (ependorf) to

**Table 1.** Primer information for real-time qRT-PCR

Name	Gene ID	Primer (5' to 3')
<i>PPAR<math>\gamma</math></i>	NM_181024	Sense: GATAGGTGTGATCTTAACTGTCGGAT Anti-sense: CGCTAACAGCTTCTCCTTCTCG
<i>FASN</i>	NM_001012669	Sense: CTGGAGCGTGAGCACAACCTG Anti-sense: GTGTGGAGTCCGTCAGCTCAT
<i>ACADM</i>	BC102989	Sense: TGTGCCTATTGTGTAACAGAACCT Anti-sense: TCCATTGGTGATCCACATCTTCTGAC
<i>GAPDH</i>	NM_001034034	Sense: CGTGTCTGTTGTGGATCTGACCTG Anti-sense: CAACCTGGTCCCTCAGTGTAGCCT



**Figure 1.** RNA analysis and PCR products detection. (A) Agarose gel electrophoresis of total RNA from different tissues using Trizol reagent. 1 and 2: Perirenal adipose tissues of Yanbian yellow cattle and Yan yellow cattle 3 and 4: Abdominal adipose tissues of Yanbian yellow cattle and Yan yellow cattle 5 and 6: Subcutaneous adipose tissues of Yanbian yellow cattle and Yan yellow cattle 7 and 8: *Longissimus dorsi* muscle of Yanbian yellow cattle and Yan yellow cattle M: 2,000 bp DNA ladder. (B) Agarose gel electrophoresis showing specific RT-PCR products with the expected size for the four genes. 1: *PPAR $\gamma$*  2: *FASN* 3: *ACADM* 4: *GAPDH* M: 2,000 bp DNA ladder.

generate the melting curves. The ratio of each signal was calculated using  $\Delta\Delta C_t$  method (Livak and Schmittgen, 2001).

### Statistical analysis

The results of the experiment are presented as means  $\pm$  SD. The statistical significance of differences found between groups was analyzed by two-way ANOVA. Statistical analysis was performed using the GraphPad Prism software (GraphPad Software, San Diego, CA, USA).  $p < 0.05$  was considered to be significant.

## RESULTS

Total RNA from different adipose tissues, LD and other organs were analyzed by qRT-PCR for the following genes: *PPAR $\gamma$* , *FASN*, *ACADM*. RNA integrity and PCR products with the expected size were shown in Figure 1. A cattle *GAPDH* gene fragment was also amplified using the same cDNA sample as the control.

### *PPAR $\gamma$* , *FASN*, and *ACADM* mRNA expression in adipose tissue and LD of Yanbian yellow cattle

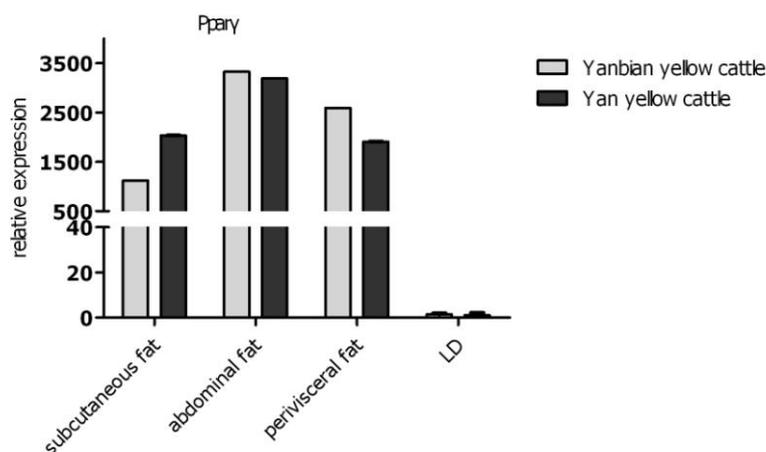
*PPAR $\gamma$* , *FASN*, and *ACADM* were expressed in adipose

tissues and LD of Yanbian yellow cattle, as shown in Figure 2, 3, and 4. The result indicated that *PPAR $\gamma$*  mRNA level was higher ( $p < 0.05$ ) in the abdominal fat and perirenal fat than that in the subcutaneous fat. However, there was no significant difference in the mRNA levels of *PPAR $\gamma$*  between abdominal fat and perirenal fat. Moreover, the *PPAR $\gamma$*  mRNA levels in both abdominal fat and perirenal fat were higher than that in LD ( $p < 0.05$ ).

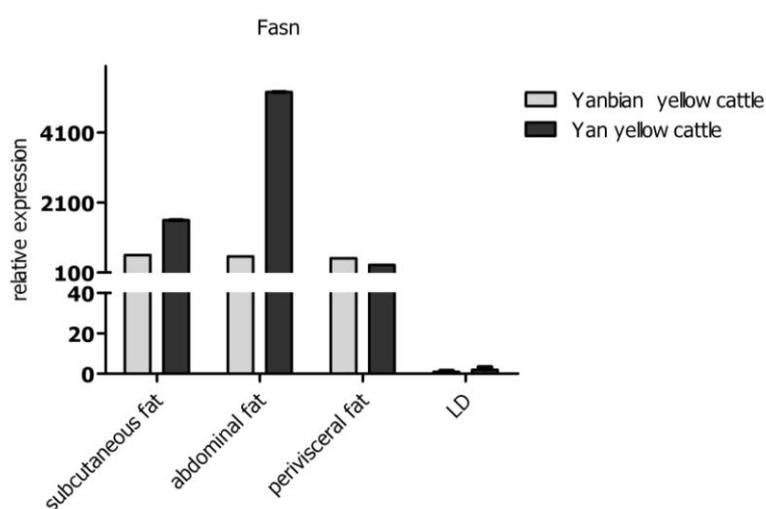
*FASN* and *ACADM* mRNA expressions were detected in the same samples, though *FASN* gene showed a trend for higher expression in adipose tissue than in LD (Figure 3); *ACADM* gene showed a trend for higher expression in adipose tissue than in LD, though the difference was not statistically significant (Figure 4).

### *PPAR $\gamma$* , *FASN*, and *ACADM* mRNA expression levels in adipose tissues and LD of Yan yellow cattle

As shown in Figure 2, 3, and 4, *PPAR $\gamma$* , *FASN*, and *ACADM* mRNA expression were detected in Yan yellow cattle. There was no significant difference between subcutaneous fat and perirenal fat in Yan yellow cattle. Although the *PPAR $\gamma$*  mRNA expression level of abdominal fat tended to be higher than subcutaneous fat and perirenal fat, these differences were not statistically significant.



**Figure 2.** The relative expression levels of *PPARγ* mRNA measured in the four tissue samples using qRT-PCR with *GAPDH* as internal standard from the two breeds of cattle. Data were expressed as means±SD. Three biological replicates were used in the experiment.



**Figure 3.** The relative levels of *FASN* mRNA measured in the four tissue samples using qRT-PCR with *GAPDH* as internal standard from the two breeds of cattle. Data were expressed as means±SD. Three biological replicates were used in the experiment.

Furthermore, *PPARγ* mRNA expression level was significantly higher in adipose tissue than in LD ( $p < 0.05$ ).

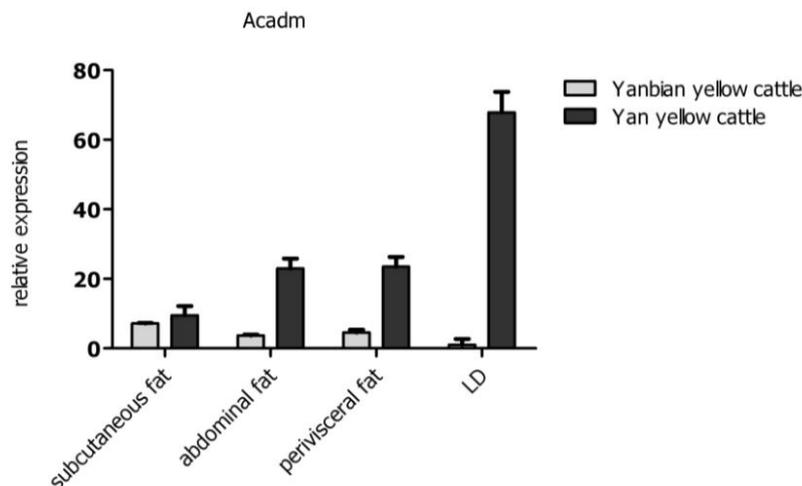
As shown in Figure 3, the *FASN* mRNA expression level in subcutaneous fat and abdominal fat were significantly higher than that in perirenal fat ( $p < 0.05$ ), and the expression levels in subcutaneous fat and abdominal fat were higher than in LD ( $p < 0.05$ ). *ACADM* gene was expressed in all the adipose tissues and LD and the expression level in LD was higher than that in adipose tissues, though the difference was not statistically significant (Figure 4).

**Comparison of *PPARγ*, *FASN*, and *ACADM* mRNA expression levels between Yanbian yellow cattle and Yan yellow cattle in adipose tissue and LD**

The mRNA expression levels of *PPARγ*, *FASN*, and

*ACADM* in adipose tissue and LD were compared between Yanbian and Yan yellow cattle (Figure 2, 3, and 4). The *PPARγ* mRNA expression level in subcutaneous fat in Yan yellow cattle was higher than that in Yanbian yellow cattle ( $p < 0.05$ ). *PPARγ* gene expression in perirenal fat of Yanbian yellow cattle was higher than that in Yan yellow cattle. In addition, the expression levels of *PPARγ* gene were high in abdominal fat in both cattle breeds.

*FASN* mRNA expression levels in subcutaneous fat and abdominal fat in Yan yellow cattle were significantly higher than that in Yanbian yellow cattle ( $p < 0.05$ )(Figure 3). The relative expression level of *ACADM* gene in different adipose tissues and LD in two different cattle breeds is shown in Figure 4, but this difference was not statistically significant.



**Figure 4.** The relative levels of *ACADM* mRNA measured in the four tissue samples using qRT-PCR with *GAPDH* as internal standard from the two breeds of cattle. Data were expressed as means $\pm$ SD. Three biological replicates were used in the experiment.

#### ***PPAR* $\gamma$ mRNA expression in other organs of Yanbian yellow cattle and Yan yellow cattle**

Figure 5A showed the relative expression levels of *PPAR* $\gamma$  in heart, liver, kidney, colon and lung of Yanbian yellow cattle and Yan yellow cattle. *PPAR* $\gamma$  gene was expressed higher in lung than that in other organs, the mRNA levels of *PPAR* $\gamma$  in the colon, heart, liver and kidney were very low. In addition, the *PPAR* $\gamma$  mRNA expression levels in abdominal fat and perirenal fat were significantly higher than that in the heart, liver, kidney, colon and lung ( $p < 0.05$ ) in both breeds.

#### ***FASN* mRNA expression in other organs of Yanbian yellow cattle and Yan yellow cattle**

As shown in Figure 5B, *FASN* mRNA levels were compared among five organs of Yanbian yellow cattle and Yan yellow cattle. In both cattle breeds the *FASN* gene was expressed higher in the lung than that in other organs. In addition, the *FASN* gene exhibited low mRNA levels in colon, heart, liver and kidney and differences among these organs were not statistically significant. *FASN* mRNA expression levels were higher in subcutaneous fat and abdominal fat than that in heart, liver, kidney, colon and lung for Yan yellow cattle ( $p < 0.05$ ).

#### ***ACADM* mRNA expression in other organs of Yanbian yellow cattle and Yan yellow cattle**

*ACADM* mRNA levels were measured in the same samples in the two breeds (Figure 5C). In contrast to *PPAR* $\gamma$  and *FASN* genes, *ACADM* gene expression in kidney and liver were significantly higher than that in other organs of Yanbian yellow cattle ( $p < 0.05$ ). The *ACADM* gene exhibited low expression levels in colon, lung, heart and LD in Yanbian yellow cattle, and comparison of *ACADM* mRNA levels among these organs, on the contrary, was not

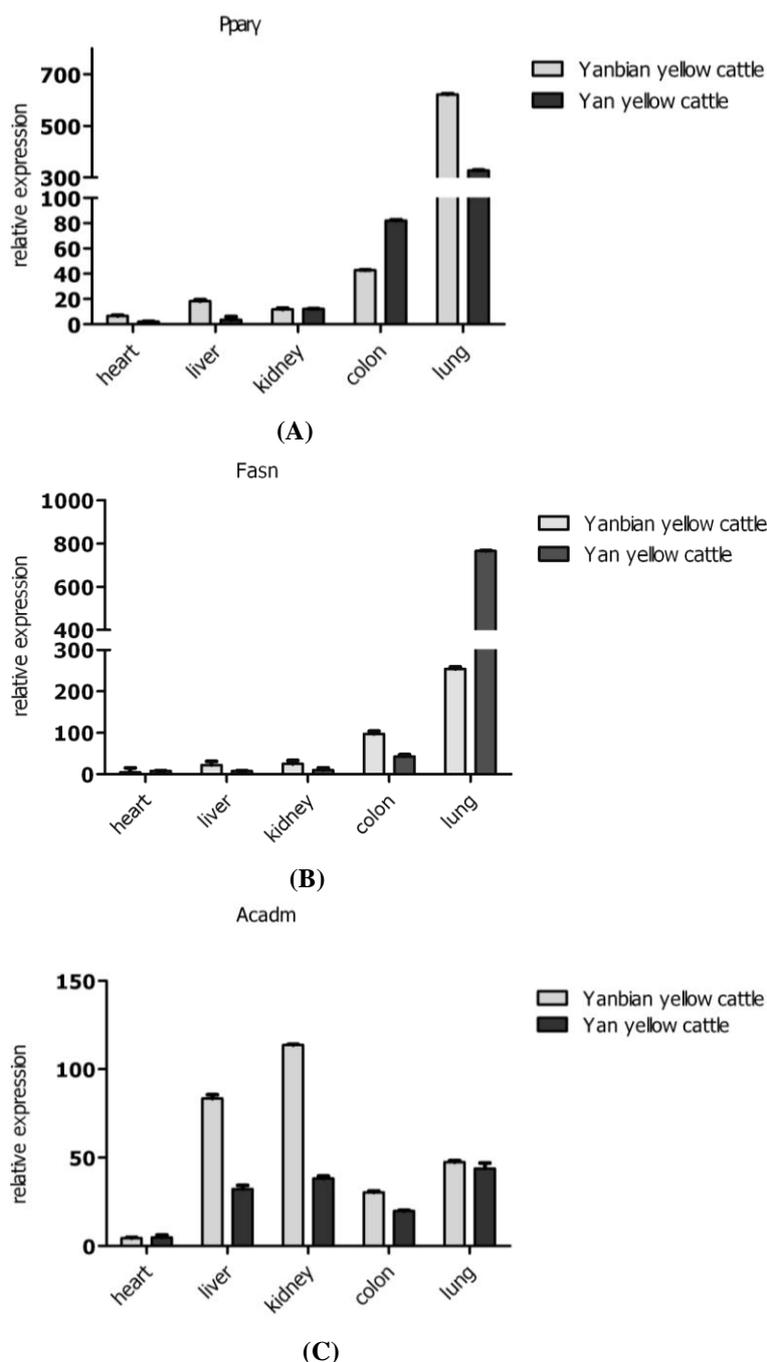
statistically significant in Yan yellow cattle.

## **DISCUSSION**

Adipose tissue is not simply lipids and an energy store organ, and it is now becoming increasingly clear that it is responsive to both central and peripheral metabolic signals. Adipose tissue is therefore able to integrate signals from other organs and capable of producing some important proteins. As shown in Figure 2 and 3, *PPAR* $\gamma$  and *FASN* mRNA levels were increased in adipose tissues, suggesting that both genes play key roles in adipose tissues. Previous studies suggest that *FASN* is a multifunctional enzyme for lipogenesis contributing to the regulation of fat depot development (Diraison et al., 2002). Different genotypes of *FASN* gene G>A SNP were significantly associated with the percentage of body fat carcass traits in Pima Indians (Kovacs et al., 2004). High levels of the *FASN* gene have been previously reported in abdominal adipose tissue (Semenkovich et al., 1995), which is in accordance with the data obtained in the present study. These data suggest that *FASN* is a candidate gene for fat storage and metabolic processing. Recent studies investigating the function of *PPAR* $\gamma$  in adipose tissue and skeletal muscle in knock out animal models have helped to further our understanding of the function of this receptor. Hevener et al. (2003) observed that *PPAR* $\gamma$  deficiency in mouse skeletal muscle results in insulin resistance (Hevener et al., 2003). Although *PPAR* $\gamma$  expression in muscle is low in the two breeds studied, it seemed *PPAR* $\gamma$  plays a central role in maintaining regular glucose processing. It is also suggested that *PPAR* $\gamma$  not only has direct effects on adipose tissue lipid metabolism, but also has secondary benefits in muscle (Rajala and Scherer, 2003). These results indicate that *PPAR* $\gamma$  plays a crucial role in the endocrine cross talk between muscle and fat.

There have not been many reports of the roles of these genes in different adipose tissues in cattle. In the present study, it was shown that the *PPAR $\gamma$*  mRNA expression of interior adipose was generally higher than that of subcutaneous adipose for the two cattle breeds. Deposited adipose depots mature at different rates since earlier deposited adipose has more time to mature, resulting in differing fatty acid composition (Lee et al., 2011). FA

composition of adipose tissue in meat animals is affected by a number of factors including adipose depot sites throughout the bovine carcass (Turk and Smith, 2009). Yokota et al. (2012) identified an SNP in the *FASN* gene in Japanese Black cattle that was associated with fat quality, affecting fatty acid composition (Yokota et al., 2012). It has been reported that fatty acid synthase is associated with fatness variability in turkeys (Sourdioux et al., 1999). Ju et



**Figure 5.** mRNA expression of three genes in the five tissue samples of Yanbian yellow cattle and Yan yellow cattle measured by quantitative RT-PCR and normalized to the level of *GAPDH*. Data were expressed as means $\pm$ SD. Three biological replicates were used in the experiment. (A) Quantitative analysis of *PPAR $\gamma$*  mRNA levels. (B) Quantitative analysis of *FASN* mRNA levels. (C) Quantitative analysis of *ACADM* mRNA levels.

al. (2011) indicated that interior adipose, coelomic adipose and perirenal adipose contain highly saturated fatty acid, whereas subcutaneous adipose contains highly unsaturated fatty acid (Lee et al., 2011). The expression of the *PPAR $\gamma$*  gene was higher in the abdominal adipose tissue in the two Chinese breeds than that in the subcutaneous adipose tissue and the *FASN* mRNA expression in subcutaneous fat and abdominal fat were expressed significantly higher than that in perirenal fat in Yan yellow cattle. These variations were partly due to location differences in the FA composition of bovine adipose tissue.

$\beta$ -Oxidation is the main process for oxidization of fatty acids via converting acyl-CoA into acetyl-CoAs units that are used by other tissues as energy source (Houten and Wanders, 2010). *ACADM* is the most important enzymes in the *ACAD* family which functions in the initial dehydrogenation step in the  $\beta$ -oxidation of phenylbutyryl-CoA for C4 to C12 medium chain fatty acids (Illig et al., 2010). QRT-PCR analysis showed that *ACADM* mRNA was expressed in a variety of cattle tissues. It seems that *ACADM* gene is very important in liver as the expression level was significantly higher than that in other organs of Yanbian yellow cattle.

Grindflek et al. (1998) reported previously that there were breed differences in *PPAR $\gamma$*  gene expression in *Duroc* and *Landrace* pigs (Grindflek et al., 1998). FA proportions in the subcutaneous and intramuscular fat of Aberdeen Angus and Wagyu steers were found to be significantly different (May et al., 1993). Previous studies also revealed that *FASN* mRNA expression is significantly higher in fat than in lean tissues (Bluher et al., 2002; Bluher et al., 2004; Berndt et al., 2007). These results imply that fatty acid composition is closely related to the differences in fat deposition among breeds in farm animals. *PPAR $\gamma$*  and *FASN* mRNA expression levels of subcutaneous fat from Yan yellow cattle was significantly higher than that from Yanbian yellow cattle. It does not appear that the *PPAR $\gamma$*  gene expression differs in other fat tissues between cattle breeds in our results. Yan yellow cattle are bred from Yanbian yellow cattle, these two breeds may have the similar genetic basis.

It is known that adipose tissues have a major endocrine function by expressing and secreting numerous factors and act on distant target tissues (Havel, 2002). Our results in current study indicated that *PPAR $\gamma$*  and *FASN* genes are abundantly expressed in adipose tissues, but at low levels in other tissues. *PPAR $\gamma$*  has emerged as a key regulator of lipid metabolism and *FASN* as a critical enzyme in biosynthesis of fatty acids. The activation of *PPAR $\gamma$*  and *FASN* may also have an indirect effect on the endocrine function of adipose tissue, which in turn may affect other organ systems. In the present study, the *ACADM* gene was detected in most tissues, which may reflect its importance in energy

production.

## CONCLUSION

In summary, this study examined the expression levels of *PPAR $\gamma$* , *FASN*, and *ACADM* genes in different adipose tissues, LD, heart, liver, kidney, colon and lung in both Chinese Yanbian yellow cattle and Yan yellow cattle breeds, *PPAR $\gamma$*  and *FASN* mRNA expression levels were significantly higher in adipose tissues than in LD, which indicated that both genes play key roles in fat deposition. *PPAR $\gamma$*  gene showed higher expression in abdominal fat and in perirenal fat than in the subcutaneous fat ( $p < 0.05$ ) in Yanbian yellow cattle, suggesting that the gene expression variation partly contributed to the differences of FA composition in different adipose tissues. The *PPAR $\gamma$*  mRNA expression level of subcutaneous fat in Yan yellow cattle was higher than that in Yanbian yellow cattle ( $p < 0.05$ ). On the other hand, the *FASN* mRNA expression levels in subcutaneous fat and abdominal fat in Yan yellow cattle were significantly higher than that in Yanbian yellow cattle, which indicated that the gene expression differ in the same fat tissues between cattle breeds. The gene expression in the subcutaneous fat correlates with fat storage. *ACADM* gene, by contrast, had a higher expression level in LD than in adipose tissue in Yan yellow cattle. Furthermore three genes expression in the lung, colon, kidney, liver, heart were also investigated. The results showed that *PPAR $\gamma$*  and *FASN* mRNA expression levels observed in the two breeds were in the following order from high to low: lung, colon and other organs. In contrast to the *PPAR $\gamma$*  and *FASN* genes, *ACADM* gene expression in kidney and liver were significantly higher than that in other organs of Yanbian yellow cattle, suggesting that liver was an important organ for energy production. Further studies are needed to determine the roles of these three genes in adipocyte differentiation in order to get candidate genes for meat quality breeding.

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