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Fatty acid synthase (FASN) gene polymorphism and early lactation milk fat composition in Xinong Saanen goats

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ABSTRACT

Fatty Acid Synthase (FASN) is a multifunctional protein, catalyzing the de novo fatty acid (FA) synthesis, its high mRNA expression in goat mammary epithelial cells (GMECs) substantiated by the medium to high heritability of milk fat (MF), makes it a candidate gene for association analysis with milk fat profile. The study aimed to develop markers of Capra hircus FASN to improve healthfulness of goat milk FAs and to investigate early lactation MF profile. 300 milk samples were collected, 30 days postpartum, from 300 Saanen does, from 2 herds sired by 73 bucks, and were analyzed with gas-chromatography. Linear mixed models, that considered the effects of herd, herd test day (HTD), parity, doe, sire and allele and haplotype effects were used to investigate the association of 3 FASN SNPs (−911 C/T intron1 SNP1, 852 A/G intron2 SNP2 and 14420 T/C exon37 SNP3) or two haplotypes (H1, C-A-T and H2, T-G-C, constructed from the three SNPs) in separate models, with 31 individual FAs, 8 FA groups, and 7 FA indices, by SPSS 20, REML function. A single test per SNP and haplotype was performed to avoid the effect of multiple testing. H1, SNP1 and SNP2 were most desirable for milk healthfulness because they were significantly associated with lower concentrations of myristic acid (C14:0) and palmitic acid (C16:0) and higher concentrations of linoleic acid (C18:2 *n*-6, *cis*). Herd followed by HTD and parity were the predominant factors affecting FA levels, indicating an effect of nutrition and management. Lower levels of de novo synthesized FAs but higher levels of C18:1 *cis*-9 were observed, indicating mobilization of body fat reserves. Although considered as de novo, butyric acid levels were consistent throughout lactation and showed negative correlations with other de novo FAs, suggesting other sources of origin. Majority of the odd chain fatty acids (OCFAs) originate from rumen bacteria, hence they may reflect rumen microbial activity. In our study this was confirmed by the observed positive correlations of all OCFAs with rumenic CLA (through its precursor C18:1 *trans*-11) and other *trans*-FAs, which are end products of biohydrogenation. The SNP markers developed will assist in marker assisted selection, and the early lactation milk FA analysis will help in deciphering factors of variation.

1. Introduction

Provision of sufficient and healthy food to the ever-growing population of the world is the primary goal of agriculture. However, despite the enormous gains in food production, food quality and its health connotations has been a big public health concern. Food borne illnesses due to saturated fat and cholesterol contained in meat and dairy products of livestock has been claiming many lives globally. Ruminant milk contributes a significant amount of this saturated fat consumed by humans, particularly C16:0 (palmitic acid) along with C14:0 (myristic acid) and C12:0 (lauric acid) have been associated with cardiovascular diseases (Von Eckardstein, 2006). Hence, there has been a growing interest in identifying the causal factors for fatty acid variation, to implement appropriate measures to modulate the fraction of the components.

Milk fat yield and composition can be considerably modified by genetic, physiological and nutritional means (Chilliard et al., 2003). Nutritional modification of ruminant milk & meat fatty acids holds the predominant plausible means, although it is limited by rumen biohydrogenation and introduction of bacterial origin fatty acids (Vlaeminck et al., 2004; Kim et al., 2007; Bou et al., 2009; Decker and Park, 2010; Jung et al., 2010; Nafikov, 2010).

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Biohydrogenation of unsaturated fatty acids in the rumen is about 85% for polyunsaturated fatty acids (Murphy et al., 1987). Hence, there is higher concentration of saturated fatty acids in tissues and milk of ruminants than the levels in the feed. High presence of saturated fatty acids in human diet is a high health risk factor. Nonetheless, medium to high heritability of milk fat composition give a niche for improving milk fat quantity and quality genetically (Bauman et al., 2004; Bastin et al., 2011).

The quality and quantity of milk fat is influenced by many candidate genes and transcription factors. A number of molecular pathways and intricate gene networks are involved in its regulation and secretion (Bionaz and Loor, 2008). Each individual gene has a limited role in modulating the expression, in which conventional animal breeding is less effective as the small effects of each gene are hard to detect. (Hayes and Goddard, 2001; Dekkers, 2004; Rhode, 2013). However dynamics of science has shifted the paradigm of conventional animal breeding to Marker assisted selection or/and Genomic selection, in which organisms can be selected based on their DNA (Hayes and Goddard, 2001; Nakaya and Isobe, 2012). Theory and simulation studies agree this method of selection has a potential to expedite genetic improvement (Raadsma, 2004). Candidate gene SNP association analysis is one method of identifying variants associated with phenotypic traits, aiding phenotype-based selection. A non synonymous mutation in the candidate genes can affect protein structure and function. Besides, synonymous mutations and intronic variants have been observed affecting traits, explained by their linkage disequilibrium with other loci, which are causal variants for the observed association (Luo and Wu, 2001; Du et al., 2007). Moreover, significant amounts of miRNAs (microRNAs) are mapped within introns of host genes. MiRNAs are short non coding RNAs which can down-regulate gene expression by silencing specific target mRNAs (Barik, 2008; Horie et al., 2009; Berillo et al., 2013). Hence, variations in the intron could affect transcription of some miRNAs which may have an impact on their regulatory activity.

Milk from a dairy goat provides a healthy source of nutrients for human consumption (Sanz Ceballos et al., 2009; Silanikove et al., 2010; Abbas et al., 2014). Small fat globules and high content of short and medium chain fatty acids ease digestion and absorption into the enterocytes, respectively, adding value for human consumption speeding up metabolism (Hui and Chandan, 2007). Besides, short and medium chain fatty acids have no effect on cholesterol concentrations (McNamara and Hillers, 1986). Goat milk is rich in vaccenic and conjugated linoleic acids, which have therapeutic effects on cancer, artherogenesis, diabetes, obesity, immune modulation and bone formation (Parodi, 1999; Belury, 2002; Bauman et al., 2004; Tyagi and Kathirvelan, 2006; Whigham et al., 2007). Moreover goat milk is naturally homogenized; artificial homogenization has a negative impact of damaging the fat globule cells, which leads to the release of superoxide Xanthine Oxidase (free radical) which is one of the agents for DNA mutation (Enig, 2003).

Due to its central role in de novo fatty acid synthesis, we hypothesized some of the observed variation in milk fat profile might be caused by polymorphism in FASN gene. FASN is a multifunctional enzyme that catalyzes the de novo fatty acid synthesis in cells. In ruminants it is involved in synthesizing all short-chain, medium chain and half of the amount of palmitic acids (C16:0) (Zhu et al., 2014). In Xinong dairy goats the gene is located in chromosome 19 and encodes the homodimeric multifunctional enzyme. It has 8217 bp comprised of 42 exons, an ORF of 7545 bp, and 5′ and 3′-UTR regions of 88 bp and 584 bp respectively with the start codon located in the 2nd exon and the stop codon is in 42nd exon. It encompasses seven active functional domains, which participate in all the processes of fatty acid synthesis. It encodes 3514 amino acids with an approximate molecular weight (MW) of 273.8 kDa (Zhu et al., 2014).

The major precursor of de novo fatty acid synthesis in ruminants is acetate and to a lesser extent beta hydroxybutyrate and propionate. Acetate is activated by the *acetyl CoA short chain* gene family to convert it to acetyl CoA. *Acetyle CoA carboxylase* (ACACA) catalyzes a two-step reaction, carboxylation of acetyle coA to malonyl coA. Malonyl CoA is a substrate for the action of FASN to catalyze the sequence of reactions to synthesis palmitate, adding two carbon atoms acetyl-CoA to the growing chain of fatty acid (Hillgartner et al., 1995). The thioesterase catalytic domain of FAS (thioesterase I) catalyzes the chain-termination reaction and results in the formation of C16:0 fatty acid (palmitic acid), the principal product of the FAS reaction in ruminants and monogastric animals (Barber et al., 1997). Fatty acids with 16 or more carbons cannot be further elongated by the mammary gland and are susceptible to hydrolysis by thioesterase I, thus short- and medium-chain fatty acids are synthesized within the mammary epithelial cells de novo. So, the proportion of these fatty acids in milk reflect the de novo fatty acid contribution to total milk fat. The majority of short and medium chain (C4:0–C14:0) fatty acids and approximately one-half of the C16:0 fatty acid in milk are derived from de novo fatty acid synthesis (Palmquist, 2006).

Higher levels of FASN gene mRNA expression were scored in adipose, intestine and mammary gland tissues followed by lungs, stomach, muscle, spleen, liver and kidney. Negligible levels of expression were recorded in heart (Zhu et al., 2014). This indicates FASN gene's role as one of the major genes which affects lipogenesis in goat mammary epithelial cells.

Several association analysis of FASN gene in bovine identified causal SNPs with significant associations with milk fatty acid profiles (Morris et al., 2007; Ordovas et al., 2008; Schennink et al., 2009; Matsuhashi et al., 2010; Matsumoto et al., 2012; Yun et al., 2012). GWAS and QTL mapping studies identified FASN gene in bovine chromosome 19, as a candidate gene responsible for some of the variations in milk fat percentage and yield (Nafikov, 2010; Bouwman et al., 2011; Li et al., 2014). The association of short and medium chain saturated fatty acids with BTA 19 around FASN region in the study, reinforce FASNs key role in the de novo biosynthesis of short and medium chain fatty acids.

In sheep a SNP in FASN 5′UTR was found to be significantly associated with milk fat yield. The predicted mRNA secondary structure suggested one of the alleles caused a stable folding (Sanz et al., 2013). To our knowledge any association studies between FASN gene and goat milk profile have not yet been done. Considering the genes central role in milk fat synthesis it is necessary to scan the region for variants associated with the indicated traits.

2. Materials and methods

2.1. Animals and sampling

488 Saanen does belonging to two herds owned by Northwest Agricultural & Forestry university farm and Qianyang county farm were genotyped for association analysis. The Northwest A&F university animals does belong to 22 half sib families generated by AI (Artificial insemination) ranging from 1 to 25 does per sire and the Qianyang farm goats belongs to 51 half sib families generated by controlled buck breeding, ranging from 2 to 12 does per sire. Samples of 5 mL of blood were collected from the does using acid citrate dextrose (ACD) as an anticoagulant around 30 days postpartum. It was immediately stored in −80 °C refrigerator until use. The data recorded were animal, milk yield (at milk sampling), herd, days in milk and parity.