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MICRORNA PROFILING IN CATTLE DIVERGENTLY SELECTED FOR RESIDUAL FEED INTAKE

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SUMMARY

MicroRNAs (miRNAs) are short non-coding RNAs that post-transcriptionally regulate expression of mRNAs in many biological pathways. Here we report comprehensive miRNA profiles by deep sequencing in Angus cattle divergently selected for residual feed intake (RFI). Two miRNA libraries were constructed from pooled RNA extracted from livers of low and high RFI cattle, and sequenced with the Illumina Genome Analyser. We identified 305 known bovine miRNAs. bta-miR-143, bta-miR-30, bta-miR-122, bta-miR -378 and bta-let-7 were the top 5 most abundant miRNA families expressed in liver, representing more than 63% of expressed miRNAs. Mir-143 is the most expressed bovine miRNA in liver, and is up-regulated in high RFI cattle. Mir-122 is the second most expressed miRNA in liver and is down regulated in high RFI animals. The differentially expressed miRNAs may play important roles in the regulation of the bioprocesses responsible for variation in RFI in cattle.

INTRODUCTION

MicroRNAs (miRNAs) are small (~ 22 nucleotides) non-coding RNA that regulate gene expression by targeting mRNA in a sequence-specific manner, leading to either translational repression or degradation of the targeted transcript. MicroRNAs are now known to repress thousands of target genes and regulate cellular processes, including cellular proliferation, differentiation and apoptosis. The aberrant expression or alteration of miRNAs also contributes to a range of human pathologies, including diabetes and cancer (Lu *et al*. 2005).

Residual feed intake (RFI) is a measure of feed efficiency in beef cattle. It is the difference between an animal's actual feed intake recorded over a test period and its expected feed intake based on its size and growth rate (Koch *et al*. 1963). Genome wide association studies have been used to identify gene markers associated with RFI in beef cattle. More than a hundred single nucleotide polymorphisms (SNP) have been reported as being associated with RFI (Barendse *et al*. 2007). However, a large proportion of these SNP are not located in annotated genic regions of the bovine genome. Some of the most significant SNP for RFI were in or close to miRNA motifs which suggests that these miRNAs could play an important role in RFI variation (Barendse *et al*. 2007).

Considerable progress has been made in the characterization of miRNAs in livestock genomes over the last decade, and a wide and diverse range of conserved and species-specific miRNAs have been identified. However, little is known about their role in regulation of key cellular and physiological pathways involved in feed efficiency and RFI. Liver is a central controller of metabolism and a major driver of whole animal oxygen consumption in mammals. In this study we profiled miRNAs abundance in liver tissue of Angus bulls from high and low RFI selection lines using a deep sequencing approach. Here we report the first liver miRNA profile of known and

putative novel bovine miRNAs. Differentially expressed miRNAs between high and low RFI selection lines are also discussed.

MATERIALS AND METHODS

Animals. Young Angus bulls resulting from approximately three generations of divergent selection for RFI were used in this study. The selection lines were established in 1993 at the Agricultural Research Centre, Trangie, NSW, Australia (Arthur *et al*. 2001). Bulls were born in 2005 and, at approximately one year-of-age, their growth and feed intake were measured. Postweaning RFI for each animal in the test group was calculated using a linear regression model of feed intake on mean metabolic live weight and average daily gain. Based on the RFI test results, liver biopsies were taken from 30 animals with the lowest RFI and 30 animals with the highest RFI as described by Chen *et al.* (2011). Total RNA from liver tissue was isolated using TRI reagent (Ambion, Applied Biosystems) according to the manufacturer's instructions.

Small RNA library construction and analysis of small RNA sequencing data. Based on availability and the quality of RNA, two pools of total RNA were constructed from 13 high RFI animals and 13 low RFI animals with equal quantities (1 µg) from each animal. Libraries of small RNAs were prepared using a Small RNA Sequencing kit (Illumina) and sequenced by Illumina Genome Analyser. Sequencing data were analysed using miRanalyzer (Hackenberg *et al*. 2011). In brief, known bovine miRNAs were identified by mapping all sequence reads to known bovine miRNAs in miRBase (version19), and reads that matched known bovine miRNAs were grouped and removed from the dataset. Reads that mapped to known miRNAs in other species were grouped as homologue miRNAs. The remaining reads were aligned to libraries of known transcripts. To identify bovine-specific novel miRNAs, the remaining sequence reads were mapped to *Bos taurus* genome (bostau6, UMD_3.1) using Bowtie. Mapped reads were first clustered into putative mature miRNAs and pre-miRNAs. The putative candidate miRNAs were reported based on at least three out of five different Random Forest models (Hackenberg *et al*. 2009). To compare the differentially expressed miRNAs between the two libraries (low and high RFI), the expression of each specific miRNA (read counts) were normalised to percentage of million mapped reads (PMMR) and fold-changes were calculated between the high and low RFI pools.

RESULTS

There were 10,820,087 and 12,808,022 high quality sequence reads for the high RFI pool and the low RFI pool, respectively. Approximately half of these reads were an exact match to known bovine mature miRNAs (Figure 1). A total of 304 known miRNAs were detected as being expressed in bovine liver. Table 1 lists the differentially expressed miRNAs between high and low RFI animals along with their means for phenotypic traits. We defined the most abundant miRNAs as those expressed in more than 1% of the mapped miRNAs. Medium abundance miRNAs were those between 0.1-1%. Two-fold expression changes between high and low RFI was considered differentially expressed. Generally, the reads of the most abundant miRNAs were more than 100,000 fold higher than those of the scarce miRNAs. The 5 most expressed miRNA families were bta-miR-143, bta-miR-30, bta-miR-122, bta-miR-378 and bta-let-7, which constituted more than 63% of the total sequence reads, suggesting that they are the most abundantly expressed miRNAs in bovine liver tissue. In total 52 miRNAs homologous with other species and not listed in the bovine miRBase (version 19) was identified based on the precursor sequence.

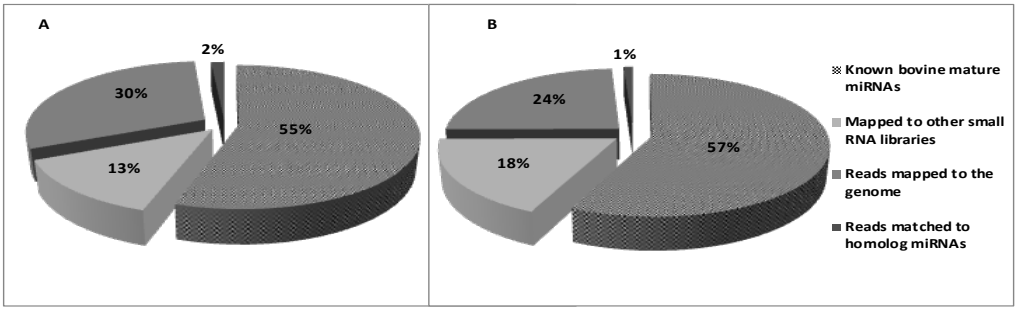
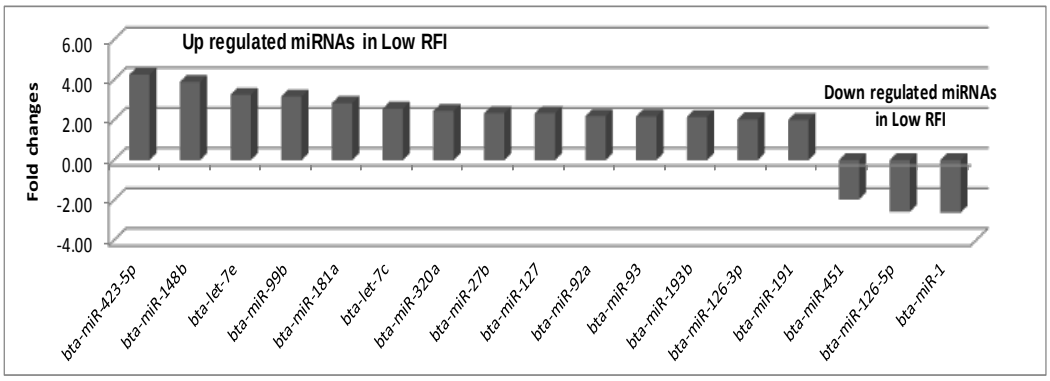


Figure 1. Distribution of mapped high quality reads in both pools: low RFI line (A), and high RFI line (B).

Table 1: Means for animal traits and differences in the top most abundant known miRNAs between low RFI pool and high RFI pool

	RFI (kg/day)	Feed intake(kg/day)	Average daily gain (kg/day)	Rib fat depth (mm)
Low RFI	-1.07	10.49	2.12	7.68
High RFI	0.99	12.63	1.98	10.73
Top 4 up regulated miRNAs in low RFI				
	bta-let-7b	bta-miR-122	bta-miR-30d	bta-let-7a-5p
Low RFI	2.25	12.29	2.25	5.96
High RFI	0.81	4.82	1.06	2.90
Fold change	2.79	2.55	2.12	2.06
Top 4 down regulated miRNAs in low RFI				
	bta-miR-143	bta-miR-192	bta-miR-21-5p	bta-miR-101
Low RFI	15.9	2.43	0.90	1.27
High RFI	31.6	5.19	2.33	4.1
Fold change	-1.99	-2.13	-2.59	-3.22

Figure 2. Fold changes of the medium abundant bovine miRNAs differentially expressed between high and low RFI cattle.



Ten putative novel bovine-specific miRNAs, based on precursor sequence and secondary structure were found. Those putative novel bovine-specific miRNAs candidates were expressed by 6,534 read counts (ranging from 2102 to 22 reads) in both RFI pools.

DISCUSSION

Accumulating evidence shows miRNAs play important regulatory roles in physiological and developmental processes in many tissues, including liver. We found that the most expressed miRNA in bovine liver is bta-mir-143 which constituted of 20% of total expressed miRNAs. This is a clear difference from the miRNAs expression pattern in human and mouse liver where the most expressed miRNA is mir-122 (Rottiers and Näär, 2012). Besides the discovery of novel miRNAs in bovine liver, we have identified 18 miRNAs up-regulated in low RFI and 7 miRNAs down-regulated in low RFI cattle. This is consistent with previous mRNA expression with microarrays, in which there were more genes up-regulated in high RFI animals than low RFI animals (Chen *et al.* 2011). These differentially expressed miRNAs may play important roles in regulation of the physiological processes involved in RFI in beef cattle. For example, bta-mir-143 is up-regulated in high RFI animals. Mir-143 was up-regulated in liver of genetic and dietary mouse models for obesity. Overexpression of miR-143 impairs insulin-stimulated AKT activation and glucose homeostasis (Jordan *et al*. 2011). The knockout mir-143 mice do not develop obesity-associated insulin resistance. MiR-122 was up-regulated in low RFI animals. Mir-122 was the first miRNA to be linked to metabolic control and it is the most expressed miRNA in human and mouse liver and affects hepatic cholesterol and lipid metabolism. Suppression of mir-122 by antisense reduced plasma cholesterol levels by 25–30% in mice. It also reduced the genes involved in lipid synthesis in liver and decreased hepatic cholesterol and fatty acids (Rottiers & Näär, 2012).

In conclusion, we have identified 305 known bovine miRNA in bovine liver. Mir-143 is the most expressed bovine miRNA in liver, and is up-regulated in high RFI cattle. Mir-122 is the second most expressed miRNA in liver and is down regulated in high RFI animals. The differentially expressed miRNAs may play important roles in the regulation of the bioprocesses responsible for the variation in RFI in cattle.

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