

## LIVER MICRORNA EXPRESSION REVEALED THE DIFFERENCE IN FAT METABOLISM FROM 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. The liver plays a critical role in the synthesis of molecules that are utilized elsewhere to support homeostasis, in converting molecules of one type to another, and in regulating energy balances. We sequenced 48 liver microRNAs from young Angus bulls divergently selected for residual feed intake (RFI). In total we obtained 135 million high quality short sequence reads and more than 93 million unique mapped sequence reads. The top 10 most abundant miRNAs families expressed in liver, represented on average 46-90% of total expressed miRNAs. We did not observe any significant miRNA expression profile for low RFI animals. However, there are distinct miRNA expression patterns separating the animals into two groups that differ significantly in P8 and rib fat thickness. This is consistent with previous finding where high RFI animals had an up-regulated AHR signalling pathway, which plays an important role in fat metabolism. This suggested that some animals have a high RFI value due to excess fat metabolism.

### INTRODUCTION

MicroRNAs (miRNAs) are small (~ 22 nucleotides) non-coding RNA that regulate gene expression by targeting messenger RNA (mRNA) in a sequence-specific manner, leading to either translational repression or degradation of targeted transcripts. In animals, miRNAs target the 3'untranslated regions of mRNA through a RNA-induced silencing complex (RISC), and subject to the accuracy of the sequence complementarities, either repression of translation or cleavage of the mRNA target is achieved (Huntzinger and Izaurralde 2011). MicroRNAs are now known to repress thousands of target genes and regulate cellular processes, including cellular proliferation, differentiation and apoptosis (Meltzer 2005). The aberrant expression or alteration of miRNAs also contributes to a range of human pathologies, including diabetes and cancer (Lu *et al.* 2005).

Feed efficiency is an economically important trait in beef production and can be assessed using residual feed intake (RFI) (Archer *et al.* 1999). This is the difference between an animal's actual feed intake recorded over a test period and its expected feed intake, predicted for its body weight and growth rate (Koch *et al.* 1963). Residual feed intake estimates the feed required by an animal for a given daily weight and for the maintenance of its metabolic weight. Therefore, understanding the molecular mechanism regulating RFI will help in breeding profitable animals in agriculture. Genome wide association studies have been carried out to identify gene markers associated with RFI in beef cattle (Bolormaa *et al.* 2011) and a large proportion of SNP markers associated with RFI are not located in annotated genes in bovine genome. Gene expression studies in cattle from high and low RFI selection lines have revealed a list of differentially expressed genes with functions related to extracellular matrix growth and fat metabolism in the liver (Chen *et al.* 2011). The liver plays a critical role in the synthesis of molecules that are utilized elsewhere to support homeostasis, in converting molecules of one type to another, and in regulating energy balances. MicroRNAs (miRNAs) are important natural regulators of global gene expression. The objective

of the study is to profile the expression of miRNA in liver by next generation sequencing and identify miRNAs related to the efficiency of feed utilization in beef cattle.

## MATERIALS AND METHODS

Ninety 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 and the animals were used for the microarray experiment previously reported by Chen *et al.* (2011). In brief, bulls were born in 2005 and feed intake was measured for each animal using an automated recording system in the Beef Research Feedlot Tullimba, NSW, Australia. During the 70-day test, the animals had ad libitum access to a barley-based feedlot ration containing 12 MJ metabolizable energy per kilogram dry matter and 15–17% crude protein. Post-weaning RFI was measured using the linear regression of daily feed intake on mean metabolic mid-test weight and average daily gain. Liver biopsies were taken at the end of the RFI test from 24 animals with the lowest RFI and 24 animals with the highest RFI. Total RNA from liver was isolated using TRI Reagent (Ambion, Applied Biosystems, Austin, TX, USA ) following the manufacturer's protocol and the quality and integrity of RNA was assessed with the RNA 6000 Nano Lab Chip Kit using the Agilent 2100 Bioanalyzer (Agilent Technologies, CA, USA).

Small RNA libraries were constructed for each animal using 1 µg total RNA with NEXTflex™ Small RNA-Seq Kit v2 (Bioo Scientific, TX, USA) following the protocols supplied by the manufacturer. The libraries were sequenced at Ramaciotti Center, University of NSW with Illumina HiSeq 2000 Sequencing System.

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 (version 21), 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). The microRNA expressed was normalized by total mapped reads from each sample and was measured as reads per million mapped reads (RPM).

Differential expression analysis was carried out with Bioconductor package DESeq (v3.1) (Anders and Huber 2010) to identify differentially expressed miRNAs between high and low RFI animals. GenStat (V17) was used for the cluster analysis and phenotype differences between clusters were evaluated using Student's t-test.

## RESULTS AND DISCUSSION

We obtained 135,042,220 high quality sequence reads and 88% of the sequence reads mapped to bovine genome. About 69% (93,336,181) of sequence reads mapped to known bovine mature miRNA (bostau6, UMD\_3.1). A total of 560 known miRNAs were detected for which at least one read was observed in the dataset. Only 224 miRNAs had more than 10 reads per million across most of the samples and were used for the subsequent analysis. There were large variations of expression of miRNAs and the top 10 most expressed miRNA (bta-miR-143, bta-miR-100, bta-miR-99a-5p, bta-miR-192, bta-miR-21-5p, bta-miR-122, bta-miR-148a, bta-miR-191, bta-miR-26a, bta-miR-30a-5p) accounted for 50-to 90 % of the total miRNA expression. Bta-miR-143 is the most abundant miRNA in bovine liver and takes up on average 17% of the total miRNA expression. Our results differ from reported liver miRNA expression in negative energy balance (NEB) dairy cows (Fatima *et al.* 2014). In NEB dairy cows 53 miRNAs were expressed and the top 10 miRNA accounted for more than 95% of the miRNAome. This difference was most likely due to the different miRNA extraction and library construction methods used for sequencing. In

this study we used the total RNA for the small RNA library construction, while in NEB dairy cows, an enriched miRNA protocol was used for the RNA extraction.

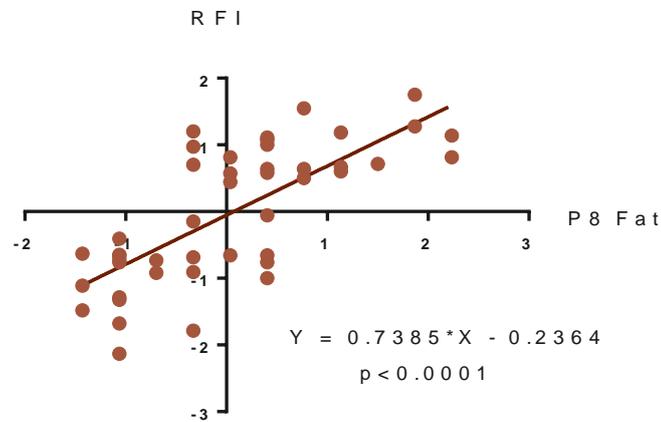
To identify differentially expressed miRNA we only used 224 miRNAs that had on average more than 10 RPM. We first divided the animals into high and low RFI groups and used DESeq (Anders and Huber 2010) to identify differentially expressed miRNAs between high and low RFI animals based on a negative binomial model. No miRNA expressed differently between high and low RFI animals when the Benjamini and Hochberg corrected P value cut-off of  $<0.05$  was applied to correct for multiple testing. Next, we carried out the cluster analysis of all animals based on miRNA profile (224 miRNAs) and top 10 miRNA only as the top 10 most expressed miRNAs accounted more than 70% of the total miRNA population. The animals were clustered in two major groups. Then we divided the animals into two groups based on cluster analysis. In Table 1 we summarize phenotypes between these two groups. The most significant difference between these two groups was in fat deposition; both P8 and rib fat were different. There was no difference in ADG, DFI and RFI.

**Table 1. Trait means ( $\pm$  standard deviation) for two groups based on the miRNA profile**

Cluster	ADG	DFI	RFI	P8	RIB	IMF%	EMA
	1.99	11.54	-0.38	11.77	9.73	5.03	82.55
Cluster 1	$\pm$ (0.05)	$\pm$ 0.30	$\pm$ 0.24	$\pm$ 0.57	$\pm$ 0.60	$\pm$ 0.10	$\pm$ 0.68
	2.04	11.16	-0.52	10.05	8.29	4.94	80.38
Cluster 2	$\pm$ 0.06	$\pm$ 0.34	$\pm$ 0.22	$\pm$ 0.54	$\pm$ 0.36	$\pm$ 0.10	$\pm$ 0.91
P	0.570	0.404	0.671	0.033	0.048	0.565	0.063

ADG: average daily gain during the 70day; RFI test. DFI: average daily feed intake; P8: P8 fat thickness (ultrasound) at the end of RFI test; RIB: RIB fat thickness (ultrasound); EMA: eye muscle area (ultrasound); P: probability of significance.

It is believed that RFI is highly associated with the energetic costs of protein turnover and basal metabolic rate and that selection for low RFI animals will reduce maintenance energy requirements (Richardson and Herd 2004). Global miRNA profiling of the current study showed clear expression patterns related to fatness instead of RFI, although the animals were from the third generation of divergent selection lines for RFI. Indeed, there was a significant correlation between P8 fat thickness and RFI (Figure 1). It is likely that animals with high P8 fat ( $>1$  sd) are high RFI animals and animals with low P8 fat ( $<1$ sd) are low RFI animals. Therefore, some proportion of RFI's variation can be explained by differences in body composition. Alternatively, at a constant weight and daily gain, some of the high RFI animals deposited more fat than the low RFI animals. There has been earlier reports on the genetic and phenotypic correlations between RFI and lipid metabolism (Robinson and Oddy 2004). Previous gene expression work with microarray identified up-regulated p450 induced xenobiotic signalling pathways in high RFI animals. P450 induced xenobiotic signalling pathway plays an important role in lipid metabolism. A recent study on miRNA in the liver of NEB dairy cows identified that mir-143 was down regulated in severe NEB cows (Fatima *et al.* 2014). One of the target genes of mir-143 is LRP2 which is involved in lipid metabolism



**Figure 1. Scatter plot of RFI and P8 fat.** The scales of X (P8) and Y (RFI) are standardized unit of standard deviation.

In conclusion, we have identified 224 known bovine miRNA expressed in bovine liver. There is distinct expression profile difference between high and low fatness animals. Some of the variation in RFI can be explained by the observed differences in lipid metabolism.

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