BRIEF COMMUNICATION: Differential expression of lactoferrin and insulin-like growth factor-1 genes in cows milked once and twice a day

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Abstract

Dairy farming in New Zealand has traditionally utilized a twice a day (TAD) milking system, but milking once a day (OAD) for the entire lactation is also practiced by a significant proportion of dairy farmers. This study reports on the differential gene expression in milk from OAD and TAD cows. Three predominant dairy breed group of New Zealand, Holstein-Friesian (F), Jersey (J) and F×J crossbred (F×J) were included in samples collected from both milking regimens. RNA was extracted from milk from 45 OAD and 42 TAD dairy cows during early lactation, and used to determine lactoferrin (LF) and insulin-like growth factor-1 (IGF-1) gene expression using qPCR. Compared to TAD cows, cows milked OAD had higher expression of the LF gene (1.38 vs 1.30 folds, P=0.08) and the IGF-1 gene (1.65 vs 1.48 folds, P=0.09). These results suggest that milking frequency affects the expression of milk composition genes at early lactation.

Introduction

Once-a-day milking (OAD) of dairy cows offers numerous benefits, including, improved animal welfare and decreased production costs (Holmes 2014). However, many New Zealand dairy farmers are hesitant to convert from twice a day (TAD) to OAD. One of the factors restricting this conversion is a limited understanding of the genetics of an optimal OAD cow, as the three predominant dairy breeds in New Zealand (Holstein-Friesian (F), Jersey (J) and F×J crossbred (F×J)) all vary within breed in their ability to optimally produce milk under OAD practices (Stelwagen et al. 2013). More information is necessary to determine what constitutes optimal OAD genetics, including understanding the expression of lactation genes. This research is the first preliminary investigation into lactation expression variation between OAD and TAD dairy cows. The cows examined in this pilot study were selected from the top yielding cow from the Massey University OAD and TAD herds. The TAD milking herd is milked under traditional TAD practices, whereas, the OAD milking herd was in the fifth season of OAD milking for the whole lactation. The genes for lactoferrin (LF) and insulin-like growth factor 1 (IGF-1) were selected for this pilot study due to their importance for lactation and downstream economic value.

Materials and methods

Milk was collected from 42 cows from the Massey University TAD herd and from 45 cows from the Massey University OAD herd during early lactation (MUAEC Protocol number 17/56). The TAD cohort comprised of 21 F, 3 J and 19 F×J cows, while the OAD cohort comprised of 15 of each three breeds. All test cows were located on two farms within 5km of each other. All test cows were initially milked under normal conditions and then left for approximately one hour, and were hand milked again to collect 50ml of fresh milk. The milk was processed and RNA was extracted as per Wickramasinghe et al. (2012).

Expression analysis of LF and the reference gene actin beta (ACTB) was performed with the primers described by Pawlik et al. (2015), while IGF-1 was performed with primers described by Murney et al. (2015). Quantitative PCR conditions were as follows; 1X Verso Enzyme Mix, 1X Verso 1 Step qPCR SYBR Mix, 1X Verso RT Enhancer (Thermo Fisher Scientific, MA, USA), 70nM of each primer (IDT, IA, USA) and 1ng of RNA in a final volume of 25µl. Thermal cycling conditions were as follows; cDNA synthesis at 50˚C for 15min, denaturation at 95˚C for 15min, followed by 40 cycles of denaturation at 95˚C for 15sec, annealing at either 55˚C for LF or 60˚C for ACTB and IGF-1 for 30sec, and elongation at 72˚C for 30 sec. A melt curve was produced with the following thermal cycling conditions, initial denaturation at 95˚C for 30sec followed by 80 cycles, beginning at 60˚C with a temperature increase of 0.5˚C per cycle. All qPCR steps were performed in a Magnetic Induction Cycler (Bio Molecular Systems, QLD, Australia).

Relative quantification of LF and IGF-1 expression was calculated using ACTB as the reference sample using the protocol as described by Pfaffl (2001). The expression of LF and IGF-1 was analyzed with a general linear model that included the fixed effects of milking frequency (OAD vs TAD) and number of lactations (1 vs. ≥2), and proportion of Holstein-Friesian as covariable. Statistical analysis was performed with SAS (SAS Institute Inc., Cary, NC).

Results and discussion

High quality RNA was successfully extracted from all milk samples and subsequently the three genes amplified under reverse transcriptase qPCR conditions. Cows milked OAD had higher expression of both, LF and IGF-1 genes than cows milked TAD (Table 1), while not highly significant, this is indicative of a trend (LF P=0.08, IGF-1 P=0.09). Similarly, first lactation cows had higher expression of both, LF (P=0.08) and IGF-1 (P=0.003) genes than multiparous cows. Proportion of Holstein-Friesian had not significant effect on the expression of either genes.

Table 1 Relative quantification (fold ratios to ACTB) of the expression of lactoferrin and insulin-like growth factor-1genes, between different milking frequencies and number of lactations.

Gene	Fixed Effect	N	Treatment	Mean	SEM	P-value
Lactoferrin	Milking frequency	45	OAD	1.38	0.03	0.08
		42	TAD	1.30	0.03	
	Lactation	43	1	1.38	0.03	0.08
		44	≥2	1.30	0.03	
Insulin-like growth factor-1	Milking frequency	45	OAD	1.65	0.05	0.09
		42	TAD	1.49	0.07	
	Lactation	43	1	1.72	0.06	0.003
		44	≥2	1.43	0.07	

These preliminary results indicate that expression differences in two genes responsible for milk production do occur at early lactation in dairy cows differing in their milking frequency. While these results have limited variation, they support a need for additional testing across the entire lactation period of LF, IGF-1 and additional lactation genes. These results provide confidence that there are lactation gene expression differences between OAD and TAD milking cows, whether these differences are epigenetic or genetic will require further investigation.

References

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