BRIEF COMMUNICATION: The relationship between milk synthesis and intracellular profiles of amino acids in the bovine mammary gland

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Introduction

There is a growing body of evidence that amino acid (AA) signalling in the mammary gland may play a key role in milk production of ruminants through modulation of translational regulation (Moshel et al. 2006; Toerien & Cant 2007). It has been postulated that the effect of nutrient signalling on elements of translational control of milk protein synthesis may contribute to the variable responses of milk protein yield observed following nutritional perturbations (Hanigan et al. 1998). Consistent with this hypothesis, we have reported that changes in the mean and variance of intracellular free AA (FAA) in the bovine mammary gland are associated with changes in milk protein synthesis in response to growth hormone (Pacheco et al. 2010). Using the same model, we have also reported that the nutrient-sensing mTOR pathway may be involved in the regulation of protein synthesis in the lactating bovine mammary gland (Hayashi et al. 2009). Knowledge of the potential for AA to act as signalling molecules to regulate milk protein synthesis may contribute to the development of nutritional regimes to improve milk production in dairy cows.

The objective of this study was to further explore the association among milk synthesis, the protein-synthetic capacity of the mammary gland and intracellular FAA profiles using an alternative model of perturbed milk protein synthesis.

Materials and methods

The experimental procedures were approved by the AgResearch Ruakura Animal Ethics Committee in accordance with the 1999 Animal Welfare Act of New Zealand.

Samples used for this experiment were derived from a trial described elsewhere in this proceedings (Singh et al. 2012). Samples from groups of five cows that were not milked for either 7-, 14- or 28-days and then re-milked for a period of seven days before being euthanized, and mammary tissue snap frozen in liquid nitrogen. Intracellular FAA profiles were determined in mammary tissue as previously described (Pacheco et al. 2010). Within and between assay variation was lower that 4%. Total RNA, DNA and protein was extracted from 100 mg of powdered mammary tissue from each cow using Tri-reagent (Invitrogen, Auckland, New Zealand) according to the manufacturer's instructions. RNA and DNA were quantified using a NanoDrop Spectrophotometer ND-1000 (Nanodrop Technologies, Willmington, DE, USA). Total protein content was estimated using Bradford reagent (Bio-Rad, Hercules, CA, USA) (Bradford, 1976).

Amino acid concentrations were expressed as nmol per gram of tissue. Percentage data were square-root transformed before the statistical analysis to fulfil the assumption of normality of the data. Back-transformed data are presented. Differences between treatments were assessed via an analysis of variance using the MIXED procedure in SAS (2002). Probability of differences between pair-wise treatment comparisons were calculated using the Tukey adjustment in the "least square means" statement of the procedure.

Results and discussion

Biochemical measures of mammary gland composition were used to evaluate the effect of non-milking period on mammary gland activity. Udder weight was reduced in cows subjected to a non-milking period of 28- versus either 7- or 14-days (Table 1). There was less total DNA following a non-milking interval of 28- versus 7-days, but not 14-days, suggests that cell loss may contribute to the observed reduction in parenchymal mass and milk output. This is in contrast to observations by Capuco et al. (1997) where no net losses of cells were observed during involution for a 60-day dry period, but agrees with the reduction in cell number observed following a 42-day non-milking period observed by Toerien & Cant (2007). The reason for these differences is unclear.

Parenchymal protein-synthetic capacity, as indicated by total RNA, was reduced in the 28-day versus 7- and 14-day non-milked glands consistent with the reduction in total protein, and a tendency for reduced protein-synthetic capacity per cell, indicated by the RNA:DNA ratio (Table 1). Translational efficiency, as indicated by the protein:RNA ratio, was reduced in the 7- versus 28-day non-milked glands with a similar numerical difference in the 14- versus 28-day non-milked galnds (Table 1). Decreased parenchymal RNA content per gland in the 14- and 28-day relative to thw 7-day non-milked glands may represent a down-regulation of the translational machinery required for milk protein synthesis. Thus,

Table 1 Effect of the length of non-milking period on mean milk and milk protein output and the biochemical indices in the mammary gland of lactating Friesian cows subject to a 7-, 14- or 28-day non-milking period followed by a 7-day milking period. Five cows per group. Relative ratios are back-transformed. Values in brackets are the range of the 95% confidence interval. Bolded P values indicate significance ($P < 0.05$). Protein:DNA indicates cell size, Protein:RNA indicates translational efficiency and RNA:DNA indicates cell protein-synthetic capacity. P values in bold indicates significance at $P < 0.05$. P values in italics indicates approaching significance with a P value between 0.5 and 0.10.

Measurement	Non-milking period			Standard error of mean	P-value		
	7 days	14 days	28 days		7 days vs 14 days	7 days vs 28 days	14 days vs 28 days
Milk yield (L)	10.3	7.8	2.4	0.9	0.16	**<0.001**	**0.003**
Protein yield (g)	39.2	33.6	10.1	3.7	0.55	**0.003**	**0.002**
Udder weight (kg)	11.2	11.1	7.3	0.8	0.99	**0.01**	**0.02**
Total DNA (g)	25.1	22.1	16.3	2.9	0.46	**0.05**	0.17
Total RNA (g)	24.7	21.8	11.8	2.5	0.42	**0.003**	**0.02**
Total protein (g)	683	672	435	79	0.92	**0.05**	**0.05**
Protein:DNA	27.4 (18.9–39.7)	31.2 (21.5–45.2)	25.6 (17.7–37.2)	-	0.60	0.79	0.43
Protein:RNA	27.6 (23.3–32.8)	31.0 (26.2–36.8)	37.4 (31.5–44.3)	-	0.32	**0.02**	0.12
RNA:DNA	1.1 (0.7–1.4)	1.0 (0.7–1.4)	0.7 (0.5–0.9)	-	0.94	*0.09*	*0.08*

while re-initiation of milk secretion can be achieved following a 14- and 28-day non-milking period, the protein-synthetic capacity of the gland is compromised compared to a 7-day non-milking period. Whether these effects are due to changes in the abundance and phosphorylation of factors involved in the regulation of mRNA translation, as observed in lactating versus non-lactating mammary tissue (Toerien & Cant 2007), or mTOR-mediated changes in ribosome biogenesis, was not an objective of this study, however further research is warranted.

Total FAA concentration did not differ among any of the groups in this study. Total essential AA concentration was greater following a 28- versus 7-day non-milking interval (Table 2), resulting from increased histidine (1.8 times) and leucine (2.1 times) with a trend for increased arginine (2.3 times), lysine (2.4 times) and methionine (1.5 times). Increased concentrations of arginine (4.7 times), histidine (1.6 times) and methionine (2.1 times), and a trend for increased isoleucine (2.2 times) were also observed following a 28- versus 14-day non-milking interval. Models of bovine mammary metabolism indicate that the mammary gland is able to match FAA inputs to synthetic activity (Volpe et al. 2010), and increasing intracellular FAA concentration may stimulate protein synthesis (Clarke et al. 1980; Hanigan et al. 2000;Volpe et al. 2010). However, the present study suggests that decreased milk output resulting from a longer non-milking interval is associated with the accumulation of essential AA in the mammary epithelial cells. Accumulation of essential AA coupled with decreased parenchymal protein-synthetic capacity, suggest that the abundance and activity of the protein synthetic machinery within mammary gland, rather than AA supply, may be an important regulator of milk protein output in the bovine mammary gland in response to increasing non-milking interval. The significance of the accumulation of only histidine, leucine, arginine, lysine and methionine warrants further investigation.

In previous studies (Pacheco et al. 2010; Shennan et al. 1997), non-essential AA made a major contribution to total intracellular FAA. However, unlike essential AA, non-essential AA concentrations were unaffected by the non-milking interval, with the exception of a tendency for decreased alanine and glutamate in the 28-day versus 7- and 14-day non-milking intervals. These observations contrast with changes in FAA in the bovine mammary gland following growth hormone administration (Pacheco et al. 2010). The mechanisms underpinning the responses between the two trials are likely to be different, therefore it is not surprising that the AA profiles also differ. Mechanisms mediating the observed difference in protein-synthetic capacity associated with non-milking interval, such as via effects on translation initiation in response to external nutrition or hormonal signals, will be the subject of further investigation.

This study indicates that the abundance of the protein-synthetic machinery within the mammary gland, rather than AA supply or signalling, may be an important regulator of milk protein output in the bovine mammary gland resulting from a longer non-milking interval. Further investigation into the mechanisms controlling ribosome biogenesis and mRNA translation in addition to the interaction between the protein-synthetic machinery and the nutritional (AA) environment is warranted. Such knowledge may contribute to development of new strategies to increase protein synthesis in the mammary gland, thereby increasing farmer profits.

Table 2 Absolute intracellular concentrations (nmol per g of tissue) of free amino acids (AA) in the mammary gland of lactating Friesian cows in response to a 7-, 14- or 28-day non-milking period followed by a seven day milking period. Five cows per group. P values in bold indicates significance at ($P < 0.05$). P values in italics indicates approaching significance with a P value between 0.5 and 0.10. Total AA includes unlisted metabolites in addition to the sum of the essential and non-essential AA.

Amino acid	Non-milking period			Standard error of mean	P-value		
	7 days	14 days	28 days		7 days vs 14 days	7 days vs 28 days	14 days vs 28 days
Total AA	8,712	9,239	8,773	380	0.60	0.99	0.67
Essential							
Arginine[a]	42	21	98	17	0.64	*0.09*	**0.02**
Histidine	39	43	70	7	0.91	**0.02**	**0.04**
Isoleucine	50	44	95	16	0.97	0.16	0.11
Leucine	80	105	171	23	0.72	**0.04**	0.15
Lysine	71	77	151	25	0.98	*0.09*	0.13
Methionine	26	19	39	4	0.49	0.12	**0.02**
Phenylalanine	26	34	55	10	0.83	0.15	0.35
Tyrosine[a]	32	42	60	14	0.87	0.33	0.60
Valine	113	208	212	40	0.25	0.22	0.99
Total	556	700	1,060	136	0.74	**0.05**	0.19
Non-essential							
Alanine	1,080	1,371	966	124	0.26	0.80	*0.09*
Aspartate	474	483	532	47	0.99	0.68	0.76
Glutamine	0	0	251	90	1.00	0.16	0.16
Glutamate	4,290	4,751	3,825	270	0.47	0.47	*0.08*
Glycine	1,948	1,605	1,741	123	0.16	0.48	0.72
Proline	95	120	150	30	0.83	0.42	0.76
Serine	242	190	208	22	0.25	0.54	0.83
Threonine	104	126	147	47	0.94	0.79	0.95
Total	8,161	8,562	7,733	383	0.75	0.72	0.31

[a]Deemed as "semi-essential" or conditionally essential.

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