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Influence of level of feeding and stage of lactation on proteolytic activity in bovine milk

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ABSTRACT

Using a Latin square cross-over design, twelve sets of identical Friesian or Friesian x Jersey twin cows during spring (early lactation: 42 ± 3 d in milk), were assigned to both a high (5 kg DM/d of a barley-based concentrate in addition to *ad lib.* pasture), or low (restricted to 70% of their metabolic energy requirements) level of feeding, separated by periods of uniformity (*ad lib.* access to pasture). The trial was repeated in summer (mid lactation: 177 ± 3 d in milk). During spring, low feed reduced milk yield by up to 30%, and during summer, high feed increased milk yield by up to 26%. Proteolytic activity (plasmin + plasminogen-derived activity) was not significantly altered by high feed during either stage of lactation, however during spring, low feed increased activity by up to 33%. The significant interaction between level of nutrition and stage of lactation should be considered when planning management strategies to maximise milk quantity and quality.

Keywords: Plasmin; plasminogen; stage of lactation; nutrition; cows; milk.

INTRODUCTION

Plasmin (EC 3.4.21.7) is responsible for the majority of indigenous protease activity in bovine milk (Fox, 1992). It is present in milk together with its inactive zymogen, plasminogen. The conversion of plasminogen in milk is modulated by plasminogen activators (tissue-type plasminogen activator and urokinase-type plasminogen activator), and plasminogen activator inhibitor-1 (Heegard *et al.*, 1994). Plasmin, plasminogen, and plasminogen activators are heat stable, so both plasmin activity and plasminogen activation continue during storage (Richardson, 1983). Reduced viscosity, bitter peptides, and poor cheese quality are all attributed to the proteolytic action of plasmin on casein in milk (Fox, 1992). In addition, contaminants from casein degradation in the whey may impede optimal use of this milk fraction.

Various genetic and environmental factors affect the level of plasmin activity in milk (Politis *et al.*, 1989b). Peak plasmin levels are observed during late lactation, possibly associated with the process of involution (Richardson and Pearce, 1981; Korycka-Dahl *et al.*, 1983; Politis *et al.*, 1989a). While nutrition is known to affect milk yield and composition (Rook and Thomas, 1983), the influence of plane of nutrition on proteolytic levels has only been investigated during late lactation (Prosser *et al.*, 1995).

In New Zealand there is increasing emphasis on the importance of adequate nutrition to maximise milk protein production. The objective of the present study was to investigate the effect of level of feeding on proteolytic activity in milk, during early (spring) and mid (summer) lactation.

MATERIALS AND METHODS

Cow Management and Trial Design

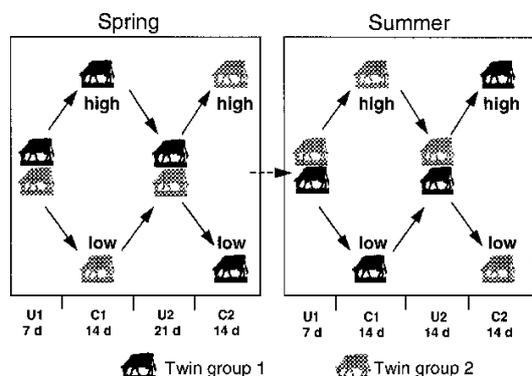
Twelve sets of multiparous, monozygotic, Friesian or Friesian x Jersey twin cows were used in a Latin square cross-over design (Figure 1). Uniformity periods were included before each nutritional challenge to minimise carry-over effects. Prior to the experiment, in addition to *ad libitum* pasture all cows were fed approximately 1 kg of a barley-based concentrate per d, for a period of 14 d, in order to increase their acceptance of the concentrate. In spring (early lactation: 42 ± 3 d in milk) all cows were grazed together with *ad libitum* access to pasture for 7 d (spring uniform [U] 1). Following this period of uniformity, one member from each twin set was assigned to the high feed challenge for 14 d (twin group 1; spring challenge [C] 1), while the other twin was assigned to a low feed challenge (twin group 2; spring C1). Cows on the high feed challenge were fed increasing amounts of concentrate, so that by d 7 of this period they were receiving approximately 5 kg/d concentrate in addition to *ad libitum* pasture. Cows on the low feed challenge were restricted on pasture to 70% of their metabolic energy requirements. A second period of uniformity followed for 21 d, during which all cows were grazing the same paddock on an *ad libitum* basis (spring U2). Following the second period of uniformity, the nutritional challenges were reversed for 14 d (spring C2). The trial was repeated in summer (mid lactation: 177 ± 3 d in milk) with the same trial design, except, the twin initially assigned to the high feed challenge in spring received the low feed challenge first in summer, and, the second period of uniformity was 14 d.

In two animals a minor pathogen was detected in one quarter of the udder, this was accompanied by low to medium somatic cell counts ($< 550,000$ cells/ml). In one

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FIGURE 1: Latin square cross-over trial design. Spring: early lactation 42 ± 3 d in milk. Summer: mid lactation 177 ± 3 d in milk. U-uniformity period (*ad lib.* access to pasture) C-challenge: high, 5kg DM/d concentrate in addition to *ad lib.* pasture; low, restricted on pasture to 70% of daily metabolic energy requirements.



animal a major pathogen was detected, accompanied by very high somatic cell counts (\bullet 700,000 cells/ml). Throughout the experiment, there was no difference in proteolytic activity, between the infected and uninfected animals.

Sample Collection

Milk yield was recorded and milk samples collected on two mornings at the end of each period and analysed for proteolytic activity (plasmin + plasminogen-derived activity). Immediately following milking an aliquot of fresh milk was centrifuged at 490 g for 15 min and the cream fraction discarded. Skim milk was incubated with 50 mM ϵ -amino-n-caproic acid (EACA; A2504, Sigma Chemical Co., St. Louis, MO), for 120 min at room temperature (25°C) to dissociate plasmin and plasminogen from casein micelles. Treated skim milk was then centrifuged at 100,000 g for 60 min at 4°C and the milk serum fraction was stored at -20°C until analysis.

Enzyme Analysis

Plasmin and plasminogen-derived activities were determined in milk serum by the method of Korycka-Dahl *et al.* (1983) as modified by Stelwagen *et al.* (1994). Briefly, each well of a 96 well microtiter plate contained 10 μ l of milk serum, 125 μ l of 50 mM Tris buffer (pH 7.4 containing 110 mM NaCl, 2.5 mM EACA), 25 μ l chromagen (6 mM H-D-valyl-L-leucyl-L-lysine-*p*-nitroanilide dihydrochloride; V7127, Sigma Chemical Co., St. Louis, MO), 25 μ l of 0.26 Sigma units/ml human urine urokinase (U8627, Sigma Chemical Co., St. Louis, MO) and deionised water to a total volume of 250 μ l, to measure total plasmin + plasminogen-derived activity. The reaction mixture was incubated for 60 min at 37°C to allow the conversion of plasminogen to plasmin. Formation of *p*-nitroanilide during cleavage of the substrate by plasmin (at 37°C) was measured by absorbance at 405 nm at 30 min intervals for up to 6 hours. The rate of *p*-nitroanilide formation was computed from the linear part of the absorbance versus time curves (between 2 to 6 h). A similar

reaction mixture, without milk serum was used as a control for the spontaneous hydrolysis of H-D-valyl-L-leucyl-L-lysine-*p*-nitroanilide dihydrochloride.

Statistical analysis

Statistical analyses were performed using SAS® (Release 6.10, 1994, Cary, NC, USA). Main effects and interactions were analysed using the PROC MIXED procedure. Results are expressed as proteolytic activity (plasmin + plasminogen-derived activity), in units/ml. One unit of activity is defined as the amount of the enzyme that produces a change in absorbance of 0.001 in 1 min at 37°C and 405 nm.

RESULTS

The effects of stage of lactation and level of feeding on milk yield are presented in Table 1. The results from the twins have not been pooled as there was a significant influence ($P < 0.01$) of the sequence of nutritional challenge on milk yield. This meant that milk yields for the twin groups were significantly different during the spring U2 period. In spring, there was a significant decrease in milk yield of 30% and 15% when cows were placed on low feed (compared to the preceding uniform period) during the first challenge (C1, twin group 2), or second challenge (C2, twin group 1) respectively. During the first challenge in spring, high feed did not significantly alter milk yield of twin group 1, but at the second challenge, milk yield increased 9% in twin group 2. During summer, low feed had no significant effect on milk yield, while high feed increased yield by 26% and 11% at the first (C1, twin group 2) and second (C2, twin group 1) challenges respectively.

There was a significant effect of stage of lactation on proteolytic activity in milk ($P < 0.01$), with activity (units/ml) increasing with advancing lactation (spring U1: 47; spring U2: 56; summer U1: 61; summer U2: 68; pooled SEM \pm 4; twin group data pooled, $n = 24$). The effects of

TABLE 1: Effect of level of feeding and stage of lactation on milk yield. Values represent milk yield (kg/d) least squares mean (LSM), pooled SEM \pm 1.0, of 12 animals.

	U1	C1	U2	C2
Spring (early lactation)				
Twin group 1	23	high 22 ¹	21 ^{1,a}	low 17 ^{1,b}
Twin group 2	23 ^a	low 16 ^{2,b}	18 ^{2,a}	high 20 ^{2,b}
Summer (mid lactation)				
Twin group 1	12	low 12 ¹	14 ^a	high 15 ^{1,b}
Twin group 2	12 ^a	high 15 ^{2,b}	14	low 13 ²

U-uniform (*ad lib.* pasture). C-challenge: high-5kg DM/d concentrate in addition to *ad lib.* pasture; low-restricted on pasture to 70% of daily metabolic energy requirements.

^{1,2}LSM with different superscripts within a period between twin groups are different $P < 0.005$.

^{a,b}Different superscripts indicate differences in LSM between the level of feed challenge and the preceding period of uniformity within a twin group $P < 0.05$.

TABLE 2: Effect of level of feeding and stage of lactation on proteolytic activity in milk. Values represent proteolytic activity (units/ml[†]) least squares mean (LSM), pooled SEM \pm 5, of 12 animals.

	U1	C1	U2	C2
Spring (early lactation)				
Twin group 1	50	high 53 ¹	56	low 60
Twin group 2	45 ^a	low 60 ^{2,b}	56	high 57
Summer (mid lactation)				
Twin group 1	61	low 66	67	high 64
Twin group 2	60	high 60	68	low 65

U-uniform (*ad lib.* pasture). C-challenge: high-5kg DM/d concentrate in addition to *ad lib.* pasture; low-restricted on pasture to 70% of daily metabolic energy requirements.

^{1,2}LSM with different superscripts within a period between twin groups are different P<0.05.

^{a,b}Different superscripts indicate differences in LSM between the dietary challenge and the preceding period of uniformity within a twin group P<0.0001.

[†] One unit of activity is defined as the amount of the enzyme that produces a change in absorbance of 0.001 in 1 min at 37°C and 405 nm.

level of feeding and stage of lactation on proteolytic activity are presented in Table 2. High feed did not significantly alter proteolytic activity in spring or summer. Low feed offered in spring at the first challenge (C1, twin group 2) resulted in a 33% increase in proteolytic activity. Although low feed offered at the second challenge in spring (C2, twin group 1) did increase proteolytic activity, the effect was not significant. Low feed did not significantly alter proteolytic activity during summer.

DISCUSSION

Uniformity periods were included in the Latin square cross-over design to minimise carry-over effects between the challenges. However, while there were no significant differences between the twin groups at the beginning of the trial, during spring U2 (after twins had been subjected to opposite nutritional challenges), there was a significant difference in milk yield. As a result, data from the twins were unable to be pooled and the analysis of the interactions between level of feeding and stage of lactation was confounded.

Nevertheless, restricting feed to 70% of metabolic energy requirements during early lactation (spring) significantly reduced milk yield in both twin groups, but there was no difference when repeated in mid lactation (summer). Supplementation with a concentrate during early lactation did not significantly alter milk yield in early spring (twin group 1; spring C1), whereas in late spring supplementation resulted in a 9% increase (twin group 2; spring C2). Although interpretation of these data is confounded by the carry-over effect from the first challenge, they suggest that in early lactation (spring) pasture intake was sufficient to support milk production, but in later lactation (summer) milk production was severely limited by pasture intake.

A major influence on plasmin activity in milk is the stage of lactation: elevated plasmin activity has been associated with the gradual process of involution during late lactation (Politis *et al.*, 1989a; Politis *et al.*, 1989b). In agreement with these studies, proteolytic activity in the present study increased with advancing lactation.

The influence of level of feeding on proteolytic activity was less consistent. Prosser *et al.* (1995) observed a significant increase in plasmin activity, but no significant effect on plasminogen activity in cows in which dry matter intake was restricted during late lactation. In the present study total proteolytic activity increased by 33% in the twin group fed low feed as the first challenge in spring and, while the level was also raised due to feed restriction in the second challenge, the increase was not significant.

In contrast to feed restriction, a high feeding level, during either stage of lactation, did not significantly alter proteolytic activity in milk, therefore, suggesting that the activity of plasmin and plasminogen in freshly secreted milk can not be manipulated by high feed. However the effect of feed intake on the activators which modulate the conversion of plasminogen to the active enzyme are not known. These activators are relatively heat stable, allowing plasmin activity to increase during storage (Richardson, 1983). Therefore, the possibility that high feed may still be used to maximise milk protein quality in stored dairy products by manipulation of one or more remaining components of the plasmin-plasminogen system can not yet be disregarded.

A further finding from this study was that the increase in proteolytic activity due to feed restriction or an advanced stage of lactation, was associated with a reduced milk yield. This may arise from a greater influx of plasminogen, a blood-derived protein, into milk, similar to changes in albumin observed in the milk of cows on restricted pasture intake (Gray and Mackenzie, 1987), or from the concentrating effect of low milk yield on plasminogen. However, in the converse, where milk yield was increased by feed supplementation, plasminogen in milk was unchanged suggesting the level of plasminogen derived protease activity in milk is not simply inversely related to milk yield.

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REFERENCES

- Fox, P.F. 1992. Indigenous enzymes in milk. *In* Advanced Dairy Chemistry-1: Proteins, P.F. Fox (editor), London, Elsevier Applied Science p310-321.
- Gray, R.M.; Mackenzie, D.D.S. 1987. Effect of plane of nutrition on the concentration and yield of whey proteins in bovine milk. *New Zealand Journal of Dairy Science and Technology*, **22**: 157-165.

- Heegaard, C.W.; Rasmussen, L.K.; Andreasen, P.A. 1994. The plasminogen activation system in milk: differential localisation of tissue-type plasminogen activator and urokinase in milk fractions is caused by binding to casein and urokinase receptor. *Biochimica et Biophysica Acta*, **1222**: 45-55.
- Korycka-Dahl, M.; Ribadeau Dumas, B.; Chene, N.; Martal, J. 1983. Plasmin activity in milk. *Journal of Dairy Science*, **66**: 704-711.
- Politis, I.; Lachance, E.; Block, E.; Turner, J.D. 1989a. Plasmin and plasminogen in bovine milk: A relationship with involution? *Journal of Dairy Science*, **72**: 900-906.
- Politis, I.; Ng Kwai Hang, K.F.; Giroux, R.N. 1989b. Environmental factors affecting plasmin activity in milk. *Journal of Dairy Science*, **72**: 1713-1718.
- Prosser C.G.; Nicholas, G.; Stelwagen, K.; Lacy-Hulbert, S.J.; Woolford, M.W.; McFadden, T.B. 1995. Influence of milking frequency and feeding level on plasmin activity and casein degradation in milk. *Proceedings of the New Zealand Society of Animal Production*, **55**: 9-11.
- Richardson, B.C. 1983. The proteinases of bovine milk and the effect of pasteurisation on their activity. *New Zealand Journal of Dairy Science and Technology*, **18**: 247-254.
- Richardson, B.C.; Pearce, K.N. 1981. The determination of plasmin in dairy products. *New Zealand Journal of Dairy Science and Technology*, **16**: 209-220.
- Rook, J.A.F.; Thomas, P.C. 1983. Milk Secretion and its nutritional regulation. *In* Nutritional physiology of farm animals. Rook, J.A.F.; Thomas, P.C. (editors). New York, Longman Inc. p314-368.
- Stelwagen, K.; Politis, I.; White, J.H.; Zavizion, B.; Prosser, C.G.; Davis, S.R.; Farr, V.C. 1994. Effect of milking frequency and somatotrophin on the activity of plasminogen activator, plasminogen, and plasmin in bovine milk. *Journal of Dairy Science*, **77**: 3577-3583.