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The effects of Insulin-nutrient supply interactions on ewe lactation

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ABSTRACT

Six lactating abomasally cannulated ewes were subjected to a hyperinsulinemic euglycemic clamp with or without casein supplementation in a two period crossover design experiment. There was a significant response in milk (1169 ± 46 g/d vs 952 ± 46 g/d, P<0.001) and milk protein (66.4 g/d ± 2.7 g/d vs 54.2 g/d ± 2.7 g/d P<0.006), yield from casein supplemented ewes. There was no overall effect of the clamp on milk and milk protein yield, while the interaction was also non-significant. However, there was a significant reduction in feed intake (P<0.001) as the clamp continued, which may have compromised milk and milk protein response. There was also a significant period effect across all the measured parameters, which could also be a stage of lactation effect, due to the rapid decline of milk production during ewe lactation. In this study, in contrast to concentrate-fed cows, the utilisation of this technique did not increase milk protein yield in pasture fed lactating ewes.

Keywords: insulin; milk protein; casein; ewe lactation.

INTRODUCTION

A major target of the New Zealand dairy industry is to increase the milk protein yield of pasture-fed dairy cows. However, our current understanding of the utilisation of pasture nutrients for milk production has not identified ways by which dairy cow metabolism could be manipulated to increase milk protein yield. An insulin-nutrient supply manipulation, utilising a hyperinsulinaemic euglycaemic clamp, has been demonstrated to increase milk protein yield in concentrate fed dairy cows (McGuire *et al.*, 1995, Grinarii *et al.*, 1997). The use of this technique alone increased milk protein yield by 7% (0.98 vs 1.05kg/d, McGuire *et al.*, 1995), whereas the clamp together with supplemental protein (in the form of casein) increased milk protein yield by 28% (0.81 vs 1.04 kg/d, Griinari *et al.*, 1997). While these studies have shown that this procedure affects the regulation of milk protein synthesis in concentrate fed dairy cows, it needs to be established if this procedure can be used to increase milk protein synthesis in the pasture-fed dairy cow.

This paper reports a preliminary trial with lactating ewes, where the objectives were to establish the methodology for future work in lactating dairy cows, and to evaluate the effect of this technique in lactating ewes.

MATERIALS AND METHODS

Animals

Six lactating ewes with abomasal cannula were housed indoors and individually fed ad libitum (offered approximately 3 kg dry matter (DM)/day) a diet of perenial ryegrass (*Lolium perenne*)-white clover (*Trifolium repens*) pasture during a 32 day experimental period. The pasture was cut daily and offered every 6 hours, at 1200, 1800, 0000 and 0600 hours, with water available *ad libitum*.

The ewes were machine milked throughout the trial period (as detailed by Peterson 1992). Two days prior to the start of infusions, jugular catheters were implanted under local anaesthesia (as detailed by Herath *et al.*, 1996) for infusions and blood sampling. Two catheters were implanted in the right jugular vein for glucose and insulin infusion and the remaining catheter in the left jugular vein for blood sampling.

Experimental Procedure

Each ewe was randomly allocated to a treatment group in a two factor crossed design; each animal was subjected to a hyperinsulinaemic euglycaemic clamp twice, with or without an abomasal infusion of casein. The experiment consisted of two 12 day periods, each comprising an initial 4 day interval for acclimatisation to casein or control (water) infusions, with basal measurements taken during days 5 to 8, to evaluate the effect of the casein. All ewes were then subjected to a hyperinsulineamic euglycaemic clamp from day 9 until day 12.

Casein (approximately 50g sodium caseinate, ICN Biomedicals Inc, Ohio.) was infused in a total volume of approximately 780 mls every 24 hours directly into the abomasum. An equal volume of water was infused into control ewes. The infusions continued for the entire 12 day experimental period at 2.1g casein/hour.

Bovine pancreas derived insulin (Sigma Chemicals, St Louis, MO.) was administered (1µg/kg $BW^{0.75}$ /hour) in a sterile filtered 0.5% Bovine serum albumin solution (Immuno Chemical Products (NZ) Ltd).

A sterile 25% w/w glucose solution was prepared using food grade dextrose monohydrate (Pure Chem Co. Ltd, Thailand), and used to maintain euglycaemia via variable speed peristaltic pumps.

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Measurements

During the basal measurements, blood samples were taken at 1200, 1400 and 1600 hours to establish basal concentrations of glucose and insulin. An average glucose concentration was determined, which was the target euglycaemia (± 10%) for each individual animal during the hyperinsulineamic euglycaemic clamp. During the clamp, euglycaemia was maintained using an Advantage Blood Glucose Meter (Boehringer Mannheim (NZ) Ltd), which allowed rapid (within 2 minutes) determination of blood concentrations for adjustment in the glucose infusion rate. To assess the effect of the clamp on circulating concentrations of insulin and glucose, blood samples were taken over the 4 days of the clamp. During the first 24 hours, blood samples were taken at 1400, 1600, 1800, 0000, and 0600 hours. Thereafter, on days 2, 3 and 4 of the clamp, samples were taken at 1200, 1400 and 1600 hours.

The ewes were milked twice daily at 0800 and 2000 hours, and the yields weighed and subsampled for storage at -20°C for further analysis. Milk protein content was measured by a scanning spectrometer (model 6500, NIRsystems Inc, Silver Spring, MD, USA) with PC software by Infrasoft International (version 3.1).

Feed offered was recorded four times daily. Refusals from each feed were collected from the bins and floor, bulked separately, weighed and the bin refusals subsampled for analysis. Dry matter was calculated on both feed offered and refused. Daily samples were analysed for crude protein (CP) and energy (ME) by a scanning spectrometer (model 6500, NIRsystems Inc, Silver Spring, MD, USA) with PC software by Infrasoft International (version 3.1).

All blood samples were collected with disodium ethylenediaminetetraacetate (Na_2.EDTA) as the anticoagulant and centrifuged at 3270g at 4°C for 15 minutes. The resulting plasma was harvested and stored at -20°C until analysed. Plasma insulin concentrations were measured using a double antibody radioimmunoassay (as detailed by Flux *et al.*, 1984). Intra- and inter-assay coefficients of variation were 8.67% and 12.9% respectively and mean sensitivity was 22.7 pg insulin/ml.

Statistical analysis

Analyses were performed using the procedure GLM from the statistical package SAS (1988). Results are expressed as least squares means ± standard errors. All treatment and time (period) effects and their interactions were tested. Where there is a significant effect of treatment, means have been generated to compare between treatments. Treatment interaction means are presented in Table 1 in the results section, whereas period effects are presented in the text.

RESULTS

Abomasal infusion of casein did not affect plasma insulin or blood glucose concentrations during the hyperinsulinaemic euglycaemic clamp (see Table 1). It also had no effect on the amount of exogenous glucose required to maintain euglycaemia. However, the amount of glucose required to maintain euglycaemia significantly increased from day 1 until day 4 of the clamp (5.0 ± 0.7 vs 7.6 ± 0.7 g/hour), and was significantly different between the 2 periods (5.1 ± 0.7 vs 7.5 ± 0.7 g/hour).

The insulin infusion raised plasma insulin concentrations over three times above basal values (see Table 1). While plasma insulin concentrations more than doubled during period 1 (+Insulin 640 ± 65 vs -Insulin 260 ± 65 pg/ml), the increase was significantly greater during period 2 (+Insulin 1481 ± 65 vs -Insulin 385 ± 65 pg/ml) despite the infusion rate being held constant for the two periods.

Blood glucose concentrations significantly increased due to the clamp (see table), particularly in the control ewes. The significant period effect (P<0.005) shows that a greater amount of exogenous glucose was required to maintain euglycaemia during period 2 (period 1, 3.2 ± 0.1 g/hour vs period 2, 3.7 ± 0.1 g/hour).

While the abomasal infusion of casein significantly increased milk yield (1169 ± 46 g/day vs 952 ± 46 g/day), there was no milk yield response to the insulin infusion, and no casein x insulin interaction. Average milk production was significantly greater in period 1 (1283 ± 46 g/day vs 838 ± 46 g/day, P<0.001) than period 2. A casein x period interaction (P=0.054) shows that the casein infused ewes in period 2 had a milk yield similar to period 1, whereas the milk yield of the control ewes was significantly less (period 1, casein 1325 ± 56 g/day vs nocasein 1241 ± 68 g/day; period 2, 1013 ± 68 g/day vs 664 ±

TABLE 1: Treatment effects during baseline (-Insulin) and hyperinsulinaemic euglycaemic clamp (+Insulin) for insulin, glucose, milk yield and intake.

	- Insulin		+Insulin			Significance		
Variable	**+ Casein**	**- Casein**	**+ Casein**	**- Casein**	**SEM**	**Casein**	**Insulin**	**CASxINS**
Insulin (pg/ml)	361	284	986	1136	65	ns	P<0.001	ns
Blood Glucose (mM)	3.3	3.3	3.4	3.8	0.1	ns	P<0.03	ns
Milk Yield (g/day)	1129	976	1208	929	60	P<0.001	ns	ns
Milk protein yield (g/day)	66.3	55.5	66.5	52.9	3.9	P<0.006	ns	ns
Intakes								
Dry Matter (kg/day)	1.26^a	1.21^a	1.09^b	0.97^c	0.02	P=0.077	P<0.001	P<0.026
Crude Protein (g/day)	236^a	239^a	215^b	193^c	5	P=0.055	P<0.001	P<0.014
ME (MJ/day)	14^a	14^a	13^b	12^c	0.3	P=0.063	P<0.001	P<0.020

Means with the same subscript do not differ at the P<0.05 level.

56 g/day). Milk protein yield also increased due to the casein infusion (66.4 ± 2.7 g/day vs 54.2 ± 2.7 g/day). However, there was no significant effect of the clamp and no casein x clamp interaction. A significant period effect shows milk protein yield differed between the two periods (74.3 ± 2.7g/day vs 43.6 ± 2.7 g/day, P<0.001).

A similar pattern was observed in DM, CP and ME (MJ ME) intake. While casein treatment tended to influence intake in the three variables measured (see Table 1), the clamp significantly reduced DM, CP and ME intake in the casein infused ewes. In addition, a significant casein x clamp x period interaction demonstrates that while the clamp reduced DMI, CP and ME intake in both periods, this decrease was particularly large in the control ewes in period 2.

DISCUSSION

The ewes in this study clearly utilised the abomasal infusion of casein to increase milk and milk protein yield. This is consistent with studies in pasture fed ewes (Barry 1980) and cows (Rogers and McLeay 1977), and concentrate fed cows (Clark *et al.*, 1977, Griinari *et al.*, 1997). In addition, that insulin infusion alone had no effect on milk yield is consistent with hyperinsulineamic euglycaemic studies of both short (less than 10 hours) (Hove 1978, Laarveld *et al.*, 1981, Tesseraud *et al.*, 1992) and long term (4 days) studies (McGuire *et al.*, 1995, Griinari *et al.*, 1997). However, in contrast to these long term studies, milk protein yield was not increased in this study when the ewes were subjected to both a hyperinsulinaemic euglycaemic clamp and abomasal infusion of casein.

Intake was decreased during the hyperinsulinaemic euglycaemic clamp and this would have compromised milk and milk protein yield. Feed intake decreased 6% in the casein infused and 16% in the water infused ewes. These are similar to the responses observed by Griinari *et al.*, (1997) where intake was reduced by 30% in the controls, but only 6% in the casein infused cows. McGuire *et al.*, (1995) also observed a decrease in intake during the clamp, with intake 29% lower in the clamped relative to the non-clamped cows. Despite these reductions in intake, milk protein yield increased.

In this study however, the ewes were compromised by being in a negative energy balance and losing weight throughout the study, which combined with the decrease in intake potentially limited milk protein synthesis. The estimated daily ME requirements of the ewes was 25-30 MJ ME/day (Geenty and Rattray 1987) and as shown in Table 1, consistently less than these requirements were consumed daily.

It is estimated that even with the additional ME supplied by the casein and glucose, the casein infused ewes required on average an extra 16.5 MJ ME and the control ewes 18 MJ ME to meet maintenance requirements. This did not change during the clamp, where both groups needed an extra 17 MJ ME to meet maintenance requirements. Although feed intake decreased in the control ewes, it appears that these ewes compensated for the reduction in intake by increasing the demand for exogenous glucose, so that on a ME basis there was no difference in intake to the casein infused ewes. This is in agreement with McGuire *et al.*, (1995), who suggested that the cows reduced intake to match the dietary energy supply and exogenous glucose infusion with their energy needs. However, although the energy intake of the ewes stayed the same, crude protein intake did not and this may have contributed (particularly in period 2) to the differences in milk yield.

The changes in glucose and insulin concentrations observed in this study may also have influenced feed intake and consequently milk and milk protein yield. The higher glucose concentrations alone probably were not responsible as Leenanuruksa *et al.*, (1988) showed in diabetic ewes, that increasing of blood glucose from 3.3mM to between 4.0 - 4.8 mM did not effect DM intake and milk yield. However, it has been demonstrated that an increase in circulating insulin concentrations and constantly high levels of glucose has the potential to activate satiety centres in ewes and depress feed intake (Rutter and Manns, 1985). This effect may have contributed to the reduction in feed intake of the ewes during the clamp.

While milk yield and milk protein yield responded to the casein treatment in this study, there was no milk protein response to the hyperinsulinaemic euglycaemic clamp. This would appear to be due to the effect on feed intake. However, the contribution of several other factors should also be considered. The data in this study are limited by the small number of animals. In addition, there was significant period interactions across all measured parameters, which could also be a stage of lactation effect due to the rapid decline of milk production during ewe lactation. Finally, it may indicate species differences between sheep and cows, and therefore we have subsequently conducted a trial to assess the effect in the pasture fed dairy cow.

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