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The effects of insulin on the milk production of pasture-fed Jersey cows

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ABSTRACT

The aim of this experiment was to determine the effects of insulin on milk protein production in lactating, pasture-fed dairy cows. Five lactating rumen-fistulated Jersey cows were subjected to a hyperinsulinaemic euglycaemic clamp (HEC) with and without an abomasal infusion of casein (310g/d) in a two period cross-over design experiment. The HEC significantly reduced milk yield in the control cows compared to casein-infused cows (9.9 ± 0.5 vs 13.2 ± 0.5 kg/d, P<0.001). Milk protein yield was significantly higher in the casein-infused cows compared to the control cows (482 ± 16 vs 421 ± 16 g/d, P<0.05). The HEC significantly reduced milk protein yield in the control cows compared to casein-infused cows (311 ± 16 vs 459 ± 16 g/d, P<0.001). The use of the HEC technique in pasture-fed cows did not result in the milk protein response reported in concentrate-fed cows.

Keywords; dairy cows; milk protein; insulin.

INTRODUCTION

Many studies have attempted to increase milk protein synthesis through increasing the supply of precursors (amino acids) to the mammary gland (Sutton, 1989), but few have examined the mechanisms that regulate milk protein synthesis with the objective of devising more effective strategies of increasing milk protein yield. Manipulating insulin status by use of the hyperinsulinaemic-euglycaemic clamp (HEC) technique in concentrate-fed cows increased milk protein yield (McGuire *et al*., 1995a). Use of this technique in conjunction with supplementary dietary protein increased milk protein yield by up to 28% (Griinari *et al.,* 1997; Mackle *et al.,* 1999, 2000; Bequette *et al.,* 2001). These results are remarkable when considered relative to the adaptations of whole body metabolism to meet the requirements for lactation, which primarily seem to be aimed at blunting the effects of insulin, particularly in adipose and muscle tissue (Hart, 1983; Grizard *et al.*, 1999).

Not all studies using the HEC in lactating ruminants have increased milk protein yield (Tesseraud *et al.,* 1992, Annen *et al.*, 1998, Back *et al.*, 1998). The reason for the variation in the milk protein response to the HEC is not known and further research is needed to understand the mechanisms involved. Pasture-fed, lactating ewes, under HEC conditions, maintained milk protein output despite a decrease in dry matter intake (DMI) and dietary crude protein intake (Back *et al.,* 1998). This indicated a change in nutrient partitioning, as shown by the change in the proportion of dietary protein used for milk protein production and indicated that the lactating ewe is a useful model for studying the effects of HEC on nutrient partitioning. However, the yield of milk protein was not increased. It is not clear if the different response compared to concentrate-fed cows and goats was because of species difference, energy balance or type of feeding. The trial described in this paper examined the response of pasture-fed dairy cows to the HEC and is to our knowledge, the only study in pasture-fed dairy cows.

MATERIALS AND METHODS

This study was approved by the Animal Ethics Committee at AgResearch, Grasslands. Five lactating Jersey cows (46 ± 14 d *post partum*, 352 ± 20 kg, (mean ± SD)) prepared with rumen fistulae (inserted 4 months prior to this study), were housed indoors. They were individually offered sufficient pasture to permit *ad libitum* intakes of a diet of perennial ryegrass (*Lolium perenne*) - white clover (*Trifolium repens*) pasture during a 6-week experimental period. The pasture was cut twice daily and offered every 6 h at 0830, 1430, 2030 and 0230 h, with water available *ad libitum*. Dry matter was calculated on both pasture offered and refused, and daily DMI was measured as offered less refused.

The cows were milked throughout the trial period with a portable milking plant (Nu Pulse Porta Milker, Hamilton, NZ). Two days prior to the start of each experimental period, two jugular catheters were inserted into each of the jugular veins for infusions and blood sampling (Back, 2002).

Each cow was randomly allocated to a treatment group in a two period cross-over design; in which each animal was subjected to a HEC twice, with or without an abomasal infusion of casein. The experiment was conducted for six weeks and consisted of an initial two-week training period. This was followed by two 12-day periods, in which (in each period) the first 4 days allowed acclimatisation to casein or control (buffer) infusions, with measurements being taken during days 5 to 8 to evaluate the effect of the casein infusion. All cows were then subjected to HEC from days 9 – 12, to allow comparison of the effect of the insulin infusion. There was a rest period of 6 days between the two experimental periods to allow for any residual effects of insulin, glucose or casein to be eliminated. Cows were grazed outdoors on pasture during the rest period and brought indoors twice daily for milking.

For each 12-d experimental period, casein (310 g/cow/d, alacid 30 mesh acid casein, New Zealand Dairy Board) was infused directly into the abomasum in a 0.1 M sodium phosphate buffer (pH 7.0, Scientific Supplies (NZ) Ltd). Control cows received an equal volume of the 0.1M

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sodium phosphate buffer. The infusions continued for the entire 12-d experimental period at approximately 3.6 ml/min (approx. 12.5 g casein/h).

For the HEC (on days 9-12) bovine pancreatic insulin (1 μg/kg BW/h, Sigma Chemicals, St Louis, MO.) in a sterile filtered 0.5% bovine serum albumin solution (Immuno Chemical Products (NZ) Ltd) was infused continuously via peristaltic pump into the jugular vein. Blood glucose concentrations were monitored using an Advantage Blood Glucose Meter (Boehringer Mannheim (NZ) Ltd). This allowed rapid determination of blood glucose concentrations for adjusting the glucose infusion via variable speed peristaltic pumps to maintain euglycaemia during the insulin infusion. A 45% w/w glucose solution prepared using food-grade dextrose monohydrate (Pure Chem Co. Ltd, Thailand) and sterilised by autoclaving (Back, 2002) was used to maintain euglycaemia.

The cows were milked twice daily at 0730 and 1930 h, milk yields were weighed and samples stored at -20°C for further analysis. Milk composition was estimated by near-infrared spectroscopy (NITS, in transmission mode, model 6500, NIRsystems Inc, Silver Spring, MD, USA) with PC software by Infrasoft International (version 3.1) that was calibrated for cows' milk (Back, 2002).

During days 5-9 of each 12 day experimental period, blood samples were taken at 1000, 1200 and 1400 to establish basal concentrations of glucose, insulin, non-esterified fatty acids (NEFA), β-hydroxybutyrate, triacylglyerols, and insulin-like growth factor-I (IGF-I). An average glucose concentration was determined, which was the target (± 10%) for each individual animal during the subsequent HEC. To assess the effect of insulin on circulating concentrations of insulin, NEFA, triacylglyerols, IGF-1, and β-hydroxybutyrate, blood samples were taken over the four days of the HEC. During the first 24 h, blood samples were taken at 1000, 1200, 1400, 2000, 0200, and 0800 h. Thereafter, on days 2, 3 and 4 of the HEC, samples were taken at 1000, 1200 and 1400 h.

All blood samples (except those for glucose concentrations) were collected with disodium ethylenediaminetetraacetate (Na_2.EDTA) as the anticoagulant. Plasma insulin concentrations were measured using a double-antibody radioimmunoassay (RIA) (Flux *et al.*, 1984). Intra- and inter-assay coefficients of variation (CV) were 8.7% and 12.9% respectively. The mean assay sensitivity was 22.7 pg insulin/ml. Plasma IGF-1 concentrations were determined by RIA using an acid/ethanol procedure as reported by Prosser *et al.* (1995). Plasma cortisol concentrations were also measured using a RIA (Back, 2002). The sensitivity of the assay was 2.0 ng/ml. For a mean cortisol concentration of 8.7 ng/ml, the intra-assay CV was 14.1%. Plasma NEFA and β-hydroxybutyrate concentrations were measured by enzymatic colorimetric methods (Back *et al.*, 1999) on a Cobas Fara II autoanalyser (Hoffman-La Roche Ltd, Basal, Switzerland).

Data were analysed with a split-plot analysis with repeated measures. The casein treatment was considered as the main plot, with the HEC as the split-plot and time was used as the repeated factor. Analyses were performed using the procedure GLM from the statistical package SAS (1988). The model used for the analyses was:

$$Y_{ijkl} = \mu + \delta k + \pi(l)k + a_i + \beta j + \varepsilon_{ijkl}$$

where μ is the mean, δ*k* is the fixed effect due to the group k, α*i* is the fixed effect due to the treatment i, β*j* is the fixed effect due to period j, and π*(l)k* is the l^{th} cow in group k. Results are expressed as least-squares means ± standard errors of the mean (SEM). All treatment and period effects and their interactions were tested. Differences between treatments were assessed by a multiple comparison test (PDIFF option of Proc GLM, SAS Institute 1996) of the calculated least-squares means. Significance levels of $P<0.05$ were taken to indicate significant effects, whereas levels of $0.05<P<0.1$ were considered to indicate trends. Normality of the data was tested by plotting the standardised residuals against the standardised predicted values of the response variables. No plots showed a pattern that would indicate that the normality assumption of the ANOVA model should be questioned and that the data should be transformed.

RESULTS

Abomasal infusion of casein did not change concentrations of plasma insulin and blood glucose or the amount of glucose required to maintain euglycaemia (Table 1). Circulating plasma insulin concentrations significantly increased in both the casein-infused and control cows during the HEC, but to a significantly greater

TABLE 1: Comparison of hormone and metabolite concentrations (LSMeans ± SEM) on day 4 of the abomasal casein infusion period and day 4 of the hyperinsulinaemic euglycaemic clamp (HEC).

	Infusion		HEC	
	Casein	**Control**	**Casein**	**Control**
Insulin (pg/ml)	265 (±487)[a]	388 (±494)[a]	3860 (±780)[b]	6653 (±712)[c]
Blood glucose (mM)[1]	3.3 (±0.2)[a]	3.4 (±0.2)[a]	3.5 (±0.2)[a]	4.1 (±0.2)[b]
NEFA (mequiv/ml)[2]	148 (± 14)[a]	160 (± 13)[a]	50 (± 13)[b]	53 (± 13)[c]
β-hydroxybutyrate (mM)	0.65 (± 0.03)[a]	0.60 (± 0.03)[a]	0.42 (± 0.03)[b]	0.37 (± 0.03)[b]
Triacylglycerols (mM)	0.09 (± 0.01)	0.11 (± 0.01)	0.11 (± 0.01)	0.10 (± 0.01)
IGF-1 (ng/ml)[2]	52 (± 5)[a]	49 (± 4)[a]	78 (± 4)[b]	87 (± 5)[b]
Cortisol (ng/ml)	6.8 (± 2.0)[a]	6.6 (± 1.9)[a]	6.2 (± 1.9)[a]	12.0 (± 1.9)[b]

Treatments: HEC = hyperinsulinaemic euglycaemic clamp, casein = casein infused (n=5), control = buffer infused (n=5).
LSMeans with different superscripts are significantly different at $P<0.05$.
[1] Values for infusion day 4 in both casein and control cows are an average calculated from samples taken over the 4 days of the infusion period.
[2] NEFA = non-esterfied fatty acids, IGF-1 = insulin-like growth factor-1.

TABLE 2: Comparison of milk yield, yield and concentration of milk protein (LSMeans ± SEM) on day 4 of the abomasal casein infusion period and day 4 of the hyperinsulinaemic euglycaemic clamp (HEC).

	Infusion		HEC		
	Casein	**Control**	**Casein**	**Control**	**± SEM**
Milk yield (kg/d)	14.3[a]	14.3[a]	13.2[a]	9.9[b]	0.5
Crude protein					
Yield (g/d)	482[a]	421[b]	459[ab]	311[c]	16
Concentration (%)	3.2[ab]	3.1[a]	3.3[b]	3.3[b]	0.1

Treatments: HEC = hyperinsulinaemic euglycaemic clamp, casein = casein infused (n=5), control = buffer infused (n=5).
LSMeans with different superscripts are significantly different at P<0.05.

extent in control cows. Overall, blood glucose concentrations were maintained within 10% of the range determined for euglycaemia during casein infusion period in the casein-infused cows. However, the concentration of blood glucose in the control cows was slightly but significantly above this range (Table 1).

Abomasal infusion of casein had no effect on feed intake (casein-infused 10.9 ± 0.9 kg/DM/d vs 12.4 ± 0.9 kg/DM/d in the control cows). Feed intake decreased gradually over the four days of the HEC so that by the final day it was significantly lower (P<0.05) than on day 4 of the casein infusion period (casein infused 9.8 ± 0.9 kg/DM/d, control cows 9.3 ± 0.9 kg/DM/d). There was a significant effect of period of insulin infusion on feed intake (P<0.01). This was caused by a larger reduction of intake during the first period of insulin infusion (average intake in period one was 9.07 ± 0.31 kg DM/d versus period two, 13.70 ± 0.31 kg DM/d).

The efficiency of CP utilisation is a measure of the gross efficiency of dietary CP utilisation for milk protein production and was calculated as g milk protein produced per g of CP intake. Casein infused and control cows had a similar efficiency of CP utilisation on day 4 of the casein infusion (0.23 ± 0.06 vs 0.21 ± 0.06). However, efficiency of utilisation increased significantly (P<0.05) in casein infused and control cows on day 4 of the HEC (0.49 ± 0.06 vs 0.38 ± 0.06).

Abomasal infusion of casein had no effect on milk yield, as there was no difference in production between the casein-infused and control cows on day 4 of the casein infusion (Table 2). On day 4 of the HEC, milk yield of the control cows was significantly lower and tended (P=0.08) to be lower in the casein-infused cows. Milk yield of the control cows was not significantly different from that of the casein-infused cows over the first three days of the HEC but dropped significantly on the fourth day (data not presented). This pattern was demonstrated in both periods of insulin infusion. There was a significant effect of period of infusion (P<0.001) on milk yield, as yield was lower during the second period of infusion.

Casein-infused cows had a significantly greater concentration of crude protein in milk than control cows (Table 2). However, crude protein concentration in milk of the control cows was significantly higher on day 4 of the HEC compared to day 4 of the casein infusion. Crude protein yield was significantly reduced in the control cows by the HEC. While crude protein yield was influenced by period of infusion (P<0.001), concentration was not.

Abomasal infusion of casein had no effect on circulating plasma concentrations of NEFA, β-hydroxybutyrate and triacylglyercol (Table 2). Concentrations of NEFA and b-hydroxybutyrate were significantly reduced by the HEC in both the casein-supplemented and control cows. The HEC did not alter triacylglycerol concentrations between treatments, although there tended to be (P=0.08) a difference between the two periods of infusion. There was a significant period-of-insulin-infusion effect on NEFA (P<0.001) and β-hydroxybutyrate (P<0.001) concentrations as concentrations were lower in the second period of infusion.

Circulating concentrations of plasma IGF-1 and cortisol on day 4 of the infusion period and day 4 of the HEC are presented in Table 1. Plasma IGF-1 concentrations were increased significantly by HEC in both the casein-supplemented and control cows, whereas cortisol concentrations did not change significantly in the casein-supplemented cows but were significantly higher on day 4 in the control cows. There was no effect of sequence or period of infusion on either IGF-1 or cortisol concentrations.

DISCUSSION

The main aim of this experiment was to test if, under HEC conditions, milk protein production could be increased in lactating, pasture-fed cows, and secondly, to compare the results with those obtained with lactating ewes under the same experimental conditions (Back *et al.,* 1998).

In contrast to other studies utilising the HEC (McGuire *et al.,* 1995a; Griinari *et al.,* 1997; Mackle *et al.,* 1999, 2000; Bequette *et al.,* 2001), neither the casein infusion, the HEC alone or HEC-plus-casein infusion increased, or in the case of the control cows, maintained milk or milk protein yield in these pasture-fed cows (Table 2). However, not all studies using a long-term (4-day) HEC have shown an increase in milk protein production (Annen *et al.,* 1998). The cows in this experiment differed in that they were able to maintain production for three of the four days of the HEC (data not shown). Yields of milk and milk components measured on day 4 of the HEC were lower, significantly so in the control cows (Table 2). There was no response to the casein infusion, therefore, it does not appear that the cows were protein deficient. However, casein may have been used as an energy source. By the end of the first period of insulin infusion, DMI was severely reduced and was calculated by an algorithm based intake model (data not presented) to be substantially

less than was required for that level of milk production (Back, 2002). However, during the second period of insulin infusion, the cows ate far more than was required for milk production. These results indicate a stage of lactation effect caused by the two periods of insulin infusion as this is consistent with what would be expected later in lactation when body reserves would be being replenished. This stage of lactation effect was also observed in ewes (Back *et al.*, 1998).

Despite decreasing milk and milk protein yields, crude protein concentration in milk was maintained in the casein-infused cows, or slightly but significantly increased in the control cows. Crude protein concentrations in the milk (Table 2) were lower than the 3.5 – 4.5% values published for Jersey cows in New Zealand (i.e. Verkerk *et al.*, 1999; Thomson *et al.*, 2001). Values for these cows pre-trial, when they were grazing pasture, ranged from 3.53 – 3.93%. A reduction in feed intake and other stressors will decrease the protein concentration in milk (Verkerk *et al.*, 1999). The present results suggest that the cows had not properly adapted to indoor housing despite a two-week training period prior to measurements being made during the infusion and HEC periods.

Circulating IGF-1 concentrations increased during the HEC and this is consistent with other studies of this type (McGuire *et al*., 1995a; Griinari *et al.*, 1997; Mackle *et al.*, 1999, 2000; Bequette *et al.*, 2001). As a result of this increase, IGF- 1 has been suggested as being the regulator for milk protein synthesis (McGuire *et al.*, 1995a,b). However, while intra-arterial and intra-mammary infusion of IGF-1 has increased milk yield in several studies (Prosser *et al.*, 1990; Prosser & Davis, 1992), it has not in others (Davis *et al*., 1989). As IGF-1 is nutritionally regulated (McGuire *et al.*, 1995b; Leonard & Block, 1997), the increase in circulating concentrations seen in HEC studies may result from high insulin concentrations plus sufficient glucose signalling to the brain that nutrients are abundant, even when intake is reduced, as in the cows in this experiment. This would not indicate a direct role for IGF-1 in increasing milk protein synthesis.

The data produced from pasture-fed cows in this experiment offer no support for the proposal that insulin (under HEC conditions) stimulates milk protein production. While there was no evidence of insulin increasing production directly, there was a change in nutrient partitioning as defined by the change in utilisation of dietary crude protein from milk protein production. However, this enabled the cows to maintain milk yield and yields of components only for three of the four days of the HEC. The use of this technique was physically demanding and this suggests that the response to the HEC is one that the cows can support for only a limited period of time.

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