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Lack of effect of short term chromium supplementation in lactating Jersey cows

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ABSTRACT

The responses of dairy cattle to chromium (Cr) supplementation reported in the literature suggest that it has the potential to reduce the incidence of both mastitis and ketosis. The effect of Cr supplementation of pasture fed dairy cows in early lactation was studied. Ten lactating Jersey cows fitted with rumen fistula were infused with either Cr-yeast (n=4), casein (n=3) or buffer only (controls; n=3) directly into the abomasum. All cows were subjected to a hyperinsulinaemic euglycaemic clamp. Preliminary results show that there was no effect of Cr supplementation on milk yield, cortisol and β -hydroxy butyrate concentrations and somatic cell counts.

Keywords: chromium; lactation; mastitis; ketosis.

INTRODUCTION

Recent studies would suggest that Chromium (Cr) supplementation may be of benefit for high producing dairy cows in times of stress such as early lactation and peak milk yield through effects on glucose metabolism and its immunoenhancing capabilities (Chang and Mowett 1992, Moonsie-Shageer and Mowett 1993, Subiyatno *et al.*, 1996, Yang *et al.*, 1996). Chromium may effect glucose metabolism through enhancing glucose tolerance and insulin sensitivity, which leads to improved regulation of blood glucose concentrations and ultimately, increased milk yield (Subiyatno *et al.*, 1996, Yang *et al.*, 1996). In addition, Cr supplementation decreased the concentration of β -hydroxybutyrate (β -HBA) in the blood in early lactation (Yang *et al.*, 1996, Subiyatno *et al.*, 1996). These effects on glucose metabolism may be beneficial to dairy cows early in lactation when they are particularly prone to development of metabolic disorders such as ketosis.

During times of stress such as early lactation, the concentration of cortisol in the blood is elevated. This elevated concentration of cortisol may suppress the immune system, contributing to the high incidence of mastitis in early lactation as these periods also coincide with impaired lymphocyte and neutrophil function (Guidry *et al.*, 1976, Kehrl *et al.*, 1989 a, b). Chromium supplementation, through its effect on the immune system and, in particular, its ability to lower blood cortisol concentrations, may enhance the resistance of the lactating dairy cow to mastitis.

Mastitis and ketosis cost the New Zealand dairy farmer millions of dollars as a result of lost productivity and associated animal health costs. If Cr supplementation was effective in reducing the incidence or severity of mastitis and metabolic disorders such as ketosis, it would be of great value. This paper reports the results of short term Cr supplementation to pasture fed dairy cows with and without the hyperinsulinaemic euglycaemic clamp. This experiment was part of a larger experiment which investigated

responses to the clamp with and without supplementary casein and responses to casein supplementation have also been presented in this paper. The clamp technique was utilised to assess if chromium supplementation had an effect on glucose uptake, and therefore glucose tolerance and metabolism.

MATERIALS AND METHODS

Ten lactating Jersey cows (approximately 46 days post-partum) fitted with rumen fistula were housed indoors and fed *ad libitum* (offered approximately 12 kg dry matter (DM)/day) a diet of perennial ryegrass (*Lolium perenne*) - white clover (*Trifolium repens*) pasture during a six week period from September - November 1997. The pasture was cut twice daily and offered every six hours at 0830, 1430, 2030 and 0230 hours, with water available *ad libitum*.

Each cow was randomly allocated to one of three treatment groups in a split plot design experiment. Each treatment consisted of a hyperinsulinaemic euglycaemic clamp, together with an abomasal infusion of either casein (n=3), chromium (n=4) or buffer (n=3). Two jugular catheters were inserted in one side for glucose and insulin infusion and one in the opposite side for blood sampling.

The experiment was 12 days in total, split into three 4-day periods, during which equal volumes of buffer, casein or chromium were infused into the abomasum. This design allowed the first four days for acclimatisation to the infusions, with the second four days for a comparison of casein, Cr and control infusions. On the final four days the effect of infusion treatments under conditions of the hyperinsulinaemic euglycaemic clamp were compared. This comparison was used to estimate the effect of the insulin treatment as well as the insulin-casein and insulin-chromium interactions.

Both casein (310 g/cow/day) and chromium (9.3g yeast/cow/day Biochrome Cr-yeast containing 1g Cr/kg yeast (Alltech Associates, Nicholasville, Kentucky)) were

infused in a 0.1 M sodium phosphate buffer (Scientific Supplies (NZ) Ltd) directly into the abomasum. Control cows received an equal volume of the 0.1 M sodium phosphate buffer.

The hyperinsulinaemic euglycaemic clamp consisted of simultaneous insulin and glucose infusions. Insulin is infused at a constant rate, whereas glucose is infused at a variable rate to maintain basal (pre insulin infusion) blood glucose concentrations (euglycaemia). The system is said to be clamped when euglycaemia is maintained for the insulin infusion. Bovine pancreas derived insulin (Sigma Chemicals, St Louis, MO) was infused constantly (1 mg/kg BW/hour) in a sterile filtered 0.5% bovine serum albumin solution (Immuno Chemical Products (NZ) Ltd). A sterile 45% w/w glucose solution (food grade dextrose monohydrate, Pure Chem Co. Ltd, Thailand) was used to maintain euglycaemia via variable speed pumps.

The cows were milked twice daily at 0730 and 1930 hours and somatic cell counts were measured on day 2 of the baseline period, and days 2 and 4 of the clamp. Analyses were performed by Livestock Improvement Corporation (Hamilton NZ.).

During the basal and clamp measurements, blood samples were taken to establish concentrations of glucose, insulin, non-esterified fatty acids (NEFA), β -HBA and cortisol. An average glucose concentration was determined over the 4 baseline days and this was the target euglycaemia ($\pm 10\%$) for each individual animal during the hyperinsulinaemic euglycaemic clamp. Blood glucose concentration was measured by an Advantage Blood Glucose meter (Boehringer Mannheim (NZ) Ltd), which allowed rapid determination (within 2 minutes) of blood concentrations for adjusting the glucose infusion rate. All blood samples were collected with disodium ethylenediaminetetraacetate (Na_2EDTA) 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 for insulin, glucose, NEFA, β -HBA and cortisol. Plasma insulin concentrations were measured using double antibody radioimmunoassay (RIA) (Flux *et al.*, 1984). Intra- and inter-assay coefficients of variation were 8.7% and 12.9%, and mean sensitivity was 22.7 pg insulin/ml. Plasma cortisol concentrations were measured by RIA (as described by T. Manley pers. comm.). The sensitivity of the assay was 2.0 ng/ml and for a mean cortisol concentration of 8.69 ng/ml, the intra-assay CV was 14.1%. Plasma glucose concentrations were measured with a Cobas Fara II autoanalyser (Hoffman-La Roche Ltd, Basel, Switzerland) using the method of Trinder (1969). Intra- and inter-assay coefficients of variation were 2.3% and 1.6% respectively. Plasma NEFA and β -HBA concentrations were measured by enzymatic colorimetric methods adapted to a Cobas Fara II autoanalyser (Hoffman-La Roche Ltd, Basel, Switzerland). The NEFA method (McCutcheon and Bauman 1986), was based on a WAKO C test kit and intra- and inter-assay coefficients of variation were 4.6% and 2.3% respectively. The β -HBA method was based on that of Williamson and Mellanby (1974) and the intra- and inter-assay coefficients of variation were 4.7% and 2.1% respectively.

Feed offered was recorded four times daily. Refusals from each feed were collected from the bins and floor, bulked separately and weighed. DM was calculated on both feed offered and refused.

The data generated from this experiment was analysed using a one way analysis of variance (ANOVA). Analyses were performed using the GLM procedure of the SAS Systems (1988). All treatment and time effects were tested. Where there was a significant effect of treatment, means and p-values associated with the corresponding tests have been generated to compare treatments. Results are expressed as least squares means \pm standard errors (SEM). The assumptions underlying ANOVA (Normality and Homogeneity of variance) were examined by plotting the standardised residuals against the predicted values of the response variables. No plots showed a pattern which would indicate that these assumptions should be questioned and that the data should be transformed.

RESULTS

Abomasal infusion of Cr did not alter milk yield. On day 8 of the 12-day experiment, the milk yield of cows infused with Cr (13.9 ± 0.8 kg/d) was not significantly ($P > 0.05$) different from that of the control group (control 14.6 ± 0.9 kg/d). It was however, lower ($P < 0.087$) than that of the casein infused group (16.8 ± 0.9 kg/d). Casein infused cows also had significantly higher milk yields than the control group ($P < 0.018$). By day 4 of the clamp, there was a significant decrease in milk yield of the control (10.8 ± 0.9 kg/d, $P < 0.001$) and Cr supplemented (10.0 ± 0.8 kg/d, $P < 0.001$) cows compared to the casein cows (15.7 ± 0.9 kg/d).

Figure 1 shows the plasma cortisol (a), NEFA (b) and insulin (c) concentrations on days 8, 9 and 12 of the trial (days 9 and 12 are days 1 and 4 of the insulin infusion).

Plasma cortisol concentrations of the Cr infused group were not significantly different from that of the controls on each of days 8, 9 and 12 of the trial. Cortisol concentrations in the casein infused cows were significantly higher than those of both the control ($P < 0.017$) or Cr ($P < 0.007$) cows on day 8. Concentrations of cortisol fell in the casein group over the insulin infusion, so that concentrations were lower than both control ($P = 0.1$) or Cr ($P = 0.086$) cows by the end of the insulin infusion on day 12 and this resulted in a significant treatment by time interaction ($P < 0.029$).

Plasma NEFA (Fig 1b) and β -HBA concentrations were not affected by Cr or casein infusion and concentrations were not significantly different between the three groups. The concentrations of NEFA and β -HBA in the plasma of all groups significantly decreased ($P < 0.001$) over the first 24 hours of insulin infusion and stayed depressed throughout the following three days of infusion.

The plasma concentrations of insulin and glucose were similar in all groups throughout the trial, and there were no differences in the amount of glucose infused to

maintain euglycaemia during the clamp. However, the amount of glucose required to maintain euglycaemia increased over time ($P<0.001$) and at a different rate between treatment groups (Fig 2). This is shown by a significant time by treatment interaction ($P<0.008$), as there was no significant difference in glucose infusion rates between the groups on day 1 and day 4 of the clamp, but significantly different increases on days 2 and 3. The concentration of insulin in the plasma rose ($P<0.001$) during the clamp, particularly on days 2 and 3 (Fig 1c). Plasma glucose concentration also increased significantly ($P<0.001$) due to a large increase in concentrations in all groups on day 4 of the glucose infusion.

Somatic cell counts increased significantly

FIGURE 1: Plasma concentrations of Cortisol, NEFA and Insulin during days 8-12 of the trial.

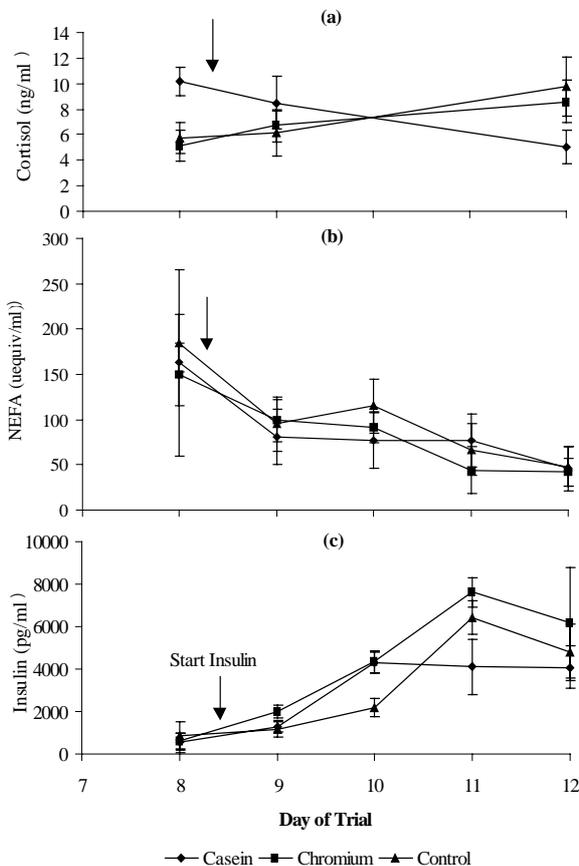
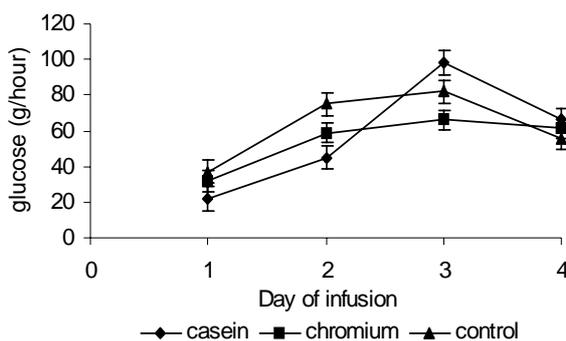


FIGURE 2: Glucose infusion rate over the hyperinsulinaemic euglycaemic clamp.



($P<0.001$) between day 2 and 4 of the insulin infusion in all groups. Casein supplemented cows had higher counts than Cr ($P<0.024$) or control ($P=0.098$) cows on days 6 and 10 (control $P=0.086$, Cr $P<0.028$), but there were no significant difference between the groups by day 12.

Feed intake was not affected by Cr supplementation. Casein supplemented cows had a higher average intake (8.9 ± 0.5 kg DM/d) over the eight days of measurements compared to control (7.4 ± 0.5 kg DM/d $P=0.06$) and Cr (7.5 ± 0.5 kg DM/d $P=0.06$) cows. Although there was no significant difference in intake between the groups over the four days before the insulin infusion commenced, there was a significant time effect ($P<0.001$). This was due to control ($P<0.01$) and Cr ($P<0.002$) cow intakes decreasing significantly over the four days of insulin infusion compared to the casein infused cows.

DISCUSSION

In this study, Cr supplementation in pasture-fed dairy cows (with or without insulin infusion) did not increase milk yield or provide any indication of enhanced resistance to mastitis. Previous studies that have reported increases in milk yields in response to Cr have primarily been in primiparous heifers whereas the cows in this study were second parity. The dose rate of Cr in the organic form as supplied by the Cr-yeast were similar to those used by Burton (1995) and equivalent to the dose used by Mallard and Borgs (1997) to obtain maximal uptake. Burton (1995) reported a 13% increase in milk yield in primiparous heifers over the first 16 weeks of lactation. This was in contrast to multiparous cows, where supplementation caused a 7% increase between weeks 11-16 of lactation. Yang *et al.*, (1996) also reported that milk yield was increased in 2 experiments with primiparous heifers (by 13% and 7%) during the first 16 weeks of lactation but there was no effect on milk yield of multiparous cows in either experiment.

In this experiment there was no differences between groups in the amount of glucose infused during the hyperinsulinaemic euglycaemic clamp, which indicates a lack of difference in glucose sensitivity of the tissues. This is in contrast with a previous study in which Cr supplementation increased glucose uptake during a clamp (Mallard and Borgs 1997). The results from plasma glucose measurements indicate that we had difficulty in maintaining target euglycaemia in all groups. In addition, insulin concentrations in the Cr and control cows did not stay constant (despite a constant infusion rate) during the clamp (Fig 1c). The response of the Cr and control cows suggests a difference in insulin clearance rate compared to the cows infused with casein but standard errors are large and would mask moderate differences.

The plasma concentrations of cortisol measured in the cows in this experiment were within the normal physiological range of 1-17mg/ml (Lefcourt *et al.*, 1993). Concentrations increased over the trial in both the Cr supplemented and control groups, but there were no differences between these two groups (Fig 1a). This is in agreement

with Yang *et al.*, (1996) who reported plasma cortisol concentrations were not different at both weeks 2 and 6 postpartum for supplemented and control cows. However, this contrasts with lower cortisol concentrations reported in non-lactating animals (stressed feeder calves) that had received dietary Cr supplementation (Chang and Mowat 1992, Mowat *et al.*, 1993). It is not clear why the casein infused group should have significantly higher cortisol concentrations on day 8.

Although there has been no direct evidence of beneficial effects of Cr supplementation on mastitis in published studies to date, it is thought to have potential to reduce mastitis through effects on the immune system. Our observations on somatic cell counts in this study provide no evidence of Cr being beneficial for mastitis prevention but it must be acknowledged that this is based on a small number of samples.

There was no effect of Cr supplementation on plasma NEFA and β -HBA. However, reports from other studies have been conflicting and indicate that parity, body condition and energy balance may influence response to Cr (Subiyatno *et al.*, 1996, Yang *et al.*, 1996).

We can report no response in milk yield, immune response or blood metabolites in supplementing Cr to pasture fed dairy cows. However, several factors may have contributed to this and should be considered. A small number of animals were involved in the trial and were supplemented over a short period of time (12 days). Studies that have reported responses have been for longer duration (16 weeks). Therefore, our period of supplementation may not have been long enough to show any benefit and consideration of this should be made for future work. In addition, using a technique such as the hyperinsulinaemic euglycaemic clamp may have made responses difficult to obtain due to confounding effects of insulin infusion and diet.

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