Performance and Glucose Metabolism in Calves Fed a Chromium-Nicotinic Acid Complex or Chromium Chloride[1]

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ABSTRACT

Twenty-four Holstein bull calves were fed a milk replacer diet to assess the effects of Cr on calf performance and metabolism of glucose. Treatments consisted of no supplemental Cr (control) or 0.4 mg/kg of dry matter of supplemental Cr from $CrCl_3$ or from a Cr-nicotinic acid complex. Supplementation with the Cr-nicotinic acid complex increased the average daily gain and feed efficiency from d 28 to 42, but not over the entire 63-d performance phase. Calves that were fed diets supplemented with $CrCl_3$ or the Cr-nicotinic acid complex had lower plasma glucose concentrations at 45 to 180 min after an i.v. infusion of insulin than did controls. Calves fed diets supplemented with the Cr-nicotinic acid complex also had lower plasma glucose concentrations from 90 to 180 min after insulin challenge than did calves that were fed diets supplemented with $CrCl_3$. After an i.v. infusion of glucose, calves that were fed diets supplemented with $CrCl_3$ had lower serum insulin concentrations at 10 to 25 min after challenge than did controls or calves that were fed diets supplemented with the Cr-nicotinic acid complex. However, the glucose clearance rate after glucose infusion was not affected by Cr supplementation. Chromium supplementation did not markedly affect the performance of calves, but the Cr-nicotinic acid complex and $CrCl_3$ did intensify the response to insulin administered i.v.
(**Key words**: chromium, calves, glucose tolerance, insulin resistance)

INTRODUCTION

Chromium is a component of a glucose tolerance factor that potentiates the actions of insulin (8). Although its structure has not been definitely determined, the glucose tolerance factor is likely made up of trivalent Cr, nicotinic acid, and amino acids (glutamic acid, glycine, and cysteine) (16). Chromium supplementation has altered glucose metabolism in humans (2), rats (13), and in calves with a developed rumen (3).

No Cr research has been conducted with nonruminating calves fed milk replacer. The major energy sources for adult ruminants are the VFA produced by microbial fermentation in the rumen; most of the glucose requirement for adult ruminants is met by gluconeogenesis. However, because calves absorb glucose from the small intestine (4), much higher concentrations of glucose are available, which must be cleared rapidly. For calves fed milk, absorption of glucose from the intestine stimulates the secretion of insulin. The tissues of calves may be more sensitive to insulin than the tissues of adult ruminants (17). Therefore, the objective of this study was to determine the effects of a milk replacer containing Cr from an organic source or from an inorganic source on calf performance and glucose metabolism.

MATERIALS AND METHODS

Care, handling, and sampling of the calves were approved by the North Carolina State University Animal Care and Use Committee (Raleigh). Twenty-four Holstein bull calves ($\bar{X} \pm SE = 39 \pm 0.6$ kg) were obtained at <1 wk of age from a local dairy. Calves were obtained in two groups 1 wk apart (replicate 1, n = 15; replicate 2, n = 9). Calves were fed a commercial milk replacer (Land O'Lakes, Fort Dodge, IA) that was reconstituted to 13% DM. The dry milk replacer contained all milk protein (22% CP), 20% crude fat, 138 mg of oxytetracycline/kg of DM, and 276 mg of neomycin base (from neomycin sulfate)/kg of DM. The milk replacer contained 0.31 mg of Cr/kg of DM. All calves were offered an equal amount of milk replacer twice daily and were fed only milk replacer throughout the study. At the start of the study, calves were offered 0.5 kg of DM/d. The amount of milk replacer offered was increased to 1.4

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kg of DM/d by the end of the study. Calves were housed individually in 1.52-m^2 plastic pens and drank the milk replacer from plastic buckets. Treatments consisted of no supplemental Cr (control) or 0.4 mg of supplemental Cr/kg of DM from either $CrCl_3$ or a Cr-nicotinic acid complex (10% Cr; ZinPro Corp., Edina, MN). Treatments were added immediately before each feeding. One calf that was fed a diet supplemented with $CrCl_3$ and one calf that was fed a diet supplemented with the Cr-nicotinic acid complex failed to adapt to drinking the milk replacer; these calves were removed from the study. One calf that was fed a diet supplemented with $CrCl_3$ died from a genetic abnormality, and one control calf died on d 52 of the study from a respiratory disease.

Calves were weighed at 14-d intervals throughout a 63-d performance phase. Blood samples were obtained by jugular venipuncture before feeding on d 28, 56, and 63 (replicate 2) or on d 70 (replicate 1). Plasma was analyzed for glucose and urea N. Blood samples were taken 1 h after feeding on d 7, 14, 28, 42, 56, and 63 (replicate 2) or 70 (replicate 1). Samples for analyses of plasma glucose and plasma urea N were collected into tubes containing potassium oxalate and sodium fluoride (Vacutainer® 6470; Becton Dickinson, Rutherford, NJ) and were kept on ice until centrifuged. Samples for analysis of serum cholesterol were collected into plain glass vacuum tubes (Vacutainer® 6431; Becton Dickinson).

On d 67 (replicate 2) or d 74 (replicate 1), calves were cannulated in the jugular vein. Over the following 3 d, blood samples were taken at –15, 0, 15, 30, 45, 60, 75, 90, 120, 150, and 180 min after the regular morning feeding. Six hours after feeding, 0.5 g of glucose (50% glucose solution)/kg of BW was infused (glucose tolerance test). Blood samples were taken at –10, 0, 5, 10, 15, 20, 25, 30, 45, 60, 90, 120, 150, and 180 min after infusion. Fourteen hours after feeding, an insulin challenge was conducted. Bovine insulin (Sigma Chemical Co., St. Louis, MO) was infused (0.1 U/kg of BW), and samples were taken at –10, 0, 5, 10, 15, 20, 25, 30, 45, 60, 90, 120, 150, and 180 min after infusion. At each sampling time, blood samples that were used for insulin determination were placed in glass tubes and kept on ice until they were centrifuged to separate serum. Blood samples used to detect plasma glucose were placed in tubes containing potassium oxalate and sodium fluoride (Vacutainer® 6470; Becton Dickinson) and kept on ice until centrifugation.

Plasma glucose was determined by a membrane-immobilized, glucose oxidase enzyme that was coupled to an electrochemical sensor (model 27 industrial analyzer; Yellow Springs Instrument Co., Inc., Yellow Springs, OH). Glucose clearance rate and half-life were calculated according to the method of Kaneko (6). Serum insulin was determined by radioimmunoassay using antibody-coated tubes (Diagnostic Products Corp., Los Angeles, CA). Mean intra-assay and interassay coefficients of variation were 5.2 and 13%, respectively. Plasma urea N (15) and serum cholesterol (14) were determined by colorimetric assays.

Data were analyzed by ANOVA using the general linear models procedure of SAS (12). The model for performance included treatment and replicate. The model for glucose, plasma urea N, and cholesterol on regular sampling dates included effects of treatment, replicate, day, and the interactions of treatment by replicate, calf nested within treatment by replicate, treatment by day, replicate by day, and treatment by replicate by day. The error term for treatment was calf nested within treatment by replicate. The model for the infusion data included effects of treatment, calf nested within treatment by replicate, time, and the interaction of time by treatment. The error term for treatment was calf nested within treatment. Treatment means were compared by an F protected t test.

RESULTS AND DISCUSSION

Performance data were analyzed at 14-d intervals and for the entire study. Performance was affected only between d 28 to 42. During this period, average daily gain and the gain to feed ratio were greater ($P <$

TABLE 1. Effect of amount and source of Cr on performance of calves fed a milk replacer diet.

Item	Control	$CrCl_3$	Cr-NA[1]	SEM
Calves, no.	7	6	7	
Initial BW, kg	39.7	39.5	38.9	1.2
Final BW, kg	82.8	83.7	83.6	1.4
Average daily gain, kg	0.66	0.66	0.67	0.02
Gain:feed	0.65	0.66	0.67	0.02

[1]Chromium-nicotinic acid complex.

TABLE 2. Effect of amount and source of Cr on plasma glucose, urea N, and serum cholesterol on the regular sampling dates.

Item	Control	$CrCl_3$	Cr-NA[1]	SEM
Calves, no.	7	6	7	
Plasma urea N, mg/dl				
Before feeding[2]	6.4	6.4	7.0	0.5
After feeding[3]	5.7	6.2	6.0	0.5
Serum cholesterol, mg/dl				
After feeding[3]	82	99	96	8
Plasma glucose, mg/dl				
Before feeding[2]	85	93	93	3
After feeding[3]	107	105	106	3

[1]Chromium-nicotinic acid complex.

[2]Mean of samples taken on d 28, 56, and 63 or 70.

[3]Mean of samples taken on d 7, 14, 28, 42, 56, and 63 or 70.

0.05) for calves that were fed diets supplemented with the Cr-nicotinic acid complex than for calves that were fed diets supplemented with $CrCl_3$. However, over the entire performance phase, average daily gain, DMI, and the gain to feed ratio were not affected by treatment (Table 1). Chromium supplementation of older cattle improved performance in some studies (9, 10), but not in others (7).

Plasma urea N, either before or after feeding, was not affected by treatment or by the interaction of treatment and day; therefore, sampling dates were combined (Table 2). Similarly, in a study by Bunting et al. (3), supplemental Cr picolinate did not affect plasma urea N of calves that were fed diets based on corn, soybean meal, and cottonseed hulls.

No treatment effects or interaction of treatment by day was observed for serum cholesterol; therefore, sampling dates were combined (Table 2). Samsell and Spears (11) also showed that supplemental $CrCl_3$ did not affect serum cholesterol in lambs fed diets with high or low concentrations of fiber. In contrast, serum cholesterol concentrations of older calves that were fed dry diets were decreased by supplementation with Cr picolinate on some, but not all, sampling dates (3). In studies with humans (19), supplemental Cr decreased serum cholesterol concentrations.

On regular sampling dates, plasma glucose concentrations were not affected by treatment or by an interaction of treatment by day; therefore, sampling dates were combined (Table 2). In studies using older cattle with functioning rumens (3, 7), plasma glucose was also unaffected by supplemental Cr. In the present study, plasma glucose concentrations before and after feeding were affected by day (data not shown). Plasma glucose concentrations of the samples taken before feeding were greatest on d 56 and lowest on d 28. As the trial continued, plasma glucose concentrations increased after feeding, which might reflect the increased dietary lactose that the calves received or indicate the development of insulin resistance as the age of the calves increased. Hostettler-Allen et al. (5) also observed that concentrations of plasma glucose after feeding increased as age increased.

When frequent samples were taken after feeding and following cannulation, neither treatment nor an interaction of treatment by time affected changes in

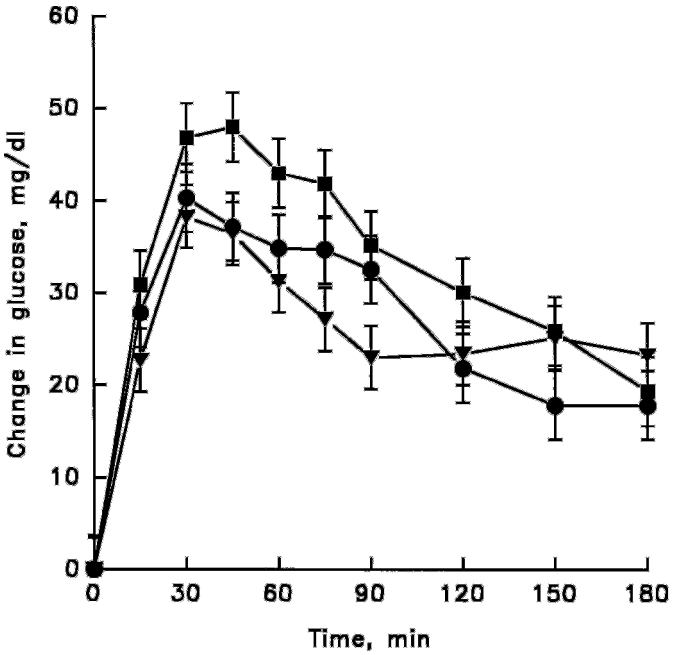

Figure 1. Effect of amount and source of Cr [control (▼), $CrCl_3$ (●), or a Cr-nicotinic acid complex (■)] on the change in plasma glucose concentrations after feeding. Bars represent standard errors.

TABLE 3. Plasma glucose and serum insulin concentrations before feeding and before glucose and insulin infusions.

Item	Control	$CrCl_3$	Cr-NA[1]	SEM
Before feeding				
Glucose, mg/dl	85	87	90	2
Insulin, μU/ml	3.2	3.2	3.2	<0.1
Before glucose infusion				
Glucose, mg/dl	105	101	100	5
Insulin, μU/ml	113[a]	29[b]	58[ab]	26
Before insulin infusion				
Glucose, mg/dl	81[b]	90[a]	90[a]	3
Insulin, μU/ml	3.2	3.4	3.2	0.1

[a,b]Means in a row with different superscripts differ ($P < 0.05$).

[1]Chromium-nicotinic acid complex.

plasma glucose (Figure 1) or serum insulin (Figure 2) concentrations. Initial glucose and insulin concentrations before feeding and before the infusions are shown in Table 3.

Glucose Infusion

After the i.v. infusion of glucose, neither treatment nor the interaction of treatment by time affected the change in plasma glucose concentrations (Figure 3). Glucose clearance rate and half-life (Table 4) also did not differ among treatments. Similarly, in a study of feeder calves (7), supplementation with the Cr-nicotinic acid complex or with $CrCl_3$ did not affect glucose clearance after the infusion of glucose. However, supplemental Cr picolinate did increase glucose clearance rate after an i.v. infusion of glucose in calves that had a developed rumen (3).

The interaction of treatment by time ($P < 0.07$) after the i.v. infusion of glucose affected the change in serum insulin concentrations (Figure 4). Calves that were fed diets supplemented with $CrCl_3$ had a lower ($P < 0.10$) peak insulin response (10 to 25 min after infusion) than did controls and calves that were fed diets supplemented with the Cr-nicotinic acid complex. Serum insulin concentrations of calves that were

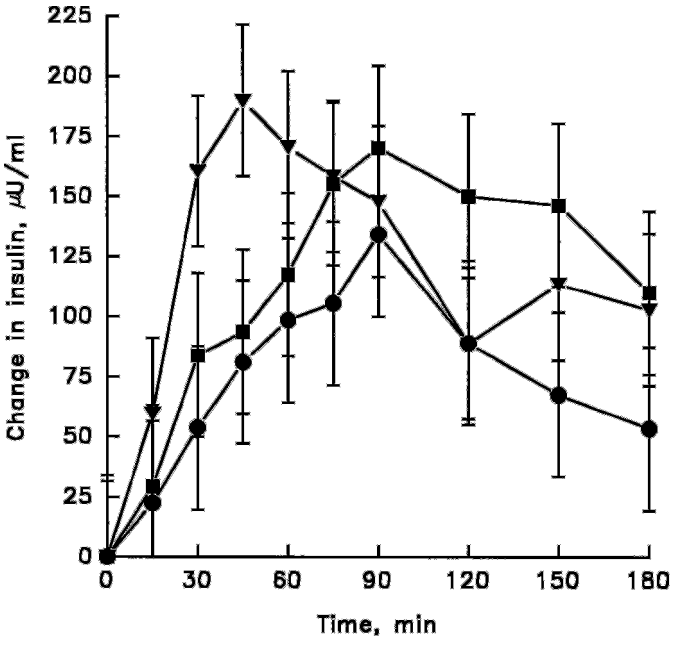

Figure 2. Effect of amount and source of Cr [control (▼), $CrCl_3$ (●), or a Cr-nicotinic acid complex (■)] on the change in serum insulin concentrations after feeding. Bars represent standard errors.

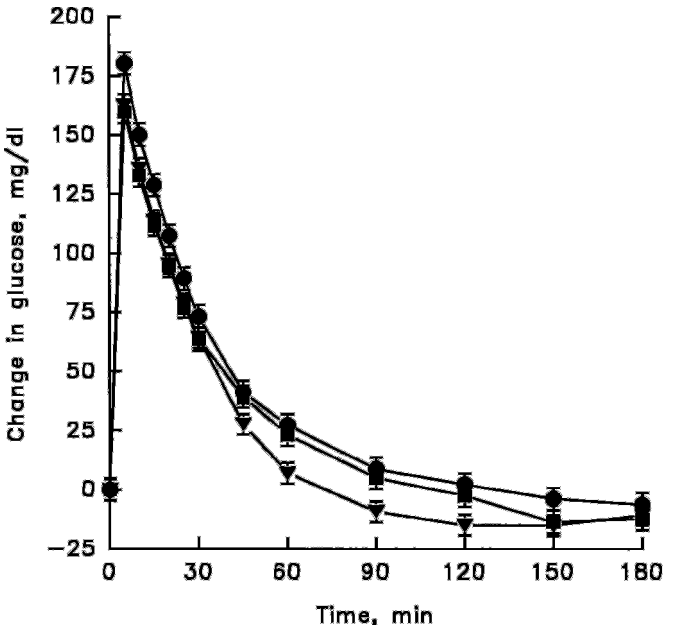

Figure 3. Effect of amount and source of Cr [control (▼), $CrCl_3$ (●), or a Cr-nicotinic acid complex (■)] on the change in plasma glucose concentrations after an i.v. infusion of glucose. Bars represent standard errors.

TABLE 4. Effect of amount and source of Cr on glucose kinetics after an i.v. infusion of glucose.

Glucose kinetics	Control	$CrCl_3$	Cr-NA[1]	SEM
Clearance rate,[2] %/min	1.67	1.66	1.43	0.20
Half-life,[2] min	44.3	49.2	52.3	7.1

[1]Chromium-nicotinic acid complex.

[2]From 15 to 45 min after infusion.

fed diets supplemented with the Cr-nicotinic acid complex did not differ ($P > 0.10$) from insulin concentrations of controls. The tissues of calves that were fed diets supplemented with $CrCl_3$ might have been more sensitive to insulin because the lower insulin concentrations in calves that were fed diets supplemented with $CrCl_3$ resulted in the same change in plasma glucose concentrations after the glucose infusion. Anderson (1) reviewed Cr effects on glucose tolerance and diabetes in humans and concluded that Cr reduced the amount of insulin required to maintain glucose tolerance. In contrast, there were no differences in insulin response between control steers that were fed a diet based on corn silage and those that were supplemented with $CrCl_3$ (7). However, in that study, steers that received the Cr-nicotinic acid complex had the greatest insulin response after an i.v. infusion of glucose (7). The difference between the insulin response that was observed in the present study and the response in the previous study might be related to the different ages of the calves and to the differences in development of rumen function. In a study of humans, Anderson (1) noted that the response of children to Cr supplementation was usually more rapid than the response of adults. Some adult humans and diabetic mice appeared to lose the ability to convert inorganic Cr to a biologically active form. In adult humans, $CrCl_3$ was less effective in decreasing glucose concentrations during feed deprivation than was brewer's yeast that contained high concentrations of organic Cr (18).

By 120 to 180 min after the glucose challenge, serum insulin concentrations of control calves had decreased ($P < 0.10$) more than those in calves that were fed a diet supplemented with $CrCl_3$. Before glucose infusion, control calves had greater ($P < 0.05$) serum insulin concentrations than did calves that were fed a diet supplemented with $CrCl_3$.

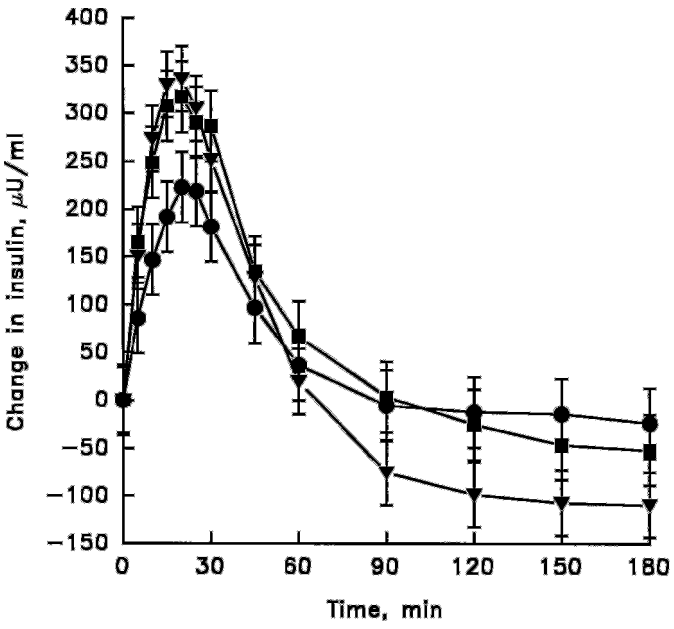

Figure 4. Effect of amount and source of Cr [control (▼), $CrCl_3$ (●), or a Cr-nicotinic acid complex (■)] on the change in serum insulin concentrations after an i.v. infusion of glucose. Interaction of time by treatment ($P < 0.07$). Bars represent standard errors.

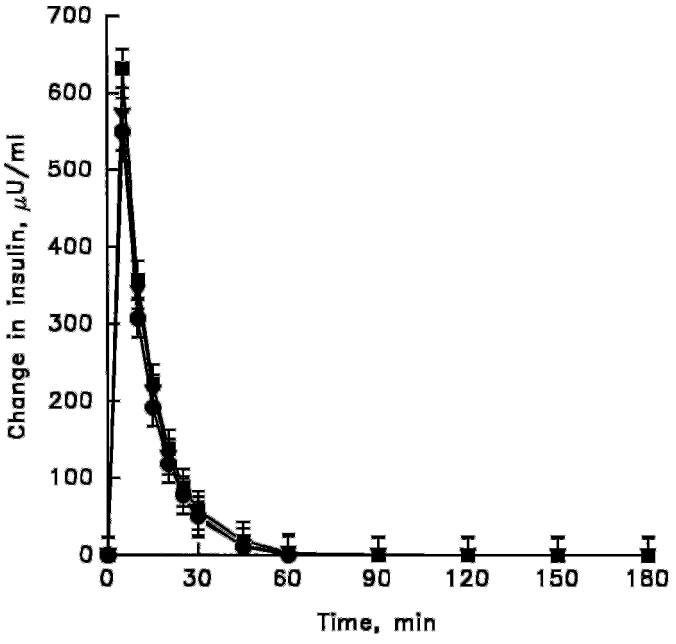

Figure 5. Effect of amount and source of Cr [control (▼), $CrCl_3$ (●), or a Cr-nicotinic acid complex (■)] on the change in serum insulin concentrations after an i.v. infusion of insulin. Bars represent standard errors.

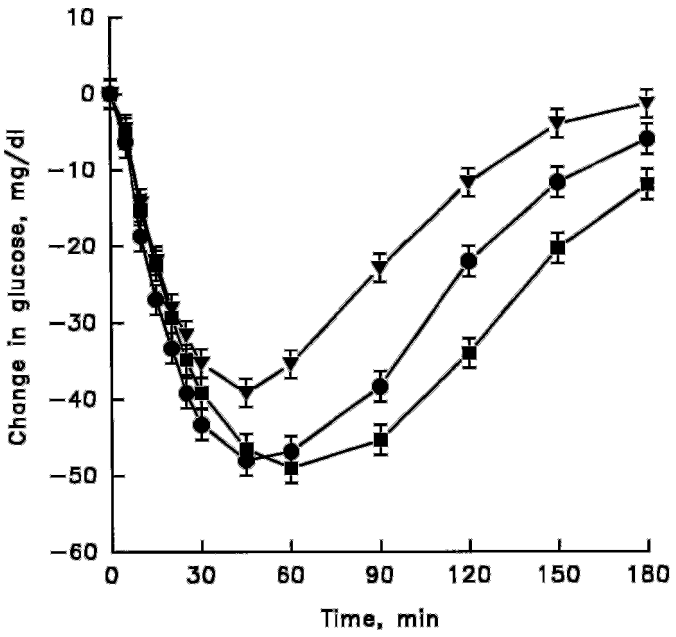

Figure 6. Effect of amount and source of Cr [control (▼), $CrCl_3$ (●), or a Cr-nicotinic acid complex (■)] on the change in plasma glucose concentrations after an i.v. infusion of insulin. Interaction of time by treatment ($P < 0.01$). Bars represent standard errors.

Insulin Infusion

Serum insulin increased rapidly after the i.v. infusion of insulin (Figure 5). Change in serum insulin concentration was not affected by treatment or an interaction of treatment by time.

Before the insulin infusion, control calves had lower ($P < 0.05$) plasma glucose than did calves that were fed diets supplemented with $CrCl_3$ or with the Cr-nicotinic acid complex (Table 3). There was an interaction of treatment by time ($P < 0.01$) on the change in plasma glucose after the i.v. infusion of insulin (Figure 6). Calves that were fed diets supplemented with $CrCl_3$ had a greater ($P < 0.05$) decrease in plasma glucose concentrations at 15 to 180 min after infusion than did controls. Calves that were fed diets supplemented with the Cr-nicotinic acid complex had lower ($P < 0.01$) plasma glucose concentrations from 45 to 180 min after insulin infusion than did controls. Calves that were fed diets supplemented with the Cr-nicotinic acid complex also had lower ($P < 0.05$) plasma glucose concentrations from 90 to 180 min after insulin challenge than did calves that were fed diets supplemented with $CrCl_3$. The return to basal glucose concentration was more gradual for calves that were fed diets supplemented with $CrCl_3$ or with the Cr-nicotinic acid complex. Tissues from calves that were fed Cr might have been more sensitive to the insulin, or the insulin might have had a longer lasting effect in these calves. In contrast, after insulin infusion, plasma glucose concentrations of calves that were fed dry diets supplemented with Cr picolinate tended to return more rapidly to baseline concentrations (3).

CONCLUSIONS

In the present study, Cr supplementation from $CrCl_3$ or a Cr-nicotinic acid complex did not improve performance of calves that were fed a limited amount of milk replacer. Chromium supplementation from $CrCl_3$ or a Cr-nicotinic acid complex did increase the sensitivity of calves to an insulin challenge.

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REFERENCES

1 Anderson, R. A. 1992. Chromium, glucose tolerance, and diabetes. Biol. Trace Element Res. 32:19.
2 Anderson, R. A., M. Polansky, N. A. Bryden, and J. J. Canary. 1991. Supplemental chromium effects on glucose, insulin, glucagon, and urinary chromium losses in subjects consuming controlled-low chromium diets. Am. J. Clin. Nutr. 54:909.
3 Bunting, L. D., J. M. Fernandez, D. L. Thompson, Jr., and L. L. Southern. 1994. Influence of chromium picolinate on glucose usage and metabolic criteria in growing Holstein calves. J. Anim. Sci. 72:1591.
4 Fahey, G. C., and L. L. Berger. 1988. Carbohydrate nutrition of ruminants. Page 269 *in* The Ruminant Animal. Digestive Physiology and Nutrition. D. C. Church, ed. Prentice Hall, Englewood Cliffs, NJ.
5 Hostettler-Allen, R. L., L. Tappy, and J. W. Blum. 1994. Insulin resistance, hyperglycemia, and glucosuria in intensively milk-fed calves. J. Anim. Sci. 72:160.
6 Kaneko, J. J. 1989. Clinical Biochemistry of Domestic Animals. 4th ed. Acad. Press, New York, NY.
7 Kegley, E. B., and J. W. Spears. 1995. Immune response, glucose metabolism, and performance of stressed feeder calves fed inorganic or organic chromium. J. Anim. Sci. 73:2721.
8 Mertz, W. 1969. Chromium occurrence and function in biological systems. Physiol. Rev. 49:163.
9 Moonsie-Shageer, S., and D. N. Mowat. 1993. Effect of level of supplemental chromium on performance, serum constituents and immune status of stressed feeder calves. J. Anim. Sci. 71: 232.
10 Mowat, D. N., X. Chang, and W. Z. Yang. 1993. Chelated chromium for stressed feeder calves. Can. J. Anim. Sci. 73:49.
11 Samsell, L. J., and J. W. Spears. 1989. Chromium supplementation effects on blood constituents in lambs fed high or low fiber diets. Nutr. Res. 9:889.
12 SAS/STAT® User's Guide, Release 6.03. 1988. SAS Inst., Inc., Cary, NC.
13 Schwarz, K., and W. Mertz. 1959. Chromium (III) and the

glucose tolerance factor. Arch. Biochem. Biophys. 85:292. (Letters to the Editors.).
14 Sigma Chemical Company. 1988. Cholesterol. Quantitative, enzymatic determination of total cholesterol in serum or plasma at 500 nm. Procedure No. 352. Sigma Chemical Co., St. Louis, MO.
15 Sigma Chemical Company. 1990. Blood urea nitrogen. Quantitative, colorimetric determination of blood urea mitogen in serum or plasma at 515-540 nm. Procedure No. 535. Sigma Chemical Co., St. Louis, MO.
16 Toepfer, E. W., W. Mertz, M. M. Polansky, E. E. Roginski, and W. R. Wolf. 1977. Preparation of chromium-containing material of glucose tolerance factor activity from brewer's yeast extracts and by synthesis. J. Agric. Food Chem. 25:162.
17 Vernon, R. G., and S. Sasaki. 1991. Control of responsiveness of tissues to hormones. Page 155 *in* Physiological Aspects of Digestion and Metabolism in Ruminants: Proceedings of the Seventh International Symposium on Ruminant Physiology. T. Tsuda, Y. Sasaki, and R. Kawashimo, ed. Acad. Press, Orlando, FL.
18 Vinson, J. A., and K.-H. Hsiao. 1985. Comparative effect of various forms of chromium on serum glucose: an assay for biologically active chromium. Nutr. Rep. Int. 32:1.
19 Wang, M. M., E. A. Fox, B. J. Stoecker, C. E. Menendez, and S. B. Chan. 1989. Serum cholesterol of adults supplemented with brewer's yeast or chromium chloride. Nutr. Res. 9:989.