

ROLE OF CHROMIUM IN DAIRY CATTLE NUTRITION

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Chromium (Cr) is an essential trace element for humans (Anderson, 1992) and laboratory animals (NRC, 1997). It is the trivalent form of the molecule that is active biologically, the hexavalent form is actually toxic to many animal cells (NRC, 1997). Chromium acts through a protein, once named glucose tolerance factor (GTF), now more commonly referred to as the glucose transporter. This protein interacts with the insulin receptor complex in facilitating the action of insulin. Although Cr is a required mineral, there had been scant evidence in past decades that supplemental Cr could benefit agricultural animals. Available data in humans using Cr as the picolinate salt do not support the view that additional Cr has benefits in energy metabolism, although many people claim such effects. About 15 years ago, more consistent reports showed that supplemental Cr, primarily in more soluble forms, benefits young pigs, and Cr-propionate (KemTRACE® brand Cr-propionate, Kemin AgriFoods North America, Des Moines, IA) was approved for all phases of swine production in 2000. There are also now sufficient data in the literature that suggest that more soluble forms of Cr such as Cr-methionine or Cr-propionate may have benefits in lactating cattle.

Mode of Action of Cr in Glucose Metabolism

Insulin is necessary for glucose uptake and anabolic reactions in several insulin-sensitive organs, the primary ones being muscle and adipose tissue. In most animals and in most physiological states, these two organs remove most of the glucose from the blood following a meal. In lactating dairy cattle, although the vast majority of glucose is taken up by the mammary gland, the uptake of glucose by other tissues is still important in regulation of metabolism (the brain still requires glucose, adipose tissue requires some glucose for energy and triglyceride synthesis, working muscle still requires some glucose). In turn, the alteration in glucose use by these non-mammary tissues can have feedback effects

on various endocrine regulatory systems. In this way, chromium may have a role in overall glucose use and metabolic regulation during lactation.

Chromium binds with low molecular weight Cr binding protein (recently suggested to be named chromodulin) (Davis and Vincent, 1997; Vincent, 2004). This molecule interacts with the insulin receptor to elicit the anabolic effects of insulin, seemingly through more efficient recruitment of glucose transporters. Insulin increases glucose transport, and thus increases pathway activity for glucose oxidation or conversion to lipid, usually very rapidly (within seconds or minutes). These effects are directly related to increased substrate and to allosteric control of enzyme activity, usually through phosphorylation/dephosphorylation mechanisms. Continued increased insulin binding increases protein synthesis and expression of genes involved in anabolic pathways such as lipogenesis and protein synthesis. Simultaneously, proteolysis and lipolysis would be reduced. Such effects would be consistent with faster rates of growth in agricultural animals.

Although the liver is not an insulin sensitive tissue for glucose uptake, the liver does respond to other anabolic actions of insulin. In this way, Cr may have a role in increasing anabolism in liver. In addition, at least until recently, the mammary gland of the cow was thought to be insulin insensitive, a direct action of Cr on the mammary gland was not thought to be a potential mode of action. With the suggestion that in some situations, the mammary gland may be insulin sensitive (Griinari et al., 1997), this thinking may need to be revisited. The serious student may wish to begin with the NRC summary of 1997 on the role of Cr in nutrition, followed by recent work of Vincent and coworkers in the reading list.

Actions of Cr in Nutrition of Young Pigs

In the NRC summary of 1997 on studies with pigs, it was stated that: “Responses of growing-finishing swine to supplemental dietary Cr have been inconsistent.” In addition, response seemed to vary with Cr status, bioavailable Cr in the diet, and exposure to various environmental stresses. Yet even at that time, there was evidence that supplemental Cr may increase insulin efficiency. At that time, it was recognized that different forms of the molecule had different effects. Basically, more absorbable salts, such as Cr-propionate seemed to be more effective than CrCl₃ or Cr-picolinate, the favorite of human body builders.

A rather large body of work from the late 1980's into the middle 1990's does support the idea that Cr, in various salts, can improve glucose use at doses in the range of 200 to 500 µg/kg of diet, or about 1 mg per day (NRC, 1997; Van de Ligt, 2002). Results on productive traits such as carcass characteristics were not forthcoming. Yet sufficient evidence existed that supplemental Cr in many situations improved growth rate and feed efficiency. One product, Cr-propionate (KemTRACE® brand Cr-propionate, Kemin AgriFoods North America, Des Moines, IA) has been approved for use in growing pigs in both the US and Canada.

Actions of Cr in Metabolism of Dairy Cattle

Much of the early work on Cr centered around the potential effectiveness to improve glucose use in young calves, and thus improve their response to environmental stressors and immune stimulators. Results in general did show effectiveness of Cr on glucose metabolism, but consistent improvements in performance remained elusive. To date, Cr is not approved for use in ruminant calves. A complete summary is available (NRC, 1997).

The period of late gestation and early lactation is one of metabolic stress for dairy animals, however, improvements in ration formulation and delivery have decreased problems and increased production. Yet it remains a challenge to increase intake in amounts sufficient to reduce deficits of amino acids and energy in several animals. We still do not fully understand why some animals can eat more or partition more nutrients toward the mammary gland. We do know that these are not single gene effects, and it is likely that metabolic regulation in all organs, not just the mammary gland, is important in development of the most efficient dairy animals. Certainly glucose use is one such aspect.

Glucose flux in the animal during this time affects many organs, not only the mammary gland, but viscera, adipose, muscle and central nervous system. A glucose lack (as well as a fatty acid deficit) increases adipose tissue lipolysis, this can lead to fat accumulation in the liver, perhaps further diminishing gluconeogenic capacity (Drackley, 1999; Overton and Waldron, 2004). Excessive lipolysis also has detrimental effects on feed intake, causing lower than expected milk production and leading to other metabolic diseases.

Supplying additional sources of absorbed VFA and/or glucose by dietary or other experimental means usually improves overall performance. The supply of gluconeogenic precursors, such as calcium salts of propionic acid, has also

improved performance in some situations (Hayirli et al., 2000; Hayirli et al., 2002; Overton and Waldron, 2004). In addition, lack of knowledge on control of glucose use and interaction among critical organs hampers development of more precise nutritional models (McNamara, 2004). If Cr has a role in the metabolism of glucose in peripheral tissues, it may have a beneficial role in nutrition of dairy cattle.

In recent years supplemental Cr in dairy diets (as picolinate or methionine salts) has provided mixed results. Supplemental Cr has increased milk production in primiparous but not in multiparous cows (Yang et al., 1996). Besong et al. (1996) observed increased milk yield in the first 60 days of lactation in cows supplemented with Cr picolinate. Popovic et al. (2000) fed two groups of six first lactation animals 4 mg/d of an organic Cr, observing a higher average daily milk yield and higher values for milk fat, protein and lactose. Jackson et al. (1994) reported no positive effect on feed intake or milk yield during the first eight weeks into the lactation when supplemented with Cr picolinate. Hayirli et al. (2000) reported altered production responses with Cr methionine supplementation; milk increased in a quadratic manner being the highest with the 0.06 mg/kg BW^{0.75}, then dropping below basal levels at the 0.12 mg Cr-Met/kg BW^{0.75}. Under grazing conditions when Cr-picolinate was bolused daily, no differences in milk production and composition was observed (Pettersen, 2000). Recently, Smith et al. (2005) found that Cr methionine at 0.03 or 0.06 mg Cr/kg BW^{0.75} increased DMI and milk yield in early lactation.

It is not yet clear how supplemental Cr may increase milk yield in early lactation, but evidence from other species provides some quite plausible hypotheses. Chromium would increase glucose uptake into the two major glucose using tissues, muscle and adipose. Even though the ruminant does not use significant amounts of glucose for fatty acid synthesis, glucose is still required to supply glycerol for esterification as well as for ATP generation. A reduction in the rate of mobilization of fatty acids from adipose tissue may help stabilize hepatic fat metabolism, reduce hepatic ketogenesis, and perhaps allow feed intake to increase more rapidly after calving. An increase in insulin efficiency in the muscle may decrease excess ketosis from ketogenic amino acids. These actions would all increase metabolic efficiency and likely increase milk production.

As a potential effector of anabolism in adipose tissue, Cr may potentially have positive effects on lactation, although at first glance this seems opposite to likely actions. Let us surmise that additional Cr increases anabolic activity in adipose tissue, either as a slight increase in lipogenesis, a decrease in lipolysis or both. As long as this effect is not so large as to limit the supply of fatty acids to

the mammary gland, an overall beneficial effect to the cow may result. Fatty acids circulating in the blood have detrimental effects on feed intake in many species. The 'fat cow syndrome' of the 1970's and 80's resulted in many studies that demonstrated that if a cow lost excess body fat in early lactation, dry matter intake was reduced compared to thinner cows and a reduction in milk production occurred perforce. If Cr is acting through glucose transporters in the adipose and/or muscle tissues, this speculation becomes a viable hypothesis.

There are some indications from work in dairy cattle that this hypothesis has merit. In the study of Besong et al. (1996), increased milk yield with Cr supplementation was accompanied by increased feed intake, however feed intake was not affected by supplemental Cr in the study of Yang et al. (1996). Hayirli et al. (2000) observed an increase in DMI (as a % BW) as lactation progressed, and the recent study of Smith et al. (2005) also demonstrated an increase in DMI. It is likely that gluconeogenic precursors (such as calcium propionate) and Cr are acting via different mechanism to elicit the same effect.

Yang et al. (1996) also postulated that increased milk yield might be the result of the indirect effects of Cr on hepatic glucose production (gluconeogenesis). Conversion of propionate to glucose has increased during i.v. propionate infusion tests in early lactation heifers (Subiyatno et al., 1996) fed supplemental Cr. Bunting et al. (2000) also observed proportional increases in glucose production following propionate infusion in animals given supplemental Cr. Moreover, gluconeogenesis is a metabolic event that is essentially opposed to increased insulin sensitivity. As suggested by Yang et al. (1996), Cr may promote the activity of IGF receptors, which have structural and functional homology to the insulin receptor. Such a direct effect on mammary synthetic capacity is more consistent with observed milk production responses. Also, trends for increased circulating IGF-I with Cr supplementation were observed in the propionate loading tests of Subiyatno et al. (1996).

Effect of Cr-Propionate in Transition Cows

In order to further test some of these hypotheses and help to confirm whether or not supplemental Cr can benefit lactating dairy cattle, research was done at Washington State University. Objectives included investigation of the effect on adipose tissue lipogenesis and lipolysis, as well as production effects, of feeding Cr propionate to supply 10 mg Cr/d and calcium propionate to supply 125 g of propionate/d to high producing dairy cattle in late pregnancy and early lactation.

Experimental Design, Animals and Diets. Forty-eight Holstein dairy cattle were blocked by parity (2nd or 3rd and greater); sorted by previous milk production, and allotted within parity and previous production to one of four treatments (12 animals each). Previous production averaged $10,623 \text{ kg} \pm 223$ (SEM) milk per 305 d at 3.62 % fat and 3.21 % true protein, with no difference among treatment allotments. Animals entered the experimental lot (freestall) 28 d prepartum and were adapted to Calan gates. Treatments began at 21 d prepartum and dry period diets were: control diet (C), [24 % alfalfa silage, 60 % grass hay and 16 % concentrate (Table 1)]; control plus 0.125 kg/d of calcium propionate (CaP; NutroCalTM; Kemin Agri Foods North America, Inc., Des Moines, IA); control plus Cr-propionate (CrP; KemTRACE ®brand Cr-propionate Cr) to supply 10 mg/d Cr; and both CaP and CrP at the same doses (Both). Treatments were mixed into the grain portion, which was included in the TMR as a pellet. Lactation diets were alfalfa and alfalfa silage based and formulated to meet NRC (2001) standards for dry cows and lactating cows and fed as a TMR (Table 1). After calving, animals were switched to this lactating ration and treatments were continued until 35 DIM at which time all animals continued on the control diet.

Adipose tissue biopsies and metabolic flux measures. Fat biopsies were removed aseptically, from a sub-set of 6 cows per treatment, from the tailhead region (3 to 4 cm lateral to coccygeal vertebrae in the depression between the ischium and vertebrae) at approximately days -7, 14, 28 and 56 around parturition as described previously (Smith and McNamara, 1990). Rates of acetate incorporation into fatty acids and rates of fatty acid release in the basal state, or stimulated with norepinephrine (10^{-5} M), adenosine deaminase (6.6 U/ml) and theophylline (1 mM) were determined as described previously (McNamara and Hillers, 1986a and b; McNamara et al., 1995).

Statistical Analysis. The experiment was analyzed as a completely random design of 4 treatments with repeated measures. Body weight, BCS, glucose, NEFA (non-esterified fatty acids) and measures of lipogenesis and lipolysis were analyzed with the following model:

$Y_{ijkl} = u + T_i + \text{Cow}(T)_j$ [main plot error] + $\text{Day}_k + T \times \text{Day}_{ikj} + \text{error}_{ijkl}$ [subplot error]. Statistical significance between treatment means was determined using the PROC MIXED protocol (SAS Users Guide, 1999).

Milk production and DMI were analyzed with a model as above, with Day being a continuous variable for day prior to and after calving. We also summarized feed intake and milk production for the 5 periods for analysis:

prepartum, 1 to 35 DIM, 36 to 56 DIM, 57 to 90 DIM and 1-90 DIM were analyzed again with this model: $Y_{ij} = u + T_i + \text{error}_{ij}$; with T_i as the treatment effect and error: cow within treatment. Contrast comparisons were also conducted for control versus each treatment (separately) and the CaP and CrP treatments versus the treatment with both. Statistical significance between treatment means for these variables was determined using the PROC MIXED protocol (SAS Users Guide, 1999).

Results. Supplementing cows with CaP tended to increase DMI over the entire period. Before calving, this was a trend ($P = 0.13$; Table 2). Dry matter intake increased after calving ($P < 0.05$) in CaP treated cows (Table 2). Mean dry matter intake of the CrP treatment group was 0.8 kg/d greater before calving than the C group, this was not significant (Table 2). Chromium propionate increased feed intake compared to Control after calving by 3.1 kg/d ($P < 0.005$; Figure 2, Table 2). There was a DIM by treatment interaction for milk yield ($P < 0.05$). The summary (mean) analysis showed that milk yield was 2.6 kg/d greater due to CrP from d 1 to d 90 (P between 0.10 and 0.13 for various periods (Table 3). For days 57 to 90 this was 4.6 kg/d greater. The apparent lag in response in milk yield compared to feed intake suggests that the feed intake effect came first.

Milk fat percentage and yield were lower on the CaP and CrP treatments by 0.16 and 0.27 percentage units and 130 and 70 g/d compared to controls, statistically these effects were trends (CrP, $P = 0.06$; CaP, $P = 0.11$; Table 4). Body weights and conditions behaved as expected for transition cows, and there were no effects of treatment on animal BW or BCS. The same was true for serum glucose and NEFA (no effects), although the animals on both treatments had a slightly (numerically) higher NEFA, consistent with the lower feed intake.

Addition of either CaP or CrP did not increase rates of lipogenesis prepartum, when the cows were in positive energy balance and rates were already high (Figure 1). After calving, all cows had a large and expected (McNamara and Hillers, 1986a; McNamara, 2004) drop in lipogenesis. However, rates in CaP and CrP treated cows were much greater than in control cows at 14, 28 and 56 d postpartum. At 56 DIM, when control cows had not yet begun their normal rebound in adipose tissue lipogenesis, cows treated with CaP or CrP were eating more, producing the same or more milk and showing increased adipose tissue lipogenesis (Figure 1).

The effects on adipose tissue lipolysis rates were more variable but consistent with the feed intake, milk yield and adipose lipogenesis data (Figure 2). We measured both basal rates and rates stimulated with norepinephrine. While it

is always a question as to the exact relation between in vitro and in vivo rates, the body of knowledge for dairy cattle (McNamara 2003; 2004) and analysis with mechanistic models compared to energy and fat input/output data (McNamara and Baldwin, 2000; McNamara, 2003; 2004) suggest that the rates in vivo are much closer to the rates measured under in vitro stimulation than basal. This is also in keeping with the known chronic stimulation of lipolysis from the sympathetic nervous system in vitro (McNamara and Murray, 2001). In any case the effects of treatment on basal and stimulated rates were similar in nature.

Prepartum there were decreases in basal and stimulated rates at 14 DIM (Figures 2). This has not been observed before, previous work in our lab and others have shown the opposite (McNamara, 2004). These cattle were eating more food overall than cattle in our previous trials, thus they may not have had to draw as much on adipose lipid. There was no difference among treatments at 14 DIM, while at 28 DIM, CaP and CrP treatments had only 60 to 70 % as fast lipolysis as controls. At 56 DIM, basal rates increased in CaP treated cows, stimulated rates of lipolysis increased in both treatment groups, this may be due to the greater milk production. Coupled with the large increase in lipogenesis in these animals, it is clear that they were able to consume more food, make the same amount of milk and begin to restore adipose lipid simultaneously. For CrP treated cows, the rates of lipolysis were close to the same as treated cows, while lipogenesis and milk production were greater. Thus, these animals were also able to eat more, make more milk and replenish adipose lipid faster than control cows. None of the means for lipolysis were statistically different, not surprising given the large normal variation in this measure, yet the results are certainly consistent within this set of data and in relation to previous known effects of feed intake and milk production (McNamara, 2004).

Effects on milk yield and intake were similar to those measured for Cr-methionine by Hayrli et al. (2000) and Smith et al (2005; published after the present trial was finished). While these data cannot prove direct effects of CaP and CrP on glucose entry or on activity of glucose transporters in adipose tissue, the effects are certainly consistent with that theory. An increased lipogenesis with a similar or decreased lipolysis would mean a net reduction in free fatty acid release to the blood. This would be consistent with allowing a greater increase in feed intake, and therefore milk production (Drackley, 1999; Overton and Waldron, 2004).

The increased supply of glucose from calcium propionate has been demonstrated and may increase glucose supply directly to the mammary gland, but this would not be consistent with the effects in this trial (increase in feed

intake but not in milk). It also cannot be completely ruled out that Cr-propionate might be having direct effects on the mammary gland. Yang et al. (1996) postulated that increased milk yield might be the result of the indirect effects of Cr on hepatic glucose production (gluconeogenesis). Conversion of propionate to glucose has increased during intravenous propionate infusion tests in early lactation heifers (Subiyatno et al., 1996) fed supplemental Cr. Bunting et al. (2000) also observed proportional increases in glucose production following propionate infusion in animals given supplemental Cr. Yang et al. (1996) suggested that Cr might promote the activity of IGF receptors, which have structural and functional homology to the insulin receptor. In addition, if Cr increases glucose transport into the mammary gland, this would be consistent with the presently observed increase in milk production. Trends for increased circulating IGF-I with Cr supplementation were observed in the propionate loading tests of Subiyatno et al. (1996). In work done in growing dairy heifers, we have demonstrated a dramatic improvement in glucose clearance rates and increased insulin sensitivity to graded doses of Cr-propionate (5, 10, 15 mg/d for two weeks) (Sumner et al, 2005), suggesting that indeed supplemental Cr does increase glucose entry into adipose and muscle cells.

In this study, there appeared to be a lag of milk yield response to CrP, such that the feed intake effect was obvious even prepartum, but the milk yield effect increased over time (0.8 kg/d for the first 35 d, increasing to 4.6 kg/d for d 57 to 90). This suggests that CrP may actually be decreasing net lipolysis, allowing an increased feed intake, reducing the use of body reserves in very early lactation, and then with the continued increase in intake, allowing more substrates for the mammary gland. Nothing in this study argues against that interpretation, of course, as yet the exact molecular mechanisms of Cr-propionate to increase intake and milk production, hasten the recovery of body fat stores, and potential direct effects on the mammary gland remain to be confirmed. Yet the data collected in this study are consistent with present understanding of metabolism in the cow and the function of Cr in glucose transport.

Implications

Chromium, in the trivalent state, is a required mineral for animal life, functioning in glucose entry to insulin-sensitive cells. Although it has been touted as an effective supplement for body-builders and athletes, no effect has been demonstrated with supplementation of human diets. Chromium has been shown to be effective in increasing glucose use and growth performance, primarily in young pigs. The propionic acid salt of Cr is approved for use in pigs. If Cr

increases effectiveness of insulin action in lactating ruminants, there may be an overall increase in metabolic efficiency, leading to improved production. Results indicate support for the concept that supplemental Cr at approximately 6 to 10 mg/day can reduce adipose lipolysis, and increase nutrient intake and milk production.

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Table 1. Dietary Ingredients and composition of feeds fed to dairy cattle in late pregnancy and early lactation

Ingredients	Dry	Lactation
	% DM	
Alfalfa haylage	24	25.6
Alfalfa hay	...	23.9
Grass Hay	60	...
Whole cotton seed	...	10.4
Wheat mill run	...	7.1
Concentrate	16	33
Corn grain, ground	...	10.6
Barley	13	5.7
Wheat	...	6.3
Peas	...	5
Soybean meal	2.0	1.5
Corn Gluten Meal	...	1.5
Molasses, cane	0.6	0.67
Limestone	...	0.5
Salt, TM w/Selenium	...	0.5
BiCarb	...	0.67
Vitamin, mineral, binder	0.4	0.24
(with MgOx)		
<u>Chemical Composition</u>		
DM, %	64	75
CP, % of DM	11	18
RUP, % of CP	34	25
NDF, % of DM	49	32
ADF, % of DM	36	25
Ash, % of DM	12	10
NE _i , Mcal/kg DM	1.34	1.69

Table 2. Dry matter intakes of cows treated with calcium propionate, Cr-propionate or both

Item	Treatment					Contrast ¹			
	<u>Control</u>	<u>CaP</u>	<u>CrP</u>	<u>Both</u>	<u>SE</u>	<u>C vs CaP</u>	<u>vs CrP</u>	<u>vs CaP and CrP</u>	
DMI, kg/d									
from									
21 to 1 day prepartum	10.6	11.8	11.4	10.3	0.45	0.12	NS	0.14	
1 to 35 DIM	17.0	18.8	18.7	15.1	0.82	NS	NS	0.10	
36 to 56 DIM	21.8	24.7	25.9	21.0	0.90	0.05	0.01	0.006	
57 to 90 DIM	22.2	25.6	26.6	23.2	0.88	0.03	0.003	0.002	
1 to 90 DIM	20.0	22.7	23.1	19.5	0.70	0.03	0.005	0.003	
n	10	9	10	12					

¹Contrasts were Control vs. Calcium Propionate; Control vs. Cr-propionate; Control vs. (CaP and CrP) treatments (average CaP and CrP) and Control vs. Both.

Table 3. Milk production from cows treated with calcium propionate, Cr-propionate or both

Item	Treatment				
	<u>Control</u>	<u>CaP</u>	<u>CrP</u>	<u>Both</u>	<u>SE</u>
Milk, kg/d, from:					
d 1 to 35	40.8	40.7	41.6	37.2	0.91
d 36 to 56	47.4	44.7	49.9	44.5	0.99
d 57 to 90	45.4	45.8	50.0	47.2	1.03
d 1 to 90	44.2	43.7	46.8	42.6	0.87
n	10	9	11	12	

Cows on either CaP or CrP: $P = 0.12$ vs. control for 1st 35 d; 0.14 for first 56 d.
Cows on CrP, $P = 0.13$ vs. control for d 57 to 90.

Table 4. Milk composition of cows treated with calcium propionate, Cr-propionate, or both

<u>Item</u>	<u>Treatment</u>		
Milk fat, %			
DIM	<u>Control</u>	<u>Ca Prop</u>	<u>Cr Prop</u>
14	4.59	4.00	3.72
28	3.83	3.25	3.18
42	3.34	3.46	3.37
56	3.14	2.84	3.16
70	3.45	3.25	3.35
84	3.27	3.44	2.62
Overall	3.56	3.32	3.25
SE milkfat %: 0.07			
Milkfat, kg/d	1.59	1.46	1.52

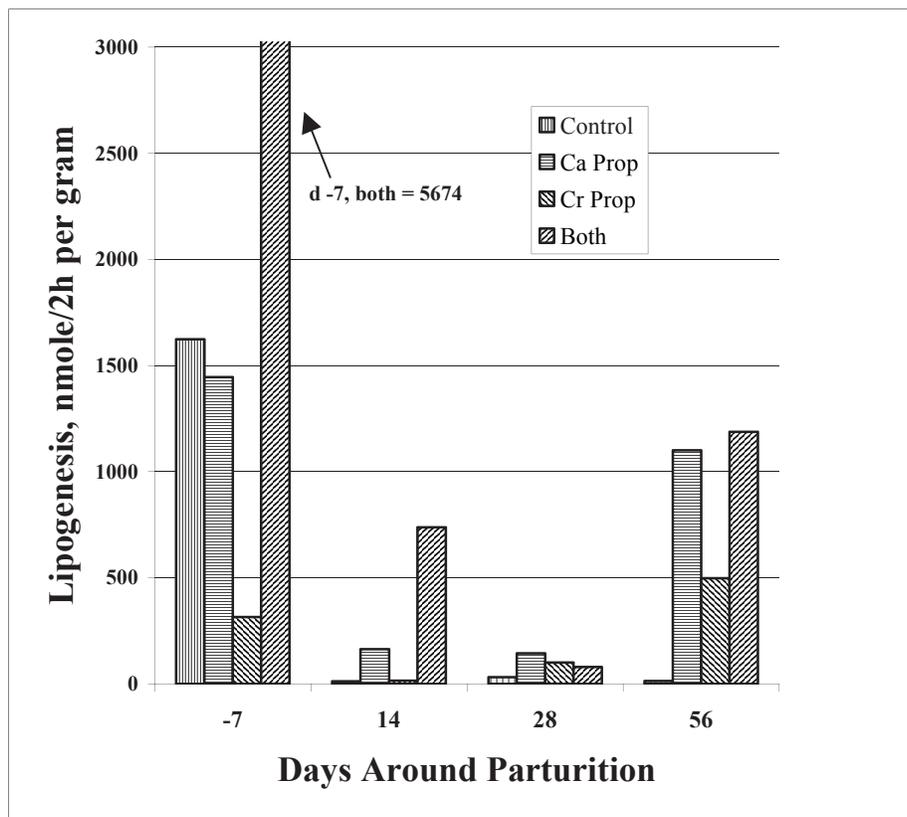


Figure 1. Rates of lipogenesis in adipose tissue in vitro from cattle fed calcium propionate or Cr-propionate from 21 days prepartum to 35 d postpartum. There was a treatment effect overall postpartum ($P < 0.001$) vs. control; there was a CrP effect by DIM interaction ($P = 0.053$).

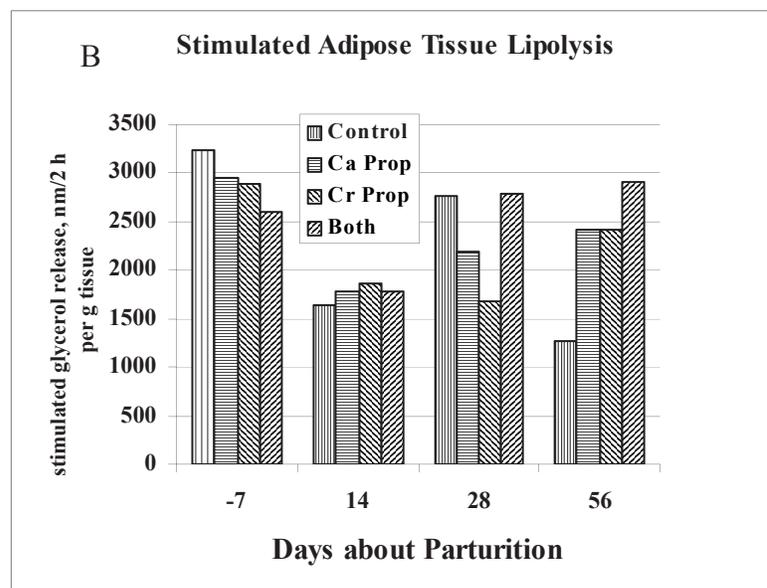
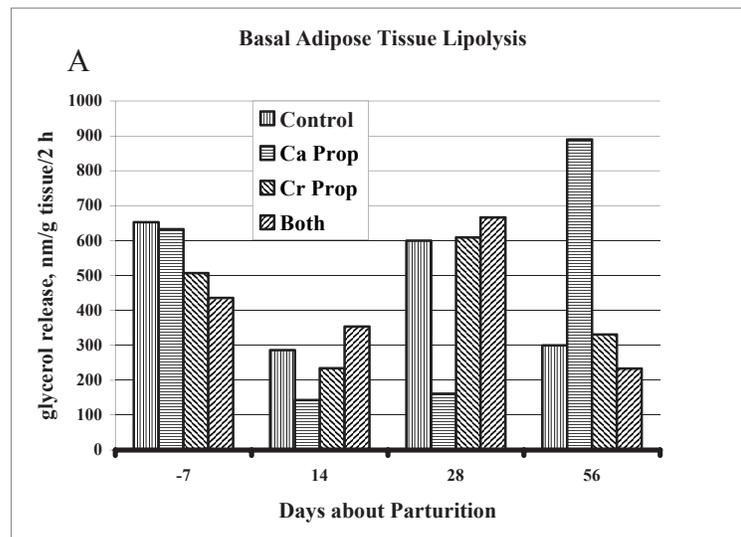


Figure 2. (A) Rates of lipolysis in adipose tissue in vitro from dairy cattle fed calcium propionate or Cr-propionate from 21 days prepartum to 35 d postpartum. For treatment overall, $P = 0.0325$; DIM, $P = 0.08$. (B) Rates of adipose tissue lipolysis stimulated with norepinephrine. Effect of norepinephrine stimulation, $P < 0.002$.