# Full length Research paper

# Effect of supplemental chromium on performance and blood serum biochemistry of dairy cows

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This study was conducted to evaluate the effect of supplemental chromium on performance and blood serum biochemistry of dairy cows. Thus, 20 multiparous Holstein cows (parity 3) were equally divided into two groups, group one (control), which received no chromium supplementation and group two (treatment) which received 5 g/day chromium methionine from week 5 prior to parturition until 12 weeks thereafter. Milk production and milk composition were evaluated on 4, 8 and 12 weeks after parturition. Serum biochemistry concentrations (serum glucose, cholesterol, triglyceride, total protein, and cortisol and insulin concentration) and blood hematology (red blood cell, hematocrit, hemoglobin concentration and percentage neutrophils, lymphocytes, monocytes, basophiles, eosinophils and ratio of neutrophils to lymphocytes) were measured on 2 and 5 weeks prior to parturition and 1 and 4 weeks thereafter. Results indicate that milk production was significantly affected by chromium-methionine supplementation during the entire period (P<0.01) but no significant effect on milk composition was found. Supplemental chromium had no significant effect on serum glucose, cholesterol, triglycerides and insulin concentration and blood hematology parameters (P<0.05). However, chromium supplementation tended to increase significantly, serum total protein concentration and decrease cortisol concentration (P<0.05). The results of this experiment showed that chromium methionine supplementation in multiparous dairy cows diet may improve their milk yield in transition period.

Key words: Dairy cow, transition period, chromium-methionine, milk yield, serum biochemistry.

#### INTRODUCTION

Chromium is a transitional element with an atomic number "24" and an atomic weight of 51.996. Chromium was first shown to be essential in swine by Schwartz and Mertz (1959) when they isolated "glucose tolerance factor" (GTF) from swine kidney. Furthermore, this element has been reported to play essential roles in activity of certain enzymes, metabolism of protein and nucleic acids, as well as impact on immune functions (Beitz and Horst, 1997). However, only its function as related to glucose metabolism is sufficiently understood. Chromium also aids in the conversion of thyroxin to triodothyronine, increasing the metabolic rate (Burton, 1995).

Potential benefits of supplementing chromium to livestock have been shown to improve performance in growing and finishing swine and ruminants (Chang and Mowat, 1992; Kegley et al., 1997a, b; Moonsie-Shageer and Mowat, 1993). In studies conducted in dairy cows, chromium supplementation has been shown to increase dry matter intake (Besong et al., 1996; Hayirli et al., 2001; Smith et al., 2002), increase milk yields (Besong et al., 1996; Hayirli et al., 2001; Smith et al., 2002), reduce blood nonesterified fatty acid (NEFA) concentration (Bryan et al., 2004; Depew et al., 1998; Hayirli et al., 2001; Yang et al., 1996), improve fertility (Yang et al., 1996; Bryan et al., 2004; Pechova et al., 2003) and decrease placental retention, and udder edema in older cows (Hayirli et al., 2001; Yang et al., 1996; Villalobos et al., 1997; Stahlhut, 2004; Bryan et al., 2004; Burton et al., 1993; Besong et al., 1996).

Since there is no adequate measure of chromium

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status, establishing dietary requirements for livestock and humans is difficult. While the recommended intake for chromium is 50 to 200 µg per day (National Research Council (NRC), 1989) in humans; currently, there is no established chromium requirement for ruminants. Improvement in impaired glucose tolerance after chromium supplementation is the most sufficient means to determine deficiency.

While the recommended intake for chromium is 50 to 200  $\mu g$  per day (National Research Council, 1989) in human; currently, there is no established chromium requirement for ruminant. Improvement in impaired glucose tolerance after chromium supplementation is the most sufficient means to determine deficiency. The aim of this study was to assess the effect of supplemental chromium for dairy cows from 5 weeks preparturient through 12 weeks postpartum on performance, and blood serum biochemistry.

#### **MATERIALS AND METHODS**

#### Animals and dietary treatments

20 multiparous Holsteins (parity 3) housed in free stalls at the Esfahan-Kesht farm (near the Esfahan - Iran), Iran, were randomly allocated across two treatment group: ten cows were supplemented once a day with 5 g/day Cr-Met via ball dough corn after the a.m. milking, whereas the other 10 cows received no chromium (Cr) supplementation. Cows received dietary treatments from 5 weeks prepartum (wk-5) through 12 weeks postpartum once a day (wk+12). From wk-5 to wk+12, a total mixed diet (NRC, 2001, Table 1) was offered to all cows (ad libitum intake). Random samples were taken of these TMR diets which were sent to laboratory for analysis. Also, the amounts of chromium of diets were determined by atomic absorption system (Table 1).

#### **Production data**

All cows were milked four times per day. At three time points, at 4 week intervals, milk yield was determined and milk samples were collected in a plastic bottle containing 2-brom-2- nitro-1, 3-propandiol (a preservative) for the determination of milk composition. Milk samples were analyzed for fat, protein, lactose, SNF (solid nonfat) and TS (total solid) by an automated near-infrared spectroscopy analyzer (Milko- Scan, 134 BN, Foss Electric, Hiller ´od, Denmark). Fat corrected milk 4% (FCM 4% = [0.4 x kg milk] + [15 x kg fat]).

## **Blood sampling**

Blood samples were also collected from all the cows, at 5 and 2 weeks before expected calving and 1 and 4 weeks post calving. Blood samples were obtained by 20 ml vein puncture of the jugular vein during the a.m. milking. After blood was collected, 2 ml of each sample was poured into tubes containing ethylene diamine tetraacetic acid for determining blood hematology and then the remaining sample was centrifuged at  $3500 \times g$  rpm for 10 min and the serum stored at  $-20^{\circ}\text{C}$  until further analysis (within 24 h). Samples were analyzed for serum glucose, total cholesterol, triglyceride, total protein, and insulin and cortisol concentrations.

#### Biochemical and hormonal assay

Glucose, total cholesterol, triglyceride, and total protein, of serum were determined automatically (Technicon RA1000 System; Technicon Instruments Corp., Tarrytown, NY) at 37°C.

Concentrations of insulin and cortisol were analyzed by radio immune-assay (RIA) kits obtained from Diagnostic System Laboratories (DSL), Corporate USA. A gamma counter (Multicrystal Gamma Counter Perthold LP 2103, Germany) was used. Insulin and cortisol were determined according to Gerich (Gerich, 1988).

Blood hematology parameters such as: Red blood cell, hemoglobin and hematocrit were evaluated by sysmex system (Sysmex K1000, TOA Ltd., Tokyo, Japan). Also, smir was made of blood samples by gimsa, staining coloring, lymphocyte, basophile and eosonophide were determined.

#### Statistical analyses

Milk production and composition and serum biochemistry parameters were analyzed by using general linear model procedures of SPSS16 in the computer for a completely randomized design with two treatments and ten repeated using the following model. Duncan multiple range test was used to test significant differences in treatments:

$$Yijk = \mu + Aj + Bk + ABjk + Eijk$$

Where, Yijk is the depended variable;  $\mu$  is the overall mean; Aj is the treatment effect; Bk is the period effect; A × Bjk is the interaction effect of treatment and period and Eijk is the residual error.

#### **RESULTS AND DISCUSSION**

### Milk yield and composition

In this study, Cr supplementation diet increased milk yield, (Table 2) which agreed to the results of Besong et al. (1996), Hayirli et al. (2001), Smith et al. (2005) and Yang et al. (1996). However, Bryan et al. (2004) and Pechova et al. (2002a, 2003) had reported Cr supplementation has no effect on milk yield. At the most, studies which increase milk production has been reported, increase at dry matter intake is also seen (Besong et al., 1996; Hayirli et al., 2001; Smith et al., 2005).

In studies where increases in milk production have been observed, increases in postpartum DMI have also been observed (Hayirli et al., 2001; Smith, 2004). It would seem logical that DMI would increase when milk production increased, especially considering body tissue mobilization, as indicated by a reduction in blood NEFA, is reduced with Cr supplementation. Reduced body tissue mobilization would provide less energy to sustain milk production (NRC, 2001).

In our study, although possibility of measuring the rate of dry matter intake was not possible, may be because Cr supplementation increased dry matter intake. Consequently, it caused increase in milk production.

As it is shown in Table 2, milk composition has not been affected by Cr supplementation. The composition of

**Table 1.** Dry matter composition of basal diets fed to non lactation or lactation cows.

Diet content	Dry off (%)	Close up (%)	Lactating (%)	
Corn silage	50 48		44.6	
Alfalfa hay	19.3	22.8	14.5	
Wheat straw	15	13.5	-	
Concentrate	15.7	16.7	28.9	
Concentrate composition (%)				
Corn grain	-	15	26	
Barely grain	16	18	23	
Cotton seed	-	8	11	
Wheat bran	54	10.4	-	
Cotton seed meal	6	-	5	
Rapeseed meal	13	10	5.5	
Soya been meal	8.4	15	20	
Fish meal	-	4	1.5	
Corn gluten meal	-	4	2	
Fat meal	-	4	2.5	
Vitamin and mineral premix	1	1	1.2	
Urea	0.3	-	-	
FeSO <sub>4</sub>	-	0.1	-	
CaCO <sub>3</sub>	0.3	-	-	
DCP	-	0.3	0.4	
NH <sub>4</sub> CI	-	1.7	-	
NaCl	0.4	0.3	0.5	
NaHCO <sub>3</sub>	_	-	1.2	
Mg <sub>2</sub> O	0.6	0.45	-	
Mycosorb	-	0.3	0.1	
Protoxin	- 6		0.01	
Calculated composition				
Protein (%)	12.97	16.09	18.87	
NDF (%)	40.25	43.26	48.12	
ADF (%)	38.29	32.23	30.26	
Ca (%)	0.27	0.31	0.35	
P (%)	0.26	0.16	0.27	
Cr (mg/kg)	9	8	8	

milk with regard to Cr supplementation was studied by relatively few authors (Pechova et al., 2003). In most cases, they found no difference between the experimental and control group (Besong et al., 1996; Yang et al., 1996; Simek et al., 1999). Hayirli et al. (2001) reported increased fat production and lactose levels in milk after Cr supplementation, which corresponds to our finding.

# **Serum biochemistry parameters**

The effects of Cr-Met supplementation on serum biochemistry parameters in the entire period of the experiment and during different experimental periods are shown in Table 3. As shown, glucose and insulin concentration had not been affected by Cr-met supplementation, whereas decreased serum cortisol

**Table 2.** The effect of Cr-Met supplementation on milk production and composition.

Lactation periods	Experimental	Milk	Fat	Protein	Lactose	TS	SNF	FCM %4
-	treatment	(kg/d)	(%)	(%)	(%)	(%)	(%)	(kg/d)
Wk 4 of Lactating	Chromium	46.94 <sup>a</sup>	3.53 <sup>a</sup>	2.68 <sup>a</sup>	4.58 <sup>a</sup>	11.68 <sup>a</sup>	8.39 <sup>a</sup>	41.7 <sup>a</sup>
	Control	42.42 <sup>a</sup>	3.79 <sup>a</sup>	2.55 <sup>a</sup>	4.47 <sup>a</sup>	11.67 <sup>a</sup>	8.16 <sup>a</sup>	41.61 <sup>a</sup>
	SEM	66.43	0.41	0.092	0.024	0.39	0.12	2.78
Wk 8 of Lactating	Chromium	54.18 <sup>a</sup>	2.78 <sup>a</sup>	2.74 <sup>a</sup>	4.61 <sup>a</sup>	11.16 <sup>a</sup>	8.48 <sup>a</sup>	42.21 <sup>a</sup>
•	Control	45.68 <sup>a</sup>	3.78 <sup>a</sup>	2.73 <sup>a</sup>	4.69 <sup>a</sup>	10.44 <sup>a</sup>	8.55 <sup>a</sup>	39.98 <sup>a</sup>
	SEM	88.7	0.14	0.093	0.016	0.52	0.122	2.54
Wk 12 of Lactating	Chromium	51.28 <sup>a</sup>	2.63 <sup>a</sup>	2.61 <sup>a</sup>	4.65 <sup>a</sup>	10.84 <sup>a</sup>	8.38 <sup>a</sup>	36.41 <sup>a</sup>
_	Control	44.66 <sup>a</sup>	2.34 <sup>a</sup>	2.69 <sup>a</sup>	4.27 <sup>a</sup>	10.68 <sup>a</sup>	8.49 <sup>a</sup>	36.8 <sup>a</sup>
	SEM	86.36	0.195	0.062	0.281	0.249	0.096	2.45
Total experimental period	Chromium	50.80 <sup>a</sup>	2.98 <sup>a</sup>	2.68 <sup>a</sup>	4.61 <sup>a</sup>	11.22 <sup>a</sup>	8.42 <sup>a</sup>	40.84 <sup>a</sup>
F 5 5	Control	44.25 <sup>b</sup>	2.97 <sup>a</sup>	2.66 <sup>a</sup>	4.48 <sup>a</sup>	10.93 <sup>a</sup>	8.40 <sup>a</sup>	38.80 <sup>a</sup>
	SEM	2.57	0.27	0.084	0.054	0.4	0.115	2.59

a-b Means in the same column with no common superscripts are significantly different (P<0.05).

concentration in the entire period of the experiment but it was not significant (P<0.05) at different experimental periods. Previous studies by Besong et al. (1996), Bryan et al. (2004), Burton et al. (1995) on cow, and Gentry et al. (1999) and Kitchalong et al. (1995) on sheep have shown that Cr supplementation has no effect on serum glucose and insulin concentration. However, Chang and Mowat (1992) and Kegley et al. (1997b, 2000) on calves, and Bunting et al. (1994) on cows reported that Cr supplementation causes decrease in glucose concentration and increase of serum insulin concentration. Chang and Mowat (1992), and Moonsie-Shageer and Mowat (1993) reported that Cr-met supplementation causes decrease in serum cortisol concentration. However, Depew et al. (1998) and Kegley et al. (1997b) did not observe any effect of Cr supplementation on serum cortisol supplementation.

In our study, glucose and insulin concentration had not been effected by Cr-met supplementation, that is, maybe because of inability of chromium on insulin secretion of pancreas gland. How chromium affects cortisol production is unknown, but it is clear that glucocorticoids inhibit excretion of insulin (Munck et al., 1984). Because GTF (glucose tolerance factor) Cr potentates the action of insulin it may inversely inhibit cortisol excretion (Chang and Mowat., 1992).

Reduced NEFA concentration was observed in response to Cr supplementation (Yang et al., 1996; DePew et al., 1998; Hayirli et al., 2001; Bryan et al., 2004). Reduced blood NEFA concentration in response to Cr supplementation may be partially attributed to reduced blood cortisol levels (Chang and Mowat, 1992; Moonsie-Shageer and Mowat, 1993; Almeida and Barajas, 2001) as cortisol acts antagonistically to insulin,

reducing glucose uptake by peripheral tissue (Burton, 1995).

As it is shown in Table 3, serum total cholesterol and triglyceride concentration were not affected by Cr supplementation. This can be because of disability of chromium on insulin concentration in our experiment. The role of insulin is confirmed by lipogenesis stimulus and lipolysis inhibition (Kegley et al., 2000).

Previous studies by Besong et al. (1996), Depew et al. (1998), Kegley et al., (1997b) and Moonsie-shageer and Mowat (1993) had shown Cr supplementation had no effect on concentration of serum total cholesterol and triglyceride. However, Bunting et al. (1994), Page et al. (1993), Riales et al. (1981) and Subiyatno et al. (1996) had reported Cr supplementation causes reduction in serum total cholesterol and triglyceride concentration.

This study, using chromium supplementation, resulted in some increase in total protein of serum (Table-3). These results are in accordance with studies reported by Bunting et al. (1994), Roginski and Mertz (1969) and Chang and Mowat (1992). However, Kitchalong et al. (1995) and Kegley et al. (1997a) reported Cr supplementation had no effect on serum total protein concentration.

Increase in total protein of serum could be due to the decrease of serum cortisol concentration or an increase of sensitivity tissue to insulin. The role of insulin is proved at increase of synthesis of proteins (Roginski and Mertz, 1969).

Moonsie-Shageer and Mowat (1993) reported increased serum albumin in response to feeding Cr-yeast to steers calves. The increase in serum albumin may be due to increased in amino acid synthesis in the liver, suggesting that Cr may improve amino acid synthesis,

TS, total solid; SNF, solid nonfat; FCM, fat corrected milk.

**Table 3.** The effect of Cr-Met supplementation on serum biochemistry parameters.

Blood sampling period	Experimental treatment	Glucose (mg/dl)	Total cholesterol (mg/dl)	Triglyceride (mg/dl)	Total protein (mg/dl)
	Chromium	49.89 <sup>a</sup>	34.22 <sup>a</sup>	6.94 <sup>a</sup>	69.11 <sup>a</sup>
5 weeks prior to parturition	Control	77.1 <sup>a</sup>	21.3 <sup>a</sup>	7.05 <sup>a</sup>	68.6 <sup>a</sup>
	SEM	5.16	23.08	0.115	5.47
	Chromium	18 <sup>a</sup>	16 <sup>a</sup>	7.09 <sup>a</sup>	55.56 <sup>a</sup>
2 weeks prior to parturition	Control	18.1 <sup>a</sup>	20.3 <sup>a</sup>	6.76 <sup>a</sup>	58.5 <sup>a</sup>
2 moone phon to partamen	SEM	2.6	2.7	0.17	5.16
	Chromium	27.89 <sup>a</sup>	7.89 <sup>a</sup>	7.43 <sup>a</sup>	36.78 <sup>a</sup>
1 week after parturition	Control	33.1 <sup>a</sup>	11.2 <sup>a</sup>	7.1 <sup>a</sup>	38.3 <sup>a</sup>
	SEM	2.5	3.5	0.2	2.5
	Chromium	17.11 <sup>a</sup>	7.89 <sup>a</sup>	8.1 <sup>a</sup>	43.22 <sup>a</sup>
4 week after parturition	Control	25.3 <sup>a</sup>	11.4 <sup>a</sup>	7.33 <sup>a</sup>	44.9 <sup>a</sup>
Two and paramion	SEM	1.46	3.95	0.21	2.2
	Chromium	26.2 <sup>a</sup>	16.5 <sup>a</sup>	a 7.39	51.17 <sup>a</sup>
Total experimental period	Control	38.4 b	16.05 <sup>a</sup>	7.06 b	52.58 <sup>a</sup>
. S.S. Oxportitional portou	SEM	11.91	3.22	0.181	4.11

 $<sup>^{\</sup>mathrm{a-b}}$  Means in the same column with no common superscripts are significantly different (p<0.05).

possibly via insulin. Schroeder et al. (1965) concluded that Cr plus insulin enhanced incorporation of several amino acids into protein in rats

Effect of Cr supplementation on blood hematology parameters are shown in Table 4. In our study, Cr supplementation had no effect on blood parameters such as; total red blood cells, hematocrit and hemoglobin. Also, Cr supplementation had no significant effect on the neutrophils, lymphocytes, monocytes, basophiles, eosinophils

percentages and ratio of neutrophils to lymphocytes.

In conclusion, results of this experiment showed that milk production was significantly affected by chromium methionine supplementation during the entire period, but it had no significant effect on milk composition. Supplemental chromium had no significant effect on serum glucose, cholesterol, triglycerides and insulin concentration and blood hematology parameters. However, chromium supplementation tended to increase serum total

protein concen concentration. T showed that supplementation may improve the

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Table 4. The effect of Cr-Met supplementation on blood hematology parameters.

Blood sampling	Experimental	RBC	Hematocrit	Hemoglobin	Neutrophil	Lymphocyte	Neutrophil to
period	treatment	(*10 <sup>6</sup> in microlitre)	(%)	(mg/dl)	(%)	(%)	Lymphocyte (%)
5 weeks prior to parturition	Chromium Control	6.89 <sup>a</sup> 6.67 <sup>a</sup>	32.3 <sup>a</sup> 33.7 <sup>a</sup>	13.2 <sup>a</sup> 9.42 <sup>a</sup>	21.6 <sup>a</sup> 20 <sup>a</sup>	61.25 <sup>a</sup> 71.78 <sup>a</sup>	0.35 <sup>a</sup> 0.29 <sup>a</sup>
	SEM	0.18	2.06	2.07	2.95	3.43	0.07
2 weeks prior to parturition	Chromium Control SEM	6.23 <sup>a</sup> 6.31 <sup>a</sup> 0.05	31.9 <sup>a</sup> 31.7 <sup>a</sup> 0.6	9.06 <sup>a</sup> 9.05 <sup>a</sup> 0.16	29.3 <sup>a</sup> 27.1 <sup>a</sup> 4.05	66.5 <sup>a</sup> 67.33 <sup>a</sup> 3.88	0.46 <sup>a</sup> 0.45 <sup>a</sup> 0.09
1 week after parturition	Chromium Control SEM	6.25 <sup>a</sup> 6.31 <sup>a</sup> 0.23	32 <sup>a</sup> 32.7 <sup>a</sup> 1	9.48 <sup>a</sup> 9.83 <sup>a</sup> 0.32	25.5 <sup>a</sup> 26.4 <sup>a</sup> 3.43	63.75 <sup>a</sup> 62.22 <sup>a</sup> 3.79	0.43 <sup>a</sup> 0.48 <sup>d</sup> 0.08
4 week after parturition	Chromium Control SEM	5.22 <sup>a</sup> 5.38 <sup>a</sup> 0.21	26.9 <sup>a</sup> 24.9 <sup>d</sup> 1.67	8.08 <sup>a</sup> 10.2 <sup>d</sup> 1.36	36.5 <sup>a</sup> 35.8 <sup>a</sup> 5.53	52.75 <sup>a</sup> 57.33 <sup>a</sup> 5.77	0.86 <sup>a</sup> 0.84 <sup>a</sup> 0.23
Total experimental period	Chromium Control SEM	6.15 <sup>a</sup> 6.17 <sup>a</sup> 0.2	30.8 <sup>a</sup> 30.8 <sup>a</sup> 1.53	9.96 <sup>a</sup> 9.62 <sup>a</sup> 1.33	28.2 <sup>a</sup> 27.3 <sup>a</sup> 3.57	62.6 <sup>a</sup> 64.7 <sup>a</sup> 4.12	0.52 <sup>a</sup> 0.51 <sup>a</sup> 0.11

a-b Means in the same column with no common superscripts are significantly different (p<0.05).

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