

## Metabolic Effects of Chromium Supplementation in Dairy Cows in the Peripartal Period

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### Abstract

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The aim of the work was to assess the effects of supplemental chromium (Cr) on metabolism of dairy cows in the peripartal period. Rations fed to dairy cows in a herd of Holstein cattle with mean milk yield of 7 500 l were supplemented with chromium-enriched yeast (Co-Factor III Chromium Yeast, Alltech, 0.1% Cr<sup>3+</sup>) at 10 mg of Cr per animal per day. The treatment was started 21 days before the expected delivery date and discontinued 30 days after the delivery. Blood and urine samples were collected from ten experimental and ten control cows at weekly intervals, the state of health was monitored by regular clinical examinations, and milk yield for the first 100 days of lactation was recorded. The results indicate favourable effects of the supplementation on energy metabolism. The Cr-supplemented cows showed significantly higher blood glucose concentrations at post-partum (p.p.) weeks 4 ( $4.25 \pm 0.21$  vs.  $3.74 \pm 0.36$  mmol·l<sup>-1</sup>;  $p < 0.01$ ) and 5 ( $4.06 \pm 0.41$  vs.  $3.64 \pm 0.28$  mmol·l<sup>-1</sup>;  $p < 0.05$ ) and lower ketone bodies concentration at p.p. week 4 ( $0.88 \pm 0.11$  vs.  $1.38 \pm 0.66$  mmol·l<sup>-1</sup>;  $p < 0.05$ ). The Cr-supplemented cows showed also significantly lower bilirubin concentration at p.p. week 2 ( $3.93 \pm 0.84$  vs.  $6.47 \pm 3.25$  μmol·l<sup>-1</sup>;  $p < 0.05$ ) and lower catalytic activities of aspartate aminotransferase at p.p. weeks 3 ( $1.37 \pm 0.14$  vs.  $1.66 \pm 0.20$  μkat·l<sup>-1</sup>;  $p < 0.01$ ) and 5 ( $1.16 \pm 0.08$  vs.  $1.47 \pm 0.18$  μkat·l<sup>-1</sup>;  $p < 0.01$ ) and lactate dehydrogenase at p.p. week 5 ( $27.35 \pm 3.76$  vs.  $33.61 \pm 5.61$  μkat·l<sup>-1</sup>;  $p < 0.05$ ). No effects on the metabolism of nitrogen substances or minerals, insulin concentration in blood serum, and blood Cr concentration were observed. Chromium excretion in urine increased after parturition; higher concentrations were found in Cr-supplemented cows at p.p. weeks 3 ( $7.14 \pm 1.72$  vs.  $5.00 \pm 1.26$  μg·l<sup>-1</sup>;  $p < 0.01$ ) and 4 ( $8.40 \pm 3.13$  vs.  $4.04 \pm 1.32$  μg·l<sup>-1</sup>;  $p < 0.01$ ). Although chromium supplementation in the peripartal period significantly improved variables characterising the energy metabolism, no effects on milk yield for the first 100 days of lactation or on the incidence of clinical diseases were demonstrable.

*Cattle, biochemical profile, liver, blood, chromium*

The relevance of the trace element chromium (Cr) to human and animal nutrition has been known for more than 40 years. Cr is present in the environment mostly in its trivalent form (Cr<sup>3+</sup>), which is more stable than the hexavalent form (Cr<sup>6+</sup>). Cr<sup>3+</sup> is known for its *in vivo* antioxidative activity and favourable effects on the stability of proteins and nucleic acids (Anderson 1994). However, its most important metabolic effect consists in an enhancement of insulin activity by its presence in an organometallic molecule known as the glucose tolerance factor (GTF). Detailed structure of GTF is unknown, but it is assumed that the factor consists of Cr<sup>3+</sup>, nicotinic and glutamic acids, glycine, and cysteine (Ducros 1992). Most of Cr present in animal tissues is bound in GTF. Data on the distribution of chromium in animal tissues is rather sporadic. Low concentrations, declining with age (Mertz 1997), can be found in almost all tissues. In the man, the concentration is the highest in lung tissue and decreases in the following order: liver → pancreas → heart → striated muscles → kidney → spleen → brain (Lentner 1981). Blood concentration does not parallel that of tissue stores (Underwood 1977), but reflects exposure to chromium (Barceloux 1999).

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Cr enhances binding of insulin to cell membrane receptors and the optimisation of insulin activity results in better regulation of glucose uptake by cells, improved control of blood glucose concentration, and maximisation of the energetic potential. Consequences of Cr deficiency, which are probably associated with disturbed interaction between Cr and insulin, include lowering of glucose tolerance, increased insulin concentration, glycosuria, growth impairment, shortening of productive age, increased concentration of cholesterol and triacylglycerols, infertility, and peripheral neuropathies (Anderson 1994). Manifestations of Cr deficiency usually develop in animals affected by metabolic stress or exposed to physical strain.

Available data on Cr concentration in rations for farm animals is insufficient. Moreover, even rations containing Cr concentrations satisfying the need during a normal production period can become deficient in critical situations, such as advanced pregnancy, parturition, onset of lactation, weaning, transport, etc. The aim of the work was to assess the effects of periparturient supplementation of Cr on metabolism of dairy cows including possible ensuing effects on milk yield for the first 100 days of lactation and health.

#### Materials and Methods

The experiment was conducted in 1999 in South Moravia in a herd of Holstein cattle with 600 heads and a mean milk yield of 7500 l (6900 l in first-calvers and 7900 l in 2<sup>nd</sup>-lactation and older cows). Twenty dairy cows, selected with regard to the expected delivery date and performance (analogous couple system), were divided into one experimental (n = 10) and one control (n = 10) groups, each including 5 first-calvers and five older cows. Chromium-enriched yeast (Co-Factor III Chromium Yeast, Alltech, 0.1% Cr<sup>3+</sup>) was mixed into supplementary feed mixture at 10 mg of Cr per animal per day mixed starting at expected delivery day -21 and discontinuing at post-delivery day 30. Rations fed during the dry period consisted of corn silage (17.0 kg), whole-plant haylage (4.0 kg), minerals (0.3 kg), and alfalfa hay (3.0 kg). Rations fed in first month of lactation were calculated for presumed milk yield 36 l and consisted of corn silage (20.0 kg), whole-plant haylage (8.0 kg), alfalfa hay (2.0 kg), and concentrates (12.5 kg).

Blood and urine samples were collected regularly at weekly intervals starting 2 weeks before and discontinuing 5 weeks after delivery. The samples for plasma and whole blood analyses were collected from *v. jugularis* into disposable heparinised test tubes and analysed for concentrations of glucose, urea, triacylglycerols, calcium (Ca), magnesium (Mg), inorganic phosphorus (P) and catalytic activities of aspartate aminotransferase (AST),  $\gamma$ -glutamyl transferase (GGT), lactate dehydrogenase (LDH) in blood plasma, and of ketone bodies and chromium (Cr) in whole blood. The samples for blood serum analyses were collected into glass tubes and analysed for total bilirubin, cholesterol, albumin, nonesterified fatty acids (NEFA), cortisol, and insulin concentrations. Urine samples were collected using a plastic catheter and tested for pH, density, concentrations of Ca, P, Mg and Cr. Chromium concentrations in blood and urine were measured in post-partum weeks 1 to 4 only.

The following parameters were measured using the automatic analyser Cobas Mira (ROCHE, Switzerland) and the tests given in parentheses: urea (Urea UV KIN 4  $\times$  50, Cat. No. 1307017), total bilirubin (BIL 100, Cat. No. 1105309), triacylglycerols (TGL 4  $\times$  100, Cat. No. 1312983), GGT (GGT KIN 100, Cat. No. 1302082) the sets supplied by LACHEMA (Czech Republic); glucose (<sup>1</sup>-Glukosa, Cat. No. 11601), total protein (<sup>1</sup>-Protein total, Cat. No. 12751), cholesterol (<sup>1</sup>-Cholesterol, Cat. No. 10851), AST (<sup>1</sup>-AST, Cat. No. 10351), LDH (<sup>1</sup>-LDH, Cat. No. 12352), P (<sup>1</sup>-Phosphorus inorganic, Cat. No. 11352) the sets supplied by BioVendor, (Czech Republic); albumin (Albumin liquicolor, Cat. No. 10560; set supplied by HUMAN, Germany); NEFA (NEFA, Cat. No. FA 115; set supplied by RANDOX, United Kingdom). The minerals Ca and Mg were determined by F-AAS using the spectrometer H 1550 (HILGER, Great Britain) apparatus. Cr was determined by ETA-AAS using the spectrometer SOLAAR 939 (UNICAM, Great Britain). Total ketone bodies (acetone, acetoacetic acid, isopropanol,  $\beta$ -hydroxybutyric acid) were determined by gas chromatography (Chrom 5, LABORATORNÍ PŘÍSTROJE, Praha, Czech Republic), as described by Hradecký et al. (1978). Cortisol (LKC01; set supplied by BioVendor, Czech Republic) and insulin (LKIN1; set supplied by BioVendor, Czech Republic) were determined by the chemiluminescence technique using the apparatus Immulite (DPC, Los Angeles, USA).

The clinical state of the cows was monitored daily by the veterinarian in charge of the herd and data from performance checks were used for the assessment of milk yield for the first 100 days of lactation.

Variance of values obtained in the individual sets was analysed by the *F*-test and subsequently by the two-sided Student's *t*-test for sets with equal or unequal variance using the EXCEL software.

#### Results

Results of biochemical analyses are shown in Tables 1, 2 and 3. As the differences between the values of metabolic profile obtained in first-calvers and older cows were nonsignificant, the data obtained in the two subgroups were pooled. Energy deficit,

Table 1  
Selected values of energetic and protein metabolic profiles in control (C; n = 10)  
and Cr-supplemented (E; n = 10) dairy cows (x = mean; s = standard deviation)

Weeks relative to delivery		-2	-1	Delivery	1	2	3	4	5
Glucose [mmol·l <sup>-1</sup> ]	E	x 3.90 s 0.27	x 3.71 s 0.23	x 3.70 s 0.24	x 4.23 s 0.34	x 4.24 s 0.55	x 4.15 s 0.42	x 4.25** s 0.21	x 4.06* s 0.40
	C	x 3.79 s 0.23	x 3.66 s 0.28	x 3.67 s 0.22	x 3.83 s 0.60	x 4.04 s 0.59	x 3.75 s 0.48	x 3.74** s 0.36	x 3.64* s 0.28
Total ketone bodies [mmol·l <sup>-1</sup> ]	E	x 0.88 s 0.22	x 0.96 s 0.25	x 0.88 s 0.09	x 0.93 s 0.20	x 1.13 s 0.47	x 0.95 s 0.30	x 0.88* s 0.11	x 0.89 s 0.19
	C	x 0.76 s 0.09	x 0.80 s 0.30	x 0.84 s 0.14	x 1.29 s 0.75	x 1.29 s 0.46	x 1.46 s 1.06	x 1.38* s 0.66	x 1.10 s 0.24
Oxidized ketone bodies [μmol·l <sup>-1</sup> ]	E	x 22.1 s 4.6	x 30.8 s 13.8	x 29.8 s 3.5	x 36.0 s 13.3	x 100.5 s 13.3	x 68.2 s 9.2	x 31.4 s 10.5	x 33.7 s 25.1
	C	x 25.1 s 3.5	x 22.9 s 6.6	x 30.7 s 7.8	x 180.9 s 388.5	x 95.2 s 110.2	x 241.3 s 542.5	x 96.3 s 126.5	x 35.9 s 20.6
NEFA [mmol·l <sup>-1</sup> ]	E	x 0.22 s 0.15	x 0.15 s 0.07	x 0.20 s 0.10	x 0.26 s 0.12	x 0.28 s 0.21	x 0.22 s 0.12	x 0.21 s 0.15	x 0.13 s 0.09
	C	x 0.12 s 0.06	x 0.25 s 0.15	x 0.18 s 0.10	x 0.24 s 0.08	x 0.33 s 0.15	x 0.24 s 0.15	x 0.19 s 0.10	x 0.35 s 0.27
Triacyl- glycerols [mmol·l <sup>-1</sup> ]	E	x 0.25 s 0.05	x 0.25 s 0.04	x 0.27 s 0.05	x 0.12 s 0.04	x 0.18 s 0.07	x 0.19 s 0.05	x 0.20* s 0.06	x 0.17** s 0.04
	C	x 0.21 s 0.05	x 0.22 s 0.08	x 0.28 s 0.07	x 0.12 s 0.05	x 0.17 s 0.09	x 0.21 s 0.06	x 0.27* s 0.07	x 0.27** s 0.04
Total cholesterol [mmol·l <sup>-1</sup> ]	E	x 3.10 s 0.37	x 3.40 s 0.33	x 3.36 s 0.63	x 3.21 s 0.99	x 3.79 s 1.03	x 4.21 s 0.80	x 4.47 s 0.79	x 4.02* s 0.57
	C	x 2.99 s 0.53	x 3.15 s 0.54	x 3.31 s 0.77	x 2.96 s 0.91	x 3.36 s 0.90	x 3.90 s 1.03	x 4.72 s 1.35	x 5.31* s 1.18
Urea [mmol·l <sup>-1</sup> ]	E	x 3.78 s 1.06	x 2.52 s 0.53	x 2.20 s 0.72	x 4.00 s 1.53	x 3.65 s 1.18	x 4.35 s 0.80	x 3.93 s 1.04	x 4.33 s 1.61
	C	x 4.45 s 1.03	x 2.37 s 1.41	x 2.14 s 0.64	x 4.12 s 1.22	x 3.84 s 1.20	x 4.58 s 1.05	x 4.65 s 0.89	x 4.22 s 0.81
Total protein [g·l <sup>-1</sup> ]	E	x 73.24 s 3.54	x 66.76 s 7.53	x 67.31 s 5.48	x 68.05 s 5.73	x 77.37 s 3.20	x 78.68 s 5.38	x 77.47 s 6.83	x 80.82 s 5.43
	C	x 73.06 s 2.66	x 69.77 s 3.05	x 66.12 s 1.9	x 67.04 s 3.31	x 73.68 s 3.91	x 80.55 s 3.53	x 78.98 s 5.05	x 78.36 s 4.35
Albumin [g·l <sup>-1</sup> ]	E	x 37.38 s 1.77	x 35.9 s 1.97	x 35.76 s 1.66	x 35.06 s 2.93	x 35.59 s 2.68	x 35.17 s 1.61	x 35.12 s 3.23	x 33.27 s 1.98
	C	x 37.46 s 1.10	x 37.16 s 1.47	x 36.45 s 1.68	x 36.40 s 2.03	x 34.91 s 2.59	x 35.44 s 2.67	x 35.82 s 3.34	x 36.00 s 2.40

\*  $p < 0.05$  (between groups E and C)

\*\*  $p < 0.01$  (between groups E and C)

manifested by low concentration of blood glucose under physiological range 3.0 – 3.9 mmol·l<sup>-1</sup> (Vrzgula et al. 1990) and increased concentration of ketone bodies above physiological 0.1 – 1.0 mmol·l<sup>-1</sup> (Vrzgula et al. 1990), was found in many cows in the first month after delivery. This metabolic disturbance was more marked in the control group. The Cr-supplemented cows showed significantly higher blood glucose concentrations at postpartum (p.p.) weeks 4 ( $p < 0.01$ ) and 5 ( $p < 0.05$ ) and significantly lower concentration of total ketone bodies at p.p. week 5 ( $p < 0.05$ ). Energy deficit was associated with damage to hepatic parenchyma manifested by increased activities of hepatic enzymes and higher concentration of total bilirubin in blood serum (Table 2). This damage was less serious in the experimental group which showed significantly lower total bilirubin concentration at p.p. week 2 ( $p < 0.05$ ) and lower catalytic activities of AST at p.p. weeks 3 and 5 ( $p < 0.01$ ) and LDH at p.p. week 5 ( $p < 0.05$ ). Marked increase in the catalytic activity of AST above physiological range 0.72 – 1.41 μkat·l<sup>-1</sup> (Pechová et al. 1997) after delivery was demonstrated in 40% cows of the Cr-supplemented and 90% of the control cows. This

Table 2  
Selected values of metabolic profiles in control (C; n = 10) and Cr-supplemented (E; n = 10) dairy cows (x = mean; s = standard deviation)

Weeks relative to delivery			-2	-1	Delivery	1	2	3	4	5
<b>Total bilirubin</b> [ $\mu\text{mol}\cdot\text{l}^{-1}$ ]	<b>E</b>	<b>x</b>	2.74	3.51	3.09	8.45	3.93*	3.67	3.50	2.47
		<b>s</b>	1.08	1.25	0.89	5.52	0.84	1.45	1.62	0.86
	<b>C</b>	<b>x</b>	2.90	3.77	4.69	7.39	6.47*	6.39	4.79	3.38
		<b>s</b>	0.67	1.21	2.79	3.51	3.25	3.56	2.08	1.28
<b>AST</b> [ $\mu\text{kat}\cdot\text{l}^{-1}$ ]	<b>E</b>	<b>x</b>	1.12	1.13	1.14	1.69	1.69	1.37**	1.34	1.16**
		<b>s</b>	0.16	0.16	0.20	0.58	0.47	0.14	0.27	0.08
	<b>C</b>	<b>x</b>	1.09	1.10	1.16	1.73	1.84	1.66**	1.58	1.47**
		<b>s</b>	0.15	0.12	0.14	0.32	0.56	0.20	0.25	0.18
<b>LDH</b> [ $\mu\text{kat}\cdot\text{l}^{-1}$ ]	<b>E</b>	<b>x</b>	27.65	25.78	24.96	30.75	29.49	28.37	31.89	27.35*
		<b>s</b>	3.86	2.46	3.18	7.42	5.11	5.92	6.52	3.76
	<b>C</b>	<b>x</b>	29.69	28.77	28.09	33.76	35.82	34.37	36.63	33.61*
		<b>s</b>	2.37	3.37	3.58	8.50	7.83	6.49	8.27	5.61
<b>GMT</b> [ $\mu\text{kat}\cdot\text{l}^{-1}$ ]	<b>E</b>	<b>x</b>	0.26	0.29	0.35	0.42	0.42	0.37	0.39	0.40
		<b>s</b>	0.04	0.06	0.08	0.10	0.09	0.09	0.07	0.07
	<b>C</b>	<b>x</b>	0.29	0.32	0.38	0.47	0.48	0.45	0.46	0.47
		<b>s</b>	0.05	0.09	0.11	0.19	0.19	0.13	0.19	0.18
<b>Cortisol</b> [ $\text{nmol}\cdot\text{l}^{-1}$ ]	<b>E</b>	<b>x</b>	53.22*	41.14	39.67	61.56	32.41	33.29	53.29	42.15
		<b>s</b>	25.46	25.48	15.43	57.40	16.77	25.73	33.40	25.28
	<b>C</b>	<b>x</b>	25.84*	30.23	33.88	35.78	36.15	34.78	35.99	36.13
		<b>s</b>	6.10	6.01	15.20	19.61	20.75	23.43	26.00	15.14
<b>Insulin</b> [ $\text{nmol}\cdot\text{l}^{-1}$ ]	<b>E</b>	<b>x</b>	4.93	5.49	6.64	5.80	3.77	2.39	3.78	3.57
		<b>s</b>	4.20	3.10	4.35	3.00	2.69	1.01	2.45	1.60
	<b>C</b>	<b>x</b>	3.80	5.31	5.48	4.58	3.84	2.18	3.25	2.52
		<b>s</b>	0.87	4.65	2.85	3.37	2.34	0.37	1.27	0.75

\*  $p < 0.05$  (between groups E and C)

\*\*  $p < 0.01$  (between groups E and C)

increase persisted in 8 of the control cows, but in only 3 Cr-supplemented cows throughout the post-partum period. More serious damage to hepatic function manifested by increased catalytic activity of GMT above physiological range  $0.14 - 0.55 \mu\text{kat}\cdot\text{l}^{-1}$  (Pechová et al. 1997) persisting for 2 to 6 weeks was found in 4 control cows. A similar, but transient increase, demonstrated on one sampling only, was observed in 2 Cr-supplemented cows. The between-group difference was nonsignificant, however. The concentration of total cholesterol in blood plasma increased progressively after delivery throughout the rest of the observation period and reached significantly higher values in the control than in the Cr-supplemented cows ( $p < 0.05$ ) at p.p. week 5. The concentration of triacylglycerols in blood moderately decreased in the post-partum period and although the concentrations found at p.p. weeks 4 and 5 were significantly ( $p < 0.05$  and  $p < 0.01$ , respectively) lower in the Cr-treated than in the control cows.

Supplemental Cr did not influence the metabolism of nitrogen substances (Table 1). The dynamics of blood urea concentration reflected the composition of rations and were similar in both the groups. Total protein and albumin concentrations were uniform throughout the observation period and did not exceed the physiological range  $60-80 \text{ g}\cdot\text{l}^{-1}$ ,  $30 - 42 \text{ g}\cdot\text{l}^{-1}$ , respectively (Vrzgula et al. 1990).

The concentration of cortisol in blood plasma of cows was monitored as an index of stress severity. However, their interpretation is rather problematic owing to large individual variations (variance coefficient 20 – 93%). The concentration rose above the normal level of  $15$  to  $19 \text{ nmol}\cdot\text{l}^{-1}$  (Kaneko 1997) in 70% of the Cr-treated and the control cows. Increase above  $100 \text{ nmol}\cdot\text{l}^{-1}$  recorded in two of the experimental cows was responsible for a high variance coefficient (93%). The concentrations tended to be higher in the Cr-treated cows, but a significant difference ( $p < 0.05$ ) was found only two weeks before delivery. The

concentration of insulin was monitored because the effects of Cr are closely associated with the activity of this hormone. The concentration of insulin decreased in the 1<sup>st</sup> and 2<sup>nd</sup> weeks after delivery and lower levels persisted up to the end of the observation period. No significant between-group differences were found.

Mineral metabolism (Table 3) was assessed on the basis of urine and blood plasma analyses. No significant between-group differences indicative of effects of supplemental Cr on the metabolism of Ca, P and Mg were demonstrable.

Table 3  
Selected values of mineral profiles in control (C; n = 10) and Cr-supplemented (E; n = 10) dairy cows  
(x = mean; s = standard deviation)

Weeks relative to delivery			-2	-1	Delivery	1	2	3	4	5
<b>Ca-plasma</b> [mmol·l <sup>-1</sup> ]	<b>E</b>	<b>x</b>	2.46	2.52	2.49	2.32	2.55	2.45	2.39	2.33
		<b>s</b>	0.22	0.13	0.18	0.37	0.22	0.12	0.23	0.11
	<b>C</b>	<b>x</b>	2.43	2.55	2.53	2.33	2.36	2.43	2.42	2.52
		<b>s</b>	0.09	0.11	0.13	0.11	0.15	0.18	0.19	0.14
<b>P-plasma</b> [mmol·l <sup>-1</sup> ]	<b>E</b>	<b>x</b>	2.40	2.45	2.18	1.66	1.92	1.88	1.89	1.90
		<b>s</b>	0.30	0.41	0.32	0.42	0.27	0.44	0.49	0.12
	<b>C</b>	<b>x</b>	2.16	2.20	1.91	1.52	1.76	1.81	1.91	1.72
		<b>s</b>	0.30	0.26	0.30	0.26	0.31	0.29	0.30	0.25
<b>Mg-plasma</b> [mmol·l <sup>-1</sup> ]	<b>E</b>	<b>x</b>	0.82	0.79	0.84	0.98	0.83	0.85	0.91	0.93
		<b>s</b>	0.09	0.08	0.07	0.20	0.10	0.09	0.07	0.08
	<b>C</b>	<b>x</b>	0.82	0.81	0.83	0.83	0.80	0.82	0.85	0.99
		<b>s</b>	0.03	0.08	0.08	0.09	0.13	0.12	0.17	0.15
<b>Ca-urine</b> [mmol·l <sup>-1</sup> ]	<b>E</b>	<b>x</b>	0.91	0.22	0.61	0.16	0.36	0.17	0.28	0.13
		<b>s</b>	1.22	0.15	0.88	0.06	0.33	0.10	0.33	0.03
	<b>C</b>	<b>x</b>	0.19	0.51	0.59	0.35	0.28	0.72	0.95	0.28
		<b>s</b>	0.18	0.50	0.79	0.46	0.42	1.16	2.45	0.14
<b>P-urine</b> [mmol·l <sup>-1</sup> ]	<b>E</b>	<b>x</b>	2.24	1.88	2.46	2.54	2.73	1.46	2.07	3.25
		<b>s</b>	1.37	1.49	3.79	2.43	3.50	1.73	2.02	3.74
	<b>C</b>	<b>x</b>	4.11	2.41	3.98	1.97	1.85	2.24	1.69	1.25
		<b>s</b>	2.93	1.79	5.41	3.01	1.54	2.81	1.43	1.54
<b>Mg-urine</b> [mmol·l <sup>-1</sup> ]	<b>E</b>	<b>x</b>	12.72	11.65	13.66	7.51	6.22	6.80	9.06	9.37
		<b>s</b>	6.23	4.11	4.27	5.24	4.50	2.62	4.09	3.48
	<b>C</b>	<b>x</b>	13.19	10.52	9.56	5.94	7.49	7.50	6.86	10.20
		<b>s</b>	4.19	3.30	4.05	3.52	3.67	5.03	3.04	3.34

Chromium concentrations in urine and blood samples are given in Table 4. The supplementation had no significant effect on whole blood concentration of Cr and no marked fluctuations were observed during the observation period. Increased urinary excretion of Cr was recorded in the Cr-treated cows throughout the postparturient period; significant between-group differences were found at weeks 3 and 4 ( $p < 0.01$ ).

Clinical examination did not reveal significant health disorders in any of the cows. One of the Cr-supplemented cows developed puerperal paresis, but responded promptly to infusing therapy. Retention of placenta with ensuing endometritis was diagnosed in 2 Cr-supplemented and 3 control cows. One of the two affected experimental cows gave birth to twins in a complicated delivery.

Performance was assessed in terms of milk yield and composition for the first 100 days of lactation (Table 5). No significant difference in any of the parameters was found between the Cr-supplemented and the control groups.

## Discussion

Investigation of health and metabolic effects of supplemental Cr in dairy cows, focused on the terminal stage of pregnancy and early lactation as a period when the animals are exposed to increased metabolic stress and physical strain, demonstrated favourable effects

Table 4  
Blood and urinary concentrations of Cr in control (C; n = 10) and Cr-supplemented (E; n = 10) dairy cows  
(x = mean; s = standard deviation)

Weeks relative to delivery			Delivery	1	2	3	4
<b>Blood</b> [ $\mu\text{g}\cdot\text{l}^{-1}$ ]	<b>E</b>	<b>x</b>	4.20	3.09	3.65	4.55	4.86
		<b>s</b>	2.32	1.20	2.64	2.62	2.68
	<b>C</b>	<b>x</b>	3.37	4.38	3.77	3.81	4.18
		<b>s</b>	2.31	1.53	1.72	0.86	2.17
<b>Urine</b> [ $\mu\text{g}\cdot\text{l}^{-1}$ ]	<b>E</b>	<b>x</b>	6.08	9.11	7.08	7.14 **	8.40 **
		<b>s</b>	2.39	4.03	3.30	1.72	3.13
	<b>C</b>	<b>x</b>	6.36	7.65	6.46	5.00**	4.04**
		<b>s</b>	4.43	2.61	1.82	1.26	1.32

\*\*  $p < 0.01$  (between groups E and C)

Table 5  
Performance of dairy cows for the first 100 days of lactation in the control (C) and the experimental (E) groups  
(x = mean; s = standard deviation, n = number of cows)

				<i>n</i>	Milk yield (l)	Fat (%)	Protein (%)	Lactose (%)
<b>First-calvers</b>	<b>E</b>	5	<b>x</b>	2277.3	3.80	3.49	5.02	
			<b>s</b>	412.3	0.49	0.25	0.06	
	<b>C</b>	5	<b>x</b>	2675.4	3.91	3.48	5.04	
			<b>s</b>	265.0	0.40	0.12	0.11	
<b>Cows</b>	<b>E</b>	5	<b>x</b>	2966.6	4.04	3.34	5.08	
			<b>s</b>	160.2	0.43	0.20	0.18	
	<b>C</b>	5	<b>x</b>	3068.0	4.26	3.23	4.95	
			<b>s</b>	404.8	0.58	0.23	0.19	
<b>Total</b>	<b>E</b>	10	<b>x</b>	2594.2	3.94	3.41	5.06	
			<b>s</b>	472.8	0.47	0.23	0.14	
	<b>C</b>	10	<b>x</b>	2871.7	4.09	3.36	5.00	
			<b>s</b>	394.4	0.53	0.22	0.16	

of this treatment on energy metabolism in the puerperal period. The latter effect was apparently associated with increased gluconeogenesis in Cr-supplemented cows (Sano et al. 1997; Subiyatno et al. 1996). Such result is indicative of a less severe and shorter lasting energy deficit. Chang et al. (1996) and Besong et al. (1996) reported a lower incidence of ketosis in multiparous cows. On the other hand, Yang et al. (1996) and Ireland (1999) did not demonstrate any decrease in NEFA and  $\beta$ -hydroxybutyrate concentrations in the blood of dairy cows. An increase in gluconeogenesis and glycogenolysis in Cr-supplemented cows was presumed also by Subiyatno et al. (1996) who demonstrated that such treatment tended to reduce the insulin:glucagon ratio. Less severe damage to the hepatic parenchyma in the postpartum period was apparently associated with a lower degree of energy deficit. We assume that this effect does not result from direct favourable action of Cr on the hepatic parenchyma, but possible toxicity of Cr at the dose used in our experiment cannot be disregarded. Similarly, Besong et al. (1996) found a decrease in hepatic triacylglycerol concentration in cows receiving Cr supplements.

Certain between-group differences were found in total blood serum cholesterol concentration. Studies of effects of Cr supplementation on the metabolism of cholesterol in humans demonstrated an increase in the concentration of HDL cholesterol and decreases in concentrations of triacylglycerols and total and LDL cholesterol (Anderson 1995). Studies of cholesterol concentration in dairy cows are encouraged by the fact that cholesterol influences the production of steroid hormones; hence, low cholesterol concentration can result in a disturbance of reproductive functions (Williams 1994). We therefore assume

that the metabolism of cholesterol in cows should be considered in studies of effects of supplemental Cr.

Blood serum concentrations of cortisol found in our experiment showed wide variations and it is therefore difficult to assess the effects of Cr thereon. Individual variability in responses of cows to stress factors apparently played a more important role than supplemental Cr. Mean cortisol concentrations were higher in the Cr-supplemented than in the control cows. Chang and Mowat (1992) and Mowat et al. (1993) demonstrated a decrease in cortisol concentration by 19 to 27% in calves exposed to transport stress. In another study, Moonsie-Shager and Mowat (1993) demonstrated in calves an inverse linear proportion between dosage of Cr and blood serum cortisol concentration. Although this finding has been confirmed in repeated studies, the situation in cows in the peripartal period is not thus unequivocal. Subiyatno et al. (1993) demonstrated a decrease of cortisol concentrations after calving in Cr-supplemented cows. On the other hand, our results confirm the data of Yang et al. (1996) and Burton et al. (1995) who reported an inconsistent increase in cortisol concentrations in animals receiving Cr supplements. It is evident that the Cr - cortisol relation is not as unequivocal as believed earlier.

A moderate decrease in insulin concentration after delivery was observed in all the cows irrespective of Cr supplementation. Similar findings were published also by Besong et al. (1996) and Ireland (1999). However, a certain relation between Cr and insulin concentration is apparent from the results published by Frank et al. (2000) who demonstrated increased insulin concentrations in Cr-deficient goats. The lower rate of lipomobilisation in the cows receiving Cr supplements may consist in the effect of Cr on the biological response to insulin. Such interpretation is supported by the finding of equal biological responses in Cr-supplemented cows showing lower insulin concentration (Mallard and Borgs 1997). However, other authors assume a higher insulin resistance in energy-deficient first-calvers in early lactation and thereby explain favourable effects of Cr supplementation on milk yield in first-calvers (Mowat et al. 1995).

Papers presenting data on Cr concentrations in tissues and body fluids are rather scarce. Our investigations included determination of blood and urinary Cr concentrations. Chromium is known to be present in blood as free Cr<sup>3+</sup> or bound to transferrin, other proteins, or complexes, such as the glucose tolerance factor GTF-Cr (Ducros 1992). Cr supplementation had no effect on its blood concentration. No significant differences were found between the Cr-supplemented and the control groups. The relationship between blood Cr concentration and the content of Cr in grazed plants in an area with an increased Cr burden was studied by Sahin et al. (1996). Blood Cr concentrations ranged from 9 to 92 µg·l<sup>-1</sup> and were dependent on the content of Cr in plants. Although blood Cr concentrations reflect to a certain extent the intake of this element, their values can be relevant only in cases of excessive Cr intake. This is evident from our studies in which supplementation with 10 mg of Cr had no effect on blood Cr concentration. Blood Cr concentration cannot be used as an indicator of Cr status, because it is not balanced with tissue Cr stores (Underwood 1977). Absorbed Cr is excreted above all in urine and the excreted amount is controlled by several factors. Our results indicate a certain increase in urinary Cr excretion in the puerperal period which was more marked in the Cr-supplemented than in the control group. The increase apparently resulted from stress and a higher proportion of saccharides in the ration as described by Mordenti et al. (1997). The difference between the Cr-supplemented and the control cows is consistent with results of other authors who tested the use of urinary excretion of Cr as a biological marker in humans exposed to toxic doses of Cr (Paustenbach et al. 1997). Considering rather high standard deviations and the small difference between the treated and the control groups, monitoring of urinary excretion of Cr in cattle is apparently of limited value only.

No unequivocal effect of Cr supplementation on the state of health was demonstrable in our study. Although the prevalence of retained placenta and endometritis was somewhat higher in the control group, the number of cases is too low for drawing any conclusions. Similar results were reported also by Chang et al. (1996) who did not observe any effects of Cr supplementation on the prevalence of mastitis, oedema, or abomasal displacement and found only a somewhat lower prevalence of retained placenta and endometritis in the treated cows. On the other hand, Villalobos et al. (1997), who started Cr supplementation nine weeks before the expected delivery date, reported a decrease in the prevalence of retained placenta to almost one fourth of the value observed in untreated cows.

No effect of Cr supplementation of dairy cows in periparturient period on performance for the first 100 day of lactation was demonstrable in our experiment. Data published by other authors are conflicting; some reported an increase in milk yield (Hayirli et al. 2001), other found such an effect only in first-calvers (Yang et al. 1996), and some were unable to demonstrate any favourable effect of Cr supplementation on milk yield (Subiyatno et al. 1996). Unlike the above authors, we did not investigate direct effects of Cr supplementation on performance of dairy cows, but rather tried to answer the question whether Cr supplementation in the periparturient period will result in an metabolism-mediated increase in milk yield for the first 100 days of lactation as a standard performance parameter. A high variance of data and low per-group number of animals complicated the assessment of performance. Responsible for the lower mean performance in the experimental group was a lower milk yield in first-calvers resulting apparently from the selection of late-pregnant heifers for which no data on previous milk yield were available.

#### **Vliv dotace chromu na metabolismus dojnic v období kolem porodu**

Cílem pokusu bylo zjistit vliv dotace chromu (Cr) na metabolismus dojnic v období kolem porodu. V chovu Holštýnského skotu s průměrnou užitkovostí 7 500 l, byl dojnícům podáván Cr ve formě kvasinek (Co-Factor III Chromium Yeast, Alltech, 0,1% Cr<sup>3+</sup>) v dávce 10 mg Cr/kus/den. Aplikace byla zahájena v průměru 21 dní před porodem (a.p.) a ukončena 30 dní po porodu (p.p.). V průběhu pokusu byly odebírány vzorky krve a moči od dojnic pokusné (E, n = 10) a kontrolní skupiny (C, n = 10) v týdenních intervalech, byl sledován zdravotní stav pravidelným klinickým vyšetřením a užitkovost dojnic byla vyhodnocena za 100 dní laktace. Byl zjištěn pozitivní vliv podávání Cr na energetický metabolismus dojnic. U dojnic pokusné skupiny byla v poporodním období vyšší koncentrace glukózy, signifikantní rozdíly byly 4 týdny p.p. ( $4,25 \pm 0,21$  vs.  $3,74 \pm 0,36$  mmol·l<sup>-1</sup>;  $p < 0,01$ ) a 5 týdnů p.p. ( $4,06 \pm 0,41$  vs.  $3,64 \pm 0,28$  mmol·l<sup>-1</sup>;  $p < 0,05$ ) a naopak nižší koncentrace ketolátů v krvi 4 týdny p.p. ( $0,88 \pm 0,11$  vs.  $1,38 \pm 0,66$  mmol·l<sup>-1</sup>;  $p < 0,05$ ). Dále byl zjištěn u skupiny dotované Cr nižší stupeň alterace jaterního parenchymu. U dojnic pokusné skupiny byla v poporodním období zjištěna nižší koncentrace bilirubinu 2 týdny p.p. ( $3,93 \pm 0,84$  vs.  $6,47 \pm 3,25$  μmol·l<sup>-1</sup>;  $p < 0,05$ ), nižší katalytická aktivita aspartátaminotransferázy 3 týdny p.p. ( $1,37 \pm 0,14$  vs.  $1,66 \pm 0,20$  μkat·l<sup>-1</sup>;  $p < 0,01$ ) a 5 týdnů po porodu ( $1,16 \pm 0,08$  vs.  $1,47 \pm 0,18$  μkat·l<sup>-1</sup>;  $p < 0,01$ ) a laktátdehydrogenázy 5 týdnů p.p. ( $27,35 \pm 3,76$  vs.  $33,61 \pm 5,61$  μkat·l<sup>-1</sup>;  $p < 0,05$ ). Dotace Cr neovlivnila metabolismus dusíkatých látek, minerální metabolismus, koncentraci inzulinu v krevním séru. Koncentrace Cr v krvi nebyla dotací Cr ovlivněna. Po porodu došlo ke zvýšení vylučování Cr močí, vyšší hodnoty byly zjištěny u dojnic dotovaných Cr 3 týdny p.p. ( $7,14 \pm 1,72$  vs.  $5,00 \pm 1,26$  μg·l<sup>-1</sup>;  $p < 0,01$ ) a 4 týdny p.p. ( $8,40 \pm 3,13$  vs.  $4,04 \pm 1,32$  μg·l<sup>-1</sup>;  $p < 0,01$ ). I přes prokázaný pozitivní vliv Cr na energetický metabolismus v období kolem porodu nebyla ovlivněna užitkovost dojnic za prvních 100 dní laktace. Rovněž výskyt klinických onemocnění nebyl dotací Cr ovlivněn.

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