

## Milk Yield and Immune Response of Periparturient and Early Lactation Friesian Cows Fed Diets Supplemented with a High Level of Amino-acid Chelated Chromium

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**ABSTRACT** : The trial was carried out on twenty-one Friesian cows at the end of eight months gestation, nine multiparous and twelve primiparous; allocated into three groups (1 control, 2 and 3 experimental). The same diet was administered to all three groups before partum (12.8 kg DM/head/day) and after partum (18.8 kg DM/head/day). The cows in groups 2 and 3 received two different daily quantities of amino-acid chelated chromium (0.6 and 1.2 mg Cr/kg DM) from 4 weeks prior to presumed parturition to 6 weeks after. The milk yield control was carried out at 15, 30, 42 and 60 days. All animals were immunised two weeks prior to the presumed parturition and two weeks after with the following antigens: ovalbumin and brucellergene. Blood samples were collected weekly to monitor humoral and cell-mediated immune responses. When analysing the results of antibody immunity (ovalbumin) in the sixth blood collection both treated groups significantly increased compared to group 1 (0.5230 and 0.4536 vs. 0.1812 OD;  $p < 0.05$ ). The results of the cell-mediated immune response (brucellergene) had significant differences ( $p < 0.10$ ) in correspondence to the third (between group 2 and control) and the fifth (between groups 3 and 2) blood collection. Significant differences in fat corrected milk were observed at 42 days between group 3 and the other two groups (31.01 vs. 26.99 and 28.66 kg/d,  $p < 0.05$ ) and at 60 days between group 3 and control (30.88 vs. 26.69 kg/d,  $p < 0.05$ ). Before partum and at partum a positive immune response was obtained with a lower dose of chromium. After partum a positive immune response, anti-OVA indicator, was obtained with the higher dose of chromium while,  $\gamma$ -IFN indicator, with the lower dose. A significant increase of the milk yield resulted at both 42 and 60 days with the highest level of chromium. (*Asian-Aust. J. Anim. Sci.* 2005. Vol 18, No. 8 : 1098-1104)

**Key Words** : Chromium, Dairy Cows, Immune Response, Milk Yield

### INTRODUCTION

Researchers have previously approached the issue of how trivalent organic chromium is essential for normal metabolism of carbohydrates, lipids and proteins (Abraham et al., 1991; Bunting et al., 1994; Kornegay et al., 1997). This trace element, with nicotinic and glutamic acid, glycine and cysteine was found to be an active component of glucose tolerance factor, GTF (Schwartz and Mertz, 1957; 1959). When the species of zootechnic interest are under stress, as with humans, the cortisol hormone increases in the blood and stimulates the glycogenesis. Simultaneously, to strengthen the insulin activity, the antagonist of cortisol, and to allow a reduction of glycemia in the blood, a greater production of GTF is obtained and mobilisation of chromium occurs from the body reserves which is not recycled but eliminated in the urine (Anderson et al., 1990).

As far as dairy cow nutrition is concerned, a deficiency of chromium can cause a negative effect both on the milk yield (Sartin et al., 1988) and the estrous cycle (Vighio and Liptrap, 1990). Concerning the immune response of cattle

to chromium supplementation Burton et al. (1993) and Moonis-Shageer and Mowat (1993) have revealed a positive response from treated animals, contrasting to results obtained by Kegley et al. (1997).

When administering chromium to dairy cows Burton (1995) and Yang et al. (1996) obtained a positive effect only in the primiparous milk yield. Without considering the category, Simek et al. (1999) and Hayirli et al. (2001) also obtained, a positive effect in the milk yield, contrasting to results obtained by Peterson (2000). The National Research Council has evaluated the results of seven trials carried out, using supplemental Cr diets on dairy cows, and concluded that the data were not sufficient to determine the requirements of chromium in diets (Hayirli et al., 2001). Therefore the literature available up to date supplies insufficient information to evaluate supplemental chromium in the diets of dairy cows (NRC, 2001). Therefore two levels of chromium were studied for lactating cows, the first was between the highest doses used by Yang et al. (1996) and Hayirli et al. (2001) while the second was the highest level ever used before.

The aim of this work was to study the effects of chromium supplementation on immune responses and milk yield with the purpose of reducing the acute physical stress of dairy cows during the last phase of the gestation up to the peak of the lactation.

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**Table 1.** Dry matter (g/kg as fed), chemical composition (g/kg DM) and net energy (MJ/kg DM) of the feedstuffs utilized in experimental diets<sup>1</sup>

	DM	CP	CF	EE	NSC	Ash	NDF	ADF	ADL	NE <sub>L</sub>
Feedstuffs:										
Alfalfa hay, 2 <sup>nd</sup> cut	862.9	140.1	302.3	14.4	303.2	75.7	466.6	366.1	90.4	4.34
Wheat straw	892.0	31.1	455.0	15.3	36.3	75.4	841.9	562.0	90.0	2.98
Maize silage	326.7	81.4	172.6	25.1	481.5	41.0	371.0	215.8	24.4	6.26
Corn ears silage	564.8	78.0	65.8	34.8	705.7	16.1	165.4	77.2	11.8	7.75
Corn meal	897.2	85.4	22.6	42.1	700.1	16.1	156.3	36.6	8.5	8.96
Prot. concentr.	902.3	329.4	122.1	18.6	275.8	118.5	257.7	173.1	38.6	6.83
Diets <sup>2</sup> for:										
Dry cows	711.7	118.2	276.8	19.4	245.5	71.9	525.9	346.2	57.7	4.98
Lactating cows	683.4	169.4	146.8	24.6	434.7	65.7	305.6	189.4	35.2	6.61

<sup>1</sup> DM = Dry matter; CP = Crude protein; CF = Crude fibre; EE = Ether extract; NSC = Non-structural carbohydrates.

NDF = Neutral-detergent fibre; ADF = Acid-detergent fibre; ADL = Acid-detergent lignin; NE<sub>L</sub> = Net energy.

<sup>2</sup> Composition on DM basis for dry cows (F:C 80:20): alfalfa hay 10%, wheat straw 37%, maize silage 30% corn ears silage 3% and prot conc 20%.

Composition on DM basis for lactating cows (F:C 53:47): alfalfa hay 14%, maize silage 34%, corn ears silage 5%, corn meal 15% and prot conc 32%.

## MATERIALS AND METHODS

### Animals and diets

The trial was carried out on twenty-one Friesian cows at the end of the eighth month of gestation, nine multiparous (605.60±41.65 kg) and twelve primiparous (531.23±21.62 kg). The animals were divided into three groups of three multiparous and four primiparous: group 1, 2 and 3. The considered parameters, in addition to the weight, for the subdivision of the animals into homogeneous groups were the following: for the multiparous, the conventional production of 305 days of the previous lactation while for the primiparous the genetic characteristics of the parents. All the cows grazed with the addition of concentrate supplement until the beginning of the trial and then were transferred, 30 days before partum, into the parturition sector. After partum the animals were moved to the lactating sector with a suitable area per head, hence excluding any stress effect induced by lack of space. Before calving all the cows were fed the same diet based on their nutritional requirement (12.8 kg DM/head/day). Following parturition all the cows of the three groups, in the same paddock, received the same diet for lactating animals (18.8 kg DM/head/day) *ad libitum* intake. The feedstuffs, using the unifeed technique, were administered once a day in the morning.

### Chemical analysis, milk yield and quality

In Table 1 the chemical analyses and net energy content of the feedstuffs are reported. Feed samples were submitted to the following chemical determinations: dry matter (DM), crude protein (CP), crude fibre (CF), ether extract (EE), ash, neutral-detergent fibre (NDF), acid-detergent fibre (ADF), acid-detergent lignin (ADL). All the analyses were performed according to the methods of Association of Official Analytical Chemists (AOAC, 1984) and of Goering and Van Soest (1970) for cell wall constituents; furthermore the non structural carbohydrates (NSC) were calculated as

reported by Van Soest et al. (1991). The above listed parameters for both diets were calculated proportionally to the percentage of the feedstuffs components. The milk yield was controlled at 15, 30, 42 and 60 days; in addition the milk yield normalised to 4% of fat (FCM) was calculated. During each control the following parameters were determined on individual samples (representative of two consecutive milkings): fat, protein and lactose by means of an infrared interference spectrometer (Milkoscan, Foss Electric, Denmark).

### Chromium supplement

The cows in group 2 and 3 were given two different quantities of chromium: group 2 received 0.6 mg Cr/kg DM and group 3 received 1.2 mg Cr/kg DM. These diverse quantities of chromium were administered to the two groups for 4 weeks prior to presumed parturition to 6 weeks after (duration of the experiment). Chromium, in the chelated form with hydrolyzed amino-acids from soybean protein, was administered using CR400 (400.7 mg Cr/kg DM) supplied by Agrolabo (Turin, Italy). The product was weighed in cylinders made from filter paper and administered via oesophageal tube as a bolus form at 7:00 a.m. before the unifeed was administered. The quantity of chromium (experimental and dietary) fed daily to each animal, according to the physiological phase, is reported in Table 2.

Samples of feeds, CR400 product and milk were ashed in a muffle furnace at 600°C, oxidated with phosphoric manganate acid and potassium bromate and analysed, to determine the quantity of chromium, by using an atomic absorption spectrometer (3100, Perkin Elmer, USA) provided with a specific lamp (358 nm).

### Immunization schedule

All the animals were immunised two weeks prior presumed parturition and two weeks after with the

**Table 2.** Daily quantity of chromium administered both with the diets and experimentally, for dry and lactating cows

	Group 1 (control)	Group 2 (0.6 mg Cr/kg DM)	Group 3 (1.2 mg Cr/kg DM)
<b>Dry cows</b>			
Experim. Cr (mg Cr/head/d)	-	7.68	15.36
Dietary Cr	13.57	13.57	13.57
Total Cr	13.57	21.25	28.93
<b>Lactating cows</b>			
Experim. Cr (mg Cr/head/d)	-	11.28	22.56
Dietary Cr	22.37	22.37	22.37
Total Cr	22.37	33.65	44.93

following antigens: 1) the ovalbumin (OVA Type VII, Sigma Chemical, St. Louis, USA) was prepared as described by Burton et al. (1989) and administered (s. c.) in Freund's Incomplete Adjuvant such that each cow received 4 mg per immunization, 2) Brucellergene (Rhone Merieux, Lyon, France) by intradermal doses of 0.1 ml/animal (at least 200 units). To monitor humoral and cell-mediated immune responses, blood samples from the jugular vein were collected weekly using two different sterile vacutainers (no anticoagulant, 5 ml of blood; lithium-heparin 0.1 N, 8 ml of blood).

### Humoral immune responses

The humoral immunity was assayed by titrating the rate of appropriate antibody development after OVA stimulation. The blood sera, obtained after centrifugation, were stored at -20°C until required. The method used was an ELISA test (direct double-antibody ELISA) in which the micro-plates (Immulon II plate) were sensitised with OVA ( $\sim 3 \times 10^{-5}$  M) for 48 h at room temperature (Burton et al., 1989). The serum diluted in phosphate buffer solution PBS+Tween 20 at 5% (1:50, 1:100 and 1:200), were added twice and the mixture was incubated again at room temperature. The second antibody was rabbit anti-bovine IgG (H+L)-alkaline phosphatase conjugate diluted at a ratio of 1:5,000 and after the second incubation p-nitrophenyl phosphate disodium, dissolved in 10% diethanolamine at pH 9.8, was added to the substrate. The value of optical density (OD: 405 nm vs. 630 nm reference filter, Reader LP 400, Sanofi Pasteur, France) was used to determine the antibody content of the test serum. The reaction was blocked when the positive control serum diluted 1:200 reached a OD value of  $\sim 1.00$ . The 1:200 dilution of the positive control serum was chosen to standardise the trial reason being that the negative control serum always maintained a OD value of  $< 0.10$  and the greatest differences in OD between positive and negative serum always occurred at this dilution.

### Cell-mediated immune responses

As indicator of the cell-mediated immune response the

increase of  $\gamma$ -IFN parameter was used, produced from lymphocytes sensitised to an allergenic subject with homologous antigens, Melitensis B115 in rugose phase (anamnestic response). To 1.5 ml of blood 100  $\mu$ l of brucellergene (diluted 1:3 in PBS sterile at pH 7.2) was added. Following 18/24 h of incubation at 37°C, in an atmosphere containing 5% CO<sub>2</sub>, the plasma was separated and subjected to a competitive ELISA reaction using the Bovigam kit of CSL (Melbourne, Australia) following the procedure suggested by the manufacturer. In the test negative, positive and blank (plasma of non stimulated cells) controls were inserted; the interpretation of the results were made from the absorbance values obtained (optical density) superior to the basal values detected before immunisation.

### Statistical analysis

With the purpose of testing the differences of anti-OVA and  $\gamma$ -IFN parameters among the three groups in each drawing, the following mono-factorial model (SAS, 1993) was adopted:

$$Y_{ij} = \mu + \alpha_i + \varepsilon_{ij}$$

where:  $\mu$  = general mean;  $\alpha_i$  = group ( $i = 1, \dots, 3$ );  $\varepsilon_{ij}$  = error of model. Considering the high individual variability of anti-OVA and  $\gamma$ -IFN, testing up to  $P = 10\%$  was considered valid for these parameters.

Furthermore to test the differences of the milk yield normalised and non, and of the fat, protein and lactose levels, the following bi-factorial model with interaction (SAS, 1993) was used:

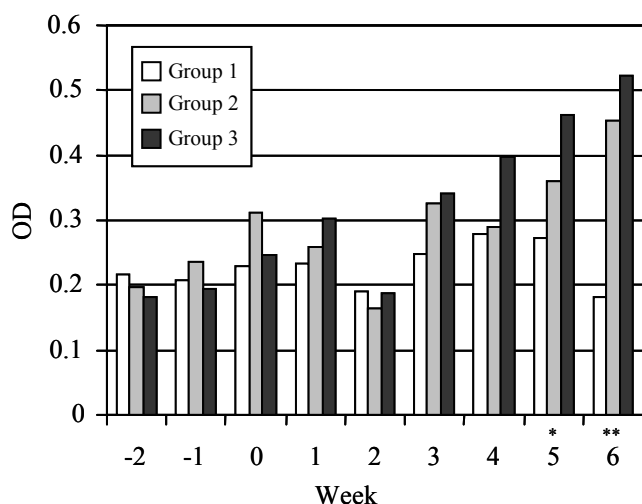
$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk}$$

where:  $\mu$  = general mean;  $\alpha_i$  = group ( $i = 1, \dots, 3$ );  $\beta_j$  = category (1, 2);  $(\alpha\beta)_{ij}$  = interaction group  $\times$  category;  $\varepsilon_{ijk}$  = error of model.

## RESULTS AND DISCUSSION

### Intake of chromium

The quantity of chromium contained in the diet of dry cows was 1.06 mg Cr/kg DM while the diet of lactating cows contained 1.19 mg Cr/kg DM. In addition to this quantity of inorganic chromium the two experimental groups were administered the following quantities of amino-acid chelated chromium: dry cows in groups 2 and 3 (0.6 and 1.2 mg Cr/kg DM), 7.68 and 15.36 mg Cr/d; lactating cows 11.28 and 22.56 mg Cr/d (Table 2). The daily quantity of chromium administered to dry cows in groups 2 and 3 was higher in both cases than that administered to the two experimental groups (4.25 and 5.50 mg Cr/d) by Yang

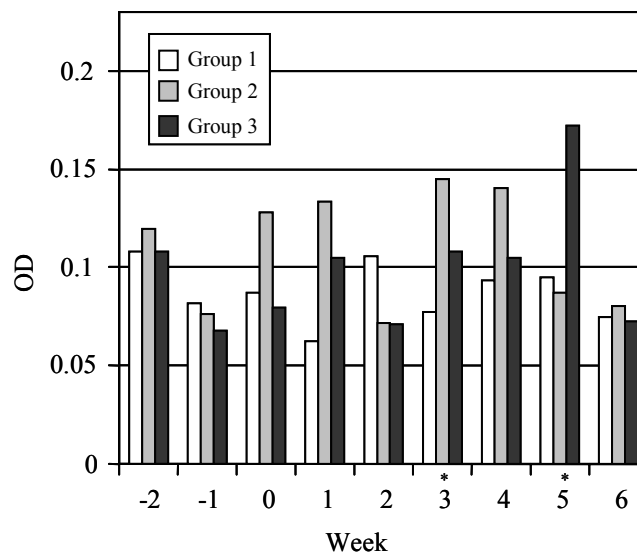


**Figure 1.** Weekly immune-enzymatic dosages (optical density, OD) developed by proteinaceous antigen stimulation (ovalbumin) of the three experimental groups (\*  $p < 0.10$ ; \*\*  $p < 0.05$ ).

et al. (1996) but similar to that administered to two experimental groups by Hayirli et al. (2001), which were given higher daily quantities of chromium (8.40 and 16.80 mg Cr/d). When considering the cows in lactation, the daily quantity of chromium administered to groups 2 and 3 was always higher than that administered by Yang et al. (1996) to the two experimental groups (7.75 and 10.25 mg Cr/d). The quantity given to group 2 was similar to that administered to the group with the higher content of chromium (12.43 mg Cr/d) by Peterson (2000) and between the higher two Cr levels (7.60 and 15.80 mg Cr/d) programmed by Hayirli et al. (2001). The quantity of chromium administered to group 3 (22.56 mg Cr/d) was 47% greater than the highest concentration (15.80 mg Cr/d) ever used before on lactating cows. When considering the chromium unit of measurement exclusively in mg/kgDM, the dose administered to group 2 was slightly higher than the single dose of 0.5 mg Cr/kgDM used for both dry and lactating cows by Burton et al. (1993) while that of group 3 was more than double. The daily quantity of chromium administered both with the diets and experimentally, for dry and lactating cows is reported in Table 2; such quantity is by far inferior to the maximum tolerable dietary level for dairy cattle, established at 3,000 ppm in oxide form (NRC, 1980). The dairy cows used for the experiments showed no health problems connected to the administration of chromium.

### Immune response

Figure 1 reports the results of the immune-enzymatic dosage to assess the antibody immunity of weekly sample. The responses of group 2 and 3 up to the second week after partum did not show relevant differences compared to group 1, while from the third week the response from group



**Figure 2.** Weekly  $\gamma$ -interferon dosages (optical density, OD) following specific stimulation (brucellergene) of the three experimental groups (\*  $p < 0.10$ ).

3 always resulted higher, with significant differences in the last two blood samples. In the fifth blood sample only the response from group 3 significantly increased compared to control (0.4611 vs. 0.2712 OD;  $p < 0.10$ ), while in the sixth both treated groups were higher (0.5230 and 0.4536 vs. 0.1812 OD;  $p < 0.05$ ). The response of the immune system to the treatment therefore results more intense in the last two weeks of lactation. The trend of data from both control and group 2 (0.6 mg Cr/kg DM) prove to be similar to the trend of results obtained by Burton et al. (1993) in control as well as the experimental group (0.5 mg Cr/kg DM): in particular a greater response of the immunity system at calving (+38% not significant, in our case; +43% significant, in the cited research).

Figure 2 reports the results from the  $\gamma$ -interferon dosages produced by specific stimulation on weekly samples. The values of the cell-mediated immune response evidence greater results, although not significant, in group 2 compared to group 3, up to four weeks after partum. Significant differences were observed in correspondence to the third and the fifth blood samples. In the third group 2 significantly increased compared to control (0.1453 vs. 0.0772 OD,  $p < 0.10$ ) while in the fifth group 3 significantly increased compared to group 2 (0.1720 vs. 0.0872 OD,  $p < 0.10$ ). Confronting the results of the cell-mediated immune response from control and group 2 with the results obtained by Burton et al. (1993) the following similarities were put into evidence. The greater response from group 2, compared to control, two weeks before and at partum, inverted the second week postpartum, coinciding with the second antigen administration, then re-establishing the same trend in both works.

From a wide-scale analysis of the anti-OVA and  $\gamma$ -IFN

**Table 3.** Effect of chromium supplementation on milk yield obtained at 42 and 60 days, also corrected to 4% fat (FCM), and milk quality parameters of the three experimental groups<sup>1</sup>

	42 days					60 days				
	Milk prod. (kg/d)	FCM (kg/d)	Fat (g/kg)	Protein (g/kg)	Lactose (g/kg)	Milk prod. (kg/d)	FCM (kg/d)	Fat (g/kg)	Protein (g/kg)	Lactose (g/kg)
Overall mean	33.08	28.76	31.27	29.81	47.82	33.10	28.67	31.05	29.70	47.23
Rmse	2.11	2.09	1.73	1.33	3.41	2.09	2.10	1.59	1.30	2.91
Group 1	31.05 <sup>b</sup>	26.99 <sup>b</sup>	31.23	29.71	47.55	30.93 <sup>b</sup>	26.69 <sup>b</sup>	30.81	29.58	47.15
Group 2	32.51 <sup>b</sup>	28.66 <sup>b</sup>	31.30	30.17	47.66	32.74 <sup>b</sup>	28.43 <sup>ab</sup>	31.23	30.08	46.95
Group 3	35.68 <sup>a</sup>	31.01 <sup>a</sup>	31.29	29.55	48.26	35.64 <sup>a</sup>	30.88 <sup>a</sup>	31.06	29.45	47.58
Primiparous	30.28 <sup>b</sup>	26.30 <sup>b</sup>	31.25	29.98	47.77	30.50 <sup>b</sup>	26.26 <sup>b</sup>	30.76	29.89	47.40
Multiparous	35.89 <sup>a</sup>	31.21 <sup>a</sup>	31.29	29.65	47.87	35.71 <sup>a</sup>	31.07 <sup>a</sup>	31.30	29.82	47.05
Group 1 * primip.	27.78 <sup>b</sup>	24.10 <sup>b</sup>	31.18	29.80	47.37	28.10 <sup>b</sup>	24.23	30.83	29.69	47.49
Group 2 * primip.	30.52 <sup>ab</sup>	26.52 <sup>ab</sup>	31.29	30.56	47.40	30.94 <sup>ab</sup>	26.72	30.91	30.43	47.15
Group 3 * primip.	32.52 <sup>a</sup>	28.29 <sup>a</sup>	31.31	29.58	48.54	32.45 <sup>a</sup>	27.84	30.53	29.54	47.57
Group 1 * multip.	34.32 <sup>b</sup>	29.88 <sup>b</sup>	31.28	29.62	47.73	33.76 <sup>b</sup>	29.15 <sup>b</sup>	30.78	29.47	46.82
Group 2 * multip.	34.50 <sup>b</sup>	30.01 <sup>b</sup>	31.32	29.79	47.91	34.53 <sup>b</sup>	30.14 <sup>b</sup>	31.54	29.72	46.75
Group 3 * multip.	38.84 <sup>a</sup>	33.73 <sup>a</sup>	31.28	29.53	47.98	38.33 <sup>a</sup>	33.93 <sup>a</sup>	31.59	29.37	47.59

<sup>1</sup> Means in the same column followed by different superscripts are significantly different (<sup>a,b</sup> p<0.05).

responses the following indications were obtained: in both cases, before and at partum, the immune system was stimulated much more by the lower quantity of chelated chromium. After partum there was a better response of the anti-OVA to a higher quantity of chromium administered while the response of  $\gamma$ -IFN was almost always higher with a lower quantity of chromium. Consequently the lower dose could be adequate before partum, the higher dose postpartum.

### Milk production

Table 3 reports the daily average milk yield obtained at 42 and 60 days and corrected to 4% fat (FCM), and the milk quality parameters of the three groups. The chromium effect on the milk yield in both treated groups was not significantly evident at 15 and 30 days when the animals are under greater stress. Considering the daily average yield of FCM at 42 days significant differences were observed between group 3 and the other two groups (31.01 vs. 26.99 and 28.66 kg/d, p<0.05). Concerning the average yield of FCM at 60 days, group 3 significantly increased compared to group 1 (30.88 vs. 26.69 kg/d, p<0.05). These data prove that through time the positive effect of the treatment remained on the milk yield of group 3. One hypothesis could be that the greater milk yield was determined by a greater intake of dry matter, even though this parameter was not measured for each group. As we asserted also Besong (1996) demonstrated that when chromium was administered the dry matter intake increased as also the milk yield. Also Hayirli et al. (2001) obtained an increase of dry matter intake and milk yield due to the effect of the chromium treatment, but up to a quantity of 7.60 mg Cr/head/d, but with 15.30 mg Cr/head/d obtained a reduction of both the dry matter intake and the milk yield. Also Simek et al.

(1999) and Popovic (2000) found an increase of the milk yield following chromium treatment, although Peterson (2000) did not verify any positive effect on the milk yield when this trace element was administered.

The significant differences (p<0.05) of the daily milk yield between primiparous and multiparous were within the expected values at both 42 and 60 days.

When considering the group $\times$ category interaction, the average daily yield of FCM obtained at 42 days from the three groups of primiparous is significantly different. The primiparous from group 3 produced more milk than control (28.29 vs. 24.10 kg/d, p<0.05); while at 60 days the effect of Cr does not provide significant results. As far as the multiparous are concerned, the FCM yield of group 3 significantly increased compared to groups 1 and 2 at both 42 and 60 days, and precisely: 33.73 vs. 29.88 and 30.01 kg/d, p<0.05; 33.93 vs. 29.15 and 30.14 kg/d, p<0.05. A diversification of the milk yield according to the category was found also by Burton (1995) in the primiparous during the first 35 days of lactation obtaining a 22.3% increase of FCM which dropped to 13.4% during the first 116 days of lactation, using 0.5 mg Cr/kg DM. By using a slightly higher dose of chromium (0.6 mg Cr/kg DM) we obtained an increase of only 10.0% of the milk yield at 42 days of lactation, this increased to 17.4% with a higher dose of chromium (1.2 mg Cr/kg DM). Burton (1995) obtained a higher milk yield up to 56 days for the multiparous from the group not treated and with a subsequent inversion tendency; in this work, an increase (3.4% for group 2 and 16.4% for group 3) of the average production of FCM at 60 days of lactation was obtained. Yang et al. (1996) also obtained a greater and significant yield in the primiparous when administering 7.75 mg Cr/d; contrarily when 10.25 mg Cr/d

was administered to the multiparous no variations in the milk yield were noticed between the experimental groups. The results from previous works, concerning the multiparous, contrast with our results, therefore it can be assumed that the doses used by other Authors were insufficient to balance the lack of chromium in the diets utilised.

Considering the quality of milk from the three groups, the fat and protein were kept almost constant, only the lactose in group 3 underwent greater variations compared to the other two values, but not significant as found by Hayirli et al. (2001).

Only traces of chromium were detected in the milk produced by animals from the three groups, confirming results obtained by Hayirli et al. (2001) where no differences of chromium content in the milk were observed between control and the experimental groups.

### CONCLUSION

The response to treatment using amino-acid chelated chromium resulted positive although diverse according to the physiological period and quantity of chromium administered. In the period before partum and at partum the positive response of the immune system is obtained with a lower dose of chromium. During lactation the positive response of the immune system is diverse: better results were obtained with a higher quantity of chromium, using the anti-OVA as an indicator; on the contrary when using the  $\gamma$ -IFN as an indicator better results were obtained with a lower quantity of chromium.

The milk yield is higher with both primiparous and multiparous when chromium is administered and results significant when a higher dose is used.

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### REFERENCES

- Abraham, A. B., B. A. Brooks and U. Eylath. 1991. Chromium and cholesterol-induced atherosclerosis in rabbits. *Ann. Nutr. Metab.* 35:203-207.
- Anderson, R. A., N. A. Bryden, M. M. Polansky and S. Reiser. 1990. Urinary chromium excretion and insulinogenic properties of carbohydrates. *Am. J. Clin. Nutr.* 55:864-868.
- AOAC. 1984. Official Methods of Analysis. 14<sup>th</sup> edn. Association of Official Analytical Chemists, Washington, DC.
- Besong, S. A. 1996. Influence of supplemental chromium picolinate on the concentrations of hepatic tryglyceride and blood metabolites in dairy cattle. Ph. D. Thesis, Kentucky Univ., Lexington.
- Bunting, L. D., J. M. Fernandez, D. L. Thompson and L. L. Southern. 1994. Influence of chromium picolinate on glucose usage and metabolic criteria in growing Holstein calves. *J. Anim. Sci.* 72:1591-1599.
- Burton, J. L. 1995. Supplemental chromium: its benefits to the bovine immune system. *Anim. Feed Sci. Technol.* 53:117-133.
- Burton, J. L., E. B. Burnside, B. W. Kennedy, B. N. Wilkie and J. H. Burton. 1989. Antybody response to human erythrocytes and ovoalbumin as marker traits of disease resistance in dairy calves. *J. Dairy Sci.* 72:1252-1265.
- Burton, J. L., B. A. Mallard and D. N. Mowat. 1993. Effects of supplemental chromium on immune responses of periparturient and early lactation dairy cows. *J. Anim. Sci.* 71:1532-1539.
- Goering, H. K. and P. J. Van Soest. 1970. Forage Fiber analysis. *Agriculture Handbook*. 379, UPSDA.
- Hayirli, A., D. R. Bremmer, S. J. Bertics, M. T. Socha and R. R. Grummer. 2001. Effect of chromium supplementation on production and metabolic parameters in periparturient dairy cows. *J. Dairy Sci.* 84:1218-1230.
- Kegley, E. B., J. W. Spears and T. T. Brown. 1997. Effect of shipping and chromium supplementation on performance, immune response, and disease resistance of steers. *J. Anim. Sci.* 75:1956-1964.
- Kornegay, E. T., Z. Wang, C. M. Wood and M. D. Lindemann. 1997. Supplemental chromium picolinate influences nitrogen balance, dry matter digestibility, and carcass traits in growing-finishing pigs. *J. Anim. Sci.* 75:1319-1323.
- Moonsie-Shageer, S. and D. N. Mowat. 1993. Effect of level of supplemental chromium on performance, serum characteristics, and immune status of stressed feeder calves. *J. Anim. Sci.* 71:232-238.
- NRC. 1980. Mineral Tolerance of Domestic Animals. National Academic Press, Washington, DC.
- NRC. 2001. Nutrient requirements of dairy cattle. 7<sup>th</sup> rev., National Academic Press, Washington, DC.
- Peterson, S. W. 2000. Effects of chromium picolinate on milk production and plasma insulin concentration in dairy cows. 60<sup>th</sup> Jubilee Conference, Hamilton, New Zealand. *Proc. New Zealand Soc. Anim. Prod.* 60:307-310.
- Popovic, Z., Z. Sinovec, S. Veselinovic, A. Ivancev, N. Ivancev, Z. Cupic and M. Vukic-Vranijes. 2000. Milk yield in heifers fed rations with chromium supplements during pregnancy. *Veterinarski Glasnik* 54:39-46.
- Sartin, J. L., R. J. Kemppainen, K. A. Cummins and J. C. Williams. 1988. Plasma concentrations of metabolic hormones in high and low producing dairy cows. *J. Dairy Sci.* 71:650-657.
- SAS. 1993. SAS/STAT<sup>®</sup> User's guide. SAS Institute Inc., Cary, NC.
- Schwartz, K. and W. Mertz. 1957. A glucose tolerance factor and its differentiation from factor 3. *Arch. Biochem. Biophys.* 72:515-518.
- Schwartz, K. and W. Mertz. 1959. Chromium (III) and the glucose tolerance factor. *Arch. Biochem. Biophys.* 85:292-295.
- Simek, M., V. Prokop, D. Vrzalova, M. Sustala and D. Zemanova.

1999. Trace elements (Cr, Se) and vitamin E in dairy nutrition. Vitamine und Zusatzstoffe in der Ernährung von Mensch and Tier: 7<sup>th</sup> Symposium Jena: 372-375.
- Van Soest, P. J., J. B. Robertson and B. A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber and nonstarch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* 74:3583-3597.
- Vighio, G. H. and R. M. Liptrap. 1990. Plasma hormone concentrations after administration of dexamethasone during the middle of the luteal phase in cows. *Am. J. Vet. Res.* 51:1711-1714.
- Yang, W. Z., D. N. Mowat, A. Subiyatno and R. M. Liptrap. 1996. Effects of chromium supplementation on early lactation performance of Holstein cows. *Can. J. Anim. Sci.* 76:221-230.