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# The Effect of Chromium Supplementation on Body Weight, Serum Glucose, Proteins, Lipids, Minerals and Ovarian Follicular Activity in Working Horses

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**Abstract:** This field trial was performed to investigate the effects of chromium ($Cr^{+3}$) on body weight, some serum parameters related to carbohydrate, lipid, protein and mineral metabolism, reproductive hormones and ovarian follicular activity of horses with poor body condition score. Twenty-four, quarter horses, aged from 4-13 years were evenly assigned into three groups. The animals received, orally, 0, 200 or 400 μg Cr daily for 45 days in the form of chromium picolinate (CrPic). All of the animals consumed the identical diets consisting of concentrate and hay throughout the study. Initial and final body weights of the animals were recorded. The number and size of the follicle developed in the ovaries were measured by an ultrasonograph and plasma estradiol and progesterone levels were determined at weekly intervals. Other blood chemistry variables were determined at the end of the study. Chromium had no effect on body weight, serum glucose, total protein, globulin, High Density Lipoprotein (HDL), Low Density Lipoprotein (LDL) and mineral levels. Chromium increased serum albumin in both groups, but the increase in 200 μg Cr supplemented group was significant ($p<0.01$). Both level of chromium slightly reduced total cholesterol concentration. A decrease was determined in triglycerides by 400 μg Cr ($p<0.05$). Serum chromium level was increased by 400 μg Cr ($p<0.01$). Follicle numbers and follicle size were slightly increased by 200 and 400 μg Cr respectively. In the treatment groups, a linear increase was determined in the estrus rate after the 4th week. In conclusion, changes in some biochemical parameters by 200 and 400 μg Cr supplementations and slight increases in follicular activity suggest that chromium may be of importance in field application. However, further detailed studies with various levels of chromium for longer period may be valuable to determine the biological functions of chromium in reproduction of horses.

**Key words:** Body weight, chromium, horse, ovarian activity, serum parameters

## INTRODUCTION

Chromium ($Cr^{+3}$) is considered as an essential nutrient for human and animals for many years (Mertz, 1969, 1993; McDowell, 1992; NRC, 1997). It is believed that chromium plays an integral role in Glucose Tolerance Factor (GTF) (Mertz, 1969), which trigger insulin activity through the formation of links between insulin and insulin receptors (Miranda and Dey, 2004, Anonim, 2005). Chromium is involved in carbohydrate, lipid, protein and nucleic acid metabolisms by enhancing the insulin signalling (Anderson, 1998, Offenbacher and Pi-Sunyer, 1988; Vincent, 2000) or through its insulin mimetic activity (Miranda and Dey, 2004).

It has been reported that growth retardation, alterations in glucose and lipid homeostasis due to the chromium deficiency (Mertz, 1969; Striffler *et al*., 1999) can be reversed by chromium supplementation (Riales and Albrink, 1981; Mossop, 1983; Striffler *et al*., 1999). In previous studies, chromium from different sources

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improved body condition in human (Clarkson,1997; Rubin *et al*., 1998), performance (Wenk *et al*., 1995; Mooney and Cromwell, 1997), carcass traits (Moonsie-Shageer and Mowat, 1993; Lindeman *et al*.,1995; Mooney and Cromwell, 1997; Uyanik, 2001) and immunity (Kegley *et al*., 1997; Uyanik *et al*. 2002a) in various animal species. However, inconsistent results have been reported concerning the effects of chromium on blood glucose, lipids, proteins and minerals (Page *et al*., 1993; Kitchalong *et al*., 1995; Ward *et al*., 1997; Mooney and Cromwell, 1997; Vincent, 2001; Uyanik, 2001; Uyanik *et al*., 2002b).

The amount of the chromium consumed through the food is low (Anderson, 1998). Furthermore, the chromium losses occur in stressed animals (Anderson *et al*., 1982, 1991; Vincent, 2001) and the beneficial effects of chromium is more pronounced in the stressed animals (Moonsie-Shageer and Mowat, 1993). Strenuously exercised horses fed high grain diets excrete more chromium in their urine than sedentary horses (Anonim, 2005). Therefore, it can be expected that horses subjected to intense exercise may benefit from chromium supplementation. However, to the authors' knowledge, only a few studies investigated the role of chromium in the metabolism of horses and controversies exist between the results. In the study by Pagan *et al*. (1995) that conducted on exercising horses, supplementation of 5 mg of chromium as Cr-enriched yeast for 14 days had beneficial effect on glucose metabolism. Ott and Kivipelto (1999) found that chromium tripicolinate increased the rate at which glucose is metabolized and reduced glucose, but they found no effect of chromium on insulin sensitivity and growth rate of yearling horses. Gentry *et al*. (1999) found no significant effects of chromium on glucose metabolism in healthy adult mares following i.v. glucose challenge. In a more recent study, Vervuert *et al*. (2005), found no beneficial effect of chromium supplementation in the form of yeast-chromium on glucose and insulin concentrations in healthy, exercising horses. However, there is no study investigating the effect of chromium on ovarian follicular activity in horses. Therefore, this study was performed to try to confirm the results of previous studies concerning the effects of chromium on body weight, selected blood metabolites and to determine the effects of chromium on ovarian follicular activity of horses with poor body condition.

## MATERIALS AND METHODS

**Animals, management and experimental design:** Twenty-four, quarter horses, with body condition score of 1-2, reared under the field condition were used in this study. The age of the animals were ranged from 4-13 years. The horses were individually housed in box stalls at feeding times and at night. Animals had free access to a sand paddock and they underwent strenuous exercise through a walking tour programme at least 3 h daily or all of the day time. Animals were evenly assigned into three groups consisting of 8 horses (1 male and 7 female) in each as control and treatments according to their initial weights and body condition scores. Each animal in all groups consumed the identical diets consisting of 4 kg of concentrate (grounded barley:oat:bran at the ratio of 1:0.5:1) and 6-7 kg of hay daily without any mineral or vitamin supplements, throughout the study, as the routine feeding regimen of the centre, on which this study was performed. The animals received orally either 0 (control group), 200 or 400 μg Cr (treatment groups) daily in the form of CrPic as bolus in gelatine capsules for 45 days.

**Body weight measurements and body condition scoring:** Initial and final body weights and body condition scores of the animals were recorded with the method described by Ellis (2000).

**Sample collection and analysis:** The number and size of the follicle developed in the both ovaries of 7 mares were measured by an ultrasonograph (Honda H3000) with 5 mHz transrectal probes at weekly intervals. At the times of the ultrasonography, blood samples from V. jugularis were collected into tubes with anticoagulant. At the end of the study, blood samples were collected into the tubes without anticoagulant following 12 h fasting. Blood samples with heparin were immediately centrifuged to separate the plasmas and blood samples without anticoagulant were centrifuged at 1500 g for 10 min. after one hour incubation at room temperature to separate the sera. The samples were transported to the laboratory in ice and stored at -20°C until the analysis.

Serum glucose, total protein, albumin, Calcium (Ca), inorganic Phosphorus (Pi), Magnesium (Mg) (Chema Diagnostica, Italy), triglycerides, total cholesterol, High Density Lipoprotein (HDL), Low Density Lipoprotein (LDL) (BioSystems, Spain) concentrations were determined with commercially available kits by a Shimadzu UV/VIS 1208 model spectrophotometer. Serum globulin levels were calculated by subtracting albumin values from total protein values. Plasma estradiol (DSL, USA, cat no:DSL4800) and progesterone (DSL, USA, cat no:DSL3900) levels were determined with Izocomp-I Gamma Counter (MGM Instruments Inc. USA) using commercially available Radioimmunoassay kits in the Department of Gynaecology and Obstetrics, Faculty of Veterinary Medicine, University of Istanbul, Istanbul,

Turkey. Sera were analyzed for chromium levels by an atomic absorption spectrophotometer (Varian AA 880) equipped with a graphite furnace (GTA-110). The sera were mixed with 0.1% of triton-X (1:1) and then chromium levels were measured.

**Statistical analysis:** Data were analysed by SPSS 9.0 version for Windows. One-way Analysis of Variance (ANOVA) was used for the differences between groups for body weight and biochemical data. When the F values were significant, Duncan's Multiple Range Test was performed The data of ovarian follicle activity were analyzed by Mann-Whitney U test. All data were expressed as means±SEMs. Differences were considered as significant at $p<0.05$.

## RESULTS

Daily supplementation of 200 and 400 μg Cr in the form of chromium picolinate had no effect on body weight (Table 1). Both levels of chromium did not affect serum glucose, total protein, globulin, HDL, LDL and mineral levels. Chromium increased serum albumin in both group, but solely the increase in 200 μg Cr supplemented group was significant ($p<0.01$). Both levels of chromium slightly reduced total cholesterol. A decrease was determined in triglycerides level with 400 μg chromium supplementation ($p<0.05$). Serum chromium concentrations were increased reaching the significance ($p<0.01$) with 400 μg Cr supplementation (Table 2). Although, it was not significant, follicle numbers (Fig. 1) in 200 μg Cr supplemented group and follicle size (Fig. 2) in 400 μg Cr supplemented group were increased. In treatment groups, a linear increase was determined in the estrus rate after the 4th week, whereas tended to fluctuate in control group. An increase was found in the estrus cycle number in one breeding season (Fig. 3 and 4).

Table 1: Effects of chromium picolinate on body weight of horses

| | Chromium supplementation | | | |
|---|---|---|---|---|
| | Control n:8 Mean±SEMs | 200 μg Cr n:8 Mean±SEMs | 400 μg Cr n:8 Mean±SEMs | p-value |
| Initial body weight (kg) | 355.75±22.44 | 362.13±22.13 | 363.13±31.64 | - |
| Final body weight (kg) | 344.50±27.05 | 341.63±23.09 | 343.13±33.74 | - |
| Difference | -11.25±10.82 | -20.50±7.87 | -23.00±8.02 | - |

-: Not significant

Table 2: Effects of chromium picolinate on blood chemistry of horses

| | Chromium Supplementation | | | |
|---|---|---|---|---|
| Parameters | Control n:8 Mean±SEMs | 200 μg Cr n:8 Mean±SEMs | 400 μg Cr n:8 Mean±SEMs | p-value |
| Glucose (mg $dL^{-1}$) | 85.2±10.83 | 88.8±6.59 | 84.6±3.91 | - |
| Total protein (g $dL^{-1}$) | 6.1±0.27 | 5.4±0.24 | 6.0±0.44 | - |
| Albumin (g $dL^{-1}$) | 2.0±0.09$^{b}$ | 2.5±0.08$^{a}$ | 2.2±0.12$^{b}$ | ** |
| Globulin (g $dL^{-1}$) | 4.1±0.28 | 3.0±0.27 | 3.7±0.52 | - |
| Triglycerides (mg $dL^{-1}$) | 13.8±1.51$^{a}$ | 14.1±1.79$^{a}$ | 7.84±1.48$^{b}$ | * |
| Total cholesterol (mg $dL^{-1}$) | 262.4±5.34 | 255.7±2.44 | 252.2±2.69 | - |
| HDL (mg $dL^{-1}$) | 17.0±1.11 | 16.4±1.15 | 14.8±0.68 | - |
| LDL (mg $dL^{-1}$) | 242.6±5.23 | 236.4±2.95 | 235.9±2.25 | - |
| Ca (mg $dL^{-1}$) | 11.2±0.44 | 10.2±0.53 | 11.2±0.26 | - |
| Pi (mg $dL^{-1}$) | 6.8±0.49 | 6.9±0.85 | 6.5±0.51 | - |
| Mg (mEq $L^{-1}$) | 1.8±0.04 | 1.8±0.05 | 1.7±0.05 | - |
| Cr (ng $mL^{-1}$) | 0.8±0.13$^{b}$ | 0.9±0.08$^{b}$ | 4.3±0.48$^{a}$ | ** |

-: Not significant *: $p<0.05$ **:$p<0.01$, $^{a,b}$ Means with different superscripts within the same row differ significantly

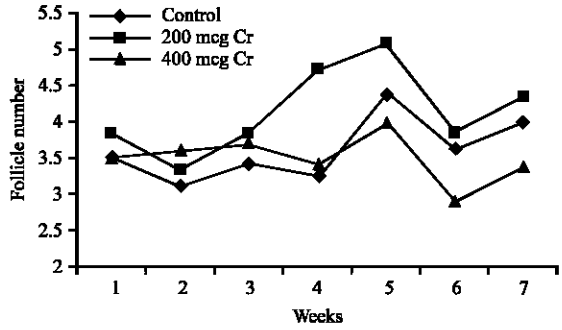


Fig. 1: Effects of chromium picolinate on follicle numbers on ovaries of mares

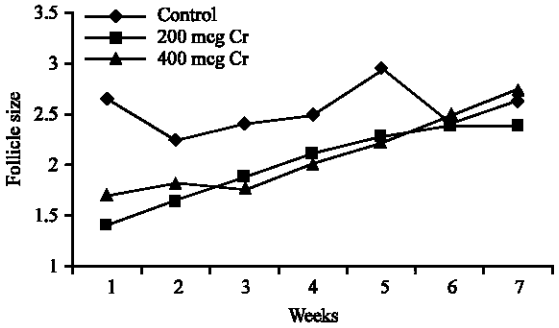


Fig. 2: Effects of chromium picolinate on follicle size on ovaries of mares

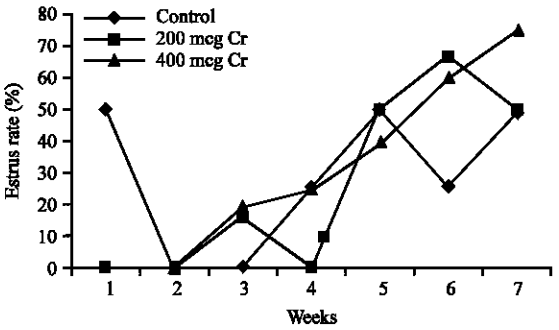


Fig. 3: Effects of chromium picolinate on estrus rate of mares

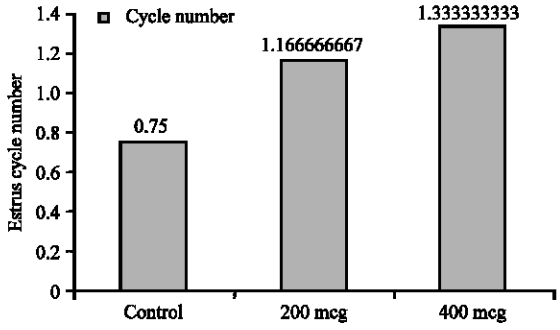


Fig. 4: Effects of chromium picolinate on estrus cycle numbers of mares in one breeding season

## DISCUSSION

Some previous studies have shown that chromium supplementation from different sources in various animal species had no effect on weight gain (Kitchalong *et al*., 1995; Kegley and Spears, 1995; Besong *et al*., 2001; Uyanik, 2001; Uyanik *et al*., 2002a). Similarly, in the present study, lack of the effect of chromium on body weight confirmed the results of Ott and Kivipelto (1999) who fed similar levels of chromium supplemented diet to yearling horse.

In the present study, no effect of chromium was seen on serum concentrations of total protein and globulin (Kitchalong *et al*., 1995; Moonsie-Shageer and Mowat, 1993; Mooney and Cromwell, 1997; Uyanik, 2001) as indicated previously. Consistent with the results of the studies conducted on stressed feeder calves, the elevation in albumin levels may result from the increased amino acid synthesis (Moonsie-Shageer and Mowat, 1993).

Chromium supplementation had no effects on glucose level consistent with the results of some previous studies (Moonsie-Shageer and Mowat, 1993; Kegley *et al*., 1997; Bryan *et al*., 2004). On the other hand, some other studies have shown that chromium from organic or inorganic chromium sources reduces the serum glucose (Uyanik, 2001; Mooney and Cromwell, 1997; Ward *et al*., 1997; Uyanik *et al*., 2002a). Pagan *et al*. (1995) conducted a study on exercising horses and they reported the beneficial effect of 5 mg of chromium as Cr-enriched yeast on glucose metabolism. In another horse study, Ott and Kivipelto (1999) found a reduction in glucose level with chromium tripicolinate, but they found no effect of chromium on insulin sensitivity. However, Gentry *et al*. (1999) found no significant effects of chromium on glucose metabolism in healthy adult mares and Vervuert *et al*. (2005) also found no beneficial effect of chromium supplementation on glucose and insulin concentrations in healthy, exercising horses as in the present study.

Chromium at the levels used in this study did not affect serum lipid variables except triglycerides, which is reduced as indicated in previous studies (Striffler *et al*., 1998; Uyanik *et al*., 2002b). In contrast, Pagan *et al*. (1995) found an elevated triglycerides level in horses after 15 and 30 min. of exercises. In the study of these authors, triglycerides levels had been measured after exercise, but in the present study, the measurement were performed after a resting period at least a night. The diversity of the results of this study from the results of Pagan *et al*. (1995) may be due to differences in the timing of the measurements as well as experimental procedures. Lack of the effects of chromium supplementation on serum macro minerals confirmed the results of Page *et al*. (1993).

To the authors' knowledge, no findings were cited in the literature concerning the effect of chromium on reproductive performance in horses. Although statistically not significant, increases in follicle numbers and sizes by 200 and 400 μg CrPic supplementations as well as a linear increase in estrus rate by 400 μg CrPic were confirmed by the hormone levels. The increases in the numbers of the follicle developed in ovaries may suggest that chromium has a positive effect on reproductive performance confirming the results of the pig studies of Lindeman *et al*. (1995) and Campbell (2004). These authors found an increased birth rate with the supplementation of chromium at a level of 200 μg in the form of CrPic. Furthermore, Aragon *et al*. (2001) also reported that chromium supplementation from a high-Cr yeast reduced the interval from calving to first estrus and tended to improve pregnancy rate. In conclusion, changes in some biochemical parameters by 200 and 400 μg Cr supplementations and slight increases in ovarian follicle numbers and sizes showed that chromium may be of importance in the horses breeding. However, further detailed studies on horses with various levels of CrPic for longer durations may be warranted.

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## REFERENCES

Anderson, R.A., 1998. Recent advances in the clinical and biochemical manifestation of chromium deficiency in human and animal nutrition. J. Trace Elem. Experim. Med., 11: 241-250.

Anderson, R.A., M.M. Polansky, N.A. Bryden, E.E. Roginski, K.Y. Patterson, C. Veillon and W. Glinsmann, 1982. Urinary chromium excretion of human subjects: Effects of chromium supplementation and glucose loading. Am. J. Clin. Nutr., 36 (6): 1184-1193.

Anderson, R.A., M.M. Polansky, N.A. Bryden and J.J. Canary, 1991. Supplemental-chromium effects on glucose, insulin, glucagon and urinary chromium losses in subjects consuming controlled low-chromium diets. Am. J. Clin. Nutr., 54: 909-916.

Anonim, 2005. Nutrition for insulin resistant horses. J. Equine Vet. Sci., 25 (7): 316.

Aragon, V.E.F., D.S. Graca and A.L. Notre, 2001. Supplemental high chromium yeast and reproductive performance of grazing primiparous Zebu cows. Arg. Bras. Med. Vet. Zool., 53: 624-628.

Besong, S., J.A. Jackson, D.S. Trammell and V. Akay, 2001. Influence of supplemental chromium on concentrations of liver triglyceride, blood metabolites and rumen VFA profile in steers fed moderately high fat diet. J. Dairy Sci., 84 (7): 1679-1685.

Bryan, M.A., M.T. Socha and D.J. Tomlinson, 2004. Supplementing intensively grazed late-gestation and early-lactation dairy cattle with chromium. J. Dairy Sci., 87: 4269-4277.

Campbell, R.G., 2004. The effects of chromium picolinate on the fertility and fecundity of sows under commercial conditions. General Manager Technical Services. Bunge Meat Industries Ltd. Corowa NSW. Australia.

Clarkson, P.M., 1997. Effects of exercise on chromium levels. Is supplementation required. Sports Med., 23 (6): 341-349.

Ellis, P., 2000. Condition scoring and weight estimation of horses. Agriculture Notes.

Gentry, L.R., D.L. Thompson, J.M. Fernandez, L.A. Smith D.W. Horohov and B.S. Leise, 1999. Effects of chromium tripicolinate supplementation on plasma hormones and metabolite concentrations and immune functions in adult mares. J. Equine Vet. Sci., 19: 259-265.

Kegley, E.B. and J.W. Spears, 1995. Immune response, glucose metabolism and performance of stressed feeder calves fed inorganic or organic chromium. J. Anim. Sci., 73: 2721-2726.

Kegley, E.B., J.W. Spears and J.H. Eisemann, 1997. Performance and glucose metabolism in calves fed a chromium-nicotinic acid complex or chromium chloride. J. Dairy Sci., 80 (8): 1744-1750.

Kitchalong, L., J.M. Fernandez, L.D. Bunting, L.L. Southern and T.D. Bidner, 1995. Influence of chromium tripicolinate on glucose metabolism and nutrient partitioning in growing Lambs. J. Anim. Sci., 73: 2694-2705.

Lindeman, M.D., C.M. Wood, A.F. Harper, E.T. Kornegay and R.A. Anderson, 1995. Dietary chromium picolinate additions improve gain: Feed and carcass characteristics in growing finishing pigs and increase litter size in reproducing sows. J. Anim. Sci., 73 (2): 457-465.

McDowell, L.R., 1992. Minerals in Animal and Human Nutrition. Academic Press, pp: 265-293.

Mertz, W., 1993. Chromium in human nutrition: A review. J. Nutr., 123: 626-633.

Mertz, W., 1969. Chromium occurrence and function in biological system. Am. Physiol. Soc., 49: 163-239.

Miranda, E.R. and C.S. Dey, 2004. Effect of chromium and zinc on insulin signalling in skeletal muscle cells. Biol. Trace Elem. Res., 101 (1): 19-36.

Mooney, K.W. and G.L. Cromwell, 1997. Efficacy of chromium picolinate and chromium chloride as potential carcass modifiers in swine. J. Anim. Sci., 75 (10): 2662-2671.

Moonsie-Shageer, S. and D.N. Mowat, 1993. Effects of level of supplemental chromium on performance, serum constituents and immune status of stressed feeder calves. J. Anim. Sci., 71: 232-238.

Mossop, R.T., 1983. Effects of chromium III on fasting blood glucose, cholesterol and cholesterol HDL levels in diabetics. Centrl. Afr. J. Med., 29: 80-82.

National Research Council (NRC), 1997. The Role of Chromium in Animal Nutrition. National Academy Press. Washington D.C.

Offenbacher, E.G. and F.X. Pi-Sunyer, 1988. Chromium in human nutrition. Ann. Rev. Nutr., 8: 543-563.

Ott, E.A. and J. Kivipelto, 1999. Influence of chromium tripicolinate on growth and glucose metabolism in yearling horses. J. Anim. Sci., 77: 3022-3030.

Pagan, J.D., S.G. Jackson and S.E. Duren, 1995. The effect of chromium supplementation on metabolic response to exercise in thoroughbred horses. Proc. Alltech 11th Ann. Symp: Biotechnology in the Feed Industry University Press, Nottingham, UK.

Page, T.G., L.L. Southern, T.L. Ward and D.L. Thompson, 1993. Effects of chromium picolinate on growth and serum and carcass traits of growing-finishing pigs. J. Anim. Sci., 71: 656-662.

Riales, R. and M.J. Albrink, 1981. Effect of chromium chloride supplementation on glucose tolerance and serum lipids including high-density lipoprotein of adult men. Am. J. Clin. Nutr., 34: 2670-2678.

Rubin, M.A., J.P. Miller, A.S. Ryan, M.S. Treuth, K.Y. Patterson, R.E. Pratley, B.F. Hurley, C. Veillon, P.B. Moser-Veillon and R.A. Anderson, 1998. Acute and chronic resistive exercise urinary chromium excretion in men as measured with an enriched chromium stable isotope. J. Nutr., 128: 73-78.

Striffler, J.S., M.M. Polansky and R.A. Anderson, 1999. Overproduction of insulin in the chromium-deficient rat. Metabolism, 48: 1063-1068.

Uyanik, F., 2001. The effects of dietary chromium supplementation on some blood parameters in sheep. Biol. Trace Elem. Res., 84: 93-101.

Uyanik, F., A. Atasever, S. Özdamar and F. Aydın, 2002a. Effects of dietary chromium chloride supplementation on performance, some serum parameters and immune response in broilers. Biol. Trace Elem. Res., 90: 99-115.

Uyanık, F., Ş. Kaya, A.H. Kolsuz, M. Eren and N. Şahin, 2002 b. The Effect of chromium supplementation on egg production, egg quality and some serum parameters in laying hens. Turk. J. Vet. Anim. Sci., 26: 379-387.

Vervuert, I., D. Cuddeford and M. Coenen, 2005. Effects of chromium supplementation on selected metabolic responses in resting and exercising horses. Equine Comp. Exerc. Physiol., 3 (1): 19-27.

Vincent, J.B., 2000. The biochemistry of chromium. J. Nutr. 130: 715-718.

Vincent, J.B., 2001. The bioinorganic chemistry of chromium (III). Polyhedron, 20: 1-26.

Ward, T.L., L.L. Southern and T.D. Bidner, 1997. Interactive effects of dietary chromium tripicolinate and crude protein level in growing-finishing pigs provided inadequate and adequate pen space. J. Anim. Sci., 75: 1001-1008.

Wenk, C., S. Gebert and H.P. Pfirter, 1995. Chromium supplements in the feed for growing pigs and meat quality. Arch. Tierernahr., 48 (1-2): 71-81.