Effect of Cr picolinate and Zn supplementation on plasma cortisol and some metabolite levels in Charolais hoggets during acclimatization

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ABSTRACT

The effect of Cr and Cr+Zn treatment on adrenal response to stress in newly imported Charolais hoggets after the quarantine period was investigated. Chromium and Cr+Zn implication in carbohydrate and protein metabolism as evidenced by the levels of some metabolites was studied, too.

Eighteen one year old Charolais hoggets were divided into three groups: control group, Cr and Cr+Zn experimental groups- given supplemental dietary Cr picolinate and $ZnSO_4 \cdot 7H_2O$ respectively. Plasma cortisol, glucose, cholesterol, urea and indole levels were measured.

Supplemental Cr+Zn caused cortisol decline by 2nd and increase by 9th day, but didn't have any influence on 5th day. Chromium alone had no significant effect on plasma cortisol level by 2nd day but increased cortisol level by 9th day. Plasma glucose level was not influenced by supplemental Cr or Cr+Zn. Supplemental Cr+Zn caused significant decline in plasma cholesterol, urea and indole levels by 2nd day. It is suggested that the enumerated effects of supplemental Cr+Zn are mediated via the hypothalamic-pituitary adrenal axis.

Key words: Chromium, zinc, Charolais hoggets, cortisol, glucose, cholesterol, urea, indole

INTRODUCTION

Trivalent Cr is an essential trace mineral that is involved in carbohydrate, lipid, and protein metabolism. Mertz et al. (1974) showed that Cr potentiates the effects of insulin and, therefore, improves carbohydrate metabolism, and they concluded that protein synthesis may be affected positively as well.

In addition, Chang and Mowat (1992) found that supplemental Cr seemed to improve the immune status of stressed ruminants; however, these effects are not consistent (Chang et al., 1994; Mathison and Engstrom, 1995). Chromium tripicolinate was used as the Cr source because previous research has suggested that Cr is more available as an organic chelate (Evans, 1982; Chang et al., 1992; Page et al., 1993).

The ability of zinc to retard oxidative processes has been recognized for many years. In general, the mechanism of antioxidation can be divided into acute and chronic effects. Chronic effects involve exposure of an organism to zinc on a long-term basis, resulting in induction of some other substance that is the ultimate antioxidant, such as the metallothioneins. Chronic zinc deprivation generally results in increased sensitivity to some oxidative stress. The acute effects involve two mechanisms: protection of protein sulfhydryls or reduction of -OH formation from H_2O_2 through the antagonism of redox-active transition metals, such as iron and copper (Afanas et al., 1995; Aiuto et al., 1995).

Most of the Cr nutrition studies have focused on the role of Cr in preventing insulin resistance, however, interactions among insulin sensitizers and antioxidants should also be evaluated as suggested by Preuss (1998) and Anderson et al. (2001).

Hence, the objectives of this article are:

- To study the effect of Cr and Cr+Zn treatment on adrenal response to stress of newly imported Charolais hoggets after the quarantine period;
- To investigate Cr and Cr+Zn implication in carbohydrate and protein metabolism as evidenced by the levels of some metabolites.

MATERIAL AND METHODS

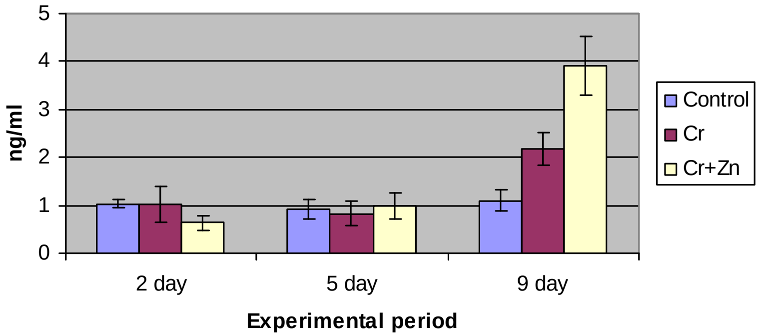
Eighteen one year old Charolais hoggets were transported from the mountainous base ”Zlatusha” after the quarantine period following importation from France to the experimental barn of the Institute of Animal Science – Kostinbrod. They were divided into three groups as follows: control group – fed according to the nutritional standards and two experimental groups. Hoggets in the first experimental group were given organic chromium as Cr picolinate (“Kromisan”®, produced by “Jastfri-Lactosfri Sockerfri”-Finland) and that in second experimental group - Cr picolinate plus Zn given as $ZnSO_4.7\ H_2O$. Basal diet was formulated according to nutritional requirements for the corresponding age (NRC). Chromium and Zinc content in basal diet was not determined since the effect of Cr depends on the intensity and duration of the applied stress stimuli.

Hoggets in the experimental groups were treated for 9 consecutive days with Cr and Cr+Zn solutions given *per os* and blood samples were taken by jugular venipuncture after 2nd, 5th and 9th treatment. Chromium concentration in both experimental groups was 100 µg per animal in the first treatment and 300 µg in the other treatments. Zinc concentration in the second experimental group was 30 mg per animal, daily.

Cortisol level was determined by radioimmunoassay (Kanchev et al., 1976). All assays were performed in duplicate. Inter- and intra-assay coefficients of variation were 9.9% and 6.7% respectively. Plasma glucose level was determined by the method of Ceriotti as modified by Profirov (1990) and plasma total cholesterol and indole levels were measured by the method of Watson (1960) and Balakovsky (Chilov, 1959), respectively. Plasma urea levels were assayed as described by Rerat et al., 1979.

The results of one factor statistical analysis are expressed as means ± S.E.M. and were analyzed by Student t-test. When the results were statistically processed by the use of two factors analysis the difference was less than 3%.

Fig. 1: Plasma cortisol level



RESULTS AND DISCUSSION

Chromium supplementation had no significant effect on plasma cortisol levels by the 2nd and 5th day after the transport and accommodation of hoggets to the new environment (Fig. 1). Presently Cr sufficiency is assessed by the ability of supplemental Cr to reduce stress-induced changes. The very fact that supplemental Cr did not influence plasma cortisol level suggests that the intensity and duration of stress-eliciting factors under the conditions of our experiment were not enough to reduce body stores of Cr. Another noteworthy point is that the increase of plasma cortisol level on the 9th day ($p<0.01$) is not consistent with the results of previous studies with Cr supplementation that have reported decreasing or no effect on plasma cortisol levels. This increase could be due to the insulin-potentiating effect of Cr that ultimately reduces plasma glucose level. Therefore the increase in plasma cortisol level could be considered as a homeostatic mechanism aimed at increasing plasma glucose level and its maintenance within normal range. The lack of significant differences in plasma glucose level between the three groups supports this hypothesis (Fig. 2).

This phenomenon was even more pronounced in 3rd experimental group supplemented with both Cr and Zn. It seems that the combination Cr+Zn had higher insulin-potentiating effect than Cr alone as suggested by the observed cortisol-reducing effect by the 2nd day. Yet, more investigations are needed to elucidate the established effect of Cr or Cr+Zn on plasma cortisol level by 9th day.

Chromium (in 1st experimental group) or Cr+Zn (in 2nd experimental group) supplementation decreased plasma cholesterol levels during the experimental period except on the 2nd day in the Cr treated group (Fig. 3). These data are in accordance with our previous study (Yanchev et al., 2003) and with most of the investigations in this field. Despite the numerous hypotheses concerning the cholesterol reducing effect of Cr and Zn, the mechanism of their effect is not fully explained.

Fig. 2: Plasma glucose level

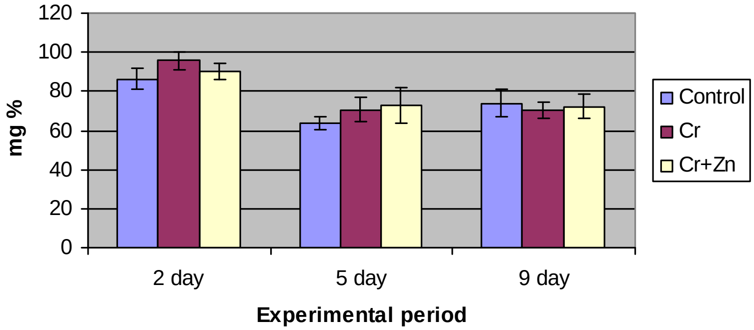
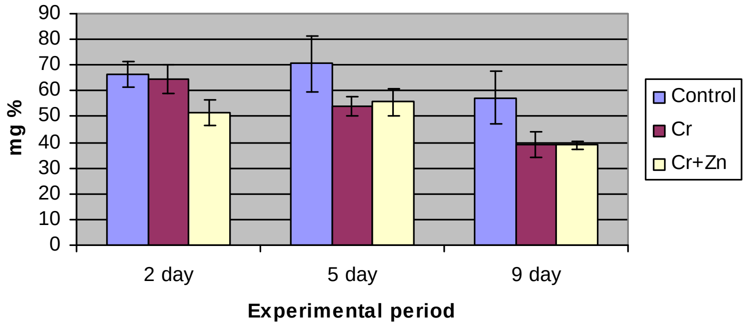


Fig 3: Plasma cholesterol level



Both Cr and Cr+Zn supplementation had no significant effect on plasma urea level by 5th and 9th day. However the combination Cr+Zn caused synergistic urea-decreasing effect by 2nd day (Fig. 4). This finding is in accordance with the above mentioned view that supplemental Cr+Zn has better stress-alleviating effect than Cr supplement, given alone. These results are in contrast with those, found in our previous study cited above, where we found higher plasma urea levels in weaned lambs given inorganic Cr. The inconsistent results reported in many studies could result from factors such as experimental design, age of the experimental animals, chemical form of the supplemental Cr, diet structure, etc.

Fig. 4: Plasma urea level

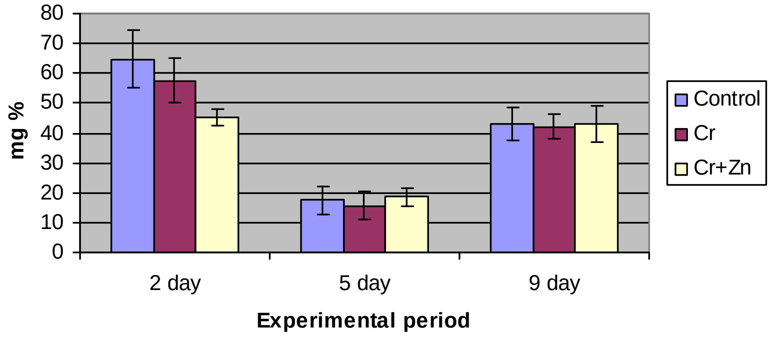
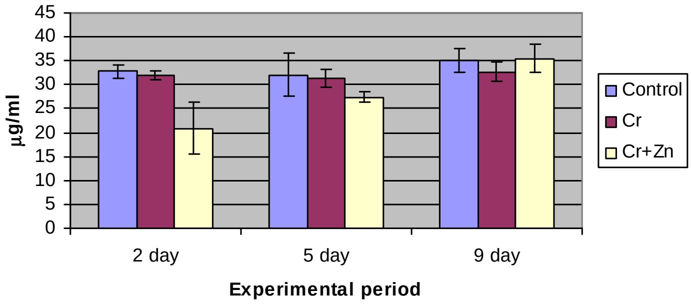


Fig 5: Plasma indol level



It is barely coincidental that Cr+Zn induced decrease in plasma cortisol level on 2nd day was accompanied by a decline in cholesterol and urea levels. Puvadolpirod and Thaxston (2000) have reported that exogenous adrenocorticotrophin increased plasma cholesterol level. Therefore the observed decline of plasma cholesterol level on 2d in 3rd group of hoggets was probably due to Cr+Zn induced decline in plasma cortisol and adrenocorticotrophin respectively. However this explanation is not consistent with the observed lower plasma cholesterol levels on 5d and 9d in groups II and III despite the unchanged or increased cortisol levels on 5 and 9d respectively. Urea level can be influenced by the activity of hypothalamic-pituitary adrenal axis as well. Adrenal secretion of cortisol is controlled by hypothalamic release of corticotrophin releasing hormone which is known to affect the rate of digesta passage. Besides it decreases feed intake (Lenz, 1987). Consequently, the decline of cortisol as a result of decreased corticotrophin releasing hormone, by 2nd day in Cr+Zn supplemented hoggets could provoke changes in the rate of feed intake and the rate of feed transition. These corticotrophin releasing hormone induced changes are directly related with the rate of ammonia release and its utilization by rumen microflora for synthesis of its own proteins.

Our results suggest that supplemental Cr+Zn may have caused reduced diet consumption, less feed protein intake as established Gentry et al. (1999), lower level of released NH_3 and better NH_3 utilization by rumen microorganisms, thus reducing the level of ammonium that has to be detoxicated as urea in the liver.

Plasma indole level in 3rd group of hoggets given supplemental Cr+Zn were significantly lower ($p<0.05$) by 2nd day, as compared to control and Cr supplemented group (Fig. 5). Indole is a toxic gas produced by some bacteria which colonize the large intestine. Therefore supplemental Cr+Zn may protect against these bacteria, perhaps by altering the intestinal flora, thus reducing indole level. Presently we don't know how supplemental Cr+Zn exert their beneficial effect on these bacteria. However the possibility cannot be excluded that supplemental Cr+Zn affect tryptophan availability to bacteria and it is known that tryptophan is predecessor of indole (Claus and Raab, 1999).

CONCLUSIONS

- Supplemental Cr+Zn caused cortisol decline by 2nd and increase by 9th day, but didn't have any influence on 5th day. Chromium alone had no significant effect on plasma cortisol level by 2nd day but increased cortisol level by 9th day;
- Plasma glucose level was not influenced by supplemental Cr or Cr+Zn ;
- Supplemental Cr+Zn caused significant decline in plasma cholesterol, urea and indole levels by 2nd day. It is suggested that the enumerated effects of supplemental Cr+Zn are mediated via hypothalamic-pituitary adrenal axis.

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