# Effect of Supplementing Chromium on the Blood Chemistry Profiles of Equines Used for Antitoxin Production

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### **Abstract**

For this study, twenty-four healthy equines, were randomly divided in three equal groups viz,  $T_0$ ,  $T_1$  and  $T_{\nu}$  each having eight animals (four mules and four ponies). Group  $T_0$  served as control and received ration as per standard feeding practice followed on the farm. Group T<sub>1</sub> and T<sub>2</sub> were fed with same ration as used for group  $T_0$  supplemented with chromium tripicolinate @ 210 and 420  $\pi$ g/kg ration, respectively. The feed treatments had no significant effect on the average blood glucose and haemoglobin values of the animals from different groups. The average PCV values recorded for group  $T_2$  were significantly (P $\leq$ 0,01) lower than that of groups T<sub>0</sub> and T<sub>1</sub> However, differences between groups T<sub>0</sub> and T<sub>1</sub> were statistically nonsignificant. The chromium supplement had no significant effect on average neutrophyls counts, average percent lymphocytes, eosinophils, monocytes and basophils counts and average total serum protein of equines from different groups. The average serum albumin values and average serum albumin: globulin ratios values of the animals from control group  $(T_0)$  were significantly  $(Pd \le 0.01)$  higher than that of other two groups. The average serum globulin values of the animals from the control group were significantly  $(P \le 0.01)$  lower than that of other two groups supplemented with chromium. However, groups  $T_1$  and  $T_2$ did not differ significantly from each other. The average serum tryglyceride values of the animals from control group were significantly ( $P \le 0.01$ ) higher as compare to other two groups. Futrther values for group  $T_1$  were significantly (P $\leq$ 0,01) higher when compared with group  $T_2$ . The chromium supplementation had no significant effect on average serum cholesterol values of the animals from various experimental groups. The average serum LDL values observed for T₁ and T₂ groups were significantly (P≤0,01) lower as compared to control group. The average serum HDL values for groups  $T_1$  and  $T_2$  were significantly (P $\leq$ 0,01) higher as compared to T<sub>0</sub> group. However, values for group T<sub>1</sub> and T<sub>2</sub> were comparable. Chromium supplementation had no effect on average serum VLDL values of the animals from different groups. Thus it is concluded from over all results of the present study that chromium tripicolinate can be supplemented to equines for better antisera production programme without affecting blood chemistry profile and animal well being.

**Keywords:** Blood glucose; Hemoglobin; Serum triglyceride; Cholesterol; LDL; HDL; VLDL.

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## Introduction

Chromium is essential trace element for normal carbohydrate metabolism which potentiates insulin action and stimulate glucose clearance and also aids in the conversion of thyroxin to tri-iodothyronine which results in increasing metabolic rate. Although lot of literature is available on the role of chromium on glucose metabolism in various species of animals; very scanty information is available on the effect of chromium on horses used for anti-snake venom production which remain under stress due to regular toxin antigen dosing and frequent bleeding. Considering the scope for chromium supplementation to reduce the stressed condition in the equines used in antivenum production programme, the present experiment was planned to study the effect of different levels of chromium supplementation on blood chemistry profile.

## **Materials and Methods**

For this study twenty four healthy equines (twelve mules and twelve ponies) of about 4-5 years of age used in routine hyperimmunization bleeding programme for antisnake venum production, were selected. The animals were divided randomly in to three equal groups namely,  $T_0$  (control),  $T_1$  and  $T_2$ group each having eight equine animals (four mules and four ponies) on the basis of species, breed, body weights, age and sex. Group T<sub>0</sub> served as control and received ration as per standard feeding practice followed on the farm. Group T<sub>1</sub>and T<sub>2</sub> were fed with same ration as used for group To supplemented with chromium tripicolinate @ 210 and 420 µg/kg ration, respectively. Measured quantity of chromium tripicolinate in the form of premix was supplemented daily through the ration of individual animal. Measured amount of concentrate mixture was fed twice daily divided in two equal parts, offered individually in the manger in the stable. The animals were let loose group wise in open paddocks for roughage feeding and were fed with greens like Lucerne

Table 1: Percent Ingredient Composition of the Farm Concentrate Mixture

Name of ingredient	Per cent level		
Maize	16.80		
Soybean meal	17.00		
Cottonseed cake	08.00		
Rice polish	10.00		
Deoiled rice bran	18.00		
Wheat bran	17.00		
Molasses	10.00		
Dicalcium phosphate	01.50		
Lime stone powder	00.50		
Mineral mixture	0020		
Salt	01.00		
Total	100.00		

and maize and dry roughage like hay. Ad lib water was made available to individual equines in the stable throughout the experiment. The percent ingredient and chemical composition of the farm concentrate mixture is given in Table 1 and 2, respectively. The average chemical composition (% DMB) of hay, green maize and Lucerne is given in Table 3.

The experimental animals were housed in ideal stables with proper ventilation and flooring. Normal methods of hygine, management, feeding practices, vaccination and deworming programmes were followed for all the experimental animals throughout the trial period. Animals were let loose daily in open paddock for roughage feeding, watering and exercise.

## Parameters Studied

Blood chemistry profiles in terms of glucose, Hb, PCV, DLC, total serum protein, albumin, globulin and albumin:globulin ratio, serum

Table 2: Per cent Chemical Composition (%DMB) of Farm Concentrate Mixture

Nutrient	Per cent		
Dry matter	90.22		
Moisture	09.78		
Crude protein	21.12		
Ether extract	04.98		
Crude fibre	10.61		
Nitrogen free extract	52.91		
Total ash	10.38		
Acid insoluble ash	02.37		
Calcium	01.32		
Phosphorus	00.65		

Table 3: The Average Chemical Composition (%DMB) of Hay, Green Maize and Lucerne

Particulars	Hay	Green maize	Lucerne	
Dry matter	88.32	26.81	22.12	
Moisture	11.68	73.19	77.88	
Crude protein	02.25	05.54	22.68	
Ether extract	02.55	01.80	01.89	
Crude fibre	37.28	26.85	22.30	
Nitrogen free extract	49.05	57.51	41.22	
Total ash	08.87	08.30	11.91	
Acid insoluble ash	05.95	03.62	00.72	
Calcium	00.95	00.65	01.48	
Phosphorus	00.28	00.15	00.35	

Table 4: Blood Chemistry Profile of Equines from Different Experimental Groups

Parameters	$T_0$	$T_1$	$T_2$	Treatment	Fortnight
Blood glucose mg/dl	97.46	88.86	88.55	NS	*
Hemoglobin g%	11.00	11.80	11.84	NS	NS
PCV %	39.63	39.51	35.40	**	*
Neutrophils%	53.68	55.50	51.83	NS	NS
Lymphocytes%	33.88	31.10	35.13	NS	NS
Eosinophils %	04.07	03.95	03.88	NS	*
Monocytes %	07.43	07.68	07.94	NS	NS
Basophils %	0.96	1.73	1.36	NS	NS
Total serum protein g/dl	07.51	07.89	07.92	NS	NS
Serum albumin g/dl	$03.58^{a}$	03.09 <sup>b</sup>	02.86 <sup>b</sup>	**	**
Serum globulin g/dl	03.93a	04.80b	05.06b	**	**
A/G ratio	0.94a	$0.67^{b}$	0.62b	**	**
Serum triglycerides mg/dl	61.13a	54.27 <sup>b</sup>	49.76 <sup>b</sup>	**	NS
Serum cholesterol mg/dl	94.45	92.43	92.91	NS	*
LDL mg/dl	48.21a	35.47 <sup>b</sup>	31.01 <sup>b</sup>	**	**
HDL mg/dl	33.96 <sup>a</sup>	46.86 <sup>b</sup>	$49.45^{b}$	**	*
VLDL mg/dl12.2	12.28	10.10	12.45	NS	NS

Note:1. The mean with at least one common superscript in the same row do not differ significantly.

2. \* Significant at 5 % level \*\* Significant at 1 % level NS Not Significant

tryglycerides, total cholesterol, LDL, HDL and VLDL were studied during experiment of 13 weeks.

and Cochran by using randomized block design to draw the conclusions.[2]

# Analytical Techniques

The blood samples were analyzed for Hb, PCV and DLC by using standard methods described by Sastry.[1] Total serum protein, serum albumin, serum globulin and albumin:globulin ratio was estimated by using Barret and BCG Dye binding method with Qualigans reagent kit on spectrophometer-104. Blood glucose and serum lipid profiling was carried out with the help of auto-analyzer. The standard laboratory procedure was followed for estimation of blood profiles. All the data collected during experimental period were subjected to statistical analysis as per Snedecor

## **Results and Discussion**

Blood chemistry profiles of equines from different experimental groups is given in Table 4. The average blood glucose values for groups  $T_{0}$ ,  $T_{1}$  and  $T_{2}$  were 97.46, 88.86 and 88.55 mg/dl, respectively. It was observed that the feed treatments had no significant effect on the average blood glucose concentration values of the animals from different groups. The numerically lower values of blood glucose concentration observed in animals supplemented with chromium might be due to enhanced glucose metabolism. Findings of

the present study are in agreement with the findings of Pagan *et al* who observed lower glucose levels in exercising horses supplemented with 5 mg of chromium.[3]

The average hemoglobin values for groups  $T_0$ ,  $T_1$  and  $T_2$  were 11.00, 11.80 and 11.84 g %, respectively. It was seen that feed treatments had no significant effect on average blood hemoglobin values of the animals from different groups. Estrada *et al* reported no significant changes in hemoglobin throughout immunization.[4] The observations recorded from different regimes in the present study are in agreement with the above findings. Angulo *et al* observed drop in hemoglobin concentration and hematocrit in horses used for anti-venom production.[5]

The average PCV values of the equines from groups  $T_0$ ,  $T_1$  and  $T_2$  were 39.63, 39.51 and 35.40 %, respectively. It was observed that PCV values recorded for group  $T_1$  were significantly (P≤0.01) lower than that of groups  $T_0$  and  $T_1$ . However, the differences in average of PCV values recorded for groups  $T_0$  and  $T_1$  were nonsignificant. The reduced PCV values in treatment groups may result in increased plasma recovery which is very much preferred in case of equines used for ASVS production.

The average percent neutrophils counts of the equines from groups  $T_0$ ,  $T_1$  and  $T_2$  were 53.68, 55.50 and 51.83, respectively. It was observed that the chromium supplement had no significant effect on average neutrophils counts for different groups. The average percent lymphocytes counts for groups  $T_0$ ,  $T_1$  and  $T_2$ were 33.88, 31.10 and 35.13 %, respectively. It was seen that the chromium supplement had no significant effect on average lymphocytes counts for different groups. The average values for groups  $T_0$  and  $T_2$  were almost comparable and that of group T<sub>1</sub> were lower. The average percent monocytes counts of the equines from groups T<sub>0</sub>, T<sub>1</sub> and T<sub>2</sub> were 07.43, 07.68 and 07.94 %, respectively. It was observed that the chromium supplementation had nonsignificant effect on average percent eosinophils counts of equines for different groups. The values being 04.07, 03.95 and 03.88 %, respectively for groups  $T_0$ ,  $T_1$  and  $T_2$ . The average basophils count values for groups  $T_0$ ,  $T_1$  and  $T_2$ 

were 0.96, 01.73 and 01.36 %, respectively. It was seen that the chromium supplement had no significant effect on average basophils counts for different groups.

The average total serum protein values for groups  $T_0$ ,  $T_1$  and  $T_2$  were 07.51, 07.89 and 07.92 g/dl, respectively. It was seen that the chromium supplement had no significant effect on average total serum protein of the animals for different groups. Estrada *et al.* and Angulo *et al.* observed a significant increment in total serum proteins in the second half of the immunization in the horses inoculated with snake venom for production of antivenom. [4,5]

The average serum albumin values of the equines from groups  $T_0$ ,  $T_1$  and  $T_2$  were 03.58, 03.09 and 02.86 g/dl, respectively. It was observed that average serum albumin values of the animals from control group ( $T_0$ ) were significantly ( $P \le 0.01$ ) higher than that of other two groups. The average serum albumin values of animals from groups  $T_1$  and  $T_2$  did not differ significantly from each other.

The average serum globulin values of the equines from groups  $T_0$ ,  $T_1$  and  $T_2$  were 03.93, 04.80 and 05.06 g/dl, respectively. It was observed that average serum globulin values of the animals from control group (T<sub>0</sub>) were significantly (P≤0.01) lower than that of other two groups. The average serum globulin values of animals from groups T<sub>1</sub> and T<sub>2</sub> did not differ significantly from each other. The average fortnightly serum albumin: globulin ratios of the equines from groups  $T_0$ ,  $T_1$  and  $T_2$  were 0,94, 0.67 and 0.62, respectively. It was observed that average serum albumin :globulin ratios of the animals from control group (T<sub>0</sub>) were significantly (P≤0.01) higher than that of other two groups. The average serum albumin globulin ratios values of animals from groups:  $T_1$  and  $T_2$  did not differ significantly from each other.

The average serum triglyceride values of the animals for groups  $T_0$ ,  $T_1$  and  $T_2$  were 61.13, 54.27 and 49.76 mg/dl, respectively. It was observed that average serum triglyceride values of the animals from control group ( $T_0$ ) were significantly ( $P \le 0.01$ ) higher than that of other two groups. Further, values for group  $T_1$  were

significantly (P $\leq$ 0.01) higher when compared with group T $_2$ . Finding of the present study are matching with Uyanik *et al* who reported significant (P $\leq$ 0.01) reduction in serum triglyceride levels of working horses receiving chromium picolinate @ 200 and 400  $\mu$ g/day for 45 days.[6]

The average serum cholesterol values of the animals for groups  $T_0$ ,  $T_1$  and  $T_2$  were 94.45, 92.43 and 92.91 mg/dl, respectively. It was seen that the chromium supplementation had no significant effect on average serum cholesterol values of the animals from various experimental groups. Similar findings were observed by Uynaik *et al* who observed slight reduction in serum cholesterol levels of working horses receiving chromium picolinate @ 200 and 400 µg/day for 45 days.[6]

The average serum LDL values from animals for groups  $T_0$ ,  $T_1$  and  $T_2$  were 48.21, 35.47 and 31.01 mg/dl, respectively. It was seen that the values observed for  $T_1$  and  $T_2$  groups were significantly (P≤0.01) lower as compared to control group. However, values for groups  $T_1$  and  $T_2$  were comparable.

The average serum HDL values from animals for groups  $T_0$ ,  $T_1$  and  $T_2$  were 33.96, 46.86 and 49.45 mg/dl, respectively. It was seen that the values observed for  $T_1$  and  $T_2$  groups were significantly (P≤0.01) higher as compared to control group. However, values for groups  $T_1$  and  $T_2$  were comparable. Uyanik et al. (2008) reported no significant effect on serum HDL levels for working horses receiving chromium picolinate @ 200 and 400 µg/day for 45 days than that of horses receiving control ration without chromium.

The average serum VLDL values from animals for groups  $T_0$ ,  $T_1$  and  $T_2$  were 12.28, 12.10 and 10.10 mg/dl, respectively. It was seen that the chromium supplementation had no effect on average serum VLDL values from animals from different groups.

### Conclusion

From the overall performance of equines

from the present study, it is summerised that chromium supplementation to ration fed to equines for anti-sera production programme resulted satisfactory blood chemistry profiles giving higher serum globuline values which is most important criteria to decide number of doses of antisera that will be produced from the equines. Supplementation of chromium in the form chromium tripicolinate @ 420 μg/kg ration, to the equines give numerically superior performance when compared with the performance that of @ 210 µg/kg ration, although the differences were not statistically significant. Thus it is concluded from the overall results of the present study that chromium tripicolinate can be supplemented @ 210 µg/ kg ration of equines for better anti-sera production programme without affecting plane of nutrition and animal wellbeing. For steady performance the supplementation of chromium tripicolinate @ 420 µg/kg ration of equines can be done.

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