Biological Forum – An International Journal **15(8a): 383-388(2023)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Effect of Dietary Chromium Supplementation on Liver Function of Crossbred Calves in Pre and Post Weaning Period

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(Received: 15 June 2023; Revised: 02 July 2023; Accepted: 26 July 2023; Published: 15 August 2023)
(Published by Research Trend)

ABSTRACT: Chromium plays novel as cofactor in many enzymatic reactions. Organic chromium lowered the serum cortisol level which improves the immune system in newborn calves. Thus, feeding trial was conducted on newborn calves to evaluate chromium role their health in terms of liver function test. A feeding trial, of 180-day duration was conducted to evaluate effect of dietary supplementation of chromium on liver profile of crossbred calves during pre and post weaning period. Eighteen newborn crossbred calves, of either sex were randomly divided into 3 groups, each consisting of 6 animals. The treatments were: T_0- control (no chromium), T_1- nano chromium @ 0.05 mg/kg BW $^{0.75}$ and T_2 chromium picolinate @ 0.05mg/kg BW $^{0.75}$. The results of current investigation indicated that the average serum AST during pre and post weaning feeding period was significantly higher ($P<0.05$) in the T_0 group compared to T_1 and T_2 treatment groups. But, during pre-weaning feeding period, serum AST level concentration was statistically similar in T_1 and T_2 groups. Furthermore, T_1 group showed lower level of serum AST concentration compared to T_0 and T_2 groups during post weaning feeding period. ALP levels were found to be statistically similar during pre-weaning feeding period. During post weaning feeding period, serum ALP concentration was significantly higher ($P<0.05$) in T_0 and T_2 groups compared to T_1 group. In both pre and post weaning feeding trial, serum ALT level was non-significantly different among different treatment groups. It is concluded that chromium supplementation in variable forms show effect on liver function test in both pre and post weaning feeding trial.

Keywords: Chromium picolinate, crossbred calf, nano chromium, liver function.

INTRODUCTION

Agriculture and livestock production are closely interconnected and play a vital role in ensuring food security for India. Livestock farming holds significant importance in the country's agricultural economy, serving as a crucial component of farmers' livelihoods and supporting ongoing farming activities. Additionally, it provides essential inputs for agriculture, contributes to household nutrition and health, supplements income, generates employment opportunities, and acts as a reliable asset during times of need. Globally, livestock accounts for 40% of agricultural output and plays a critical role in sustaining the livelihoods and food security of approximately 1.3 billion people. Minerals are essential inorganic elements that play a crucial role in the physiological functions and metabolic processes of animals. They constitute approximately 4% of an animal's body weight and perform vital functions within the body. Consequently, a deficiency in these elements can lead to suboptimal performance and hinder the animal's potential (Ospina *et al.*, 2010). Minerals are more intricately involved in biological functions within the body than any other category of nutrients. Their functions encompass a wide range, including the regulation of gene expression and enzyme systems that govern cellular activities, interaction with vitamins for enhanced functionality, maintenance of osmotic balance, detoxification processes, immune system support, regulation of cell membrane functions, acid-base balance, and facilitation of structural growth, particularly in bones (i.e., bone development). The scientific literature enumerates 21 essential minerals, which are categorized into two groups: macro minerals and micro (trace) minerals. This classification is based on the quantities required in the diet rather than their physiological importance. Macro minerals are present in significant amounts within the animal body and are

needed in larger quantities in the diet (greater than 0.01%). They include calcium, phosphorus, magnesium, sulfur, and electrolytes such as sodium, potassium, and chloride. On the other hand, micro minerals are required in trace amounts (less than 0.01%), typically measured in milligrams, micrograms, or parts per million. Micro minerals encompass chromium, manganese, zinc, iron, copper, selenium, iodine, cobalt, and molybdenum (Cherian, 2020). To mitigate the adverse effects of environmental stress, various supplements and additives are incorporated into dairy animal rations. One of these supplements is chromium (Cr). The primary role of Cr in metabolism is to enhance the effects of insulin by being part of an organometallic compound called the glucose tolerance factor (GTF) (Sahin *et al.*, 2002).

Chromium (Cr) is a micronutrient and transition metal that continues to generate interest among scientists and consumers, particularly as a dietary supplement. The name "chromium" originates from the Greek word "chroma," meaning color, due to the vibrant hues exhibited by chromium salts and minerals. Within the Earth's crust, chromium is found at an approximate concentration of 102 parts per million (ppm), primarily in the form of chromite and crocoite minerals, in various oxidation states ranging from -2 to +6. In living organisms, chromium exists predominantly in two valence states: trivalent chromium (Cr^{3+}) and hexavalent chromium (Cr^{6+}) (Soltan, 2010). Cr (III) is highly stable within biological systems and does not readily traverse cell membranes. It is significantly less toxic to living cells than Cr (VI), which is a potent oxidant and toxic to both humans and animals. However, Cr (VI) can be easily reduced to the trivalent state within living organisms (Kosla *et al.*, 2018).

MATERIAL AND METHODS

The present study was conducted in two phases. In phase I, pre weaning period (0– 13 weeks) and Phase II, post weaning period (13-26 weeks) carried out on crossbred calves at Instructional Dairy Farm (IDF), Nagla, College of Veterinary and Animal Sciences, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India. Eighteen newborn crossbred calves with an average body weight 25.5 ±4.39 kg of 3 days old have been selected for a period of 180 days to investigate the effect of dietary chromium supplementation of through milk for 3 d after birth up to week 13 during pre-weaning feeding period and for post weaning feeding (13-26 weeks) chromium was supplemented through concentrate. The treatment assigned for pre and post weaning feeding trial as per follows:

T_0- Basal diet (Control)

T_1- Nano chromium @ 0.05 mg/kg BW $^{0.75}$

T_2- Chromium picolinate @ 0.05mg/kg BW $^{0.75}$

Feeds and Feeding Schedule

Pre-Weaning Feeding Phase-1

During the study, the calves were provided with a specific feeding schedule, as outlined in Table 1. The calves were divided into three groups: control (T_0), nano chromium (T_1), and chromium picolinate (T_2), with six calves in each group. The grouping was done in a manner to ensure that the average body weight in all groups was approximately similar.

In the control group (T_0), no chromium supplement was given in the milk. In the T_1 group, nano chromium was provided through the milk (Proximate analysis of milk (on fresh basis) fed to the calves contained 12.98% dry matter, 3.14% crude protein, 4.04% ether extract, 0.72% total ash, 0.45%, 5.08% lactose, 8.94% solid not fat)., while in the T_2 group, chromium picolinate was given via milk supplementation.

The calculated quantity of whole milk was offered in divided doses at 6:00 a.m. and 5:00 p.m. daily until the calves reached 13 weeks of age. Calf starter feed was provided in pre-measured amounts twice daily, and a weighed quantity of mixed green fodder (consisting of green oats, green berseem, and naiper in approximately a 1:1:1 ratio) was offered once daily for *ad libitum* feeding. The remaining calf starter if any and fodder residues were recorded daily in the morning. The dry matter content in the feeds and residue samples was determined weekly using a hot air oven at a temperature of 100±1°C. Fresh and clean drinking water was provided to the calves twice daily. The feeding trial was continued until the calves reached 13 weeks of age.

Table 1: Chemical compositions of dietary feed ingredients fed to crossbred calves in pre weaning feeding period.

Feeding stuffs	DM	On dry matter basis (%)							
		CP	EE	CF	NFE	TA	AIA	OM	TCHO
Concentrate	90.64	22.08	3.06	7.81	57.03	10.02	8.32	89.98	64.84
Mixed fodder (Oat+ Naiper + Berseem)	22.37	14.65	2.72	26.73	44.80	11.10	7.36	88.90	71.53
Wheat straw	90.67	3.32	1.30	33.74	50.49	11.15	6.55	88.85	84.23

DM- dry matter, OM- organic matter, CP- crude protein, CF- crude fiber, EE- ether extract, TA- total ash, NFE- nitrogen-free extract, TCHO- total carbohydrates, AIA- acid insoluble ash,
*Total CHO = 100- CP+EE+T Ash

Post Weaning Feeding Phase- 2

This phase closely resembled Phase 1 and spanned from (13 - 26 weeks) of age. During this phase, the feeding and treatment protocols remained similar to Phase 1, except for the addition of chromium to the calf starter instead of milk and milk was withdraw from daily diet. However, the composition of the calf starter had slight variations, as specified in Table 2, and the ingredients were adjusted to meet the nutrient requirements recommended by (BIS, 2009) for calf starter. The calves were provided with calf starter at a rate of 0.75 kg, 1 kg, and 1.5 kg per calf during the 4th, 5th, and 6th months of age, respectively. Additionally, they were given a mixture of green fodder (consisting of green

oats, green berseem, and green mustard in approximately equal proportions) along with wheat straw. The mixture of green fodder and wheat straw was prepared at a ratio of 10:1 based on fresh weight.

Table 2: Chemical compositions of dietary feed ingredients fed to crossbred calves in post weaning feeding period.

Feeding stuffs	DM	On dry matter basis (%)							
		CP	EE	CF	NFE	TA	AIA	OM	TCHO
Concentrate	90.04	22.74	3.00	7.00	58.86	8.40	5.26	91.60	67.96
Mixed fodder (Oat + Berseem + Green Mustard)	22.67	14.40	2.85	26.87	45.07	10.80	7.17	89.20	71.30
Wheat straw	90.54	2.85	1.27	38.71	49.12	8.05	4.65	92.95	88.81

DM- dry matter, OM- organic matter, CP- crude protein, CF- crude fiber, EE- ether extract, TA- total ash, NFE- nitrogen-free extract, TCHO- total carbohydrates, AIA- acid insoluble ash,
*Total CHO = 100- CP+EE+T Ash

Collection and analysis of blood samples. Blood samples were obtained from all the calves at specific time points (0, 45th, 90th, 135th, and 180th day of calf age) through puncture of the jugular vein. Approximately 10 ml of blood was collected from each calf. Collected blood was transferred to vials for serum separation and subsequently analyzed. The analysis of serum alkaline phosphatase (ALP), serum alanine aminotransferase (ALT), and serum aspartate aminotransferase (AST) concentration (U/L) was determined using the ERBA diagnostic kit based on the method provided by the International Federation of Clinical Chemistry (IFCC) as described by Tietz (1986) and Bradley *et al.* (1972).

Statistical Analysis. The experimental data obtained in the present study was analyzed statistically applying one way ANOVA by using SPSS software version 21 (Snedecor and Cochran 1994). The significant mean difference was separated by Tukey post hoc analysis with significance level defined at P< 0.05.

RESULTS AND DISCUSSION

Liver Function Test. The result of liver function test as affected by chromium supplementation in crossbred calves during pre and post weaning feeding period is depicted in Table 3.

Table 3: Average values of serum ALT, AST and ALP concentration of crossbred calves ration supplemented with nano chromium and chromium picolinate during pre-weaning and post weaning period.

Parameters	Days	T_0 (Control)	T_1 (Nano Chromium)	T_2 (Chromium Picolinate)	SEm±	Sig. 5%
	Pre-Weaning Period					
ALT (IU/L)	**0**	23.54±0.55	23.76±0.81	23.96±0.93	0.43	0.930
	45	23.60±0.76	23.84±0.86	24.22±0.60	0.41	0.844
	90	23.00±1.40	24.26±0.87	24.38±1.05	0.63	0.644
	Overall Mean	23.38±0.53	23.96±0.46	24.19±0.48	0.28	0.499
	Post Weaning Period					
	90	23.00±1.40	24.26±0.87	24.38±1.05	0.63	0.644
	135	22.88±0.80	23.04±0.93	24.51±0.71	0.47	0.330
	180	23.85±1.00	22.82±0.95	24.98±0.54	0.51	0.237
	Overall Mean	23.24±0.60	23.38±0.52	24.62±0.43	0.31	0.134
	Pre-Weaning Period					
AST (IU/L)	**0**	80.55±1.27	81.25±1.68	79.59±1.23	0.78	0.715
	45*	83.64±0.95 ^{a}	76.92±0.75^{b}	79.12±0.87^{b}	0.82	0.000
	90*	79.95±0.74 ^{a}	74.30±0.58^{c}	77.32±0.52^{b}	0.65	0.000
	Overall Mean*	81.38±0.67^{a}	77.49±0.42^{b}	78.68±0.55^{b}	0.47	0.002
	Post Weaning Period					
	90*	79.95±0.74 ^{a}	74.30±0.58^{c}	77.32±0.52^{b}	0.65	0.000
	135*	83.88±1.96 ^{a}	71.89±0.49^{b}	74.85±0.58^{b}	1.4	0.000
	180*	88.05±1.29 ^{a}	68.00±1.87^{b}	71.62±0.94^{b}	2.25	0.000
	Overall Mean*	83.96±1.11^{a}	71.40±0.89^{c}	74.60±0.68^{b}	0.89	0.000
	Pre-Weaning Period					
ALP (IU/L)	**0**	225.37±2.96	227.00±2.70	224.82±2.14	1.44	0.832
	45	244.96±1.14	238.96±2.01	243.66±2.98	1.33	0.158
	90*	239.82±1.65 ^{a}	230.85±1.72^{b}	237.81±2.49 ^{a}	1.42	0.016
	Overall Mean	236.72±2.30	232.27±1.69	235.43±2.36	1.23	0.327
	Post Weaning Period					
	90*	239.82±1.65 ^{a}	230.85±1.72^{b}	237.81±2.49 ^{a}	1.42	0.016
	135*	242.79±1.68 ^{a}	222.91±2.44^{b}	240.16±1.61 ^{a}	2.38	0.000
	180*	237.25±2.72 ^{a}	215.93±0.66^{c}	230.84±1.29^{b}	2.37	0.000
	Overall Mean*	239.96 ^{a} ±1.25	223.24^{b} ±1.76	236.27 ^{a} ±1.39	1.29	0.000

Alanine Aminotransferase (ALT)/ Serum Glutamic Pyruvic Transaminase (SGPT) Activity. During the pre-weaning feeding period (0-13 weeks), Serum ALT activities were estimated on 0, 45th and 90th day of age of crossbred calves and the mean values were 23.54±0.55, 23.60±0.76 and 23.00±1.40 (IU/L) in group T_0, 23.76±0.81, 23.84±0.86 and 24.26±0.87 (IU/L) in group T_1, while in group T_2 it was 23.96±0.93, 24.22±0.60 and 23.96±0.93 (IU/L), respectively. ALT/SGPT did not differ significantly between all the groups at 0, 45th and 90th day of feeding trial.

The overall average Serum ALT concentration of crossbred calves during pre-weaning feeding period (0-13 weeks) was 23.38±0.53, 23.96±0.46 and 24.19±0.48 IU/L in group T_0, T_1 and T_2 respectively. The serum ALT was similar in all the groups.

During post weaning feeding period (13-26 weeks), Serum ALT/SGPT activities in crossbred calves of groups T_0, T_1 and T_2, were 23.96±0.93, 24.22±0.60 and 23.96±0.93 (IU/L) at 90th day; 23.88±0.88, 23.04±0.93 and 24.51±0.71 and 23.85±1.00, 22.82±0.95 and 24.98±0.54 (IU/L) on day 135th and 180th, respectively. Serum ALT of crossbred calves in group T_0, T_1 and T_2 was not significantly (P>0.05) different between all the groups at 90th, 135th and 180th day of feeding trial.

The overall mean value of serum ALT concentration during post weaning feeding period (13-26 weeks) in group T_0 was 23.24±0.60 IU/L, in group T_1 it was 23.38±0.52 IU/L and group T_2 it was 24.62±0.43 IU/L, respectively. There were no significant (P>0.05) differences among the groups. The findings of present study are in agreement with (Wang *et al.*, 2007); (An-Qiang *et al.*, 2009) who conducted experiments on pigs and Holstein cows, respectively, and concluded that the administration of chromium supplements did not result in any significant alterations in ALT activities. Uyanik, (2001) reported that the administration of supplements containing chromium chloride over a period of 40 days did not result in any detectable which remained within the normal range for sheep.

Aspartate Aminotransferase (AST)/Serum Glutamic-Oxaloacetic Transaminase (SGOT) Activity. During the pre-weaning feeding period (0-13th weeks), Serum AST at 0 day were 80.55±1.27 IU/L, 81.25±1.68 IU/L and 79.59±1.23 IU/L in group T_0, T_1 and T_2, respectively. On the 45th day, Serum AST concentration was 83.64±0.95 IU/L, 76.92±0.75 IU/L and 79.12±0.87 IU/L in group T_0, T_1 and T_2, respectively. On the 90th day the serum AST concentration was 79.95±0.74 IU/L, 74.30±0.58 IU/L and 77.32±0.52 IU/L in groups T_0, T_1, and T_2, respectively. On 0 day, AST of crossbred calves in group T_0, T_1 and T_2 indicated not significantly (P>0.05) different between all the groups of feeding trial. On the 45th day, Serum AST was higher in the T_0 (control) group compared to T_1 and T_2 groups but statistically, similar serum AST concentration was observed in T_1 and T_2 fed groups. Serum AST concentration in the T_0 (control) group increased during the pre-weaning period at 90th days. In contrast, during the same period, the T_1 (nano chromium) group exhibited lower serum AST. Moreover, the T_2 (Chromium Picolinate) group demonstrated AST higher than the T_1 (nano chromium) group but still lower than the T_0 (control) group. This occurred within the same duration of the feeding trial.

The overall mean value of serum AST concentration during pre-weaning feeding period (0-13 weeks) in group T_o was 81.38±0.67 IU/L, in group T_1it was 77.49±0.42 IU/L and in group T_2, it was 78.68±0.55 IU/L, respectively. The serum AST concentration was higher in the T_0(control) group as compared to T_1 (nano chromium) and T_2 (chromium picolinate) groups but statistically similar serum AST concentration was observed in T_1 (nano chromium) and T_2 (chromium picolinate) fed groups.

During post weaning feeding period (13-26 weeks). The Serum AST of crossbred calves at 90 day were 79.95±0.74, 74.30±0.58 and 77.32±0.52 IU/L in group T_0, T_1 and T_2, respectively. Whereas, Serum AST concentration at 135th day was 83.64±1.96, 71.89±0.49 and 74.85±0.58 IU/L in groups T_0, T_1, and T_2, respectively. Similarly, at 180th day the serum AST was 88.05±1.29, 68.00±1.87 and 71.62±0.94 in groups T_0, T_1, and T_2, respectively. At 90 days, there was a significant (P<0.05) difference in serum AST as it was higher in T_0 group as compared with T_1 and T_2 supplemented group. However, Serum AST was significantly higher in T_0 group compared to the supplemented groups. At 135th and 180th days, the T_0 (control) group exhibited a higher serum AST level as compared to the T_1 and T_2 fed groups. However, there was no statistically significant difference in serum AST concentration between the T_1 and T_2 fed groups.

The overall average serum AST concentration in crossbred calves during post-weaning feeding period (13-26 weeks) was 83.96±1.11 IU/Lin group T_0 fed basal ration, 71.40±0.89 IU/L in group T_1 fed nano chromium and 74.60±0.68 in group T_2 fed chromium picolinate. The serum AST concentration was higher in T_0 (control) group, in the same duration T_1 (nano chromium) group showed lower level of serum AST concentration. Followed by T_2 (Chromium Picolinate) group as compared to T_1 (nano chromium) group but lower as compared to T_0 (control group) in the same duration of feeding trial.

The present result corroborated with the finding of (Srikandakumar *et al.*, 2003) as they reported a significant decrease in plasma AST activity in Merino and Omani sheep, while (Bahga *et al.*, 2009) found a statistically significant reduction (P<0.05) in AST activity in crossbred calves during summer. However, reduction in serum AST (aspartate aminotransferase) concentration in calves might be due to its potential role in promoting liver health and function. Chromium is thought to support glucose and lipid metabolism, which in turn can positively impact liver function. A healthier liver might result in lower levels of enzymes like AST being released into the bloodstream. In contrary, (Pechova *et al.*, 2003) who investigated the metabolic effects of chromium supplementation in Holstein cows during the peripartum period and observed a significantly higher AST activity of 90% in the untreated group of animals compared to 40% in those

that received chromium treatment, According to several studies, including those by (Nazafi *et al.*, 2003), (Rasooli *et al.*, 2004); (Al-Saeed *et al.*, 2009) (Chandra *et al.*, 2012); (Rathwa *et al.*, 2017); (Giri *et al.*, 2017) found elevated ambient temperature causes an elevation in serum AST levels in cattle and sheep. In addition, these investigations found that the rise in plasma AST activity caused hepatic cellular damage in these animals.

Alkaline Phosphatase (ALP). During the pre-weaning feeding period (0-13 weeks), the average serum ALP activities was estimated on 0, 45th and 90th day of age of crossbred calves and the mean values were 225.37±2.96, 244.96±1.14 and 239.82±1.65 IU/L in group T_0, 227.00±2.70, 238.96±2.01 and 230.85±1.72 IU/L in group T_1, while in group T_2 it was 224.82±2.14, 243.66±2.98 and 237.81±2.49 IU/L, respectively. At 0 and 45th day, serum ALP did not differ significantly between all the groups T_0, T_1 and T_2 respectively. At the 90th day, crossbred calves in the T_0 (control) and T_2 (chromium picolinate) groups displayed elevated serum ALP, whereas the T_1 (nano chromium) group showed a decrease in serum ALP concentration.

The overall average serum ALP concentration of crossbred calves during pre-weaning feeding period (0-13 weeks) was 236.72±2.30, 232.27±1.69 and 235.43±2.36 IU/L in group T_0, T_1 and T_2 respectively. The serum ALP concentration was not significantly ($P>0.05$) different between all the groups.

During post weaning feeding period (13-26 weeks), the serum ALP in calves of group T_0, T_1 and T_2, were 239.82±1.65, 230.85±1.72 and 237.81±2.49 IU/L at 90th (Initial) day; 242.79±1.68, 222.91±2.44 and 240.16±1.61 and 237.25±2.72, 215.93±0.66 and 230.84±1.29 IU/L respectively. On the 135th day and 180th day of feeding trial, serum ALP levels were higher in crossbred calves from the T_0 and T_2 group, whereas the T_1 group exhibited a reduction in serum ALP concentration. At the 180th day post-weaning period, the T_0 (control) group observed a rise in serum ALP concentration. Conversely, the T_1 (nano chromium) group saw a decline in serum ALP concentration during the same duration. Moreover, within the timeframe of the feeding trial, the T_2 (Chromium Picolinate) group exhibited higher ALP levels than the T_1 (nano chromium) group, yet these levels remained lower than those of the T_0 (control) group.

The overall mean value of serum ALP concentration during post weaning feeding period (13-26 weeks) in group T_0 was 239.96±1.25 IU/L, in group T_1it was 223.24±1.76 IU/L and in group T_2 it was 236.27±1.39 IU/L, respectively. The serum ALP concentration of crossbred calves was higher in T_0 and T_2 groups, while serum ALP concentration was lower in the T_1 group. The findings of present study are in agreement with Sultana *et al* (2022) who revealed that liver enzymes activity ALP didn't vary significantly ($P> 0.05$) between the experimental group on chromium with or without enzyme supplementation in calves, similar results were also observed by (Nejad *et al.*, 2016); (Patil *et al*., 2017) with chromium supplementation and (Shinde *et al.*, 2009); (Hala *et al.*, 2014) with vitamin E and selenium in calves and kids, respectively.

CONCLUSIONS

It can be concluded that chromium supplementation in different forms does not show any effect on serum ALT concentration during pre and post weaning feeding period. Furthermore, both serum AST and ALP levels were affected by chromium supplementation during post weaning feeding trial in crossbred calves. Collectively, it was concluded that chromium supplementation of nano chromium and chromium picolinate could exert effect on liver function in crossbred calves.

FUTURE SCOPE

Chromium is a trace mineral that has potential benefits in improving glucose metabolism and insulin sensitivity in animals. In calves, as in other livestock, maintaining proper glucose metabolism is important for growth and overall health. Chromium supplementation have the potential to improve glucose utilization, which could positively affect the metabolic health of calves by reducing the risk of metabolic disorders. Improved glucose metabolism could potentially lead to enhanced growth and performance in calves and can be used as a strategy to optimize calf growth. Calves can experience stress during various stages, such as weaning or transportation. Chromium supplementation's potential effects on stress reduction and better energy utilization could be explored. Chromium supplementation should be considered as part of a holistic approach to calf nutrition. It's important to ensure that calves receive a balanced diet that meets their overall nutritional needs. Depending on the jurisdiction, regulatory agencies may set guidelines for the use of chromium in animal feed. The future scope will also depend on how these regulations evolve.

Acknowledgment. This study was granted financial assistance by the College of Veterinary and Animal Sciences, G. B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India.

Conflict of Interest. None.

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How to cite this article: Mohit Bharadwaj, B.C. Mondal, Anshu Rahal and Jyoti Palod (2023). Effect of Dietary Chromium Supplementation on Liver Function of Crossbred Calves in Pre and Post Weaning Period. *Biological Forum – An International Journal*, *15*(8a): 383-388.