

## Growth performance and nutrient utilization in black Bengal bucks (*Capra hircus*) supplemented with graded doses of chromium as chromium chloride hexahydrate

Tarun K. Paul, Sudipto Haldar, Tapan K. Ghosh\*

Department of Animal Nutrition, Faculty of Veterinary and Animal Sciences, West Bengal University of Animal and Fishery Sciences, Kolkata 700037, India

Black Bengal bucks (*Capra hircus*) were supplemented with 0, 0.2 or 0.4 mg elemental chromium (Cr) as chromium chloride hexahydrate per day for 70 days. Intake of dry matter ( $p < 0.001$ ), crude protein ( $p < 0.001$ ) and neutral detergent fiber ( $p < 0.01$ ) increased due to Cr supplementation. The apparent total tract digestibility of dry matter ( $p < 0.01$ ), organic matter ( $p < 0.05$ ), crude protein ( $p < 0.001$ ) and acid detergent fiber ( $p < 0.01$ ) improved and the total body weight gain and the live weight gain to feed intake ratio also increased ( $p < 0.001$ ) due to supplemental Cr feeding. The intake and apparent absorption ( $p < 0.001$ ) of Cr was enhanced due to its supplementation. The intake of copper, zinc, manganese and iron was also more ( $p < 0.001$ ) in the Cr supplemented bucks. As supplementation progressed, plasma glucose concentration was elevated particularly in 0.4 mg Cr supplemented bucks and a significant day  $\times$  dose interaction effect ( $p < 0.001$ ) with this parameter. The activity of plasma alkaline phosphatase increased ( $p < 0.001$ ) and that of glutamate pyruvate transaminase in plasma decreased ( $p < 0.01$ ) in the Cr supplemented bucks. Supplemental Cr had minimal ( $p > 0.05$ ) effect on the plasma half life ( $k$ ) and clearance rate of glucose ( $T_{1/2}$ ) during an intravenous glucose tolerance test. Area under the response curve from 0 to 180 minutes after glucose loading was lower ( $p < 0.001$ ) in the control group of bucks. The study revealed that Cr supplementation might promote growth and nutrient utilization in black Bengal bucks. However, little difference between the 0.2 and 0.4 mg Cr supplemented bucks suggested limited benefit of increasing the level of supplementation beyond 0.2 mg per day under the normal management regimes.

**Key words:** Black Bengal bucks, chromium, nutrient utilization, trace elements

### Introduction

Chromium (Cr) was first shown to be an essential nutrient for normal glucose metabolism in rats [16]. It is a component of the glucose tolerance factor and was believed to be composed of trivalent Cr, nicotinic acid and amino acids, and potentiates the action of insulin [10]. Organic Cr in the form of Cr-yeast complex or Cr-nicotinic acid complex appeared beneficial for ruminants as well [17]. However, it is unknown whether supplemental inorganic Cr would have similar beneficial effects in ruminants as the tissue availability of Cr depends largely on its chemical form and nature [3].

Black Bengal bucks (*Capra hircus*), one of the goat breeds producing quality meat in the world, was used as experimental units in this study because goat population in the developing nations of South-East Asia and Africa increased by 26% between 1980 and 1996 adding a lot to the national economy [12]. Promotion of goat meat production, therefore, may enhance the supply of quality protein to the human population in these countries. Cr, which augments nutrient utilization at the tissue level and has a growth stimulatory effect in the livestock [17], may be of help in this regard. The objectives of the present experiment were to investigate the effects of inclusion of Cr in graded doses in the form of Cr-chloride hexahydrate in diets on the growth measurements, nutrient utilization, mineral metabolism and selected blood metabolites in castrated black Bengal bucks.

### Materials and Methods

#### Animals and treatments

Eighteen black Bengal bucks (*Capra hircus*) of 15 to 18 months age (initial mean body weight  $18.9 \pm 0.18$  kg) were used as experimental units. The bucks were housed in a dry, clean, well ventilated and hygienically maintained shed. After de-worming with fenbendazole (5 mg/kg body weight) the bucks were adapted with a basal diet comprising of concentrate mixture (broken rice 400 g/kg, gram 350 g/kg and

\*Corresponding author  
Tel: +91-33-25569234; Fax: +91-33-25571986  
E-mail: tapan1232@rediffmail.com

**Table 1.** Chemical composition of the dietary components of the black Bengal bucks supplemented with graded doses of chromium

	Paragrass hay ( <i>Brachiaria mutica</i> )	Berseem hay ( <i>Trifolium alexandrium</i> )	Concentrate mixture <sup>a</sup>
Dry matter (g/kg)	808.5	830.4	883.0
Chemical composition (g/kg dry matter)			
Organic matter	901.3	903.5	946.2
Crude protein (N × 6.25)	126.0	145.6	176.8
Neutral detergent fiber	433.7	339.8	306.9
Acid detergent fiber	328.5	283.8	244.0
Trace elements (mg/kg dry matter)			
Chromium	0.89	1.78	1.18
Copper	13.18	18.94	10.24
Zinc	37.16	35.24	69.20
Iron	194.76	185.08	147.27
Manganese	72.65	61.64	98.64

<sup>a</sup>Composed of broken rice, gram and mustard oil cake.

mustard oil cake 250 g/kg), sun cured berseem (*Trifolium alexandrium*) and paragrass (*Brachiaria mutica*) hays for a period of 15 days. Concentration of Cr in the concentrate mixture, paragrass and berseem hay was 1.18, 0.89 and 1.78 mg per kg dry matter respectively (Table 1). Subsequently the bucks were weighed and randomly assigned to three experimental treatment groups with six animals per group. During the experimental feeding, the individual bucks in each treatment group was supplemented with 0 (control), 0.2 and 0.4 mg elemental Cr per day as chromium chloride hexahydrate for a period of 70 days. The concentrate mixture (400 g) and the berseem-paragrass hay mixture (1 : 2 ratio, 600 g) were offered individually every day at 9 a.m. and 2 p.m. to secure the nutrients required for maintenance and growth [1]. De-mineralized water was provided *ad libitum* in plastic buckets throughout the experiment.

### Supplementation of the bucks with graded doses of chromium

Laboratory reagent grade chromium chloride hexahydrate ( $\text{CrCl}_3 \cdot 6\text{H}_2\text{O}$ ; Hi-Media Lab, India; molecular weight 266.45; minimum assay 97%) was the source of supplemental Cr (molecular weight 52) in this study. A solution containing 1.0248 mg  $\text{CrCl}_3 \cdot 6\text{H}_2\text{O}$  equivalent to 0.2 mg elemental Cr was prepared by dissolving 512.4 mg  $\text{CrCl}_3 \cdot 6\text{H}_2\text{O}$  in 500 ml triple distilled water. 1 ml and 2 ml of this solution was drenched to the individual animals of two treatment groups (0.2 and 0.4 mg Cr) with the sterilized syringes everyday at 8 a.m.. The control animals were not drenched with this solution and thus received no supplemental Cr.

### Digestibility trial

A digestibility trial was conducted between days 56 and 60 to ascertain the utilization of dry matter (DM), organic

matter (OM), crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF) and that of Cr and trace elements like copper (Cu), zinc (Zn), iron (Fe) and manganese (Mn). During the digestibility trial, the bucks were kept in separate wooden metabolic cages and fed individually. The dietary residues were quantified after 24 h. The total amount of feces voided by a buck during a 24 h period was recorded. Approximately 100 g of individual fecal samples was kept everyday for estimation of DM, OM, NDF, ADF, Cr and other trace elements mentioned above. A fraction (20 g) was preserved in dilute sulfuric acid (20 % v/v) for the estimation of nitrogen.

### Collection and analyses of blood samples

Blood samples were collected on day 0 and 65 by jugular vein puncture at 8 : 30 a.m. (preprandial) and 4 p.m. (2 h postprandial) in separate blood collection tubes containing either sodium fluoride (10 mg) or heparin (100 units per 10 ml). Immediately after collection, the blood tubes were placed in an ice-bath and centrifuged subsequently. Plasma thus harvested was stored at  $-20^\circ\text{C}$  for further analyses. Plasma samples obtained from the tubes containing sodium fluoride were analyzed for glucose, alkaline phosphatase and glutamate pyruvate transaminase (GPT). Concentration of Cr, Cu, Zn, Fe, Mn and calcium (Ca) in the plasma samples was determined in the heparinized samples by an atomic absorption spectrophotometer after suitable dilution with triple distilled water.

### Intravenous glucose tolerance test (IVGTT)

An intravenous glucose tolerance test [8] was conducted taking three bucks from each group on day 70 to investigate the effects of Cr supplementation on glucose metabolism. The bucks were fitted with sterile 14 gauge × 5.1 cm jugular vein catheters and their patency was maintained with a 6%

sodium citrate-saline solution. After a 60 minutes rest period the initial pre-loading blood samples (0 h) were collected. This was followed by an intravenous dose of glucose (50% glucose saline, weight/volume, 500 mg/kg body weight) administered at ambient temperature through the jugular vein catheter over a 2 minutes period and this was quickly followed by a 10 ml saline flush. Additional samples were collected in tubes containing sodium fluoride (10 mg) after 5, 15, 30, 45, 90 and 180 minutes of dosing and placed immediately in ice-bath before centrifugation and separation of plasma. The plasma was stored at  $-20^{\circ}\text{C}$  and analyzed for glucose.

Glucose clearance rates ( $k$ ) were determined using the incremental plasma glucose concentration above the base line values between 15 ( $t_1$ ) and 45 ( $t_2$ ) minutes post dosing using the following equation:

$$k = [(\ln_1 - \ln_2)/(t_2 - t_1)] \times 100 = \text{per cent/minute,}$$

where,  $k$  is the fractional turn over rate (or clearance rate) of plasma glucose as calculated from the natural logarithms of their concentration between  $t_1$  and  $t_2$ . The plasma half-life was calculated as  $T_{1/2}$  (minutes) =  $[0.693/k] \times 100$ . Responses of plasma glucose to IVGTT was further evaluated by calculating areas under the curve (AUC) relative to basal levels using trapezoidal geometry for the time period of 0 to 180 minutes.

### Analyses of samples

The dietary ingredients and fecal samples, dried at  $70^{\circ}\text{C}$  for 12 h in a hot air oven and ground to pass through a 1 mm sieve, were analyzed for DM, ash and OM [4], NDF and ADF [7]. The N fraction of the feeds and feces was estimated by Kjeldahl distillation method and the CP content was determined by multiplying the N value with 6.25.

For estimation of Cr [21] in feeds and feces, oven dried samples (10 g) were ground through 0.5 mm sieve and transferred to glazed ceramic crucibles. Calcium oxide (10 g) was mixed with the samples which, were then moistened with 10 ml of triple distilled water and ignited at  $600^{\circ}\text{C}$  for 4 h. Cooled samples were treated with 10 ml concentrated nitric acid and filtered through ash less filter paper and diluted to 100 ml with triple distilled water. Cr was estimated in atomic absorption spectrophotometer (Analyst 100: PerkinElmer, USA). A 2% solution of ammonium chloride was added in the standards and samples to reduce interference caused by presence of Fe in the samples. For analysis of Cu, Zn, Fe, Mn and Ca, 1 ml of the aliquot was further diluted to 40 ml with triple distilled water.

Cr was analyzed directly from the plasma samples in an atomic absorption spectrophotometer. For estimation of Cu, Zn Fe and Mn, however, plasma samples were diluted (1 : 40) with triple distilled water.

Glucose (mmol/l), alkaline phosphatase (units/l) and

glutamate pyruvate transaminase (GPT, units/l) were determined photometrically in an automatic blood analyzer (Microlab 200®) using commercial kits obtained from Merek India Ltd, Mumbai, India.

### Statistical analyses

Effects of Cr supplementation on the measured parameters were analyzed by the generalized linear model of Systat standard version (6.0.1) using the level of supplemental Cr level as the independent variable. When a significant F value ( $p < 0.05$ ) was detected, the individual treatment means were compared by the least significant difference test. In cases of data related to day and time (plasma glucose and plasma enzymes) or day only (trace element concentrations in plasma), day and time or both were included in the model as independent variables along with levels of Cr and the interaction between the day and dose level of supplemental Cr was included. The sums of squares in the latter analyses were further partitioned by orthogonal contrasts to analyze the linear and quadratic effects of the levels of supplemental Cr on the measured parameters.

## Results

### Nutrient utilization and body weight gain

The mean values of intake and apparent total tract digestibility of DM, OM, CP, NDF, ADF, and those related to the live weight changes are presented in Table 2. Intake of DM ( $p < 0.001$ ), CP ( $p < 0.001$ ) and NDF ( $p < 0.01$ ) was higher in the Cr supplemented bucks compared to the control ones though the difference between the 0.2 and 0.4 mg Cr supplemented bucks was not significant ( $p > 0.05$ ). Intake of OM ( $p < 0.01$ ) and ADF ( $p < 0.001$ ), on the other hand, was higher in the 0.4 mg Cr supplemented group only.

The apparent total tract digestibility of DM ( $p < 0.01$ ), OM ( $p < 0.05$ ), CP ( $p < 0.001$ ) and ADF ( $p < 0.01$ ) increased and that of NDF showed an increasing trend ( $p < 0.1$ ) when the diet was supplemented with Cr. However, the effect of increasing the level of supplemental Cr beyond 0.2 mg on the apparent digestibility of nutrients was not evidenced ( $p > 0.05$ ) in this study.

The total body weight gain and live weight gain to DM intake ratio was higher ( $p < 0.001$ ) in the Cr supplemented animals. However, the performance of the 0.2 and 0.4 mg Cr supplemented bucks was similar ( $p > 0.05$ ) in these cases also.

### Intake and apparent absorption of Cr and other trace elements

The data related to the intake and apparent absorption of Cr and that of Cu, Zn, Fe and Mn are presented in Table 3. The intake and apparent absorption of Cr in the bucks increased ( $p < 0.001$ ) in almost a parallel fashion as the level

**Table 2.** Intake and total tract apparent digestibility of nutrients and live weight gain in black Bengal bucks supplemented with graded levels of chromium (mg/animal)<sup>a</sup>

	Supplemental Cr as chromium chloride (mg)			SEM	Probability
	0	0.2	0.4		
Nutrient intake g/day per animal <sup>b</sup>					
Dry matter	644.4	710.3	734.1	10.47	0.00001
Organic matter	594.4	612.4	661.5	9.47	0.004
Crude protein	86.7	95.6	96.6	1.33	0.0003
Neutral detergent fiber	229.2	252.8	256.7	4.11	0.005
Acid detergent fiber	187.8	188.7	211.9	3.27	0.0002
Total tract apparent digestibility of nutrients (g/g intake)					
Dry matter	0.702	0.788	0.750	0.012	0.003
Organic matter	0.744	0.805	0.777	0.009	0.011
Crude protein	0.649	0.717	0.722	0.010	0.0001
Neutral detergent fiber	0.581	0.653	0.642	0.014	0.063
Acid detergent fiber	0.557	0.650	0.609	0.013	0.005
Initial body weight kg	18.9	18.8	19.1	0.09	0.56
Live weight gain (kg) <sup>c</sup>	1.08	2.03	1.94	0.13	0.0004
Live weight gain: DM intake <sup>b</sup>	0.026	0.044	0.041	0.003	0.0006

<sup>a</sup>Animals were supplemented for a period of 70 days.<sup>b</sup>Mean values of 5 days.<sup>c</sup>Represents mean values of 70 days.**Table 3.** Intake and apparent absorption of chromium and other trace elements in black Bengal bucks supplemented with graded doses of chromium (mg/animal)<sup>a</sup>

	Supplemental Cr as chromium chloride (mg) <sup>b</sup>			SEM	Probability
	0	0.2	0.4		
Chromium					
Intake <sup>c</sup>	0.783	0.842	0.903	0.013	0.00001
Apparent absorption	0.139	0.174	0.207	0.008	0.00001
Copper					
Intake	8.7	12.9	13.5	0.52	0.00001
Apparent absorption	1.16	1.65	1.73	0.07	0.00014
Zinc					
Intake	30.7	35.0	37.9	0.79	0.00001
Apparent absorption	5.19	6.72	7.08	0.226	0.00001
Iron					
Intake	111.8	114.7	126.7	2.00	0.131
Apparent absorption	17.4	16.7	20.3	2.13	0.103
Manganese					
Intake	50.9	55.2	60.6	1.09	0.00001
Apparent absorption	10.76	1.27	11.85	0.223	0.136

<sup>a</sup>Represents mean of 5 days.<sup>b</sup>Animals were supplemented for a period of 70 days.<sup>c</sup>From diet only; does not include the supplemental chromium.

of supplemental Cr increased in the respective treatment groups. The intake of Cu, Zn, Fe and Mn ( $p < 0.001$ ) and the apparent absorption of Cu and Zn ( $p < 0.001$ ) increased due to Cr supplementation though the increment in the dose level from 0.2 to 0.4 mg did not appreciably affect their

apparent absorption ( $p > 0.05$ ). Apparent absorption of Mn was similar among the treatment groups. Neither the intake nor the apparent absorption of Fe in the bucks was affected by Cr supplementation.

**Table 4.** Preprandial and postprandial glucose and enzymes activities in plasma of black Bengal bucks supplemented with graded levels of chromium (mg/animal)

Day and Time of sampling		Supplemental Cr as chromium chloride (mg) <sup>a</sup>			SEM	ANOVA		Contrast <i>P</i> -value	
Day	Time	0.0	0.2	0.4		Effect	Probability	Linear	Quadratic
Plasma glucose (mmol/l)									
0	Preprandial	2.57	2.68	2.34	0.06	Day	<i>p</i> >0.1	0.239	0.588
	Postprandial	2.65	2.70	2.46	0.04	Dose	<i>p</i> >0.1	0.368	
65	Preprandial	2.50	2.57	2.74	0.09	Time	<i>p</i> >0.1	0.228	
	Postprandial	2.51	2.54	2.96	0.07	Day x Dose	<i>p</i> <0.001		
Plasma alkaline phosphatase (units/l)									
0	Preprandial	267.0	183.5	165.8	14.3	Day	<i>p</i> <0.001	0.00001	0.092
	Postprandial	285.8	185.4	147.3	19.6	Dose	<i>p</i> >0.1	0.864	
65	Preprandial	197.9	344.8	326.8	24.8	Time	<i>p</i> >0.1	0.865	
	Postprandial	206.5	344.3	329.0	23.9	Day x Dose	<i>p</i> <0.001		
Plasma glutamate pyruvate transaminase (units/l)									
0	Preprandial	15.7	16.3	17.0	0.83	Day	<i>p</i> <0.001	0.0002	0.001
	Postprandial	15.7	16.2	21.3	0.85	Dose	<i>p</i> <0.01	0.353	
65	Preprandial	17.3	14.3	13.0	1.12	Time	<i>p</i> <0.05	0.049	
	Postprandial	18.9	12.8	12.8	1.21	Day x Dose	<i>p</i> <0.001		

<sup>a</sup>Supplementation continued for 70 days

### Concentration of glucose and enzyme activities in plasma

The preprandial and postprandial concentration of glucose and the activities of alkaline phosphatase and GPT in the experimental bucks are presented in Table 4. The preprandial and postprandial plasma glucose on days 0 and 65 was similar ( $p > 0.05$ ) in all the experimental groups. However, a day x dose interaction ( $p < 0.001$ ) suggested that as supplementation progressed plasma glucose concentration got elevated especially in the bucks supplemented with 0.4 mg Cr. Though Cr supplementation *per se* had no effect on plasma alkaline phosphatase activity ( $p > 0.01$ ), it was augmented with time in the Cr supplemented bucks (day x dose interaction,  $p < 0.001$ ). Activity of plasma GPT was similar ( $p > 0.05$ ) in all the experimental groups on day 0. However, its activity was lower ( $p < 0.01$ ) in the Cr supplemented bucks on day 65. However, increasing the dose level of Cr beyond 0.2 mg had no apparent effect on plasma alkaline phosphatase and GPT activities.

### Cr and other trace elements in plasma

The plasma Cr concentration, which was similar ( $p > 0.05$ ) in all the experimental groups prior to the start of the experiment, increased ( $p < 0.01$ ) in the supplemented bucks (Table 5). The difference between the 0.2 and 0.4 mg Cr supplemented animals was not conspicuous ( $p > 0.05$ ), however. Concentration of Cu, Zn and Mn in plasma was similar ( $p > 0.05$ ) in all the treatment groups prior to the start of the experiment. Though Cr supplementation had no apparent effect on the plasma Cu concentration, levels of plasma Zn and Mn were elevated due to Cr supplementation.

No definite effect of supplemental Cr feeding on plasma Fe concentration could be ascertained though on day 65 it was found to be higher ( $p < 0.05$ ) in the 0.4 mg Cr group of bucks.

### Intravenous glucose tolerance test

Cr supplementation exerted variable effects on different parameters of the intravenous glucose tolerance test (Table 6). Basal level of plasma glucose and that after 15 and 45 minutes post infusion showed a dose dependent linear increment ( $p < 0.01$ ). Plasma glucose concentration returned to the basal level 180 minutes after the infusion in all the treatment groups. Plasma half life ( $k$ ) and clearance rate ( $T_{1/2}$ ) was not affected. Area under the response curve from 0 to 180 minutes after glucose loading was lower ( $p < 0.001$ ) in the control group of bucks compared to the Cr supplemented ones.

### Discussion

Enhancement in nutrient intake in Cr supplemented animals was reported earlier [11,19] especially during the periods of stress [9]. Similar improvements in the present study were observed despite absence of any stress factor *per se*. The improved live weight gain to DM intake ratio suggested an enhanced efficiency of feed utilization in the Cr supplemented bucks. However, similar performance of the 0.2 and 0.4 mg Cr supplemented bucks suggested that there was not much benefit of supplementing these animals with Cr above the level of 0.2 mg. The dose level of 0.4 mg

**Table 5.** Plasma concentration of chromium and other trace elements in black Bengal bucks supplemented with graded level of chromium for 70 days (mg/animal)

Trace element ( $\mu\text{g/ml}$ )		Supplemental Cr as chromium chloride (mg)			Pooled SEM	ANOVA		Contrast <i>P</i> -value	
		0.0	0.2	0.4		Effect	Probability	Linear	Quadratic
Chromium	Day 0	0.098	0.093	0.096	0.0037	Day	$p < 0.001$	0.00001	0.069
	Day 65	0.098	0.128	0.131		Dose	$p < 0.01$	0.006	
						Day x Dose	$p < 0.001$		
Copper	Day 0	0.997	0.950	0.945	0.015	Day	$p > 0.1$	0.593	0.842
	Day 65	1.01	1.00	0.98		Dose	$p > 0.1$	0.065	
						Day x Dose	$p > 0.1$		
Zinc	Day 0	0.93	0.94	0.95	0.012	Day	$p > 0.1$	0.538	0.017
	Day 65	0.92	1.00	1.01		Dose	$p < 0.01$	0.033	
						Day x Dose	$p < 0.05$		
Manganese	Day 0	0.087	0.105	0.108	0.011	Day	$p < 0.01$	0.004	0.414
	Day 65	0.100	0.170	0.195		Dose	$p < 0.05$	0.013	
						Day x Dose	$p > 0.1$		
Iron	Day 0	3.23	3.65	3.57	0.07	Day	$p > 0.1$	0.807	0.367
	Day 65	3.23	3.54	3.81		Dose	$p < 0.05$	0.011	
						Day x Dose	$p > 0.1$		

**Table 6.** Influence of chromium supplementation on plasma glucose kinetics during an intravenous glucose tolerance test (mg/animal)

Item	Supplemental Cr as chromium chloride (mg)			SEM	Probability
	0.0	0.2	0.4		
Glucose concentration (mmol/L)					
Basal level (Time 0 min)	1.81	2.13	2.28	0.075	$p < 0.01$
15 min (post infusion)	7.24	9.96	11.11	0.597	$p < 0.001$
45 min (post infusion)	3.98	5.26	6.17	0.327	$p < 0.001$
Clearance rate ( $k$ % per minute)	3.07	2.9	2.74	0.145	$p > 0.1$
Half life ( $T_{1/2}$ ; min)	22.62	24.62	26.02	1.40	$p > 0.1$
Area under curve (0-180 min)	634.9	895.8	970.4	51.9	$p < 0.001$

Cr might be sufficient to bring down the circulatory insulin level as a part of the glucose counter regulatory mechanism [6] leading to a widened molar ratio between the glucagons and insulin in that group. This might have offset, at least in part, the anabolic effect of insulin and contained further increase in their body weight.

The higher intake of Cr and other trace elements from the basal diet in the Cr supplemented bucks might be due to the difference in DM intake among the treatment groups. DM intake in the Cr supplemented bucks was higher resulting more Cr intake in these animals compared to the control ones. It was reported that [2] inorganic trivalent Cr is absorbed at very low levels (0.4 to 3 per cent) though in this experiment the apparent absorption of Cr was higher than these values. In spite of the higher apparent absorption of Cr, the bucks supplemented with 0.4 mg Cr failed to outperform those supplemented with 0.2 mg Cr and a widened molar ratio between the glucagons and insulin, as discussed earlier, might explain this phenomenon.

The difference in the intake of Cu, Zn, Mn and Fe in the treatment groups might be explained as a function of the DM intake that was augmented due to Cr supplementation. The intake of these trace elements in all the three experimental groups was within the normal range [1].

The elevated plasma glucose in the 0.4 mg Cr supplemented bucks apparently contradicted the general hypothesis governing the mode of Cr action. Taking into account the effect of Cr on the potentiation of insulin action a lower plasma glucose concentration in this group was expected. However, in Cr supplemented ruminants, an enhanced gluconeogenesis from propionate was reported [18], which at the dose level of 0.4 mg, might have had overridden the effect of insulin on the cellular uptake of glucose and resulted an elevated plasma glucose concentration. Moreover, a widened glucagon to insulin ratio in the 0.4 mg Cr supplemented group might trigger off glycogenolysis and raised the plasma glucose concentration in these animals. However, it is not possible to be certain about which effect

operated actually in this case.

Alkaline phosphatase is primarily responsible for the uphill transportation of calcium ions across the membranes that bar the movement of soluble mineral crystals and their conversion to hydroxyapatite [20]. The higher alkaline phosphatase activity in the supplemented bucks suggested that Cr augmented new bone crystal formation by enhancing the uptake of circulatory Ca into the bone matrix. This was supported by the lower ( $p < 0.01$ ) concentration of Ca in the plasma of the Cr supplemented bucks (2.18 and 2.17 mmol/L respectively in the 0.2 and 0.4 mg groups respectively) compared to the control group of bucks (2.34 mmol/L).

Circulatory GPT level increases due to enhanced liver function upon exposure to stress and it was reported that supplemental Cr has stress alleviating effects in ruminants [11,13]. Though no stress *per se* was imposed upon the experimental bucks, partial confinement and exposure to different experimental procedures inevitably exerted some stress upon them. It appeared that Cr alleviated these inevitable stresses as well, which obviously helped in better body weight gain and health conditions in the supplemented bucks.

The present experiment, however, did not indicate any appreciable effect of Cr supplementation beyond a dose level of 0.2 mg. However, a particular level of supplementation during the normal management regime might appear deficient in critical situations such as transportation, vaccination, weaning and stress associated with production [14]. The present experimental design might not be sensitive enough to detect the differences between the two doses owing to the absence of the factors mentioned above.

The higher plasma concentrations of Cr, Zn and Mn in the supplemented bucks indicated towards an enhanced retention of these elements, which might have occurred under the influence of Cr supplementation. Cr supplementation has been shown in mice to protect against stress-induced losses of several trace elements [15]. It appeared from the present observations that supplemental Cr might have an overriding effect by reducing the need for supplemental Zn, Mn and possibly other micro minerals.

The IVGTT suggested that the major effects of Cr in ruminants might not be mediated through glucose tolerance [19]. Ruminants meet up their glucose requirements from hepatic gluconeogenesis with little or no absorption of glucose taking place from the small intestine. Thus, the ruminant tissues are less sensitive to insulin [9]. As a result, unlike the non-ruminants, ruminants seem to be refractory to the exogenous glucose loading despite enhancement of insulin action due to Cr supplementation [5]. This might be the most plausible explanation for the present observations as well.

It was concluded that Cr supplementation at the dose level of 0.2 mg Cr per day in the form of chromium chloride hexahydrate might augment nutrient utilization and body

weight gain in the black Bengal bucks. The activity of alkaline phosphates and glutamate pyruvate transaminase in the Cr supplemented bucks corroborated their growth performance. However, in absence of any specific stress factor Cr supplementation above the level of 0.2 mg/day might not yield much beneficial effect in terms of growth performance and nutrient utilization in the black Bengal bucks.

## Acknowledgments

The authors are grateful to the Super Agro (India) Limited for their material assistance and for providing help in sample analysis.

## References

1. **Agriculture and Food Research Council.** The Nutrition of Goats. Agriculture and Food Research Council Technical Committee on Responses to Nutrients, Report No. 10. pp. 74-81. CAB International, 1998.
2. **Anderson RA.** Chromium. In: Smith KT (ed.). Trace Minerals in Foods. pp. 225-244. Marcel Dekker, New York, 1988.
3. **Anderson RA, Bryden NA, Polansky MM.** Form of chromium affects tissue chromium concentration. FASEB J 1993, **7A**, 204 (Abstract).
4. **Association of Official Analytical Chemists.** Official Methods of Analysis, 16th ed. AOAC, Arlington, 1995.
5. **Brockman RP, Laarveld B.** Hormonal regulation of metabolism in ruminants; a review. Livest Prod Sci 1986, **14**, 313-334.
6. **Depew CL, Bunting LD, Fernandez JM, Thompson DL Jr., Adkinson RW.** Performance and metabolic responses of young dairy calves fed diets supplemented with chromium tripicolinate. J Dairy Sci 1998, **81**, 2916-2923.
7. **Goering HK, Van Soest PJ.** Forage Fiber Analyses (Apparatus, Reagents, Procedures and some Applications). Agriculture Handbook No. 379. ARS, USDA. Washington D.C. 1970.
8. **Kaneko JJ, Harvey JW, Bruss ML.** Clinical Biochemistry of Domestic Animals. 5th ed., pp. 890-894. Academic Press, Washington DC, 1997.
9. **Kitchalong L, Fernandez JM, Bunting LD, Southern LL, Binder TD.** Influence of chromium tripicolinate on glucose metabolism and nutrient partitioning in growing lambs. J Anim Sci 1995, **73**, 2694-2705.
10. **Mertz W.** Chromium occurrence and function in biological systems. Physiol Rev 1969, **49**, 163-239.
11. **Moonsie-Shageer S, Mowat DN.** Effect of level of supplemental chromium on performance, serum constituents and immune status of stressed feeder calves. J Anim Sci 1993, **71**, 232-238.
12. **Morand-Fehr P, Boyazoglu J.** Present status and future outlook of the small ruminant sector. Small Rumin Res 1999, **34**, 175-188.

13. **National Research Council.** Nutrient Requirements of Beef Cattle. 7th ed. National Academies Press, Washington DC, 1996.
14. **Pechova A, Podhorsky A, Lokajova E, Pavlata L, Illek J.** Metabolic effects of chromium supplementation in dairy cows in the peripartal period. *Acta Vet Brno* 2002, **71**, 9-18.
15. **Schrauzer GN, Shrestha KP, Molenaar TB, Meade S.** Effects of chromium supplementation on food energy utilization and the trace element composition in the liver and heart of glucose exposed young mice. *Biol Trace Elem Res* 1986, **9**, 79-87.
16. **Schwarz K, Mertz W.** Chromium (III) and the glucose tolerance factor. *Arch Biochem Biophys* 1959, **85**, 292-295.
17. **Spears JW.** Reevaluation of the metabolic essentiality of minerals- review. *Asian-Australian J Anim Sci* 1999, **12**, 1002-1008.
18. **Subiyatno A, Mowat DN, Yang WZ.** Metabolite and hormonal responses to glucose or propionate infusions in periparturient dairy cows supplemented with chromium. *J Dairy Sci* 1996, **79**, 1436-1445.
19. **Swanson KC, Harmon DL, Jacques KA, Larson BT, Richards CJ, Bohnert DW, Paton SJ.** Efficacy of chromium yeast supplementation for growing beef steers. *Anim Feed Sci Technol* 2000, **86**, 95-105.
20. **Wasserman RH, Kallfelz FA, George L.** Bones, joints and synovial fluid. In: Swenson MJ, Reece WO (eds.). *Dukes' Physiology of Domestic Animals*. pp. 549-550, Panima Publishing, New Delhi & Bangalore, 1996.
21. **Watson CA.** Official and Standardized Methods of Analysis. 3rd ed. pp. 277-279, The Royal Society of Chemistry, London, 1994.