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Effect of Dietary Chromium Supplementation in Transition Calves on Insulin Sensitivity and Biomarkers of Rumen Development

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ABSTRACT

A study of 100 days using calves in transition phase of growth (15 to 115 days) was conducted. 24 Hariana calves were randomly assigned to four groups of six each and fed on diets without supplemental chromium (Cr) as control or 0.05 mg, 0.10 mg, and 0.15 mg Cr/kg $BW^{0.75}$ groups. Treatment had no significant effect on the average daily gain (ADG) and body condition score (BCS). Plasma glucose, insulin, insulin: glucose ratio, and insulin receptor substrate – (IRS-1) as biomarkers of insulin sensitivity while â-hydroxy butyrate (BHBA) and insulin like growth factor -1 (IGF-1) as biomarkers of rumen development were studied on 0, 30, 60, 70, 80, 84, 86, 87, 88, 89, 90, 95, and 100 days post-Cr supplementation. Plasma glucose and insulin concentrations decreased ($p < 0.05$) with the advancement of age of calves and their levels were lowest ($p < 0.01$) in the calves supplemented with highest Cr level (0.15 mg of Cr/kg $BW^{0.75}$). The insulin: glucose ratio was higher ($p < 0.05$) in the calves supplemented with Cr. Treatment, period and treatment × period interaction had a significant effect on plasma IRS-1 concentration as its concentration increased with the increase in supplementation level of Cr. Treatment and treatment × period interaction had non significant on plasma BHBA and non-esterified fatty acids (NEFA) levels while period had significant effect on BHBA ($p<0.05$ and NEFA ($p<0.01$) concentrations. However, a significant effect of treatment, period, and treatment × period interaction on plasma IGF-1 concentrations was observed with higher ($p < 0.01$) plasma IGF-1 concentration in 0.15 mg of Cr/kg $BW^{0.75}$ supplemented calves. In conclusion, the dietary supplementation of Cr was beneficial in improving insulin sensitivity and modulating biomarkers of rumen development of calves during the transition period from pre-ruminant to ruminant phase.

KEYWORDS: Biomarkers, Chromium, Rumen development, Transition calf,

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INTRODUCTION

The process of transitioning of dairy calves from their pre-ruminant to the ruminant stage results in various metabolic ramifications (Baldwin et al., 2004). The energy metabolism of dairy calves during this phase experiences major changes with the shift from a pre-ruminant glucose, cholesterol, and â-hydroxybutyric acid (BHBA) dependency to a functional ruminant metabolism with volatile fatty acids (VFAs) dependency in adult animals (Nussio et al., 2003). An antagonistic relationship between glucose and BHBA concentrations is observed during this phase, showing a decrease in glucose and an increase in BHBA concentrations (Khan et al., 2011). BHBA is considered as an indicator of rumen maturation and its VFA utilization capability (Deelen et al., 2016). BHBA also maintains functions involved in cellular signalling to regulate rumen cell growth by activating or inhibiting various signal pathways (Han et al., 2020). A decline in insulin sensitivity during the transition phase is a normal homeorhetic metabolic adaptation that helps in this transformation by making glucose-dependent tissues of young calves into glucose-resistant tissues in adult ruminants (Bauman et al., 2000; Ingvartsen and Andersen, 2000). However, a progressive decrease in insulin sensitivity during this phase predisposes calves towards various metabolic ramifications, such as neonatal calf diarrhea (Pantophlet et al., 2016),

negative energy balance, promoting lipolysis, and other metabolic disorders occurring in later life (Contreras et al., 2017). The promoting effect of BHBA on rumen epithelial proliferation was associated with improved insulin sensitivity (Kato et al., 2011). Studies claimed that an increase in insulin sensitivity or infusion of insulin significantly stimulated cell proliferation in the rumen epithelium (Sakata et al., 1980). Insulin like growth factor 1 (IGF-1) also acts as a growth promoter and regulates the proliferation of many cell types, including the epithelial cells of the rumen (Wang et al., 2017). Calves weaned earlier showed significantly more ruminal epithelial growth and had higher circulating levels of IGF-1 than calves weaned later (Zitnan et al., 2005). During this transitioning, increase in blood non-esterified fatty acid (NEFA) concentration was observed which may reflect the initiation of lipolysis (Stanley et al., 2002).

Several studies and clinical trials with humans and animals have provided confirmation in favour of the beneficial role of chromium (Cr), which has been shown to improve insulin sensitivity more effectively than other nutritional strategies (Wang et al., 2022). Cr as a low-molecular-weight Cr (LMWCr) binding substance enhances insulin sensitivity by potentiating the binding of insulin to its receptor (Wada et al., 1993). LMWCr enhances communication between insulin to its receptors by activating insulin receptor kinase and by increasing phosphorylation rates of insulin binding receptors, thus facilitating the expression of insulin receptor substrate 1 (Wang et al., 2009; Kooshki et al., 2021). Several studies concluded a better glucose clearance during the glucose challenge test, indicating greater insulin sensitivity in Cr supplemented animals (Hayirli et al., 2001; Kumar et al., 2023; Khare et al., 2023). Enhanced insulin action upon Cr supplementation results in variation in plasma NEFA and liver triglyceride concentrations in transition cows (Hayirli et al., 2001). The activity of IGF-1 that has functional homology to insulin receptors and BHBA is increased in Cr supplemented animals (Al-Saiady et al., 2004).

Recent studies (Khare et al., 2023; Kumar et al., 2023) provided evidence that Cr supplementation in calves during the transition period stimulates insulin sensitivity. Various studies have been conducted to fasten the rumen development using nutritional approaches like feeding of milk replacer, calf starter, liquid vs. solid feed etc. (Górka et al., 2009; Górka et al., 2011). Nevertheless, an association study on the role of Cr and age of calves on insulin sensitivity and biomarkers of rumen development has not yet been documented. To develop nutrition strategies for the smooth transitioning of dairy calves by regulating insulin sensitivity will be helpful in controlling various metabolic disorders. We tested the hypothesis that the Cr supplementation may assist in smooth transitioning of young calves from pre-ruminant to ruminant stage by increasing insulin sensitivity and modulating biomarkers of rumen development.

MATERIALS AND METHODS

Ethics approval, animals and experimental design

All animal procedures and protocols were approved by the article number 13 of Institutional Animal Ethic Committee (IAEC) of DUVASU, Mathura (approval number: IAEC/21/22), in compliance with the guidelines set forth by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) rules laid down by the Government of India.

24 healthy Hariana calves were selected from the Livestock Farm Complex (LFC), assigned at random to four groups (n=6 in each group), and fed on diets without supplemental Cr (control) or basal diet supplemented with 0.05, 0.10, 0.15 mg Cr per kg $BW^{0.75}$. The daily dose of Cr as Cr-picolinate (Research Lab, Fine Chemicals Industries, Mumbai, India) was calculated per kg $BW^{0.75}$, weighed and encapsulated in 500 mg capacity gelatinized capsules, and fed orally in the morning at 7.00 h to the experimental calves of respective groups. The daily nutrient requirement of the experimental calves was met by feeding milk, calf starter, green maize fodder, and wheat straw (ICAR, 2013). The fresh milk and calf starter were offered at 10% and 1% of BW individually at 8.00 h and 10.00 h, respectively. The calves were dewormed before start of the study and access to fresh water, green maize fodder, and wheat straw was *ad libitum*. The ingredient and nutrient composition of the calf starter, maize fodder, and wheat straw are presented in Table 1.

Table 1. Composition of calf starter, maize fodder and wheat straw

Items	Calf starter	Maize fodder	Wheat straw
Ingredients, g/kg			
Soybean meal (solvent extracted)	380		
Maize grain (yellow)	350		
Wheat bran	150		
Gram husk	100		
Mineral and vitamin premix[α]	10		
Salt	5		
Dicalcium phosphate	5		
Analyzed composition (% DM, except for DM)			
Dry matter	90.4	17.9	89.0
Crude protein	23.0	9.8	3.9
Ether extract	40.3	3.3	1.4
Total ash	14.9	4.5	13.3
Neutral detergent fibre	33.6	47.2	78.1
Acid detergent fibre	11.2	27.3	53.3
Acid detergent lignin	3.5	2.9	8.7
Calcium	1.4	0.5	0.4
Phosphorus	0.6	0.2	0.2
Chromium, mg/kg DM	0.32	0.19	0.15

[α]Premix per kg composed of vitamin A: 10,000,000 IU; vitamin E: 80,000 IU; vitamin D: 1,500,000 IU; Fe: 50 g; Zn: 60 g; Mn: 50 g; Co: 0.1 g; Cu: 12 g; Se: 0.15 g; I: 0.5 g

Observation recording, blood sampling, and laboratory analysis

The experimental calves were monitored fortnightly for growth performance and BCS (Anitha et al., 2010). The representative samples of feeds and fodders offered were collected and analysed for their chemical composition. AOAC (2005) procedures were used for dry matter (DM; methods 967.03), ash (method 942.05), ether extract (EE; method 920.39), and crude protein (CP; method 984.13) determination. Amylase treated neutral detergent fibre (aNDFom), acid detergent fibre (aADFom), and acid detergent lignin (ADL) were determined (Mertens, 2002). Peripheral blood samples were collected on 0, 30, 60, 70, 80, 84, 86, 87, 88, 89, 90, 95, and 100 days post-Cr supplementation for the determination of variation in biomarker of rumen development by venipuncture of anterior vena cava in heparinized vacuutainer tubes (BD Franklin, USA). The vacuutainer tubes with blood were immediately placed in an ice box and transferred to the laboratory for centrifugation at 3000 × g at 4°C for 30 min for plasma isolation. Plasma samples were stored at 20°C until further analysis of biomarkers, i.e., glucose, insulin, IRS-1, BHBA, NEFA, IGF-1, and Cr level. Glucose in plasma was measured using the "Endpoint Assay Test Kit" (Span Diagnosis Ltd. Surat, Gujrat). Serum insulin, IRS-1, BHBA, and NEFA concentrations were quantified using a "Bovine Specific ELISA Test Kit" (Bioassay Technology Laboratory, China). The mineral analysis in feedstuffs and plasma was carried out by using Agilent Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES-5800, USA).

Statistical analysis

The data were analyzed using the MIXED procedure of SPSS (Version 21.0, Inc., Chicago, IL). Plasma variables determined during IVGTT were analyzed by using one-way ANOVA, while other blood variables were analyzed by using the following model of repeated measures.

$$Yijk = \mu + Ti + Dj + (T \times D)\, ij + eijk$$

Where Yijk is the dependent variable, ì is the overall mean of the population, Ti is the mean effect of the treatment, Dj is the mean effect of period (j=0, 30, 60, 70, 80, 84, 86, 87, 88, 89, 90, 95, and 100 days of dietary treatment) of sampling, (T×D)ij is the effect of the interaction between the effect of Cr supplementation and the day or period of sampling, and eijk is the unexplained residual element assumed to be independent and normally distributed. The effects of treatment, period, and treatment by period interaction were considered fixed while experimental calves as a random effect. If the statistical analysis revealed a significant effect ($p < 0.05$), the differences between treatment, period, and treatment by period interaction were then determined by Duncan's post hoc test.

RESULTS AND DISCUSSION

There was no significant effect of treatment, period, and treatment × period interaction on ADG, BCS, and calf starter intake (Table 2).

Table 2. Effect of Cr on growth performance during experimental period

Particulars	Supplemental Cr (mg/kg $BW^{0.75}$)				Pooled SEM	P value		
	0.00	0.05	0.10	0.15		Treatment (T)	Period (P)	T×P
Initial BW (kg)	29.24	30.52	30.67	30.89	1.42	0.554	0.839	0.994
Final BW (kg)	45.88	46.96	48.44	50.45	2.24	0.550	0.878	0.996
Calf starter intake (g/day)	369.50	387.5	392.2	407.5	15.28	0.189	0.277	0.582
ADG (g/day)	184.81	175.28	201.37	217.30	12.23	0.357	0.662	1.000
BCS	2.88	2.88	2.92	2.90	0.060	0.648	0.719	0.772

Calves are born with a functional monogastric stomach that relies on nutrients from milk or milk replacer. During the transition from pre-ruminant to ruminant stage, various physiological and metabolic adaptations take place. The change from functional monogastric to ruminant not only relies on VFA production in the rumen to supply energy but also on well-functioning endocrine and biochemical features such as ruminant specific insulin homeostasis and hepatic gluconeogenesis (Schwarzkopf et al., 2019). In the present study the dietary supplementation of Cr did not exert any significant effect on ADG and BCS. Accordingly, no effect of 400 and 800 µg Cr/kg as Cr-L-methionine supplementation on ADG was noticed in calves (Kegley et al., 2000). A similar body weight gain was observed in winter-exposed buffalo calves receiving diets supplemented with different levels of inorganic Cr (Kumar et al., 2017). Others also did not observe any influence on weight gain in Cr supplemented calves (Swanson et al., 2000; Yari et al., 2010; Mousavi et al., 2019). In contrast to the findings of this study, better weight gain was found in Cr supplemented Holstein calves (Kegley et al., 1997; Ghorbani et al., 2012; Kargar et al., 2018). No effect of 1.0 mg Cr/kg DM from different Cr sources (Cr-picolinate, Cr-polynicotinate, and Cr-yeast) was noticed by Keshri et al. (2019) in Hariana calves. No effect of Cr supplementation on growth and higher BCS was observed in post-partum cows fed diet supplemented with Cr as Cr-methionine (Hayirli et al., 2001).

Insulin sensitivity

The changes in plasma glucose, insulin, insulin: glucose ratio, and IRS-1 from pre-ruminant to ruminant stage were used as biomarkers of insulin sensitivity (Table 3).

Table 3. Effect of Cr on biomarkers of insulin sensitivity and rumen development

Biomarker	Supplemental Cr (mg/kg $BW^{0.75}$)				Pooled SEM	Significance		
	0.00	0.05	0.10	0.15		Treatment (T)	Period (P)	T×P
Biomarkers of insulin sensitivity								
Glucose concentration (mMol/L)	4.34[b]	4.02[ab]	3.68[a]	3.72[b]	0.15	0.045	0.028	0.049
Insulin concentration (mIU/L)	1.76[b]	1.73[a]	1.72[a]	1.71[a]	0.02	<0.001	0.016	0.0289
Insulin: glucose ratio	0.41[a]	0.43[ab]	0.47[b]	0.46[b]	0.13	0.027	0.047	0.898
IRS-1 level (ng/ml)	16.31[a]	17.75[a]	22.19[c]	19.48[b]	1.31	<0.001	0.006	0.041
Biomarkers of rumen development and energy balance								
BHBA concentration (nMol/ml)	434.32	455.54	487.06	500.67	15.13	0.272	0.031	0.994
IGF-1 level (ng/ml)	198.83[a]	219.49[ab]	241.34[b]	263.29[b]	13.97	<0.001	0.038	0.039
NEFA concentration (µMol/L)	224.62	259.76	262.18	267.11	5.08	0.073	<0.001	0.899
Cr level (µg/L)	113.37[a]	209.68[b]	246.89[bc]	292.58[c]	10.10	<0.001	0.493	0.918

As the age of the experimental calves advanced, plasma glucose concentration decreased, with the lowest value ($p < 0.05$) in the calves fed on a diet supplemented with 0.15 mg of Cr/kg $BW^{0.75}$ (Fig. 1). A similar trend of lower ($p < 0.01$) plasma insulin concentration was observed in Cr supplemented calves than in calves of the control group (Fig. 2). The period and treatment x period interaction showed a significant ($p < 0.05$) effect on insulin concentration while interaction of treatment × period had non-significant effect on insulin: glucose ratio and the ratio was higher in Cr supplemented calf compared to non Cr supplemented calves.

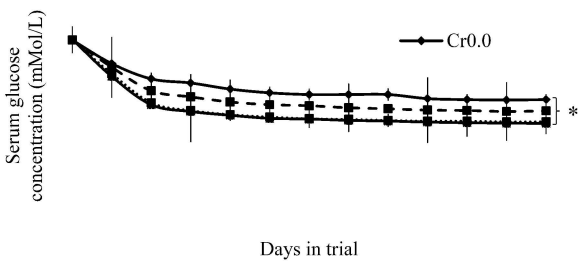

Fig. 1 The effect of Cr supplementation on plasma glucose concentrations in transition calves. * ($p < 0.05$)

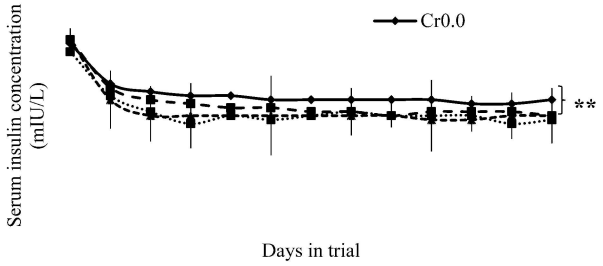

Fig. 2 The effect of Cr supplementation on plasma insulin concentrations in transition calves. ** ($p < 0.001$)

The better insulin sensitivity in this study was in accordance with other reports. The serum glucose concentrations in calves administered with 400 µg supplemental Cr per kg of diet were lower at 5 and 10 min after glucose infusion than in control and in calves supplemented with 800 µg supplemental Cr/kg of diet (Kegley et al., 2000). Similarly, calves supplemented with 400 µg Cr-nicotinic acid complex/kg of feed had higher plasma glucose concentration after 15 min of an IVGTT, and that their serum glucose concentrations declined faster than those of non Cr-supplemented calves (Kegley and Spears, 1995). However, no effect of Cr supplementation on the serum insulin response during glucose challenge was noticed in calves fed on diet supplemented with Cr as Cr-Pic (Bunting et al., 1994). The amount of insulin released was considerably lower in heifers fed a diet supplemented with Cr as compared to the un-Cr-supplemented heifers (Spears et al., 2012). A tendency for a higher glucose clearance rate in calves receiving 0.05 mg of supplemental Cr-Met/kg of body weight was reported while insulin sensitivity remained unaltered (Mousavi et al., 2019). In other reports also the supplementation of Cr improved insulin sensitivity and glucose kinetics following the glucose challenge test (Hayirli et al., 2001; Stahlhut et al., 2006; Yari et al., 2010; Spears et al., 2020) whereas insulin concentrations and insulin: glucose ratio did not differ among heifers supplemented with 0.47, 0.94, and 1.42 mg Cr/kg DM (Spears et al., 2016).

Treatment, period and treatment × period had a significant ($p < 0.05$) effect on plasma IRS-1 concentration (Table 3 and Fig. 1). As the level of Cr supplementation and age of calves advanced, plasma IRS-1 concentration increased due to better insulin sensitivity in Cr supplemented calves. IRS-1 is a substrate of the insulin receptor tyrosine kinase and appears to have a central role in the insulin-stimulated signal transduction pathway (De Meyts, 2016). A study on transition Hariana calves supplemented with Cr-Pic reported better IRS-1 response in treatment than in control group (Kumar et al., 2023). However, work to date in dairy cattle has not attempted to determine the effects of Cr supplementation on IRS-1 response. Turgut et al., (2018), reported that Cr supplementation can increase the expression level of IRS-1 mRNA in skeletal muscles. The results of the study corroborate previous report (Jain et al., 2010), who reported that IRS-1 expression in the liver tissues of type 2 diabetic rats increased after Cr supplementation. In other studies, Cr-Pic supplementation improved glucose disposal rates and IRS-1 expression in skeletal muscles (Wang et al. 2006). In contrast, an inhibitory effect of Cr supplementation was observed on IRS-1 in hepatoma cells (Yurkow and Kim, 1995). Enhanced insulin-mediated tyrosine phosphorylation of IRS-1 after Cr exposure could be one of the explanations for this finding (Chen et al., 2006).

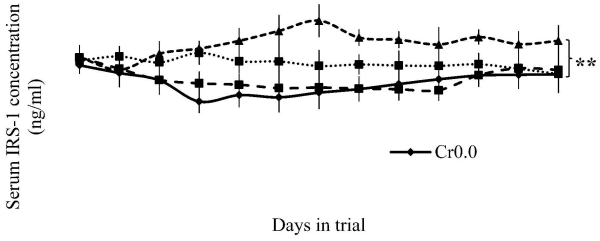

Fig. 3 The effect of Cr supplementation on plasma IRS-1 response in transition calves. ** ($p < 0.001$)

Dynamics of biomarkers of rumen development and energy balance

The changes in plasma BHBA and IGF-1 levels from pre-ruminant to ruminant stage were used as biomarker of rumen development whereas, variation in plasma NEFA concentration were used as biomarker of energy balance (Table 3).

The effect of treatment on plasma BHBA concentration was not significant whereas, plasma BHBA concentration increased ($p < 0.05$) with the advancement of age of the calves. Recent research suggested that circulating BHBA levels may be a meaningful indicator of rumen development and a surrogate measure of rumen function in pre-ruminant calves (Deelen et al., 2016). Before the rumen develops, glucose is used as a primary energy source by the calf. When calves are offered a starter concentrate and fermentation occurs, a large amount of BHBA is produced; afterward, the calf is adapted to this new nutrient as a source of energy (Quigley et al., 1991; Klotz and Heitmann, 2006). The serum BHBA concentration increased after weaning for the early-weaned calves, whereas it remained low in the late-weaned calves and increased after their weaning period (Schwarzkopf et al., 2019) and BHBA concentration was negatively correlated with glucose concentration. Following supplementation with organic Cr for 63 days, no significant changes were detected between treatments in plasma BHBA

in young calves (Earley et al., 2002). However, lower blood BHBA levels in Cr supplemented calves were reported by Ghorbani et al. (2012).

There was a significant effect of treatment ($p < 0.01$), period ($p<0.05$), and treatment × period interaction ($p < 0.05$) on plasma IGF-1 concentrations (Table 3 and Fig. 4). As the level of Cr supplementation and age of calves increased, plasma IGF-1 concentrations also increased (Fig. 4).

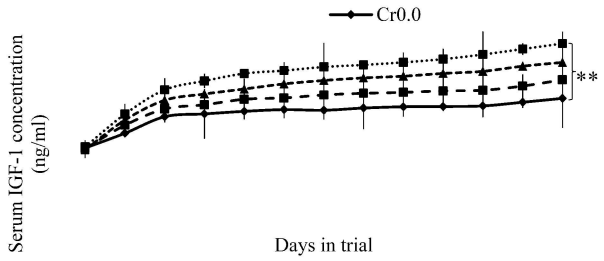

Fig. 4 The effect of Cr supplementation and period on plasma IGF-1 response in transition calves ($p<0.05$)

IGF-1 is an anabolic hormone that plays an important role in cell proliferation (Obradovic et al., 2019; Yoshida and Delafontaine, 2020). IGF-1 can stimulate epithelial cell proliferation and differentiation to enhance ruminal papillae development by regulating IGF-1 binding proteins (Hayashi et al., 2005). Cr is involved in the up-regulation of mRNA IGF-1 expression (Peng et al., 2010). In the present study, as the age of calves and level of Cr supplementation increased, plasma IGF-1 concentration also increased. Increased serum IGF-1 concentrations due to Cr-supplemented diets were shown to play a role in regulating protein and fat metabolism in pigs (Wang et al., 2014). Subiyatno et al. (1996) reported a tendency for increased circulating IGF-1 in response to Cr supplementation periparturient dairy cows. The offspring of Cr-treated male mice showed increased serum IGF-1 serum concentrations (Cheng et al., 2002). Modulation of IGF-1 signalling after Cr supplementation is well-recognised by others also (Peng et al., 2010; Chen et al., 2014; Ullah Khan et al., 2014; Morvaridzadeh et al., 2022). However, Depew et al. (1998) reported that blood IGF-1 concentrations were not affected by Cr-methionine supplementation in young calves. A trend of decrease in insulin and insulin IGF-1 concentrations with advancement of age was observed in calves (Breier et al., 1988; Abdelsamei et al., 2005; Kesser et al. 2017).

Statistical analysis of the data revealed a non significant effect of the treatment and treatment × period interaction while period showed a significant effect ($p < 0.001$) on plasma NEFA concentrations. Mostly, variation in NFEA concentrations was more often observed in animals having a state of negative energy balance (Spears et al., 2012; Leiva et al., 2017). In the present study, plasma NEFA concentration was not significantly different among groups and similar observation was reported with, no significant variation in NEFA concentrations in animals with positive energy balance and supplemented with Cr-amino acid chelates in Holstein cows (Yang et al., 1996) or Cr-Pic in Holstein steers (Besong et al., 2001). However, lower serum NEFA concentrations were noticed in Cr-Pic or chelated Cr supplemented animals (Kitchalong et al., 1995; Subiyatno et al., 1996). Lower serum NEFA concentration was noticed in calves fed milk replacer supplemented with Cr, indicating indirect evidence of enhanced insulin sensitivity in calves fed milk replacer or starter supplemented with Cr (Depew et al., 1998).

Cr supplementation resulted in a significant ($p < 0.01$) increase in plasma Cr concentration (Table 3) while the period and the interaction of treatment × period was not-significant and it was highest in the calves supplemented with 0.15 mg of Cr/kg $BW^{0.75}$. However, the serum concentration of Cu, Zn and Fe in Cr supplemented summer exposed Buffalo calves Kumar et al. (2013) remained unaltered. In opposite to findings of the present study, Cr supplemented heifer have high intake of Zn, Cu, Fe, and Mn in compared to non Cr supplemented cross-bred dairy heifers (Biswas et al., 2006). Kumar et al. (2013), Deka et al. (2015), and Kumar et al. (2023) also observed a dose-dependent increase in serum Cr concentration while the level of other minerals remained comparable in treatment as well as in the control group.

CONCLUSION

The results of the study indicated that the dietary supplementation of Cr may be beneficial in improving insulin sensitivity and modulating biomarkers of rumen development in calves during the transition period from pre-ruminant to ruminant phase.

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