



Short Communication
Chromium and *In Vitro* Rumen Fermentation

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Assessing Effect of Chromium Treatment on *In Vitro* Rumen Fermentation Attributes

R. S. Deka¹, V. Mani², S. M. Shete² and M. Kumar^{3*}

Dairy Cattle Nutrition Division, ICAR-National Dairy Research Institute, Karnal-132 002 Haryana, India

¹Krishi Vigyan Kendra, Morigaon, Assam Agricultural University-782410, Assam, India

²Dairy Cattle Nutrition Division, ICAR-National Dairy Research Institute, Karnal-132001, Haryana, India

³Animal Nutrition Department, College of Veterinary Science and Animal Husbandry,

DUVASU, Mathura-281001, UP, India

*Correspondence: muneendra82@gmail.com

ABSTRACT

The present study was designed to investigate the effect of different levels (0.25, 0.5, 0.75, 1.0, 1.5, 2.0, 2.5 and 3.0 ppm) of inorganic chromium (Cr) as $\text{CrCl}_3 \cdot 6\text{H}_2\text{O}$ on *in vitro* microbial fermentation using ruminal fluid. The substrate sample used for the study consisted of a mixture of compounded concentrate, maize fodder and wheat straw in the ratio of 40:30:30, respectively. Three sets of *in vitro* trials in triplicate were conducted along with respective blanks to estimate the various rumen fermentation attributes such as *in vitro* dry matter degradability (IVDMD), *in vitro* organic matter degradability (IVOMD), individual volatile fatty acid (IVFA), total VFA (TVFA), partitioning factor (PF), methane (CH_4) and short chain fatty acid (SCFA) to obtain the comprehensive picture of rumen fermentation pattern. Treatment with Cr did not exert any effect on IVDMD and PF values. The molar proportion of propionate at 24 h of incubation was lower ($P < 0.05$) with 2.5 ppm of Cr compared with 1.5 ppm of Cr. However, TVFA, CH_4 and SCFA did not differ across the dietary treatments. It was concluded that the addition of Cr up to 3.0 ppm did not exert any adverse effect on the studied *in vitro* rumen fermentation attributes.

Key words: Chromium, Methane, Rumen fermentation, Volatile fatty acids

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INTRODUCTION

Mineral content in feeds and fodder is decreasing over a period of time due to intensive agricultural practices (López-Alonso, 2012). Simultaneously, every attempt is being made to explore the full production potential of dairy animals. Therefore, there is a scope for modifying the existing mineral mixture composition with newer elements having physiological role, to get better returns. In this regard, mineral supplementation, particularly the trace minerals are supposed to be natural and safer strategy to increase the production as well as alleviate the stress and enhance the immune status of the animals (Echeverry et al., 2016).

Chromium (Cr) is the newer trace element which can be supplemented to yield better results under various stress conditions (NRC, 1997). The essentiality of Cr was first demonstrated in rats by Schwarz and Mertz (1959). Cr as low-molecular-weight Cr (LMWCr) binding oligopeptide enhances communication between insulin and its receptors, therefore, reducing the risk of insulin resistance (Wada et al., 1983). Furthermore, Cr is an integral module in various enzymes involved in the metabolism of carbohydrates, lipid, protein, nucleic acids as well as the regulation of hormones, reproduction, growth and immune functions (Pechova and Pavlata, 2007). Therefore, Cr insufficiency results in impaired nutrient

metabolism, decreased insulin sensitivity in peripheral tissues, weakened immunity, and reduced weight gain and health status, particularly under stressful conditions (Kargar et al., 2018a&b; Mousavi et al., 2019b). NRC (2001) has also indicated the addition of Cr in the stressed animals exerts beneficial effects, but supplementation level is not recommended. Over the past, several well-designed clinical trials have provided evidence in favor of a beneficial effect of Cr but still now there is no clear-cut recommended level of Cr is identified. Considering these facts, the present study was therefore designed to investigate the effect of different treatment levels of Cr on rumen fermentation parameters.

In this *in vitro* study, rumen liquor was collected from two donor buffalo bulls, fitted with permanent rumen fistula. Donor animals were maintained on roughage and concentrate based diet (1.0 kg concentrate mixture, 5 kg green fodder and ad libitum wheat straw). The ruminal fluid was collected before morning feeding and watering of animals into a pre-warmed thermos flask and was brought to the laboratory in anaerobic condition. The rumen liquor was bubbled with CO₂ and filtered through four layers of muslin cloth. Different levels (0.25, 0.5, 0.75, 1.0, 1.5, 2.0, 2.5 and 3.0 ppm) of Cr from inorganic Cr source (CrCl₃•6H₂O) were added to the substrate to determine effects on *in vitro* microbial fermentation using ruminal fluid. Substrate used for *in vitro* study consisted of concentrate mixture, maize fodder and wheat straw mixed in the ratio of 40:30:30. Measurement of gas production *in vitro* was used to study the extent and

rate of digestion of feedstuffs (Hungate, 1966; as referenced by Stern et al., 1997). The three sets of *in vitro* trials in triplicate were conducted to estimate various parameters such as individual volatile fatty acid (IVFA) (Erwin et al., 1961), total VFA (TVFA) (Barnett and Reid, 1957), methane (CH₄) production (Gas chromatograph; Nucon 5700, India), *in vitro* gas production test (IVGPT), and *in vitro* dry matter degradability (IVDMD) and *in vitro* organic matter degradability (IVOMD) to obtain the comprehensive picture of rumen fermentation pattern. Gas produced (ml/200 mg substrate) by fermentation during 24 h was measured after correcting corresponding blank values. True degradable DM (TDDM) and true degradable OM (TDOM) of samples were estimated as per Van Soest et al. (1991). Short chain fatty acid (SCFA) production was calculated as per Getachew et al. (1999). The feedstuffs were analyzed for dry matter (DM), crude protein (CP), ether extract (EE), ash and acid insoluble ash (AIA) content by using methodology of AOAC (2005). However, neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) were estimated as per the procedure described by Van Soest et al. (1991). Chemical composition of different feeds and fodders used as substrate in the *in vitro* study is presented in Table 1. The partitioning factor (PF) was also calculated at 24 h of incubation as the ratio between mg of TDOM (True digestible OM) and gas volume (mL) to evaluate the microbial degrading effectivity (Blümmel et al., 1997). The generated data for studied attributes were analyzed by using the one-way ANOVA procedure of SPSS (V20.0; SPSS Inc., Chicago, IL, USA).

Table 1. Chemical composition of feed ingredients (% , dry matter basis)

Parameters	Concentrate mixture	Maize fodder	Wheat straw
Dry matter	86.8	16.8	86.3
Organic matter	91.3	95.6	92.3
Crude protein	21.3	10.1	3.50
Ether extract	3.70	2.60	1.00
Neutral detergent fibre	36.0	48.6	73.1
Acid detergent fibre	11.2	25.3	50.65
Total ash	8.50	4.40	7.70

The effect of different levels of Cr on IVDMD, IVOMD, IVGPT, PF and CH₄ production is presented in Table 2. Addition of Cr showed non-significant effect on the degradability of DM. IVOMD was similar in all the treatment groups and observed in between the range of 67-68% in different groups. PF calculated by concomitant true DM degradability and gas volume measurements after 24 h incubation have been used to assess the partitioning of nutrients between gases and microbial

cells (Blümmel et al., 1997) and the high PF value (low gas production per unit of substrate degraded) is indicative of proportionally higher microbial yield. The PF values obtained by different levels of Cr addition ranged from 3.46 to 3.62. Estimation of CH₄ production expressed as % of total gas, ml CH₄/200 mg DM and mg CH₄/g truly digestible OM found to be similar in all groups. It was concluded that the CH₄ production was not altered notably due to Cr supplementation in an *in vitro* rumen system.

Table 2. Effect of Cr treatment on *in vitro* degradability, gas production and PF (n=6)

Cr level (ppm)	IVTDMMD (%)	IVTOMD (%)	IVGPT (ml/200 mg DM)	PF	CH ₄ production		
					% of total gas DM	ml/200 mg	ml/g TDOM
0.00	66.6±1.37	67.2±0.03	37.3±0.88	3.5±0.08	32.3±0.13	10.9±0.05	81.3±0.29
0.25	66.6±0.55	67.2±0.02	37.3±0.88	3.5±0.08	32.2±0.13	10.7±0.02	79.7 ±0.20
0.50	66.1±0.35	67.2±0.04	37.4±0.44	3.5±0.04	32.3 ±0.82	10.8±0.38	79.9±3.13
0.75	66.6±0.61	67.7±0.04	37.4±0.17	3.6±0.02	32.3±0.08	10.6±0.16	78.5±1.19
1.00	67.4±0.48	67.4±0.18	39.0±1.00	3.6±0.09	32.2±0.34	11.1±0.31	81.9±0.85
1.50	67.4±0.99	67.5±0.18	38.2±0.44	3.6±0.05	32.3±0.17	10.9±0.06	81.4±0.63
2.00	67.8±0.23	67.5±0.18	38.3±0.33	3.6±0.03	32.1±0.86	11.1±0.37	81.9±2.59
2.50	68.2±0.55	67.5±0.18	37.8±1.01	3.6±0.09	32.1±0.37	10.7±0.41	79.5±1.10
3.00	68.1±0.28	67.5±0.36	37.3±0.28	3.6±0.05	32.3±0.16	10.8±0.04	79.6±0.68

Cr, chromium; IVDMD, *in vitro* dry matter degradability; IVOMD, *in vitro* organic matter degradability; IVGPT, *in vitro* gas production test; DM, dry matter; PF, partitioning factor; CH₄, methane; TDOM, true degradable OM.

Table 3. Effect of Cr treatment on *in vitro* volatile fatty acid and short chain fatty acid production

Cr level (ppm)	Molar concentration (mM/100ml)				Fractions of volatile fatty acid (%)				SCFA (µmol/g DM)
	Total volatile fatty acid	Acetate	Propionate	Butyrate	Acetate	Propionate	Butyrate	Acetate: Propionate ratio	
0.00	80.0±1.42	57.5±1.06	15.3±0.16 ^{cd}	7.18±0.52 ^b	71.8±0.05 ^{cd}	19.1±0.55 ^{dc}	8.71±0.50 ^b	3.76±0.11 ^{bc}	0.82±0.02
0.25	80.3±0.88	57.6±0.20	15.0±0.14 ^{de}	6.10±0.02 ^c	71.7bcd±0.58 ^{bcd}	18.7±0.03 ^d	7.60±0.08 ^c	3.95±0.00 ^{ab}	0.82±0.02
0.50	80.8±1.83	56.8±1.56	15.9±0.33 ^{bcd}	8.12±0.05 ^a	70.2±0.34 ^e	19.6±0.04 ^{cd}	10.0±0.3 ^a	3.57±0.02 ^{cd}	0.82±0.01
0.75	81.3±1.64	57.5±1.42	16.4±0.25 ^{bc}	7.31±0.14 ^b	70.7±0.41 ^{de}	20.2±0.39 ^{bc}	8.99±0.02 ^b	3.50±0.09 ^{de}	0.82±0.00
1.00	81.5±1.69	59.1±0.99	15.8b±0.50 ^b	6.55±0.20 ^{bc}	72.5±0.30 ^{bc}	19.3±0.22 ^{cde}	8.05±0.08 ^{cd}	3.75±0.06 ^{bc}	0.86±0.02
1.50	81.8±0.60	58.0±0.50	17.9±0.25 ^a	6.37±0.18 ^c	70.9±0.59 ^{de}	21.2±0.56 ^a	7.79±0.26 ^{dc}	3.34±0.11 ^e	0.84±0.01
2.00	82.1±1.73	58.4±1.21	16.98±0.35 ^{ab}	6.71±0.19 ^{bc}	71.1±0.07 ^{de}	20.6±0.02 ^{ab}	8.16±0.10 ^{cd}	3.44d±0.00 ^{de}	0.85±0.01
2.50	80.0±3.59	58.6±2.63	14.6±0.66 ^e	6.69±0.30 ^{bc}	73.3±0.00 ^{ab}	18.2±0.00 ^f	8.36±0.00 ^{bc}	4.02±0.00 ^a	0.83±0.02
3.00	79.0±1.30	58.2±0.96	14.7±0.24 ^{de}	5.96±0.10 ^c	73.7±0.00 ^a	18.6±0.00 ^{ef}	7.54±0.00 ^d	3.95±0.00 ^{ab}	0.82±0.01

a-f Means with different superscript in a column differ significantly (P<0.05)

The average concentration of TVFA, fractions of VFA (acetate, propionate and butyrate), acetate: propionate ratio and SCFA under in vitro incubation is presented in Table 3. The TVFA concentration was 80.00 mM/100 ml in control and ranged from 79.00 to 82.17 mM/100 ml in substrate incubated with different levels of Cr thus revealing no significant effect of Cr addition. These results are in accordance with those obtained by Samanta et al. (1998). Besong et al. (1996) and Rikhari et al. (2010) also reported that in vivo addition of Cr in diet of ruminants had no effect on TVFA concentration and CH₄ production. VFA fraction of acetate, propionate and butyrate in control was 71.88, 19.16 and 8.71%, respectively. However, fractions of acetate, propionate and butyrate in substrates incubated with varying levels of Cr ranged from 70.25-73.79, 18.27-21.24 and 7.54-10.06, respectively. The molar proportion of propionate at 24 h of incubation was lower (P<0.05) with 2.5 ppm Cr compared with 1.5 ppm Cr group. Results reveal no detrimental effects of Cr supplementation on microbial activity. However, different levels of Cr may alter individual molar proportions of VFA in rumen fluid. Similar to the findings of the present study, Besong et al. (1996) also observed no or little effects of in vitro Cr treatment (up to 26.5 ppm) on VFA fractions. Samanta et al. (1998) concluded from their in vitro study that incremental dose level of 10, 25, 50 and 100 ppm of Cr did not exert any significant effect on VFA fractions. In this study, acetate-to-propionate ratio was 3.76 in control group and ranged from 3.44 to 4.03 in different Cr treated groups. However, SCFA values for control were 0.83 $\mu\text{mol/g}$ DM and varied from 0.82 to 0.85 $\mu\text{mol/g}$ DM in different treatment groups. Although, SCFA provides a better insight into the roles of various nutrients with respect to production of fermentative gases but in this study, findings are not discussed due to lack of literature.

The findings of this in vitro study revealed that addition of Cr up to 3.0 mg/kg DM did not exert any adverse effect on studied rumen fermentation attributes. However, obtained results did not indicate any conclusive picture to draw any clear cut

inference on addition of Cr therefore; further studies are required for confirmation.

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