

Effect of dietary supplemental chromium on nutrient utilization, rumen metabolites and enzyme activities in fistulated crossbred male cattle

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Received: 16 December 2008; Accepted: 23 August 2009

ABSTRACT

The experiment was conducted to ascertain effect of different levels of dietary supplementation of chromium (Cr) on nutrient utilization, ruminal enzyme activities and fermentation pattern. Adult fistulated male crossbred cattle (3) were supplemented with chromium as chromium picolinate in a 3×3 latin square switch-over design, viz. treatment 1, control (without chromium supplementation), treatment 2 (0.5 mg chromium/kg diet dry matter as chromium picolinate), and treatment 3 (1.0 mg chromium/kg diet dry matter as chromium picolinate). There was no significant effect on dry matter, total digestible nutrients and digestible crude protein intakes and digestibility of different nutrients due to supplementation of chromium. The mean concentration of total nitrogen and non-protein nitrogen in SRL was significantly higher in T₂ supplemented with 0.5 mg Cr/kg DM than in T₃ supplemented with 1.0 mg Cr/kg DM, while TCA-precipitable protein nitrogen and ammonia nitrogen concentrations did not differ significantly among the treatments. No significant difference was observed in TVFA concentration among the treatments. However, all the rumen biochemical parameters differed significantly due to time interval of sampling of rumen liquor. The different fractions of whole rumen content, viz. particulate material (PM), cellular fraction (C) and extra cellular fraction (EC) exhibited no significant effect on activity of carboxymethyl cellulase, α -amylase, xylanase, α -glucosidase and β -glucosidase in rumen content with different levels of chromium supplementation. Activity of all enzymes was higher in particulate material fraction as compared to cellular fraction and extra cellular fraction. It is concluded that 0.5 to 1.0 mg chromium supplementation per kg diet dry matter as chromium picolinate had no effect on feed intake, nutrient utilization, ruminal metabolic profile and enzyme activities in crossbred male cattle.

Key words: Chromium, Crossbred male cattle, Nutrient utilization, Ruminal enzyme activities, Rumen metabolites

Most grains and feedstuffs are deficient in chromium (Giri *et al.* 1990). The chromium nutrition is a relatively new concept for livestock. NRC (1997) evaluated the results of chromium supplementation from several studies performed in dairy cattle, beef cattle and in sheep and reported that dietary supplementation of chromium could be beneficial for the health and well being of ruminants during time of stress. Reports exist that dietary supplementation of chromium tended to increase glucose and glucagons and decrease non-esterified fatty acids concentrations in plasma of dairy cows (Smith *et al.* 2008), increase neutralizing antirabies antibody titres in heifers (Giometti *et al.* 2006), and to decrease plasma cortisol in lactating cows (Li *et al.* 2007). Chromium supplementation also improved fertility and decreased postpartum body weight loss in beef cows

(Stahlhut *et al.* 2006b) and dairy heifers (Biswas *et al.* 2006), lowered plasma glucose (Stahlhut *et al.* 2006a) and increased glucose utilization in growing heifers (Sumner *et al.* 2007). However, there is lack of reports on the effect of supplemental chromium on ruminal metabolites and enzyme activities. The present experiment was, therefore, conducted to discern the effects of dietary supplementation of different levels of chromium as chromium picolinate on the rumen metabolism and nutrient utilization in crossbred adult male cattle.

MATERIALS AND METHODS

The experiment was conducted on 3 fistulated adult male crossbred cattle (bullocks) in a 3×3 latin square switch-over design for 3 periods on 3 dietary treatments. The treatments were allotted randomly in the first instance and thereafter these were switched over in such a way that each animal received all the 3 treatments at one time or other. The maintenance ration was fed to all the bullocks during experiment. Each period lasted for 22 days consisting of 15 days adjustment period followed by 5-day digestion trial

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during which records of feed offered, residue left and faeces voided were maintained. Thereafter in each period sampling of rumen liquor and rumen contents was done in last 2 consecutive days of experimental feeding period. The 3 dietary treatments were T_1 , control (without chromium supplementation), T_2 (0.5 mg chromium/kg diet dry matter as chromium picolinate) and T_3 (1.0 mg chromium/kg diet dry matter as chromium picolinate). For preparing the treatments, the mustard cake was ground finely and chromium picolinate containing 12% chromium was mixed in it according to the levels of chromium in different treatments, viz. 36 mg chromium picolinate was added to 100 g ground mustard cake to supplement 0.5 mg Cr/kg DM (T_2) and 72 mg chromium picolinate was added to 100 g mustard cake to supplement 1.0 mg Cr/kg DM (T_3). The concentrate mixture was given to the animals as per their maintenance requirements (NRC 2001). The respective chromium enriched mustard cake (100 g) was given as top dress after offering the concentrate mixture in T_2 and T_3 groups, whereas the animals in control group (T_1) were given 100 g mustard cake without chromium. All the animals were offered with weighed quantity of mixed fodder (oat straw + green maize or sorghum after chaffing) *ad lib.* once a day at 8.00 AM. The weight of feed offered and residue left was recorded daily. All the animals were offered fresh and clean drinking water twice daily. Body weight of each animal was recorded at the start and end of each period for 3 consecutive days. The rumen liquor (SRL) samples were collected on day 21 and 22 of the experimental feeding in each period, from each animal at 0, 2, 4 and 6 h postprandial for the estimation of total nitrogen, TCA-precipitable protein nitrogen, non-protein (soluble) nitrogen, ammonia nitrogen and total volatile fatty acids as per Sastry *et al.* (1999). The rumen liquor samples were preserved by adding 20% sulphuric acid (1 drop/10 ml) in clean dry plastic bottles of 100 ml capacity at 4°C in refrigerator till analyzed. The enzymes were assayed in the whole rumen contents collected at 2 and 4 h post-feeding. The whole rumen content was fractioned into particulate material (PM), cellular fraction (C) and extra cellular fraction (EC) by lysozyme treatment. Rumen contents (100 g) were strained through 2-layered muslin cloth by squeezing to separate particulate material. The filtrate was further processed to separate protozoal and bacterial fractions. The filtrate was centrifuged at 450×g at ambient temperature for 5 min and the pellet was marked as P (protozoal fraction). Thereafter supernatant was centrifuged at 27 000×g at 4°C for 20 min and the pellet was marked as B (bacterial fraction). The supernatant was used as source of extra cellular enzymes. The particulate material (2 g) was suspended in 10 ml phosphate buffer (0.1 M, pH 6.8), 2 ml of 0.4% lysozyme solution (0.4 g dissolved in 100 ml phosphate buffer) and 2 ml carbon tetrachloride. The suspension was incubated for 3 h at 39°C and the reaction was terminated by keeping it in ice followed by centrifugation

Table 1. Chemical composition of experimental feeds and fodder (% DM basis)

Particulars	Mixed fodder (oat straw + green sorghum/maize)	Concentrate mixture	Mustard cake
Dry matter	28.65	89.00	93.50
Organic matter	92.61	88.46	93.69
Crude protein	8.28	21.35	35.00
Ether extract	1.10	1.41	7.59
Crude fibre	27.81	5.61	3.12
Nitrogen-free extract	55.41	60.09	47.98
Total ash	7.40	11.42	6.31
Acid insoluble ash	3.71	2.34	0.52

at 27 000×g for 30 min at 4°C to get clear supernatant, which was used as enzyme source in PM fraction of rumen content. For the extraction of enzymes from P and B fractions, both pellets were collectively suspended in the amount of phosphate buffer equal to amount of extra cellular (EC) fluid. To this suspension, each of 0.4% lysozyme solution and carbon tetrachloride were added @ 1 ml/6 ml of suspension. Further processing was same as in particulate material. The clear supernatant thus collected was used as sources of cellular enzymes (C). Different fractions of rumen contents were used for estimation of different enzyme activities, viz. carboxymethyl cellulase, α -amylase, xylanase, α -glucosidase and β -glucosidase activities as per Sastry *et al.* (1999). Carboxymethyl cellulase, α -amylase and xylanase activities were determined by using assay mixture, respectively, containing 1 ml of 0.1 M phosphate buffer (pH 6.8), 0.5 ml carboxymethyl cellulose (1%), 0.5 ml enzyme; 0.5 ml buffer, 0.25 ml soluble starch (1%), 0.5 ml enzyme; and 1 ml buffer, 0.5 ml xylan (0.25%), 0.5 ml enzyme and incubated for 60, 30 and 15 min. The reducing sugars in all the above tests were estimated by dinitrosalysilic acid method (Miller 1959) taking glucose and xylose as standards. The enzyme activity was expressed as μ mol of reducing sugars produced per hour under assay conditions. The activities of α -glucosidase and β -glucosidase were measured as per Shewale and Sadana (1978) using p-nitrophenyl β -D-glucopyranoside as substrates. The activity was expressed as μ mol of reducing sugars produced per hour under assay conditions. The feeds offered, residue left and faeces voided were analyzed for proximate principles (AOAC 1990). The data were analyzed for variance and treatment means, and were compared using latin square switchover design (Snedecor and Cochran 1980).

RESULTS AND DISCUSSION

The chemical composition of experimental feeds and fodder is presented in Table 1. The dry matter intake/100 kg body weight was 1.66, 1.70 and 1.67 kg for T_1 , T_2 and T_3 , respectively (Table 2). There was no significant difference

Table 2. Average daily dry matter and nutrients intake and digestibility in fistulated crossbred male cattle fed ration supplemented with different levels of chromium

Attributes	T ₁ (Control)	T ₂ (0.5 mg Cr/kg DM)	T ₃ (1.0 mg Cr/kg DM)	SEm
<i>Dry matter and nutrients intake</i>				
Total dry matter intake (kg)	8.02±0.30	8.09±0.85	8.05±0.76	0.02
Dry matter intake/100 kg body weight (kg)	1.66±0.23	1.70±0.17	1.67±0.18	0.01
Dry matter intake/kg W ^{0.75} (g)	77.60±7.77	78.97±4.42	77.80±5.53	0.38
DCP intake (kg)	0.59±0.09	0.55±0.04	0.57±0.04	0.02
DCP intake/100 kg body weight (kg)	0.122±0.02	0.117±0.01	0.126±0.03	0.003
DCP intake/kg W ^{0.75} (g)	5.68±0.82	5.44±0.47	5.76±1.14	0.17
TDN intake (kg)	4.67±0.40	4.09±0.36	4.69±0.28	0.20
TDN intake/100 kg body weight (kg)	0.97±0.18	0.86±0.08	0.99±0.15	0.04
TDN intake/kg W ^{0.75} (g)	45.27±6.88	40.11±2.16	45.94±5.21	2.07
<i>Nutrient digestibility (%)</i>				
Dry matter	56.27±5.59	54.88±8.36	54.94±4.51	2.88
Organic matter	61.55±4.63	58.76±5.19	62.49±2.09	1.36
Crude protein	56.52±6.27	56.10±4.98	55.76±5.18	1.45
Ether extract	58.05±1.74	54.12±2.84	57.91±3.18	2.80
Crude fibre	52.65±7.78	48.65±9.13	58.55±0.92	4.16
Nitrogen-free extract	58.64±4.23	55.68±4.72	58.31±2.24	0.80

in intakes of DM, TDN and DCP among the treatments. Chang and Mowat (1992) and Wu *et al.* (2006) observed increased dry matter intake when stressed beef calves and heat stressed dairy cows, respectively, were fed a Cr-yeast supplemented diet. However, Bunting *et al.* (1994) did not observe any significant effect on dry matter intake in Holstein calves due to chromium picolinate supplementation of 370 µg Cr per kg diet dry matter. Similarly, Depew *et al.* (1998) did not find difference in DM intake by feeding 0.5 mg/kg diet DM of Cr as chromium picolinate to lambs. Biswas *et al.* (2006) found increased TDN intake in anoestrus dairy heifers supplemented with chromium. The digestibilities of different nutrients, viz. dry matter, organic matter, crude protein, ether extract, crude fibre and nitrogen-free extract were also not affected due to chromium supplementation in the diet (Table 2).

The average concentration of total volatile fatty acids (TVFA), total nitrogen, TCA-precipitable protein nitrogen, non-protein nitrogen and ammonia nitrogen in strained rumen liquor (SRL) of crossbred bullocks are given in Table 3. The rumen TVFA concentration ranged from 7.38 to 12.19, 6.94 to 12.73 and 6.62 to 10.51 mmol/100 ml SRL in treatments T₁, T₂ and T₃, respectively, in different hours of collection, with an average value of 10.07, 10.70 and 9.19 mmol/100 ml SRL and the values were not affected due to chromium supplementation. Samanta *et al.* (1998) and Besong *et al.*

(2001) who also reported that addition of chromium in the diet of ruminants had no effect on TVFA concentration. Significantly ($P<0.01$) lower concentration of TVFA was recorded at 0 h than at 2, 4 and 6 h post-feeding. The TVFA concentration in SRL of crossbred cattle indicated peak production at 4 h post-feeding followed by 2, 6 and 0 h post-feeding, which corroborated with the findings of Gupta *et al.* (2006) and Pandey *et al.* (2009) who also reported the maximum TVFA concentration in rumen liquor at 4 h post-feeding in crossbred male cattle.

The total nitrogen, TCA-precipitable protein nitrogen, non-protein nitrogen and ammonia nitrogen concentration in SRL of crossbred cattle indicated peak production at 2 h post-feeding beyond, which there was a progressive decline in all the treatment groups. The mean values of total nitrogen and non-protein nitrogen concentration in SRL were 56.94, 61.91 and 52.94 mg/100 ml and 23.02, 28.03 and 21.09 mg/100 ml, respectively, in T₁, T₂ and T₃. There was significantly ($P<0.05$) higher concentration of total nitrogen and non-protein nitrogen in SRL of cattle fed ration supplemented with 0.5 mg Cr/kg DM (T₂) than those fed ration supplemented with 1.0 mg Cr/kg DM (T₃) and also in cattle fed ration without chromium supplementation (T₁). The TCA-precipitable protein nitrogen and ammonia nitrogen concentration in SRL did not differ among the treatments. Samanta *et al.* (1998) observed no effect of chromium

Table 3. Effect of chromium supplementation on rumen metabolites in fistulated crossbred male cattle fed ration supplemented with different levels of chromium

Treatments	Post-feeding time interval (h)				Mean*
	0	2	4	6	
<i>TVFA (mMol/100 ml)</i>					
T ₁	7.38±0.53	10.44±1.24	12.19±1.34	10.28±1.69	10.07±0.99
T ₂	6.94±0.53	11.94±1.29	12.73±0.26	11.20±0.45	10.70±1.29
T ₃	6.62±0.14	10.28±0.16	10.51±0.07	9.34±0.38	9.19±0.89
SEm	0.43	0.33	0.59	0.54	0.35
Mean**	6.98 ^b ±0.22	10.89 ^a ±0.53	11.81 ^a ±0.67	10.27 ^a ±0.54	9.99±0.44
<i>Total nitrogen (mg/100 ml)</i>					
T ₁	49.56±3.32	71.94±6.02	55.99±5.04	50.25±5.17	56.94 ^{xy} ±5.21
T ₂	52.23±5.66	74.82±7.98	64.46±7.37	56.13±3.99	61.91 ^x ±5.00
T ₃	49.30±4.37	65.13±2.63	51.97±2.02	45.38±0.66	52.94 ^y ±4.28
SEm	1.76	2.09	2.16	1.34	0.86
Mean**	50.36 ^b ±0.94	70.63 ^a ±2.87	57.47 ^b ±3.68	50.59 ^b ±3.11	57.26±2.59
<i>TCA-precipitable protein nitrogen (mg/100 ml)</i>					
T ₁	24.43±4.94	43.03±5.20	36.36±4.41	31.17±2.79	33.75±3.94
T ₂	25.05±4.48	40.06±5.68	37.67±6.05	32.76±2.91	33.89±3.31
T ₃	24.73±3.57	39.44±3.15	35.84±3.12	27.44±3.92	31.85±3.46
SEm	1.24	1.28	2.21	2.49	1.81
Mean**	24.74 ^d ±0.18	40.84 ^a ±1.11	36.62 ^b ±0.54	30.46 ^c ±1.58	33.16±0.66
<i>Non-protein nitrogen (mg/100 ml)</i>					
T ₁	25.13±6.16	28.91 ^{xy} ±4.31	18.97±1.15	19.07±2.79	23.02 ^y ±2.43
T ₂	27.18±3.19	34.76 ^x ±2.99	26.79±1.31	23.37±1.33	28.03 ^x ±2.40
T ₃	24.56±2.06	25.69 ^y ±1.55	16.17±3.23	17.94±4.35	21.09 ^y ±2.37
SEm	2.22	0.87	1.34	2.72	0.86
Mean**	25.62 ^{ab} ±0.79	29.79 ^a ±2.65	20.64 ^b ± 3.18	20.13 ^b ±1.65	24.05±2.07
<i>Ammonia nitrogen (mg/100 ml)</i>					
T ₁	12.46±1.22	19.93±2.05	14.00±0.70	10.73±1.04	14.28±1.99
T ₂	10.69±0.78	19.74±2.66	15.17±2.35	10.27±0.34	13.97±2.22
T ₃	10.97±0.93	19.37±2.15	14.19±0.53	11.01±0.93	13.89±1.98
SEm	0.63	0.92	1.12	0.41	0.67
Mean**	11.37 ^c ±0.55	19.68 ^a ±0.16	14.45 ^b ± 0.36	10.67 ^c ± 0.22	14.05±0.12

Values bearing different superscripts in a row or column differ significantly from each other, * P<0.05, ** P<0.01.

supplementation on total nitrogen concentration but reported significant reduction in ammonia nitrogen concentration as the level of chromium was increased in the diet of ruminants. Our results also indicated reduced concentration of ammonia nitrogen in SRL due to supplemental chromium.

There was no significant effect on activities of ruminal carboxymethyl cellulase, α -amylase, xylanase, α -glucosidase and β -glucosidase among the treatments (Table 4). Thus chromium supplementation up to 1.0 mg/kg diet dry matter did not influence the ruminal enzyme activities in particulate

material, cellular and extra cellular fractions and whole rumen content. The particulate fraction of rumen contents exhibited highest ruminal enzyme activities followed by cellular and extra cellular fraction of rumen contents. Rumen bacteria bound with feed particles were responsible for higher enzyme activity in the particulate material (Minato *et al.* 1966, Gupta *et al.* 2006).

It can be concluded that supplementation of chromium from 0.5 to 1.0 mg/kg dry matter as chromium picolinate had no significant influence on dry matter intake, nutrient

Table 4. Activities of enzymes (Units/100 g whole rumen contents) in fistulated crossbred male cattle fed ration supplemented with different levels of chromium

Treatments	Fractions of whole rumen contents			Total
	Particulate material	Cellular	Extra-cellular	
<i>Carboxymethyl cellulase</i>				
T ₁	56.85±1.21	26.05±1.91	4.10±0.53	86.99±1.23
T ₂	54.90±0.00	26.34±5.14	4.21±1.25	85.45±6.39
T ₃	53.60±5.85	30.51±3.19	4.35±0.19	88.45±8.85
SEm	9.29	2.59	1.29	10.31
<i>α-amylase</i>				
T ₁	174.81±33.34	113.33±4.45	70.92±11.29	359.07±49.08
T ₂	173.52±44.26	106.67±10.01	69.44±14.26	349.63±68.52
T ₃	152.59±35.19	98.33±7.22	71.67±14.26	322.59±56.66
SEm	12.63	1.78	1.30	11.98
<i>Xylanase</i>				
T ₁	78.83±16.70	27.63±7.30	13.42±4.19	119.55±28.52
T ₂	80.77±15.84	26.30±6.23	13.43±2.30	120.50±24.37
T ₃	83.55±13.12	23.23±4.64	12.60±3.4	119.38±21.15
SEm	3.46	1.33	0.99	2.31
<i>α-glucosidase</i>				
T ₁	17.88±3.01	9.97±1.04	4.98±1.35	32.82±5.38
T ₂	17.99±3.93	10.46±1.17	5.71±0.99	34.16±6.09
T ₃	14.49±3.46	9.64±0.91	6.08±0.69	30.20±5.06
SEm	1.66	0.26	0.18	1.84
<i>β-glucosidase</i>				
T ₁	15.45±3.65	4.72±1.89	4.79±0.25	24.95±2.01
T ₂	12.06±3.30	5.97±1.08	5.69±1.05	23.72±3.27
T ₃	14.13±2.39	4.35±0.33	4.59±1.69	23.06±3.76
SEm	0.74	0.52	0.96	2.05

digestibility, rumen metabolites and enzyme profile in crossbred adult male cattle.

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