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Effect of Dietary Supplementation of Chromium and Yeast on *in vitro* Dry Matter Degradability and Rumen Fermentation Pattern

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ABSTRACT

Background: Yeast (*Saccharomyces cerevisiae*), widely used probiotics, was found to increase the utilization of fiber and improve the production in ruminants. The concept of chromium (Cr) supplementation has also received a great deal of attention since past decade, as Cr could have supra-physiological benefits for domestic livestock. The present *in vitro* experiment was conducted to investigate the effect of dietary chromium and yeast supplementation on *in-vitro* dry matter degradability and rumen fermentation by rumen simulation technique.

Methods: The yeast (*Saccharomyces cerevisiae*) (Y) (5×10^9 CFU /kg) and chromium (Cr) (1.5 mg/kg) supplementation to sorghum stover based complete diets (Basal diet: 10.58% CP) either alone or in combination on *in vitro* nutrient degradability and rumen fermentation was assessed by rumen simulation technique.

Result: Chromium supplementation alone did not affect the cumulative degradability and effective dry matter degradability *in vitro* compared to BD. Yeast supplementation enhanced ($P<0.01$) *in vitro* DM degradability on 12, 24 and 48 h, effective DM degradability and lowered ($P<0.01$) ammonia nitrogen level in comparison to control. When compared to control, combination of yeast and Cr positively affected rumen fermentation pattern and *in vitro* nutrient degradability but no further improvement was observed compared to only yeast supplementation in diet.

Key words: Chromium, *In-vitro* degradability, Rumen simulation technique, Supplementation, Yeast.

INTRODUCTION

In the present scenario of scarcity of feed resources, dry roughages are mostly used for feeding ruminants in developing countries. Yeast (*Saccharomyces cerevisiae*), widely used probiotics, was found to increase the utilization of fiber and improve the production in ruminants (Khan *et al*., 2016). The concept of chromium (Cr) supplementation has also received a great deal of attention since past decade, as Cr could have supra-physiological benefits for domestic livestock. Chromium is biologically active as part of a biomolecule called chromodulin, which is part of an insulin-signaling pathway and appears to affect carbohydrate and lipid metabolism via the action of insulin (Vincent, 2000). The ability to increase glucose tolerance could lead to more efficient use of glucose and ultimately improvement in growth and feed efficiency. There is still lack of studies regarding the effect of chromium on nutrient utilization and rumen fermentation pattern in ruminants. Looking at the beneficial effects of yeast and chromium, the present *in vitro* experiment was conducted to investigate the effect of dietary chromium and yeast supplementation on *in vitro* dry matter degradability and rumen fermentation by rumen simulation technique.

MATERIALS AND METHODS

Source of yeast and chromium

Chromium propionate or organic chromium propionate (4% Cr) and Yeast (*Saccharomyces cerevisiae* @ 50×10^9 CFU/g) were procured from Kemin Asia Pvt Ltd., Chennai, Tamil nadu.

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Dietary treatments

A basal diet (BD) was formulated with sorghum stover as the sole roughage source along with other concentrate ingredients for adult sheep. The ingredient composition is given in Table 1. From these 4 diets were formulated as given below:

Diet 1: BD: Negative Control (No yeast or Cr supplementation).
Diet 2: 1.5Cr: Chromium Propionate (1.5 mg/kg) supplemented to BD.
Diet 3: Y: Dry Yeast (5 billion CFU/Kg) supplemented to BD
Diet 4: 1.5Cr+Y: Yeast and chromium (1.5 mg/kg of Cr + 5 billion CFU/kg of yeast) supplemented to BD.

The study was conducted in the Department of Animal Nutrition, College of Veterinary Science, Rajendranagar,

Hyderabad in 2019. About 10 kg of each experimental diet was prepared in triplicate at feed plant. The feed samples from each replicate were collected and were ground to uniform size to pass through 1mm sieve with Wiley mill and finally stored for *in vitro* studies.

Rumen simulation technique

All the feed samples were analyzed for proximate constituents (AOAC, 2012) and neutral detergent fibre (Van Soest *et al.,* 1991). The *in-vitro* DM degradability, gas production and rumen fermentation pattern was assessed by Rumen simulation apparatus as per Czerkawski and Breckenridge (1977). About 500 ml of the pooled strained rumen liquor (collected from 5 freshly slaughtered sheep), 200 ml of artificial saliva (McDougall, 1948) and 100 ml of distilled water were added in each reaction vessel with continuous flushing of CO_2. About 80 g cud was tied tightly in a nylon bag and placed in the feed container of reaction vessel before setting in the groove. The flow rate of saliva (0.55 ml/min) and temperature (39°C) was adjusted properly with the frequent observation on microbial density. After 24 h, 5 g of feed sample was incubated along with cud for next 24 h. Then the cud was replaced with another nylon bag containing the feed sample. So each feed sample was incubated for 48 h in their respective reaction vessels. After 48 h of incubation, the gas bags were analyzed quantitatively for the amount of gas produced by water displacement method and the quantity of effluent collected in the effluent vessels was recorded. At initial period, gas and effluent were found to be high and then became constant after 5-6 days as the digestibility of DM remained constant after 4 to 6 days of incubation (standardization) (Czerkawski and Breckenridge 1977). Then these bags were washed and dried in the spin drier and the samples were dried in a hot air oven at 65°C for a period of 3 days and the final weight was taken. Five grams ground dried sample were placed in separate nylon bags and incubated at 0, 3, 6, 12, 24 and 48 h in six reaction vessels of Rusitec (Czerkawski and Breckenridge, 1977). The feed samples were analyzed in triplicate for the *in vitro* study. The total volatile fatty acid (TVFA) (mmol/100ml) was estimated using Markham's distillation apparatus as per the method of (Barnett and Reid, 1957) and the ammonia nitrogen (NH_3-N) (mg/100 ml) was estimated using Gerhardt, Germany by steam distillation procedure including blank (Makkar and Becker, 1996).

The DM disappearance was calculated at 0, 3, 6, 12, 24 and 48 h and the percentage of *in vitro* degradability of samples in Rusitec were calculated using the following formula:

$$\textit{In vitro}\ \text{digestibility (\%)} = \frac{\text{Weight of nylon bag with sample before incubation} - \text{Weight of nylon bag with sample after incubation}}{\text{Weight of sample}} \times 100$$

The results of dry matter degraded at various time intervals were fitted to the exponential equation of Mc Donald (1981) using NAAWAY (1992) software.

$$P = a + b\ (1 - e^{-ct})$$

Where;
P= Effective degradability.
t= Time of incubation.
a + b= Potential degradability.
c= Rate of degradability.
a, b and c are constants in exponential equations.

Statistical analysis

All the statistical procedures were done as per Snedecor and Cochran (1994) and Duncan (1955) with a significance at $P<0.05$.

RESULTS AND DISCUSSION

The chemical composition of the four complete diets is given in Table 2. The CP and NDF content in all the diets varied from 10.51% to 10.76% and 53.13% to 54.52%, respectively. No significant ($P>0.05$) difference was observed in amount of gas produced among four dietary treatments (Table 4). The Cr supplementation did not affect the *in vitro* gas production and these results were in accordance with those obtained by Sarma (2013) and Keshri (2016) when added at the dose rate of 0 to 3 and 0 to 2.5 ppm, respectively. Sarma (2013) noticed no statistical difference in total gas production in range of 37.33 to 39 (ml/200 mg DM) in all Cr supplemented group (0-3 ppm). Supplementation of yeast (5×10^9 CFU/kg) also had no significant ($P>0.05$) effect on gas production compared to control (Table 4). which could be attributed to reduced CH_4 production as a result of suppressed methanogenic bacteria in the rumen with yeast supplementation (Wang *et al.*, 2016). Besharati (2015) also noticed similar gas production *in vitro* when *S. cerevisiae* was added @ 2.5 and 5 g/kg DM (355.82 and 327.52 ml/g DM respectively) in comparison to control (no supplementation) (340.61 ml/g DM), while a reduction in gas production was observed @ 7.5 g/kg DM (163.44 ml/g DM) on 48hr of incubation. Similar results were also observed when Cr was added to yeast (1.5Cr+Y) (Table 4). Chen *et al*. (2018) reported 13.23% higher gas production with yeast supplemented @ 0.25% and Cr 2000 ppm as chromium enriched yeast to maize stover than control, however no significant ($P>0.05$) difference was observed in rice straw based diet as the chromium level increased in the yeast.

The effect of yeast and Cr on cumulative DM degradation at 0 h, 3 h, 6 h, 12 h, 24 h and 48 h given in Table 3. Cumulative DM degradability at various intervals and effective DM degradability were not affected due to supplementation of only chromium @ 1.5Cr and were comparable to control (Table 3 and 4). Similar results were reported by Sarma (2013) and Keshri (2016) with regard to Cr supplementation on *in vitro* DM and OM degradability. The *in vitro* DM and OM degradability ranges around 66 to

68% and 67% respectively (Sarma, 2013). In Rusitec, the yeast supplementation increased the cumulative DM degradation at 12 h, 24 h and 48 h interval and the effective degradability than BD (Table 3 and 4). Higher degradability of nutrients with yeast supplementation could be associated with stimulation of growth of rumen microbial population, attributed to its oxygen scavenging ability and maintaining optimum environment for anaerobic bacteria (Jouany, 2001). Secondly, *S. cerevisiae* could provide certain vitamins i.e. biotin and thiamine, required for the growth and activity of microbes in the rumen (Akin and Borneman, 1990). Significant (P<0.05) increase in IVDMD and IVOMD with yeast supplementation was also observed by Harikrishna *et al*. (2012), Elmasry *et al.* (2016) and Elanthamil *et al*. (2018). Elanthamil *et al*. (2018) noticed highest IVDMD in 0.5×10^8 CFU level (56.42%) against non- supplemented group (48.09%). Regarding the effect of added chromium to yeast (1.5Cr+Y), higher cumulative degradability (at 12 h, 24 h and 48 hr) and effective DM degradability was observed in comparison to control and the values were comparable with yeast supplemented group (Y) (Table 3, 4). While Chen *et al.* (2018) did not notice any effect on IVDMD and IVNDFD when supplemented Cr enriched yeast (yeast @ 0.10, 0.25, 0.40 and 0.55% of fermentation medium and Cr at 2000 ppm). The reason may be regarding the variation in dose level of chromium and yeast as well as type of substrates used in the experiment.

Table 1: Ingredient composition (%) of basal diet.

Ingredient	Basal diet
Sorghum stover	50.0
Maize	10.0
Cotton seed cake	11.6
De-oiled rice bran	27.0
Mineral mixture[1]	0.2
Calcite powder	0.8
Salt	0.4
Vitamin A (g/100 kg)*	2.15
Vitamin E (g/100 kg)*	32.7
Total	100*

[1]Mineral and vitamin premix provided (mg/kg diet): Calcium 440, Phosphorus 200, Magnesium 80, Iron 12, Zinc 4.4, Copper 4, Iodine 0.4, Cobalt 0.2, Vitamin B_1 0.65, Vitamin B_6 0.26, Vitamin B_{12} 6, Vitamin A 1500IU, Vitamin D_3 75 IU and Vitamin E 1.95 IU.
*Vitamin E was added to provide 181.831 IU/kg diet and Vitamin A was added to provide 1775.353 IU/kg diet added to meet the requirement according to NRC (2007).

The rumen fermentation metabolites *viz*. pH, NH_3-N and TVFA due to supplementation of yeast, Cr and their combination to sorghum stover based complete diets assessed by Rusitec is presented in Table 4. In the present study, there was no significant variation in pH, TVFA and NH_3-N level among Cr supplemented groups than BD (Table 4). Samanta *et al.* (1998) and Sarma (2013) also

Table 2: Chemical composition of chromium and yeast based complete diets (% on dry matter basis).

Diet	Dry matter	Organic matter	Crude protein	Ether extract	Crude fibre	Nitrogen free extract	Total ash	Neutral detergent fibre
BD	92.21	89.19	10.51	2.86	23.72	52.10	10.81	53.13
1.5Cr	91.90	90.04	10.76	2.80	24.80	51.69	9.96	53.56
Y	93.28	90.46	10.61	2.79	24.11	52.95	9.54	54.17
1.5 Cr +Y	90.52	88.88	10.74	2.84	24.65	50.65	11.12	54.52

All figures are average of two observations.
BD- Basal diet with no supplementation, 1.5Cr- 1.5 ppm Cr, Y- Yeast (*Saccharomyces cerevisiae* @ 5×10^9CFU/kg, 1.5Cr + Y- 1.5 ppm Cr and yeast (*Saccharomyces cerevisiae* @ 5×10^9 CFU/kg.

Table 3: Effect of dietary supplementation of Cr and yeast on cumulative DM degradation (%) of sorghum stover based complete diet in Rusitec.

Diet	Time (h)					
	0h	3h	6h	12h	24h	48h
BD	10.56	26.46	31.78	38.54^b	43.35^b	54.45^b
1.5Cr	10.67	27.12	32.12	39.53^b	44.48^b	55.12^b
Y	9.86	27.87	35.64	44.94^a	52.96^a	63.04^a
1.5Cr+Y	10.06	28.50	35.31	44.52^a	49.84^a	61.12^a
SEM	0.487	0.594	0.760	0.915	1.147	1.125
P	0.938	0.687	0.134	0.004	0.001	0.001

All figures are average of three observations.
^{abc}Means with different superscripts in a column differ significantly: P<0.05, P<0.01.
BD- Basal diet with no supplementation, 1.5Cr-1.5 ppm Cr, Y- Yeast (*Saccharomyces cerevisiae* @ 5×10^9 CFU/kg, 1.5Cr+Y- 1.5 ppm Cr and yeast (*Saccharomyces cerevisiae* @ 5×10^9 CFU/kg).
SEM: Standard Error Mean; P: Probability value.

Table 4: Effect of dietary supplementation of Cr and yeast on (%) on *in vitro* rumen fermentation of sorghum stover based complete diet in Rusitec.

Diet	Attribute					
	Gas production (ml/24 h)	Effluent production (ml/24 h)	pH	TVFA (mmol/100 ml)	NH3-N (mg/100 ml)	Effective DM degradability (NAAWAY)
BD	146.75	710.00	7.13	5.18	19.18[a]	38.53[b]
1.5Cr	156.25	670.00	7.18	5.00	18.97[a]	39.35[b]
Y	139.25	657.50	7.20	5.66	14.14[b]	44.65[a]
1.5Cr+Y	154.50	675.00	7.15	5.48	13.58[b]	43.50[a]
SEM	33.36	18.758	0.02	0.129	0.786	0.734
P	0.263	0.818	0.634	0.274	0.001	0.001

All figures are average of three observations.

[abc]Means with different superscripts in a column differ significantly: P<0.05, P<0.01

BD- Basal diet with no supplementation, 1.5Cr-1.5 ppm Cr, Y- Yeast (*Saccharomyces cerevisiae* @ 5×10^9 CFU/kg, 1.5Cr + Y- 1.5 ppm Cr and yeast (*Saccharomyces cerevisiae* @ 5×10^9CFU/kg).

SEM: Standard error mean; P: Probability value.

reported non-significant effect on *in vitro* TVFA concentration due to Cr supplementation (0, 0.25, 0.5, 0.75, 1.00, 1.50, 2.00, 2.50, 3.00 ppm Cr) and (10, 25, 50, 100 ppm Cr), respectively. In the Cr supplemented groups Rikhari *et al.* (2010) also observed no significant (P>0.05) effect on NH_3-N level than control similar to present study. In contrast, Sarma (2013) and Samanta *et al.* (1998) found decreasing trend of NH_3-N on higher level of Cr supplementation, however, the levels tried by Samanta *et al.* (1998) were higher upto 100 ppm. In yeast supplemented diets (Y) no significant difference was observed in the levels of pH and TVFA when compared to control (Table 4). The possible explanation for constant pH could be the enhanced utilization of lactic acid by *Selenomonas ruminantium* by yeast supplementation (Martin and Nisbet, 1992). The present results were corroborative with results obtained by Rodriguez *et al.* (2015) who also found no effect of live cells and cell extract of yeast on pH level in the rumen. Lila *et al.* (2006) also reported no effect of twin strain of live cells of *S. cerevisiae* on rumen pH. However inconsistent results were observed regarding TVFA concentration with yeast supplementation compared to previous studies. Enjalbert *et al.* (1999) and Bayat *et al.* (2015) observed no effect of yeast on TVFA concentration and their molar proportions whereas higher concentration of TVFA was reported by Mao *et al.* (2013), Elghandour *et al.* (2014a) and Elanthamil *et al.* (2018) on forage based diet. As per Wallace and Newbold (1992), the variable response in VFA production and patterns is a consequence of the effects of yeast culture on microbial numbers in the rumen rather than a direct effect on ruminal fermentation. The substrates or diets influence the growth of different species of rumen microbes that are responsible for the VFA production and pattern when yeast culture was supplemented (Lascano and Heinrichs, 2009). A significant (P<0.01) decreased level of NH_3-N was observed in Y group than BD (Table 4). This could be due to increased incorporation of ammonia into microbial protein which was reflected by the higher microbial protein synthesis in the concerned group. When supplemented with yeast by Chaucheyras-Durand and Fonty (2001) and Krizova *et al.* (2011) reported lower NH_3-N at non-significant (P>0.05) level at all sampling times compared to control. The 1.5Cr +Y diet also did not affect *in vitro* pH and TVFA level and values were similar to BD and Y groups (Table 4). Chen *et al.* (2018) also observed similar pH level *in vitro* when chromium enriched yeast (0, 0.10, 0.25, 0.40 and 0.55% yeast and 2000 ppm Cr) was added to maize stover based diet whereas increased level of VFA was noticed for maize stover and decreased trend was observed in case of rice straw. Significantly (P<0.01) lower level of NH_3-N was found in Cr+Y combination groups than control and the level was similar to yeast supplemented groups (Table 4). Similar to our experiment, Chen *et al.* (2018) also reported lower (P<0.05) NH_3-N level due to addition of Cr enriched yeast in maize stover and rice straw diet which coincides with present results. The highest NH_3-N concentration of maize stover obtained at the level of 0.1% of Cr+Y which was 24.85% higher than the lowest concentration level obtained at the level of 0.55% (P<0.05) (Chen *et al.* 2018).

CONCLUSION

From the results obtained from the present *in vitro* study, it could be inferred that the supplementation of Cr (1.5 mg/kg) did not affect the *in vitro* effective dry matter degradability, cumulative degradability and rumen fermentation pattern when compared with control. However, yeast supplementation positively affected the *in vitro* dry matter degradability and rumen fermentation pattern. The supplementation of yeast along with chromium at a concentration of 1.5 mg/kg resulted in higher *in vitro* rumen fermentation pattern as compared to control but the values were comparable with yeast supplementation.

Conflict of interest: None.

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