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Influence of 10 ppm Zinc With or Without Enzyme/Yeast Supplementation on Rumen Fermentation

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Abstract: As 10 ppm zinc (Z) in rumen liquor retards urea (U) hydrolysis and prevents ammonia toxicity, its effect and the supplemental value of enzyme (E)/yeast (Y) on rumen fermentation were studied in Hohenheim gas production test. Treatments includes control (C), CU, CUZ, CUE, CUEZ, CUY, CUYZ experimented in duplicate in two sequential runs with two replications in each run. CUZ had significantly ($p<0.01$) lowest gas production at 24 h (15.33 mL) where as significantly ($p<0.01$) highest gas production was recored in CUY (43.83 mL) and CUYZ (41.08 mL) that maintained untill 48 h of incubation. The molar proportion of propionate was increased whenever zinc supplemented but at non significant level. The acetate to propionate ratio ranged from 3.35 to 4.00. Energetic efficiency was significantly ($p<0.01$) increased in CUEZ (30.75%) over (C), (CU), (CUZ) and was inturn significantly ($p<0.01$) lower than CUY (36.59%). Maximum energetic efficiency ($p<0.01$) was recorded in CUYZ (38.67%) than the rest of treatments. Zinc consistently improved energetic efficiency over those treatments that do not have Zinc. It is thus concluded that while supplementing Zinc at 10 ppm to retard urea hydrolysis, 1 CFU of yeast needs to be included to offset the ill effects of Zinc on rumen fermentation. The projected equivalent in the diet of adult cattle weighing 500 kg is 1.31 g Zinc/day and 3,000 CFU of yeast.

Key words: Zinc, urea, ammonia toxicity, yeast, enzymes, *in vitro* gas production

INTRODUCTION

Urea is the cheapest source of non-protein nitrogen source to ruminants that can be effectively converted as protein source by rumen micro-organisum. Exceeding permissible level of urea inclusion in diet or improper mixing of ration containing urea can lead to ammonia toxicity leading to death. Hence many attempts have been made to improve the utilization of urea by reducing its rate of ammonia production to match the rate of assimilation by rumen microbes. Decreasing rate of ammonia release from urea can prove beneficial (1) to avoid ammonia spikes and consequent loss from the rumen and (2) to maintain ruminal ammonia at the adequate level for a longer postfeeding period. The rate of ammonia release can be controlled either by decreasing the activity of rumen urease, (by the use of the specific urease inhibitors) or by modification of urea into products which would release ammonia slowly.

Various methods to modulate urea degradation have been developed. These include complexing or coating urea with a variety of compounds such as oils, carbohydrates and treatments with formaldehyde or acids. Some of these studies showed inconclusive results or no marked difference with feeding untreated urea (Doyle, 1987). Hence the other alternative approach of decreasing the urease activity in rumen release was studied. One such alternative is that the elevated concentration of zinc could inhibit ammonia accumulation from urea (Spears and Hatfield, 1978). Earlier work conducted in

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this laboratory (Kathirvelan and Balakrishnan, 2006) has proved that Zinc supplementation at 10 ppm in rumen liquor could delay urea hydrolysis and hence can be used to counter urea toxicity as rapid hydrolysis of urea in the rumen is the principle cause for urea toxicity in cattle.

However, detailed investigation report does not exist on the influence of zinc at 10 ppm on rumen fermentation pattern. Hence a study was proposed to examine influence of zinc at 10 ppm on rumen fermentation pattern. In an effort to over come any ill effects of 10 ppm zinc on rumen fermentation, the effect of with or without enzyme/yeast supplementation was also examined.

MATERIALS AND METHODS

The effect of 10 ppm zinc and the supplemental value of enzyme/yeast on rumen fermentation characteristics were studied in Hohenheim gas production test as per the procedure of Menke and Steingass (1988) at Department of Animal Nutrition, Madras Veterinary College, Tamil Nadu, India during 2005. The experimental design included seven treatments as listed in Table 1. The rumen liquor was obtained from three cattle maintained on grazing to ensure that cellulolysis was optimum. The entire laboratory handling involving rumen fluid was carried out using continuous flushing with CO_2. In this study, 500 mg sample and 40 mL rumen buffer volume were taken in 100 mL glass syringe as described by Blummel and Becker (1997). In addition 3 mL were added out of which 1 mL was allocated for urea supplementation, 1 mL for zinc supplementation, one millilitre for either enzymes/yeast supplementation. The syringes were pre-warmed at 40°C prior to incubation and were incubated at 39°C in shaking water bath, specially designed to accommodate 100 syringes in vertical position in a perforated stand. The perforated stand was mechanically shaken at the rate of 5 times/min.

Forty three millilitre was considered based on the daily intake equivalent in the live animal by taking into account of the total rumen volume that include calculation of turn over rate of an animal weighing 500 kg and eating roughages at 1.5% of body weight (Arelovich *et al*., 2000). As 35 mg of urea per 43 mL was equivalent to 106 g/head/day that is considered to produce toxicity (Arelovich *et al*. 2000), the treatments containing urea were added at this level. Zinc chloride was used to achieve 10 ppm of Zinc in 43 mL. enzyme mixture (containing 40 units of cellulase and 50 units xylanase/mL) and yeast (1 colony forming unit mL^{-1}) were added as per the recommended level.

Table 1: Treatments subjected to examine the effect of 10 ppm zinc on fermentation characteristics and their detail

Treatments	Details	Abbreviations
Control	0.5 g paddy straw + 40 mL buffered rumen liquid + 3 mL distilled water	C
Control + urea	0.5 g paddy straw + 40 mL buffered rumen liquid + 1 mL urea solutions [35 mg mL^{-1}] + 2 mL distilled water	CU
Control + urea + zinc	0.5 g paddy straw + 40 mL bufferedrumen liquid + 1 mL urea solutions [35 mg mL^{-1}] + 1 mL zinc solution (10 ppm) + 1 mL distilled water	CUZ
Control + urea + enzyme	0.5 g paddy straw + 40 mL buffered rumen liquid + 1 mL urea solutions [35 mg mL^{-1}] + 1 mL enzyme solution *[contains cellulase 40 units, xylanase 50 units per mL] + 1 mL distilled water	CUE
Control + urea + enzyme + zinc	0.5 g paddy straw + 40 mL buffered rumen liquid + 1 mL urea solutions + 1 mL enzyme solution + 1 mL zinc solution	CUEZ
Control + urea + yeast	0.5 g paddy straw + 40 mL buffered rumen liquid + 1 mL urea solutions + 1 mL yeast solution** [contains 1 cfu mL^{-1}] + 1 mL distilled water	CUY
Control + urea + yeast + zinc	0.5 g paddy straw + 40 mL buffered rumen liquid + 1 mL urea solutions + 1 mL yeast solution + 1 mL zinc solution + 1 mL distilled water	CUYZ

*1 mL enzyme solution contains cellulase 40 units + xylanase 50 units; **1 mL yeast solution contains 1 Colony Forming Unit (CFU)

The syringes were incubated in duplicate in two sequential runs with two replications in each run. Gas production was recorded at 6, 12, 18, 24, 36 and 48 h. The results of gas volume at various time intervals were fitted to exponential equation (Blummel and Orskov, 1993) and modified for lag phase as suggested by Krishnamoorthy *et al.* (1991).

The equation is $p = a+b\ (1-e^{-ct})$
Where, p = Gas production, t = time, a+b = potential gas production,
c = Rate of gas production, a, b and c are constant in exponential equation.
With lag phase: $p = a+b\ (1-e^{-c(t-l)})$
Where l = Initial lag for on set of fermentation.

Chemical Analysis

At the end of each incubation hour, the whole contents of the syringes were transferred into 45 mL capped centrifuge tubes. Residues were centrifuged in an ultracentrifuge (HIMAC, model SCR 20BA, Hitachi) at 20,000 g for 30 min at 4°C. 2.5 mL of supernatant was collected for Volatile Fatty Acid (VFA) analysis and added into the tubes, which already contained 0.5 mL metaphosphoric acid (25%) and analysed as per Chase (1990).

Stoichiometry Derivations

$$\text{Acetate to propionate ratio} = \frac{\text{Acetate}}{\text{Propionate}} \quad (1)$$

$$\text{Non glucogenic ratio (NGR)} = \frac{\text{Acetate} + (2 \times \text{Butyrate})}{\text{Propionate}} \quad (2)$$

$$\text{Energetic efficiency of VFA} = 0.622A + 1.092P + 1.56B/A+P+2B \quad (3)$$

(where A, P and B represent acetic, propionic and butyric acids (Mol percent) (Orskov *et al.*, 1968)

The data obtained in different parameters were subjected to statistical analysis as per the procedure of Snedecor and Cochran (1994).

RESULTS

Cumulative Gas Production

The cumulative gas produced (mL) for 500 mg of paddy straw with 10 ppm zinc for various treatments for various h of incubation is furnished in Table 2. The description of gas produced was furnished through exponential equation as follows:

C = Y = −4.50±84.14 [1−EXP(-0.0215t−1)] lag. time 4.4 h, RSD 1.5
CU = Y = −4.76±92.94 [1−EXP(-0.0121t−1)] lag. time 4.4 h, RSD 1.65
CUZ = Y = −3.92±50.17 [1−EXP(-0.0191t−1)] lag. time 4.2 h, RSD 0.81
CUE = Y = −7.11±70.76 [1−EXP(-0.0240t−1)] lag. time 4.4 h, RSD 1.99
CUEZ = Y = −5.58±78.13 [1−EXP(-0.0181t−1)] lag. time 4.3 h, RSD 1.25
CZ = Y = −14.30±101.68 [1−EXP(-0.0342t−1)] lag. time 4.4 h, RSD 4.87
CUYZ = Y = −10.86±92.75 [1−EXP(-0.0295t−1)] lag. time 4.0 h, RSD 2.6

Table 2: The cumulative volume of gas (mL) produced, volatile fatty acids production and stoichiometric derivations due to various treatments imposed to elicit the effect of zinc on rumen fermentation characteristics (Mean±SE)

Treatments	6	12	18	24	36	48
	(h)					
C	2.22±0.15^{a}	5.92±0.83^{a}	12.50±0.79^{b}	19.00±1.33^{ab}	25.00±1.27^{ab}	34.00±2.12^{a}
CU	2.57±0.20^{a}	6.25±0.96^{a}	12.75±1.34^{b}	20.67±1.45^{ab}	27.13±1.38^{ab}	36.25±2.14^{a}
CUZ	2.10±0.10^{a}	5.50±0.50^{a}	10.25±0.77^{a}	15.33±1.12^{a}	21.25±1.40^{a}	31.25±2.06^{a}
CUE	3.40±0.31^{a}	8.83±0.91^{a}	17.08±1.41^{d}	26.33±1.77^{b}	32.63±2.41^{b}	41.25±1.49^{a}
CUEZ	3.00±0.26^{a}	8.08±0.88^{a}	15.17±1.03^{c}	23.00±1.55^{ab}	31.50±2.35^{ab}	39.25±2.06^{a}
CUY	6.42±0.82^{b}	16.08±1.90^{b}	31.00±2.43^{e}	43.83±3.54^{c}	54.25±4.49^{c}	68.50±7.98^{b}
CUYZ	5.73±0.62^{b}	15.92±1.79^{b}	30.33±2.21^{e}	41.08±3.21^{c}	50.38±4.62^{c}	64.25±8.87^{b}

Table2: Continued

Treatments	Acetic acid (mmol L^{-1})	Propionic acid^{NS} (mmol L^{-1})	Butyric acid^{NS} (mmol L^{-1})	A:P ratio^{NS}	NGR^{NS}	Energetic efficiency (%)
C	18.96±1.46^{a}	4.74±1.78	1.23±0.77	4.00±0.58	4.52±0.04	16.74±0.58^{a}
CU	23.37±0.86^{ab}	5.92±2.22	1.92±0.40	3.95±0.26	4.60±0.12	21.47±0.27^{b}
CUZ	22.14±1.69^{abc}	6.01±2.23	2.26±0.31	3.69±0.18	4.45±0.09	21.88±0.58^{b}
CUE	30.29±0.95^{bcd}	7.97±2.71	3.71±0.75	3.80±0.12	4.73±0.13	30.49±0.24^{c}
CUEZ	30.33±0.96^{cd}	8.32±2.89	3.56±0.89	3.65±0.09	4.50±0.06	30.75±0.29^{c}
CUY	33.81±3.27^{d}	9.92±2.14	4.70±1.58	3.41±0.06	4.35±0.06	36.59±0.34^{d}
CUYZ	34.45±4.10^{d}	10.22±2.22	5.45±1.29	3.35±0.05	4.44±0.02	38.67±0.10^{e}

Mean of four observations, Mean bearing different superscript (s) within a column differ significantly $p<0.01$), NS: Non Significant, C : Control, U: Urea equivalent to produce toxicity, Z: Zinc at 10 ppm (sufficient enough to retard urea hydrolysis), E: enzyme mixture (cellulase 40 units + xylanase 50 units mL^{-1}), Y: Yeast (1olony forming unit mL^{-1})

The initiation of gas production did not vary with treatments. The lag time for gas production ranged from 4.0 to 4.4 h for various treatments. The cumulative gas volume at 48 h of incubation from various treatments ranged from 31.25 to 68.50 mL.

In 6 h, the yeast supplementation with or without zinc had significantly ($p<0.01$) highest amount of gas production compared to other treatments. The C, CU, CUZ, CUE and CUEZ did not vary among themselves. The same trend was noticed in 12 h also. Whereas in 18 h the total gas production scenario changed where in CUZ recorded significantly ($p<0.01$) lowest value compared to the rest. While the CUE had the gas production that was significantly ($p<0.01$) higher than CUEZ, the yeast supplementation (CUY, CUYZ) had significantly ($p<0.01$) highest gas production compared to the other treatments. The results thus showed that enzyme and yeast supplementation increased total gas production from 18h of incubation onwards.

In 24 h, CUZ had significantly ($p<0.01$) lowest gas production but was comparable with C, CU and CUEZ. The yeast supplementation continued to have significantly ($p<0.01$) highest value. The same trend was noticed in 36 h of incubation also. In 48 h, yeast supplementation had significantly ($p<0.01$) highest value of gas production compared to other treatments.

Studies on End Products and Their Stoichiometric Derivations

The effect of supplemental zinc including remedial measures experimented to overcome the ill effect of zinc on the *in vitro* synthesis of short chain fatty acids (mmol L^{-1}), Acetate to propionate ratio, Non glucogenic ratio, energetic efficiency (%) at 48 h of incubation with paddy straw as a substrate are presented in Table 2.

The molar proportion of VFA across the various treatments at 48 h ranged from 18.96 to 34.45 mmol for acetate, 4.74 to 10.22 mmol for propionate, 1.23 to 5.45 mmol for butyric acid.

The acetate production varied significantly ($p<0.01$) among the treatments experimented. Maximum acetate was produced in CUYZ and was comparable to yeast and enzyme supplementation groups viz., CUY, CUE and CUEZ. The enzyme supplementation treatment (CUE and CUEZ) was comparable to CUZ whereas C, CU and CUZ did not vary among themselves.

The molar proportion of propionate was increased whenever zinc supplemented. The mmol of propionate was increased to 1.50% when zinc was supplemented to CU ration, 4.21% over CUE and 2.94% over CUY. However, they were all at nonsignificant level. No trend was noticed in butyrate production and variations among treatments were at nonsignificant level.

The acetate to propionate ratio ranged from 3.35 to 4.00. The acetate to propionate ratio shows that zinc supplementation (CUZ) reduces the ratio comparing to CU (3.69 vs 3.95). The same trend noticed in CUEZ and CUYZ. However, these variations were not statistically significant. Reduction in acetate and acetate/propionate ratio and increase in propionate level in steers fed with yeast was also reported by Dawson *et al.* (1990).

The NGR of the treatments imposed did not reveal any significant difference and the values ranged from the 4.35 to 4.73. The results of energetic efficiency showed that urea supplementation increases ($p<0.01$) the energetic efficiency when compared to the control. Enzyme further enhanced ($p<0.01$) the energetic efficiency and yeast supplementation maximized ($p<0.01$) it. Curiously it was only in the yeast supplementation zinc could further enhance ($p<0.01$) the energetic efficiency.

DISCUSSION

Cumulative Gas Production

The results revealed that across treatments zinc supplementation reduced the gas production except in the case of yeast supplementation. The lower gas production in zinc-supplemented treatments may be due to reduced digestibility (Kathirvelan and Balakrishnan, 2006).

Highest gas production in yeast supplementation could be due to increased fermentation rate from initial h of incubation. It is possible that increased anaerobic bacterial count in yeast supplementation groups might have lead to increased total gas production. Harrison *et al.* (1987), Dawson *et al.* (1990) and Newbold *et al.* (1995) have reported that anaerobic bacterial counts were increased due to yeast supplementation. It could thus be argued that yeast supplementation is able to resist the ill effects of zinc supplementation. However, digestibility and fermentation characteristics would throw more light on this aspect.

Studies on End Products and Their Stoichiometric Derivations

The result of VFA production shows that enzymes or yeast supplementation increased ($p<0.01$) the acetate production whereas zinc supplementation increased the propionic acid production at nonsignificant level. Arelovich *et al.* (2000) reported that molar production propionate was increased linearly by added zinc and decreased the acetate-propionate ratio. Froetschel *et al.* (1990) noted that zinc supplementation at 1,142 ppm in 50% concentrate diet when fed to steers increases the molar proportions of propionate and decrease acetate-propionate ratio.

Elevated zinc concentration may alter microbial species in a fashion similar to ionophores. Various dietary compounds including ionophores (Monensin, lasalocid and salinomycin) change the molar proportions of ruminal VFA through inhibition of certain gram-negative, hydrogen producing ruminal microbes (Van Soest, 1982). It appears that high concentration of added zinc in the rumen can act selectively on the growth or the metabolic activity of a single group of microbes. It may depress the activity of fibrolytic and ureolytic bacteria, but may stimulate propionic acid producing bacteria (Arelovich *et al.*, 2000) No trend could be identified from NGR results.

The energetic efficiency results leads to conclude that ill effects of zinc can be overcome by yeast supplementation. In support of this argument Arelovich *et al.* (2000) reported that zinc supplementation at a concentration of 250 ppm equivalent to 5.81 ppm *in vitro* concentration increase the energetic efficiency of ruminal fermentation. Further, Cecava *et al.* (1993) substantiated the benefits obtained by feeding chelated zinc supplements to the increased proportion of propionate in ruminal

VFA that lead to an increased energetic efficiency of ruminal fermentation. In an adult cattle weighing 500 kg the equivalent of 10 ppm Zinc is 1.31 g/day and one CFU of yeast in 43 mL is equivalent to 3,000 CFU of yeast (Kathirvelan, 2003).

This study reveals that zinc depressed gas production while the enzyme and yeast supplementation enhanced the gas production. Zinc with or without enzyme/yeast supplementation reduced acetate to propionate ratio over non zinc group. Energetic efficiency being one of the main stoichiometric derivation revealed that Zinc consistently improved energetic efficiency over those treatments that do not have Zinc. It is thus concluded that while supplementing Zinc at 10 ppm to retard urea hydrolysis, 1 CFU of yeast needs to be included to offset the ill effects of Zinc on rumen fermentation. The projected equivalent in the diet of adult cattle weighing 500 kg is 1.31 g Zinc/day and 3,000 CFU of yeast.

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