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Effect of Supplemental Zinc at 10 ppm on Apparent, True Digestibility, Microbial Biomass Production and Exploring Means to Overcome Ill Effects in Cattle

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Abstract: A study was conducted to examine effect of supplemental 10 ppm zinc on *in vitro* dry matter degradability and fibre digestibility of paddy straw at 48 h of incubation in 4 replications. The results indicated that supplemental zinc at 10 ppm significantly ($p<0.01$) reduced dry matter digestibility as well as neutral detergent fibre digestibility compared to control. Another experiment was conducted to study the efficacy of supplemental enzyme/yeast to overcome ill effect of 10 ppm zinc on apparent, true digestibility and microbial biomass by using Hoheinhem gas production test. Treatments include control, urea (35 mg mL^{-1}), urea + zinc (10 ppm), urea + enzyme (cellulase 40 units, xylanase 50 units mL^{-1}), urea + enzyme + zinc, urea + yeast (1 cfu mL^{-1}), urea + yeast + zinc. Experiment was replicated in duplicate in two sequential runs with two replications in each run. The urea + zinc lowered the apparent digestibility, true digestibility and percent microbial biomass yield compared to urea. The urea + yeast had the highest apparent digestibility (30.87%), true digestibility (35.70%) and maximum percent microbial biomass yield (4.83%). These results were however comparable to urea + enzyme. It is concluded that the ill effect of 10 ppm zinc on dry matter degradability, neutral detergent fibre digestibility, apparent and true digestibility can be countered by supplementing enzyme (cellulase 40 units, xylanase 50 units mL^{-1}) or yeast (1 cfu mL^{-1}).

Key words: Zinc, *in vitro* dry matter degradability, neutral detergent fibre, yeast, enzyme

INTRODUCTION

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 to avoid ammonia spikes and consequent loss from the rumen and to maintain ruminal ammonia at the adequate level for a longer post feeding 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 this laboratory (Kathirvelan and Balakrishnan, 2006) has proved that zinc supplementation at 10 ppm 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.

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However, conflicting report exists on the effect of zinc on fibre digestibility Hence a study was proposed to examine influence of zinc at 10 ppm on fibre digestibility as well as to assess the effect of enzyme/yeast supplementation in alleviating ill effects if any.

MATERIALS AND METHODS

Studies on the Effect of 10 ppm Zinc on the *in vitro* Dry Matter Degradability and Neutral Detergent Fibre Digestibility

The experiment was conducted following Tilley and Terry method (1963) in which two groups were assessed for *in vitro* dry matter degradability and Neutral detergent fibre digestibility of paddy straw at 48 h of incubation in four replications. While one group did not contain zinc supplementation (control), the other group contained 10 ppm zinc. Each tube contained 0.5 g of paddy straw, 40 mL of rumen buffered solutions, 1 mL of urea solution (35 mg of urea), 1 mL of zinc solution (10 ppm), 1 mL of distilled water (to serve as vehicle for later experiment). Forty three milliliters was considered based on the equivalence of 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^{-1} day^{-1} that is considered to produce toxicity, the treatments containing urea were added at this level. Zinc chloride was used to achieve 10 ppm of Zinc in 43 mL.

At the end of incubation, the contents were transferred to centrifuge tube and centrifuged at 25000 rpm. The residue was dried at 105°C in forced draft hot air oven for 24 h. Thus dried samples were subjected to Neutral detergent fibre estimation following Georing and Vansoest (1970). The dry matter and Neutral detergent fibre were also determined in the test material (Paddy straw) to calculate its digestibility.

Assessing the Effect of Supplemental Zinc at 10 ppm on percent Apparent, True Digestibility and Microbial Biomass at 48 h of Incubation and Exploring Means to Overcome Ill Effects

The effect of 10 ppm zinc and the supplemental value of enzyme/yeast on per cent apparent, true digestibility and microbial biomass at 48 h of incubation were studied in Holenheim gas production test as per the procedure of Menke and Steingass (1988) following eight treatments as shown in Table 1.

The rumen liquor was obtained from three cattle maintained on grazing to ensure that cellulolysis was optimum. All the laboratory handling involving rumen fluid was carried out using continuous flushing with carbon dioxide. 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) to minimize the analytical error in gravimetric determinations of apparent and truly degraded substances. The syringes were

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
Urea	0.5 g paddy straw + 40 mL buffered rumen liquid + 1 mL urea solutions (35 mg mL^{-1}) + 2 mL distilled water.	CU
Urea + Zinc	0.5 g paddy straw + 40 mL buffered rumen liquid + 1 mL urea solutions (35 mg mL^{-1}) + 1 mL zinc solution (10 ppm) + 1 mL distilled water.	CUZ
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
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
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
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 yeast solution contains 1 Colony Forming Unit (CFU) (Dutta *et al.*, 2001)

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 that were made to shake 5 times min^{-1}.

The experimental design included seven treatments as shown in Table 1 to explore the effect of zinc with or without Non Starch Polysaccharide enzymes or yeast on percent apparent, true digestibility and microbial biomass at 48 h of incubation The total volume was 43 mL, out of which 1 mL was allocated for urea supplementation, one ml for zinc supplementation, 1 mL for either Non starch polysaccharide enzymes/yeast supplementation and the rest 40 mL was allocated to rumen buffered solutions containing 0.5 g of paddy straw. Forty three milliliter was considered based on the equivalence of 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, the treatments containing urea were added at this level. Zinc chloride was used to achieve 10 ppm of zinc in 43 mL.

The composition of media solution was essentially as specified by Menke and Steingass (1988). The syringes were incubated in duplicate in two sequential runs with two replications in each run.

At the end of 48 h of incubation, the whole contents of the syringes were transferred into 45 mL capped centrifuge tubes. The *in vitro* apparent digestibility was determined as per Blummel *et al*. (1997b). True degradability was calculated as the weight of substrate incubated minus the weight of the residue after neutral detergent fibre treatment (Van Soest and Robertson, 1985). The microbial biomass was calculated from the equation quoted by Blummel *et al*. (1997a).

Paired T Test for experiment 1 and completely randomised design for experiment 2 were carried out to examine the statistical validity.

RESULTS

Studies on the Effect of 10 ppm Zinc on the *in vitro* Dry Matter Degradability and Neutral Detergent Fibre Digestibility

The results of *in vitro* dry matter degradability and Neutral Detergent Fibre digestibility (NDF) indicate that zinc at 10 ppm reduced the *in vitro* dry matter degradability of paddy straw at 48 h of incubation significantly ($p<0.01$) to 21.34% in 10 ppm from 25.05% in the unsupplemented group. Similarly the neutral detergent fibre digestibility was also affected ($p<0.01$) to 28.51% in 10 ppm zinc from 33.15% in unsupplemented group.

Assessing the Effect of Supplemental Zinc at 10 ppm on per cent Apparent, True Digestibility and Microbial Biomass at 48 h of Incubation and Exploring Means to Overcome Ill Effects

The apparent digestibility of control, urea, urea + zinc did not vary significantly among themselves. However, urea + zinc lowered the apparent digestibility by 13.32% compared to urea. The treatments viz., urea + enzyme, urea + enzyme + zinc as well as urea + yeast + zinc group enhanced the apparent digestibility and were comparable. The urea + yeast had the highest apparent digestibility. Interestingly with enzyme supplementation, zinc reduced the apparent digestibility to the extent of 4.71% and with yeast supplementation to the extent of 7.81% over their respective group without zinc (Table 2).

The profile in true digestibility becomes much clearer with no significant variation among control, urea as well as urea + zinc. However, the urea + zinc has lowered true digestibility by 12.40% compared to urea. While urea + enzyme, urea + enzyme + zinc, urea + yeast as well as urea + yeast + zinc were comparable; zinc reduced the true digestibility to the extent of 5.70% with enzyme supplementation and 8.10% with yeast supplementation over their respective groups without zinc.

Table 2: The per cent apparent and true digestibility and microbial biomass production as affected 10 ppm zinc at 48 h incubation (Mean±SE)

Treatments	Apparent digestibility (%)	True digestibility (%)	Microbial biomass (%)
C	24.86±1.16^{ab}	26.64±1.49^{ab}	1.78±0.33^{a}
CU	25.67±1.29^{abc}	27.98±1.61^{abc}	2.31±0.36^{a}
CUZ	22.25±1.48^{a}	24.51±1.78^{a}	2.26±0.31^{a}
CUE	30.57±1.34^{cd}	35.27±1.60^{d}	4.70±0.48^{b}
CUEZ	29.13±1.81^{bcd}	33.26±1.98^{cd}	4.13±0.59^{b}
CUY	30.87±1.35^{d}	35.70±1.59^{d}	4.83±0.49^{b}
CUYZ	28.44±1.47^{bcd}	32.81±1.93^{cd}	4.37±0.54^{b}

Mean of four observations; Means bearing different superscripts within a column differ significantly ($p<0.01$)

The per cent microbial biomass yield revealed that control, urea as well as urea + zinc were significantly ($p<0.01$) lower compared to the enzyme and yeast supplemented treatments.

DISCUSSION

Studies on the Effect of 10 ppm Zinc on the *in vitro* Dry Matter Degradability and Neutral Detergent Fibre Digestibility

The 10 ppm zinc lowered 12.65% of *in vitro* dry matter degradability and 13.82% of Neutral detergent fibre digestibility at 48 h of incubation whereas Arelovich *et al*. (2000) reported that *in vitro* dry matter degradability in 10 ppm zinc supplemented group was 2.01% lower than in unsupplemented group in *in vitro* results of 24 h incubation. The decreased *in vitro* dry matter degradability of zinc supplemented group closely agreed with Martinez and Church (1970). They also reported that elevated zinc concentration in *in vitro* from 20 to 30 ppm decreased *in vitro* cellulose digestibility by 31%.

In agreement with the result of this study Chamberlain and Borroughs (1962) found that higher amount of added zinc decreased cellulose digestibility *in vitro*.

These results indicate that an elevated concentration of zinc can depress fibre digestibility. It could also be the reason for non significant effect in the daily weight gain and feed efficiency in finishing cattle due to 15 or 65 ppm of zinc along with urea in the experiment conducted by Clark *et al*. (1970).

Decreased *in vitro* dry matter degradability and NDF digestibility with high zinc concentrations supports the concept that zinc can selectively inhibit the growth or metabolic activity of microbes (Arelovich *et al*., 2000). Ivan and Grieve (1975) reported that NDF digestibility was not affected at zinc supplementation up to 500 ppm. The variability in digestibility could possibly due to difference in substrate or species of experimental animal used.

Assessing the Effect of Supplemental Zinc at 10 ppm on per Cent Apparent, True Digestibility and Microbial Biomass at 48 h of Incubation and Exploring Means to Overcome Ill Effects

The apparent digestibility of control obtained in this study, concurs with the result of Reddy and Siviah (2001), who noticed 17.12 to 32.36% by *in vitro* dry matter digestibility of paddy straw.

The zinc supplementation group (urea + zinc) results obtained in this study for true digestibility was similar to that of the study conducted by Arelovich *et al*. (2000), who reported zinc supplementation reduced the digestibility and also dry matter disappearance of prairie hay had decreased linearly with added zinc *in vitro*. Similarly, Martinez and Church (1970) reported that 20 to 80 ppm of added zinc resulted in significant decrease in *in vitro* cellulose digestion. The highest true digestibility observed in enzyme and yeast supplementation with urea viz., urea + enzyme and urea + yeast were consistent with the hypothesis that fibre digestibility can be increased by providing a supplement which provides sufficient nutrients to stimulate the activity of rumen micro organism (Prasad *et al*., 1994).

The urea + zinc treatment lowered microbial biomass production by 2.61% compared to urea. The microbial biomass yield was reduced to 12.13% with enzyme and 9.52% with yeast supplementation over their respective groups with out zinc. The results revealed that zinc supplementation reduced the per cent apparent, per cent true digestibility and per cent microbial biomass.

The results of the study revealed that the ill effect of 10 ppm zinc on dry matter degradability, neutral detergent fibre digestibility, apparent and true digestibility can be countered by supplementing enzyme (cellulase 40 units, xylanase 50 units mL^{-1}) or yeast (1 cfu mL^{-1}).

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