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Effects of three slow-release urea inclusions in rice straw-based diets on yearling Bali bull performances

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Abstract

The effects of slow-release zinc-urea complexes (ZnU), urea-impregnated zeolite (UZ) and zinc-urea-impregnated zeolite (ZnUZ) on the performance of yearling Bali bulls were assessed using 20 Bali bulls (145.3 ± 2.5 kg bodyweight (BW)), which were allocated to five treatments and four replications in a completely randomized design. The treatments were: Diets supplemented with no urea (NU) and with urea (U), ZnU, UZ and ZnUZ. The results of the *in vivo* study revealed that both ZnU and UZ might replace urea effectively by increasing feed intake. Moreover, substituting urea with ZnU, UZ or ZnUZ increased crude protein total tract apparent digestibility whereas ZnU or UZ replacing urea, improved fibre total tract apparent digestibility. Furthermore, inclusion of UZ in the diet improved live weight gain and feed efficiency in Bali bulls above that of the U and NU diets. Thus, the inclusion of ZnUZ in rice straw-based diets showed slow-release urea had positive impacts on feed intake and nutrient digestibility, and increased the efficiency of feed utilization in Bali bulls.

Keywords: Apparent digestibility, feed intake, urea-impregnated zeolite, zinc
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Introduction

The use of urea as an effective and inexpensive nitrogen (N) source for ruminants has increased in recent years. However, it is generally accepted that urea is rapidly hydrolysed into ammonia by rumen microbes and generates negative influences on the rumen system. Therefore, many investigations focused on reducing its negative effects. Ludden *et al*. (2000a, 2000b) used N-(n-butyl) thiophosphoric triamide; Kardaya *et al*. (2000) and Kathirvelan & Balakrishnan (2006) experimented with zinc compounds, Golombeski *et al*. (2006) tried a Ca-urea compound and Taylor-Edwards *et al*. (2009a, 2009b) used polymer-coated urea as slow-release urea agents. Other researchers (Bosi *et al*., 2002; Koknaroglu *et al*., 2006; Migliorati *et al*., 2007) used zeolite to reduce the rate of urea hydrolysis in the rumen system. In addition, Mentz *et al*. (2015) applied Optigen® II as a slow-release N source for sheep. Most tropical forages are regarded as being of low quality. This is especially true of agriculture waste products such as rice straw. Ruminal carbohydrate degradation of rice straw is a slow process, which needs synchronizing with rumen microbial N usage. To achieve greater synchrony of these processes, newer forms of slow-release urea compounds (zinc-urea complex, urea-impregnated zeolite and zinc-urea impregnated zeolite (ZnUZ)) were introduced by Kardaya *et al*. (2009; 2010) to reduce the rate of urea release in the rumen system in *in vitro* studies. However, no investigation has examined the effects of these slow-release urea compounds on ruminant performance as such. Therefore, it is imperative that the slow-release urea compounds should be evaluated under *in vivo* situations. An investigation was conducted to determine the effects of supplementing rice straw-based diets with slow-release urea on feed intake, apparent digestibility of nutrients and growth performances of yearling Bali bulls.

Materials and Methods

Twenty yearling Bali bulls *(Bos sundaicus)* with an initial body weight (BW) of 145.3 ± 2.5 kg were used in a completely randomized experimental design with five dietary treatments and four replicates, to

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measure their intake, digestibility of nutrients and performance in a feedlot. The bulls were randomly assigned to five treatments (four bulls per treatment), and housed in 20 pens with one bull per pen (3.15 m^2). The basic experimental diet (Table 1) contained 45% chopped rice straw (8–10 cm in length) and 55% concentrates (dry matter (DM) basis). Five experimental treatments were used: A control containing no urea (NU) but soybean meal and coconut meal and as the main sources of protein, and four treatments where urea replaced some of the plant protein sources, and was included as urea (U), a slow-release zinc-urea complexes (ZnU), urea-impregnated zeolite (UZ) and zinc-urea-impregnated zeolite (ZnUZ). These slow-release urea compounds have been evaluated in *in vitro* studies (Kardaya *et al*., 2009; 2010). All urea containing diets were formulated to contain the same crude protein (CP) level with a urea content of 1.43% which supplied 33% of total CP.

Table 1 Feed composition and nutrient composition of the experimental diets fed to yearling Bali bulls

Item	Diets (DM basis, %)*				
	NU	U	ZnU	UZ	ZnUZ
Ingredients, % DM basis					
Rice straw	45.00	45.00	45.00	45.00	45.00
Corn, crack	2.00	4.00	4.00	4.00	4.00
Rice bran	13.00	13.00	10.00	9.59	9.59
Soybean meal	5.00	-	-	-	-
Cassava meal	10.48	15.05	16.12	15.00	15.00
Coconut meal	24.00	15.00	16.00	16.12	16.14
Molasses	-	6.00	6.00	6.00	6.00
Premix†	0.10	0.10	0.10	0.10	0.10
Dicalcium phosphate	0.40	0.40	0.40	0.40	0.40
ZnSO4	0.02	0.02	-	0.02	-
Urea	-	1.43			
Zinc-urea	-	-	2.38		
Urea-impregnated zeolite	-	-		3.77	
Zinc-urea-impregnated zeolite	-	-			3.77
Nutrient composition, % DM basis					
Crude protein	12.91	11.66	12.20	12.18	12.10
Extract ether	6.03	4.95	4.17	4.52	3.81
Crude fibre	18.00	17.73	17.55	17.37	16.60
Nitrogen-free extracts	50.58	53.07	53.67	53.00	54.78
Ash	12.48	12.59	12.41	12.93	12.72
Calcium	0.10	0.12	0.11	0.13	0.13
Phosphorus	0.04	0.06	0.07	0.07	0.07
Zinc, mg/kg	34.85	33.75	34.30	33.75	34.85
Neutral detergent fibre	52.68	50.81	48.61	51.48	48.84
Acid detergent fibre	34.45	34.88	33.81	34.47	34.05
Cellulose	21.89	22.33	21.66	21.98	21.41
Lignin	4.56	4.05	4.03	4.65	4.08
Hemicellulose	18.23	15.94	14.80	17.01	14.79
Silica	8.17	8.11	8.13	8.27	8.29
Total digestible nutrients	60.07	59.44	60.47	59.73	59.62

* NU: no urea; U: urea; ZnU: zinc-urea; UZ: urea-impregnated zeolite; ZnUZ: zinc-urea-impregnated zeolite; urea N represented 33% of total N; ‡ Total digestible nutrients = 0.67 x DM (NRC 1985)

† Composition (per kg): vit. A 500 000 IU, vit. D 100 000 IU, vit. E 150 mg, L-lysine 3 750 mg; DL-methionine 5 000 mg, magnesium 1 700 mg, iron 1 250 mg, manganese 2 500 mg, copper 25 mg, iodine 5 mg, zinc 500 mg

The experiment was conducted over 67 days (14 days for diet adaptation, 56 days for feed intake and growth trial, and 7 days a nutrient digestibility study). Bulls were weighed biweekly before morning feeding. Feed refusals (rice straw and concentrates) were weighed every morning at 07:00 before morning feeding. The bulls were fed twice daily (08:00 and 16:00) at 3% BW (DM basis). The amount of the feed was adjusted biweekly for changes in BW. Water was provided ad libitum.

Feed and feed refusals were collected, sub-sampled (100 g) and dried (55 °C, 96 h) each week. The weekly samples were composited for each bull, sub-sampled again (100 g), ground through a 1-mm screen in a Willey mill and frozen (-4 °C), pending analysis. Samples were analysed for DM (AOAC, 1990), organic matter (OM) (determined by ash), CP (obtained by total N determination using the micro-Kjeldahl technique and calculated as N x 6.25) and ether extract (EE) (determined gravimetrically after extraction using petroleum ether in a Soxhlet instrument) (AOAC, 1990). Furthermore, neutral detergent fibre (NDF) was determined according to the procedures of Van Soest *et al*. (1991), acid detergent fibre (ADF), using the AOAC method (1990) and sulphuric acid lignin according to the procedure of Robertson & Van Soest (1981).

Feed was weighed daily just before morning feeding. During a seven-day collecting period (from days 61 to 67) feed refusals and faeces were collected daily at 07:00 before morning feeding and was used to calculate nutrient digestibility. Total daily feed refusals were weighed, sub-sampled, dried (55 °C, 96 h), and stored (-4 °C) for analysis. Faeces was collected several times daily and stored in plastic containers. Total daily faecal output was weighed, sub-sampled (5%), dried (55 °C, 96 h) and stored (-4 °C) for analysis. Feed, feed refusals and faecal samples were analysed in duplicate for DM (AOAC, 1990), OM, CP, EE, NDF, ADF and sulphuric acid lignin according to the methods used in the feed analysis. Digestibility of nutrients was calculated as:

Digestibility, % = {100 × (g of dietary intake – g of faecal nutrient)/(g of dietary intake)}

All data were subjected to a one-way ANOVA and Duncan's multiple range test (Steel & Torrie, 1980) using an SPSS application (Release 16.0, Chicago, IL, USA). Significant differences were measured within *P* <0.05.

Results

Experimental diets (Table 1) supplied nutrients in relatively similar quantities to all bulls. However, the use of urea as a non-protein nitrogen (NPN) source to replace some of the N of soybean meal and coconut meal resulted that the EE and NDF levels of the NU diet were slightly higher than in the urea containing diets. Adding molasses to all urea supplemented diets increased their nitrogen free extract (NFE) levels slightly. Thus, all diet are assumed to supply equivalent levels of energy to the bulls.

The experimental diets affected (*P* <0.05) dry matter intake (DMI) of the bulls: Including slow-release urea in the diets did not affect DMI, though including urea in treatment U decreased (*P* <0.05) DMI compared to the control (Table 2). The replacing U with ZnU and UZ increased (*P* <0.05) DMI, but not when ZnUZ replaced the urea in treatment U.

When diet ingredients were separated individually into those of rice straw and concentrate, it was found that the concentrate intake fluctuated across the experimental diets, whereas rice straw intakes were similar. The DMI responses of the bulls, which were measured as percentage of BW (% BW) and metabolic weight (g DM/kg $BW^{0.75}$) showed no difference across experimental diets. However, nutrient intakes, except for ADF, were influenced (*P* <0.05) by experimental diet. Intakes of OM, CP, NDF and hemicellulose were higher in the control (NU) than in the U treatment (*P* <0.05), but not cellulose intake. The OM intake was higher (*P* <0.05) in the ZnU treatment than in the U treatment, whereas CP intake was found to higher again in the slow-release urea diets. Both NDF and hemicellulose intakes were higher (*P* <0.05) when U was replaced with UZ, while hemicellulose intake decreased (*P* <0.05) when urea (U) diet was replaced with ZnU and ZnUZ in the diets.

In the present study the experimental diets affected (*P* <0.05) the apparent digestibility of the nutrients in the bulls (Table 3). Compared to the control diet (NU) the inclusion of urea (U treatment) did not change the digestibility of DM, OM, cellulose, hemicellulose and CP, but decreased NDF and ADF digestibility. Hemicellulose and CP digestibility increased (*P* <0.05) in the zinc-urea (ZnU) treatment compared to the control (NU), but not the digestibility of the other nutrient. The digestibility of all nutrients except that of NDF and ADF was higher (P <0.05) in urea-impregnated zeolite (UZ) treatment than in the control. In the zinc-urea-impregnated zeolite (ZnUZ) diet CP digestibility was higher than in the NU diet, while DM, OM, cellulose and hemicellulose digestibility were similar, but NDF and ADF digestibility were lower. Comparing the digestibility of nutrients in the U treatment with those in the diets containing slow-release urea compounds, the digestibility of all nutrients except that of OM were higher in the SRU and ZnU treatments than in the U treatment. All nutrients showed higher digestibility in the UZ treatment than in the urea (U) treatment but in the ZnUZ treatment only DM and CP digestibility were higher, and not that of the other nutrient.

Table 2 Dry matter and nutrient intake of yearling Bali bulls during the experimental period

Dry matter intake†	Diets (DM basis)*					Mean	SEM	*P*
	NU	U	ZnU	UZ	ZnUZ			
Feed, g/d	4,164[b]	4,053[a]	4,230[b]	4,156[b]	4,140[ab]	4,149	15.61	0.001
Rice straw, g/d	1,936	1,940	1,987	1,924	1,919	1,941	8.66	0.077
Concentrate, g/d	2,228[b]	2,113[a]	2,243[b]	2,232[b]	2,221[b]	2,207	13.70	0.003
% BW	2.30	2.25	2.27	2.26	2.23	2.26	0.01	0.088
g/kg $BW^{0.75}$	82.44	82.47	83.27	83.74	84.29	83.24	0.25	0.053
Nutrient intakes†, g/d								
Organic matter	3,637[bc]	3,529[a]	3,695[c]	3,612[abc]	3,606[ab]	3,616	14.51	0.000
Crude protein	537.41[d]	472.76[a]	516.01[c]	506.36[bc]	501.08[b]	506.72	4.94	0.000
NDF	2 193[c]	2 059[a]	2 056[a]	2 139[b]	2 022[a]	2 094	15.03	0.000
ADF	1 434	1 413	1 430	1 432	1 410	1 424	3.78	0.095
Cellulose	911[b]	905[ab]	916[b]	913[b]	886[a]	906	3.08	0.003
Hemicellulose	759[d]	646[b]	626[a]	707[c]	612[a]	670	12.70	0.000

† Different superscripts within rows differed significantly (*P* <0.05)
*NU: no urea; U: urea; ZnU: zinc-urea; UZ: urea-impregnated zeolite; ZnUZ: zinc-urea-impregnated zeolite
SEM: standard error of mean; NDF: neutral detergent fibre; ADF: acid detergent fibre

Table 3 Apparent digestibility of nurients of experimental diets fed to yearling Bali bulls

Apparent digestibility†, %	Diets (DM basis)*					Mean	SEM	*P*
	NU	U	ZnU	UZ	ZnUZ			
Dry matter	67.30[ab]	65.85[a]	69.35[bc]	71.07[c]	69.16[bc]	68.55	0.461	0.000
Organic matter	68.79[a]	68.75[a]	70.60[ab]	72.23[b]	70.13[ab]	70.10	0.378	0.004
NDF	63.27[b]	58.91[a]	63.33[b]	65.32[b]	58.63[a]	61.89	0.657	0.000
ADF	55.36[b]	49.96[a]	54.24[b]	56.25[b]	50.36[a]	53.23	0.667	0.000
Cellulose	72.03[ab]	71.37[a]	73.51[bc]	75.36[c]	73.03[ab]	73.06	0. 361	0.000
Hemicellulose	78.22[a]	78.51[a]	84.09[b]	83.71[b]	77.65[a]	80.44	0.663	0.000
Crude protein	71.19[a]	71.79[a]	75.13[b]	74.93[b]	74.27[b]	73.46	0.426	0.000

† Different superscript within rows differ significantly (*P* <0.05)
*NU: no urea; U: urea; ZnU: zinc-urea; UZ: urea-impregnated zeolite; ZnUZ: zinc-urea-impregnated zeolite.
SEM: standard error of mean; NDF: neutral detergent fibre; ADF: acid detergent fibre

The experimental diets influenced (*P* <0.05) final weight, live weight gain (LWG), average daily gain (ADG) and feed efficiency (Table 4). Compared to the control (NU), the urea containing diet (U) did not change the performance parameters of the bulls. In the case of slow-release urea compounds, both ZnU and ZnUZ improved (*P* <0.05) weight gain above that of the urea (U) containing diet, but did not change other performance parameters. Live weight gain, ADG and feed efficiency in the urea-impregnated zeolite (UZ) treatment were improved (*P* <0.05) above that of the urea (U) containing diet.

Discussion

Total feed intake differed between treatments owing to differences in amounts of concentrates consumed. Animals receiving the diet containing urea (U) had a lower DMI than those on the control (NU) diet. It is suggested that the poor palatability of urea could be the reason for this reduced intake, because Golombeski *et al.* (2006) previously reported that a decrease in feed intake in a urea treatment was caused by the bitter taste of urea. In the current study, the increased DMI responses of the bulls receiving the ZnU and UZ containing diets compared with those on the urea (U) diet suggested that these complexes improved the palatability problem of untreated urea, as found by Golombeski *et al*. (2006).

Table 4 Performances of yearling Bali bulls fed different sources of urea

Performances†	Diets (DM basis)*					Mean	SEM	*P*
	NU	U	ZnU	UZ	ZnUZ			
Initial weight, kg	147.25	145.75	150.50	146.50	148.75	147.75	0.615	0.087
Final weight, kg	181.25[ab]	180.00[a]	186.75[c]	183.75[abc]	185.25[bc]	183.40	0.723	0.004
Live weight gain, kg	34.00[a]	34.25[a]	36.25[ab]	37.25[b]	36.50[ab]	35.65	0.399	0.013
Average daily gain, g	606.75[a]	611.25[a]	646.75[ab]	664.50[b]	651.25[ab]	636.10	7.092	0.013
Feed efficiency:								
kg LWG/kg DMI	0.146[a]	0.151[ab]	0.153[abc]	0.160[c]	0.157[bc]	0.153	0.002	0.017

† Different superscripts within the same row differ significantly ($P < 0.05$)
*NU: no urea; U: urea; ZnU: zinc-urea; UZ: urea-impregnated zeolite; ZnUZ: zinc-urea-impregnated zeolite.
SEM: standard error of mean. LWG: liveweight gain; DMI: dry matter intake

Other studies (Forero *et al*., 1978; Galina *et al*., 2003) reported increased feed intakes when coated urea replaced pure urea. However, Galo *et al.* (2003) and Taylor-Edwards *et al*. (2009b) reported that polymer-coated urea substituting urea did not alter feed intake significantly. It was more likely that the slow-release urea properties of ZnU and UZ in this study improved fibre fermentability in the rumen, which allowed ruminal fluid and solid particles to flow rapidly into the post-rumen digestive tracts. As a result, the rumen fill became empty and this promoted the bulls to consume more of the diet. This explanation is consistent with the study of Karsli & Russell (2001), who reported that an increase in ruminal fluid and flow rate of solid particles into the small intestine enhance feed intake.

Further analyses of DMI responses of the bulls relative to their weight (percentage of BW) and metabolic weight (g DM/kg $BW^{0.75}$) showed similar DMI responses in all yearling bulls in the current study. Differences in DMI in yearling bulls were in proportion with the differences in their BW and physiological state. The DMI range in the current study (2.23–2.27% BW) was similar to that of bulls (2.1–3% BW) reported by Galina *et al*. (2003) and steers (2.13–2.26% BW) reported by Chizzotti *et al.* (2008).

Changes in nutrient intake as a result of experimental treatments followed the changes in feed intake patterns. In addition, nutrient composition fluctuated less across the experimental diets. This allowed nutrient intake to follow the feed intake pattern. It was not surprising that OM and CP intakes were higher in the ZnU, treatment because its OM and CP contents were slightly higher than in the U treatment. Furthermore NDF and hemicellulose intakes were higher in UZ than in ZnU and ZnUZ treatments, because the levels of NDF and hemicellulose were higher in UZ than in the ZnU and ZnUZ treatments. On the other hand, ADF intake did not change across the experimental rations because ADF levels in the diets were similar.

Galo *et al.* (2003) reported that when polymer-coated urea substituted urea in a diet, apparent digestibility of DM and CP improved, but ADF digestibility decreased. Taylor-Edwards *et al.* (2009b) reported that when slow-release urea substituted urea, the apparent digestibility of DM, OM, NDF and ADF did not change, but N digestibility decreased. In the present study, a decrease in NDF or ADF digestibility occurred only when NU was replaced with urea (U). In contrast, when urea was replaced with slow-release urea compounds, fibre (NDF, ADF, cellulose and hemicellulose) digestibility increased, especially with ZnU and UZ. In ruminants, fibre digestion takes place mainly in the rumen, where rumen microbes ferment fibre fractions of ingested feed. For this purpose, rumen microbes require available energy and nitrogen sources in synchronous amounts. Apparently, the energy to N ratio available to rumen microbes becomes less synchronized when protein N from plant protein sources (NU) was replaced with urea-N, and this resulted in lower fibre (NDF or ADF) digestion. However, in this study, the synchrony of available energy and N improved when slow-release urea replaced urea because slow-release urea provided N in continuous supply for rumen microbes, which resulted in improvement of fibre digestibility. This finding could support the observation that slow-release urea compounds improved DMI.

An increase in apparent digestibility of CP when slow-release urea compounds replaced U and NU was probably because these slow-release urea compounds could supply nitrogen continuously in optimum amounts for rumen microbial protein synthesis. As a result, the microbial protein supply to the small intestine increased, followed by an increase in CP digestibility. This increase in apparent CP digestibility was in line with the results of an *in vitro* study by Kardaya *et al*. (2010), who reported that *in vitro* rumen microbial protein synthesis increased when urea was replaced with ZnU, UZ or ZnUZ, combined with 6% molasses.

It was expected that *in vivo* DM or OM digestibility would correlate with *in vitro* values. For this purpose, *in vitro* DM and OM degradability data (Kardaya *et al*., 2009) were used. Both *in vivo* DM and OM digestibility correlated with *in vitro* DM and OM degradability, respectively, and could best be predicted with linear regression. The equation: $Y_{dmd} = 28.762 + 0.795\ X_{dmd}$; (r = 0.83; p = 0.03), and $Y_{omd} = 44.504 + 0.453\ X_{omd}$ (r = 0.83; p = 0.03), where Y_{dmd} = predicted *in vivo* dry matter apparent digestibility, Y_{omd} = predicted *in vivo* organic matter apparent digestibility; X_{dmd} = *in vitro* dry matter degradability and X_{omd} = *in vitro* organic matter degradability.

The final weight of the bulls after 56 days ranged from 181 to 187 kg (Table 4). The range was narrower than that reported by Panjaitan *et al*. (2003), who found that the live weight of 18-month-old Bali bulls was in the range of 146–185 kg. Meanwhile, the ADG of Bali bulls (607–651 g/head) was higher than that of Sariubang *et al*. (2002), who reported that the ADG of Bali bulls fed urea-supplemented rice straw combined with rice bran was 370–410 g/head. Apparently, the higher final weight of bulls fed UZ was related to their initial weight, which was slightly higher (P = 0.087) than that of bulls fed other experimental diets.

Improved LWG, ADG and feed efficiency when urea-impregnated zeolite (UZ) replacement urea (U) agree with results of a study of Ortiz *et al.* (2002), who reported that slow intake urea supplement increased (P <0.05) the LWG of Zebu cattle. On the other hand, Taylor-Edwards *et al.* (2009a) found that polymer-coated urea (1.2% DM) did not significantly increase the LWG of steers. It was suggested that the increase in ADG and feed efficiency of Bali bulls fed UZ in the current study was the result of its slow-release property, which improved ration palatability and nutrient utilization, as reflected in nutrient digestibility. Feeding UZ ration to Bali bulls improved feed efficiency and ADG.

Conclusions

The inclusion of zinc-urea-impregnated zeolite (ZnUZ) in rice straw-based diets showed slow-release urea characteristics that had positive effects on feed intake, nutrient digestibility, feed efficiency and ADG of Bali bulls.

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Authors' Contributions

DK designed the study, executed the project and analysed data. KGW assisted in the design of the study and data analyses. AP designed the research and HMW edited the draft version of the manuscript.

Conflict of Interest Declaration

The authors declare that there is no conflict of interest.

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