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Effect of Feeding Nano and Neem Coated Urea on Nutrient Intake, Digestibility and Blood Chemistry of Lactating Crossbred Cow

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ABSTRACT

Background: With diminishing communal grazing lands, cost-effective protein supplementation for ruminant livestock is crucial. Utilizing nano-formed urea and a novel nano-formed nitrogen source, in conjunction with balanced energy sources, is anticipated to decrease the feeding costs for ruminant livestock. Nano-urea alone or with neem-coated urea supplementation may be used as good economic source of nitrogen, if found suitable.

Methods: Two doses of Nano-urea (160 µL Nu) and combined with neem-coated urea (160 µL Nu+1% Ncu) were selected for lactation trial based upon *in vitro* rumen fermentation system.

Result: Addition of Nano-urea and combination of Nano-urea and neem-coated urea enhanced voluntary intake of different nutrients and increased DCP value in crossbred dairy cows. TDN intake pattern was similar among all treatment groups. However, DCP intake was greater ($p<0.001$) and increased blood urea nitrogen in T1 and T2 compared to T0 groups. However, Feeding nano-urea and combination of nano-urea + neem-coated urea marginally benefitted in terms of feeding economics for milk production.

Key words: Blood chemistry, Dairy cattle, Digestibility, Nano urea, TMR.

INTRODUCTION

Nano-Urea as a source of non-protein nitrogen may be a potential strategy to reduce the overall emission intensity of nitrogenous compound through milk production system of dairy animals, since nano-urea is prepared with nano-technology. Soybean meal (SBM), groundnut cake (GNC), mustard cake, tiloil (sesame) cake has long been used as a prominent source of crude protein for ruminants (Koukolová *et al.*, 2020). However, with its increasing price, the use of these protein sources results in ultimately higher cost of production. Therefore, the use of urea as a protein (NPN source) replacement is attractive in ruminant diets because of its low cost compared with other protein feeds such as soyabean meal with high rumen degradability (Wanapat, 2009; Xin *et al.*, 2010; Muralidharan *et al.*, 2016). Driven by the gradual loss of community pastureland in India, progressive farmers are increasingly adopting intensive dairy farming systems. This shift necessitates a focus on cost-effective agribusiness, where a primary challenge lies in optimizing rumen function. Specifically, farmers must synchronize the availability of energy and nitrogen from feed to maximize nutrient utilization, thereby overcoming the traditionally high costs associated with protein nutrition for ruminant livestock (Alipour *et al.*, 2020). As a non-protein nitrogen source, urea is a popular, low cost and easily obtained protein supplement (Wahyono *et al.,* 2022). Slow-release urea (SRU) is a coated non-protein nitrogen (NPN) source for ruminant nutrition (Salami *et al.* 2020; Manju *et al.,* 2022). These products are usually available for feeding to all ruminant species (cattle, buffalo, sheep and goat).Rumen bacteria can convert NPN sources into true protein (Simoni *et al.*, 2023). Therefore, use of urea (NPN compound) is cost effective as ruminant animal can convert non protein nitrogen into true protein that can be utilized by host animal for production purpose.

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MATERIALS AND METHODS

The research was conducted during the period of August, 2023 to December, 2024 at the experimental facility within the laboratories of animal nutrition department, Instructional livestock cattle farm ILF©, college of veterinary science which is located in Khanapara, Assam, India. Eighteen crossbred

(Jersey × Holstein Friesian) cow (weight between 350 kg to 400kg were equally divided into 3 dietary treatment groups denoted as T0, T1 and T2. Animals were distributed to each square based on parity and lactation stage. Live weights were measured using electronic/electrical balance. Prior to commencement of the experiment, the animal underwent deworming. Intake parameters was recorded at the fortnight basis of each experimental period. The animals were randomly allocated to receive one of the experimental diets which are TMRs based diet *i.e.* Concentrate: Green roughage: Dry roughage (40:45:15), where T0 (control; only TMR diet); T1 (addition of 160 µL Nano urea on the diet DM basis in the TMR based diet); T2 (addition of 160 µL Nano urea+1%neem coated urea in the TMR based diet DM basis). Nano urea product produced by IFFCO which is available in the local market. Diets were formulated to meet the nutrient requirement of cows according to NRC (2001) recommendations. Amounts of feed offered and orts of each cow were weighted daily and were restricted to 5 to 10% of intake as-fed basis.

Throughout the 120days of duration of the experiment, the animal in each group adhered to their respective feeding schedules and had access to their allocated feed.So, there was continuous monitoring of growth performance and nutrient intake of the experimental animal were using established protocols. Throughout the experiment, experimental animals received consistent access to clean drinking water and their daily feed consumption was precisely measured using a double pan balance. Bi-weekly, between 8:00 and 9:00 AM, the weight of each animal was recorded to monitor growth. For the estimates of digestibility of the diets total 6day collection period was fixed. This analysis aimed to estimate the intake, digestibility and availability of various nutrients, as per the procedures laid out in AOAC, (2012). Analysis of minerals were done using atomic absorption spectroscopy (AAS). Blood samples were collected from the jugular vein of all experimental cows at 0, 60 and 120 days of feeding under aseptic conditions. Some relevant blood metabolites (Protein, albumin, globulin, glucose, blood urea nitrogen) estimated by commercially available kit.

Statistical analysis

Data related to the chemical and mineral composition of different samples were statistically analyzed by using one way analysis of variance (ANOVA). The intake of nutrients during digestion trial, blood parameters were analyzed by a mixed ANOVA with repeated measures, where treatment was a fixed factor (between subject effect), time period (within-subject) was a random factor having subject (cow) nested within it and their (period treatment) interaction was evaluated. A computerized IBM SPSS 26.0 software program was used for ANOVA. Tukey's HSD test was used to measure the differences of means.

RESULTS AND DISCUSSION

Chemical composition and mineral profile of experimental diet

The chemical and mineral compositions of various feed components including maize grain, wheat bran, mustard oil cake, soya bean cake, rice polish, paddy straw and maize fodder are provided in Table 1. Mustard oil cake had comparatively higher protein content than soyabean cake (32.99 vs. 26.65%).Samples of total mixed ration collected from study area had maximum CF (13.95%) and AIA (2.58%). Wheat bran had the maximum NDF and hemicelluloses content while soyabean oil cake contained highest ADF, cellulose and lignin levels. Maize grain contained least ADF, cellulose and lignin among all the concentrates. The crude protein content of mustard cakes and soyabean oil cakes observed in the present study were well within the range reported by earlier workers (Chowdhury *et al.,* 2010 and Kumar *et al.,* 2002). The proximate compositions and fibre fractions of feedstuffs analyzed in the present study are also within the normal range (Yadav *et al*., 2018). The diverse chemical compositions and fiber fractions observed in feedstuffs can be attributed to variations in oilseed varieties and agronomic factors, including fertilizer application, harvest timing and field drying practices.The DM, OM, CP, EE, total ash, T-CHO, NFC, NDF, ADF, hemicelluloses, cellulose and lignin content of concentrate mixture and green fodders were within the normal range as reported by Ranjhan, (1991) for Indian feeds and fodders. The results on macro and micro mineral profile of concentrate feeds collected from dairy farms are presented in Table 2, respectively. Compounded cattle feeds exhibited the optimum concentrations of major minerals (Ca, P, Mg) and micro minerals (Cu, Zn, Mn, Fe, Co) compared to other concentrate feeds as per Ranjhan, (1991).

Evaluation of chemical and mineral composition in different TMR based feeds

Table 2 outlines the chemical and mineral composition of the rations. While DM, OM, EE, TCHO, ADF, hemicellulose and lignin were consistent among the three TMRs, NDF and cellulose were higher in T1 and T2 compared to T0. However, three TMRs had a similar concentration of Ca and P. TMR T1 and TMR T2 contained greater amounts of Mg, Cu, Zn, Mn, Co and Fe compared to T0, which may be due to addition of nano urea may influence the bioavailability of some of major and trace minerals.

Compounded cattle feeds provided an optimal balance of essential minerals, showing the highest concentrations of major minerals (Ca, P, Mg) and ideal levels of micro minerals (Cu, Zn, Mn, Fe, Co) among all concentrate feeds (Yadav *et al.*, 2018). Of all the concentrates, maize grain demonstrated the lowest concentrations of several macro and micro minerals, specifically Mg, Cu, Zn, Mn and Co.

Intake, apparent digestibility and nutritive value of feeds

Table 3 summerizes intake performance of digestion trial. Although total DM intake was significantly ($p<0.001$) lower in T2 than T0 and T1, but DMI per 100 kg BW and g/kg $W^{0.75}$ was statistically similar among three treatment groups. Table 3 also depicts the value of DMI (g)/kg $W^{0.75}$ was 101.88, 105.69 and 98.21 g in crossbred dairy cows under T0, T1 and T2 groups, respectively. However, diets containing

Table 1: Chemical composition on (%DM basis) and mineral contents of different feed ingredients used for the preparation of total mixed ration.

Parameters	Maize grain	Wheat bran	Mustard oil cake	Soyabean oil cake	Rice polish	Paddy straw	Maize fodder
Chemical composition (%)							
DM	90.58±0.45	89.24±1.07	92.65±0.91	92.65±0.91	89.24±1.07	90.69±0.32	21.15±0.65
CP	8.39±0.39	13.25±0.69	32.99±0.28	26.65±0.61	13.25±0.69	3.02±0.09	8.60±0.37
EE	2.36±0.08	2.33±0.12	9.04±0.11	8.04±0.46	2.33±0.12	1.35±0.07	2.44±0.12
Total ash	7.90±0.15	5.58±0.29	7.92±0.15	7.92±0.15	5.58±0.29	11.72±0.26	10.34±0.11
T-CHO	81.34±0.32	78.83±1.08	50.05±0.32	57.38±1.03	78.83±1.08	83.89±0.19	78.61±0.44
NFC	68.41±0.45	36.20±2.86	21.71±0.65	29.05±0.68	36.20±2.86	11.75±0.50	15.26±1.08
OM	92.10±0.15	94.42±0.29	92.08±0.15	92.08±0.15	94.42±0.29	88.28±0.26	89.65±0.11
NDF	22.93±0.22	42.63±1.84	28.6±0.48	28.33±0.35	41.6 3±1.82	72.14±0.63	63.34±0.65
ADF	5.77±0.05	12.04±0.27	20.97±0.40	21.04±0.31	11.95±0.21	50.67±1.65	33.50±0.32
Hemicellulose	17.16±0.17	30.59±2.08	7.63±0.88	7.29±0.61	30.68±2.03	21.46±1.37	29.84±0.39
Cellulose	3.77±0.06	8.38±0.15	16.74±0.13	17.12±0.43	8.61±0.23	43.42±1.12	29.54±0.32
Lignin	1.78±0.06	3.10±0.06	2.63±0.09	2.69±0.04	3.10±0.06	6.04±0.46	3.17±0.01
Macro mineral composition (%)							
Ca	0.12±0.04	0.16±0.08	0.55±0.00	0.48±0.04	0.31±0.03	0.24±0.011	0.34±0.01
P	0.22±0.08	0.34±0.01	1.02±0.03	0.93±0.02	1.09±0.06	0.06±0.01	0.17±0.01
Mg	0.3±0.01	0.23±0.01	0.35±0.02	0.38±0.02	0.32±0.02	0.23±0.02	0.23±0.01
Trace mineral composition (ppm)							
Fe	144.67±1.45	181.26±2.39	246.04±6.00	198.11±1.42	267.21±5.78	267.22±6.38	252.00±9.73
Cu	8.61±0.84	20.87±1.30	16.54±0.29	32.26±1.32	12.07±0.96	10.73±1.03	15.36±0.41
Mn	20.33±0.88	79.33±2.63	56.57±1.83	57.47±4.46	50.91±1.08	94.92±1.16	61.25±2.96
Zn	29.41±1.67	64.66±1.63	65.74±2.59	54.99±0.52	43.14±2.09	33.77±1.96	62.30±1.93
Co	0.05±0.01	0.91±0.01	0.40±0.06	0.23±0.01	0.03±0.03	0.03±0.003	0.02±0.03

DM = Dry matter, CP = Crude protein, EE = Ether extract, TCHO = Total carbohydrate, NFC = Nitrogen free extract, OM = Organic matter, NDF = Neutral Detergent Fibre, ADF = Acid detergent fibre.

Table 2: Chemical composition and mineral profile of total mixed ration diets used during experimental period.

Parameter	TMR T0	TMR T1	TMR T2	Pooled mean±SE
Chemical composition (%)				
DM	35.13	35.96	35.79	35.63±0.70
CP	12.02	13.27	13.51	12.93±0.13
EE	2.53	2.57	2.45	2.52±0.38
TA	10.36	10.11	10.45	10.31±0.17
OM	89.64	88.89	89.55	89.36±0.17
NDF	48.89	49.4	49.84	49.38±0.18
ADF	28.95	28.92	29.30	29.05±0.39
Hemicellulose	19.94	19.89	19.54	19.79±0.52
Cellulose	25.44	25.14	25.86	25.48±0.45
Lignin	3.20	3.06	3.13	3.09±0.11
Macro mineral composition (%)				
Ca	1.28	1.29	1.28	1.28±0.002
P	0.61	0.62	0.61	0.61±0.001
Mg	0.25	0.26	0.26	0.22±0.0001
Trace mineral composition (ppm)				
Cu	16.36	20.17	21.44	18.51±0.79
Zn	86.72	89.90	88.09	88.24±0.485
Mn	46.56	51.47	51.85	49.96±0.863
Fe	59.26	63.78	65.85	62.96±0.978
Co	0.51	0.59	0.61	0.57±0.03

*Mineral mixture: Grominvet (1 kg)+ supplevite-M(0.25 kg+ZydusAHL).

Nano-urea and combination of Nano-urea and neem-coated urea resulted in increased ($p<0.001$) CP intake in T1 and T2 compared to T0. CPI (g)/kg $W^{0.75}$ was estimated 10.45, 13.61 and 13.83 g in T0, T1 and T2 treatments, respectively. In contrast to our findings, Salami *et al.* (2020) reported no significant effect on overall dry matter intake (DMI) and crude protein intake (CPI) in beef cattle fed slow-release urea. Similarly, neutral detergent fiber (NDF) intake and apparent dry matter digestibility remained consistent across all treatment groups. Consistent with our findings, Salami *et al.* (2021) reported a significant decrease ($P<0.05$) in dry matter intake (DMI, -500 g/d) in slow-release urea treated groups compared to controls. These results can be explained by the increased ruminally available nitrogen, following a rise in ammonia nitrogen concentration. This optimal nitrogen availability likely stimulated microbial growth, leading to an apparent increase in dietary intake (Wahyono *et al.,* 2022). Conversely, McGuire *et al.* (2013) and Xu *et al.* (2019) stated that dry matter intake (DMI) and crude protein intake (CPI) are higher in sheep who receive urea supplementation than non-supplemented animals. Different results were shown by Kozloski *et al.* (2007) and Currier *et al.* (2004), who reported that supplemental urea to ruminants consuming low-quality roughage does not affect an animal's dry matter intake (DMI). Despite consistent dry matter intake (DMI), we hypothesize that urea supplementation altered the intake of other nutrient components. Therefore, it can be enumerated that nano urea likely created the most favorable conditions for rumen function compared to other treatments in the present study. We did not find any report in relation to the utilization of nano-urea in ruminant species. Based on the current observations it may be interpreted that feeding nano-urea has resulted increased feed intake pattern compared to control (T0) and combination of both (Nu + Unc). Probably, neem-coated urea did not produce any tangible impact on the intake performance in lactating crossbred cows.

These variations in nutrient intake, observed with urea supplementation in sheep, may stem from several factors, including differences in crude protein (CP) concentration and the quality of the basal diets (Wang *et al.,* 2015) differences in the chemical and/or physical characteristics of the forage/feedstuff (Sano *et al.,* 2011) differences in the slow-fast degradable carbohydrate and/or forage-concentrate composition of the basal diets (Zhao *et al.,* 2007; Galina *et al.,* 2007).The digestibility's of organic matter (OM) ($p<0.001$) and crude protein (CP) ($p<0.001$) were notably higher in T1 and T2 compared to control T0 (Table 3). Despite supplementation with Nano urea and a combination of Nano-urea + neem-coated urea, the digestibility coefficients of dry matter, ether extract, nitrogen-free extract, NDF and ADF remained unchanged.These outcomes indicated that rumen degradable nature of Nano and neem coated urea may enhance nitrogen availability in the rumen in the treatment groups compare to the control groups. Comparing our findings with earlier workers is difficult, because no published reports are available on

Table 3: Nutrients intake and nutrients digestibility during digestion trial in lactating cows.

Attributes (%)	T0	T1	T2	S.E.M.	pvalue
Nutrients intake during digestion trial					
DMI (kg/d)	8.79^{A}	8.75^{A}	8.58^{B}	0.02	<0.001
DMI (% BW)	2.31	2.42	2.21	0.04	0.14
DMI (g)/kg $W^{0.75}$	101.88	105.69	98.21	1.46	0.11
CPI (kg/d)	0.90^{A}	1.13^{B}	1.21C	0.03	<0.001
CPI (%BW)	0.24^{A}	0.31^{B}	0.31^{B}	0.01	<0.001
CP I (g)/kg $W^{0.75}$	10.45^{A}	13.61^{B}	13.83^{B}	0.41	<0.001
NDFI (kg/d)	3.39	3.41	3.47	0.02	0.44
NDFI, %BW	0.89	0.94	0.89	0.02	0.37
NDFI (g)/kg $W^{0.75}$	39.34	40.84	39.67	0.54	0.53
Apparent digestibility co-efficient of nutrients(%)					
DM	63.18	64.24	64.9	0.66	0.563
OM	60.48^{A}	67.23^{B}	67.46^{B}	0.87	<0.001
CP	53.67^{A}	63.55^{B}	66.82^{B}	1.53	<0.001
EE	64.32	64.85	65.31	0.44	0.692
NFE	68.21	67.61	67.42	0.84	0.930
NDF	50.31	50.68	52.44	1.13	0.739
ADF	38.61	39.73	38.54	1.05	0.886

*Values bearing different superscripts (A, B, C etc. and a, b, c) in a row and column differ significantly. (T0 = Control; T1 = T0 +160 µL Nu /kg of TMR diet DM; T2 = T0 +1% neem coated urea+160 µL Nu /kg of TMR diet DM.

DMI = Dry matter intake, OMI = Organic matter intake, CPI=Crude protein intake, NDFI = Neutral detergent fiber intake, CP = Crude protein, EE = Ether extract, NFE = Nitrogen free extract, NDF = Neutral detergent fibre, ADF = Acid detergent fiber.

the utilization of Nano-urea in ruminant diets.However, Cherdthong *et al.* (2011) found that the urea and slow-release urea (urea-calcium sulfate and urea-calcium chloride) did not influence dry matter intake. However, improved CP and OM digestibility was found in the Nano urea and Nu +Ncu added groups, which also supported by Phillip *et al.* (2023); Alizadeh *et al.* (2019); Sevim *et al.* (2019). However, the effect of urea and non-protein nitrogen sources on digestibility depends on their concentration, physical structure and adaptation period. Increased digestibility of optigen and coated urea could be due to these treatments supplied enough nutrients for microorganisms in the rumen. Earlier it was reported that SRU in rations had no effect on DM, NDF and ADF digestibility (Xin *et al.,* 2010). With some exceptions, feeding FGU and SRU did not affect nutrient intake and digestibility on lactating dairy cow (Simoni *et al.*, 2023).

Availability of nutrients and nutritive value of TMR feeds

Data pertaining to nutrients intake in all the treatment groups (T0, T1 and T2) was presented in the Table 4. TDN intake pattern was similar among all treatment groups. However, DCP intake was greater ($p<0.001$) in T1 and T2 compared to T0 control. The results demonstrated that DCP percentage was significantly higher ($p<0.001$) in treatment groups (T1 and T2) compared to control T0. Digestible NDF value of TMR feeds remained similar in the groups, indicating that dairy animals likely adjusted their feed intake to maintain a consistent digestible NDF intake in all treatment groups. Phillip *et al.* (2023) reported that there was no significant ($p<0.05$) differences found for total digestible nutrients (TDN) value among tested groups; Acetic acid addition to urea-treated rice straw in dairy ewes resulted in a significant ($p<0.05$) increase in digestible crude protein (DCP). This enhancement, coupled with the fulfillment of daily dry matter (DM), total digestible nutrients (TDN) and DCP intake recommendations outlined by NRC (2001), which specifies an energy requirement of 1.67 Mcal/kg for dairy cows, demonstrates effective nutritional management.

Blood chemistry of the experimental animal

Plasma total protein concentration found significant in terms of treatment effect (P=0.032) and period effect ($p<0.001$) and no significant difference found for interaction between treatment and period (T×P). Notably, we did not find any difference among treatment groups in relation to plasma albumin, globulin and albumin: globulin ratio (Table 5). The periodic effect was highly significant ($p<0.001$) for globulin, but the interaction (T×P) effect was statistically similar for albumin, globulin and albumin/globulin ratio. Moreover, plasma glucose concentration increased significantly ($p<0.001$) with different period of time. The BUN (mg/dL) value was significant (P=0.002) increased in T2 Groups compare to the other. Periodic effect was also higher ($p<0.04$) for this parameter. Moreover, significant (p=0.027) interaction effect was also found for treatment and period. But, for bilirubin, there was no significant ($p<0.05$) difference found for treatment effect (T), period effect (P) and interaction between treatment and period effect (T×P).

However, our findings are in close agreement with the finding of Alizadeh *et al.* (2019) who investigated the blood glucose concentration in plasma increased by soya bean meal replacement with slow-release urea in the diet. Similarly, Huntington *et al.* (2006) suggested that feeding urea increases plasma glucose concentration. Increased in glucose concentration in the T2(Nu+Ncu) treatment might be due to greater DM and CP intake per unit body weight and OM digestibility compared to control diet (T0). However, current study supported the observation of Xin *et al.* (2010); Mazinani *et al.* (2021); Abyane *et al.* (2020). Consistent with our findings, Gonçalves *et al.* (2015) also demonstrated that blood urea nitrogen concentrations were elevated in lactating dairy cows receiving slow-release urea and soybean meal compared to regular urea.Therefore, it could be enumerated that higher BUN value (mg/dL) concentration might be due to rapid hydrolysis of urea in the rumen leads to high ammonia levels. This excess ammonia can be absorbed into the bloodstream and potentially contribute to negative health effects. The rumen

Table 4: Availability of different nutrients in lactating cows fed with different TMR based rations.

Attributes	T0	T1	T2	S.E.M.	p value
Availability of different nutrients					
TDNI (kg/d)	5.92	5.93	5.90	0.10	0.563
TDNI (%BW)	1.55	1.58	1.52	0.02	0.79
TDNI (g)/kg W0.75	68.64	68.56	67.54	0.70	0.79
DCPI (kg/d)	0.50^{A}	0.64^{B}	0.68^{C}	0.02	<0.001
DCPI (%BW)	0.13^{A}	0.18^{B}	0.17^{B}	0.01	<0.001
DCPI (kg)/kg W0.75	5.83^{A}	7.70^{B}	7.78^{B}	0.24	<0.001
Nutritive value of feed					
DCP, %	5.72^{A}	7.08^{B}	7.87^{C}	0.24	<0.001
TDN, %,	67.37	67.71	68.33	0.94	0.92
DNDF %	20.32	20.63	21.61	0.47	0.54

TDNI = Total digestible nutrient intake, DCPI = Digestible crude protein intake, DCP = Digestible crude protein, CPI = Crude protein intake, NDFI = Neutral detergent fiber intake.

Table 5: Blood metabolites in animals fed with different TMRs.

Days of feeding	T0	T1	T2	Pooled mean ± SEM	p value
Plasma protein (mg/dL)					
0th day	7.31	7.30	7.42	7.34^{A}±0.08	Treatment effect (T), p=0.032 Period effect (P), p<0.001; T×P, p =0.431
60th day	8.12	8.65	8.48	8.42^{B}±0.11	
120th day	7.98	8.58	8.66	8.41^{B}±0.16	
Mean±S.E.M	7.80^{A}±0.13	8.18^{b}±0.18	8.19^{c}±0.18	8.06±0.09	
Plasma albumin (mg/dL)					
0th day	2.30	2.34	2.35	2.34±0.06	Treatment effect (T), p=0.065 Period effect (P), p=0.081 T×P, p=0.705
60th day	2.40	2.53	2.62	2.52±0.04	
120th day	2.30	2.62	2.56	2.49±0.07	
Mean±S.E.M	2.33^{a}±0.03	2.51^{b}±0.07	2.51^{b}±0.06	2.45±0.03	
Plasma globulin (mg/ dL)					
0th day	5.01	4.93	5.07	5.01^{A}±0.12	Treatment effect (T), p=0.454 Period effect (P), p<0.001 T×P, p=0.748
60th day	5.71	6.12	5.86	5.90^{B}±0.12	
120th day	5.68	5.96	6.10	5.92^{B}±0.14	
Mean±S.E.M	5.47±0.12	5.67±0.18	5.67±0.17	5.61±0.09	
Plasma albumin: Globulin ratio (mg/dL)					
0th day	0.46	0.49	0.47	0.48±0.02	Treatment effect (T), p=0.662 Period effect (P), p=0.141 T×P, p=0.899
60th day	0.42	0.42	0.45	0.43±0.01	
120th day	0.41	0.44	0.43	0.43±0.02	
Mean±S.E.M	0.43±0.1	0.45±0.18	0.45±0.15	0.45±0.01	
Blood glucose (mg/ dL)					
0th day	45.66	45.78	45.83	45.76^{A}±0.82	Treatment effect (T), p=0.01 Period effect (P), p<0.001 T×P, p=0.183
60th day	46.66	52.83	55.66	51.72^{B}±1.08	
120th day	57.33	60.16	61.83	59.78^{C}±1.39	
Mean±S.E.M	49.88^{a}±1.69	52.93^{ab}±1.67	54.44^{b}±1.82	52.42±1.02	
Blood urea nitrogen (mg/dL)					
0th day	11.67	11.28	11.38	11.44^{A}±0.45	Treatment effect (T), p<0.002 Period effect(P), p=0.04 T×P, p=0.027
60th day	11.76	12.67	14.52	12.98^{AB}±0.64	
120th day	10.92	14.33	16.83	14.03 ±0.73	
Mean±S.E.M	11.45^{a}±0.46	12.76^{ab}±0.58	14.24^{b}±0.75	12.82±0.38	
Bilirubin (mg/dL)					
0th day	0.32	0.33	0.3	0.32±0.1	Treatment effect (T), p=0.217 Period effect (P), p=0.165 T×P, p=0.693
60th day	0.31	0.36	0.38	0.35±0.02	
120th day	0.33	0.38	0.37	0.36±0.01	
Mean±S.E.M	0.32±0.01	0.36±0.01	0.34±0.02	0.34±0.01	

* The values with different superscripts (A, B, C) among different treatments differ significantly (T0 = Control; T1 = T0 +160 µL Nu /kg of TMR diet DM; T2 = T0 +1% neem coated urea+160 µL Nu /kg of TMR diet DM.

itself can recycle some of this ammonia back into urea, but significant amounts may still be excreted in the urine.

CONCLUSION

Addition of Nano urea and neem coated urea in TMR based diet enhanced voluntary intake of different nutrients and increased DCP value of the TMR feeds in crossbred dairy cows. At the feeding rates used in these trials, our findings suggest there is no improvement in animal performance when a ruminal nitrogen met with Nano urea and slow release urea. However current studies demonstrated measurable effects of Nano-urea, these effects were minimal in lactating crossbred cows. Further research is warranted to validate these findings across different ruminant species (cattle, buffalo, goats and sheep) at various physiological stages (growth, pregnancy, lactation).

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Author contribution

Mamata Joysowal: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Visualization, Original draft writing Tapas K. Dutta: Conceptualization, Funding acquisition, Project administration, Writing – review and editing, Formal analysis. Bibeka N. Saikia: Writing-review and editing, Software. AD: Writing-review and editing, Software. Anupam Chatterjee: Funding acquisition, Resources, Supervision. AM: Conceptualization, Software. SR: Funding acquisition, Resources, Supervision. All authors read and approved the final manuscript.

Data availability

All data generated or analyzed during this study are included in this published article.

Declarations

Ethics approval

All procedures performed in studies involving animals were in accordance with the ethical standards of the Institutional Animal Ethics Committee (IAEC), The Institute research granted approval for the experiment with reference no.770/GO/Re/S/03/CPCSEA/FVSc/AAU/IAEC/23-24/1065.

Conflict of interest

The authors declare that they have no conflict of interests.

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