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In vitro evaluation of Niacin Supplementation on Total Mixed Rations with Different NPN Sources

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ABSTRACT

The present was aimed to study the effect of varying levels of niacin supplementation (0, 200, 400 and 600ppm, respectively) on medium urea based total mixed ration (TMR) replacing 20% of total crude protein (CP) of ration with different non protein nitrogen (NPN) sources by *in vitro* gas production technique. All the rations were iso-nitrogeneous in nature. On the basis of higher partition factor, TD%, OMD%, microbial mass production and efficiency of microbial mass production. Supplementation of niacin in low urea based TMR did not have any significant effect on microbial mass production and its efficiency. The *in vitro* pH and NH_3 concentration was significantly reduced ($p<0.05$) at 600 ppm level of niacin supplementation. The TVFA concentration was significantly comparable in control and urea based TMR and lowest in uromol based TMR. Niacin supplementation produced significantly higher ($p<0.05$) TVFA at 400 ppm level and lowest at 600 ppm in TMR. It can be concluded that slow release urea seems to be better option than urea and uromol as NPN supplement in the diets of ruminants when medium (20% of total CP) urea based TMR is to be prepared.

Keywords: NPN sources, total mixed ration, nitrogen, *in vitro* gas production

Microorganisms present in rumen can synthesize niacin. Niacin is a water-soluble B-vitamin, consists of a pyrimidine ring with either an amide or carboxylic acid side group attached to position 5. Side groups distinguish the two biological forms of niacin: nicotinamide (NAM) and nicotinic acid (NA) respectively. Both NA and NAM can be incorporated into nicotinamide adenine dinucleotide (NAD). NAD is an essential coenzyme for many oxidation reactions in energy metabolism. Consequently niacin plays a critical role in mitochondria respiration and the metabolism of carbohydrate, lipids and amino acids. Nicotinic acid is utilized or degraded in the rumen by bacteria and protozoa, helping to enhance microbial protein synthesis directly (Riddell *et al.,* 1980; Riddell *et al.,* 1981), or enhance protozoal populations that maintain rumen environments favorable for bacteria (Horner *et al.,* 1988, Doreau and Ottou 1996). Niacin present in the feeds is generally in bound form and is unavailable to animals and human beings. But, recent research findings suggest that microbial production of niacin in the rumen is not as per the requirements of calves and high producing dairy cows in early lactation (Campbell *et al.,* 1994). The niacin content in feedstuffs for ruminants can vary widely. Plant proteins and animal protein are some of the best protein sources to be used but are very expensive and not always economically justified. Non-protein nitrogen (NPN) sources are most commonly used as protein supplements due to the ability of ruminants to utilize the nitrogen, its high nitrogen density and low cost per unit nitrogen. As niacin play an important role in various biological oxidation, carbohydrate, protein and lipid metabolism are especially important in the metabolic reactions that furnish energy to the animal (Erickson *et al.,* 1990). Hence this study was being planned to see the effect of varying levels of niacin supplementation on nitrogen utilization from different NPN sources in total mixed rations (TMR) by *in vitro* gas production technique.

MATERIALS AND METHODS

The total mixed rations (TMR) were prepared by using various NPN sources i.e. urea, urobran and slow release urea replacing at 20 % of the total CP of TMR in 60 :40 ration (R:C). All the TMR prepared were iso-nitrogenous having approximately 15% CP.

Animal feeding and rumen analysis

Rumen liquor was collected in morning (6 am) from fistulated animals before feeding and watering into a pre-warmed thermo-flask and brought to the laboratory. Donor animals were fed on basal diet (concentrate @ 3kg and wheat straw ad libitum).

The *in vitro* gas production was done according to Menke *et al*. (1979). The amount of net gas produced (NGP) was used to calculate the metabolizable energy (ME) value. Neutral detergent fibre (NDF) of the residue was also determined. Total degradable sample (TDS), organic matter degradability (OMD), partition factor (PF), % organic matter degradability (% OMD), % neutral detergent fiber degradability (% NDFD), microbial biomass production (MBP), efficiency of microbial mass production (EMMP), true digestibility (TD) and short chain fatty acids (SCFA) were calculated according to formulae suggested by Makkar (2004). Volatile fatty acids (VFAs) were estimated by (Cottoyn and Boucque, 1968) using gas liquid chromatography (GLC) technique using Net Chrom-9100 model. The gas column (6 ft length and 1/8 inch diameter) packed with chromosorb 101 was used for the estimation of VFA. The gas flows for nitrogen hydrogen and zero air were 30, 30, and 320 µl/min, respectively. Temperature of injector oven, Column oven and detector were 270ºC and 172ºC, respectively.

Statistical analysis: Data found from *in vitro* study were analyzed 1×3×4 factorial design (Snedecor and Cochran, 1994), by using SPSS Version 19. The differences in means were tested by Tukey B.

RESULTS AND DISCUSSION

The chemical composition of different TMR's containing natural protein, slow release urea, uromol and urea with varying niacin levels is shown in Table 1.

The effect of different NPN sources irrespective of different niacin levels and level of replacement as 20 % of total CP of TMR in 60:40 ratio was studied on *in vitro* utilization of nutrients and presented in Table 2. The net gas production was significantly (P<0.05) lower in slow release urea ration (78.88ml) and was comparable in TMR and control. No significant effect was seen on amount of truly degraded substrate (TDS) in all different NPN sources TMR and control TMR.

In this study PF values were significantly lower (P<0.05) in urea, uromol and control based TMR and higher PF value was observed in slow release urea based TMR (3.54). The OMD% was significantly lowest in urea (76.47%) and uromol based rations (78.79%) and significantly higher (P<0.05) in slow release urea based rations (82.27%), whereas no significant effect has been seen on NDFD %. Microbial mass production (165.54mg) as well as efficiency of microbial mass production (59.37%) was significantly higher (P<0.05) in TMR containing slow release urea as NPN source. Metabolizable energy (ME) was significantly lower (9.10) in uromol based TMR; however it was comparable in other NPN and control TMR's. The short chain fatty acids (SCFA) was significantly lower (P<0.05) in uromol based ration and was comparable in other NPN sources and control TMR's. The concentration of ammonia was significantly lower (P<0.05) in slow release urea based total mixed ration (22.37mg/dl) and control TMR, where as it was highest in urea TMR.

The amount of fermentable methane was significantly lowest (0.338mMol) in urea based TMR and highest in slow release urea based TMR. The fermentable carbon dioxide was significantly lower (0.52mMol) in TMR having uromol as NPN source and significantly higher in urea based TMR (0.538mMol).

The effect of various levels of niacin supplementation, irrespective of NPN source and level of replacement (20% of total CP of ration) is presented in Table 3. The results revealed that niacin supplementation did not have any significant effect on various *in vitro* digestibility parameters. The ME values varied from 9.68 (0ppm) and 9.90 (200 ppm) levels of niacin supplementation but the results were non-significant at different levels of niacin supplementation. The pH value was significantly lowest (P<0.05) at 600 ppm level of supplementation whereas at other levels of supplementation it was statistically comparable. The concentration of ammonia was increased with niacin supplementation at 400 ppm level (25.93mg/

Table 1: Chemical Composition of niacin supplemented total mixed rations with different NPN sources

Parameters	Control				Urea (20%)				Uromol (20%)				Slow releasing urea (20%)			
	Level of niacin (ppm)				Level of niacin (ppm)				Level of niacin (ppm)				Level of niacin (ppm)			
	0	200	400	600	0	200	400	600	0	200	400	600	0	200	400	600
CP	14.63	14.60	14.70	14.47	14.78	14.64	14.68	14.87	14.77	14.57	14.63	14.73	14.8	14.7	14.73	14.73
ASH	6.60	6.67	6.77	6.75	6.80	6.85	6.40	6.50	6.67	6.6	6.75	6.77	6.95	6.5	6.65	6.35
OM	93.40	93.32	93.22	93.25	93.20	93.15	93.60	93.50	93.32	93.4	93.25	93.23	93.05	93.50	93.35	93.65
NDF	37.50	37.20	37.40	37.90	44.8	44.2	44.60	44.40	35.40	36.0	35.90	35.50	42.5	42.25	42.40	42.30
ADF	23.40	23.80	23.60	23.90	22.10	22.00	22.10	22.90	19.8	19.6	19.40	19.70	20.85	20.10	19.80	19.70
HC	14.10	13.40	13.80	14.00	22.70	22.20	22.50	21.45	15.80	16.40	16.40	15.80	21.65	22.15	22.60	22.40
FAT	3.10	3.15	2.93	3.05	2.75	2.80	2.60	2.65	2.70	2.8	2.70	2.80	2.90	2.92	2.75	2.70
CELLULOSE	13.60	13.80	13.40	13.20	15.10	14.70	14.70	14.60	14.30	13.90	14.30	13.70	16.2	16.6	16.5	16.8
T CHO	75.67	75.57	75.59	75.72	75.66	75.70	76.31	75.98	75.88	76.02	75.92	75.69	75.35	75.87	75.87	76.22
NFC	38.17	38.37	38.19	37.82	30.86	31.50	31.71	31.58	40.45	40.02	40.02	40.19	32.85	33.62	33.47	33.92

CP-crude protein, OM-organic matter, NDF-nutrient detergent fibre, ADF-acid detergent fibre, TCHO-total carbohydrate NFC-non CP fibre carbohydrate, HC-hemicellulose

Table 2: Effect of different NPN sources on in vitro substrate degradation of graded levels of niacin supplemented TMR

Parameters	Sources			Control	SEM
	Uromol	Slow release Urea	Urea		
NGP, ml	88.75^{b}	78.88^{a}	92.50^{b}	90.75^{b}	1.06
TDS ,mg	338.87	339.06	339.63	339.27	0.568
PF	3.01^{a}	3.54^{b}	2.80^{a}	2.99^{a}	0.054
OMD, %	78.79^{ab}	82.27^{c}	76.47^{a}	79.96^{bc}	0.48
NDFD, %	54.59	54.94	52.81	52.02	0.752
MMP, mg	143.62^{a}	165.54^{b}	136.13^{a}	139.62^{a}	2.31
EMMP,%	53.80^{a}	59.37^{b}	52.39^{a}	51.49^{a}	0.67
TD,%	78.01^{ab}	82.00^{c}	76.50^{a}	79.66^{bc}	0.46
SCFA, (m mole)	1.74^{a}	1.96^{b}	2.04^{b}	2.01^{b}	0.023
pH	6.52^{a}	6.74^{b}	6.85^{c}	6.81^{bc}	0.027
ME,MJ/kg DM	9.10^{a}	9.85^{b}	10.08^{b}	10.15^{b}	0.083
NH3-N, mg/dl	27.12^{b}	22.37^{a}	27.87^{c}	22.35^{a}	0.65
Ferm.CO_2	0.520^{a}	0.532^{c}	0.538^{d}	0.530^{b}	0.0005
Ferm.CH_4	0.342^{c}	0.344^{d}	0.338^{a}	0.341^{b}	0.0008

Means bearing different superscripts in a row differ significantly ($P<0.05$)

NGP-net gas production, TDS-truly degraded substrate, PF-partition factor, OMD-organic matter degradability, NDFD-neutral detergent fiber degradability, MBP-microbial biomass production, EMMP-efficiency of microbial mass production, TD-true degradability, SCFA-short chain fatty acids, ME-Metabolizable energy

Table 3: Effect of graded levels of niacin on *in vitro* substrate degradation of TMR with different NPN sources

Parameters	Levels of niacin supplementation (ppm)				SEM
	0	200	400	600	
NGP, ml	85.63	89.25	87.87	88.13	1.06
TDS, mg	337.84	340.33	338.63	340.03	0.568
PF	3.16	3.02	3.06	3.10	0.054
OMD, %	79.52	78.85	79.11	80.01	0.48
NDFD,%	53.64	51.67	53.84	55.21	0.752
MMP, mg	149.47	143.98	145.30	146.16	2.31
EMMP, %	55.53	53.61	54.17	53.73	0.67
TD, %	79.27	78.52	78.82	79.55	0.46
SCFA, m mole	1.89	1.97	1.94	1.95	0.023
pH	6.76^{b}	6.78^{b}	6.73^{b}	6.65^{a}	0.027
ME, MJ/kg DM	9.68	9.90	9.81	9.79	0.083
NH_3-N mg/dl	24.42^{a}	25.09^{b}	25.93^{c}	24.28^{a}	0.65
Ferm. CO_2	0.532^{a}	0.534^{c}	0.533^{b}	0.532^{a}	0.0005
Ferm. CH_4	0.342^{d}	0.340^{b}	0.342^{c}	0.340^{a}	0.0008

Means bearing different superscripts in a row differ significantly (P<0.05)

Table 4: Effect of NPN sources irrespective of graded level of niacin on volatile fatty acids fractions (mMol/dl)

Parameters	Urea	Slow release urea	Uromol	Control	SEM
Acetic acid	4.07^{d}	3.78^{b}	2.89^{a}	4.04^{c}	0.127
Propionic acid	1.12^{d}	1.01^{b}	0.836^{a}	1.12^{c}	0.033
Iso butyric acid	0.0447^{c}	0.0431^{b}	0.0319^{a}	0.0476^{d}	0.001
Butyric acid	0.546^{c}	0.528^{b}	0.444^{a}	0.563^{d}	0.0137
Iso valeric acid	0.0875^{b}	0.089^{c}	0.061^{a}	0.093^{d}	0.002
Valeric acid	0.056^{c}	0.055^{b}	0.045^{a}	0.063^{d}	0.0016
TVFA	5.93^{c}	5.51^{b}	4.30^{a}	5.93^{c}	0.179
Relative proportion, %					
Acetate	68.59^{c}	68.57^{c}	67.05^{a}	68.13^{b}	0.128
Propionate	18.96^{c}	18.43^{a}	19.41^{d}	18.89^{b}	0.079
Iso butyrate	0.75^{b}	0.78^{c}	0.74^{a}	0.80^{d}	0.006
Butyrate	9.25^{a}	9.58^{c}	10.33^{d}	9.51^{b}	0.086
Iso-valerate	1.48^{b}	1.62^{d}	1.41^{a}	1.58^{c}	0.021
Valerate	0.95^{a}	0.99^{b}	1.03^{c}	1.06^{d}	0.011
A:P ratio	3.61^{c}	3.72^{d}	3.45^{a}	3.60^{b}	0.021

Means bearing different superscripts in a row differ significantly (P<0.05)

dl) and lowest at 600 ppm level (24.28 mg/dl) of niacin supplementation in TMR. The fermentable methane mMol was significantly higher at 0 ppm level (0.342) followed by 400, 200 ppm, respectively and lowest at 600 ppm level of niacin supplementation (0.340) in TMR; however, the fermentable CO_2 was significantly lowest at 600 ppm level (0.532 mMol) and highest at 200 ppm level of niacin supplemented TMR.

The effect of different NPN sources, irrespective of niacin levels and level of replacement on total and individual volatile fatty acids is presented in Table 4. The TVFA was significantly lowest in uromol based TMR (4.30 mMol/dl) and was significantly higher (P<0.05) in control and urea based TMR (5.93 mMol/dl). The relative percent of acetate was significantly lowest (67.05%) in uromol and highest in slow release urea and urea based TMR (68.57%). The propionate percent was statistically higher (P<0.05) in uromol (19.41 %) and lowest in slow release urea based TMR (18.43%).The percent iso-butyric was significantly higher (P<0.05) in control TMR (0.80%) whereas it was significantly lower in uromol based TMR (0.74%).

The butyrate percent was observed highest in uromol based ration (10.33 %) followed by slow release urea based and control TMR's. The lowest percent of butyrate was found in urea based ration. The acetate to propionate ratio was significantly lowest in uromol (3.45) and highest in slow release urea based TMR (3.72)

The effect of varying levels of niacin supplementation, irrespective of different NPN source and level of replacement on volatile fatty acids is presented in Table 5. The TVFA concentration mMol/dl was significantly lowest (P<0.05) at 600 ppm level (4.87 mMol/dl) and highest at 400 ppm level of niacin supplementation in TMR (5.78 mMol/dl). The relative percent of acetate was significantly lowest at 200 ppm and 600 ppm level however, it was significantly highest (P<0.05) at 0 ppm level of niacin. The percent butyrate was significantly lowest at 0 ppm and highest at 200 ppm level of niacin supplementation but it was statistically comparable at 400 and 600 ppm level of niacin supplementation. The branched chain fatty acids iso-valeric and valeric percent was significantly highest at 600 ppm and lowest at 400 ppm level of niacin supplementation. The A: P ratio was observed significantly lowest (P<0.05) at 600 ppm (3.56) and highest at 0 ppm level of niacin supplementation (3.65).

The effect of varying levels of niacin supplementation on in vitro volatile fatty acids, irrespective of different NPN source and level of replacement is presented in Table 5. The TVFA concentration mMol/dl was significantly lowest (P<0.05) at 600 ppm level (5.12 mMol/dl) and highest (P<0.05) at 400 ppm level of niacin supplementation in TMR (5.90 mMol/dl). The percent acetate was significantly lowest (P<0.05) at 400 ppm level but it was statistically comparable at 200 ppm and 600 ppm level of niacin supplementation in TMR, however, it was

Table 5: Effect of graded level of niacin on volatile fatty acids fractions irrespective of NPN sources

Parameters	Level of niacin (ppm)				SEM
	0	200	400	600	
Acetic acid	3.89^{c}	3.63^{b}	3.95^{d}	3.31^{a}	0.127
Propionic acid	1.06^{c}	1.01^{b}	1.09^{d}	0.93^{a}	0.033
Iso butyric acid	0.044^{c}	0.042^{b}	0.044^{c}	0.0374^{a}	0.001
Butyric acid	0.533^{c}	0.522^{b}	0.556^{d}	0.470^{a}	0.0137
Iso valeric acid	0.087^{d}	0.082^{b}	0.086^{c}	0.076^{a}	0.002
Valeric acid	0.056^{c}	0.055^{b}	0.057^{c}	0.050^{a}	0.0016
TVFA	5.67^{c}	5.35^{b}	5.78^{b}	4.87^{a}	0.179
	Relative proportion, %				
Acetate	68.36^{c}	67.89^{a}	68.21^{b}	67.89^{a}	0.128
Propionate	18.72^{a}	19.00^{c}	18.91^{b}	19.07^{d}	0.079
Iso butyrate	0.781^{d}	0.771^{b}	0.762^{a}	0.768^{ab}	0.006
Butyrate	9.57^{a}	9.78^{c}	9.66^{b}	9.66^{b}	0.086
Isovalerate	1.54^{c}	1.52^{b}	1.47^{a}	1.56^{d}	0.021
Valerate	1.01^{b}	1.02^{c}	0.980^{a}	1.03^{d}	0.011
A:P ratio	3.65^{d}	3.57^{b}	3.60^{c}	3.56^{a}	0.021

Means bearing different superscripts in a row differ significantly (p<0.05)

significantly highest (P<0.05) at 0ppm level of niacin. The branched chain fatty acids iso-valeric and valeric percent was significantly higher (P<0.5) at 400 ppm and lowest at 0 ppm level of niacin supplementation. The Acetate: Propionate ratio was significantly lowest (P<0.05) at 400ppm (3.54) and highest (P<0.05) at 0 ppm level of niacin supplementation (3.67) whereas, Zimmerman *et al*. (1992) reported no change in the ruminal acetate: propionate ratio. On the basis of higher partition factor, TD%, OMD%, microbial mass production and efficiency of microbial mass production it can be concluded, that slow release urea seems to be better option than urea and uromol as NPN supplement in the diets of ruminants when medium (20% of total CP) urea based TMR is to be prepared which was in the accordance of Nangia *et al*. (2000) also found that supplementation of niacin to buffaloes @6 g and 12 g/day increased the microbial protein nitrogen from 67.75 to 88.29 and 114.71 mg/100 ml SRL, respectively. Supplementation of niacin in low urea based TMR did not have any significant effect on microbial mass production and its efficiency. The *in vitro* pH and NH_3 concentration was significantly reduced (P<0.05) at 600 ppm level of niacin supplementation. TVFA concentration was significantly comparable in control and urea based TMR and lowest in uromol based TMR. Niacin supplementation produced significantly higher (P<0.05) TVFA at 400 ppm level and lowest at 600 ppm in TMR.

CONCLUSION

From the present study, it was concluded that significant (P<0.05) increase was reported in PF, TD%, OMD%, microbial mass production and efficiency of microbial mass production in slow release urea group. The *in vitro* pH and NH_3 concentration was significantly reduced (P<0.05) at 600 ppm level of niacin supplementation. Niacin supplementation produced significantly higher (P<0.05) TVFA at 400 ppm level and lowest at 600 ppm in TMR. It can be concluded that slow release urea seems to be better option than urea and uromol as NPN supplement in the diets of ruminants when medium (20% of total CP) urea based TMR is to be prepared.

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