Journal of Animal Research: v.5 n.4, p. 729-735. December 2015

DOI: 10.5958/2277-940X.2015.00121.7

Effect of Graded Levels of Niacin Supplementation on Total Mixed Ration Containing Different Non-Protein Nitrogen Sources *in vitro*

Tejbeer Singh, Jaspal Singh Lamba*, Ravinder Singh Grewal and Shashi Saijpaul

Department of Animal Nutrition, College of Veterinary Sciences, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, INDIA

**Corresponding author: Jaspal Singh Lamba; Email: jaspalsinghgadvasu@gmail.com*

Received: 14 October, 2015 **Accepted:** 22 October, 2015

ABSTRACT

The present study was undertaken to study the effect of varying levels of niacin supplementation(0, 200, 400 and 600 ppm, respectively) on low urea based total mixed ration (TMR)replacing 10% 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, neutral detergent fibre degradability (NDFD%), organic matter degradability (OMD%), microbial mass production and efficiency of microbial mass productionfrom different NPN sources. Supplementation of varying levels 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 ($P<0.05$) reduced at 600ppm level of niacin supplementation. The total volatile fatty acids (TVFA) concentration was significantly higher ($P<0.05$) in control TMR and lowest in uromol based TMR. Niacin supplementation produced significantly higher ($P<0.05$) TVFA at 200ppm level and lowest ($P<0.05$) at 600ppm 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 low (10% of total CP) urea based TMR is to be prepared.

Keywords: *In vitro* gas production, niacin, NPN sources, total mixed ration

Rumen microorganisms can synthesize niacin. Niacin is a water-soluble B-vitamin that 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), which 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. 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. A variety of protein sources can be used as the main source of protein in the diet of ruminants. Plant proteins and animal protein are some of the best protein sources to be used but are very expensive and not always economically justified. The 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. 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 *invitro* gas production technique.

MATERIALS AND METHODS

Animal feeding and sample analysis: Rumen liquor was collected in the morning 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 accordingto 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 fibre 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). Volaltile 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 ml/min, respectively. Temperature of injector oven, column even and detector were 270 C, 172 C respectively.

Statistical analysis

Data found from*in-vitro* study were analyzed1x3x4 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 10 % of total CP of TMR in 60:40 ratios 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 (83.88ml) and was highest ($P<0.05$) in urea based TMR (94.75ml). The amount of truly degraded substrate (TDS) was significantly lower ($P<0.05$) in slow release urea ration (338.88 mg) and was highest in urea based TMR (342.78 mg).

The PF is the ratio of organic matter degraded (mg) *in vitro* to the volume of gas (ml) produced. A higher PF means that proportionally more of the degraded matter is incorporated into microbial mass i.e. the efficiency of microbial protein synthesis is higher. The PF calculated *in vitro* provides useful information for predicting the dry matter intake, MBP in the rumen and the methane emission of the whole ruminant animal. In this study PF value was significantly lower ($p<0.05$) in urea based TMR (2.83) followed by control TMR (2.99) and higher PF value was observed in slow release urea based TMR (3.39). The OMD% was significantly lowest ($P<0.05$) in uromol (78.08 %) and urea based rations (78.15 %) and significantly higher ($P<0.05$) in slow release urea based rations (83.43%), where as NDFD % was significantly lowe r($P<0.05$) in control TMR(52.02 %) and highest in slow release urea TMR(57.91%). MBP (154.36mg) as well as EMMP (54.61 %) was significantly higher ($p<0.05$) in TMR containing slow release urea as NPN source. ME was significantly lower (9.47) in uromol based TMR; however it was comparable in other TMR's and in control TMR. The SCFA was significantly lower ($p<0.05$) in uromol based ration and was observed significantly higher in urea based TMR, but it was comparable in both control and slow release urea based TMR. The concentration of ammonia was significantly lower ($P<0.05$) in slow release urea based TMR(21.69mg/dl) and highest($P<0.05$) in uromol followed by urea and control TMR.

The amount of fermentable methane (0.529mmole) and fermentable carbon dioxide (0.338mmole) was significantly lower ($P<0.05$) in TMR having uromol as NPN source where as both these were significantly higher ($P<0.05$) in urea based TMR.

Effect of niacin supplementation at varying levels in TMR having 60:40 roughage: concentrate ratio replacing 10% of total protein, irrespective of different NPN sources and level of replacement on *in vitro* utilization of nutrients is given in (Table 3). Net gas production was significantly reduced ($P<0.05$)with higher level of niacin supplementation at 600ppm level (88.13 ml) and was observed higher at 200ppm level of niacin supplementation.. The PF in TMR at all levels of niacin supplementation varied from 2.96 to 3.09 although the results were non-significant. The OMD % varied from 79.90% at 0ppm TMR and 80.13% at 600ppm level of

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

Parameters	Control				Urea (10%)				Uromol (10%)				Slow releasing urea (10%)			
	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	14.85	14.61	14.57	14.61	14.62	14.47	14.83	14.56	14.54	14.51	14.65	14.80
ASH	6.60	6.67	6.77	6.75	7.0	6.7	6.6	7.10	6.50	6.52	6.77	6.80	6.73	6.93	6.71	6.67
OM	93.40	93.32	93.22	93.25	93.0	93.3	93.40	92.90	93.50	93.47	93.22	93.20	93.27	93.05	93.29	93.32
NDF	37.50	37.20	37.40	37.90	44.30	43.8	44.4	44.1	35.80	35.50	35.30	35.40	43.5	43.80	43.60	43.00
ADF	23.40	23.80	23.60	23.90	19.50	20.2	19.70	20.30	18.35	18.15	17.85	18.05	19.65	19.35	19.40	19.95
HC	14.10	13.40	13.80	14.00	24.8	23.6	24.7	23.75	17.45	17.35	17.45	17.35	23.85	24.15	24.20	23.40
FAT	3.10	3.15	2.93	3.05	2.72	2.82	2.70	2.62	2.85	2.90	2.90	2.87	2.92	2.95	2.87	2.77
CELLULOSE	13.60	13.80	13.40	13.20	14.00	14.40	14.00	14.30	13.70	14.0	14.6	13.90	16.8	16.4	16.9	16.6
T CHO	75.67	75.57	75.59	75.72	75.42	75.86	76.13	75.66	76.02	76.10	75.49	75.76	75.81	75.58	75.76	75.75
NFC	38.17	38.37	38.19	37.82	36.12	32.06	31.73	31.56	40.22	40.60	40.19	40.36	32.31	31.78	32.16	32.75

CP-crude protein, OM-organic matter, NDF-nutrient detergent fibre, ADF-acid detergent fibre, TCHO-total carbohydrate, NFC-non 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	91.00^{b}	83.88^{a}	94.75^{c}	90.75^{b}	1.03
TDS ,mg	340.01^{ab}	338.88^{a}	342.78^{b}	339.27^{ab}	0.582
PF	2.92^{a}	3.39^{b}	2.83^{a}	2.99^{b}	0.047
OMD, %	78.08^{a}	83.43^{b}	78.15^{a}	79.96^{b}	0.44
NDFD, %	54.42^{a}	57.91^{b}	55.81^{ab}	52.02^{a}	0.63
MBP, mg	139.81^{b}	154.36^{b}	134.33^{a}	139.62^{a}	2.02
EMMP,%	52.65^{b}	54.61^{b}	50.14^{a}	51.49^{a}	0.63
TD,%	77.65^{a}	82.40^{b}	77.75^{a}	79.66^{b}	0.39
SCFA,(m mole)	2.01^{b}	1.85^{a}	2.09^{c}	2.01^{b}	0.022
pH	6.72^{b}	6.53^{a}	6.85^{c}	6.81^{c}	0.02
ME,MJ/kg DM	10.05^{b}	9.47^{a}	10.16^{b}	10.15^{b}	0.076
NH_3-N, mg/dl	21.69^{a}	26.54^{c}	25.63^{b}	22.35^{b}	0.72
Ferm.CO_2	0.529^{a}	0.530^{b}	0.544^{d}	0.530^{c}	0.009
Ferm.CH_4	0.338^{a}	0.340^{b}	0.345^{c}	0.341^{b}	0.001

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

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

niacin supplementation. The MBP at 0ppm level was 141.68 mg however, at 600ppm niacin level MBP was theoretically higher (144.41mg) although results were non-significant. The efficiency of microbial mass production was observed higher(53.27%) at 600ppm niacin level and lowest at 200ppm level but they were statistically non-significant.

ME was significantly lower (P<0.05) at 600ppm (9.82) level and significantly higher(P<0.05) at 200ppm level of niacin supplementation(10.08). The SCFA was significantly lower (p<0.05) at 600ppm level of niacin supplementation(1.95 m Mole) and was significantly higher (P<0.05) at 200ppm level (2.03 mmol) where as at 0ppm and 400ppm level of niacin supplementation were statistically similar. Riddell *et al.* (1980) observed a signifi cant reduction of SCFA in the nicotinic acid (NA) supplemented groups 6 h after feeding, when a ration containing 50 % forage was fed. The pH value was significantly lower (P<0.05) at 600ppm (6.67) and was statistically same at other levels of niacin supplementation in TMR.

A decrease of pH in rumen fluid was observed by Riddell *et al.* (1980) when niacin was supplemented with 1 g/L to the fermentation vessel, which was a considerably higher supplementation. The concentration of ammonia was significantly higher (P<0.05) at 0 ppm level (25.80 mg/dl) followed by 400ppm (24.42 mg/dl) and lowest at 600ppm and 200ppm level of niacin supplementation (22.97mg/dl). Mean ammonia-N concentration (mg/dl SRL) was significantly (p<0.01) lowest in 600ppm and highest in non supplemental niacin groups. Similar lower ammonia-N concentration was reported earlier in cattle (Riddell *et al.* 1980, 1981; Horner *et al.* 1988) and buffaloes (Nangia *et al.* 2000) that received supplemental niacin in their diet.

The effect of varying levels of niacin supplementation in different TMR,s on fermentable methane was significantly higher (P<0.05) at 0ppm level followed by 200ppm and was observed lowest at 400ppm level of niacin supplementation. The amount of fermentable

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	90.25^{ab}	92.00^{b}	90.00^{ab}	88.13^{a}	1.03
TDS, mg	340.23	341.65	340.78	338.28	0.582
PF	3.01	2.96	3.05	3.09	0.047
OMD, %	79.90	79.65	79.94	80.13	0.44
NDFD,%	54.67	54.23	55.37	55.88	0.63
MMP, mg	141.68	139.25	142.78	144.41	2.02
EMMP,%	52.17	51.11	52.33	53.27	0.63
TD, %	79.37	79.14	79.16	79.80	0.39
SCFA, m mole	1.99^{ab}	2.03^{b}	1.99^{ab}	1.95^{a}	0.022
pH	6.74^{b}	6.75^{b}	6.75^{b}	6.67^{a}	0.02
ME,MJ/kg DM	9.99^{ab}	10.08^{b}	9.95^{ab}	9.82^{a}	0.076
NH_3-N mg/dl	25.80^{c}	23.01^{a}	24.42^{b}	22.97^{a}	0.72
Ferm. CO_2	0.534^{c}	0.533^{b}	0.534^{c}	0.532^{a}	0.009
Ferm. CH_4	0.343^{d}	0.342^{c}	0.339^{a}	0.341^{b}	0.001

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

carbon dioxide was significantly lowest (P<0.05) at 600ppm level of niacin supplementation (0.532 m mole) however it was statistically similar at 400ppm and 0ppm level of supplementation. The effect of different NPN sources, irrespective of niacin levels and level on total and individual volatile fatty acids is presented in (Table 4). The TVFA was significantly lowest(P<0.05) in uromol based TMR (5.18mM/dl) and was significantly higher(P<0.05) in control TMR (5.93 mMole/dl). The percent acetate was significantly lowest (P<0.05) (67.59%) in uromol and highest in slow release urea (68.27%) followed by control and urea based TMR. The propionate percent was statistically higher (P<0.05) in urea and lowest in uromol based TMR. The percent is obutyric was significantly higher (P<0.05) in slow release TMR (1.59%) whereas it was significantly lower in urea based TMR(1.49%). The acetate to propionate ratio was significantly lowest(P<0.05) in urea (3.52) and highest in uromol based TMR.(3.70)

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 600ppm level (5.12

Table 4: Effect of NPN sources irrespective of graded level of niacin onvolatile fatty acids fractions (mMole/dl)

Parameters	Urea	Slow release urea	Uromol	Control	SEM
Acetic acid	3.87^{c}	3.63^{b}	3.52^{a}	4.04^{d}	0.141
Propionic acid	1.09^{c}	1.01^{b}	0.93^{a}	1.12^{d}	0.034
Iso butyric acid	0.043^{c}	0.0406^{b}	0.0403^{a}	0.0476^{d}	0.001
Butyric acid	0.530^{b}	0.499^{a}	0.5516^{c}	0.563^{d}	0.018
Iso valeric acid	0.0848^{c}	0.0843^{b}	0.0783^{a}	0.0933^{d}	0.002
Valeric acid	0.057^{c}	0.048^{a}	0.056^{b}	0.063^{d}	0.001
TVFA	5.67^{c}	5.31^{b}	5.18^{a}	5.93^{d}	0.198
Relative proportion, %					
Acetate	68.02^{b}	68.27^{d}	67.59^{a}	68.13	0.154
Propionate	19.29^{d}	19.03^{c}	18.31^{a}	18.89^{b}	0.122
Iso butyrate	0.75^{a}	0.76^{b}	0.77^{c}	0.80^{d}	0.008
Butyrate	9.40^{a}	9.41^{a}	10.68^{c}	9.51^{c}	.0108
Isovalerate	1.49^{a}	1.59^{d}	1.52^{s}	1.58^{c}	0.104
Valerate	1.02^{b}	0.92^{a}	1.11^{d}	1.06^{c}	0.020
A:P ratio	3.52^{a}	3.58^{b}	3.70^{d}	3.60^{c}	0.030

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

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

Parameters	Levels of niacin (ppm)				SEM
	0	200	400	600	
Acetic acid	3.96^{c}	4.02^{d}	3.58^{b}	3.48^{a}	0.141
Propionic acid	1.07^{c}	1.11^{d}	1.00^{b}	0.96^{d}	0.034
Iso butyric acid	0.044^{c}	0.045^{d}	0.042^{b}	0.039^{a}	0.001
Butyric acid	0.56^{c}	0.57^{d}	0.51^{b}	0.49^{a}	0.018
Isovaleric acid	0.088^{c}	0.090^{d}	0.082^{b}	0.079^{a}	0.002
Valeric acid	0.056^{c}	0.059^{d}	0.055^{b}	0.053^{a}	0.001
TVFA	5.79^{c}	5.90^{d}	5.28^{b}	5.12^{a}	0.198
Relative proportion, %					
Acetate	68.33^{c}	68.09^{b}	67.51^{a}	68.07^{b}	0.154
Propionate	18.68^{a}	18.87^{b}	19.05^{c}	18.92^{b}	0.122
Iso butyrate	0.759^{a}	0.771^{b}	0.808^{c}	0.761^{a}	0.008
Butyrate	9.72^{b}	9.72^{b}	9.93^{c}	9.64^{a}	.0108
Isovalerate	1.51^{a}	1.52^{b}	1.58^{b}	1.55^{c}	0.104
Valerate	0.98^{a}	1.00^{b}	1.09^{d}	1.03^{c}	0.020
A:P ratio	3.67^{c}	3.61^{b}	3.54^{a}	3.59^{b}	0.030

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

mMole/dl) and highest(P<0.05) at 400ppm level of niacin supplementation in TMR (5.90 mMole/dl). The percent acetate was significantly lowest (P<0.05) at 400ppm level but it was statistically comparable at 200ppm and 600ppm level of niacin supplementation in TMR,s., however it was significantly highest (P<0.05) at 0ppm level of niacin . The branched chain fatty acids isovaleric and valeric percent was significantly highest (P<0.05) at 400ppm and lowest at 0ppm level of niacin supplementation. The A:P ratio was significantly lowest (P<0.05) at 400ppm (3.54) and highest (P<0.05) at 0ppm level of niacin supplementation (3.67).

CONCLUSION

On the basis of higher PF, NDFD%, OMD%, MBP and EMMP 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 low (10% of total CP) urea based TMR is to be prepared.

Supplementation of varying levels of niacin in low urea based TMR did not have any significant effect on MBP and its efficiency. The *in vitro* pH and NH_3 concentration was significantly (P<0.05) reduced at 600ppm level of niacin supplementation. The TVFA concentration was significantly higher (P<0.05) in control TMR and lowest in uromol based TMR. Niacin supplementation produced significantly higher (P<0.05) TVFA at 200ppm level and lowest (P<0.05) at 600ppm in TMR.

REFERENCES

Campbell, J M., Murphy. M.R., Christensen, R.A. and Ovetton, T.R.1994. Kinetics of niacin supplements in lactating dairy cows. *J. Dairy Sci.,* **77**: 566-575.

Cottyn, B.G. and Boucque, C.V. 1968.Rapid methods for the gas chromatographic determination of volatile acids in rumen fluid. *J. Agric. Food Chem.,* **16**: 105-107.

Horner, J.L., Coppock, C.E., Moya, J.R, Labore, J.M. and Lanham, J.K. 1988. Effects of Niacin and Whole Cottonseed on Ruminal Fermentation, Protein Degradability, and Nutrient Digestibility. *J. Dairy. Sci.,* **71**: 1239-1247.

Makkar, H.P.S. 2004. Recent advances in the in vitro gas method for evaluation of nutritional quality of feed resources. In: Assessing Quality and Safety of Animal Feeds. FAO Animal Production and Health Series 160. FAO, Rome, pp. 55–88

Menke, K.H., Raab, L., Salewski, A., Steingass, H., Fritz, D. and Scheinder, W. 1979. The estimation of the digestibility and metabolizable energy content of ruminant feedstuffs from the gas production when they are incubated with rumen liquor in vitro. *J. Agric. Sci.Camb.,* **92**: 217-222.

Nangia, O.P., Gupta, M., Sindhu, S. and Garg, S.K. 2000. Influence of niacin supplementation on rumen fermentation and microbial protein synthesis in buffaloes. *Ind. J. Anim. Sci.* **70**: 764-765.

Riddell, D.O., Bartley, E.E. and Dayton, A.D. 1980. Effect of nicotinic acid on rumen fermentation in vitro and in vivo. *J. Dairy Sci.,* **63**(9): 1429-1436.

Riddell, D.O., Bartley, E.E. and Dayton, A.D. 1981. Effect of nicotinic acid on microbial protein synthesis in vitro and on dairy cattle growth and milk production. *J. Dairy Sci.,* **64**: 782.

Snedecor, G.W. and Cochran, W.G. 1994. Statistical Methods, 11th Edn. The Iowa State University Press, Ames, IA, p. 267.