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Effect of Niacin Supplementation on *in-vitro* Rumen Fermentation Pattern in crossbred Cattle

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ABSTRACT

An *in vitro* experiment was conducted to study the effect of supplementation of different levels of niacin (0, 300, 400, 500, 600, 700 and 800 ppm) on rumen fermentation and digestibility. The substrate comprised of concentrate mixture, maize fodder and wheat straw (40:20:40). Results revealed that TCA-ppt. N (mg/100 ml incubation media) and TVFA concentration (meq/100 ml incubation media) were significantly ($P<0.05$) higher at 600 ppm (17.56; 7.28) as compared to control (12.12; 6.38). The molar proportion of propionate was also higher at 600 ppm (26.52%) as compared to control (25.87%). The total gas (ml) production increased in a linear fashion whereas methane level decreased significantly ($P<0.05$) with graded levels of niacin. The NH_3-N (mg/100 ml incubation media) decreased significantly ($P<0.05$) from 15.26 (control) to 10.71 (600 ppm). The IVDMD (%) and IVOMD (%) also increased from 44.04 to 48.04 and 53.91 to 57.38, at 0 and 600 ppm niacin supplementation, respectively. The three higher levels of niacin viz. 600, 700 and 800 ppm had comparable fermentation parameters viz. digestibility, total gas, methane, TCA-ppt. N, TVFA, acetate, propionate and butyrate. It was concluded that 600 ppm niacin level is comparatively better than other niacin levels.

Keywords*:* i*n-vitro*, rumen fermentation, niacin supplementation.

Niacin is a direct precursor of the important coenzymes Nicotinamide Adenine Dinucleotide (NAD) and Nicotinamide Adenine Dinucleotide-phosphate (NADP), which act in the transfer of hydrogen, a basic function in the synthesis and degradation of fatty acids, carbohydrate and amino acids and are consequently involved in mitochondrial respiration (Bender, 2003). However, the niacin synthesis in the rumen depends on the composition of diet and microbial activity. Also research findings suggest that microbial production of niacin in the rumen is not adequate as per the requirements of calves and high producing dairy cows in early lactation (Girard, 1988). Therefore, supplementing ruminant diet with niacin becomes necessary. The supplementation of niacin has been reported to influence the rumen fermentation and increased ruminal microbial protein synthesis, milk production and weight gain of growing ruminants. Keeping in view the above facts the present study was designed to study the effect of supplementation of niacin on *in vitro* parameters.

MATERIALS AND METHODS

The *in vitro* gas production technique was employed to estimate the parameters such as total gas production, methane production, ammonia nitrogen (NH_3-N) concentration, TVFA and individual VFA's (Menke *et al.*, 1979 and Menke and Steingass, 1988). The *in vitro* DM/OM degradability was estimated by the method as described by Tilley and Terry (1963). Three trials were done for each treatment and each treatment has three replicates. TVFA was estimated as per method described by Barnett and Reid (1957). Whereas niacin content of feed sample was estimated by the method of Toma and Tabehkia (1979).

Preparation of substrate

Concentrate mixture, wheat straw and maize fodder were dried in hot air oven at 70 ^{0}C for overnight. They were ground separately to 0.5 mm size by laboratory grinder machine and mixed in the ratio of 40:40:20, respectively to make homogenous mixture for avoiding sample variation. A stock solution of niacin was prepared by dissolving 10 mg pure niacin in 100 ml of distilled water and diluted further to have different niacin concentrations viz. 0, 300, 400, 500, 600, 700 and 800 ppm. These were added to the syringes containing feed substrate. The rumen liquor was collected before feeding and watering in the morning from a bull having permanent rumen fistula. The animal was fed concentrate mixture, wheat straw and chopped green maize fodder as per the requirements (NRC, 2001). After three hours of feeding, rumen liquor sample was collected through rumen canula.

Statistical Analysis

The data obtained from the *in vitro* study was analyzed and subjected to one way ANOVA as described by Snedecor and Cochran (1989). The test of significance among different treatments was also analyzed (SPSS, 1999).

RESULTS AND DISCUSSION

The results obtained from the present study are discussed below in the light of chemical composition and *in vitro* studies.

Chemical composition of ration

The feed offered to the animal's viz. concentrate mixture, wheat straw and maize fodder were analyzed for their proximate composition and fibre fractions (Table 1). The chemical composition of the ingredients of concentrate mixture is presented in Table 2. The concentration of niacin was also estimated in samples of concentrate mixture, its ingredients (wheat bran, deoiled rice bran), wheat straw and maize fodder by HPLC. The Niacin content (ppm) of wheat straw, maize fodder, concentrate mixture, wheat bran and deoiled rice bran was 355.38, 273.92, 538.16, 711.81 and 181.47, respectively. Similar to our findings, Ghosh (2002) reported that niacin content of wheat straw, wheat bran and GNC was 211.67, 400 and 330 ppm respectively. However, the minor variations observed may be due to the variation in agro-climatic conditions, season, stage of maturity of cultivars, genetic makeup, soil fertility, harvesting methodology, post harvest storages and processing conditions like grinding and drying before analysis.

Table 2. Chemical composition of concentrate mixture (on % dry matter basis)

Ingredients	DM	CP	OM	EE	ASH	NDF	ADF	Lignin
Maize grain	87.0	9.2	98.1	4.0	1.9	21.5	5.3	1.2
Wheat bran	92.1	14.1	94.0	5.2	6.0	32.4	18.0	2.5
DORB	90.9	15.1	91.4	1.2	8.6	30.9	15.1	3.4
GNC	90.2	41.9	94.1	6.9	5.9	27.0	16.1	4.2
Mustard cake	89.0	34.0	93.4	8.1	6.6	28.1	18.4	5.5

In vitro studies

IVDMD and IVOMD

The results of IVDMD and IVOMD are presented in Table 3. At 300 ppm niacin level, the IVDMD increased significantly from 44.04% (control) to 46.06% ($P<0.05$), however there is no further significant ($P>0.05$) increase in IVDMD with increasing levels of niacin. However,

Table 1. Chemical composition of maize fodder, wheat straw and concentrate mixture (on % dry matter basis)

Feed ingredients	DM	CP	OM	EE	ASH	NDF	ADF	Niacin content (ppm)
Maize fodder	15.42	8.45	90.33	1.95	9.67	51.20	28.44	273.92
Wheat straw	89.33	3.23	86.02	0.95	13.98	66.30	37.30	355.38
Concentrate mixture	90.56	21.22	92.82	4.10	7.18	32.19	16.33	538.16

IVOMD was highest at 400 ppm level (56.93%), which was significantly higher (P<0.05) than control (53.91%) and 300 ppm level (55.13%) respectively. Samanta *et al.* (2000) also stated that IVDMD (%) values increased from 62.37 to 68.41% by supplementing niacin (0 to 400 ppm). Similar results were reported by Ghosh *et al*. (2003) who found that supplementation of 50 ppm of nicotinic acid (NA) and 100 ppm of nicotinamide (NM) in buffaloes significantly increased IVDMD. It might be due to shift in microbial population resulting in greater digestion of cellulose (Horner *et al.*, 1988)

Table 3. Effect of different levels of niacin supplementation on IVDMD *(%) and IVOMD*(%)

Levels of Niacin (ppm)	IVDMD (%)	IVOMD (%)
0	$44.04^{a}\pm 0.25$	$53.91^{a}\pm 0.56$
300	$46.06^{b}\pm 0.15$	$55.13^{ab}\pm 0.49$
400	$46.54^{ab}\pm0.33$	$56.93^{c}\pm 0.43$
500	$46.84^{ab}\pm0.30$	$56.36^{bc}\pm 0.16$
600	$48.04^{b}\pm 0.56$	$57.38^{c}\pm 0.40$
700	$47.96^{b}\pm 0.79$	$57.01^{c}\pm 0.33$
800	$47.18^{ab}\pm0.67$	$56.98^{c}\pm 0.73$

a,b,cValues with different superscripts in a column differs significantly (P<0.05)

* Each value is an average of 9 observations

Total gas production

The total gas production (ml/200 mg substrate) at 24 h of incubation at different levels of niacin supplementation is presented in Table 4. It was observed that the total gas production significantly increased with the graded level of niacin used, being higher at 800 ppm niacin level (P<0.05). The present results are in agreement with the findings of Doreau and Ottou (1996) who reported that supplementation of 150 ppm niacin resulted in significantly high total gas production after 60, 180 and 300 minutes of incubation as compared to control (304, 864 and 1492 ml in niacin supplemented group vs. 255, 710 and 1264 ml in control group respectively). The increased total gas production may be attributed to decreased ammonia production in the present study, which has an inhibitory effect on the release of indirect gas (Schofield, 2000).

Methane production

The *in vitro* methane production (% of total gas) at different levels of niacin used is presented in Table 4. The results revealed that methane production decreased significantly (P<0.05) with supplementation of niacin in comparison to control. This might be attributed to an increased production of propionate due to altered NADH/NAD ratio in microbes that inhibits methanogenesis (Flachowsky, 1993). The acetate formation releases large amounts of CO_2 and CH_4, whereas propionate production does not release CH_4 (Stern *et al.*, 1997). However in the present study, niacin supplementation promoted propionate production. These results are contradictory to the findings of Doreau and Ottou (1996) who reported that supplementation of 150 ppm niacin resulted in no significant effect on methane production. The information on this aspect that how the supplementation of niacin affects the methane production is scanty, so the results cannot be discussed due to lack of literature.

In vitro NH_3-N production

The concentration of NH_3-N content decreased significantly (P<0.05) from 15.3 (control) to 10.7 at 600 ppm level of niacin, however at 800 ppm niacin level, the NH_3-N was 12.2 (Table 4). It may be due to the increased utilization of ammonia for the synthesis of microbial protein (Horner *et al.*, 1986 and Doreau and Ottou, 1996). The above results are in line with the findings of Samanta *et al*. (2000), who reported that NH_3-N decreased significantly with increased levels of niacin. Ghosh *et al.* (2003) also reported that NH_3-N decreased at 50 ppm level of NA and 100 ppm level of Nicotinamide (NM) in *in vitro* studies. Kumar and Dass (2005) conducted a study on 3 male rumen fistulated buffaloes and concluded that the mean NH_3-N concentration (mg/dl SRL) was significantly (P<0.01) lower at 100 ppm (16.38) and 200 ppm (15.42) of niacin supplementation than control (18.14). The decreased NH_3-N concentration on supplementation of NA and NM might be because of its more uptake by rumen microbes, which prefer ammonia to peptides or amino acids as nitrogen source.

In vitro TCA – ppt. N production

The concentration of TCA-ppt. N was significantly (P<0.05) higher at 600, 700 and 800 ppm niacin level (17.6, 16.1 and 16.9 mg/100 ml incubation media respectively) as compared to control, 300, 400 and 500 ppm (12.1, 13.4, 14.8 and 15.2 mg/100 ml incubation media). However, there is gradual increase in TCA-ppt. N with the increased levels of niacin. These results corroborate with the findings of Samanta *et al.* (2000) who found that supplementation of niacin increased *in vitro* TCA-ppt. N from 25.79 mg/100 ml (control) to 28.80 mg/100 ml (400 ppm) on substrates containing wheat straw: concentrate mixture (60:40). Ghosh *et al.* (2003) also found that TCA-ppt. N increased with the addition of NA and NAM (P<0.05). Similarly, Dass and Kumar (2005) also reported an increase in TCA-ppt. N with supplementation of niacin in buffaloes.

Table 4: Effect of different levels of niacin supplementation on *in vitro* ruminal fermentation pattern

Levels of Niacin (ppm)	Total gas* (ml)	Methane* (% of total gas)	NH_3-N* (mg/100ml incubation media)	TCA-ppt. N* (mg/100ml incubation media)
0	$17.50^{a}\pm0.43$	$34.29^{d}\pm0.14$	$15.26^{d}\pm0.46$	$12.12^{a}\pm0.71$
300	$23.50^{b}\pm0.43$	$30.84^{c}\pm0.23$	$14.35^{d}\pm0.56$	$13.43^{ab}\pm0.53$
400	$25.67^{c}\pm0.42$	$29.57^{b}\pm0.11$	$12.95^{c}\pm0.48$	$14.84^{bc}\pm0.28$
500	$26.50^{c}\pm0.56$	$29.14^{ab}\pm0.11$	$10.85^{ab}\pm0.53$	$15.25^{c}\pm0.22$
600	$26.67^{cd}\pm0.33$	$29.11^{ab}\pm0.12$	$10.71^{a}\pm0.24$	$17.56^{d}\pm0.35$
700	$26.83^{cd}\pm0.60$	$29.03^{a}\pm0.25$	$11.97^{abc}\pm0.31$	$16.14^{cd}\pm0.49$
800	$28.00^{d}\pm0.26$	$29.27^{ab}\pm0.21$	$12.18^{bc}\pm0.37$	$16.90^{d}\pm0.75$

[a,b,c]Values with different superscripts in a column differs significantly (P<0.05)

* Each value is an average of 9 observations

Total volatile fatty acids

TVFA production (m mol/100ml) increased with an increase in the level of niacin level showing highest concentration (P<0.05) at 600 ppm level (7.3**±0.1). However, above this level of niacin, there was no significant difference observed (**Table 5). It may be because of stimulatory effect of niacin on rumen microorganisms which might have increased cellulose digestion and resulted in more TVFA production (Nangia and Sharma, 1994). The above results are also in agreement with the findings of Ottou and Doreau (1996), Samanta *et al.* (2000) and Ghosh *et al.* (2003) who also reported an increasing trend for TVFA production while supplementing graded levels of niacin. Kumar and Dass (2005) also observed a significant (P<0.01) increase in TVFA concentration in niacin supplemented groups (10.97, 11.44 meq/dl for 100 and 200 ppm niacin supplementation respectively) as compared to control (9.75 meq/dl) in buffaloes.

In vitro VFAs production

The *in vitro* IVFA (acetate, propionate and butyrate) production (molar % of TVFA) by the fermentation of substrate containing different levels of niacin is presented in Table 5. There was no significant difference (P>0.05) between means of acetate, however, propionate concentration increased significantly (P<0.05) at and over 600 ppm niacin level, whereas butyrate concentration was significantly low at 800 ppm niacin level. An increased propionate production might have occurred due to altered NADH/NAD ratio in microbes with niacin supplementation probably due to inhibition of methanogenesis (Flachowsky, 1993; Samanta *et al.*, 2000). Another reason for increased propionate production might be due to increased rumen protozoa population with addition of niacin. It has been suggested that increased protozoal numbers contributed to higher rumen propionic acid level (Erickson *et al.*, 1990).

Table 5. Effect of different levels of niacin supplementation on TVFA*(meq/100ml incubation media) and IVFA*(molar %)

Levels of Niacin (ppm)	TVFA (%)	IVFA (molar %)		
		Acetate	Propionate	Butyrate
0	$6.36^{a}\pm0.17$	68.58±0.20	$25.87^{a}\pm0.15$	$5.55^{b}\pm0.10$
300	$6.59^{a}\pm0.16$	68.90±0.10	$25.76^{a}\pm0.10$	$5.35^{b}\pm0.13$
400	$6.66^{a}\pm0.06$	68.94±0.20	$25.65^{a}\pm0.18$	$5.41^{b}\pm0.07$
500	$6.73^{ab}\pm0.05$	68.94±0.38	$25.67^{a}\pm0.33$	$5.39^{b}\pm0.11$
600	$7.28^{c}\pm0.14$	68.29±0.50	$26.52^{b}\pm0.28$	$5.19^{ab}\pm0.26$
700	$7.11^{bc}\pm0.14$	68.17±0.18	$26.62^{b}\pm0.12$	$5.21^{ab}\pm0.13$
800	$7.16^{c}\pm0.16$	68.38±0.22	$26.74^{b}\pm0.15$	$4.88^{a}\pm0.13$

[a,b,c]Values with different superscripts in a column differs significantly (P<0.05)

* Each value is an average of 9 observations

However, Christenson *et al.* (1998) reported that acetate was increased relative to propionate while total ruminal VFA in the ruminal fluid remained constant with supplementation of niacin. But most of the studies examining the impact of niacin supplementation on VFA concentrations in the rumen found that it increased propionate relative to acetate (Arambel *et al.,* 1982; Schwab *et al.,* 2006).

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