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IN VITRO UTILIZATION OF NPN SOURCES BY INCREASING LEVELS OF CORN STARCH IN STRAW BASED DIETS

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ABSTRACT

This study was conducted to investigate the effect of replacement of 50% cottonseed meal (CSM) nitrogen with various non protein nitrogen (NPN) sources i.e. urea (CU), biuret (CB) and diammonium phosphate (CD). The four energy sources were: wheat straw with no corn starch (WS), WS + 20% corn starch, WS + 30% corn starch and WS + 40% corn starch. These substrates were fermented with rumen liquor to measure *in vitro* dry matter digestibility (DMD), bacterial count and ammonia nitrogen (NH_3-N) concentrations. The protein sources provided 2% nitrogen (12.5% CP). The control substrate contained CSM as the sole source of nitrogen and ground wheat straw as the sole sources of energy. The *in vitro* DMD increased to 49.10, 40.06 and 31.52% in substrates containing CB, CU and CD compared to 23.10% for CSM ($P<0.01$). Similarly, supplementation of straw with 20, 30 and 40% corn starch gave 24.31, 38.03 and 45.48% DMD compared to 23.10% for control ($P<0.01$). Increase of corn starch from 20 to 30% resulted in 13.72 units higher DMD which increased to a mere 7.45 units when the corn starch was raised from 30 to 40%. The interactions between nitrogen sources and starch levels revealed that substrate CB x 40% corn starch yielded 70.73% DMD, followed by 49.66% DMD with CU x 40% starch ($P<0.01$). The NH_3-N increased due to 50% replacement of CSM with NPN sources on isonitrogenous basis. It was maximum with CU as nitrogen source, followed by CD, CB and CSM. The differences among the four nitrogen sources were significant ($P<0.01$). The substrates containing CU resulted in highest bacterial counts of $33.78x10^8$ compared to $20.41x10^8$, $17.06x10^8$ and $11.34x10^8$ for CB, CSM and CD, respectively ($P<0.01$). Addition of corn starch up to 20 and 30% to straw based substrates increased the bacterial counts to $23.25x10^8$ and $23.12x10^8$ and 40% corn starch yielded $15.58x10^8$ bacterial counts which was significantly ($P<0.01$) lesser than $17.06x10^8$ for substrates containing 0% corn starch. Bacterial count of $33.72x10^8$ was significantly higher than $8.51x10^8$ and $19.72x10^8$ at 0 and 48 hours fermentation, respectively. The ruminal NH_3-N concentration was highest (16.97 mg/dl) for CU, followed by 15.75, 14.31 and 12.98 mg/dl for CD CB and CSM, respectively. The ruminal NH_3-N concentrations were 15.69, 15.14, 14.18 and 12.98 mg/dl for 30, 20, 40 and 0% corn starch supplementation in the substrates, respectively. All these values were significantly ($P<0.01$) different from each other. During *in vitro* fermentation the NH_3-N progressively increased till 2 hour of fermentation and declined thereafter.

Key words: *in vitro*, NPN, corn starch, digestibility, ammonia nitrogen concentration, bacterial population.

INTRODUCTION

Wheat straw is the most abundantly available and important feed ingredient of ruminants in arid regions of Pakistan. However, its use is limited due to its high lignocelluloses, low protein contents and poor digestibility. Previous work (Ali *et al.*, 1997) has revealed that partial replacement of cottonseed meal (CSM) with non protein nitrogen (NPN) sources in wheat straw based substrates improves dry matter digestibility (DMD), bacterial counts and ammonia nitrogen (NH_3-N) during *in vitro* fermentation with rumen inoculums.

The cost of oilseed cakes and other conventional protein sources for ruminants is increasing thus affecting the production of milk and meat from cattle and buffaloes. The dietary supply of nitrogen in ruminants ration must be adequate to support a sufficiently dense population of rumen bacteria to digest fibrous constituents of the fodder plants. The microbial growth in rumen is dependent on the availability of nitrogen in the form of peptides, amino acids and ammonia (Russel *et al.*, 1992). The microbial protein synthesized in the rumen is a major source of amino acids for the host animal. The ruminal microbial population is diversified and has capability to ferment

structural and non structural carbohydrates (Hungate, 1966).

Most extensively studied NPN sources in ruminants include: urea, diammonium phosphate (DAP) and biuret which contain 46, 21 and 41% nitrogen, respectively. Urea, biuret and DAP are hydrolyzed to ammonia by the ruminal bacteria. The *in vivo* and continuous culture techniques are being used extensively to study bacterial physiology. However, *in vitro* technique is easy to manage and generates useful information regarding digestibility of nutrients and various parameters of rumen fermentation. This project was undertaken to explore the efficiency of *in vitro* utilization of combinations of urea, biuret and DAP with CSM and increasing levels of corn starch in straw based substrates by urea adapted rumen bacteria obtained from cannulated buffaloes.

MATERIALS AND METHODS

This experiment was planned to investigate the effect of 50% replacement of cottonseed meal (CSM) nitrogen with various NPN sources added to wheat straw on *in vitro* fermentation parameters. The CSM and CSM + urea (CU), CSM + biuret (CB) and CSM + DAP (CD) were four nitrogen sources. The four energy sources were: wheat straw with no corn starch (WS), WS + 20% corn starch, WS + 30% corn starch and WS + 40% corn starch. The CSM in the control substrate provided 2% nitrogen (12.5% CP) which was replaced with urea, biuret and DAP to replace 50% nitrogen of the CSM. The biuret was prepared by heating urea crystals at boiling temperature in a water bath for 5 minutes.

Single step *in vitro* fermentation suggested by Johnson (1966) was used with some modification. The apparatus consisted of a conical glass flask of 250 ml capacity with rubber stopper fitted with Bunsen valves, CO_2 gas cylinder and water bath at 39°C with shaker. Two fistulated buffaloes kept at the Animal Nutrition Research Center, University of Agriculture, Faisalabad, Pakistan were used as donor of rumen inocculum. The animals were fed green fodder *ad libitum* and 70g urea in solution form was poured in the rumen daily for 14 days before start of the experiment and maintained during the experimental period. Well mixed contents of ventral sac of rumen were collected in a plastic bottle and strained through four layers of cheese cloth. Artificial saliva (McDougall, 1948) was prepared and bubbled with CO_2 to attain pH of 6.9. For *in vitro* fermentation environment maintained included carbonic atmosphere, pH 6.5 to 7.0, temperature 39 °C and gentle shaking in water bath. Duplicate samples of CSM, CU, CB and CD containing 1g of oven dried and finely ground (1 mm mesh) wheat straw, biuret and corn starch were placed in 250 ml glass flask along with 50 ml of strained rumen fluid and 50 ml of artificial saliva. The flasks were capped with rubber stopper fitted with Bunsen valves to allow escape of gasses.

Experiment I

In this experiment, *in vitro* dry matter digestibility (DMD) was determined to examine the effect of CSM, CU, CB and CD as nitrogen sources with 0, 20, 30 and 40% corn starch as energy sources. After 48 hours of incubation, microbial activity was stopped by immediate cooling of flasks in ice cold water. The filtrate was used for the microbial count. The residue was centrifuged at 3000 rpm for five minutes and was dried at 70°C for 24 hours in an oven. The DMD was measured by difference methods (Hopson *et al*., 1963), using the following formula:

$$DMD = \frac{\text{Sample dry matter} - \text{residual dry matter}}{\text{Sample dry matter}} \times 100$$

Experiment II

The effect of four nitrogen sources i.e. CSM, CU, CB and CD and four energy sources i.e. 0, 20, 30 and 40% corn starch supplementation of wheat straw on bacterial count were studied. The fermentation technique was same as in experiment I. The bacterial count was determined at pre-fermentation, 4 and 48 hours after incubation using modified technique of Knaysi and Ford (1938). The rumen contents were strained through four layers of muslin cloth and the fluid thus obtained was diluted with artificial saliva solution up to 10 times. From the final dilution, 0.01 ml fluid was transferred to a thoroughly cleaned slide, upon which an area of 1 cm^2 was marked. The sample was spread evenly and fixed over a flame. It was stained with Gram's stain and examined under oil immersion lens. The counts were made from 5 microscopic fields and calculated according to following formula:

Number of organisms per ml = MF x N x Dilution factor, where:

Microscopic field (MF) = $10^8/11304$

N = average number of organisms per field

Experiment III

In this experiment, effect of four nitrogen sources i.e. CSM, CU, CB and CD and four energy levels i.e. 0, 20, 30 and 40% corn starch on NH_3-N concentration was studied.. For this purpose, eight sets of duplicate flasks were fermented. The NH_3-N was measured at 10, 20, 40 minutes and 1, 2, 4, 24 and 48 hours of

fermentation according to Chaney and Marbach (1962). The fermented samples were centrifuged for 5 minutes at 4000 rpm and supernatants were used for NH_3-N measurement. One ml of supernatant was added to 9 ml of distilled water, thoroughly mixed and to one ml of this mixture 3.6 ml of distilled water and 0.04 ml of Nesseler reagent were added. This was thoroughly shaken and its absorbance was measured within 10 minutes with Spectoronic at 420 nm wave length.

A standard curve was made to determine whether a linear relationship existed between varying concentrations of ammonium sulphate standard solution and intensity of colour produced by Nesselerizatoin. Ten test tubes containing 0.10 to 1.0 ml of standard solution were prepared. To each test tube 0.04 ml of Nessler's reagent was added and volume was made up to 5 ml with distilled water. The intensity of colour thus developed was measured at a wavelength of 420 nm on Spectronic 21 within 5 to 10 minutes after setting it at 0 absorbance with blank. The NH_3-N concentration was calculated as below:

$$NH_3\text{-N (mg/dl)} = \frac{\text{Absorbance of samples}}{\text{Absorbance of standard}} \times 0.01$$

The data were analyzed in a completely randomized design by using analysis of variance (Steel and Torrie, 1981) to find the effect of four nitrogen sources, four levels of corn starch as well as interactions. The significance of differences between means was tested by Duncan multiple range test (Duncan, 1955). For bacterial counts and NH_3-N concentrations, the effects of fermentation hours and their interactions with nitrogen sources and corn starch levels were also tested.

RESULTS AND DISCUSSION

Dry matter digestibility

The *in vitro* DMD increased to 49.10, 40.06 and 31.52% in substrates containing CB, CU and CD compared to 23.1% for CSM ($P<0.01$). Similarly, supplementation of straw with 20, 30 and 40% corn starch gave 24.31, 38.03 and 45.48% DMD compared to 23.1% for control ($P<0.01$; Table 1). This experiment revealed that different nitrogen sources and readily available energy from increasing levels of corn starch enhanced *in vitro* DMD in experimental substrates ($P<0.01$). This indicates that rumen microorganisms from the buffalo have the capability to hydrolyze CB favorably and incorporate NH_3-N thus released for multiplication of bacterial population to increase DMD. Because of slow nitrogen releasing, CB proved to be the best nitrogen source, followed by CU and CD. Increase of corn starch from 20 to 30% resulted in 13.72% units higher DMD which increased to a mere 7.45% units when the corn starch was raised from 30 to 40%.

The interactions (Table 1) between nitrogen sources and starch levels also revealed that interactions of substrate CB x 40% corn starch yielded 70.73% DMD, followed by 49.66% DMD with CU x 40% starch. This difference of 21.07% units ($P<0.01$) is an indicator of highly beneficial effect of comparatively lesser soluble CB and corn starch synergism. The 47.40% DMD with CB x 30% corn starch level was 2.26% units less than the 49.66% DMD for CU x 40% corn starch, however, this difference was statistically significant ($P<0.01$). The DMD due to interactions CD x corn starch levels of 20, 30 and 40% were 22.65, 30.13 and 41.78%, respectively (Table 1). These

Table 1: Percent DMD as influenced by replacement of wheat straw with 0, 20, 30 and 40% corn starch in diets containing CSM, CU, CD and CB as nitrogen sources

Nitrogen sources	DMD (%)	Starch (%)	DMD (%)	Nitrogen sources x starch levels	DMD (%)
CB	49.10 [a]	40	45.48 [a]	CB x 40%	70.73 [a]
CU	40.06 [b]	30	38.03 [b]	CU x 40%	49.66 [b]
CD	31.52 [c]	20	24.31 [c]	CB x 30%	47.40 [b]
CSM	23.10 [d]	0	23.10 [d]	CU x 30%	47.03 [b]
				CD x 40%	41.78 [c]
				CD x 30%	30.13 [d]
				CB x 20%	29.16 [d]
				CSM x 30%	27.57 [de]
				CU x 20%	23.54 [ef]
				CD x 20%	22.65 [f]
				CSM x 20%	21.89 [f]
				CSM x 40%	19.84 [f]

Means within a column with different superscripts differ significantly ($P<0.01$).

Table 2: Total bacterial counts as influenced by replacement of wheat straw with 0, 20, 30 and 40% corn starch in diets containing CSM, CU, CB and CD as nitrogen sources

Nitrogen sources	Bacterial count (x 10^8)	Starch (%)	Bacterial count (x 10^8)	Fermentation time (Hours)	Bacterial count (x 10^8)
CU	33.78 [a]	20	23.25 [a]	04.00	33.72 [a]
CB	20.41 [b]	30	23.12 [a]	48.00	19.72 [b]
CSM	17.06 [b]	40	15.58 [c]	00.00	8.51 [c]
CD	11.34 [c]	0	17.06 [b]		

Means within a column with different superscripts differ significantly (P<0.01).

digestibilities were though higher than CSM x starch levels 20 and 40% but were less than CB and CU with starch levels of 30 and 40%. Efficient coupling of NH_3-N from relatively lesser soluble CB with available energy from starch provided suitable environment for degradation of cellulosic matter by rumen bacteria.

The maximum *in vitro* DMD in this experiment was revealed by CB, followed by CU and CD. However, the linear increase of DMD from 47.40 to 70.73% with increasing energy level of corn starch from 30 to 40% with NPN sources can be explained by the fact that starch being a non structural carbohydrate was easily fermented by rumen microbes and the type of the bacterial population in these cultures could equally digest straw cellulose as well as starch. Rather high DMD of 70.73% with CB x 40% starch may also be explained by the fact that non structural carbohydrate (starch) was 100% digested and the DMD in our experiment was not due to straw only. Hannon and Trenkle (1990) reported that *in vitro* DMD by rumen microbes increased (P<0.05) by addition of cottonseed molasses soluble (a fermentation product), an effluent of lysine production system, than by addition of only urea in cellulose. Ammonia is an important nitrogen source for cellulose degrading bacteria in the rumen (Russel *et al.*, 1992) which explains why NPN sources improved the *in vitro* DMD with substrates containing wheat straw.

Bacterial counts

The total bacterial counts due to various nitrogen sources revealed that CU showed higher bacterial population compared to CB, CSM and CD (P<0.01). The difference in bacterial count between CB and CSM was not significant (P>0.01). However, the bacterial count was minimum when CD was the nitrogen source. The bacterial counts were $33.78x10^8$, $20.41x10^8$, $17.06x10^8$ and $11.34x10^8$ for CU, CB, CSM and CD, respectively (Table 2).

In a trial with steers, the effect of supplementation of NPN sources on bacterial efficiency in the rumen (g bacterial N/kg organic matter digested in rumen) was greater when biuret was used as nitrogen source compared to urea (Currier *et al.,* 2004). However, duodenal nitrogen flow increased with crude protein supplementation but no differences were observed between urea and biuret as NPN sources.

The depressing effect of CD as nitrogen source on bacterial counts is probably due to the fact that fertilizer grade DAP used in this experiment contained toxic material like fluorides which proved detrimental for bacterial growth. It seems that bacteria killed due to this toxicity were decomposed and thus contributed to the NH_3-N concentration which was significantly (P<0.01) higher for CD than that due to CB or CSM (Table 4).

The three energy levels showed interesting effects on bacterial counts which were $23.25x10^8$, $23.12x10^8$ and $15.58x10^8$ for 20, 30 and 40% starch level, respectively (Table 2). These results indicate that starch level of 20 and 30% had a similar effect on the bacterial counts which were significantly greater (P<0.01) than those with 0 and 40% starch levels. However, the bacterial counts with 0% starch were significantly higher than those with 40% starch (P<0.01), indicating that 40% starch level had a depressing effect on the bacterial counts. The interactions between the NPN source and starch level revealed that CU x 30% corn starch resulted in significantly higher bacterial count of $43.33x10^8$ compared to other combinations of NPN sources and corn starch levels (Table 3).

Higher bacterial counts with urea compared with those of biuret can be explained by the fact that the inoculum used was obtained from urea adopted buffaloes only. It is logical to assume that ureolytic activity present in the culture resulted in a fast release of NH_3-N. This also indicates that ureolytic bacteria do not degrade biuret with similar efficiency.

The bacterial counts were measured at 0, 4 and 48 hours of fermentation. The bacterial counts at 4 hours were maximum and significantly (P<0.01) higher than those at 0 and 48 hours. The lower bacterial counts at 48 hours can be explained by the depressing effects of the closed *in vitro* system where nutrients required for bacterial growth were exhausted.

The effects of interactions between CU x 4 hours yielded maximum bacteria ($61.27x10^8$) which was significantly higher (P<0.01) than $35.06x10^8$ due to CU x 48 hours. The differences among other groups are

Table 3: Total bacterial counts (10^8) as influenced by interactions between starch levels and NPN sources during *in vitro* fermentation

NPN x starch (%)	Bacterial count (x 10^8)	NPN x fermentation time	Bacterial count (X $10^{8)}$	Starch (%) x fermentation time	Bacterial count (x 10^8)
CU x 30	43.33 [a]	CU x 4 h	61.27 [a]	20 x 4 h	40.43 [a]
CU x 20	33.87 [b]	CU x 48 h	35.06 [b]	30 x 4 h	36.45 [a]
CB x 20	28.27 [4bc]	CB x 4 h	30.63 [b]	30 x 48 h	24.75 [b]
CU x 40	24.13 [cd]	CSM x 4 h	25.90 [bc]	40 x 4 h	24.28 [b]
CB x 30	19.13 [cde]	CB x 48 h	19.13 [cd]	20 x 48 h	21.63 [b]
CSM x 30	18.84 [cdef]	CD x 4h	17.07 [cd]	40 x 48 h	12.78 [c]
CSM x 20	17.08 [def]	CSM x 48 h	14.72 [de]	40 x 0 h	9.68 [c]
CSM x 40	15.27 [def]	CB x 0 h	11.47 [de]	30 x 0 h	8.16 [c]
CB x 40	13.83 [ef]	CSM x 0 h	10.57 [de]	20 x 0 h	7.70 [c]
CD x20	13.80 [ef]	CD x 48 h	997 [de]		
CD x 30	11.17 [ef]	CD x 0 h	07.00 [e]		
CD x40	09.07 [f]	CU x 0 h	05.00 [e]		

Means within a column with different superscripts differ significantly (P<0.01).

shown in Table 3. The interactions between starch levels x fermentation time were also considered. The bacterial count of $40.43x10^8$ was maximum with 20% starch x 4 hours, followed by $36.45x10^8$ with 30% starch x 4 hours. However, the counts were not statistically different from each other (Table 3). It was also evident that bacterial count for 40% corn starch x 4 hours ($24.28x10^8$) was significantly lower than the bacterial counts obtained with 20% corn starch x 4 hours and 30% corn starch x 4 hours, indicating that higher level of corn starch had depressing effect on the bacterial counts (Table 3).

Ammonia Nitrogen

The NH_3-N increased due to replacement of CSM with NPN sources on isonitrogenous basis. It was maximum with CU, followed by CD, CB and CSM (Table 4). The differences among four nitrogen sources with respect to NH_3-N were significant (P<0.01). Similarly, 30% corn starch level proved to be the optimum for NH_3-N concentration, followed by 20 and 40% corn starch levels (Table 4). The concentrations of NH_3-N due to three levels of corn starch were significantly (P< 0.01) different from each other and from 0% corn starch. The NH_3-N was also measured at 10, 20, 40 minutes and 1, 2, 4, 24 and 48 hours post fermentation. It was maximum at 1 hour (16.83 mg/dl), remained almost static till 2 hours but reduced to 15.96 mg/dl at 4 hours (P<0.01). It showed increasing trend from 10 minutes to 40 minutes post fermentation. The concentration of 12.48 mg/dl at 24 hours was minimum which increased to 13.57 mg/dl after 48 hours (Table 4).

Slyter *et al*. (1971) reported that NH_3-N concentration of rumen culture contents collected throughout the day were higher when urea was fed than when biuret was fed. During *in vitro* fermentation, the NH_3-N levels in ruminal contents were similar at 4 hours after

Table 4: Ruminal NH_3-N concentration (mg/dl) as influenced by replacement of wheat straw with 0, 20, 30 and 40% corn starch in diets containing CSM, CU, CD and CB as nitrogen sources

Nitrogen sources	NH_3-N (mg/dl)	Starch level (%)	NH_3-N (mg/dl)	Fermentation time	NH_3-N (mg/dl)
CU	16.97 [a]	30	15.69 [a]	1 h	16.83 [a]
CD	15.75 [b]	20	15.14 [b]	2 h	16.75 [a]
CB	14.31 [c]	40	14.18 [c]	4 h	15.96 [b]
CSM	12.98 [d]	0	12.98 [d]	40 min	15.75 [b]
				20 min	15.00 [c]
				10 min	13.67 [d]
				48 h	13.57 [d]
				24 h	12.48 [e]

Means within a column with different superscripts differ significantly (P <0.01).

Table 5: Ruminal NH_3-N concentration (mg/dl) as influenced by replacement of wheat straw with 0, 20, 30 and 40% corn starch in diets containing CSM, CU, CD and CB as nitrogen source x starch levels

N source x starch level	NH_3-N (mg/dl)	N source x fermentation time	NH_3-N (mg/dl)	Starch level x fermentation time	NH_3-N (mg/dl)
CU x 20%	18.69 [a]	CU x 60 min	19.83 [a]	20% x 60 min	17.63 [a]
CU x 30%	18.13 [b]	CD x2 h	18.67 [b]	20% x 2 h	17.63 [a]
CD x 30%	16.44 [c]	CU x 4 h	18.50 [b]	30% x 60 min	17.38 [a]
CD x 40%	15.75 [d]	CU x 40 min	18.33 [b]	30% x 2h	16.50 [b]
CD x 20%	15.05 [e]	CU x 20 min	17.33 [c]	30% x4 h	16.38 [bc]
CB x 30%	14.88 [e]	CU x 2 h	17.00 [cd]	20% x 40 min	16.25 [bc]
CB x 40%	14.44 [f]	CB x 2 h	17.00 [cd]	30% x 40 min	16.25 [bc]
CU x 40%	14.10 [f]	CD x 60 min	16.83 [cde]	20% x 4 h	16.25 [bc]
CB x 20%	13.63 [g]	CB x60 min	16.67 [cde]	40% x 2 h	16.13 [bcd]
CSM x 20%	13.31 [g]	CD x 4 0 min	16.50 [def]	30% x20 min	15.75 [cde]
CSM x30%	13.19 [g]	CD x 4 h	16.33 [defg]	40%x 60 min	15.50 [de]
CSM x40%	12.44 [h]	CU x 10 min	16.17 [efg]	20% x20 min	15.50 [de]

Means within a column with different superscripts differ significantly ($P< 0.01$).

feeding the two diets containing urea and biuret as the two NPN sources. Addition of starch slowed biuret hydrolysis. During *in vivo* studies with lactating cows fed diets containing fish meal supplemented with urea and starch, the ruminal NH_3-N increased by supplementation of urea and decreased by addition of starch (Cameron *et al.*, 1991).

The addition of corn starch in straw based substrates indicates that 40% starch level released minimum ammonia nitrogen compared to 20 and 30% starch levels. This indicates that in fermentation systems containing readily degradable NPN sources, the losses in the form of ammonia can be minimized with addition of suitable starch levels This also indicates that NPN sources continued to contribute to ammonia pool till 48 hours though with a decreasing trend after 2 hours fermentation. The increase in ammonia concentration from 24 to 48 hours post fermentation is presumably due to lysis of the dead bacterial population.

The interactions between nitrogen sources x corn starch levels, nitrogen sources x fermentation times and corn starch levels x fermentation times are shown in Table 5. These results indicate that CU x 20% corn starch and CU x 60 minutes fermentation time liberated significantly ($P<0.1$) higher NH_3-N than all other combinations. The 20% corn starch x 60 minutes and 20% corn starch x 2 hours had similar values of NH_3-N and were not different from that of 30% corn starch x 60 minutes fermentation time.

In summary, addition of NPN and corn starch (readily available nitrogen and energy) in the substrate triggered the bacterial population growth in the *in vitro* system up to 4 hours which is also revealed by NH_3-N release that is an essential requirement of the cellulosic bacteria. This indicates the possibility of using these nutrients in the maintenance and production rations of cattle and buffaloes with a strategy of frequent feeding after every four hours. This may be optimal for bacterial cell production for ultimate use post ruminally as high quality protein (cell mass) and possibly production of volatile fatty acids at a sustainable rate.

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