Asian-Aust. J. Anim. Sci.
Vol. 19, No. 12 : 1722 - 1727
December 2006
www.ajas.info

Non-traditional Straws: Alternate Feedstuffs for Ruminants

S. Kaushal, M. Wadhwa and M. P. S. Bakshi*
Department of Animal Nutrition, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana-141004, India

ABSTRACT : The nutritive value of 4 straws, obtained after thrashing of seeds from fodder crops, was assessed as complete feed for ruminants. Sixteen male Murrah buffaloes (liveweight 365.8±19.5 kg), were divided into 4 equal groups and offered *ad lib*. straw of either *Trifolium resupinatum, Trifolium alexandrium, Medicago sativa* or *Lolium perenne,* supplemented with minerals and vitamin A, for 40 days in a completely randomized design. Simultaneously, each straw was offered to 3 rumen fistulated male buffaloes in order to assess the biochemical changes in the rumen. Compared to other straws *M. sativa* straw had higher ($p<0.05$) organic matter (OM), crude protein (CP), acid-detergent fiber (ADF) and cellulose content. *L .perenne* had the highest ($p<0.05$) hemicellulose and lowest ($p<0.05$) CP and acid-detergent lignin (ADL) content. *T. resupinatum* had the lowest concentration of cell wall constituents (CWC). The digestibility of nutrients of *T. resupinatum* and *L. perenne* straw was similar, but higher ($p<0.05$) than that of other straws. *M.sativa* straw showed highest ($p<0.05$) digestibility of CP. The highest OM digestibility of *T. resupinatum* and CP digestibility of *M. sativa* were responsible for highest ($p<0.05$) total volatile fatty acids and trichloroacetic acid precipitable nitrogen in the strained rumen liquor. The digestible crude protein (DCP) was highest ($p<0.05$) in *M. sativa* followed by that in *T. alexandrium*. The total purine derivatives excreted in urine varied from 0.22-0.32 mmol/kg $W^{.75}$/d. The efficiency of microbial protein synthesis indicated that OM of straws of *M. sativa* and that of *T. alexandrium* was used more ($p<0.05$) efficiently. The microbial protein synthesized was highest in *T. resupinatum*, but statistically similar to other groups. The values for N-retention and apparent biological value were highest for *L. perenne*, though comparable with that of *M. sativa* and *T. alexandrium*. The available metabolizable energy (ME) was highest ($p<0.05$) in *T. resupinatum* followed by that in *L. perenne* and lowest in *M. sativa*. It was concluded that all the straws, supplemented with minerals and vitamin A, could be fed exclusively to adult ruminants with no adverse affect, as animals were able to maintain body weight (372±20.1 kg). (**Key Words :** Straws, *T. alexandrium*, *T. resupinatum*, *M. sativa*, *L. perenne*, Purine Derivatives, Microbial Nitrogen, Nutrient Utilization, Nutritive Value, Buffaloes)

INTRODUCTION

India proudly occupies first position in livestock population (464 million) and milk production (88MT) and to sustain this position, 300×10^6 adult cattle units (1 adult unit = 450 kg live weight) are to be fed, but there is shortage of 26 million tons (MT), 280 MT and 44 MT of concentrates, green forages and straws/stovers, respectively (Bakshi and Wadhwa, 2004). Due to competing pressures on land, ironically the green revolution and diversified use of agricultural crop residues, the gap between the demand and supply of feedstuffs is increasing.

The nutritive value of feeds and fodder has a significant bearing on productivity of livestock. In most of the developing countries, the conventional cereal straws and stovers constitute the bulk of DM consumed by low and medium yielders under field conditions. These straws generally have poor nutritive value (Ranjhan and Pathak, 1979). In this situation, there is a need to either improve the efficiency of utilization of existing feed resources or to tap the newer non-conventional feed resources.

There is little scope for increasing the area under fodder production. Efforts, therefore, have been made to improve the quality of existing green forages, to increase per acre yield of fodder and to develop new fodder varieties, to ensure sufficient availability of green fodder to the livestock. The leftover material i.e. the straw (obtained after thrashing/collection of seeds) from the leguminous/non-leguminous fodder crops, currently being used as soil conditioner or burnt in the field, thereby causing environmental pollution. The average straw production from *T. alexandrium*, *T. resupinatum*, *M. sativa* and from *L. perenne* was 30, 37, 15 and 62 Q/ha, respectively. Such straws if utilized judiciously, can bridge the gap between demand and supply. Such byproducts of forage crops could

* Corresponding Author: M. P. S. Bakshi. Tel: +91-161-3298253, Fax: +91-161-2400945, E-mail: bakshimps@yahoo.com
Received August 4, 2005; Accepted March 23, 2006

be exploited as livestock feed. This study was therefore, planned to assess the nutritional value of leguminous and non-leguminous straws for buffaloes.

MATERIALS AND METHODS

Feedstuffs

The straws of 4 different multi-cut, *rabi* fodder crops of Punjab viz. *Trifolium resupinatum* (shaftal), *Trifolium alexandrinum* (berseem), *Medicago sativa* (alfalfa) and *Lolium perenne* (rye grass), sown in febrile loamy soil with good drainage in October (when the ambient temperature and relative humidity is around 24°C and 67%, respectively), were procured from the Plant Breeding Department of Punjab Agricultural University, Ludhiana. The seeds are usually ready by the end of May-June (when average ambient temperature and relative humidity is around 31°C and 49%, respectively) and were thrashed mechanically (*Trifolium alexandrinum* and *Medicago sativa*) or manually (*Trifolium resupinatum* and *Lolium perenne*).

Digestibility trial

Each straw was offered *ad lib.*, supplemented with mineral mixture (calcium 16%, phosphorus 9%, magnesium 4%, sulphur 1.4-2.3%, iron 0.3%, copper 0.078%, zinc 0.64%, manganese 0.10%, cobalt 0.009%, iodine 0.02%, acid insoluble ash 2.4%, lead 16 mg/kg and arsenic 5 mg/kg), common salt and vitamin A, as a complete feed once-a-day at 9 h, individually to 4 male Murrah buffaloes (live weight 365.8±19.5 kg). Fresh water was offered twice a day. The animals were adapted for 30 days followed by a 7-day metabolism trial. During the trial, the animals were kept in metabolism cages and a record of feed intake, orts, faeces and urine voided was maintained. The animals were weighed at the beginning and at the end of the experimental period for 3 consecutive days. Samples of feedstuffs and orts were collected at 24 h interval and dried in duplicate at 80°C in a forced air oven. Urine voided by the experimental animals was collected in plastic cans (containing 500 ml of 20% sulphuric acid in order to maintain the pH below 3) attached to a urine collection duct underneath the floor of metabolism cages. An aliquot of 5 ml was diluted to 25 ml with distilled water and kept in a deep freezer at -20°C until analyzed for purine derivatives (PD) and creatinine (CRT). For nitrogen estimation, 1% of the total urine was preserved in bottles daily which were kept in a refrigerator till analyzed. The faeces voided were collected manually and kept in iron drums (30 kg capacity) for 24 h, weighed and mixed; 0.5% duplicate samples were weighed into aluminum trays for DM determination. For nitrogen estimation, 0.25% of total faeces was preserved in previously tared, wide-mouth plastic bottles daily, containing 25 ml sulphuric acid (20 %v/v).

Rumen studies

Each straw supplemented with minerals and vitamin A was offered as complete feed to 3 rumen fistulated male Murrah buffaloes (live weight 451.2±12.5 kg) *ad lib.* for a 30 day adaptation period. Thereafter, samples of rumen contents were collected before feeding and then at 2 h interval up to 10 h post-feeding. The rumen contents were strained through 4-layered muslin cloth and pH was determined (digital pH meter) immediately after collection. The strained rumen liquor (SRL) was preserved with a few drops of saturated mercuric chloride and stored at 4°C till analyzed.

Analytical methods

Finely ground (1 mm) samples of straws, feeding residues and faeces were analyzed for moisture, CP and total ash (AOAC, 1990), cellulose (Crampton and Maynard, 1938) and other cell wall constituents (Robertson and Van Soest, 1981). The SRL was analyzed for total-N, ammonia-N (AOAC, 1990), trichloroacetic acid precipitable-N (TCA-N; Cline et al., 1958), and total volatile fatty acids (TVFA; Barnet and Reid, 1957). The NPN was calculated by subtracting TCA-N from Total-N. The ME was determined from apparent digestible OM using the relationship given by Broster and Oldham (1981). The urine samples were analyzed for total-N (AOAC, 1990), allantoin (Young and Conway, 1942), uric acid (Trivedi et al., 1978) and creatinine by the method of Folin and Wu described by Hawk et al. (1976). Purines absorbed were calculated from the urinary PD excreted using the equation of Chen et al. (1990). Efficiency of microbial protein synthesis (EMPS) was expressed as microbial nitrogen production (g) /kg digestible OM fermented in the rumen (DOMR); DOMR was taken as 0.65 of the apparent digestible OM intake and purine nitrogen index (PNI) presents the ratio between purine-N and total -N in urine.

Statistical analysis

The data were analyzed according to a completely randomized design (Snedecor and Cochran, 1994) using STATGRAPHICS Version 5.0. The differences between means were compared by Duncan's multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

The CP content of non-conventional straws ranged from 64 (*L. perenne*) to 86.9 (*M. sativa*) g/kg DM. *M. sativa* straw had higher ($p<0.05$) OM, CWC and CP content (Table

Table 1. Chemical composition of the straws (g/kg DM)

Constituent	*Trifolium resupinatum*	*Trifolium alexandrium*	*Medicago sativa*	*Lolium perenne*	Pooled SE
Total ash	87.5[b]	85.0[b]	45.0[a]	92.5[b]	2.1
OM	912.5[a]	915.0[a]	955.0[b]	907.5[a]	2.1
CP	81.4[b]	85.6[c]	86.9[c]	64.0[a]	0.6
NDF	650.0[a]	680.0[a]	810.0[b]	800.0[b]	18.0
ADF	470.0[a]	515.0[a]	620.0[b]	525.0[a]	14.6
Hemi-cellulose	120.0[a]	165.0[a]	160.0[a]	275.0[b]	15.4
Cellulose	355.0[a]	360.0[a]	435.0[c]	405.0[b]	4.3
ADIN	7.3[c]	6.0[b]	4.4[a]	5.6[b]	0.10
ADL	105.0[b]	120.0[c]	150.0[d]	65.0[a]	3.5

NDF: Neutral detergent fiber, ADF: Acid detergent fiber, ADIN: Acid detergent insoluble nitrogen, ADL: Acid detergent lignin.
Figures with different superscripts in a row differ significantly, p<0.05.

Table 2. Voluntary dry matter intake and digestion parameters in Murrah buffaloes fed four different straws

Parameters	*Trifolium resupinatum*	*Trifolium alexandrium*	*Medicago sativa*	*Lolium perenne*	Pooled SE
DMI (kg/d)	6.51	6.83	6.15	6.95	0.42
DMI (as % LW)	1.78	1.86	1.66	1.89	0.10
Digestible content (g/kg DM)					
DM	556.8[c]	485.8[b]	428.7[a]	505.9[bc]	19.5
OM	539.8[c]	458.2[ab]	420.4[a]	486.7[bc]	17.4
CP	26.0[a]	43.5[b]	56.7[c]	33.2[b]	2.1
NDF	339.7[b]	270.3[a]	302.8[a]	461.9[b]	15.2
ADF	292.0[b]	202.1[a]	251.1[a]	259.9[b]	11.6
Cellulose	237.9[b]	203.2[a]	230.1[a]	277.0[b]	6.8

DMI: Dry matter intake, NDF: Neutral detergent fiber, ADF: Acid detergent fiber.
Figures with different superscripts in a row differ significantly, p<0.05.

1). Nath et al. (1990) and Dahiya et al. (1998) reported much lower CP content (58 to 61 g/kg DM) in berseem straw. The CP content in these straws was much higher than that reported (28-47 g/kg DM) for traditional cereal (wheat and paddy) straws, pearl millet or sorghum stalks or maize stovers (Bakshi and Langar, 1994), but comparable with urea-treated naturally fermented wheat straw and other crop residues (Bakshi et al., 1986; Bakshi and Langar, 1994; Bakshi and Wadhwa, 2001). The NDF content in *M. sativa* (810 g/kg DM) and *L. perenne* (800 g/kg DM) was similar but higher (p<0.05) than the straws of *Trifolium* spp. (650 to 680 g/kg DM). The straw of *L. perenne*, being non-leguminous in nature, had the highest (p<0.05) hemicellulose, lowest (p<0.05) CP and ADL content as compared to other straws. The bound nitrogen or acid detergent insoluble-N (ADIN) was lowest (p<0.05) in *M. sativa* and highest (p<0.05) in *T. resupinatum*. The straw of *T. alexandrium* had higher ADL content than *T. resupinatum*, resulting in low digestibility of nutrients (DM, CWC and cellulose). The ADL content in leguminous fodder straws was much higher (5.1 to 7.6%) but that of *L. perenne* was comparable to that of traditional cereal straws, millet stalks and stovers (Bakshi and Langar, 1994).

DM intake, expressed on liveweight basis, was highest (1.89%) for *L. perenne* and lowest (1.66%) for *M. sativa*; however, the differences were statistically non-significant (Table 2). The DM consumption of *T. alexandrium* was higher (1.9 vs. 1.4% of body weight) than reported by Dahiya et al. (1998). The digestibilities of DM, OM and that of ADF were highest (p<0.05) for *T. resupinatum* followed by the digestibility values for *L. perenne* straw. NDF and cellulose of *L. perenne* straw was highly digestible. The CP of *M. sativa* was more (p<0.05) digestible than that of other straws. The digestibilities of most of the nutrients in *T. alexandrium* and *M. sativa* were similar, but lower (p<0.05) than those of *T. resupinatum* and *L. perenne* straws. The digestibilities of DM and CP of these straws were similar, though that of CWC was much lower than that of naturally fermented wheat straw (Bakshi and Langer, 1994; Bakshi and Wadhwa, 2001). Nath et al. (1990) reported DM digestibility within a comparable range, although CP digestibility was above the expected value for *T. alexandrium* straw. The higher digestibility of nutrients (OM) in animals fed *T. resupinatum* straw resulted in higher (p<0.05) TVFA production in the rumen (Table 3) as compared to that of *T. alexandrium*. The high VFA concentration in the rumen for *T. resupinatum, L. perenne* and *M. sativa* suggested a higher microbial activity. The total-N concentration in the SRL was higher in animals fed *L. perenne* straw, though differences between straws were statistically non-significant. The highest concentration of TCA-N was observed in animals fed *M. sativa* straw, which could be due to significantly higher CP content and digestibility. *L. perenne* straw showed similar results to those of *M. sativa*. Minimum ammonia-N concentration was observed in the SRL of animals fed *L. perenne* straw.

Table 3. pH, volatile fatty acids and N fractions in the rumen liquor of Murrah buffaloes fed four different straws

Parameters	*Trifolium resupinatum*	*Trifolium alexandrium*	*Medicago sativa*	*Lolium perenne*	Pooled SE
pH	6.88 [b]	6.95 [b]	6.69 [a]	6.65 [a]	0.05
TVFA (mmol/L)	96.87 [b]	87.30 [a]	91.00 [ab]	94.07 [ab]	2.78
Nitrogen fractions (mg/100 ml)					
Total	55.88	51.18	55.57	60.40	3.54
NH_3-N	12.08 [b]	12.43 [b]	12.37 [b]	5.90 [a]	0.49
NPN	23.48 [b]	22.93 [b]	17.70 [a]	23.0 [b]	1.27
TCA-N	32.40	28.25	37.87	37.40	3.91
Nitrogen fractions (as % of total-N)					
NPN	42.52[ab]	45.39[b]	32.17[a]	38.05[ab]	3.76
TCA	57.24[ab]	54.61[a]	67.83[b]	61.95[ab]	3.83

TVFA: Total volatile fatty acids, NH_3-N: Ammonia nitrogen, NPN: Non protein nitrogen, TCA-N: Trichloroacetic acid precipitable nitrogen.
Figures with different superscripts in a row differ significantly, $p<0.05$.

Table 4. Urinary excretion of purine derivatives (mmol/kg $W^{0.75}$/d) in Murrah buffaloes fed four different straws

Parameters	*Trifolium resupinatum*	*Trifolium alexandrium*	*Medicago sativa*	*Lolium perenne*	Pooled SE
Allantoin	0.24	0.21	0.21	0.17	0.02
Uric acid	0.08[b]	0.08[b]	0.06[ab]	0.05[a]	0.01
PD	0.32	0.29	0.27	0.22	0.03
CRT	0.77 [b]	0.74 [b]	0.62[a]	0.61[a]	0.04
PD:CRT	0.42	0.40	0.44	0.37	0.05
PD:CRT/$W^{0.75}$	34.54	33.47	36.61	31.28	3.66
Purines absorbed	88.14	65.47	45.15	20.82	22.67
MNS (g/d)	64.08	47.60	32.83	15.13	16.48
EMPS (g N/kg DOMR)	10.00[a]	15.62[b]	22.80[c]	7.40[a]	1.10
PNI	0.085[b]	0.057[a]	0.041[a]	0.084[b]	0.005

PD: Purine derivatives, CRT: Creatinine, MNS: Microbial nitrogen synthesized, EMPS: Efficiency of microbial protein synthesis.
N: Nitrogen, DOMR: Digestible organic matter fermented in rumen, PNI: Purine nitrogen index.
Figures with different superscripts in a row differ significantly, $p<0.05$.

The N-fractions, expressed as percent of total-N, showed that TCA-N constituted 54.2% in *T. alexandrium* and 67.8% in *M. sativa*.

Topps and Elliott (1965) and Kanjanapruthipong and Leng (1998) showed that urinary excretion of PD provide an indicative and noninvasive method for predicting synthesised microbial protein that leaves the rumen and is digested in the intestines. Urinary allantoin excretion was similar in all the groups (Table 4). Uric acid excretion was higher ($p<0.05$) in animals fed *T. resupinatum* or *T. resupinatum* straw than that in animals fed either *M. sativa* or *L. perenne* straw. However, urinary excretion of total PD was similar (0.22 to 0.32 mmol/kg $W^{0.75}$/d) in all the groups. Allantoin constituted 72-78% of PD excreted. Allantoin has been reported to be the main constituent of total PD excreted as also observed in cattle urine (Chen et al., 1995; Susmel et al., 1994; Chen and Gomes, 1995). Allantoin and uric acid are the PD present in cattle urine because of high xanthine oxidase activity which, converts xanthine and hypoxanthine into uric acid prior to excretion (Valadares et al., 1999; Pimpa and Liang, 2002). PNI indicates the efficiency with which degradable nitrogen is converted to microbial protein in the rumen (Chen and Jayasuria, 1997; Chen et al., 1999). The PNI values were lowest (0.041) in animals fed *M. sativa* straw and highest (0.084) in those fed *L. perenne* and *T. resupinatum* straw, clearly indicating poor conversion efficiency of *M. sativa* straw (Table 5); however, the supply of microbial protein to the host animal was higher in animals fed *M. sativa* straw and lowest in animals fed *L. perenne* straw.

The urinary excretion of creatinine ranged between 0.61-0.77 mmol/kg $W^{0.75}$/d. It was higher ($p<0.05$) in animals fed *T. resupinatum* or *T. alexandrium* straw than in animals fed either *M. sativa* or *L. perenne* straw. The ratio of PD to creatinine concentration in urine ranged from 0.37 (*L. perenne*) to 0.44 (*M. sativa*). The ratio of PD to creatinine corrected for metabolic weight was lowest for *L. perenne* (31.3) and highest for animals fed *M. sativa* straw (36.6). Moscardini et al. (1999) reported similar values for buffaloes. The allantoin/creatinine ratio, an index of total allantoin excretion in urine and an indicator of rumen microbial protein synthesis (Gonda, 1995), was not affected by dietary treatments and averaged 2.92 ($p>0.05$). The daily N intake was highest ($p<0.05$) for *T. alexandrium* straw followed by that for *M. sativa* straw (Table 5). The excretion of N in faeces was greater ($p<0.05$) in animals fed *Trifolium* straw than on the other straws. On the contrary, the animals fed *M. sativa* straw excreted more urinary-N. The minimum urinary-N excretion was observed in animals fed *L. perenne* straw. High excretion of urinary N (22.7 to

Table 5. Nitrogen retention (g/d) and body weight changes in Murrah buffaloes fed four different straws

Parameters	*Trifolium resupinatum*	*Trifolium alexandrium*	*Medicago sativa*	*Lolium perenne*	Pooled SE
N- Intake	74.24[a]	96.21[b]	89.12[ab]	72.99[a]	6.78
N-Outgo					
Total	69.09[ab]	80.16[b]	71.51[b]	52.27[a]	5.74
Fecal	46.40[bc]	48.75[c]	33.47[a]	35.86[ab]	4.07
Urinary	22.69[a]	31.41[b]	38.04[b]	16.41[a]	2.24
Excretion (as % of NI)	94.91[c]	83.30[ab]	80.19[a]	71.78[a]	3.89
N-Retained	5.15[a]	16.05[b]	17.61[b]	20.72[b]	3.11
Apparent BV (%)	12.33[a]	33.27[ab]	31.64[ab]	55.20[b]	8.41
Body weight changes (kg)					
Initial	364.0	369.0	369.0	372.0	19.5
Final	369.0	372.2	372.2	376.2	20.1
Gain/loss	5.0	3.2	3.2	4.2	3.5
Gain (g/d)	125.0	81.2	81.2	106.2	87.9
Nutritive value					
ME (MJ/kg DM)	8.20[c]	6.84[ab]	6.38[a]	7.38[b]	0.24
DCP (%)	3.02[a]	4.25[b]	5.49[c]	3.33[a]	0.16

N: Nitrogen, BV: Biological value. ME: Metabolizable energy, DCP: Degestible crude protein.
Figures with different superscripts in a row differ significantly, p<0.05.

38.0 g/d) on feeding these fodder straws, other than *L. perenne*, might be indicative of inefficient capture of ruminal NH_3-N (Table 3) for microbial protein synthesis. The Total-N excretion was higher in animals fed *T. alexandrium* straw, but when expressed as a percent of N intake it was greater in animals fed *T. resupinatum* straw. The higher TCA-N in *M. sativa* and *L. perenne* was responsible for minimum N-excretion resulting in higher N-retention and apparent biological value. The poor digestibility and inefficient utilization of N in *T. resupinatum* straw by the buffaloes could be due to high ADIN content in this straw. The N-retention from these straws was comparable to that obtained with naturally fermented cereal straws, millet stalks and maize stovers fed to buffaloes (Bakshi and Langar, 1994).

The nutritive value of different straws revealed that the straw of *M. sativa* had the highest (p<0.05) DCP content followed by that in *T. alexandrium* (Table 5). The *T. resupinatum* straw had the lowest DCP value, N-retention and apparent biological value, but it had higher (p<0.05) available ME as compared to other straws. The DCP content in traditional cereal (wheat and rice) straws is negligible and the ME availability varies between 5.8 and 6.6 MJ/kg DM (Mc Donald et al., 1977; Ranjhan and Pathak, 1979). Animals fed these non-traditional straws maintained body weights (Table 5) indicating the capability of these feedstuffs to meet the basal requirements of ruminants, unlike the traditional straws such as wheat and rice.

CONCLUSION

The results conclusively revealed that nutritionally, all four evaluated un-conventional straws of fodder crops could meet the basal maintenance requirements of adult buffaloes.

REFERENCES

AOAC. 1990. Official Methods of Analysis. Association of Official Analytical Chemists, Arlington, Virginia, USA.

Bakshi, M. P. S., V. K. Gupta and P. N. Langar. 1986. Fermented straw as a complete basal ration for ruminants. Agric. Wastes, 16:37-46.

Bakshi, M. P. S. and P. N. Langar. 1994. Utilization of naturally fermented urea-crop residues in buffalo production. Buffalo J. 10:19-27.

Bakshi, M. P. S. and M. Wadhwa. 2001. Nutritive evaluation of inoculated fermented wheat straw as complete feed for buffaloes. Indian J. Anim. Sci. 71:710-711.

Bakshi, M. P. S. and M. Wadhwa. 2004. Evaluation of forest tree leaves of semi-hilly arid region as livestock feed. Asian-Aust. J. Anim. Sci. 17:777-783.

Barnett, A. J. G. and R. L. Reid. 1957. Studies on the production of volatile fatty acids from the grass in artificial rumen. 1. Volatile fatty acid production from fresh grass. J. Agric. Sci. 48:315 Biosphere Programme. pp. 131-161.

Broster, W. H. and P. D. Oldham. 1981. Recent developments in ruminant nutrition. In: (Ed. W. Hariesign and D. J. A. Coles), Butterworths, London, pp. 194-199.

Chen, X. B., E. R. Ørskov, F. D. Hovell and B. De. 1990. Excretion of purine derivatives by ruminants: endogenous excretion, differences between cattle and sheep. Br. J. Nutr. 63:121-129.

Chen, X. B., A. T. Mejia, D. J. Kyle and E. R. Ørskov. 1992. Evaluation of the use of purine derivative:creatinine ratio in spot urine and plasma samples as an index of microbial protein supply in ruminants: Studies in sheep. J. Agric. Sci. 125:137-143.

Chen, X. B. and M. J. Gomes. 1995. Estimation of microbial protein supply to sheep and cattle based on urinary excretion of purine derivatives- An Overview of the Technical details.

Occasional Publication. Rowette Research Institute, Aberdeen. pp. 1-21.

Chen, X. B. and M. C. N. Jayasuria. 1997. “Purine Nitrogen Index, a potentially new parameter for rapid feed evaluation in ruminants”. *In vitro* techniques for measuring nutrient supply to ruminants, Proc. International Symposium of Br. Soc. Anim. Sci. University of Reading, UK.

Chen, X. B., D. B. Subba, E. R. Ørskov and M. C. N. Jayasuria. 1999. Purine Nitrogen Index, a potentially new parameter for rapid feed evaluation in ruminants. IAEA-TECDOC-1093.

Cline, J. H., T. V. Hershberger and O. G. Bentley. 1958. Utilization and/or synthesis of valeric acid during digestion of glucose, starch and cellulose by rumen micro-organisms *in vitro*. J. Anim. Sci. 17:284-292.

Crampton, E. W. and L. A. Maynard. 1938. The relation of cellulose and lignin content to the nutritive value of animal feeds. J. Nutr. 16:383-395.

Dahiya, S. S., B. S. Punia and T. R. Chauhan. 1998. Voluntary feed intake and nutrient utilization in buffalo bullocks fed untreated and urea (ammonia) treated berseem and oat straw. Indian J. Anim. Nutr. 15:93-96.

Duncan, D. B. 1955. Multiple range and multiple F tests. Biometrics 11:1-42.

Gonda, H. L. 1995. Nutritional status of ruminants determined from excretion and concentration of metabolites in body fluids. Ph.D. Dissertation. Swedish Univ. of Agricultural Sciences. Department of Animal Nutrition and Management, Uppsala.

Hawk, P. B., B. L. Oser and W. H. Summerson. 1976. Physiological Chemistry. (14th Ed). McGraw Hill Publishing Company Ltd., London.

Kanjanapruthipong, J. and R. A. Leng. 1998. Purine derivatives excreted in urine as an indicator estimating microbial yield from the rumen: A review. Aust. J. Agic. Res. 11:209-216.

McDonald, P., R. A. Edward and J. F. D. Greenhalgh. 1977. Nutritive value of foods for ruminants. In Animal Nutrition. English Language Book Society ang Longman. pp. 432-435.

Moscardini, S., M. L. Haddi, B. Stefanon and P. Susmel. 1999. Measurement of purine derivatives in the urine of some ruminant species. IAEA-TECDOC-1093.

Nath, D. K., C. S. Barsaul and M. L. Meheshwari. 1990. Chemical composition and nutritive value of berseem straw in Haryana calves. Indian J. Anim. Nutr. 7:305-306

Pimpa, O. and J. B. Liang. 2002. Estimation of microbial protein supply of lactating dairy cows under small-holder farms in north-east Thailand using urinary purine derivatives technique. Paper presented at the joint IAEA/FAO 4th RCM, 6-10 May 2002, University of Agriculture and Forestry, Hue, Vietnam.

Ranjhan, S. K. and N. N. Pathak. 1979. Average nutritive value of common feeding stuffs. In ‘Management and feeding of buffaloes’. Vikas Publishing House, New Delhi. pp. 255-256.

Robertson, J. A. and P. J. Van Soest. 1981. The detergent system of analysis and its application to human food. In: (Ed. W. P. T. James and O. Theander), the analysis of Dietary Fibre in Food. Marcel Dekker Inc., New York, pp. 123-158.

Snedecor, G. W. and W. G. Cochran. 1994. Statistical Methods. Oxford and IBH Publications, New Delhi.

Susmel, P., B. Stefanon, E. Plazzota, M. Spanguero and C. R. Mills. 1994. The effect of energy and protein intake on the excretion of purine derivatives. J. Agric. Sci. 123:257-266.

Topps, J. H. and R. C. Elliott. 1965. Relationship between concentrations of ruminal nucleic acids and excretion of purine derivatives by sheep. Nature 205:498-499.

Trivedi, R. C., L. Rebar, E. Berta and L. Stong. 1978. New enzymatic method for serum uric acid at 500 nm. Clin. Chem. 24:1908-1911.

Valadares, R. F. D., G. A. Broderick, S. C. Valadares Filho and M. K. Clayton. 1999. Effect of replacing alfalfa silage with high moisture corn on ruminal protein synthesis estimated from excretion of total purine derivatives. J. Dairy Sci. 82:2686-2696.

Young, E. G. and C. F. Conway. 1942. On the estimation of allantoin by Rimini-Schryver reaction. J. Biol. Chem. 142:839-852.