Energy Metabolism and Methane Production in Faunated and Defaunated Sheep Fed Two Diets with Same Concentrate to Roughage Ratio (70:30) but Varying in Composition

Chandramoni*, S. B. Jadhao[1], C. M. Tiwari[2], N. Haque, Murarilal and M. Y. Khan
Energy Metabolism and Respiration Calorimetry Laboratory, Animal Nutrition Division
Indian Veterinary Research Institute, Izatnagar 243122, U. P., India

ABSTRACT : Two calorimetric experiments were performed to investigate the effects of two diets with same concentrate: roughage ratio (70:30) but varying in composition on energy metabolism and methane production in faunated (F) and defaunated (DF) Muzaffarnagari sheep. For experiment I, ten animals were divided equally into two groups of which one was kept normally F as such while other was DF using 10% sodium lauryl sulphate. All the animals were offered diet I which comprised of oat hay and concentrate mixture I (CM I) containing maize grain (93%) as a major ingredient in 70:30 ratio. Similarly, the experiment II was conducted for which four F and four DF sheep (same as used for experiment I) were switched to diet II that consisted of maize hay and CM II (maize grain 59% + molasses 36%). Through diet II, DM intake in DF sheep was significantly ($p<0.05$) lower. Intake of GE through both the diets was similar in F and DF sheep. Digestibility of DM, OM, CP and GE and also metabolisability (ME/GE) was similar in F and DF sheep on both the diets. Total urinary energy loss did not differ in F and DF on both the diets, but methane energy loss as a percent of GE in DF was significantly ($p<0.05$) lower on diet I (3.75 vs 2.48), while it did not differ on diet II (3.20 vs 3.60). Heat production was significantly ($p<0.01$) reduced in DF on both the diets. Although, efficiency of utilisation of ME for maintenance calculated as per ARC (1984) did not differ in F and DF on both the diets, efficiency for maintenance and growth was higher (0.60 vs 0.672) on diet I in DF. It was inferred that methane production in DF sheep reduces on good quality hay-based diet supplemented with slowly fermentable carbohydrate (maize grain) but supplementation of molasses(rapidly fermentable CHO) nullify this effect when sheep were fed diets with concentrate: roughage ratio of 70:30. ***(Asian-Aust. J. Anim. Sci. 2001. Vol 14, No. 9 : 1238-1244)***

Key Words : Energy Metabolism, Sheep, Defaunation, Two Diets, Concentrate Roughage Ratio, Methane

INTRODUCTION

Ciliate protozoa play a significant role in the digestion of nutrients, methane production and utilisation of energy (Hungate, 1966). Defaunation i.e. removal of ciliate protozoa is an important tool not only to study role of cilliate protozoa in rumen metabolism but is also one of the way to reduce methane production (see review Moss, 1994; Islam and Begum, 1997; Lee et al., 2000). Manipulation of rumen fermentation through removal of ciliate protozoa appears to have considerable practical potential in improving ruminant productivity under certain feeding situation (Bird and Leng, 1984; Nahn et al., 2001).

Diet is the single most important factor, which influences the number and relative proportion of different species of microbes in the rumen. The magnitude of effect of protozoal population on overall rumen fermentation and energy utilisation depends on constitution of the diet i.e. slow or rapidly fermentable as well as the animal, which affects utilisation of energy. Effective utilisation of metabolisable energy depends on the nature of energy sources (starch vs cellulose) (Kreuzer and Kirchgessner, 1989). Many studies have been done which investigated the effects of protozoa on ruminal metabolism using different types of diets (Demeyer et al., 1982; Demeyer, 1989; Nangia and Shrivastava, 1989; Vermorel and Jouany, 1989; Santra et al., 1994). However, studies on the effects of supplementing good quality forages with concentrates on methane production are few and inconsistent (Moss, 1994) as is also the case with defaunated animals (Itabashi et al., 1984; Kurar and Mohini, 1989; Vermorel and Jouany, 1989). Moreover, diets with high concentrate to roughage (good quality roughage like oat or maize hay as used in this study) ratio have been rarely used to study methane production in both faunated (Chandramoni et al., 2000a; Islam et al., 2000) and defaunated animals.

Keeping in view the above facts, the experiment reported herein was conducted to compare the effects of feeding two diets with same concentrate to roughage ratio (70:30) but varying in composition (mainly quantity of fermentable CHO) on energy metabolism and methane production in faunated and defaunated sheep.

* Corresponding Author: Chandramoni. Qr. 11, Road 9, Bihar Veterinary College, Patna 800 014, India. E-mail: chandramoni@hotmail.com.
[1] Central Institute of Fisheries Education (Indian Council of Agricultural Research), 7 Bunglows, Versova, Mumbai 400061, India.
[2] Department of Animal Nutrition, Rajiv Gandhi college of Veterinary and Animal Sciences, Pondicherry 605 009, India.
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MATERIALS AND METHODS

Experimental animals, feeding and housing

The study was conducted in healthy rams (uncastrated) of Muzaffarnagari sheep which is sturdy and meat type breed and an important animal resource in the area. The animals were dewormed, and housed in a well-ventilated room with individual feeding and watering arrangements. Concentrate mixture was offered to individual animals between 9 to 10 A.M. and hay was offered in afternoon in the same manger. Fresh water was provided *ad libitum*. Sheep were fed as per NRC (1985) at maintenance level.

In experiment I, ten sheep were divided into two groups of five each. Animals in group II were defaunated by drenching sodium lauryl sulphate (9 g/100 kg body weight) orally by stomach tube for three consecutive days. Before the fist dose, animals were fasted for 24 h. Only drinking water was provided during this period and after 8 h post treatment 33.3% of normal requirement of energy was supplied through concentrate. The treatment with sodium lauryl sulphate was repeated twice with 24 hrs interval. The DF sheep were kept in a separate room to avoid contact with faunated animals and presence of protozoa in the rumen was checked periodically, every week. All the animals were offered Diet I which comprised of oat hay and concentrate mixture I (CM I) which contained maize 93%, deoiled groundnut cake (GNC) 3.5% and wheat bran 3.5%.

In experiment II, the same animals but instead of five F and five DF, four F and four DF sheep were shifted to diet II. The diet II comprised of maize hay plus CM II in which 30% energy was replaced by molasses. The composition of CM II was maize 59%, GNC 2.5%, wheatbran 2.5% and molasses 36%. To every 100 kg CM, 2 kg mineral mixture (contained moisture maximum 5%, Calcium minimum 28%, Phosphorus minimum 12%, Iodine as KI 0.026-0.13%, Copper 0.077-0.13 and Fluorine maximum 0.04%) and 1 kg common salt was added.

Metabolism trial

For metabolic and energy metabolism study, after feeding for a month, sheep were transferred to metabolism cage and adapted for three days followed by metabolism trials of seven days wherein quantitative collection of urine and feces voided by individual animal was done. All the animals were weighed before and after trial. Representative samples of feed offered and feed refusals were taken daily for dry matter (DM) estimation and chemical analysis.

Total amount of feces voided by individual animal was collected quantitatively at 9 A.M. daily. It was weighed and representative sample of each animal was drawn in sample bottles after crushing and mixing. Representative samples were brought to the laboratory in properly marked and well-stoppered sample bottles. Aliquoting of feces from each animal equal to 1/20th of the total faces voided were taken and dried in weighed petridish in hot air oven maintained at 100±5°C for DM estimation. Similar aliquots were dried in oven maintained at 40°C for energy estimation.

The dried aliquots of all the seven days were pooled and kept for analysis of proximate principles, except N and gross energy. For the analysis of crude protein (N×6.25) another aliquot equal to 1/40th of the total faces voided by each animal was preserved with diluted H_2SO_4 (1:4) in wide mouth air-tight stoppered weighed bottles. The aliquots of seven days were composited for each animal in separate bottles. The composite weighed sample was mixed thoroughly and suitable aliquots were taken for nitrogen estimation. Urine excreted daily by individual animal was collected separately in bucket for 24 hrs, acidified and was measured. Duplicate aliquots equal to 1/100th of the total urine excreted daily were taken in 500 ml Kjeldahl's flask containing 30 ml of concentrated H_2SO_4 for nitrogen determination. Another aliquot equal to 1/100th of total urine excreted daily was pooled in brown coloured bottles for seven days. The bottle was air tightened and preserved in a refrigerator for estimation of energy. Proximate principles of the experimental feeds, refusals and feces were analysed as per AOAC (1980) and the GE content was determined using Gallenkamp adiabatic bomb calorimeter (CBA 301 Series).

Energy balance

Complete energy balance trials were conducted on individual sheep for two consecutive days one after another, in an open circuit respiration chamber. The sheep were adapted to the cages for three days which were later shifted to the respiration chamber one by one. The chamber was maintained at 20-25°C with relative humidity of about 65%. Details and calibration procedure of the respiration chambers are described elsewhere (Khan and Joshi, 1983; Chandramoni et al., 2000b). The animals were *per se* trained for calorimetry studies. Heat production was calculated following the equation of Brouwer (1965). The efficiency of utilization of ME (k_m) was calculated using equation of ARC (1984) which relates to k_m to q_m. It was assumed that qm =q. Efficiency of ME utilisation for maintenance and gain was calculated using fasting heat production (Chandramoni et al., 2000b), ME intake and energy balance i.e. k_{mg} = (energy balance + FHP) / ME intake.

Statistical analysis

The significance between faunated and defaunated sheep fed a particular diet was tested with analysis of variance using Student's t tests (Snedecor and Cochran, 1989).

RESULTS AND DISCUSSION

Methane production as a result of enteric fermentation in ruminants has assumed considerable significance in recent years due to its contribution to global warming. Sustainable ruminant production systems henceforth require that the methane produced per unit output of animal should be less. In this context, accurate evaluation of locally available animal and feed resources using respiration calorimetry technique for their optimum and economic utilisation in sustainable animal production systems is the prime objective of this laboratory. Thus, present report follows many recently published unique reports (eg Haque et al., 1996, 1997 and 1998; Sahoo et al., 2000; Tiwari et al., 2000a,b) from this laboratory on this very important aspects of animal production in India.

Chemical composition

The composition of oat and maize hay, molasses and concentrate mixtures is given in table 1. Oat hay contained 5% more crude fiber than maize hay. Concentrate mixture (CM) II contained 2% less protein and 0.143 kcal/g less gross energy than CM I.

Defaunation of sheep

Defaunation was achieved by drenching 10% sodium lauryl sulphate after overnight fasting of sheep. Like previous studies of defaunation (Bird and Leng, 1984; Santra et al., 1994), in the present study too, it was observed that starvation of animals (overnight fasting) do help in speeding up the process of defaunation, perhaps because of the lower particulate material in the rumen and therefore, better contact of detergent with the protozoa which resulted in faster abolition of ciliates from rumen. Starvation of animals for 3 days did not created any difficulty as is also reported by Jouany et al. (1981). It is indicative of better physiological condition of sheep prior to fasting and sturdiness of the breed, which is governed by genetic makeup to bear stress following starvation and defaunation. The defaunated sheep were housed separately and were maintained so throughout experiment. There was no contamination. Sheep have been maintained devoid of protozoa for 2.5 years by preventing their contact with other ruminants (Church, 1988).

Digestibility of nutrients

Dry matter intake (DMI) and digestibility of nutrients on diet I (oat hay plus concentrate devoid of molasses) was similar in faunated (F) and defaunated (DF) sheep (table 2). Similar results were found by Rowe et al. (1985) and Santra (1992). Negative effects of defaunation on OM digestbility was observed by Abou Akkada and El Shazly (1965). Jouany et al. (1981), Demeyer et al. (1982) and Kreuzer et al. (1986) reported higher OM digestibility in DF animals.

However, DMI through diet II (maize hay + concentrate with molasses) in DF sheep was significantly ($p<0.05$) lower than F. But digestibility of DM, OM and N were similar in F and DF sheep fed diet II. Nonsignificantly lower DMI and OMI by DF animals was also reported by Santra (1992).

Energy utilisation

Energy metabolism data of F and DF sheep fed two diets are presented in table 3. Total GE intake was similar in F and DF sheep on both the diets. Variation in the type of the supplemental carbohydrate could not cause any significant effect on the N digestibility, energy digestibility and metabolisability in F and DF sheep. Despite similar ME intakes, methane energy (total and as % of GE, DE or per unit digestible nutrient) loss was significantly ($p<0.05$) reduced in DF sheep on diet I while no effect was observed with diet II meaning that the change of diet in DF sheep nullified the effect (reduced methane production) of defaunation (table 3 and 4). The results obtained with diet I are on expected lines and agrees with earlier reports (Itabashi et al., 1984; Kreuzer et al., 1986; Ushida, 1986; Santra et al., 1994). These reports which support findings on diet I (table I) states that defaunation increases fecal energy loss but reduces methane energy loss because of shift of OM digestion from rumen to hindgut. Vermorel and Jouany (1989) found decreased methane production in DF sheep fed with 71% ammoniated straw plus 18% maize grain but it was unaffected when sheep were fed diet with 89% ammoniated straw.

Protozoal activity results in H_2 gas production and it is

Table 1. Chemical composition of ingredients and diets (%DM basis)

Particulars	Organic matter	Crude protein	Ether extract	Crude fiber	N free extract	Total CHO	Total Ash	GE (kcal/g)
Oat hay	91.89	9.85	2.60	34.14	45.29	79.43	8.11	4.09
Maize hay	92.41	8.65	1.85	28.84	53.07	81.91	7.59	4.16
Molasses	89.60	5.60	0.14	0.0	83.86	83.86	10.40	3.65
Concentrate mixture-I	93.98	11.43	2.05	2.67	77.81	80.48	6.02	4.17
Concentrate mixture - II	92.42	9.41	1.36	1.70	80.05	81.75	7.58	4.03

Table 2. Intake and digestibility of feeds in faunated (F) and defaunated (DF) sheep fed two different diets

Particulars	Diet : Oat hay + (conc. mix - molasses)				maize hay + (conc. mix + molasses)			
	F	DF	SEM	Significance	F	DF	SEM	Significance
Live Weight (W, kg)	48.1	51.2	1.6	NS	49.2	54.6	1.1	NS
Metabolic (kg $W^{0.75}$)	18.2	19.1	0.7	NS	18.5	20.1	0.3	NS
Concentrate mix. (g/d)	841	892	28.6	NS	937	890	26.4	NS
Hay (g/d)	264	294	26.6	NS	459.7	305	43.6	*
Total DM intake (g/d)	1,105	1,186	30.0	NS	1,397	1,195	60.6	*
DM intake (g/kg$w^{0.75}$/d)	60.7	62.1	1.31	NS	75.1	59.5	2.8	*
DM digestibility (%)	68.3	62.2	1.20	NS	58.6	61.0	1.4	NS
OM intake (g/kg$w^{0.75}$/d)	56.5	58.1	1.12	NS	69.4	55.0	2.57	*
OM digestibility (%)	72.2	67.0	1.2	NS	61.8	63.3	1.1	NS
CP intake (g/kg$w^{0.75}$/d)	6.67	6.85	0.12	NS	6.87	5.47	0.25	*
CP digestibility (%)	64.5	66.1	2.13	NS	66.5	69.0	8.9	NS

* $p<0.05$, NS - Nonsignificant, SEM-Standard error of mean

Table 3. Energy metabolism(kcal/d) of faunated (F) and defaunated (DF) sheep fed two diets with 70:30 ratio of concentrate to roughage

Diet	Oat hay + (conc. mix –molasses)				maize hay + (conc. mix + molasses)			
Particulars	F	DF	SEM	Significance	F	DF	SEM	Significance
Gross energy intake	4,651	4,995	136.2	NS	5,690	4,855	249.6	NS
Fecal energy	1,385	1,809	81.9	*	2,384	1,956	148.3	NS
% Digestibility	70.1	63.7	1.6	NS	58.2	59.6	1.3	NS
Urinary energy	137	148	3.7	NS	202	170	10.6	NS
Methane energy	176	124	10.4	*	181	177	17.4	NS
Metabolisable energy	674.1	639.3	23.0	NS	657.3	528.5	20.9	*
Metabolisability (%)	63.2	58.0	1.7	NS	51.5	52.5	0.8	NS
RQ (fed)	0.97	1.04	0.003	*	0.96	0.97	0.002	NS
Heat production	2,129	1,959	33.8	*	2,141	1,868	45.3	*
Energy balance	824	954	107.7	NS	782	666	108.5	NS
k_m	0.720	0.706	0.006	NS	0.683	0.687	0.005	NS
k_{mg}	0.600	0.672	0.012	*	0.609	0.672	0.020	NS

* $p<0.05$, NS-Non-significant, SEM-Standard error of Mean, k_m calculated as per ARC (1984).

Table 4. Methane production in sheep fed two diets with same concentrate to roughage ratio (70:30) but varying in composition

Methane production	Oat hay + (conc. mix - molasses)				maize hay + (conc. mix + molasses)			
	F	DF	SEM	Significance	F	DF	SEM	Significance
g/d	13.3	9.3	0.85	*	13.7	13.3	1.31	NS
as a % of GE	3.75	2.48	0.20	*	1.72	1.93	0.19	NS
as % of DE	5.4	3.9	0.31	*	5.5	6.2	0.18	NS
g/100 g DOM	1.78	1.27	0.10	*	1.72	1.93	0.19	NS
g/100 g digestible CHO	2.07	1.40	0.38	*	1.72	1.93	0.19	NS

* $p<0.05$, NS-Non-significant, SEM-Standard error of Mean.

used for methane production by methanogenic bacteria (Hungate, 1967) which has got ectosymbiotic relationship with rumen ciliate protozoa (Stumm et al., 1982; Krumholtz et al., 1983). In DF sheep, methanogenic bacteria loose their symbiotic partners resulting in reduced methane production. However, nonsignificant effects on methane production in DF animals on diet II can be explained on following grounds. Protozoa attached to ruminal wall may be behaving in different manner from the freely suspended protozoa in rumen fluid. Methanogens are attached to rumen protozoa and frequency of attachment between these and protozoa appears to increase during fasting and decrease with feeding of animal (Stumm et al., 1982). There may be rapid multiplication of methanogenic bacteria not attached to protozoa and are free in rumen liquor and so eventhough protozoa are removed there may be an alternative mechanism for their survival (in rumen niche) and growth. Thus, change in the nature of fermentation (partition of organic matter between microbial synthesis: fermentation) is contributing factor for methane production (Beever, 1993). It has been earlier reported that apparent rumen digestibility is higher in dextrose as compared to starch even when pH and protozoa are unaffected (Piwonka et al., 1994). This may be a factor, which increase methane production. It has been found that feeding diet with higher fermentable carbohydrate (like molasses) can result in different manner a change in the microbial population and fermentation in rumen. Under these conditions, where there is low liquid turnover and rapid fermentation, there is production of VFA in large quantities, which undergo secondary fermentation. Bacteria not normally seen in such high number will increase in rumen. Methanosarcina sometimes proliferate and cleaves acetate to carbon dioxide and methane. β-oxidation of fatty acids to acetate and methane may also occur (Rowe et al., 1971; Demeyer et al., 1982). Other reason for increased methane production in DF sheep on diet II (+ molasses) may be due to possible and variable contribution of hind gut fermentation to total methane production (Mbanzamihigo et al., 1996) as there may be shift in the digestion from rumen to hind gut because of defaunation especially in sheep (Ushida et al., 1990). Methane production as such is related to FOM and OM escaped from rumen fermentation might have been fermented in the hindgut. With aging methanogenesis prevails in the hindgut (Piattoni et al., 1996). It is to mention that sheep used were matured (body weight range 48 to 55 kg).

The type of the roughage and also concentrate (starchy/oil cake) can substantially influence on methane production (Moss and Givens, 1993) and in many instances rumen VFA stoichimetry was reported to be not correlating well with methane stoichiometry (Moss et al.,1993). They also found that the methane production (l/kg FOM) increase significantly and linearly with decreasing forage: concentrate ratio for rolled barley diets, but was nonlinear for soyabean diets. Rumen stoichiometry was reported to be unable to explain non linearity of methane production for soyabean diet. The mean total VFA on two diets in F and DF were nonsignificantly different (Chandramoni et al., unpublished data).

Total heat production in DF animals was significantly lower than F. Although energy balances and efficiency of utilisation of ME for maintenance (k_m) calculated using formula of ARC (1984) were similar between F and DF sheep, efficiency for maintenance and gain (k_{mg}) was higher ($p<0.05$) in DF on diet I. This was supported by higher ($p<0.05$) respiratory quotient in DF animals on diet I.

The heat production (HP) is probably related to an improved duodenal bacterial protein supply. Rumen fermentation revealed higher TCA-soluble and amino-N indicative of increased bacterial synthesis (Chandramoni et al., unpublished data). An improved partitioning of digested OM towards more protein and less VFA is indeed a characteristic of defaunation (Jouany et al., 1988; Jouany and Ushida, 1999) leading to improvement in k_{mg}. These results corroborate with the earlier findings (Kreuzer et al., 1986; Santra et al., 1994) in which HP in DF animals was found to be less. Contrary to these, Whitelaw et al. (1984) found higher HP in ciliate free steers. The reduction in HP might be attributed to low microbial fermentation in the rumen or reduced tissue metabolism. Rowe et al. (1985) observed higher carbon dioxide production in rumen of DF sheep with reduced performance.

CONCLUSIONS AND PERSPECTIVES

Thus the study concludes that defaunation reduces methane production and significantly increases the efficiency of utilisation of metabolisable energy on diet with 70:30 concentrate (containing maize grain) to roughage ratio but change over to the similar diet which contained molasses (a rapidly fermentable CHO) nullify the effect in defaunated animals as evinced by increased methane production with no effect on efficiency of utilisation of energy. Further research is necessary to precisely quantity the response of rumen microbial ecology of defaunated animals of different species in terms of methane production on different types of diets (particularly straw-based) supplemented with varying quantities of rapidly fermentable CHO such as molasses which is easily available with many small farmers in the region.

REFERENCES

Abou Akkada A. R. and K. El Shazly. 1965. Effect of absence of cilliate protozoa on some blood components, nitrogen retention and digestibility of food constituents in lambs. J. Agric. Sci., Cambridge 64:251-261.

Agriculture Research Council. 1984. The nutrient requirements of Livestock. Slough: Commonwealth Agricultural Bureaux.

AOAC. 1980. Official methods of analysis (12th Ed.). Association of Official Analytical Chemist, Washington, D. C.

Beever, D. E. 1993. Rumen function. In: Quantitative Aspects of Ruminants Digestion and Metabolism (Ed. J. M. Forbes and J. France). Wallingford: CAB International, pp. 187-215.

Bird, S. H. and R. A. Leng. 1984. Absence of protozoa in the rumen and production of grazing sheep. Proc. Austr. Soc. Anim. Prod. 15:654-658.

Brouwer. E. 1965. Report of subcommittee on constants and factors. In: Proc. 3rd Symp. Energy Metabolism (Ed. K. L. Blaxter).

Troon, EAAP. Publ. 11, London, Academic press, pp. 441-443.

Chandramoni, S. B. Jadhao, C. M. Tiwari and M. Y. Khan. 2000a. Energy metabolism with particular reference to methane production in Muzaffarnagari sheep fed rations varying in roughage to concentrate ratio. Anim. Feed Sci. Technol. 83(3-4):287-300.

Chandramoni, C. M. Tiwari, S. B. Jadhao and M. Y. Khan. 2000b. Fasting heat production of Muzaffarnagari sheep. Small Rumin Res. 36:43-47.

Church, D. C., 1988. The Ruminant Animal Digestive Physiology and Nutrition, New Jersey, Prentice Hall, P. 134

Demeyer, D. I. 1989. Effect of defaunation of rumen fiber digestion and digesta kinetics. In: The Role of Protozoa and Fungi in Ruminant Digestion (Ed. J. V. Nolan, R. A. Leng and D. I. Demeyer). Armidale, Penambul Books, pp 171-180.

Demeyer, D. I., C. J. Van Nevel and G. van de voorde.1982. The effect of defaunation on the growth of lambs fed three urea-containing diets. Arch. Tieren. 32:595-602.

Haque, N., V. P. Varshney, M. Y. Khan and M. Lal. 1996. Effect of feeding *Leucaena leucocephala* supplemented rations on thyroid hormones and fasting heat production in Jamunapari goats. Small Rumin. Res. 19:29-33.

Haque, N., M. Y. Khan and Murarilal. 1997. Effect of level of *Leucaena leucocephala* in the diets of Jamunapari goats on carbon, nitrogen and energy balances. Asian-Aus. J. Anim. Sci. 10: 455-459.

Haque, N., Murarilal, M. Y. Khan, J. C. Biswas and P. Singh. 1998. Metabolisable energy requirements for maintenance of pashmina producing Chegu goats. Small Rumin. Res. 27:41-45.

Hungate, R. E. 1966. The rumen and its microbes. Academic Press, Newyork, NY.P p. 553.

Hungate, R. E. 1967. Hydrogen as an intermediate in the rumen fermentation. Archives Microbiol. 59:158-167.

Islam, M. R. and J. Begum. 1997. Short review of global methane situtaions and of facillities to reduce in ruminants in third world countries. Asian-Aus. J. Anim. Sci. 10:157-163.

Islam, M., H. Abe, F. Terada, K. Iwasaki and R. Tano. 2000. Effect of level of feed intake and inclusion of corn on rumen environment, nutrient digestibility, methane emission and protein utilisation by goats fed alfalfa pellets. Asian-Aus. J. Anim. Sci. 13(9):948-956.

Itabashi, H., T. Kobayashi, M. Matsumoto. 1984. The effects of rumen ciliate protozoa on energy metabolism and some constituents in rumen fluid and blood plasma of goats. Jpn. J. Zootech. Sci. 55:248- 25.

Jouany, J. P. and K. Ushida. 1999. The role of protozoa in feed digestion. A review. Asian-Aus. J. Anim. Sci. 12(1):113-128.

Jouany, J. P., D. I. Demeyer and J. Grain. 1988. Effects of defaunating the rumen. Anim. Feed. Sci. Technol. 21:229-265.

Jouany, J. P., B. Zainab, J. Senaud, C. A. Groteirs, J. Grain and P. Thivend. 1981. Role of the rumen ciliate protozoa *Polyplastron multivesiculatum*, *Entodinium* sp. and *Isotricha prostoma* in the digestion of a mixed diet in sheep. Reprod. Nutr. Develop. 21:871-884.

Khan, M. Y. and D. C. Joshi. 1983. A new simplified open-circuit respiration equipment for sheep-a note. Indian J. Anim. Prod. 15:34-36.

Kreuzer, M. and M. Kirchessner. 1989. Effect of rumen protozoa on metabolism and retention in ruminants, with special reference to diet characteristics. In: The Role of Protozoa and Fungi in Ruminant Digestion (Ed. R. A. Leng and J. V. Nolan). OCED/UNE International Seminar. September 1988. Armidale, Australia.

Kreuzer, M., M. Kirchgessner and H. L. Muller. 1986. Effect of defaunation on the loss of energy in wethers fed different quantities of cellulose and normal or steam flaked maize starch. Anim. Feed Sci. Technol. 16:233-241.

Krumholtz, L. R., O. W. Forsberg and D. M. Veira. 1983. Association of methanogenic bacteria with rumen protozoa. Canadian J. Microbiol. 29:676-680.

Kurar, C. K. and M. Mohini. 1989. Effect of defaunation on the availability of energy and nitrogen in Murrah buffalo calves. Indian J. Anim. Sci. 59:896-898.

Lee, S. C., H. J. Lee and Y. K. Oh. 2000. Methane production from enteric fermentations in ruminants. Asian-Aus. J. Anim. Sci. 13 (Special Issue):171-181.

Mbanzamihigo, L., C. J Van Nevel and D. I. Demeyer, 1996. Lasting effects of monensin on rumen and caecal fermentation in sheep fed a high grain diet. Anim. Feed Sci.Technol. 62:215-218.

Moss, A. R. 1994. Methane production by ruminants -literature review of I. Dietary Manipulation to reduce methane production and II. Laboratory procedures for estimating methane potentials of diets. Nutr. Abstr. Rev. (Series B) 64(12):796-797.

Moss, A. R. and D. I. Givens. 1993. Effect of supplement type and grass silage: concentrate ratio on methane production by sheep. Proc. Bri. Soc. Anim. Prod. Paper no. 52.

Moss, A. R., E. R. Deaville and D. I. Givens. 1993. Effect of supplementing grass silage with sugarbeet feed on methane production by sheep. Proc. Bri. Soc. Anim. Prod. Paper no. 53

Nahn, Nguyen Thi Hong, Nguyen van Hon, Nguyen Trong Ngu, Nguyen Tien Von, T. R. Preston and R. A. Leng. 2001. Practical application of defaunation of cattle on farms in Vietnam: Response of young cattle fed rice straw and grass to a single drench of groundnut oil. Asian-Aust. J. Anim. Sci. 14(4):485-490.

Nangia, O. P. and P. N. Shrivastava. 1989. Effect of presence or absence of protozoa on rumen digestive functions in buffaloes on straw-based diets. Asian-Aus. J. Anim. Sci. 2(3):493-496.

National Research Council.1985. Nutrients Requirements of sheep, National Academy of Sciences, Washington. D. C.

Piattoni, F., D. Demeyer and I. Mertens. 1996. *In vivo* study of the age-dependent fermentation pattern and methanogens in young rabbits. Reprod. Nutr. Develop. 35:375-386

Piwonka, E. J., J. L. Firkins and B. L. Hull. 1994. Digestion in the rumen and total tract of forage based diets with starch or dextrose supplement fed to Holstein heifers. J. Dairy Sci. 77:1570-1579.

Rowe, J. B., A. Davies and A. W. J. Brooms. 1985. Quantitative effects of defaunation of rumen fermentation and digestion in sheep. Br. J. Nutr. 54:105-119.

Rowe, J. B., M. L. Loughan, J. V. Nolan and R. A. Leng. 1971. The Ruminant Animal Digestive Physiology and Nutrition (Ed. D.C. Church), New Jersey, Prentice Hall.

Sahoo, B., M. L. Sarswat, N. Haque and M. Y. Khan. 2000. Energy balance and methane production in sheep fed chemically treated straw. Small rumin. Res. 35:13-19.

Santra, A.. 1992. Effect of defaunation on nutrient digestibility and enzyme-profile in the rumen of Buffalo. M.V. Sc. Thesis, Indian Veterinary Research Institute (Deemed University), Izatnagar, India.

Santra, A., D. N. Kamra, N. N. Pathak and M. Y. Khan. 1994. Effect of protozoa on the loss of energy in Murrah buffalo (*Bubalus bubalis*) calves. Buffalo J. 3:249-253.

Snedecor, G. W. and W. G. Cochran. 1989. Statistical methods, Iowa State Univ. Press. USA.

Stumm, C. K., H. J. Gijzen and G. D. Vogels. 1982. Association of methanogenic bacteria with ovine rumen ciliates. Brit. J. Nutr. 47:95-99.

Tiwari, C. M., Chandramoni, S. B. Jadhao, M. Y. Khan. 2000a. Fasting heat production of growing buffalo calves. Asian-Aus. J. Anim. Sci. 13:307-312.

Tiwari, C. M., S. B. Jadhao, Chandramoni, Murarilal and M. Y. Khan. 2000b. Comparative calorimetric evaluation of ammoniated straw-based ration supplemented with low levels of untreated, formaldehyde treated groundnut cake and fishmeal with respect to growing buffalo calves. Asian-Aus. J. Anim. Sci. 13:761-763.

Ushida, K. 1986. Studies on protozoal contribution to the ruminant digestion. Ph.D. Thesis, Tokyo University, p. 115.

Ushida, K. C., Kayouli, S. DeSmet and J. P. Jouany. 1990. Effect of protein and fiber digestion in sheep fed on ammonia-treated straw-based diets with or without maize. Br. J. Nutr. 64:765-755.

Whitelaw, F. G., J. M. Eadie, L. A. Bruce and W. Shand. 1984. Methane formation in faunated and ciliate free cattle and its relationship with rumen VFA proportions. Br. J. Nutr. 52:261-275.

Vermorel, M. and J. P. Jouany. 1989. Effects of rumen protozoa on energy utilisation by wethers of two diets based on ammonia-treated straw supplemented or not with maize. Asian-Aus. J. Anim. Sci. 2:475-476.