Effects of Formalin Treated Soy Bean as a Source of Rumen Undegradable Protein on Rumen Functions of Non-lactating Dairy Cows on Concentrate Based-diets**

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ABSTRACT : An objective of this study was to determine the effects of increasing contents of rumen undegradable protein (RUP) from formalin treated soy bean (FSBM) on rumen functions. Four rumen canulated non-lactating cows were randomly allocated to total mixed rations (TMR) containing different proportions of soy bean meal (SBM) and FSBM. Of rumen fermentation characteristics, concentrations of ruminal fluid ammonia and molar proportions of isoacids decreased with increasing contents of RUP in diets ($p<0.01$). The animals on TMR containing only SBM gained less weight and had smaller rumen volume than those on TMR containing RUP from FSBM ($p<0.05$). Organic matter and neutral detergent fiber digestibility *in sacco* were not different ($p>0.05$). The density of protozoa particularly small Entodinium sp. in ruminal fluid was higher in animal fed TMR containing SBM:FSBM (34:66) and FSBM than those fed TMR containing SBM:FSBM (66:34) and SBM ($p<0.01$). Total viable count, and net microbial protein synthesis as indicated by purine derivatives in urine increased with increasing contents of RUP from FSBM ($p<0.01$). It can be concluded that a reduction in net microbial protein synthesis in the rumen with increasing contents of RUP in the diet can be due to the reduction of preformed protein available for microbial growth as well as an increased turnover rate of microbial cells by predatory activity of protozoa. ***(Asian-Aust. J. Anim. Sci. 2002. Vol 15, No. 10 : 1439-1444)***

Key Words : Rumen Undegradable Protein, Rumen Functions

INTRODUCTION

Insufficient supply of protein to dairy cows intestines for digestion and absorption is one of the major problems for dairy production under tropical conditions (Leng, 1982b). To meet the protein demands of lactating cows, the flow of microbial protein should be maximized prior to supplementing the bypass protein. Efficiency of substrate utilization by rumen microbes involves a strong interaction between the carbohydrate and protein fractions in the diet (Oldham, 1984). Rate of starch digestion in the rumen is the major factor controlling the energy available for microbial growth (Oldham, 1984), while an adequate supply of nitrogenous sources (ammonia, peptides and amino acids) in the rumen will increase microbial growth efficiency (Leng, 1982b). Therefore the substitution of rumen undegradable protein (RUP) for rumen degradable protein (RDP) can reduce the microbial protein entering the host intestines, which is presumably due to the lack of supply of peptides and amino acids to the rumen microbes (Clark et al., 1992).

Ammonia is the primary nitrogen source for rumen microbes (Bryant and Robinson, 1962). Microbial protein can be mostly synthesized from the nitrogen of RDP, passing through the ammonia pool in the rumen (Nolan, 1993). A number of *in vivo* studies (Redman et al., 1980; Cruz Soto et al., 1994; Fujimaki et al., 1994) indicate that peptides and amino acids are not limited for microbial growth in the rumen. However, the outcomes of the balance between RDP and RUP are dependent on interactions among rumen microbes. Changes in availability of nitrogenous substrates which may influence any change in interactions among rumen microbes were examined in the rumen of dairy cows fed TMR containing various levels of RUP from formalin treated soybean (FSBM).

MATERIALS AND METHODS

Four Holstein×indigenous (93.75×6.25) non-lactating dairy cows, weighing 460-480 kg with permanent rumen canulas were held in individual pens and randomly allocated to dietary treatments according a 4×4 Latin square design with 21 d periods.

Dietary treatments consisted of 0, 6.1, 12.1 and 18.2 g as FSBM (37.5 ml of 37% formaldehyde/100 g of soy protein). Paragrass hay was made from perennial paragrass. The paragrass was cut, chopped into 2-3 cm length and sun dried for total mixed rations (TMR). Diets were isonitrogenous and isocaloric as shown in Table 1.

Experimental diets were offered ad-libitum at 08:00 and 19:00 h and were sampled weekly and bulked for later analysis of chemical compositions. Within the 21 d

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Table 1. Feed ingredients of total mixed rations (TMR)

Ingredients	TMR containing			
	SBM	FSBM-6.1	FSBM-12.1	FSBM-18.2
Soy bean meal (SBM)	18.2	12.1	6.1	-
Formalin treated soy bean meal (FSBM)	-	6.1	12.1	18.2
Extracted rice bran	14.7	14.7	14.7	14.7
Cassava chips	28	28	28	28
Molasses	5.6	5.6	5.6	5.6
Urea	1.1	1.1	1.1	1.1
Minerals+vitamins	2.4	2.4	2.4	2.4
Paragrass hay	30	30	30	30
Total	100	100	100	100

experimental periods, the first 16 d was regarded as an adaptation period and within the last 5 d, intensive sampling was undertaken. On day 17, ruminal fluid was sampled via a probe covered with a double layers of nylon stocking material for total viable and cellulolytic bacteria counts prior to incubation of nylon bags. Digestibilities of organic matter (OM), neutral detergent fiber (NDF) and crude protein (CP) of the diet was assessed. A nylon bag containing 10-12 pieces of rice straw samples was suspended for 24 h in the rumen to allow fungi to colonize the rice straw blades and produce sporangia. On day 18, Cr-EDTA (I mg Cr/kg BW) used as a ruminal fluid marker was injected into the rumen at 05:00 h. From 3-27 h after injection, 10 samples of ruminal fluid were taken periodically via the rumen canula with a probe covered with the stocking material. Prior to acidification, samples were removed to a vial for enumeration of protozoa and for measurement of pH. The rest was acidified for later analysis of Cr, VFA and NH_3-N. On days 19-21, daily urine voided by individual animal was collected into a container with 2 litres of 2% (V/V) CH_3COOH and 1% (V/V) H_2SO_4. The urine sample was diluted by 3-4 times just after collection and then stored at -20°C. Equal portions of the daily urine samples from each animal from each day were pooled prior to analysis of purine derivatives.

Crude protein, ether extract (EE), DM, OM and ash contents of the experimental diets were determined according to the AOAC (1980). Neutral detergent fiber (NDF) was measured following the method of Van Soest et al. (1991).

Fresh ruminal fluid (0.1 ml) was used as inoculum and serially diluted in a bicarbonate buffer containing L-α cystine –HCL as a reducing agent. Medium 98-5 agar roll tubes (Bryant and Robinson, 1961) and cellulose broth tubes (Halliwell and Bryant, 1963) were used for the determination of the densities of total viable and cellulolytic bacteria (most probable numbers) in ruminal fluid.

Enumeration of fungal sporangia followed the technique that recommended by Bauchop (1979). The rice straw leaves were removed from the nylon bags after 24 h incubation in the rumen and then dipped in 4% formal saline. The leaves were randomly sampled and stained with lactophenol cotton blue (Gurr, 1965) for 2 min and then washed with deionized water to remove the excess stain. Sporangial counts were made under a light microscope. The technique of enumeration of protozoa was similar to that recommended by Bird and Leng (1984). Protozoa were counted and also classified into three groups, namely small and large Entodinium sp. and Holotrich sp. under a light microscope.

Prior to analysis of Cr, VFA and NH_3-N, the acidified sample was thawed and centrifuged at 3,000 g and 4°C for 10 min. The supernatant was kept for analysis. The concentration of Cr was measured according to the method of Downes and McDonald (1964). The concentration and molar proportions of VFA were determined according the method of Erwin et al. (1961). Iso-caproic acid was used as an internal standard (Geissler et al., 1976). The concentration of NH_3-N was analyzed according the method of Weatherburn (1967).

Allantoin in urine was analyzed according to the method of Borchers (1977) and uric acid was measured following the method described by Fujihara et al. (1987).

The statistical significance of the data was analyzed by SAS (1989). The difference between treatments means was assessed by the Least squares means.

RESULTS

Dietary treatments were isonitrogenous and isocaloric and contained similar levels of nutrients. Values of nutrient composition on a DM basis for TMR were as follows: CP, 15.6%, EE, 2.3%; NDF, 30%, TNFC, 39.4% and ash, 9.9%.

Effects of TMR containing formalin treated soy bean (FSBM) on dry matter intake (DMI) and average daily gain (ADG) were shown in Table 2. Voluntary intake was not influenced by the contents of rumen undegradable protein (RUP) from FSBM in the diet ($p>0.05$). A significant increase in ADG was observed in the animals fed TMR containing RUP from FSBM compared with those fed TMR containing only SBM ($p<0.05$).

Effects of TMR containing FSBM on ruminal

Table 2. Effects of total mixed rations (TMR) containing formalin treated soy bean (FSBM) on dry matter intake (DMI) and average daily gain (ADG)

Item	TMR containing				S.E
	SBM	FSBM-6.1	FSBM-12.1	FSBM-18.2	
DMI					
- kg/d	13.4	13.0	13.8	13.3	0.46
- %BW	3.42	3.38	3.52	3.38	0.12
ADG, kg	0.643[1]	0.875[2]	0.964[2]	0.946[2]	0.19

[1,2] Mean within a row without a common superscript letter differ ($p<0.05$).

fermentation characteristics were shown in Table 3. The pH of ruminal fluid was not influenced by dietary treatments ($p>0.05$). Concentrations of ruminal fluid ammonia (NH_3-N) were decreased with increasing contents of RUP form FSBM in the diet ($p<0.01$). Of the pattern of VFA in ruminal fluid, only isoacids were decreasing with increasing contents of RUP from FSBM in the diet ($p<0.01$).

Effects of TMR containing FSBM on the kinetics of ruminal fluid and 24 h *in sacco* OM, NDF and CP digestibilities were shown in Table 4. Rumen volume in the animals fed TMR containing RUP from FSBM was larger than those fed TMR containing only SBM ($p<0.05$). Outflow rate and turnover rate of ruminal fluid were affected by the different contents of RUP from FSBM in the diet ($p<0.01$). The 24 h *in sacco* (OM) and (NDF) digestibility was not influenced by dietary treatments ($p>0.05$). The 24 h *in sacco* CP digestibility decreased with increasing contents of RUP from FSBM ($p<0.01$).

Effects of TMR containing FSBM on microbial ecosystem in the rumen and net microbial protein synthesis as indicated by purine derivatives in urine were shown in Table 5. Of microbial ecosystem in the rumen, the populations of total viable bacteria ($p<0.05$) and protozoa particularly Entodinium sp. ($p<0.01$) were affected by the contents of RUP from FSBM in the diet. Net microbial protein synthesis in the rumen as indicated by purine derivatives in urine was decreasing with increasing contents of RUP from FSBM in the diet ($p<0.05$ and $p<0.01$).

DISCUSSION

Rumen undegradable protein (RUP) is one of the limiting factors affecting milk yield and its composition and ovarian functions during early lactation of dairy cows in the tropics (Kanjanapruthipong and Buatong, 2002). Changes in availability of nitrogenous substrates will result in a change in microbial ecology in the rumen due primarily to differences in the capabilities of substrate assimilation among microbial species. The outcomes of the level and balance of RDP and RUP in the rumen are dependent on interaction between bacteria, protozoa and fungi in the rumen. Changes in these interactions can result in changes in either the specific growth rate of microbes or the turnover of microbial cells within the rumen as implied by a changing protozoal density in ruminal fluid. The contribution of the microbial cells that leave the rumen was assumed to follow closely the relative changes in purine derivatives excreted in urine (Chen and Gomes, 1992).

Table 3. Effects of total mixed rations (TMR) containing formalin treated soy bean (FSBM) on ruminal fermentation characteristics

Item	TMR containing				S.E
	SBM	FSBM-6.1	FSBM-12.1	FSBM-18.2	
pH	6.38	6.29	6.15	6.25	0.19
HN_3-N, mgN/l	221.4[a]	165.4[b]	154.0[c]	129.3[d]	7.61
Acetate, %	56.0	56.1	56.5	57.0	0.79
Propionate, %	28.0	28.6	28.4	28.7	0.62
Butyrate, %	10.7	10.6	11.0	10.8	0.15
Isobutyrate, %	1.8[a]	1.6[b]	1.3[c]	0.9[d]	0.06
Valerate, %	1.4	1.2	1.3	1.4	0.03
Isovalerate, %	2.1[a]	1.9[b]	1.5[c]	1.3[d]	0.04
Total VFA, μm/ml	139.0	137.8	138.2	136.9	3.2

[a,b] Mean within a row without a common superscript number and letter differ ($p<0.01$).

Table 4. Effects of total mixed rations (TMR) containing formalin treated soy bean (FSBM) on the kinetics of ruminal fluid and 24 h *in sacco* digestibility

Item	TMR containing				S.E
	SBM	FSBM-6.1	FSBM-12.1	FSBM-18.2	
Kinetics of ruminal fluid					
Rumen volume, l	60.0[1]	62.2[2]	63.9[2]	63.9[2]	1.6
Outflow rate, l/d	101.1[1,c]	95.1[2,a,b]	93.7[1,2,a,b]	91.1[1,a]	3.7
Turnover rate, /d	1.58[2,b]	1.59[2,b]	1.50[1,2,a,b]	1.44[1,a]	0.12
24 h *In sacco* digestibility, %					
Organic matter	82.2	81.6	80.1	79.8	3.01
Neutral detergent fiber	48.2	48.5	47.8	47.5	0.05
Crude protein	75.5[a]	71.6[b]	66.2[c]	62.7[d]	2.90

[1,2, a,b] Mean within a row without a common superscript number and letter differ. $p<0.05$ and $p<0.01$.

Table 5. Effects of total mixed rations (TMR) containing formalin treated soy bean (FSBM) on microbial ecosystem of in the rumen and net microbial protein synthesis in the rumen indicated by purine derivatives in urine

Item	TMR containing				S.E
	SBM	FSBM-6.1	FSBM-12.1	FSBM-18.2	
Total viable count, 10^{-11}/ml	9.41[1,a]	9.35[1,a]	9.17[2,a,b]	9.02[2,b]	0.58
Cellulolytic bacteria count, 10^{-7}/ml	0.94	0.91	0.89	0.90	0.05
Sporangia, No/mm^2	19	19	18	17	2.02
Large entodinium, 10^{-4}/ml	1.15[a]	0.47[b]	0.57[b]	0.42[b]	0.29
Small entodinium, 10^{-4}/ml	7.29[a]	8.80[a]	14.79[b]	12.83[b]	0.41
Dasytricha sp., 10^{-4}/ml	0.47	2.63	0.83	0.84	0.09
Isotricha sp., 10^{-4}/ml	0.52	0.63	0.58	0.42	0.08
Protozoa, 10^{-4}/ml	9.43[a]	10.53[a]	16.77[b]	14.51[b]	0.65
Purine derivatives, mmol/d	270.9[1,a]	269.1[1,a]	264.2[2,a,b]	262.3[2,b]	8.9
Microbial N supply, g/d	196.9[1,a]	195.6[1,a]	192.1[2,a,b]	190.7[2,b]	4.2

[1,2 and a,b] Mean within a row without a common superscript number and letter differ $p<0.05$ and $p<0.01$.

These changes were examined in the rumen of dairy cows fed on concentrate based-diets containing various contents of RUP from FSBM.

Santos et al. (1998) reported that there were no statistically significant differences in DMI when SBM was replaced by the high RUP sources in any of the comparisons. A similar result was also observed in this study.

When the rate of protein proteolysis exceeds the rate of small peptide and amino acid assimilation, excessive RDP undergoes hydrolysis and deamination to form ammonia, VFA and CO_2 (Annison, 1956). In this study, there was a decrease in ruminal ammonia concentrations with increasing contents of FSBM in diets. The lower concentrations of ruminal ammonia can be reflective of the less rapidly degradable protein in FSBM compared with that in SBM.

Isoacids appear to be essential and required by cellulolytic bacteria in the rumen (Van Gylswyk, 1970). However, a decrease in isoacids with increasing contents of FSBM in diets reported in this study did not appear to influence populations of cellulolytic bacteria in the rumen. This indicates that isoacids did not limit cellulolytic bacteria growth in the rumen on these diets.

Feed intake, rumen digestion, salivary secretion and retention time of the feed in the rumen, all affect the kinetics of rumen digesta. An increase in rumen volume is generally accompanied by an increase in digesta in the rumen. Increasing feed intake results in increasing pools of rumen digesta (Owen et al., 1984) and, at constant feed intake, the opposite would be expected with increasing the digestibility in the rumen (Colucci et al., 1982). Organic matter digested in the rumen reported here tended to decrease with increasing levels of RUP in the diets and thus it may be assumed that decreasing degradability of protein did not influence digestion in the rumen. However, increasing degradability of protein in the rumen will increase concentrations of ruminal fluid ammonia (Tamminga, 1983). Hume et al. (1970) reported that ruminal fluid ammonia concentrations can influence rumen volume. An increase in concentrations of ruminal fluid ammonia above 200 mgN/l were associated with a decrease in volume of ruminal fluid (Kanjanapruthipong and Leng, 1998). The smaller volume of ruminal fluid in the animal on the diet containing SBM as RDP, reported in this study, is likely influenced by the concentration of ruminal fluid ammonia.

Digestibility primarily determines the amounts of nutrients extracted from the feed and also the level of intake and therefore the amounts available for absorption by the host animal. The availability of microbial protein and VFA for absorption is dependent on the availability of the monomers required for microbial cell synthesis in the rumen. Regardless of diet, it has been reported that peptides and amino acids added to the rumen appear to give no benefit of OM digested in the rumen over urea (Redman et al., 1980; Cruz Soto et al., 1994; Fujimaki et al., 1994). A decrease in OM digested in the rumen with increasing FSBM reported in this study was partly due to an increase in contents of RUP in diets.

Various species of rumen microbes are all responsible for proteolysis of RDP (Nolan, 1993). Proteolytic activity of bacteria in the rumen may be more active for soluble protein (Nugent and Mangan, 1981) while that of protozoa and fungi is apparent for insoluble particulate protein (Wallace and Munro, 1986). In this study, a decrease in ruminal fluid bacteria as indicated by total viable count with increasing contents of RUP from FSBM in diets would be possibly due to lower soluble peptides and amino acids available for assimilation (Annison, 1956). Michalowski (1989) reported that numbers of protozoa in ruminal fluid appeared to increase with increasing RUP. A similar result was also observed in this study.

Any excess of peptides and amino acids from RDP over that are required for microbial protein synthesis can be utilized as ATP-yielding substrates. Amino acids catabolized anaerobically are low ATP-yielding substrates and could contributed to energetic-spilling reactions occurring under the condition that the rate of ATP

production by catabolism is in excess of the rate of ATP utilization by anabolism (Stouthamer. 1979). On the other hand. a supply of a small amount of peptides and amino acids in addition to ammonia to a sugar or starch but not cellulose diet *in vitro* studies resulted in a substantial increase in net bacterial protein synthesis (Maeng and Baldwin. 1976). A lack of response in the majority of *in vivo* studies (Redman et al., 1980; Cruz Soto et al., 1994; Fujimaki et al., 1994) can be due primarily to a large diversity of rumen microbes and their interactions. In this study. a decrease in net microbial protein synthesis in the rumen (as indicated by purine derivatives in urine) with increasing contents of RUP from FSBM was associated with increasing numbers of protozoa in ruminal fluid. The possible explanation is that the predatory activity of protozoa appeared to be the main factor causing considerable turnover of bacterial and fungal cells within the rumen (Wallace and McPherson. 1987). Whereas the majority of protozoa lyse and are degraded within the rumen and only 10-30% enter the intestine (Leng. 1982a).

It was likely that reduction in net microbial protein synthesis in the rumen with increasing contents of RUP in diets may be due to the reduction of preformed protein available for microbial growth as well as an increased turnover rate of bacterial cells by predatory activity of protozoa. However. a substantial increase in average daily gain in dairy cows fed TMR containing RUP from FSBM reported in this study appeared to be due to on increase in dietary protein available for digestion and absorption by the animals.

REFERENCE

Annison, E. F. 1956. Nitrogen metabolism in the sheep: Protein digestion in the rumen. Biochem. J. 64:705-714.

Association of official Analytical chemists. 1980. Official Methods of Analysis 13th Ed. AOAC. Washington, DC.

Bauchop, T. 1979. Rumen anaerobic fungi of cattle and sheep. Appl. Env. Microbiol. 38(1):148-158.

Bird, S. H. and R. L. Leng. 1984. Further studies on the effects of the presence or absence of protozoa in the rumen on live-weight gain and wool growth of sheep. Br. J. Nutr. 52:607-611.

Borchers, R. 1997. Allantoin determination. Anal. Biochem. 79:612-613.

Bryant, M. P. and I. M. Robinson. 1961. An improved non-selective culture medium for ruminal bacteria and its use in determining diurnal variation in numbers of bacteria in the rumen. J. Dairy Sci. 44:1446-1456.

Bryant, M. P. and I. M. Robinson. 1962. Some nutritional characteristics of predominant culturable ruminal bacteria. J. Bacteriol. 84:605-614.

Chen, X. B. and M. J. Gomes. 1992. Estimation of microbial protein supply to sheep and cattle based on urinary excretion of purine derivatives: A overview of the technical details. International Feed Resources Unit, Rowett Research Institute, Backsburn, UK. Occasional Publication. p. 19.

Clark, J. H, T. H. Klusmeyer and M. A. Cameron. 1992. Microbial protein synthesis and flows of nitrogen fractions to the duodenum of dairy cows. J. Dairy Sci. 75:2304-2323.

Colucci, P. E., L. E. Chase and P. J. Van Soest. 1982. Feed intake, apparent diet digestibility, and rate of particulate passage in dairy cattle. J. Dairy Sci. 65:1445-1456.

Cruz Soto, R., A. Samirah, S. A. Muhammed, C. J. Newbold, C. S. Stewart and R. J. Wallace. 1994. Influence of peptides, amino acids and urea on microbial activity in the rumen of sheep receiving grass hay and on the growth of rumen bacteria *in vitro*. Anim. Feed Sci. Technol. 49:151-161.

Downes, A. M. and I. W. McDonald. 1964. The chromium-51 complex of ethylenediamine tetraacetic acid as a soluble rumen marker, Br. J. Nutr. 18:153-162.

Erwin, E. S., G. J. Macro and B. M. Emesy. 1961. Volatile fatty acid analysis of blood and rumen fluid by gas chromatograply. J. Dairy Sci. 44:1768-1771.

Fujahara T., E. R. Orskov and P. J. Reeds. 1987. The effect of protein infusion on urinary excretion of purine derivatives in ruminants nourished by intragastric nutrition. J. Agric. Sci. Camb., 109:7-12.

Fujimaki, T., Y. Kobayashi, M. Wakita and S. Hoshino. 1994. Influence of amino acid supplements to a straw-maize-based urea diet on duodenal digesta flow and digestion in sheep. Asian-Aus. J. Anim. Sci. 7(1):137-145.

Geissler, C., M. Hoffman and B. Hickel. 1976. Ein beitrag zur gas chromatographischen bestimmung flüchtiger fettsäuren. Arch. Tierernährung. 26:123-129.

Gurr, E. 1965. The Rational use of dyes in biology and general staining methods. J. W. Arrowsmith Ltd. Great Britain. p. 422.

Halliwell, G. and M. P. Bryant. 1963. The cellulolytic activity of pure strains of bacteria from the rumen of cattle. J. Gen. Microbiol. 32:441-448.

Hume, I. D., R. J. Moir and M. Somers. 1970. Synthesis of microbial protein in the rumen. I. Influence of the level of nitrogen intake. Aust. J. Agric. Res. 21:283-296.

Kanjanapruthipong, J. and N. Buatong. 2002. Effects of rumen undegradable protein and minerals proteinate on early lactation performance and ovarian functions of dairy cows in the tropics. Asian-Aust. J. Anim. Sci. 15(6):806-811.

Kanjanapruthipong, J. and R. A. Leng. 1998. The effects of dietary urea on microbial populations in the rumen of sheep. Asian-Aust. J. Anim. Sci. 11(6):661-672.

Leng, R. A. 1982a. Dynamics of protozoa in the rumen of sheep. Br. J. Nutr. 48:399-415.

Leng, R. A. 1982b. Modification of rumen fermentation. In: Nutritional Limits to Animal Production from Pastures. Ed. J. B. Hacker. CAB, Farnham Royal, UK. pp. 427-453.

Maeng, W. J. and R. L. Baldwin. 1976. Factors influencing rumen microbial growth rates and yields: Effects of amino acids additions to a purified diet with nitrogen from urea. J. Dairy Sci. 59(4):648-655.

Michalowski, T. 1989. The importance of protein solubility and nature of dietary nitrogen for growth of rumen ciliates *in vitro*. In: The Role of Protozoa and Fungi in Ruminant Digestion (Ed. J. V. Nolan, R. A. Leng and D. I. Demeyer). University of New England, Armidale, Australia. pp. 223-232.

Nolan, J. V. 1993. Nitrogen kinetics, In: Quantitative Aspects of

Ruminant Digestion and Metabolism (Ed. J. M. Forbes and J. France) The University Press, Cambridges, UK. pp. 123-143.

Nugent, H. A. and J. L. Mangan. 1981. Characteristics of rumen proteolysis of fraction I (188) leaf protein from Lucerne (Medicago sative L). Br. J. Nutr. 46:39-59.

Oldham, J. D. 1984. Protein-energy interrelationships in dairy Cows. J. Dairy Sci. 67:1090-1114.

Owens, F. N., D. C. Weakley and A. L. Goetsch. 1984. Modification of rumen fermentation to increase efficiency of fermentation and digestion in the rumen. In: Herbivore Nutrition in the Subtropics and Topics. (Ed. F. M. C. Gilchrist and R. I. Mackie). The Science Press (Pty) Ltd., Graighall, South Africa. pp. 435-454.

Redman, R. G., R. G. Kellaway and J. Leibholz. 1980. Utilization of low quality roughages: effects of urea and protein supplements of differing solubility on digesta flows, intake and growth rate of cattle eating oaten chaff. Br. J. Nutr. 44:343-354.

Santoes, F. A. P., J. E. P. Santos, C. B. Theurer and J. T. Huber. 1998. Effects of rumen-undegradable protein on dairy cow performance: A 12-year literature review. J. Dairy Sci. 81:3182-3213.

SAS/STAT® User's Guide, Version 6, 4 th Edition. Vol 2. 1989. SAS Inst., Cary, NC.

Stouthamer, A. H. 1979. The search for correlation between theoretical and experimental growth yields. In: International Review of Biochemistry. Microbial Biochemistry (Ed. J. R. Guayle). University Park Press, Baltimore. 21:1-47.

Tamminga, S. 1983. Recent advances in our knowledge on protein digestion and absorption in ruminants. In: 4th International Symposium Protein Metabolism and Nutrition Clemond-Ferrand. Sept. pp. 1-25.

Van Gylswyk, N. O. 1970. The effect of supplementing a low-protein hay on the cellulolytic bacteria in the rumen of sheep and on the digestibility of cellulose and hemicellulose. J. Agric. Sci. Camb. 74:169-180.

Van Soest, P. J., J. B. Robertson and B. A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3583-3597.

Wallace, R. J. and C. A. McPherson. 1987. Factors affecting. The rate of breakdown of bacterial protein in rumen fluid. Br. J. Nutr. 58:313-323.

Wallace, R. J. and C. A. Munro. 1986. Influence of rumen anaerobic fungus Neocallimastix frontalis on the proteolytic activity of a defined mixture of rumen bacteria growing on a solid substrate. Let. Appl. Microbiol. 3:23-26.

Weatherburn, M. W. 1967. Phenol-hypochlorite reaction for determination of ammonia. 39:971-974.