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Effect of Mineralized Solid Palm Fat and Feeding Pattern on Ruminal Ecology and Digestibility of Nutrients in Dairy Steers Fed on Urea-Treated Rice Straw

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Abstract: Four, rumen fistulated crossbred dairy steers were randomly assigned according to a 2 x 2 Factorial arrangement in a 4 x 4 Latin square design to investigate the effect of feeding pattern and supplementation of mineralized solid palm fat (MSPF) on feed intake, digestibility of nutrients, ruminal fermentation and ruminal microbial protein synthesis. The dietary treatments were as follows: T1 = feeding with roughage and followed by concentrate after 4 h (FRC); T2 = feeding with roughage and followed by concentrate after 4 h with mineralized solid palm fat (FRC+MSPF); T3 = total mixed ration (TMR); T4 = total mixed ration with mineralized solid palm fat (TMR+MSPF), respectivity. The animals were offered urea-treated rice straw as a roughage source. Results revealed that feed-intake, digestibility of nutrients and volatile fatty acids in the rumen were similar among all treatments. As for ruminal pH and urinary purine derivatives they were significantly different among treatments and were higher in animals with roughage feeding and followed by concentrate after 4 h than with TMR diets; however, supplementation of MSPF were not significantly different among treatments. In addition, supplementation of MSPF resulted in reducing protozoal population while population of ruminal bacteria and fungal zoospores were enhanced. In conclusion, the combined use of feeding with roughage and followed by concentrate after 4 h with MSPF could improve rumen pH, microbial protein synthesis but reduced protozoal population in dairy steers.

Key words: Feeding pattern, rumen fermentation, protozoa, urea-treated rice straw, dairy steers

Introduction

Ruminants primarily digest fibrous feeds in the rumen with the aid of rumen microbial enzymes, which are mostly cell bound. Therefore, attachment of rumen microbes to feed particles is thought to be a critical phase of fiber digestion (Olubobocum and Craig, 1990). Bacteria have long been considered to be the major degraders of plant cell walls in the rumen (Chesson and Orskov, 1984) and bacterial species of the rumen are considered more important than protozoa and fungi in determining the extent and rate of feed degradation and utilization for the production of microbial protein and VFA (Stewart *et al.,* 1997). Rumen ciliated protozoa plays diverse and important roles in ruminal metabolism of nutrients (Williams and Coleman, 1992). Earlier reports indicated that grain fed feedlot cattle are virtually free (Eadie *et al.,* 1970; Lyle *et al.,* 1981) or have dramatically reduced populations of protozoa (Slyter *et al.,* 1970; Vance *et al.,* 1972).

The efficiency of microbial protein synthesis was not affected by supplemental fat in some studies (Elliott *et al.,* 1997). Microbial efficiency appears to be increased by fat because of reduced protozoal counts and predation (Stern *et al.,* 1994). In contrast, increased feeding frequency increased protozoal counts (Dehority and Orpin, 1988).

Mineralized solid palm fat is a palm-extracted powder and high-density rumen by-pass vegetable fat with high-energy content. It contained organic trace and major minerals, and claimed to act as a rumen buffer and fermentation enhancers. Therefore, the objectives were to determine the effects of mineralized solid palm fat together with feeding pattern on efficiency of microbial protein synthesis when dairy steers were fed urea-treated rice straw as the roughage source.

Materials and Methods

Animals, diets and experimental design: Four-fistulated dairy steers (Holstein-Friesian crossbred cows, 75%) of 250±10 kg body weight, were randomly assigned according to a 2 x 2 Factorial arrangement in a 4 x 4 Latin square design to investigate the effect of feeding pattern and comparing feeding roughage for 4 h and followed by concentrate with that of total mixed ration diet (TMR). Both feeding patterns were followed with and without supplementation of mineralized solid palm fat (MSPF) at 400 g/day. Diets were fed to dairy steers to assess feed-intake, digestibility of nutrients, ruminal fermentation and ruminal microbial protein synthesis. The dietary treatments were as follows: T1 = feeding with roughage and followed by concentrate after 4 h (FRC); T2 = feeding with roughage and followed by concentrate after 4 h with mineralized solid palm fat (FRC+MSPF); T3 = total mixed ration (TMR); T4 = total mixed ration with mineralized solid palm fat (TMR+MSPF).

All animals received diets at 3 % of BW with roughage to concentrate at 40:60 ratio with urea-treated rice straw (5%) fed ad lib as prepared according to Wanapat (1990) as the roughage source. All animals were kept in individual pens and receiving free access to water. The experiment was conducted for four periods; each period lasted for 21 days. Chemical composition of concentrate, mineralized solid palm fat (MSPF) and UTS used are shown in Table 1.

Table 1: Chemical compositions of concentrate, mineralized solid palm fat (MSPF) and urea-treated rice straw (UTS) used in the experiment (%DM basis)

Item	Concentrate	MSPF	Urea – treated rice straw (UTS)
Ingredient (%DM)			
Cassava chips	80.0		
Fine rice bran	5.0		
Brewer's grains	5.0		
Molasses	3.5		
Urea	3.5		
Sulphur	0.5		
Salt	0.5		
Limestone	1.0		
Mineral mix	1.0		
Analyzed composition (%)			
OM	88.6	86.0	83.9
CP	14.2	1.0	8.5
EE	1.4	61.0	1.4
NDF	21.2	1.5	71.2
ADF	4.0	0.5	53.3

DM = dry matter, CP = crude protein, OM = organic matter, NDF = neutral detergent fiber, ADF = acid detergent fiber, EE = ether extract

Data collection, analysis and sampling procedures: Feeds were regularly sampled and fecal samples were collected by rectal sampling from each individual steer on each treatment during the last 7 days of each period. UTS, feces and concentrate were sampled to be analyzed for chemical compositions (DM, OM, Ash, CP and EE (AOAC, 1985); NDF, ADF (Georing and Van Soest, 1970).

Rumen fluid and jugular blood samples were collected at 0, 2, 4, 8, 12 h-post feeding. Approximately 200 ml of rumen fluid was taken from the mid part of the rumen by using a 60 ml hand syringe during each time. Rumen pH was measured immediately after withdrawal and temperature using a portable pH and temperature meter (HANNA instrument HI 8424 microcomputer). Rumen fluid samples were then filtered through four layers of cheesecloth. Samples were divided into two portions; one portion was used for VFA analysis where 5 ml of H_2SO_4 solution (1M) were added to 50 ml of rumen fluid. The mixture was centrifuged at 16,000 x *g* for 15 minute and supernatant stored at -20 °C prior to and VFA analyses using a high-performance liquid chromatography (HPLC) according to Zinn and Owens (1986). Another portion was fixed with 10% formalin solution in normal saline (0.9% NaCl, Galyean, 1989). The total direct count of bacteria, protozoa and fungal zoospores based on the use of a haemocytometer (Galyean, 1989).

Samples of blood (about 10 ml) were drawn from the jugular vein at the same time as rumen fluid sampling, separated by centrifugation at 500 x *g* for 10 minute and stored at -20°C until analysis of blood urea nitrogen (BUN) according to Crocker (1967). Urine samples were analyzed for allantoin in urine by HPLC as described by (Chen *et al*., 1993).

Statistical analysis: The means of each parameter measured in the digestibility studies, nutrient intake and rumen microorganisms were analyzed by the analysis of variance (ANOVA) techniques using the General Linear Model (GLM) procedures of the Statistical Analysis System Institute (1998). Mean separations with a significant F ($P<0.05$) for treatment were statistically compared using Duncan's New Multiple Range Test (DMRT) (Steel and Torrie, 1980).

Results and Discussion

Feed intake and apparent digestibility: Dry matter intakes of UTS and concentrate did not vary significantly between treatments. Apparent digestibility (%) of ether extract (EE) were significantly ($P<0.05$) influenced by mineralized solid palm fat supplementation while digestibility of DM, OM, NDF and ADF were similar in all groups (Table 2). Although the mean EE digestibility in dairy steers was not significantly affected by feeding pattern, animals which received mineralized solid palm fat (MSPF) had significantly higher EE digestibility.

Rumen fermentation parameters: Temperature, pH, BUN and VFA in the rumen fluid were used to monitor rumen fermentation pattern (Table 3). The rumen temperature was similar in all treatments, whilst rumen pH was significantly ($P<0.05$) affected by feeding pattern of diets but not by mineralized solid palm fat supplementation. The dairy steers fed T1 and T2 had higher rumen pH than those dairy steers fed T3 and T4. However, the rumen pH in dairy steers T1 and T2 were found in the optimal pH range (6.7 ± 0.5) to maintain normal cellulolytic organisms (Van Soest, 1994). Nevertheless, previous reports suggested that most ruminal bacteria prefer pH near neutrality for growth, although some species (e.g., *Streptococcus bovis* and *Prevotella ruminicola*) can grow in the pH 5 to 6 range (Weimer, 1996). Moreover, Stewart (1977) suggested an optimum pH range of 6.7 to 7.0 for cellulolysis, and greater than 5.7 for microbial protein synthesis. Other studies (Melaku *et al*., 2004) demonstrated inhibitory effects of rumen pH on cellulolysis only at values below

Table 2: Influence of mineralized solid palm fat and feeding pattern on feed - intake and digestibility of nutrients in dairy steers

Items	Treatment[1/]					Contrast[2/]		
	T1	T2	T3	T4	SEM	D	E	D x E
DM intake (kg/hd/d)								
UTS	3.9	3.7	3.7	4.1	0.32	NS	NS	NS
Conc.	2.4	2.3	2.4	2.7	0.25	NS	NS	NS
Apparent total-tract digestibility (%)								
DM	68.7	69.9	68.4	69.5	1.66	NS	NS	NS
OM	77.6	77.0	77.0	76.0	1.47	NS	NS	NS
CP	73.7	70.5	71.2	72.3	1.37	NS	NS	NS
NDF	55.1	54.6	55.0	54.7	1.88	NS	NS	NS
ADF	49.4	48.8	48.9	47.6	1.76	NS	NS	NS
EE	69.2[a]	81.9[b]	68.9[a]	79.4[b]	0.76	NS	**	NS

[ab]Values on the same row with different superscripts differed (p<0.05). [1/]T1=feeding with roughage and followed by concentrate after 4 h (FRC). T2=feeding with roughage and followed by concentrate after 4 h with mineralized solid palm fat (FRC+MSPF). T3=total mixed ration (TMR). T4=total mixed ration with mineralized solid palm fat (TMR+MSPF). [2/]Probability of main effects of Diets (D) (Feeding with roughage and followed by concentrate after 4 h vs TMR), MSPF (E) (without vs MSPF 400 g/d), or the D x E interaction. * = P<0.05, ** = P<0.01, NS = P>0.05. Conc. = concentrate, UTS = urea-treated rice straw, SEM = Standard error of the mean

6.1. Reducing ruminal pH can have great impact on fiber digestion (Mould and Orskov, 1984). Cheng *et al.,* 1984) reported that low ruminal pH appeared to prevent a strong attachment of bacteria to plant cell walls, resulting to lower fiber digestion. This was also supported by Shriver *et al.* (1986) who reported that at pH 5.8, the quantity of microbes associated with fiber particles was reduced by 43% and NDF digestibility at this pH was 8.1% as compared to an average of 32.5% at a higher pH. A fall in pH below 6.0 resulted in a precipitous loss of fibrolytic activity and complete cessation of fiber digestion between 4.5 and 5.0 (Hoover *et al.,* 1984) in view of reduced growth of several species of ruminal bacteria and to a flush out of cellulolytic microbes from continuous cultures (Russell and Dombrowski, 1980).

Blood-urea nitrogen (BUN) concentrations were significantly different (P<0.05) between treatments and were in the range of optimal blood-urea-nitrogen (5 to 25 mg/dl, Lewis, 1975). The differences in BUN concentrations among treatments may have been related directly to CP levels of concentrate. Preston *et al.* (1965) reported that concentrations of BUN were highly correlated to protein intake and reflected the level of ammonia production in the rumen. This study revealed that incorporation of concentrate has increased NH_3-N concentration with ammonia being the main nitrogen source for growth and protein synthesis by ruminal bacteria to achieve maximum fermentation (Satter and Slyter, 1974). Purine derivatives in urine were significantly (P<0.05) affected by feeding pattern of diets as presented in Table 3 and was highest in roughage feeding (T1).

The influence of feeding pattern of diets with mineralized solid palm fat supplementation on total VFA concentration, proportion of acetic acid, propionic acid, butyric acid, acetic to propionic ratio and acetic + butyric to propionic ratio are shown in Table 3. Mean total VFA concentration increased from 79.5 to 92.3 m*M*/L and proportions of acetic acid and propionic acid ranged from (69.4 to 73.3 and 18.9 to 22.5 mol/100mol, respectively) and were significantly (P<0.05) affected by interaction between feeding pattern and mineralized solid palm fat supplementation, whist proportion of butyric acid was unaffected. In addition, acetic to propionic ratio and acetic+ butyric to propionic ratio were significantly (P<0.05) influenced by the interaction between feeding pattern and mineralized solid palm fat supplementation. However, the total VFA concentrations in all diets were in the normal concentrations and agreed with values of 70 to 130 m*M*/L (France and Siddons, 1993). Furthermore, Hoover (1986) reported that ruminal fermentation of structural carbohydrates such as cellulose and hemicellulose in diets exceeding 60% roughage would yield high proportions of acetate and butyrate, respectively. While acetate and butyrate production from lactate was pH-dependent, with acetate production maximal at higher pH and butyrate production at lower pH. The inverse relationship between acetate to propionate ratio and the amount of concentrate in the diet has often been explained by the tendency of fiber fermenting bacteria to produce acetate and starch fermenting bacteria to produce propionate (Satter and Slyter, 1974).

Rumen microorganism populations: Table 4 presents rumen microorganism populations. For ruminal bacteria, there were significant differences between treatments as influenced by both feeding pattern and mineralized solid palm fat (MSPF) supplementation. However, populations of ruminal bacteria in all treatments were in the normal range (10^{11} to 10^{12} cell/ml of rumen fluid) (Hungate, 1966). The populations of ruminal bacteria in T2 was highest and might have been

Table 3: Influence of mineralized solid palm fat (MSPF) and feeding pattern on rumen ecology and fermentation characteristic in dairy steers

Items	Treatment[1/]					Contrast[2/]		
	T1	T2	T3	T4	SEM	D	E	D x E
Temperature (^{0}C)	40.2	39.8	40.3	40.4	0.29	NS	NS	NS
Ruminal pH	6.6^a	6.6^a	6.5^{ab}	6.3^b	0.08	0	NS	NS
BUN (mg/dl)	9.9^a	8.6^{ab}	8.1^{ab}	6.7^b	0.89	0	NS	NS
Purine derivative (m*M*/ml)	37.3^a	36.1^a	26.9^b	28.1^b	4.76	**	NS	**
Total VFA (m*M*/L)	88.8^a	79.5^b	84.2^b	92.3^c	1.48	0	NS	**
VFA (mol/100mol)								
Acetate (C2)	73.3^a	69.4^b	70.2^b	72.9^a	0.48	NS	NS	*
Propionate (C3)	18.9^a	22.5^b	21.5^b	19.0^a	0.40	NS	NS	*
Butyrate (C4)	7.8	8.2	8.2	8.1	0.54	NS	NS	NS
C2 : C3 ratio	3.8^a	3.1^b	3.2^b	3.8^a	0.12	NS	NS	*
C2+C4 : C3 ratio	4.3^a	3.4^b	3.6^b	4.2^a	0.15	NS	NS	*

^{abc}Values on the same row with different superscripts differed (p<0.05). $^{1/}$T1=feeding with roughage and followed by concentrate after 4 h (FRC). T2=feeding with roughage and followed by concentrate after 4 h with mineralized solid palm fat (FRC+MSPF). T3=total mixed ration (TMR). T4=total mixed ration with mineralized solid palm fat (TMR+MSPF). $^{2/}$Probability of main effects of Diets (D) (Feeding with roughage and followed by concentrate after 4 h vs TMR), MSPF (E) (without vs MSPF 400 g/d), or the D x E interaction

Table 4: Influence of mineralized solid palm fat (MSPF) and feeding pattern on ruminal bacteria, fungi and protozoa population in dairy steers

Items	Treatment[1/]					Contrast[2/]		
	T1	T2	T3	T4	SEM	D	E	D x E
Ruminal Bacteria (x 10^{11} cell/ml)	1.7^a	2.5^b	1.7^a	1.9^a	0.16	0	**	*
Fungi zoospores (x 10^5 cell/ml)	5.0	4.3	5.2	4.6	0.99	NS	NS	NS
Ruminal protozoa								
Holotrich (x 10^5 cell/ml)	2.9^a	1.9^b	2.8^a	2.1^b	0.19	NS	**	NS
Entodiniomorph (x 10^6 cell/ml)	6.7	4.6	6.7	5.7	0.82	NS	NS	NS

^{ab}Values on the same row with different superscripts differed (p<0.05). $^{1/}$T1=feeding with roughage and followed by concentrate after 4 h (FRC). T2=feeding with roughage and followed by concentrate after 4 h with mineralized solid palm fat (FRC+MSPF). T3=total mixed ration (TMR). T4=total mixed ration with mineralized solid palm fat (TMR+MSPF). $^{2/}$Probability of main effects of Diets (D) (Feeding with roughage and followed by concentrate after 4 h vs TMR), MSPF (E) (without vs MSPF 400 g/d), or the D x E interaction

influenced by the reduced protozoal numbers, while populations of ruminal fungi zoospores were similar among treatments (P>0.05). Populations of ruminal protozoa especially holotrich protozoa decreased significantly in animals that received mineralized solid palm fat supplementation, while entodiniomorph populations were not significantly different between treatments (P>0.05). It was also found that fat may decrease intraruminal N recycling of microbial nitrogen because of decreased protozoal counts (Tesfa, 1993). Additionally, decreased protozoal number due to high dietary fat has been reported in several studies (Sutton *et al.,* 1983; Ruiz *et al.,* 2004). Previously, Ivan *et al.* (2001) observed that Holotricha were the most susceptible to toxic effects of oils, especially with oils rich in unsaturated fatty acids, and similar result was found under this experiment. Furthermore, microbial efficiency appears to be increased by fat because of reduced protozoal counts and predation as also reported by (Stern *et al.,* 1994). Moreover, increased feeding frequency resulted in increased protozoal counts (Dehority and Orpin, 1988). Although the degree of fatty acid saturation did not affect the efficiency of microbial protein in the study with frequently fed cattle (Elliott *et al.,* 1997), tallow was the most saturated fat source fed; in frequently fed situations the effects of fat might be reduced, and fat might need to be more unsaturated to elicit the same response. In addition, Jouaney and Ushida (1999) reported that the number of protozoa per ml rumen fluid depends on the rate of soluble sugars and starches in the ration and also pH. Moreover, if the ration is based on grain, protozoa engulfment of starch grains can modulate pH and protect the animal from acidosis (Huntington, 1997). However, the decrease in protozoal count may be attributed to the increase in fungal zoospores per ml rumen fluid, as removal of protozoa has been associated with an increase in the concentration of fungi (Demeyer, 1981). Therefore, the combined use of feeding with roughage and followed by concentrate after 4 h with MSPF could not only improve rumen pH, microbial protein synthesis but also reduced protozoal population in dairy steers.

Conclusion: Based on this experiment it could be concluded that differences in feeding pattern of diets by feeding of roughage and followed by concentrate after 4

h could bring beneficial effects on ruminal bacterial populations as compared with TMR diets. Moreover, supplementation with mineralized solid palm fat (MSPF) with diets can lead to decreased ruminal protozoal populations and might possibly reduced methanogenic bacteria in the rumen. Therefore, the use of MSPF should be supplemented in ruminant feeding, however further studies with the use of energy source especially cassava chip should be conducted.

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References

AOAC., 1985. Official Methods of Analysis. Association of Official Analysis Chemists, DC, U.S.A.

Chen, X.B., D.J. Kyle and E.R. ørskov, 1993. Measurement of allantoin in urine and plasma by high-performance liquid chromatography with pre-column derivatization. J. Chromatogr., 617: 241-247.

Cheng, K.J., C.S. Stewart, D. Dinsdale and J.W. Costerton, 1984. Electron microscopy of bacteria involved in the digestion of plant cell walls. Anim. Feed Sci. Tec., 10: 93-120.

Chesson, A. and E.R. Orskov, 1984. Microbial degradation in the digestive tract. In: Straw and Other By-products as Feed (Ed. F. Sundstol and E. OwenElsevier), , Amsterdam, The Netherlands, pp: 305-339.

Crocker, C.L., 1967. Rapid determination of urea nitrogen in serum or plasma without deproteinzation. Am. J. Med. Tec., 33: 361-365.

Dehority, B.A. and C.G. Orpin, 1988. Development of and natural fluctuations in rumen microbial populations. In: The Rumen Microbial Ecosystem (Ed. P.N. Hobson), Elsevier, New York, pp: 51-184.

Demeyer, D.I., 1981. Rumen microbes and digestion of plant cell walls. Agric. Environ., 6: 295-337.

Eadie, J.M., J.S. Hyldgaard-Jensen, O.R. Mann, S. Reid and F.G. Whitelaw, 1970. Observations on the microbiology and biochemistry of the rumen in cattle given different quantities of a pelleted barley ration. Br. J. Nutr., 24: 157-177.

Elliott, J.P., J.K. Drackley, C.G. Aldrich and N.R. Merchen, 1997. Effects of saturation and esterification of fat sources on site and extent of digestion in steers: Ruminal fermentation and digestion of organic matter, fiber, and nitrogen. J. Anim. Sci., 75: 2803-2812.

France, J. and R.C. Siddons, 1993. Volatile fatty acid production. In: Quantilitive Aspects Ruminant Digestion and Metabolisim (Ed. J.M. Forbes and J. France), International, Willingford, UK., pp: 107-122.

Galyean, M., 1989. Laboratory procedure in animal nutrition research. Department of Animal and Life Science. New Mexico state University, USA.

Goering, H.K. and P.J. Van Soest, 1970. Forage Fiber Analysis (apparatus, reagent, procedures and some application). Agric. Handbook No. 379, ARS, Washington, D.C.

Hoover, W.H., 1986. Chemical factors involved in ruminal fiber digestion. J. Dairy Sci., 69: 2755-2766.

Hoover, W.H., C.R. Kincaid, W.V. Varga, J.R. Thayne and L.L. Junkins, 1984. Effects of solids and liquid flows on fermentation in continuous cultures. IV. pH and dilution rate. J. Anim. Sci., 58: 692-699.

Hungate, R.E., 1966. The Rumen and It Microbe. (Ed. R.E. Hungate). Academic Press, New York, NY.

Huntington, G.B., 1997. Starch utilization by ruminants: from basics to bunk. J. Anim. Sci., 75: 852-867.

Ivan, M., P.S. Mir, K.M. Koenig, L.M. Rode, L.T. Neil and Z. Mir, 2001. Effects of dietary sunflower seed oil on rumen protozoa population and tissue concentration of conjugated linoleic acid in sheep. Small Rumin. Res., 41: 215-227.

Jouaney, J.P. and K. Ushida, 1999. The role of protozoa in feed digestion. Asian-Aust. J. Anim. Sci., 12: 113-126.

Lewis, D., 1975. Blood urea concentration in relation to protein utilization in the ruminant. J. Agri. Sci., 48: 438-446.

Lyle, R.R., R.R. Johnson, J.V. Wilhite and W.R. Backus, 1981. Ruminal characteristics in steers as affected by adaptation from forage to all-concentrate diets. J. Anim. Sci., 53: 1383-1390.

Melaku, S., K.J. Peters and A. Tegegne, 2004. Microbial nitrogen supply, nitrogen retention and rumen function in Menz sheep supplemented with dried leaves of multipurpose trees, their mixtures or wheat bran. Small Rum. Res., 52: 25-36.

Mould, F.L. and E.R. Orskov, 1984. Manipulation of rumen fluid pH and its influence on cellulolysis in sacco, dry matter degradation and the rumen microflora of sheep offered either hay or concentrate. Anim. Feed Sci. Tec., 10: 1-14.

Olubobocum, J.A. and W.M. Craig, 1990. Quantity and characteristics of microorganisms associated with ruminal fluid or particles. J. Anim. Sci., 68: 3360-3370.

Preston, R.L., D.D. Schnakanberg and W.H. Pfander, 1965. Protein utilization in ruminants. I. Blood urea nitrogen as affected by protein intake. J. Nutr., 86: 281-287.

Ruiz, D.R., A.I. Garcia, A. Moumen and E. Alcaide, 2004. Ruminal fermentation and dgradation patterns, protozoa poppulation and urinary purine derivatives excretion in goats and weathers fed diets based on olive leaves. J. Anim. Sci., 82: 3006-3014.

Russell, J.B. and D.B. Dobrowski, 1980. Effect of pH on the efficiency of growth by pure culture of rumen bacteria in continuous culture. Appl. Environ. Microbiol., 39: 604-610.

SAS., 1998. SAS/STAT User's Guide: Version 6.12. SAS Inst., Inc., Cary, NC.,USA.

Satter, L.D. and L.L. Slyter, 1974. Effect of ammonia concentration on rumen microbial protein production in vitro. Br. J. Nutri., 32: 199-208.

Shriver, B.J., W.H. Hoover, J.P. Sargent, J.R. Crawford and W.V. Thayne, 1986. Fermentation of a high concentrate diet as affected by ruminal pH and digesta flow. J. Dairy Sci., 69: 413-419.

Slyter, L.L., R.R. Oltjen, D.L. Kern and F.C. Blank, 1970. Influence of type and level of grain and diethylstilbestrol on the rumen microbial populations of steers fed all concentrate diets. J. Anim. Sci., 31: 996-1002.

Steel, R.G.D. and J.H. Torrie, 1980. Principles and Procedure of Statistics. A Biometrical Approach. 2nd edn. McGraw-Hill Book Company, New York, New York.

Stern, M.D., G.A. Varga, J.H. Clark, J.L. Firkins, J.T. Huber and D.L. Palmquist, 1994. Evaluation of chemical and physical properties of feeds that affect protein metabolism in the rumen. J. Dairy Sci., 77: 2762-2786.

Stewart, C.S., 1977 Factors affecting the cellulolytic activity ofumen contents. Appl. Environ. Microbiol., 33: 497-502.

Stewart, C.S., H.J. Flint and M.P. Bryant, 1997. The Rumen Bacteria. In: The Rumen Microbial Ecosystem (Ed. P.N. Hobson and C.S. Stewart), Blackie Academic and Professional Publishers, London. pp: 10-72.

Sutton, J.D., R. Knight, A.B. McAllan and R.H. Smith, 1983. Digestion and synthesis in the rumen of sheep given diets supplemented with free and protected oils. Br. J. Nutr., 49: 419-427.

Tesfa, A.T., 1993. Effects of rapeseed oil supplementation on digestion, microbial protein synthesis and duodenal microbial amino acid composition in ruminants. Anim. Feed Sci. Tec., 41: 313-328.

Vance, R.D., R.L. Preston, E.W. Klosterman and V.R. Cahill, 1972. Utilization of whole shelled and crimped corn grain with varying proportions of corn silage by growing-finishing steers. J. Anim. Sci., 35: 598-605.

Van Soest, P.J., 1994. Nutrition Ecology of The Ruminant. 2nd edn. Comstock Publishing Associates a division of Cornell University Press, Ithaca and London.

Wanapat, M., 1990. Nutritional Aspects of Ruminant Production in Southeast Asia with Special Reference to Thailand. Funny Press, Ltd., Bangkok, Thailand.

Weimer, P.J., 1996. Why don't ruminal bacteria digest cellulose faster. J. Dairy Sci. 79: 1496-1502.

Williams, A.G. and G.S. Coleman, 1992. The Rumen Protozoa. Springer-Verlag, New York.

Zinn, A.R. and F.N. Owen, 1986. A rapid procedure for purine measurement and its use for estimating net ruminal protein synthesis. Can. J. Anim. Sci., 66: 157-163.