

THE EFFECTS OF STRAW WITHDRAWAL ON SOME RUMEN PARAMETERS IN SHEEP FED HIGH CONCENTRATE DIET.

¹Mlay, P. S. and ²Leek, B. F. ¹Department of Physiology, Biochemistry, Pharmacology & Toxicology P. O. Box 3017, Morogoro, Tanzania. ²Department of Physiology and Biochemistry, Veterinary College, Ballsbridge, Dublin 14, Ireland.

SUMMARY

A study was conducted to investigate the effects of sudden withdrawal of straw in a feeding regime comprising of high concentrate and straw. Five Suffolk sheep, with average weight of 69 ± 5 kg (Mean + standard deviation) were fed a high concentrate diet with straw *ad libitum* for six weeks. At the end of the sixth week, ruminal fluid sampling was done to determine the diurnal variation in ruminal pH, ammonia and volatile fatty acids. Straw was withdrawn from the feeding regime for the following three weeks when the sheep were maintained on high concentrate alone. At the end of the three weeks, ruminal fluid sampling was done and the samples were analysed for the same parameters as before. The two sets of data (before and after the withdrawal of straw) were compared by two-tailed students' t-test using a Statview computer application. It was found that, removal of straw from the diet led to significantly lower ruminal pH ($P < 0.01$), total VFAs ($P < 0.05$), Acetate ($P < 0.05$), butyrate ($P < 0.05$), ciliate protozoa counts ($P < 0.001$) at various sampling hours. Also, there was significantly higher propionate ($P < 0.05$) and ammonia ($P < 0.001$) when straw was withdrawn compared to when straw was offered. It is hereby concluded that long term adaptation to high concentrate feeding alone may not be sufficient in the maintenance of a stable intra-ruminal condition and that incorporation of low quality roughage in high concentrate feeding regime is desirable.

INTRODUCTION

The diet fed to ruminants exerts a great influence in the establishment of the various microbial species in the rumen (Hungate, 1966), which in turn influence the intra-ruminal environment through their (microbes) end products of fermentation. The one factor that exerts the greatest influence on the activities of ruminal microbes and can even affect the general health of the animal is the ruminal pH.

Ruminal pH is determined by the concentration of acids (VFA and Lactate) and the neutralising effects of saliva and ammonia (Svendson, 1974). Animals maintained on a diet with high proportion of roughage can easily maintain a stable intraruminal condition due the slower rate of plant fibre fermentation and therefore less acid concentration in the rumen (Schwart and Gilchrist 1984) and buffering effect of saliva produced in

large amounts during chewing (Iggo and Leek, 1967, Leek, 1993).

Consumption of high amounts of concentrates by ruminants results into high production rate of acids (VFAs and Lactate) which places a great load on the rumen buffering systems (Broster et al, 1981, Van Soest, 1994). Reports show that, when ruminant animals are gradually adapted to high concentrate feeding, they develop the ability to consume large quantities of rapidly fermentable carbohydrates without causing the breakdown of the mechanisms to maintain optimal acid-base balance (Leek, 1993, Allison, 1984). The aim of this study was to investigate whether long term adaptation to high concentrate feeding can lead to optimal ruminal fermentation in the absence of materials that necessitates chewing.

MATERIALS AND METHODS

Experimental Animals

Five Suffolk sheep weighing between 69±5kg (Mean + standard deviation) and previously ruminally cannulated by the method of Leek, (1976) were used during the experimental period. The animals were kept in individual pens of 2m x 3m size. They were routinely de-wormed and vaccinated against clostridial diseases and pasteurellosis.

Feeding regime

The sheep were gradually introduced to the concentrate by giving 400g per day per sheep for the first 2 weeks.

The amount was increased to 600g for the next 2 weeks and finally to a to 1000g per day, with this high level being maintained for six weeks. The composition of the concentrate used in this trial was: 19% Crude protein (of which 11% is rumen degradable), 3% crude oil, 9.2% crude fibre, 7.3% crude ash, 14% Moisture, Vitamin D3 (2000 iu/kg), alpha-tocopherol (10 iu/ kg), and vitamin A (7000 iu/kg). Barley straw was provided *ad libitum* for the initial six weeks. All sheep had free access to mineral lick blocks (Yellow Stocklick, Tithebarn Limited, Southport, Merseyside) with the following composition: 1000mg/kg Magnesium, 100mg/kg Manganese and Cobalt, 50 mg/kg Iodine, 130 mg/kg Zinc, 200 mg/kg Iron, 20 mg/kg Selenium and 38% Sodium. Fresh drinking water was provided each morning and evening.

Rumen fluid sampling

On the last day of the sixth week, ruminal fluid sample was taken just before feeding (at 8 am). The sheep were then given concentrates to intake limit determined by providing 2 kg of concentrates and allowing the sheep to feed to replete. Further rumen samples were taken at the following times: 10 am, 11am, 12 noon, 2pm, 6pm, 10pm, 12 Midnight, 2 am, 4 am, 6am and 9 am. Same procedure was done at the end of three weeks following the withdrawal of straw from the feeding regime.

Sampling procedure and sample handling

After opening the cannula, a long (12 inch) intestinal forceps was used to remove a portion of raft material so as to gain access to the soupy

materials in the ventral ruminal sac. An allowance was made for two waves of ruminal contractions to take place so as to ensure a fairly representative sample was taken (Czerkawski, 1986). About 200g rumen contents were withdrawn and squeezed through four layers of cheesecloth material to obtain about 40 ml of reasonably clear rumen liquor. The pH of the sample was quickly taken and the rumen fluid was divided into two portions. To one half, about 1 ml of 70mM mercuric chloride was added and the sample was deep-frozen, later to be analysed for volatile fatty acids (VFAs). The other half was quickly centrifuged at 3000 revolutions per minute so as to sediment protozoa and feed particles. The supernatant was used for ammonia assay as will be described. The supernatant of samples taken 1 and 3 hours after feeding was also analysed for lactate concentration. Samples for ciliate protozoa counts were obtained 3 hours after feeding by straining rumen contents through a one layer of coarse muslin to obtain about 20 ml of rumen liquor. The rumen liquor was thoroughly mixed by shaking and immediately, 1 ml was pipetted into a test tube containing 19ml methyl green formalin solution to obtain the mixture (1:20 dilution) that was used in the estimation of ruminal fluid protozoal density.

Ruminal fluid pH

Ruminal fluid pH was measured immediately upon taking the sample using a pH meter (pH μ -Sensor, HANNA, Lennox Laboratory Apparatus Scientific glassware and Instruments).

Ruminal fluid ammonia

The concentration of ammonia was estimated by a standard procedure as described by Chaney and Marbach, (1962) using alkaline hypochlorite/phenol nitroprusside.

Ruminal fluid VFAs

The concentration VFAs in the ruminal fluid were determined by using a gas liquid chromatography (Unicam 610 Gas Chromatograph, Brennan & Company) coupled to peak integrator (Chromjet integrator, SP 4400, Spectra Physics, San Jose, California USA) by the method described by Ryan, (1980).

Estimation of DL- Lactate in ruminal fluid

A UV method for the enzymatic determination of D- and L- lactic acid in foodstuffs and other material (Boeringer Mannheim cat. No. 1112821) was used in the determination of the two lactic acid isomers in the ruminal fluid.

Ciliate protozoal density.

The mixture for protozoa count was thoroughly shaken and immediately 5 μ l were pipetted on a slide with 1-mm rulings. The slide was mounted on a microscope stage and all cells counted at X 40 magnification. The number of cells in 5 μ l was multiplied by 200 (to get the count in 1 ml) and finally the result was multiplied by 20 (the dilution) so as to get the protozoa concentration in the rumen liquor.

RESULTS

Feed intake

The average consumption of concentrates was 1.4 ± 0.18 kg on as fed basis and the straw consumption per day was 600 ± 91 g. This gave a ratio of concentrate to straw of 71:29 respectively.

Diurnal changes in ruminal pH, ammonia and VFAs

The diurnal trends of pH and ruminal fluid ammonia were as indicated in Figure 1 and 2 respectively. Ruminal fluid pH was severely depressed reaching lowest values of 5.3 and 5.1 three and 6 hours after feeding with and without straw respectively. After attaining these low values, there was a steady rise in pH around 6.5 and 6.8 at 9 am the following day (Figure 1)

Ruminal fluid ammonia levels rose sharply during both periods (Figure 2) reaching a peak value of about 28 mM three hours after feeding when straw was given, and 26 mM two hours after feeding when straw was withdrawn. Thereafter, changes in the ruminal fluid ammonia concentration took different patterns. During the time when straw was available, there was a sharp fall in ruminal ammonia from 2pm to minimum levels of about 10-15 mM between 6pm and 9 am (Figure 2). In the absence of straw, ruminal ammonia remained rather high (20-22 mM) between 6pm to 4 am followed by a drop to about 17 mM at 9 am.

Diurnal trends in ruminal total VFAs are indicated in Figure 3. During the period when straw was given, total ruminal VFA concentration rose to peak value of 110 mM at 12 noon (Figure 3) followed by a sharp fall to 100 mM at 2pm and this level remained steady until 4 am when there was a fall to 55 mM. In the absence of straw, there was a very sharp to peak level of 100 mM two hours after feeding. This level (100mM) was almost maintained till 2 am after which there was a sharp fall to 60 mM at 9 am.

Statistical comparisons of ruminal pH, ammonia and VFAs

Table 1 shows the summary of a paired student's t -test results for ruminal fluid pH, ammonia and volatile fatty acids at the indicated sampling hours. The data were compared by Statview 512 TM \pm 1.0 computer programme. The fall in pH was non-significantly ($P < 0.1$) lower at 12 noon and significantly ($P < 0.01$) lower between 2pm and 6pm. For the remaining hours, there was a non-significantly lower pH (by about 0.2 units) when straw was denied compared to the period when straw was given.

Ruminal fluid ammonia concentration was significantly ($P < 0.001$) higher in the samples taken between 2pm and 6pm when straw was withdrawn compared to the period when straw was available. The total ruminal VFA concentration were significantly ($P < 0.05$) higher three hours after feeding when straw was given compared to the period when straw was denied (Table 1).

Ruminal fluid acetic acid were significantly ($P < 0.05$) lower at 12 noon, 10pm and 4 am when straw was withdrawn compared to when straw was given. Propionate concentration in the ruminal fluid was significantly higher ($P < 0.05$) in the absence of straw between 2pm and 2 am compared to the period when straw was given. Butyric acid level was significantly ($P < 0.05$) higher 3 hours after feeding when straw was available compared to when straw was not offered.

There were non-significant increases in the concentrations of L-, D- and total lactate in the rumen of sheep when straw was denied in the samples taken one and three hours after feeding (Table 2). The withdrawal of straw led to significantly ($P < 0.001$) lower number of ciliate protozoa counts in the ruminal fluid three hours after feeding.

DISCUSSION

It appeared that slow introduction of concentrate to the animals allowed a high intake of the concentrate without any obvious adverse effects. Despite the fact that the concentration of total volatile fatty acids in the rumens of the sheep were relatively higher when they had free access to straw compared to the period when straw was denied, the sheep were able to maintain a relatively higher ruminal pH. This was most probably due to the presence of higher buffering ability from increased saliva production during chewing straw. Chewing

during eating and rumination are processes primarily aimed at causing particle breakdown and injuring the cuticles to facilitate bacteria entrance (Murphy and Kennedy 1993). However, there is the secondary but equally important aspect of stimulation of saliva production from salivary glands during chewing (Ørskov and Ryle, 1990, Iggo and Leek, 1967). The severe depression of pH in the sheep when straw was denied was probably due to fall in the buffering processes in the rumen due to limited saliva production and high lactate. Lactic acid has a higher dissociation constant, and therefore a strong acid (Ogimoto and Giesecke, 1974). Very low ruminal pH (below 5.2) has a negative effect on the growth and survival of microbes in the rumen especially protozoa and cellulolytic bacteria (Nagaraja et al., 1992). This explains the observed fall in the number of ciliate protozoa when straw was withdrawn compared to when straw was available to the sheep.

Several factors may have contributed to the significantly ($P < 0.001$) higher levels of ammonia in the sheep when the straw was withdrawn. One factor might be the longer stay in the rumen of proteins in fed concentrates, probably in accordance to the findings of Ledoux et al., (1985) that with decreasing of fibre content in ruminant diet, both fluid and particle passage rates are decreased. There was also the possibility that most protozoa were killed due to the drastic fall in pH and thus, protein from the dead protozoa may have acted as an added source of ammonia through

proteolytic and deaminative activities in the rumen. The contribution of ammonia in the buffering systems in the rumen was not manifested in this study due to the fact that despite the higher ruminal ammonia in the sheep when straw was not given, ruminal pH remain depressed most of the time. This implies that ammonia plays an almost insignificant role in the neutralisation of acids in the rumen when the rate of acid production is high.

The observed higher total VFAs in the rumens of sheep most of the day when straw was available was due to a higher total dry matter intake (concentrates plus straw) compared to the low DM intake when straw was denied. In conjunction with this, was the maintenance of a more favourable condition for fermentation as a result of buffering effects of saliva. This could have allowed a certain degree of increased cellulolytic activities as evidenced by the higher ruminal acetic acid concentration during that period. The increase in the propionic acid in the absence of straw was probably an indication that with depressed activity of cellulolytic and methanogenic bacteria, more reducing equivalent were diverted to propionate production. These findings were in agreement with those by Obara et al, (1989) that increasing the ratio of concentrates in the diet fed to a ruminant animal results into a VFAs profile with relatively lower proportion of acetate and higher propionate. However increased proportion of propionate absorption relative to other acids has been incriminated as of the

factors causing the low butter fat syndrome in lactating cows (Maynard and Loosli, 1962) and the deposition of undesirable branched-chain soft fat in lambs (Ørskov et al, 1974).

CONCLUSION

From the findings of this work, it seems that long term high concentrate feeding does not lead to the development of an ability to sustain stabilised ruminal environment in the absence of materials that necessitates chewing. Provision of a fibre source to act as a stimulus for increased saliva production may be necessary in high concentrate fed animals so as to ensure a more stabilised ruminal pH. The complete absence of any sign of systemic acidosis in the sheep for the whole period despite the high intake of concentrates is a proof that slow introduction of concentrates to ruminants can lead to high intake without serious effects. However, these results should be treated with care when extrapolating them to cattle since other studies have shown that small ruminants are less prone to develop acidosis compared to cattle (Mgasa et al., 1994).

REFERENCES

- Allison, M. J. (1984). Microbiology of the rumen and small and large intestines. In: *Dukes Physiology of Domesticated Animals* 10th edition. Edited by Melvin, Swenson. Comstock

- Publishing Associates Ithaca and London. pp 340-350.
- Broster, W. H., Sutton, J. D., and Bines, J. A. (1981). Concentrate : Forage ratios for high yielding animals. In: *Recent Developments in Ruminant Nutrition*. Edited by Haresign, W., and Cole, D. J. A. Butterworth London, Boston, Sydney, Durban Toronto. pp 325-349
- Chaney, A. L. and Marbach E. P. (1962). Modified reagents for determination of urea and ammonia. *Clin. Chem.* 8 NO. 2 , 130.
- Czerkawski, J. W. (1986). An Introduction to Rumen studies. Pergmon Press Ltd, Headington Hill Hall, Oxford, England.
- Hungate, R. E. (1966). The rumen and its microbes. Academic Press, New York, USA and London, UK.
- Iggo, A and Leek, B. F. (1967). An electrophysiological study of some reticulo-ruminal and abomasal reflexes in sheep. *Journal of Physiology*, 193:95-119.
- Ledoux, D. R, Williams, J. E., Stroud, T. E., Garner, G. B. and Peterson, J. A. (1985) Influence of forage level on passage rate, digestibility and performance of cattle. *Journal of Animal Science*, 61:6, 1559-1566
- Leek, B. F. (1976). A simple and effective ruminal cannulation technique in sheep. *Journal of Physiology*, 263:233P-234P.
- Leek, B. F. (1993). Digestion in the ruminant stomach In: *Dukes Physiology of Domesticated Animals* 11th edition. Edited by Melvin, Swenson, J. and William O. Reece. Comstock Publishing Associates Ithaca and London. pp 387-416.
- Mgasa, M. N., Arnbjerg, J. and A. Basse (1994). Aetiological and epidemiology of laminitis in intensive bulls. *Zimbabwe Veterinary Journal* Vol. 25 Nos 3&4 103-120.
- Maynard, L. A. and Loosli, J. K. (1962). *Animal Nutrition*. 5th Edition. Macgraw-Hill Book Company, Inc. 1962. New York, Toronto London pp 43-63.
- Murphy, M. R., and Kennedy, P. M. (1993). Particle Dynamics In: *Quantitative Aspect of ruminal digestion and metabolism*. Edited by Forbes, J. M and France, J. C.A.B International, Wallingford, UK pp 87-101
- Nagaraja, T. G., Townen, G. and Beharka, A. A. (1992). Moderation of ruminal protozoa in cattle fed a high grain diet. *Applied and Environmental Microbiology*, 58: 8, 2410-2414.
- Obara, Y., Dellow, D. W. and Nolan, J. V. (1991). The influence of energy rich supplements on nitrogen kinetics in the rumen. In: *Physiological aspects of digestion and Metabolism in Ruminants* Edited by Tsuda, T., Sasaki, Y. and Kawashima, R. Academic Press Inc. San Diego and UK. pp 484-533.
- Ogimoto, K. and Gieseckle, D. (1974). The genesis and biochemistry of rumen acidosis 2. Micro-

- organisms and metabolism of lactic isomers. *Untersuchungen zur Genes und Biochemie der Pensenacidose*. (1) Stärke, Amylase-Activitan und Aciditat. *Zentralblatt für Veterinärmedizin*, A 21: 7, 532-538
- Ørskov E. R. and Ryle, M. (1990). *Energy Nutrition in Ruminants*. Elsevier Applied Science Harcourt Brace Jovanovich, London, UK and New York, USA. pp 1-123
- Ørskov E. R., Fraser, C. and Gordon, J. G. (1974). The effect of processing of cereals on rumen fermentation, rumination time, and firmness of subcutaneous fat in lambs. *British Journal of Nutrition*, 32: 59
- Ryan, P. (1980). Determination of volatile fatty acids and some related compounds in ovine rumen, abomasum, urine, and blood plasma by gas liquid chromatography. *Anal Biochem.*, 108: 374-384.
- Schwartz, H. M. and Gilchrist, F. M. C. (1974). Microbial interaction with the diet and the host animal. In: *Digestion and metabolism in the ruminant*. Proceedings of the IV International Symposium on Ruminant Physiology. Edited by McDonald, I. W. and Warner, A. C. I. Sydney, Australia, August, 1974.
- Svendson, P. E. (1974). Experimental studies of gastrointestinal atony in ruminants. In: *Proceedings of the IV International Symposium on Ruminant Physiology* Edited by McDonald, I. W. and Warner, A. C. I. Sydney, Australia. August 1974
- Van Soest, P. J. (1994). *Nutritional ecology of the ruminant*. 2nd edition. Comstock Publ. Associates. London, UK pp1-6

Table 1 Ruminal fluid pH, ammonia, volatile fatty acids , ciliate protozoal density and before and after straw was withdrawn form the diet.

Item	With straw (n=5)		Without straw (n=5)		Statistical significance
	Mean	SE	Mean	SE	
Ruminal fluid pH (hours A. F.)					
3	5.50	0.093	5.20	0.172	NS
5	5.42	0.044	5.10	0.068	**
9	5.62	0.070	5.38	0.030	**
Ruminal fluid ammonia (mM).(hrs A. F.)					
9	13.00	1.580	22.60	1.230	***
13	11.00	0.970	22.00	1.200	***
15	12.20	1.220	22.40	2.970	***
17	11.93	1.539	21.40	1.699	***
19	15.10	0.630	23.10	2.000	***
Ruminal fluid VFA (mM) Hrs after feeding 3					
Total	112.77	6.240	88.13	6.480	*
Acetic	74.36	4.060	57.15	2.920	*
Butyric	16.49	0.990	11.00	1.300	*
Propionic	20.18	2.19	16.82	3.020	NS
Hrs after feeding 9					
Total	93.47	3.250	98.32	3.700	NS
Acetic	67.34	3.23	63.94	3.110	NS
Butyric	11.57	0.780	11.25	1.670	NS
Propionic	12.94	1.210	21.55	2.210	*
Hrs after feeding 13					
Total	106.10	3.960	103.97	4.910	NS
Acetic	78.74	3.650	65.40	1.940	*
Butyric	11.98	0.710	15.47	1.500	NS
Propionic	13.23	1.280	18.86	1.190	*

VFA=Volatile fatty acid, mM= concentration in millimoles per litre

Statistical significant levels (paired student's t test), *** P <.001, ** P <.01 *P < 05

Table 2 Ruminal lactic acid concentration before and after straw was withdrawn from the feeding regime.

Item	With straw (n=5)		Without straw (n=5)		Statistical significance
	Mean	SE	Mean	SE	
L-Lactate (mM)					
1 hour after feeding	6.05	0.735	10.93	2.077	NS
3 hours after feeding	11.0	2.725	9.53	1.996	NS
D-Lactate (mM)					
1 hour after feeding	7.70	1.485	11.63	2.276	NS
3 hours after feeding	13.20	2.758	16.50	3.611	NS
Total Lactate (mM)					
1 hour after feeding	13.75	1.193	22.55	3.003	NS
3 hours after feeding	24.22	4.830	26.03	3.819	NS
Total ciliate protozoa count/ml ruminal fluid					
1 hr after feeding	1495000	49916	562500	9639	***
3 hr after feeding	1066000	53128	106500	5377	***

mM= concentration in millimoles per litre

Statistical significant levels (paired student's t test),

*** P <.001, ** P <.01 * P < .05

Fig. 1 The effect of straw on ruminal pH

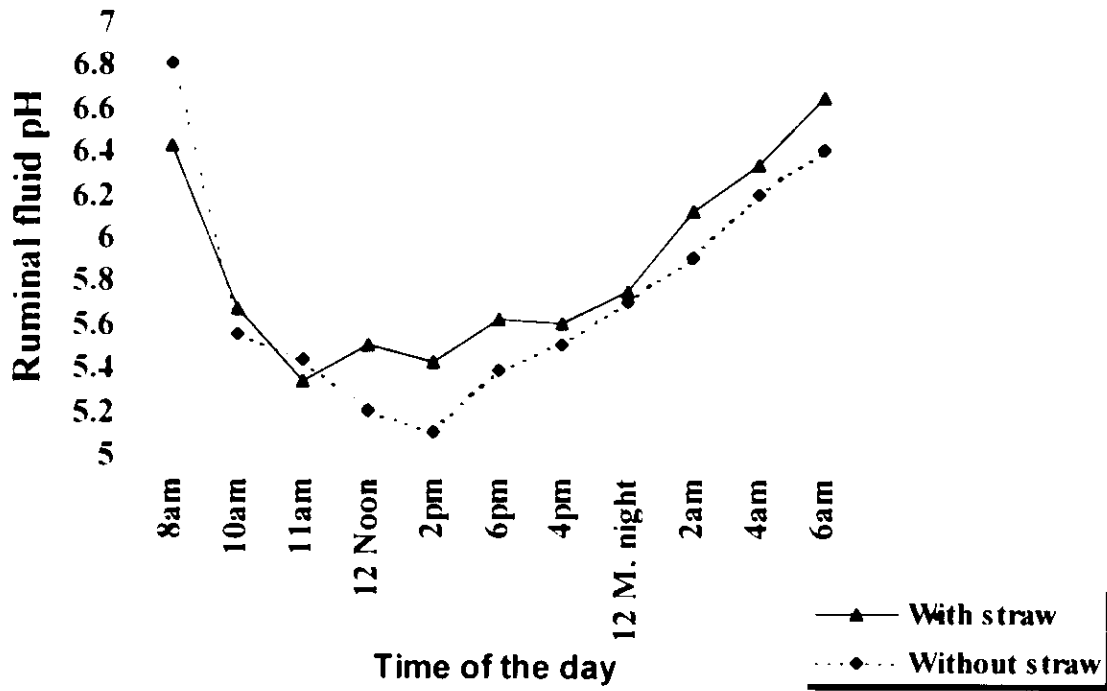


Fig. 2 The effect of straw on ruminal ammonia

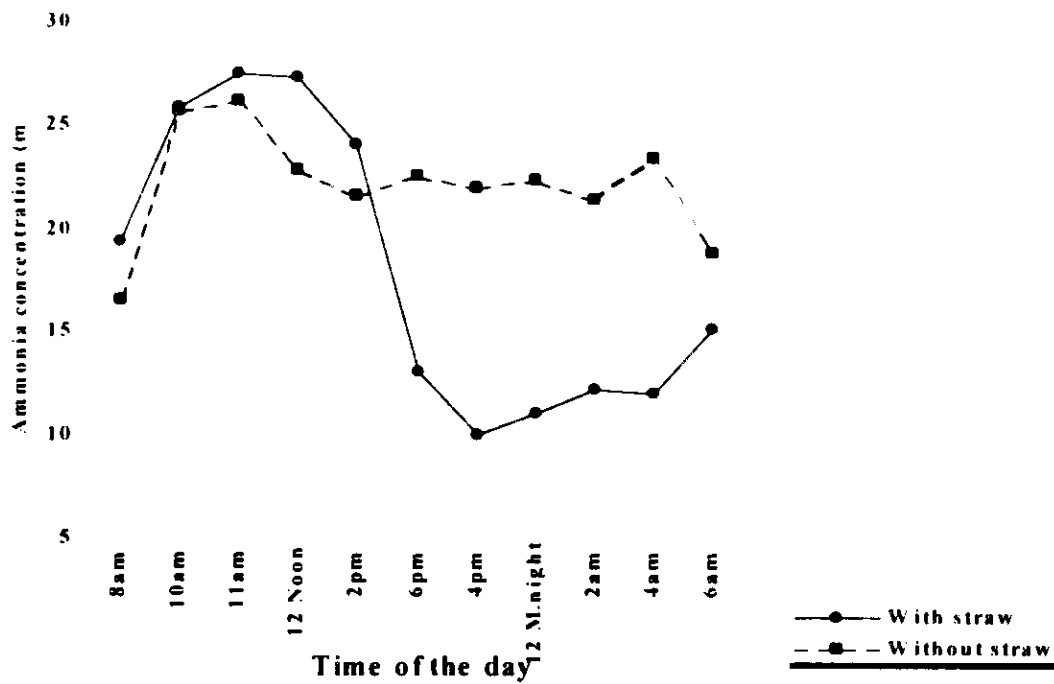


Fig. 3 The effect of straw on ruminal total VFAs

