

## RUMEN ACTIVITY AND MICROBIAL PROTEIN SYNTHESIS AS AFFECTED BY DIETARY PROTEIN AND FIBER LEVELS

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### SUMMARY

Four rumen fistulated Ossimi rams were used in 4 x 4 Latin square arrangement, to investigate the effect of dietary levels of protein and fiber on some rumen fermentation parameters (pH, ammonia -nitrogen, total volatile fatty acids VFA, rate of solid and liquid passage from the rumen and microbial protein synthesis.

Rations were formulated and pelleted to include four different levels of CP and CF i.e. high protein low fiber (HPLF), high protein high fiber (HPHF), low protein low fiber (LPLF) and low protein high fiber (LPHF). Results indicated that: No differences in rumen pH values were noted between the dietary treatments at any time before or after feeding. Rumen ammonia - N was higher for the HP diets regardless of the CF level. Ruminal total VFA concentration increased for the high protein diets and the low fiber ones. Dry matter and OM digestibility based on in vitro (IVDMD & IVOMD) were 67.45 & 66.33 %, 60.52 & 60.44 %, 69.82 & 69.19 % and 68.05 & 68.16 %, respectively. Both rates of solid and liquid passage were significantly and negatively affected by CF but not by CP. The rates of passage for solid phase were 3.39, 3.90, 4.85 and 3.91% hr and for liquid phase 20.57, 13.85, 17.38 and 13.68 % hr respectively. Values of retention time (RT) of solid phase were 22.78, 25.66, 20.61 and 25.55 hr and for liquid phase 4.86, 7.22, 5.72, and 7.31 hr respectively. The high protein diets showed higher rate of digestion than the low protein diets. Differences in RNA concentration between dietary treatments were not significant. The overall averages were higher for the low fiber diets than for the high-fiber diets. Total non-ammonia - nitrogen (TNAN) was higher for diets of high protein than diets of low protein. More microbial Protein (MCP) was synthesized with high protein and low fiber diet. It is concluded that a level of dietary protein as low as 13 % and a level of dietary fiber as high as 21 % may be used in sheep nutrition without any adverse effects on ruminal microbial activity as long as diets satisfy the energy requirements under similar condition.

**Keywords:** Sheep, protein level, fiber levels, Rumen activity, Microbial protein synthesis.

### INTRODUCTION

In previous two conferences, one of them held in Alexandria (1992) under a title of Manipulation of Rumen Micro-organisms and the other held in Ismailia (The 5th

Scientific Conference on Animal Nutrition, 1995), it was suggested that more work is needed to gain more information on microbial protein synthesis and rumen fermentation and factors affecting them (Borhami *et al.*, 1992; Mehrez, 1992 and 1995). Mehrez (1995) discussed in detail some of these factors i. e., dietary protein and extent of its degradation, adequacy of available nitrogen in the rumen to satisfy microbial nitrogen needs, dietary fiber, starch and sugars, rumen environment factors such as pH, additives, temperature...etc.

Borhami *et al.* (1992) stated that the rate and extent of microbial growth in the rumen could be studied using a variety of laboratory procedures. However, measurement of microbial yield *in vivo* is not an easy task. The estimation of microbial yield *in vivo* presents problems involving measurement and calculations. While literature values for microbial yield reflect problems in measurements, therefore, no doubt that the real variations in yield is due to various diets and feeding conditions. Averages of microbial yields for different classes of diets and/or method of estimation showed marked variations (Van Soest, 1982).

The present study was conducted to investigate the effect of varying levels of both protein and fiber in the diet on some rumen fermentation parameters (pH,  $\text{NH}_3\text{-N}$ , VFA). Rates of solid and liquid passage from the rumen along with microbial protein synthesis were also determined.

## MATERIALS AND METHODS

This study was conducted at the Animal Production Experimental Farm and Animal Nutrition Lab. of the Faculty of Agriculture, Menofiya University. Two roughages (berseem hay and wheat straw), and two concentrates (soybean meal and ground corn grain) were used to prepare four isocaloric rations. Main effects were level of crude protein (CP) and level of crude fiber (CF). Four tested rations were prepared to contain high protein high fiber (HHPF), high protein low fiber (HPLF), low protein high fiber (LPHF) and low protein low fiber (LPLF) respectively. Vitamins and minerals were provided to meet the requirements (NRC., 1985). Ingredients and chemical composition of the experimental diets are shown in Table (1).

### *In vitro* DM and OM digestibilities

Four, 2 g samples of each ration were taken to determine *in vitro* dry matter (IVDM) and organic matter (IVOM) digestibilities by the two stage technique of Tilly and Terry (1963) with slight modification (Ahmed, 1989) that the first stage of incubation was extended to 72 hr. The rumen liquor for the *in vitro* analysis, was obtained from four fistulated Ossime rams (average BW of 40 kg) fed a good quality berseem hay based diet.

### *In Situ* DM and OM disappearance

*In situ* dacron bag technique (ISDMD & ISOMD) of Mehrez and Ørskov (1977) was used to determine DMD & OMD in the rumen of sheep at different incubation intervals (0, 6, 24, and 72 hr). Bags were suspended into the rumen of fistulated sheep maintained on basal diet of good quality berseem hay. Four replicated samples were removed at 0, 6, 24, and 72 hr. After removal, bags were then washed according to Crawford *et al.* (1978). The bags then were dried in a forced air oven at 80 °C until constant weight was achieved. Contents of bags were transferred

carefully and quantitatively and ashed in a muffle furnace at 600 °C for 2 hr. The dry matter and organic matter disappearances were calculated. Rates of digestion were calculated as regressions of natural logarithmic transformation of potentially digestible DM remaining at 0, 6, 24, and 72 hr. of fermentation.

#### Rumen activity

Four rumen fistulated Ossimi rams weighing approximately 40 kg were placed in individual metabolic cages (1.6 m x .53 m) as described by Maynard *et al.* (1979). Rams were allowed 10 - day to adapt to the cages before the initiation of the experiment.

Table 1. Formulation and chemical analysis of the experimental diets.

| Ingredients, %                | HPLF  | HPHF  | LPLF  | LPHF1 |
|-------------------------------|-------|-------|-------|-------|
| Soy bean meal                 | 16.8  | 23.4  | 7.0   | 13.3  |
| Corn grains                   | 23.63 | 23.5  | 32.4  | 33.1  |
| Berseem hay                   | 54.54 | 10.0  | 55.6  | 10.0  |
| Wheat straw                   | 5.03  | 43.1  | 5.0   | 43.6  |
| M+V <sup>2</sup> (0.31%)      | +     | +     | +     | +     |
| Molasses (4.0%)               | ++    | ++    | ++    | ++    |
| Chemical Composition (%)      |       |       |       |       |
| Dry matter (DM)               | 91.92 | 88.79 | 92.97 | 89.33 |
| On DM basis                   |       |       |       |       |
| Organic matter (OM)           | 90.99 | 90.83 | 91.83 | 90.75 |
| Crude protein (CP)            | 17.92 | 17.29 | 13.81 | 13.76 |
| Crude fiber (CF)              | 16.53 | 21.36 | 17.03 | 21.78 |
| Ether extract (EE)            | 3.26  | 3.08  | 3.45  | 3.09  |
| Nitrogen free extract (NFE)   | 53.28 | 49.10 | 57.54 | 52.12 |
| Ash                           | 9.01  | 9.17  | 8.17  | 9.25  |
| TDN <sup>3</sup> (calculated) | 61.99 | 61.83 | 62.00 | 62.02 |

1- HPLF, high protein low fiber; HPHF, high protein high fiber; LPLF, low protein low fiber; LPHF, low protein high fiber. 2- M+v, Minerals and Vitamins. 3- TDN, Total digestible nutrients

The experimental diets (Table 1) were fed to the animals according to NRC requirements (1984). The experimental design was 4 x 4 Latin square.

Each experimental period consisted of a 15-day adjustment and 4-day ruminal sampling periods. Water was available all the times.

On d-16 of each period, ruminal samples from each animal were collected at hourly interval for 7 hr. after feeding. Ruminal digesta were strained through four layers of cheesecloth, ruminal pH was immediately measured using a pH - meter with a glass electrode. A part of each rumen sample was acidified with 10% HCl and frozen at -20 °C unanalyzed for ammonia - N and total volatile fatty acids. The other part of rumen sample was centrifuged at 500 x g for 5 min and frozen at -20 °C until analyzed for bacterial RNA.

Extemarkers of polyethylene glycol (molecular weight 4000, PEG) and chromic oxide ( $\text{Cr}_2\text{O}_3$ ) were used to determine ruminal passage rates for liquid and solid phases of rumen content, respectively. On the 17th d of each collection period, a mixture of 20 g of PEG (dissolved in 100 ml of water) and 5 g of chromic oxide was dosed into the rumen via the ruminal fistula of each animal immediately before feeding at 0800 hr. Samples of ruminal contents were taken at 0, 2, 4, 6, 8, 24, 30, 48, and 72 hr after dosing; an aliquot of each sample was centrifuged at 500 x g for 20 min and the supernatant was kept for analysis of PEG. The rest of the sample was dried and ground (1-mm screen) for chromium analysis.

The change in ruminal concentrations of PEG and  $\text{Cr}_2\text{O}_3$  over time were described by the equation  $Y = Ae^{-kt}$ , where Y is the concentration of marker at time T, A is the (extrapolated) initial (zero - time) concentration of the marker at dosing, and k is the fractional outflow rates of liquid (PEG) or solid ( $\text{Cr}_2\text{O}_3$ ).

#### Analytical Methods:

Dry matter (DM), crude protein (CP), crude fiber (CF), ether extract (EE), nitrogen free extract (NFE) and ash were determined according to A.O. A.C. (1980). Ammonia nitrogen was determined using MgO distillation method (Al-Rabbat *et al.*, 1971). Total volatile fatty acids was estimated by steam distillation as described by Warner (1964) using the markham distillation apparatus. RNA was determined in this supernatant by the orcinol procedure using hydrolyzed yeast RNA as a standard (Munro and Fleck, 1969). Protein was determined by the Hartrees modification of lowry (folin-phenol) procedure. Bovine serum albumin served as protein standard (Hartree, 1972). Polyethylene glycol was measured by the turbidimetric method of Ulyatt (1964). Chromium concentration in feed and ruminal digesta was determined by the method of Williams *et al.* (1962) using an atomic absorption spectrophotometer.

#### Statistical Analysis:

Experimental data were statistically analysed according to Snedecor and Cochran (1967) using one or two way classification followed by Duncan's multiple range test (Duncan, 1955) to examine the significance between means.

## RESULTS AND DISCUSSION

Dry matter and OM disappearance were evaluated in vitro and in situ, and data are shown in Table (2). The lowest values of IVDMD and IVOMD were reported for HPHF diet. In situ values were higher than in vitro with all diets. Differences between dietary treatments were not significant. The underestimation of the in vitro method could be attributed to the accumulation of the fermentation end-products which change the media and affect the microbial activity and shorten the protozoal life (Ahmed, 1989). De Faria and Huber (1984) studied the effect of dietary protein and energy on DM disappearance of three forage types (corn silage, alfalfa hay and grass hay) from dacron bags suspended in the rumen of fistulated steers and found that neither protein levels nor energy concentrations significantly affected DMD of any of the studied forages at 24, 48 and 72 hr of suspension.

Table 2. *In vitro* and *in situ* dry organic matter disappearance (%) as affected by dietary protein and fiber levels (mean + SE)

| Item* | HPLF                     | HPHF                     | LPLF                     | LPHF                     |
|-------|--------------------------|--------------------------|--------------------------|--------------------------|
| IVDMD | 67.45 <sup>b</sup> ±0.84 | 60.52 <sup>a</sup> ±1.05 | 69.82 <sup>b</sup> ±0.99 | 68.05 <sup>b</sup> ±1.28 |
| IVOMD | 66.33 <sup>b</sup> ±0.60 | 60.44 <sup>a</sup> ±1.09 | 69.19 <sup>b</sup> ±0.64 | 68.16 <sup>b</sup> ±1.01 |
| ISDMD | 74.12±1.52               | 73.62±1.29               | 76.98±1.47               | 74.53±1.08               |
| ISOMD | 76.13±2.35               | 75.43±1.32               | 77.34±1.71               | 73.09±1.44               |

\*HPLF, high protein low fiber; HPHF, high protein high fiber; LPLF, low protein low fiber; LPHF, low protein high fiber. IVDMD, *in vitro* DM digestibility; IVOMD, *in vitro* OM digestibility; ISDMD, *in situ* DM digestibility; ISOMD, *in situ* OM digestibility. a, b, c Means not sharing the same superscript within each row are significantly different (p < 0.05).

Rumen pH was high before feeding in all groups and sharply declined to reach the lowest values at 3 hr post-feeding and then increased thereafter. No differences were noted between the dietary treatments at any time before or after feeding (Fig. 1).

Van Beukelen *et al.* (1981); Orma and Eassa, (1988); Bakr, (1991) observed the lowest values of rumen pH at 2-4hr post-feeding with different dietary treatments.

Those investigations along with others reported pH values quite similar to those obtained in this study.

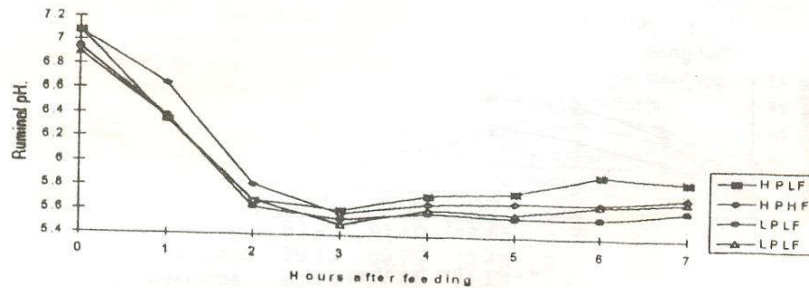


Figure 1. Ruminal pH as effected by dietary treatments.

Ruminal ammonia -N was almost equal in all treatments before feeding being on the average 22 mg/dl. as shown in Fig. 2.

In general, NH<sub>3</sub> - N reached the highest values in all diets at 1 hr after feeding. It declined thereafter sharply to reach the lowest level at 7 hr. Animals fed the high protein diets, regardless of the fiber level, showed the highest (P < 0.05) concentration at all times. Roffler and Satter (1975) showed a positive correlation of ruminal NH<sub>3</sub> -N concentration to concentration of protein in the diet. Earlier reports by Abou-Akkada and El-Shazly (1964) and El-Ashry (1971) showed that ruminal NH<sub>3</sub> was increased by increasing dietary CP level.

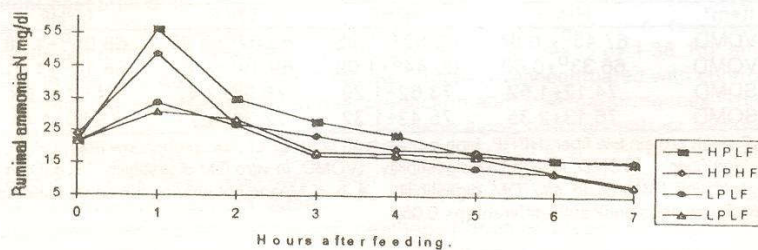


Figure 2. Ruminal ammonia-N concentration as affected by dietary treatments.

Figure (3) illustrates the total VFA concentration. The lowest values were reported before feeding for all groups. At one hour post-feeding sheep fed the LPHF diet showed the lowest VFA concentration than the other 3 groups which were nearly similar.

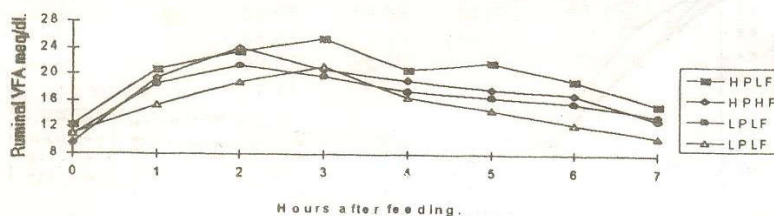


Figure 3. Ruminal volatile fatty acids production as affected by dietary treatments.

The same trend was observed at all times after feeding. The high protein diets and the low fiber ones had higher fermentable materials which would be degraded to VFA by the rumen micro-organisms during digestion. The energetic efficiency of rumen microbial production was related to the fermentable materials degraded to VFA (Walker and Nader, 1968; Mathison and Milligan, 1971). Johanson *et al.* (1961) stated that VFA are the end products of complex carbohydrates fermentation.

RNA concentration was determined (Fig. 4) at different times after feeding as affected by the dietary protein and fiber levels. Values were high before feeding and started to decline to reach the lowest values at 2 h after feeding then it increased to reach a maximum at 3-4 h then it levelled off. The overall average showed a non-significantly higher values for the low fiber diets than for the high-fiber diets. Dietary protein did not show any effect on the overall average of RNA. The use of RNA as a

marker assumes the complete ruminal degradation of dietary RNA. Schelling *et al.* (1980) reported that a significant amount of dietary nucleic acid passing from the rumen.

McAllan and Smith (1968) concluded that nucleic acids in the rumen fluid were almost entirely of microbial origin. Accordingly, data obtained in this study (Fig. 4) may be divided into three phases: 1) from 0-2 hr, 2) from 2-4 h and 3) from 4 to 7 h. The decline in the 1<sup>st</sup> phase may indicate the RNA degradation rate, while the increase in the 2<sup>nd</sup> phase reveals the rate of microbial protein synthesis, and the 3<sup>rd</sup> phase shows the rate of RNA outflow. As it could be seen, interpretation of the results is difficult, because RNA and ratio of RNA to protein in bacteria may vary relative to feeding time of the animal (Smith and McAllan, 1974).

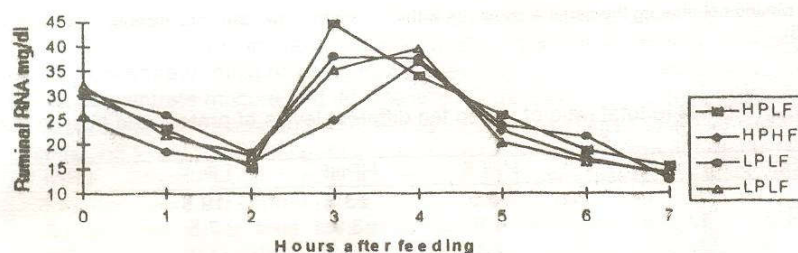


Figure 4. Ruminal RNA concentration as affected by dietary treatments.

Total non ammonia -N (TNAN) in the rumen of sheep fed different levels of protein and fiber were determined and presented in Table (3).

The higher TNAN values were obtained with diets of high protein at either fiber levels. Elliot *et al.* (1965) showed that extra protein intake increased the total nitrogen (TNAN) of rumen liquor. El-Ashry (1971) reported similar results. The decline in TNAN may indicate different flow rates out of the rumen. This is in good agreement with the data of the flow rates in the present study.

Data of TNAN positively correlated with the NH<sub>3</sub>-N. Many early reports suggested the usefulness of the ratio between RNA to total NA-N as a predictor of rumen microbial growth (Ellis and Pfander, 1965; Smith *et al.*, 1968; Schelling *et al.*, 1980).

Data in Table (4) show the calculated ratios of RNA - N to total NA-N. In general, the ratio was high before feeding which may have been due to the high RNA concentration with lower total NAN. Ratios of RNA / TNAN declined at 1-2 h post-feeding. During this period RNA concentration was low due to the degradation rate while total N was high due to the high protein intake.

Table 3. Total non - ammonia-N in the rumen of sheep fed different levels of protein and fiber.

| Sampling Time (h)      | HPLF                | HPHF               | LPLF               | LPHF*              |
|------------------------|---------------------|--------------------|--------------------|--------------------|
| 0                      | 24.23               | 16.21              | 23.44              | 19.61              |
| 1                      | 78.30 <sup>b</sup>  | 72.00 <sup>b</sup> | 51.88 <sup>a</sup> | 59.96 <sup>a</sup> |
| 2                      | 52.13 <sup>a</sup>  | 71.97 <sup>b</sup> | 58.32 <sup>a</sup> | 46.01 <sup>a</sup> |
| 3                      | 49.71 <sup>ab</sup> | 67.65 <sup>b</sup> | 43.72 <sup>a</sup> | 36.89 <sup>a</sup> |
| 4                      | 45.80 <sup>ab</sup> | 57.33 <sup>b</sup> | 34.14 <sup>a</sup> | 36.26 <sup>a</sup> |
| 5                      | 30.18               | 25.34              | 28.06              | 36.26              |
| 6                      | 26.75               | 21.80              | 27.86              | 34.75              |
| 7                      | 25.56               | 19.53              | 26.92              | 25.65              |
| x <sup>-</sup>         | 41.38               | 43.98              | 36.79              | 36.63              |
| ± SE                   | 6.22                | 5.93               | 4.32               | 4.16               |
| Total protein (NX6.25) | 258.6               | 274.9              | 229.9              | 228.9              |

HPLF, high protein low fiber; HPHF, high protein high fiber; LPLF, low protein low fiber; LPHF\*, low protein high fiber.

a, b, c Means not sharing the same superscript within each row are significantly different (p<0.05).

Table 4. RNA-N to total ratio of sheep fed different levels of protein and fiber.

| Sampling Time (h) | HPLF | HPHF | LPLF | LPHF* |
|-------------------|------|------|------|-------|
| 0                 | 18.5 | 23.7 | 19.8 | 24.5  |
| 1                 | 4.5  | 3.9  | 7.5  | 5.3   |
| 2                 | 4.4  | 3.4  | 4.7  | 5.8   |
| 3                 | 13.5 | 5.5  | 13.1 | 14.3  |
| 4                 | 14.1 | 9.6  | 16.5 | 16.4  |
| 5                 | 12.9 | 13.5 | 12.6 | 8.4   |
| 6                 | 10.5 | 11.8 | 11.6 | 7.0   |
| 7                 | 9.1  | 10.5 | 7.1  | 8.4   |
| x <sup>-</sup>    | 10.9 | 10.2 | 11.6 | 11.3  |
| ± SE              | 1.8  | 2.4  | 1.9  | 2.5   |

HPLF, high protein low fiber; HPHF, high protein high fiber; LPLF, low protein low fiber; LPHF\*, low protein high fiber. a, b, c Means not sharing the same superscript within each row are significantly different (p< 0.05).

At 3 to 6 hr the ratio was almost constant in all diets with no differences between dietary treatment or time of feeding. Ellis and Pfander (1965) reported that a relatively constant amount (14 -18 %) of the total MCP-N could be attributed to nucleic acid - N. Of this total, RNA - N was reported to comprise 10.4 to 14.8 %, which is near to that obtained in this study. These workers reported highly significant correlation of total nucleic acids - N (r = 0.80) and RNA (r = 0.72) with total MCP - N.

Almost identical results were reported by Smith *et al.* (1968) in their work with rumen fluid from calves fed diets of various roughage to concentrate ratios. They reported that a relatively constant protein (approximately 19%) of the total MCP - N was in the form of MCP nucleic acid - N. Cole *et al.* (1976) reported a ratio of MCP-N to RNA - N to be 10 : 1. Bergen *et al.* (1980) found a ratio of RNA - N to total N to be



8.4 to 9.1 %. Schelling *et al.* (1980) found that this ratio is constant irrespective of the N content of the diet. While others (Theurer, 1980 and Gillett *et al.*, 1982) reported that the ratio of MCP-N to RNA - N can change with dietary conditions. In this respect, it could be seen clearly that this ratio is fairly constant only between 3 to 6 h post feeding regardless of the dietary factors studied. Microbial protein synthesized in the rumen was calculated depending upon ruminally digested OM (*in situ*, Table 2) along with some published figures and assumptions *i.e.*:

1. One hundred g OM fermented in the rumen produces 2.2 mole ATP (Henderickx *et al.*, 1972).
2. YATP is 15.2 g microbial cells (produced per one mole of ATP, (Van Nevel *et al.*, 1975).
3. Rumen bacteria contains 53 % of their DM as MCP (Hutton *et al.*, 1971).
4. One hundred g OM digested in the rumen produces 38 g microbial cells (Bucholtz and Bergen, 1973).
5. One hundred g OM digested in the rumen produces 21 g MCP (Smith, 1975).

Data calculated are presented in Table (5). Microbial protein produced per day depending on the amount of OM digested in the rumen (Smith, 1975) was 215 g for HPLF, 206 g for HPHF, 224 g for LPLF and 201 g for LPHF; differences were not significant.

Table 5. Calculated microbial protein synthesis in the rumen of sheep fed different levels of protein and fiber.

| Item                             | HPLF    | HPHF    | LPLF    | LPHF*   |
|----------------------------------|---------|---------|---------|---------|
| DM intake, g                     | 1481    | 1433    | 1502    | 1444    |
| OM, %                            | 90.99   | 90.83   | 91.83   | 90.75   |
| OM intake, g                     | 1348    | 1302    | 1379    | 1310    |
| ISOMD                            | 76.13   | 75.43   | 77.34   | 73.09   |
| DOM, rumen                       | 1026    | 982     | 1066    | 957     |
| MCP (x 0.21), g                  | 215     | 206     | 224     | 201     |
| MCP cells (0.38), g              | 390     | 373     | 405     | 364     |
| MCP (cells x 0.53), g            | 207     | 198     | 215     | 193     |
| ATP, mol                         | 22.6    | 21.6    | 23.5    | 21.1    |
| Y ATP (x 15.2)                   | 344     | 328     | 357     | 321     |
| MCP (Y <sub>ATP</sub> x 0.53), g | 182     | 174     | 189     | 170     |
| MCP synthesis, range, g          | 182-215 | 174-206 | 189-224 | 170-201 |

HPLF, high protein low fiber; HPHF, high protein high fiber; LPLF, low protein low fiber; LPHF, low protein high fiber. DMI, dry matter intake; OM, organic matter; OMI, OM intake; ISOMD, *in situ* OM digestibility; DOM, ruminally digested OM; MCP microbial protein.

When it was calculated as microbial cells x 0.53 (Hutton *et al.*, 1971 and Bucholtz and Bergen, 1973) values were 207, 198, 215 and 193 for the same respective diets. The lowest estimate was that according to the YATP (Henderickx *et al.*, 1972 and Van Nevel *et al.*, 1975) being 182, 174, 189 and 170 g / d for HPLF, HPHF, LPLF and LPHF, respectively.

The similarity of MCP synthesis per day may indicate that all diets used in this study had enough N and energy to satisfy the microbial growth factors and requirements. Satter and Roffler (1977) reported that MCP synthesis peaked when the diet contained approximately 12 to 13 % CP. Above this level (Burroughs *et al.*,

1975; Satter and Roffler, 1975)  $\text{NH}_3$  - N concentration will increase without a concurrent increase in MCP protein production. Tarakanov (1985) reported that synthesis of bacterial protein in the rumen of lactating cows was not affected by increasing in CP from 13 to 20 % in iso - energetic diets. The none significantly higher MCP synthesis in the rumen of sheep fed the low fiber diets may have been due to the higher flow rate of the digesta. Firkins *et al.* (1986) found that extent of fermentation and efficiency of MCP growth are associated with ruminal rate of passage.

Bergen *et al.* (1980) observed that increasing dilution rate of particulate or fluid digesta may increase efficiency of MCP synthesis. Mees and Merchen (1984) obtained highly significant relationship between flow rate and efficiency of MCP synthesis.

Data in Table (6) present the flow rate and retention time of the rumen solid and liquid phases as affected by the dietary protein and fiber levels.

Table 6. Flow rates and retention time of rumen and liquid phases as affected by dietary protein and fiber levels (mean  $\pm$  SE )

| Phases <sup>a</sup>     | HPLF                          | HPHF                          | LPLF                          | LPHF                          |
|-------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|
| <b>Solid</b>            |                               |                               |                               |                               |
| Retention time, RT (hr) | 22.78 <sup>b</sup> $\pm$ 0.63 | 25.66 <sup>c</sup> $\pm$ 0.94 | 20.61 <sup>a</sup> $\pm$ 0.32 | 25.55 <sup>c</sup> $\pm$ 1.14 |
| Flow rate (%hr)         | 4.39 <sup>b</sup> $\pm$ 0.46  | 3.90 <sup>a</sup> $\pm$ 0.44  | 4.85 <sup>b</sup> $\pm$ 0.26  | 3.91 <sup>a</sup> $\pm$ 0.26  |
| <b>Liquid</b>           |                               |                               |                               |                               |
| Retention time, RT (hr) | 4.86 <sup>a</sup> $\pm$ 0.27  | 7.22 <sup>b</sup> $\pm$ 0.29  | 5.72 <sup>a</sup> $\pm$ 0.41  | 7.31 <sup>b</sup> $\pm$ 0.34  |
| Flow rate (%hr)         | 20.57 <sup>b</sup> $\pm$ 0.96 | 13.85 <sup>a</sup> $\pm$ 1.14 | 17.38 <sup>b</sup> $\pm$ 1.60 | 13.68 <sup>a</sup> $\pm$ 1.36 |

<sup>a</sup> HPLF, high protein low fiber; HPHF, high protein high fiber; LPLF, low protein low fiber; LPHF, low protein high fiber.

<sup>a, b, c</sup> Means not sharing the same superscript within each row are significantly different ( $p < 0.05$ ).

Both rates of solid and liquid phases were significantly and negatively affected by dietary fiber levels. The low fiber diets (HPLF and LPLF) had faster rates of passage for solid and for liquid. Dietary CP had no effect on neither rates of solid nor liquid passages. Retention time (RT) within the rumen of both phases had the opposite figures being more for high fiber diets and less for low fiber diets.

Firkins *et al.* (1986) reported that fluid and particulate phase dilution rates were highly correlated ( $r = 0.72$ ;  $P < 0.01$ ). Soluble and small particulate feed components were also reported to have increasing out flow (Teeter and Owens, 1983). This may explain the higher flow rate obtained in this study with low fiber diets (with smaller particulate feed components and more soluble fraction).

Values of passage rate and RT of solid phase reported in this study agree with those of many published articles (Harrison and McAllan, 1980; Mees and Merchen, 1984 and Baraghit *et al.* 1995). Rate of passage and RT of liquid phase reported herein are slightly higher than those reported by Bergen *et al.* (1980) who found that liquid turnover rate to be 3 to 12 %  $\text{h}^{-1}$  while Tamminga (1979) reported a rate of 20 %  $\text{h}^{-1}$  for liquid phase turnover.

The high rate of passage of rumen liquor obtained in this study may be due to use of PEG measuring the rate of outflow. Harrison *et al.* (1975, 1976) mentioned that rumen fluid turnover rate in sheep can be increased by infusing PEG in the rumen.

Rate of digestion (Rd, % hr<sup>-1</sup>) was estimated in this study (*in situ*) and data are shown in table (7).

Table 7. Digestion rates (*in situ*) and fractional digestion of DM as affected by dietary protein and fiber levels (mean + SE)

| Item                         | HPLF                     | HPHF                      | LPLF                      | LPHF                     |
|------------------------------|--------------------------|---------------------------|---------------------------|--------------------------|
| Solid Rd, % h                | 7.11 <sup>c</sup> ± 0.69 | 6.07 <sup>bc</sup> ± 0.62 | 5.84 <sup>ad</sup> ± 0.46 | 4.72 <sup>a</sup> ± 0.26 |
| Rate DM passage (Rp% h)      | 4.39                     | 3.9                       | 4.85                      | 3.91                     |
| Fraction DMD, rumen %        | 61.83 ± 4.31             | 60.88 ± 3.72              | 53.05 ± 1.58              | 54.69 ± 2.25             |
| Fraction DMD, post ruminal % | 38.17 ± 0.83             | 39.12 ± 0.77              | 46.95 ± 0.79              | 45.31 ± 1.13             |

HPLF, high protein low fiber; HPHF, high protein high fiber; LPLF, low protein low fiber; LPHF, low protein high fiber.

a, b, c Means not sharing the same superscript within each row are significantly different (p<0.05).

The high protein diets showed higher Rd than the low protein diets. Differences were significant (P < 0.05). Data in Table (7) showed that more DM was digested within the rumen for diets with high protein content than with low protein ones.

The DMD post ruminally followed the opposite trend being low for high - protein diets and high for low - protein diets. This may indicate that N is a limiting factor affecting digestion in the rumen, which is in complete agreement with the NH<sub>3</sub>-N concentration. Hopson *et al.* (1963) reported greater DM digestion rate from bags suspended in the rumen of animals fed high protein hay (alfalfa) than those fed low protein hay (grass) or straw. Aerts *et al.* (1977) maximized DMD when rumen NH<sub>3</sub> reached 23 mg / dl.

Ganev *et al.* (1979) and Weakley *et al.* (1983) reported that the faster rate of digestion may be due to changes in protein solubility or shift in microbial types in response to chemical or physical characteristics of the ruminal medium.

From the results reported herein it is concluded that a level of dietary protein as low as 13 % and a level of dietary fiber as high as 21 % may be used in sheep nutrition without any adverse effects on ruminal microbial activity as long as diets satisfy the energy requirements under similar condition.

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## تأثير تغذية مستويات مختلفة من البروتين و الألياف على نشاط الكرش وبناء البروتين الميكروبي

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استخدمت في هذه الدراسة أربعة كباش ذات (فستولا) - في تصميم مربع لاتيني. غذيت هذه الحيوانات على أربع علائق ذات مستويات مختلفة في البروتين والألياف لبحث تأثير هذه العلائق على نشاط الكرش وبناء البروتين الميكروبي، وذلك بقياس الحموضة، تركيز الأمونيا، الأحماض الدهنية الطيارة. كما تم قياس معدل هضم المادة الجافة داخل وخارج الكرش وفترة بقاء الغذاء داخل الكرش ومعدل تدفق المادة الصلبة والسائلة من الكرش وقياس الحامض النووي RNA لتقدير كمية البروتين الميكروبي. تم خلط وتكميع علائق التجربة كالتالي: عليقة مرتفعة البروتين منخفضة الألياف، عليقة مرتفعة البروتين مرتفعة الألياف، عليقة منخفضة البروتين منخفضة الألياف، عليقة منخفضة البروتين مرتفعة الألياف.

أظهرت النتائج مايلي:

لم يلاحظ إختلافات في قيم رقم حموضة سائل الكرش بين المعاملات الأربع سواء قبل أو بعد التغذية. أدت التغذية على العلائق المرتفعة في البروتين إلى إرتفاع تركيز أمونيا الكرش. إنداد متوسط تركيز الأحماض الدهنية الطيارة الكلية في سائل الكرش بالتغذية على العلائق المرتفعة البروتين ومنخفضة الألياف. كانت نسبة المهضوم معمليا من المادة الجافة والمادة العضوية هي ٦٧,٤٥ - ٦٦,٣٣، ٦٠,٥٢ - ٦٠,٤٤، ٦٩,٨٢ - ٦٩,١٩ و ٦٨,٠٥ - ٦٨,١٦ % لكل عليقة على الترتيب. وجد أن معدل مرور السائل والجزء الصلب من الكتلة الغذائية في الكرش تأثر سلبيا ومعنويا بمستوى الألياف الخام ولكن لم يتأثر بمستوى البروتين الخام. وأن معدل مرور الجزء الصلب هو ٤,٣٩، ٣,٩٠، ٤,٨٥ و ٣,٩١ % كل ساعة على الترتيب. مدة بقاء الغذاء في الكرش بالنسبة للجزء الصلب كانت ٢٢,٧٨، ٢٥,٦٦، ٢٠,٦١ و ٢٥,٥٥ ساعة بينما في حالة السائل كانت ٤,٨٦، ٧,٢٢، ٥,٧٢ و ٧,٣١ ساعة على الترتيب.

إزداد معدل الهضم في الكرش بالتغذية على العلائق المرتفعة في البروتين عن العلائق المنخفضة فيه. كانت الإختلافات بين العلائق في تركيز الحمض النووي الريبوزي RNA في سائل الكرش غير معنوية ولكن ظهر زيادة غير معنوية في متوسط تركيز RNA في حالة العلائق المنخفضة الألياف عن العلائق مرتفعة الألياف.

تركيز النتروجين الكلي غير الأمونيا (T-NAN) كان أعلى في حالة العلائق مرتفعة البروتين عن العلائق منخفضة البروتين.

إنداد بناء البروتين الميكروبي عند التغذية على العلائق المرتفعة البروتين و منخفضة الألياف. يستنتج من هذا البحث أنه لم يحدث تأثير معاكس على النشاط الميكروبي في الكرش للأغنام المغذاة على علائق ذات مستوى بروتين لا يقل عن ١٣% ومستوى ألياف لا يزيد عن ٢١%.