IN SITU RUMINAL DEGRADATION KINETICS OF FORAGES AND FEED BYPRODUCTS IN MALE NILI-RAVI BUFFALO CALVES

M. Sarwar[1], S. Mahmood, W. Abbas and C. S. Ali[2]

Department of Animal Nutrition, University of Agriculture, Faisalabad, Pakistan

Summary

The rate and extent of digestion of dietary carbohydrates has a tremendous impact on ruminal fermentation and the productivity of the animals. The objective of the study was to determine the dry matter (DM) and neutral detergent fiber (NDF) degradabilities and rate and extent of feed byproducts (cotton seed cake, wheat bran), legumes [berseem (Egyptian clover), lucern (Medicago sativa), cowpeas (Vigna sinensis)], grasses [maize (Zea mays), millet (Panicum miliaceum), sorghum (Sorghum vulgare)] and wheat straw in ruminally fistulated male buffalo calves. By using nylon bags, 10 grams sample was exposed to the ruminal fermentation for 0, 1, 2, 4, 6, 10, 16, 24, 36, 48 and 96 hours. Dry matter and NDF degradability was measured at 48 hours. Extent of DM and NDF disappearance was determined at each time point. Rates of disappearance of DM and NDF were determined by regressing the natural logarithm of the percentage of original DM and NDF remaining in the bags between 1 and 96 hours. The dry matter digestibility (DMD) of the feed byproducts (FBP) and legume forages when incubated in the rumen of male buffalo calves were greater ($p < 0.05$) than grasses. Extent of digestion followed similar pattern as DMD. Rate of DMD was higher in FBP than in legumes and was the lowest in the wheat straw. The NDF degradability (NDFD) of FBP, legumes and grasses did not differ, however, wheat straw had the lowest NDFD from all the feeds tested. The lowest NDFD of wheat straw may have been due to the depressing effect of lignin on fiber digestion. The FBP and legumes had higher ($p < 0.05$) rates and lower extents of NDF digestion than grasses.

(**Key Words**: Ruminal, Degradation, Forages, Buffalo)

Introduction

The rate and extent of digestion of dietary carbohydrates has a tremendous impact on ruminal fermentation and the productivity of the animals (Varga and Whitesel, 1991). The fermentation rate of fiber is dependent upon the source of feedstuff (Varga and Hoover, 1987), species, maturity, and morphology (Cherney et al., 1990; Bowman et al., 1991; Weiss and Shockey, 1991; Cherney et al., 1993). The rate of neutral detergent fiber (NDF) digestion is negatively related to NDF content of forages stored at different DM (Nocek and Grant, 1987). This indicates that ruminal digestibility of NDF is not uniform across different forages.

The NDF content of forages is used to predict DM intake for ration formulation in dairy cattle. However, the NDF content of FBP is not as effective as forages (Sarwar et al., 1992). Briceno et al. (1987) have summarized the results of 20 experiments and concluded that the use of NDF to formulate dairy rations is limited to within roughage sources. That is why the results of using NDF to predict intake have been variable. This variable intake from comparable amounts of dietary NDF may be due to the difference in rate and extent of cell wall degradation of different forages.

The *in situ* (dacron bag) technique is widely used to determine the rate and extent of degradation of feeds because it is inexpensive (Van Keuren and Heinman, 1962; Weakley et al., 1983), simple, rapid and reproducible (Mehrez and Orskov, 1977). Information on the rate and extent of digestion of feed stuffs is used to assess their nutritive value. Information on the rate and extent of fermentation of commonly available forages,

[1] Address reprint requests to Dr. Sarwar, Department of Animal Nutrition, University of Agriculture, Faisalabad, Pakistan.

[2] Department of Physiology and Pharmacology, University of Agriculture, Faisalabad, Pakistan.

Received December 12, 1995
Accepted April 30, 1996

FBP and crop residues in Pakistan is limited. The use of feedstuffs of unknown nutritive value to formulate rations may be a wrong approach as it is neither beneficial for the farmers nor for the animals. The latest approach to fine-tune rations for greater performance is to feed a balance of ingredients of slow and fast digestible carbohydrates to get the maximum out of ruminal bugs (Nikolic et al., 1981). This can avoid uncoupling of essential nutrients at the ruminal level which consequently results into an effective and profitable livestock production. The objectives of the present project was to determine the lag time, rate and extent of NDF digestion and the slow and fast digestible feedstuffs in the rumen of buffalo calves.

Materials and Methods

Animals and diets

Two Nili-Ravi male buffalo calves, average weight 200 kg, fitted with ruminal cannulae were used to evaluate nine different feedstuffs *in situ*, in three batches. In the first batch, cotton seed cake, wheat bran and wheat straw were incubated in the rumen. During this period the animals were fed total mixed ration (50% concentrates plus 50% wheat straw) twice daily at one percent of body weight for each feeding. The concentrate consisted of 30% cotton seed cake and 70% wheat bran. In the second batch, berseem, lucern and cowpeas were incubated in the rumen and the animals were fed fresh berseem only twice daily, at the rate of one percent (on DM basis) of their body weight for each feeding. In the third batch, maize, millet and sorghum were incubated in the rumen and the animals were fed fresh grass only twice daily at the rate of one percent (on DM basis) of their body weigth per each feeding.

The animals were fed the same diets as were being incubated in their rumen. This was done to avoid the effects of diet on the ruminal fermentation of the feed stuffs (Clark and Davis, 1990, 1983 and Clark et al., 1981).

The animals were housed on a concrete floor in separate pens and the water was offered twice daily. For each batch, the animals were given 10 days of adaptation period to the diets at the start of the experiment followed by 4 days of incubation period for the *in situ* bags.

Sample preparation and analysis

The legumes (berseem, lucern and cowpeas) used in this experiment were harvested at mid bloom stage; the grasses (maize, millet and sorghum) were harvested at early heading stage; and the by-products (cotton seed cake, wheat bran and wheat straw) were purchased from the market. The legume and grass samples were first chopped in a locally manufactured chopper and then dried in the forced air oven at 55℃. All the feed samples (legumes, grasses and feed by products) were, then, ground through a Wiley mill (2-mm screen) and analyzed for DM, Nitrogen (AOAC, 1984), NDF and ADF (Van Soest et al., 1991). Chemical composition of all the feed samples is given in table 1.

TABLE 1. COMPOSITION OF FEED STUFFS

Items	Cottonseed cake	Wheat bran	Barseem	Lucern	Cowpeas	Maize	Millet	Sorghum	Wheat straw
	% DM								
CP	19.0	15.0	16.0	15.5	17.0	8.0	7.5	8.0	4.0
NDF	56.0	54.0	52.0	54.0	51.0	72.0	73.0	72.6	86.7
ADF	17.0	16.0	38.0	38.0	37.0	45.0	44.0	42.9	54.0
ADL	2.5	2.0	10.5	11.0	9.5	8.12	8.31	7.98	14.0
Cellulose	14.5	14.0	27.5	27.0	26.5	37.0	35.70	35.10	40.0
H. Cellulose	39.0	38.0	14.0	16.0	14.0	27.0	29.0	29.60	32.7

Incubation procedure

Nylon bags measuring 13 × 21 cm, with an average pore size of 50 μm, were used to determine the rate and extent of DM and NDF disappearance *in situ*. For each time point, 10 grams of each feed sample were weighed into bags, in triplicate. Two bags were used to determine DM and NDF disappearance and the third bag served as blank. The bags were closed and tied with braided nylon fishing line. To remove soluble and (or) 50 μm filterable material, the bags were soaked in specific amount of tap

water for 15 minutes, just before the ruminal incubation. Weight loss due to soaking was expressed as preruminal disappearance of DM. On day 11 of each experiment at 08:00, 3 bags for each fermentation time were placed in the rumen for 0, 1, 2, 4, 6, 10, 16, 24, 36, 48 and 96 hours, in reverse order and removed all at the same time. After removal from the rumen, bags were washed in running tap water until the rinse was clear. The bags were then dried in a forced air oven at 55°C for 48 hours. After equilibration with air for 8 hours, the bags were weighed back and the residues were transferred to 100 ml cups and stored for later analysis of NDF. Digestibility coefficients of DM and NDF were calculated at 48 hours of incubation. Rate and extent of DM and NDF disappearance was calculated at 96 hours, according to the procedure of Sarwar et al. (1991).

Statistical analysis

The data on each parameter (lag time, rate and extent of digestion of DM and NDF) were analysed according to Completely Randomized Design. The means were compared using Duncan's Multiple range test (Steel and Torrie, 1981).

Results and Discussion

Dry matter disappearance kinetics

The DM disappearance (DMD) coefficients of cotton seed cake, wheat bran, berseem, lucern and cowpeas were higher ($p < 0.05$) than those of maize, millet, sorghum and wheat straw (table 2). The reduced DMD of maize, millet and sorghum compared to cotton seed cake, wheat bran, berseem, lucern and cowpeas may be due to their higher NDF content. Earlier studies (Mertens, 1980, 1982, 1985, 1985; Varga and Hoover, 1987) have shown that the NDF contents were negatively correlated to the apparent digestibility of the forages. The higher DMD of cotton seed cake, wheat bran, berseem, lucern and cowpeas than maize, millet, sorghum and wheat straw could be attributed to their faster rate of disappearance (as DMD was measured at 48 hours). Glenn et al. (1989) reported a higher ($p < 0.05$) DMD of alfalfa hay than orchard grass hay fed to Holstein steer which was explained by the higher digestion rate of the former.

The extent of digestion (calculated at 96 hours) of the feedstuffs followed the same trend as DMD. The greater extent of digestion of cotton seed cake, wheat bran, berseem, lucern and cowpeas could be attributed to the quality of feedstuffs. Weiss and Shockey (1991) and Slabbert et al. (1992) reported that the extent of DMD *in vivo* decreased by lowering the quality of the diet fed to feedlot steers.

The rate of DMD disappearance was higher ($p < 0.05$) in cotton seed cake and wheat bran than in berseem, lucern and cowpeas (table 2). However, maize, millet and sorghum had a lower DM digestion rate than berseem, lucern and cowpeas. The wheat straw had the lowest rate of DM disappearance *in situ*. These results are in agreement with the study of Varga and Hoover (1987) in which legumes (alfalfa, 7.8%/h; clover, 6.2%/h) had a higher rate of DM disappearance than grasses (timothy, 5.8%/h; orchard grass, 5.5%/h). However, *in situ* or *in vitro* digestion rate give no information on residence time in the rumen and therefore, provide no direct estimate of nutrient availability (Nocek and Russell, 1988).

TABLE 2. *IN SITU* DRY MATTER DIGESTIBILITY (DMD), LAGTIME, RATE AND EXTENT OF DIGESTION OF FEED BY PRODUCTS, LEGUMINOUS AND NON-LEGUMINOUS AND WHEAT STRAW IN RUMINALLY CANNULATED BUFFALO MALE CALVES

Items	Feed by products		Leguminous forages			Grasses			Crop residue	SE
	Cotton-seed cake	Wheat bran	Bar-seem	Lucern	Cow-peas	Maize	Millet	Sor-ghum	Wheat straw	
DMD (%)*	73.60^{a}	72.56^{a}	76.69^{a}	74.26^{a}	77.00^{a}	60.46^{b}	54.52^{bc}	52.41^{c}	41.16^{d}	1.85
Lag (h)	0.11^{a}	0.09^{a}	1.18^{b}	1.55^{b}	1.40^{b}	2.80^{c}	3.01^{c}	3.05^{c}	3.95^{d}	0.12
Rate (%/h)	7.25^{a}	7.50^{a}	6.00^{b}	6.00^{b}	6.25^{b}	4.50^{c}	4.25^{c}	4.50^{c}	3.00^{d}	0.57
Extent (%)**	76.55^{a}	75.60^{a}	80.51^{a}	78.41^{a}	78.51^{a}	69.86^{bc}	69.67^{bc}	67.51^{c}	46.01^{d}	1.88

Means in the same row with different superscripts differ ($p < 0.05$).

* Drymatter digestibility was determined at 48 hours of incubation.

** Extent of digestion was determined at 96 hours of incubation.

Neutral detergent fiber disappearance kinetics

The NDF disappearance (NDFD) of cotton seed cake, wheat bran, berseem, lucern, cowpeas, maize, millet and sorghum were not different (table 3). The reason for similar NDFD of all feedstuffs except wheat straw may be longer incubation period (48 hours) which may have unabled to detect the expected differences due to the quality of different feeds. This notion is confirmed by the greater extent of digestion of maize, millet and sorghum. However, the lowest NDFD of wheat straw may be due to the depressing effect of lignin on fiber digestion (Jung and Fahey, 1983).

TABLE 3. *IN SITU* NEUTRAL DETERGENT FIBRE DIGESTIBILITY (NDFD), LAGTIME, RATE AND EXTENT OF DIGESTION OF FEED BY PRODUCTS, LEGUMINOUS AND NON-LEGUMINOUS AND WHEAT STRAW IN RUMINALLY CANNULATED BUFFALO MALE CALVES

Items	Feed by products		Leguminous forages			Grasses			Crop residue	SE
	Cotton-seed cake	Wheat bran	Bar-seem	Lucern	Cow-peas	Maize	Millet	Sor-ghum	Wheat straw	
NDFD (%)*	55.87[a]	54.74[a]	54.23[a]	52.50[a]	50.19[a]	56.49[a]	52.65[a]	50.51[a]	39.57[b]	1.91
Lag (h)	0.16[a]	0.15[a]	1.43[b]	1.84[b]	1.75[b]	3.05[c]	3.19[c]	3.21[c]	4.15[d]	0.12
Rate (%/h)	7.15[a]	7.50[a]	5.50[b]	5.10[b]	5.50[b]	4.25[c]	4.15[c]	4.22[c]	3.00[d]	0.31
Extent (%)**	60.69[a]	59.72[a]	54.19[a]	59.70[a]	58.55[a]	68.51[b]	65.50[b]	67.51[b]	44.90[c]	1.76

Means in the same row with different superscripts differ ($p < 0.05$).
* Neutral detergent fibre digestibility was determined at 48 hours of incubation.
** Extent of digestion was determined at 96 hours of incubation.

The cotton seed cake, wheat bran, berseem, lucern and cowpeas have higher rates and lower extent of NDF digestion than in maize, millet and sorghum. These results are in agreement with previous works (Varga and Hoover, 1987; Shaver et al., 1988; Buxton, 1989; Fisher et al., 1989; Grant and Mertens, 1992; Andrighetto et al., 1993) in which legumes have higher rates and lower extents of NDF digestion than grasses. Shaver et al. (1988) evaluated bromegrass and alfalfa and reported that fractional rate of NDF digestion *in situ* was higher (7.5%/h) and extent of digestion was lower (43.3%) in alfalfa than in bromegrass (4.3%/h; 61.7%). Generally extent of NDF digestion of legume is lower than that of grasses because of lower cell wall content and higher lignification of the former (Van Soest, 1994; Jung, 1989). Rate of cell wall digestion seems to be related to the anatomical and histological structure of plant tissues (Akin, 1979, 1989) or greater microbial colonization of legumes versus grasses (Bowman and Firkins, 1993). Firkins et al. (1991) evaluated carboxymethylcellulase (CMCase) activity as a marker of cellulolytic bacterial colonization and concluded that CMCase activity gave a quantitative assessment of mass of cellulolytic bacteria colonizing plant fiber. Using CMCase as an indicator of cellulolytic bacteria, it has been shown that cellulolytic bacteria, as a proportion of total particle associated bacteria, were attached in higher concentration to red clover than grasses (Gammagrass and Orchardgrass) during early (3-18 hours) incubation (Bowman and Firkins, 1993). This difference in colonization rate of forage particle by cellulolytic bacteria was implicated as a reason for more rapid rate of fiber degradation for legumes than in grasses. Huhtanen and Khalili (1992) also reported that the CMCase activity was highly correlated with disappearance rate of NDF *in situ*. This could be a possible explanation of why the legumes have a higher NDF digestion rate than grasses.

Conclusion

The DMD of the FBP and leguminous forages were similar but higher than that of grasses. However, the NDFD of the FBP and forages did not differ among themselves. It can be explained that the slowly degradable grasses had compensated their NDF disappearance due to the longer incubation period (48 hours), as is confirmed by the highest extent of digestion of grasses. An inverse relationship between lag time and rate of disappearance was observed in all feed stuffs. However, *In vivo* study needs to be conducted to estimate the nutrient availability of the feed stuffs because *in situ* experiments provide limited information on the ruminal residence time of the feed stuffs.

Literature Cited

Akin, D. E. 1979. Cell wall research to determine forage quality. Forage and geassland Conf. Amer. For Grassld. Council p. 29.

Akin, D. E. 1989. Histological and physical factors affecting digestibility of forages. Agron. J. 81:12.

Andrighetto, I., L. Baliloni, G. Coozi, H. F. Tolosa, D. Hartman, M. Hinds and D. Sapienza. 1993. Observation *in situ* degradation or forage cell components in alfalfa and Italin rye grass. J. Dairy Sci. 76:2624.

AOAC. 1984. Official methods of analysis (4th Ed.) Association of Analytical Chemists, Washington, DC. U.S.A.

Bowman, J. G. P., C. W. Hunt, M. S. Kerley and J. A. Patterson. 1991. Effect of grass maturity and legume substitution on large particle size reduction and small particle flow from the rumen of cattle. J. Anim. Sci. 69:369.

Bowman, J. G. P. and J. L. Firkins. 1993. Effect of forage species and particle size on bacterial cellulolytic activity and colonization *in situ*. J. Anim. Sci. 69:67.

Briceno, J. V., H. H. Van Horn, B. Harris, Jr. and C. J. Wilcox. 1987. Effects of neutral detergent fiber and roughage source on dry matter intake and milk yield and composition of dairy cows. J. Dairy Sci. 70:298.

Buxton, B. R. 1989. *In vitro* digestion kinetics of temperate perennial forage legume and grass stems. Crop Sci. 29:213.

Cherney, D. J. R., D. R. Mertens and J. E. Moore. 1990. Intake and digestibility by wethers as influenced by forage morphology at three levels of forage offering. J. Anim. Sci. 68:8387.

Cherney, D. J. R., J. H. Cherney and R. Lucey. 1993. *In vitro* digestion kinetics and quality of perennial grasses as influenced by forage quality. J. Dairy Sci. 76:790.

Clark, J. H. and C. L. Davis. 1990. Some aspects of feeding high producing dairy cows. J. Dairy Sci. 68:873.

Clark, J. H. and C. L. Davis. 1983. Future improvement of milk production potential for nutritional improvement. J. Anim. Sci. 57:750.

Clark, J. H., C. L. Davis and J. A. Rogers. 1981. Manipulation of rumen fermentation and its effects on performance of ruminants. Proc. 40th semi annual meeting, AFMA Nutrition. Published by American Feed Manufacturers Association. 1701 North Myer Drive, Arlington, Virginia.

Firkins, J. L., J. G. P. Bowman, W. P. Weiss and J. Naderer. 1991. Effects of protein, carbohydrate and fat sources on bacterial colonization and degradation of fiber *in vitro*. J. Dairy Sci. 74:4273.

Fisher, D. S., J. C. Burns and K. R. Pond. 1989. Kinetics of *in vitro* cell wall disappearance and *in vivo* digestion. Agron. J. 81:25.

Glenn, B. P., G. A. Varga, G. B. Huntington and D. R. Waldo. 1989. Duodenal nutrient flow and digestibility in holstein steers fed formaldehyde and formic acid treated alfalfa orchard grass silage at two intakes. J. Anim. Sci. 67:513.

Grant, R. J. and D. R. Mertens. 1992. influence of buffer pH and raw corn starch addition on *in vitro* fiber digestion kinetics. J. Dairy Sci. 71:2762.

Huhtanen, P. and H. Khalili. 1992. The effect of sucrose supplements on particle associated carboxymethylcellulase and xylonase activities in cattle given grass silage based diet. Brit. J. Nutr. 67:242.

Jung, N. G. and G. C. Fahey, Jr. 1983. Nutritional implication of phenolic monomers and lignin: A review. J. Anim. Sci. 57:206.

Jung, N. G. 1989. Forage lignin and their effects on fiber digestibility. Agron. J. 81:33.

Mehrez, A. Z. and E. R. Ørskov. 1977. A study of the artificial fiber technique for determining the digestibility of feeds in the rumen. J. Agric. Sci. (Camb.) 88:645.

Mertens, D. R. 1980. Fiber content and nutrient density in dairy ration. Proc. Distillers Feed Conf. 35:35.

Mertens, D. R. 1982. Using neutral detergent fiber to formulate dairy rations. pp. 116-126 in Proc. Ga. Nutr. Conf. for the Feed Industry. Athens: University of Georgia.

Mertens, D. R. 1985. Effect of fiber on feed quality for dairy cows. pp. 209-244 in Proc. 46th Minn. Nutr. Conf. St. Paul : University of Minnesota.

Mertens, D. R. 1985. Factors influencing feed intake in lactating cows: from theory to application using neutral detergent fiber. pp. 1-18 in Proc. 46th Ga. Nutr. Conf. Athens: University of Georgia.

Nikolic, J. A., Jovanoic, M. and Djordjevic, D. 1981. Influence of readily digestible carbohydrates on the utilization of ammonia and sulfur for protein synthesis in rumen contents. Acta Vet. (Beogred), 31:289.

Nocek, J. E. and A. L. Grant. 1987. Characterization of *in situ* nitrogen and digestion and bacterial nitrogen contamination of hay crop forages preserve at different dry matter percentages. J. Anim. Sci. 64:552.

Nocek, J. E. and J. P. Russell. 1988. Protein and energy as an integrated system. Relationship of ruminal

protein and carbohydrate availability to microbial synthesis and milk production. J. Dairy, Sci. 71:2070.

Sarwar, M., J. L. Firkins and M. L. Eastridge. 1991. Effect of replacing neutral detergent fiber of forage with soyhulls and corn gluten feed for dairy heifers. J. Dairy Sci. 74:1006.

Sarwar, M., J. L. Firkins and M. L. Eastridge. 1992. Effect of varying forage and concentrate carbohydrates on nutrient digestibilities and milk production by dairy cows. J. Dairy Sci. 75:1533.

Shaver, R. D., L. D. Satter and N. A. Jorgensen. 1988. Impact of forage fiber content on digestion and digesta passage in lactating cows. J. Dairy Sci. 71:1556.

Slabbert, N., J. P. Campher, T. Shelby, G. P. Khun and H. H. Meissner. 1992. The influence of dietary concentration and feed intake level on feedlot steers. 1. Digestibility of diets and rumen parameters. S. Afr. J. Anim. Sci. 6:101.

Steel, R. G. D. and J. H. Torrie. 1981. Principles and procedures of statistics. Biometerical Approach. International Student Ed. McGraw Hill International Book Co. London.

Van Keuren, R. W. and W. W. Heinman. 1962. Study of a nylon bag technique for *in vivo* estimate of forage digestibility. J. Anim. Sci. 21:340.

Van Soest, P. J. 1994. Nutritional ecology of the ruminants. O and B Books, Inc. Corrvalis, OR.

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

Varga, G. A. and W. H. Hoover. 1987. Rate and extent of neutral detergent fiber degradation of feedstuffs *in situ*. J. Dairy Sci. 66:2109.

Varga, G. A. and T. J. Whitesel. 1991. Effect of nonstructural to structural carbohydrate ratio on rate and extent of nutrient utilization *in situ*. Anim. Feed Sci. Technol. 32:275.

Weakley, D. C., M. D. Stern and L. D. Satter. 1983. Factors affecting disappearance of feedstuffs from bags suspended in the rumen. J. Anim. Sci. 56:493.

Weiss, W. P. and W. L. Shockey. 1991. Value of orchardgrass and alfalfa silages fed with varying amounts of concentrates to dairy cows. J. Dairy Sci. 74:1933.