Indian Journal of Animal Sciences **80** (10): 993–97, October 2010

Effects of rumen protected fat on *in vitro* dry matter degradability of dairy rations

S SAIJPAUL[1], P K NAIK[2] and NEELAM RANI[3]

Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab 141 004 India

Received: 10 January 2009; Accepted: 26 July 2010

ABSTRACT

Rumen protected fat (PF) was incorporated in 12 concentrate mixtures (CM_1-CM_{12}) and total mixed rations (TMR_1-TMR_{12}) to study the effect of different levels of PF in dairy rations and the effect of urea in dairy rations containing limited grains and high PF on *in vitro* dry matter degradability (IVDMD) and *in vitro* fermentation. The CM_1 contained 6% natural fat and no PF whereas, in CM_2, CM_3, CM_4, CM_5, CM_6 and CM_7, the natural fat was replaced @ 30, 40, 50, 60, 70 and 80% by PF, respectively. The CM_8-CM_{12} contained limited cereal grains (5–10%), high deoiled rice polish (50–60%) without (CM_8-CM_{10}) or with 1% urea (CM_{11}-CM_{12}), in which 80% natural fat was replaced by PF to increase the fat content. The 12 total mixed rations (TMR_1-TMR_{12}) contained respective CMs (CM_1-CM_{12}) and green sorghum in 50: 50 ratios on DM basis. Increasing the deoiled ingredients in CM_1-CM_7 increased the CP and NDF content but, IVDMD remained similar. Limited grain, high PF based CMs (CM_8–CM_{12}) with or without urea had comparable chemical composition but, IVDMD was lower in urea containing CMs. The chemical composition of TMR_1-TMR_7 showed significant variation and lower IVDMD when PF replaced more than 40% natural fat. The IVDMD in TMR_8-TMR_{12} showed inconsistent variations. No difference (P>0.05) in *in vitro* fermentation (TVFA, TN, TCA-N, NPN and NH_3-N) was observed between CM_1-CM_7, CM_8-CM_{12}, TMR_1-TMR_7 and TMR_8-TMR_{12}. It was concluded that the PF can substitute up to 40% of the natural fat of the CMs (6% natural fat) contained in TMRs (50: 50, R: C). In rations with limited grain (5–10%) and high PF, 1% urea can reduce IVDMD.

Key words: Concentrate mixture, Fermentation, IVDMD, Low grain, Protected fat, Total mixed rations

High yielding dairy animals need energy dense rations for optimum productivity, in which cereals and fat play crucial role. As cereals are mostly consumed by human beings and monogastric animals and recently being used for production of biofuel, the alternate source of energy in dairy ration is supplemental fat. Generally, inclusion of fat in dairy ration is limited to 3% of dry matter (DM) intake (Palmquist 1988) as beyond this, DM and fibre digestibilities are reduced (Chalupa *et al*. 1986). However, if supplementation of fat is through rumen protected fat (PF), the fat content of the ration can be increased up to 6–7% of the DM intake (NRC 2001). Calcium salt of long chain fatty acids (Ca-LCFA) can be used in ruminants ration as PF to increase the energy density of the ration without adversely affecting the DM intake and digestibility (Naik *et al*. 2009a). Besides, as per BIS specifications, 1% urea is permissible in cattle feed containing 10% easily available carbohydrate source. Compounded cattle feeds available in the market mostly contain limited (5–13%) grains (Shelly *et al*. 2008) and consequently, bulk of the ration contains low energy deoiled rice polish (Dhakad and Garg 1998).

The effect of higher level of PF (Ca-LCFA) as a means to increase the energy density of deoiled ingredient based concentrate mixtures (CMs) and total mixed rations (TMRs) needs to be explored. Therefore, this study was executed with 2 objectives, first to find out the effect of different levels of PF in dairy rations and second the effect of urea in dairy rations containing limited grains and high PF on *in vitro* dry matter degradability (IVDMD) and *in vitro* fermentation.

MATERIALS AND METHODS

Preparation of rumen protected fat

The PF i.e. calcium salts of long chain fatty acids (Ca-LCFA) was prepared as per Naik *et al*. (2007a,b). Concentrated H_2SO_4 (120 ml) in 500 ml tap water was mixed in 4 kg hot rice bran fatty acid oil (RBO). After a few minutes, when effervescence had almost subsided, 1.6 kg of technical grade calcium hydroxide dissolved in 10 liters of water was added and boiled for 30 min without cover on medium heat. When the PF became granular and non-sticky, it was filtered through cloth with repeated washings under running tap water and then sun dried. The PF was kept in airtight containers in

Present address: [1]Nutritionist, Department of Animal Nutrition (e mail: sksaijpaul@redffmal.com); [2]Animal Scientist, Directorate of Extension Education; [3]Department of Animal Nutrition.

a cool place after mixing with butylated hydroxy toluene (BHT) @ 0.5 g/kg as an antioxidant (Naik *et al*. 2009 [b]).

Preparation of concentrate mixtures (CM) and total mixed rations (TMR)

Twelve CMs (CM_1-CM_{12}) were prepared using feed ingredients ground to pass through 1 mm sieve size (Table 1). The CM_1 to CM_7 specially designed for animals having 450–500 kg body weight (BW) with daily milk yield of 15–20 kg, contained grain 36% and deoiled rice polish 15%. The CM_1 contained natural fat from feed sources and no PF (Ca-LCFA) whereas in CM_2, CM_3, CM_4, CM_5, CM_6 and CM_7, the natural fat was replaced @ 30, 40, 50, 60, 70 and 80% by PF, respectively. The CM_8–CM_{12} contained limited cereal grains (5–10%), high deoiled rice polish (50–60%) without (CM_8–CM_{10}) or with 1% urea (CM_{11}-CM_{12}), in which 80% natural fat was replaced by PF to increase the fat content.

Twelve TMRs (TMR_1-TMR_{12}) were also prepared in which the respective CMs (CM_1-CM_{12}) were mixed with green sorghum in 50: 50 ratios on DM basis.

Preparation for in vitro *fermentation*

Equal proportions (250 ml) of rumen liquor as inoculums was collected in the morning (9.0–9.30 AM), 2 h post feeding from 3 fistulated buffaloes (400 kg BW) fed 6.5 kg wheat straw (WS), 2 kg green sorghum and 3.5 kg CM (maize grain 35, rice bran 20, deoiled rice bran 12, mustard cake 20, soybean meal 10, mineral mixture 2 and salt 1 part by weight), maintaining roughage: concentrate ratio 65: 35. Exactly 500 mg sample (CM/TMR) was incubated at 39 °C with 40 ml buffer (Mc Dougall 1949) and 10 ml strained rumen liquor (SRL) in an *in vitro* fermentation system (Tilley and Terry 1963) in triplicate. After 48 h of incubation, the fermentation was arrested using 1 ml mercuric chloride (5%) and 2 ml Na_2CO_3 (1N). The tubes were centrifuged and the supernatant was collected for analysis for total volatile fatty acids (TVFA), ammonia nitrogen (NH_3–N), total nitrogen (TN), TCA-precipitable nitrogen (TCA-N) and non-protein nitrogen (NPN). All the samples were stored at –20°C till analyzed. The residues were incubated with 50 ml of 0.2% pepsin in 0.1 N HCl for further 48 h at 39°C with intermittent shaking. The residues were filtered through pre-weighed sintered glass crucible, dried at 100°C for 24 h and weighed after cooling in the desiccator to determine the IVDMD.

Analytical procedures

All the CMs and TMRs were analyzed for proximate analysis (AOAC 2000). The method of Van Soest *et al.* (1991) with 2% diastase (Alderman 1985) was used to determine the cell wall constituents (NDF and ADF). The EE content of the CMs and TMRs containing PF were determined after acid (6N-HCl) hydrolysis. The TVFA content of the supernatant was determined as per Bernett and Reid (1957) in a Markham distillation apparatus. Conway (1957) method was used for the determination of NH_3–N. The TN was estimated by the micro Kjeldahl digestion method described in AOAC (2000). The TCA-N of the supernatant was estimated by the method provided by Cline *et al*. (1958). The NPN was calculated as the difference between the TN and TCA-N.

Data were analyzed for one way ANOVA with Tukey's-b test (Snedecor and Cochran 1994) to find out the test of significance for different levels of PF (CM_1-CM_7, TMR_1-TMR_7) and effect of urea (CM_8–CM_{12} and TMR_8–TMR_{12}) in dairy rations.

RESULTS AND DISCUSSION

The CP of the CMs (CM_1-CM_7) ranged from 20.34 to 21.66 (Table 2). There was general variation in the CP, NDF

Table 1. Physical composition of the concentrate mixtures

Ingredients	Concentrate mixture (%)											
	CM_1	CM_2	CM_3	CM_4	CM_5	CM_6	CM_7	CM_8	CM_9	CM_{10}	CM_{11}	CM_{12}
Wheat	26.0	26.0	26.0	26.0	26.0	26.0	26.0	5.0	5.0	–	5.0	–
Maize	10.0	10.0	10.0	10.0	10.0	10.0	10.0	5.0	–	5.0	–	5.0
Mustard cake (MC)	20.0	20.0	20.0	15.0	10.0	5.0	–	–	–	–	–	–
Deoiled MC				5.0	10.0	15.0	20.0	11.0	12.0	12.0	15.0	15.0
Soybean meal	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	–	–
Wheat bran	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0
Rice bran	15.0	5.0										
Deoiled rice polish	–	10.0	15.0	15.0	15.0	15.0	15.0	50.0	54.0	54.0	60.0	60.0
Urea											1.0	1.0
Mineral mixture	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Salt	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0

CM_2, CM_3, CM_4, CM_5, CM_6 and CM_7: The natural fat was replaced @ 30, 40, 50, 60, 70 and 80% by PF, respectively.
CM_8–CM_{12}: The natural fat was replaced @ 80% by PF.

Table 2. Chemical composition, *in vitro* dry matter degradability and fermentation of the concentrate mixtures (CM)

Parameters	CM_1	CM_2	CM_3	CM_4	CM_5	CM_6	CM_7	SEM	P
Chemical composition (on% DM basis)									
OM	90.37^{a}	91.75^{b}	91.84^{b}	91.74^{b}	92.17^{b}	91.48^{b}	91.41^{b}	0.135	0.001
CP	20.34^{a}	21.44^{bc}	21.44^{bc}	20.78^{ab}	21.66^{c}	21.00^{abc}	21.22^{bc}	0.108	0.001
EE	5.95	6.10	5.95	6.10	6.10	5.90	6.00	0.024	0.087
NDF	31.09^{a}	32.62^{a}	30.76^{a}	32.4^{a}	32.73^{a}	37.06^{b}	36.34^{b}	0.560	<0.001
ADF	12.01^{ab}	12.81^{ab}	12.38^{ab}	11.48^{a}	13.64^{b}	11.73^{ab}	12.95^{ab}	0.207	0.037
Cellulose	8.12	9.01	9.47	7.58	9.10	8.61	9.82	0.233	0.116
In vitro degradability and fermentation									
IVDMD (%)	71.27	72.47	72.00	73.00	73.10	75.13	75.70	0.463	0.067
T VFA (meq/dl)	8.30	7.03	8.40	8.22	8.90	8.59	8.57	0.257	0.627
TN (mg/dl)	30.61	31.08	31.45	30.33	30.29	29.35	30.29	0.264	0.528
TCA-N (mg/dl)	15.87	16.38	16.75	16.05	17.03	15.82	16.38	0.274	0.921
NPN (mg/dl)	14.75	14.70	14.70	14.28	13.25	13.53	13.91	0.211	0.322
NH_3–N (mg/dl)	13.53	11.48	12.60	12.60	11.29	12.23	12.41	0.393	0.843

*P<0.05; Figures with different superscripts in a row differ significantly.
CM_2, CM_3, CM_4, CM_5, CM_6 and CM_7: The natural fat was replaced @ 30, 40, 50, 60, 70 and 80% by PF, respectively.

Table 3. Chemical composition, *in vitro* dry matter degradability and fermentation of concentrate mixtures (CM) containing less grain with and without urea

Parameters	CM_8	CM_9	CM_{10}	CM_{11}	CM_{12}	SEM	P
Chemical composition (on% DM basis)							
OM	87.02	87.28	87.46	87.54	87.96	0.235	0.833
CP	21.00	21.22	21.88	21.22	21.66	0.123	0.120
EE	5.90	6.30	6.10	6.05	5.85	0.075	0.207
NDF	40.04	40.28	43.3	43.21	41.48	0.578	0.213
ADF	18.29	17.18	17.96	18.16	18.90	0.253	0.304
Cellulose	12.88	11.56	12.55	11.24	12.63	0.271	0.213
In vitro degradability and fermentation							
IVDMD (%)	72.27^{c}	70.60^{bc}	71.87^{c}	67.27^{ab}	66.03^{a}	0.750	0.002
T VFA (meq/dl)	5.24	5.37	5.62	5.27	5.20	0.167	0.958
TN (mg/dl)	29.82	29.73	30.10	29.96	29.96	0.114	0.903
TCA-N (mg/dl)	14.56	14.09	14.56	14.28	13.77	0.162	0.543
NPN (mg/dl)	15.26	15.63	15.54	15.68	16.19	0.140	0.347
NH_3–N (mg/dl)	13.91	13.81	14.19	14.09	14.28	0.221	0.974

*P<0.05; Figures with different superscripts in a row differ significantly.
CM_8–CM_{12}: The natural fat was replaced @ 80% by PF.

and ADF content due to replacement of rice bran by deoiled rice polish, and mustard cake by deoiled mustard cake. The EE content of all the CMs was similar. There was no difference in IVDMD, TVFA, TN, TCA-N, NPN and NH_3–N of CMs from CM_1 to CM_7. This clearly indicated that with sufficient grain, replacement of fat from feed ingredients by PF did not influence the IVDMD and *in vitro* fermentation.

The chemical composition, IVDMD and *in vitro* fermentation of CMs (C_8–C_{12}) containing limited grains and high level of deoiled ingredients and PF with or without urea are presented in Table 3. The CP, EE and cell wall fractions in all the CMs remained similar probably due to the high level of deoiled ingredients. There was no change in IVDMD from CM_8 to CM_{10} but decrease (P<0.05) in IVDMD was observed in urea based rations (CM_{11} to CM_{12}), which were similar. This revealed that in CMs with limited grain, urea can be utilized as nitrogen source only in the presence of easily fermentable carbohydrate. *In vitro* fermentation data indicated no difference in TVFA, TN, TCA-N, NPN and NH_3–N in a limited grain (5–10%) with or without urea based CMs (CM_8–CM_{12}).

There was variation in the CP content of the TMRs (TMR_1-TMR_7) but EE, NDF, ADF and cellulose content remained similar (Table 4). The IVDMD of the TMR_4, TMR_5, TMR_6 and TMR_7 reduced (P<0.05) in comparison to TMR_1,

Table 4. Chemical composition, *in vitro* dry matter degradability and fermentation of total mixed rations (TMR)

Parameters	TMR_1	TMR_2	TMR_3	TMR_4	TMR_5	TMR_6	TMR_7	SEM	P
Chemical composition (on% DM basis)									
OM	90.38^{c}	90.99^{d}	91.36^{e}	91.49^{e}	91.92^{f}	88.40^{a}	89.59^{b}	0.257	<0.001
CP	15.22^{ab}	15.66^{b}	14.74^{a}	14.74^{a}	15.66^{b}	15.66^{b}	15.66^{b}	0.109	0.006
EE	4.25	4.50	4.50	4.40	4.50	4.55	4.55	0.038	0.373
NDF	54.67	56.79	55.51	57.63	55.08	55.61	52.39	0.468	0.053
ADF	27.08	28.76	28.06	28	27.44	25.51	27.15	0.379	0.416
Cellulose	21.28	20.87	21.07	20.78	19.05	18.04	18.98	0.467	0.395
In vitro degradability and fermentation									
IVDMD (%)	76.27^{b}	74.00^{b}	76.00^{b}	68.00^{a}	68.93^{a}	68.07^{a}	67.57^{a}	0.842	<0.001
T VFA (meq/dl)	6.62	5.87	5.87	5.83	6.07	5.77	5.70	0.089	0.060
TN (mg/dl)	24.08	24.64	24.64	24.41	24.78	23.85	23.75	0.274	0.950
TCA-N (mg/dl)	10.36	11.00	10.59	10.83	11.81	11.01	10.41	0.280	0.878
NPN (mg/dl)	13.72^{abc}	13.31^{c}	14.05^{bc}	13.58^{abc}	12.97^{ab}	12.83^{a}	13.35^{abc}	0.132	0.010
NH_3–N (mg/dl)	11.29	11.26	12.23	11.29	10.83	10.83	11.29	0.414	0.989

*P<0.05; Figures with different superscripts in a row differ significantly.

TMR_2, TMR_3, TMR_4, TMR_5, TMR_6 and TMR_7: The natural fat of the respective CMs was replaced @ 30, 40, 50, 60, 70 and 80% by PF, respectively.

Table 5. Chemical composition, *in vitro* dry matter degradability and fermentation of total mixed rations (TMR) containing less grain with and without urea

Parameters	TMR_8	TMR_9	TMR_{10}	TMR_{11}	TMR_{12}	SEM	P
Chemical composition (on% DM basis)							
OM	88.11^{a}	88.09^{a}	88.86^{b}	88.84^{b}	89.0^{b}	0.117	0.001
CP	15.58	14.74	15.66	14.74	14.74	0.141	0.020
EE	4.50	4.25	4.55	4.55	4.50	0.048	0.255
NDF	57.11	58.39	59.24	54.59	55.92	0.681	0.192
ADF	25.93^{a}	26.84^{ab}	29.28^{b}	26.03^{a}	26.47^{a}	0.499	0.184
Cellulose	18.03	18.64	19.73	17.96	18.56	0.434	0.770
In vitro degradability and fermentation							
IVDMD (%)	66.40^{ab}	64.93^{a}	67.87^{ab}	66.40^{b}	64.67^{a}	0.364	0.008
TVFA (meq/dl)	5.68	5.73	5.80	5.60	5.40	0.054	0.145
NH_3–N (mg/dl)	11.29	11.29	10.36	11.29	11.76	0.414	0.910
TN (mg/dl)	24.08	23.24	23.85	23.75	24.08	0.156	0.470
TCA-N (mg/dl)	11.25	10.59	11.81	11.62	11.53	0.250	0.642
NPN (mg/dl)	12.83	12.65	12.04	12.13	12.55	0.162	0.529

*P<0.05; Figures with different superscripts in a row differ significantly.

$TMR_{8–12}$: The natural fat of the CM was replaced @ 80% by PF.

TMR_2 and TMR_3 with the supplementation of PF suggesting that replacement of fat from feed ingredients with PF may be done only up to 40% in the CM used in dairy ration. Tangendjaja *et al.* (1993) also reported significant reduction in IVDMD with increase in the level of PF. The TVFA, TN, TCA-N, NPN and NH_3–N remained alike. Similarly, Ohajuruka *et al*. (1991) and Garg (1998) observed no change in the NH_3–N concentration in dairy ration supplemented with Ca soap.

There was no variation in the OM, CP, EE, NDF, ADF and cellulose content of the TMR_8–TMR_{12} (Table 5). The IVDMD exhibited inconsistent variation being lower in TMR_9 and TMR_{12} in comparison to TMR_8, TMR_{10} and TMR_{11}, which could not be explained. Previous reports indicated that grain less rations did not have adverse effect on the digestibility of nutrients *in vivo* (Sahoo *et al*. 2000, Mili *et al*. 2003). The TVFA, TN, TCA-N, NPN and NH_3–N remained unchanged. The present findings revealed that when the CMs contained sufficient grain, the replacement of natural fat by PF had no effect on IVDMD and in fermentation (Table 2), however, the IVDMD was affected in TMRs when more than 40% of the natural fat in its CMs was replaced by PF (Table 4). Further, CMs with limited grains and high PF, 1% urea lowered IVDMD than similar rations without urea. The NH_3–N, TN and TCA-N of the CMs and TMRs were not influenced by the PF supplementation.

It was concluded that the PF can substitute up to 40% of the natural fat of the CMs (6% natural fat) contained in TMRs (50: 50, R: C). In rations with limited grain (5–10%) and high PF, 1% urea can reduce IVDMD.

ACKNOWLEDGEMENT

The authors are thankful to the Indian Council of Agricultural Research (ICAR), New Delhi, India for providing financial support in the form of ad-hoc research project to conduct the study.

REFERENCES

Alderman G. 1985. Predicting energy value of compound feeds. *Recent Advances in Animal Nutrition*. pp 3–52. (Eds) Haresign W and Cole D J A. Butterworths, London.

AOAC. 2000. *Official Methods of Analysis of AOAC International*, 17th ed. AOAC International, Maryland.

Bernett A J G and Reid R L. 1957. Studies on the production of volatile fatty acids from grass by rumen liquor in an artificial rumen. 1. The volatile fatty acids production from grass. *Journal of Agricultural Science* **48**: 315–21.

Chalupa W, Vecchiarelli B, Elser A E and Kronfeld D S. 1986. Ruminal fermentation *in vivo* as influenced by long chain fatty acids. *Journal of Dairy Science* **69**: 1293.

Cline J H, Hershberger T V and Bentley O G. 1958. Utilization and/or synthesis of valeric acid during the digestion of glucose, starch and cellulose by rumen micro-organisms *in vitro*. *Journal of Animal Science* **17**: 284–92.

Conway E J. 1957. *Micro-diffusion analysis and volumetric error*, 4th edn. Crosby Lockwood, London.

Dhakad A R and Garg A K. 1998. Effect of replacing maize grain with wheat bran in the concentrate mixtures on intake and utilization of nutrients in sheep. *Indian Journal of Animal Nutrition* **15**: 250–54.

Garg M R. 1998. Effect of feeding bypass fat on rumen fermentation, DM digestibility and N balance in sheep. *Indian Veterinary Journal* **75**: 800–02.

Mc Dougall E I. 1948. Studies on ruminant saliva. I. The composition and output of sheep's saliva. *Biochemical Journal* **43**: 99–109.

Mili R M S, Amonge T K, Baruah K K and Mili D C. 2003. Growth performance of crossbred female calves on low grain and grainless diets. *Indian Veterinary Journal* **80**: 437–40

Naik P K, Saijpaul S and Rani Neelam. 2007[a]. Evaluation of rumen protected fat prepared by fusion method. *Animal Nutrition and Feed Technology* **7**: 95–101.

Naik P K, Saijpaul S and Rani Neelam. 2007[b]. Preparation of rumen protected fat and its effect on nutrient utilization in buffaloes. *Indian Journal of Animal Nutrition* **24**: 212–15.

Naik P K, Saijpaul S and Rani Neelam. 2009[a]. Effect of ruminally protected fat on *in vitro* fermentation and apparent nutrient digestibility in buffaloes. *Animal Feed Science and Technology* (In press).

Naik P K, Saijpaul S, Sirohi A S and Raquib M. 2009[b]. Lactation response of cross bred dairy cows fed on indigenously prepared rumen protected fat – a field trial. *Indian Journal of Animal Science* **79** (In press).

NRC. 2001. *Nutrient Requirements for Dairy Cattle*. National Research Council, Washington, D.C.

Ohajuruka O A, Zhigou W U and Palmquist D L. 1991. Ruminal metabolism, fibre and protein digestion by lactating dairy cows fed calcium soap or animal vegetable fat. *Journal of Dairy Science* **74**: 2601–09.

Palmquist D L. 1988. Using rumen inert fats in dairy diets. *Proceedings of Pacific North West Nutrition Conference*. Page 71 Spokane, W A.

Sahoo A, Chaudhary L C, Agarwal N, Kamra D N and Pathak N N. 2000. Performance of crossbred cows fed on wheat straw based grain less diet. *Indian Journal of Animal Nutrition* **17**: 189–94.

Shelly M, Saijpaul S and Naik P K. 2008. Prediction of grain content of compounded feeds. *Animal Nutrition and Feed Technology* **8**: 97–104.

Snedecor G W and Cochran W G. 1994. *Statistical Methods*. 8th ed. Oxford and IBH Publishing Co. Calcutta, India.

Tangendjaja Budi, Santoso Budi and Wina Elizabeth. 1993. Protected fat: Preparation and digestibility. Proceedings of workshop on advances in small ruminant research in Indonesia, pp. 165–78. August 3–4, 1993, Ciawi, Indonesia.

Tilley J M A and Terry R A. 1963. A two stage technique for the *in vitro* digestion of forage crop. *Journal of British Grass Land Society* **18**: 104–11.

Van Soest P J, Roberson J B and Lewis V A. 1991. Methods for dietary fiber, neutral detergent fiber and non starch polysaccharides in relation to animal nutrition. *Journal of Dairy Science* **74**: 3583–97.