The influence of conservation methods on digestion and utilization of forages by ruminants

By P. McDonald and R. A. Edwards, *Edinburgh School of Agriculture, West Mains Road, Edinburgh EH9 3JG*

Biochemical changes during conservation

Drying. During field drying the cut crop respires and sugars, malate, citrate and succinate are oxidized (Sullivan, 1973). Plant protease activity increases the soluble nitrogenous components, peptides, amides and amino acids, with marked increases in proline, glutamine and asparagine (Kemble & Macpherson, 1954). The extent of these changes is influenced by the length of the drying period, which is dependent upon the plant resistances, environment and mechanical treatment. In barn-drying, baling is carried out earlier and oxidation and nutrient losses are reduced. In high-temperature drying of forage, biochemical changes are minimal and the composition of the dried herbage is almost identical to that of the original material.

Ensiling. The fermentations in the silo can be classified broadly into five types: lactate, acetate, butyrate, wilted and chemically restricted. The lactate type of silage, in which the lactic acid bacteria have dominated the fermentation, is characterized by a low pH (*c.* 3·7–4·2), a high concentration of lactate (*c.* 80–120 g/kg dry matter (DM)), with smaller amounts of formic, acetic, propionic and butyric acids (Table 1). Variable quantities of mannitol and ethanol derived from the activities of heterofermentative lactic acid bacteria and yeasts are present. The nitrogen fraction of lactate silage is mainly in a non-protein, soluble form with low concentrations (<12%) of ammonia-N. Residual water-soluble carbohydrates (WSC) in the DM are low (usually <2%. The buffering capacity (Bc) within the pH range 4–6 is about three to four times that of the original herbage.

Under certain conditions, acetic acid-producing bacteria may dominate the fermentation. Such acetate-type silages have been reported by Catchpoole (1972) working with tropical grass and Henderson & McDonald (1975) in the UK. Apart from low lactate and high acetate values the fermentation characteristics appear to be similar to those of lactate silages.

The conditions under which butyrate-type silages are produced have been well defined (McDonald & Whittenbury, 1973). Lactic acid and residual WSC are converted to butyric acid, resulting in silages with pH values normally between 5 and 6. Proteolytic clostridia are usually active; decarboxylation of amino acids leads to the formation of amines, while deamination results in free ammonia, keto-acids and higher volatile fatty acids (VFA) (Table 1).

Table 1. *Typical compositions of grass silages produced by five different types of fermentation*

| | Silage type | | | | |
	Lactate	Butyrate	Acetate	Wilted	Chemically restricted*
pH	3·9	5·2	4·8	4·2	5·1
Dry matter (DM) (g/kg)	190	170	176	308	212
Bc†	1120	nd	1090	890	560
DM composition					
Protein-nitrogen (g/kg total N)	235	353	440	289	740
Ammonia-N (g/kg total N)	78	246	128	83	30
Lactic acid (g/kg)	102	1	34	59	26
Acetic acid (g/kg)	36	24	97	24	10
Butyric acid (g/kg)	1	35	2	1	1
Water-soluble carbohydrates (g/kg)	10	6	3	48	133
Mannitol (g/kg)	41	nd	2	36	nd
Ethanol (g/kg)	12	nd	8	6	4

nd, not determined.

*Treated with formalin–formic acid (3:1, w/w) mixture, 10 g/kg.
†Buffering capacity, mequiv./kg DM.

Prewilting restricts fermentation increasingly as DM content increases. In such wilted silages clostridial activity is minimal but some growth of lactic acid bacteria usually occurs, even in silages wilted to 500 g DM/kg. Total fermentation acids and Bc are reduced but the composition of the N fraction is similar to that in lactate silages.

In recent years, chemically restricted silages have been produced using formic acid and formaldehyde. When applied at the normal commercial level (2·3 g/kg fresh herbage), formic acid does not inhibit completely the growth of the lactic acid bacteria (Woolford, 1975), complete inhibition requiring about two to three times this concentration (Henderson & McDonald, 1971). In studies with formalin Wilkins, Wilson & Woolford (1974) showed that a minimum application of 4·9 g/kg to ryegrass (*Lolium perenne* L.) was necessary to achieve satisfactory preservation. Formalin is more effective as a fermentation inhibitor when applied with an acid (Wilson & Wilkins, 1975). The effect of a formalin–formic acid mixture (3:1, w/w) applied to ryegrass at 10 g/kg is shown in Table 1. Typical results in such treated silages include low levels of fermentation acids, low Bc values, low proportions of non-protein-N and high residual WSC.

Of the fermentation characteristics of these five types of silage, the concentrations of fermentation acids, residual WSC and N components are particularly relevant to the ruminant. Table 2 summarizes our data on N components of silages. There is little difference in the proportions of protein-N between lactate, butyrate and wilted silages. Where formic acid is added directly to forage harvested grass, some inhibition of proteolysis occurs, but when applied

after wilting the effect on proteolysis is reduced. The inhibiting action of formaldehyde on protein breakdown is clear.

Table 2.   *Nitrogenous components of sixty-two silages (g/kg total nitrogen)*

Silage	No. of samples	Protein-N		Ammonia-N	
		Mean	SE	Mean	SE
Lactate	26	371	17·0	95	5·5
Butyrate	4	354	31·8	242	36·7
Wilted	13	357	21·1	71	4·8
Chemically restricted					
Formic (direct)	7	487	18·3	93	15·7
Formic (wilted)	4	359	45·2	62	3·6
Formaldehyde	8	645	32·5	38	5·8

Nutritive value of conserved forages

Gross energy (GE). Conservation by drying results in little change in energy concentration. Morgan (personal communication) quoted a mean GE value of 18·5 MJ/kg DM for forty-seven hay samples and 18·4 MJ/kg DM for twenty-six high-temperature-dried grasses. The more extensive changes associated with ensilage result in increases in GE. Alderman, Collins & Dougall (1971) reported a mean GE value of 20·2 MJ/kg DM for forty-five grass silages and in our own studies mean GE values of 18·4, 20·0, and 19·1 MJ/kg DM were obtained for twenty-three grasses, eighteen lactate silages and seven wilted silages. Consideration of the metabolic pathways of fermentation suggests that the increases in GE are due to losses of DM without concomitant losses of energy (McDonald, Henderson & Ralton, 1973).

Metabolizable energy (ME). Of the energy deductions (faecal, urinary, methane) made from GE in the calculation of ME, faecal energy is the greatest. During haymaking digestible carbohydrates are dissipated, the proportions of indigestible cell wall components increase (Jarrige, Demarquilly & Dulphy, 1974) and hays have lower digestibilities than the original herbage. Thus Shepperson (1960) quotes values of 0·725, 0·691 and 0·644 respectively for the organic matter (OM) digestibilities of nine cuts of frozen grass, barn-dried hay and swath-made hay. A slight reduction in digestibility of OM has been reported for high-temperature-dried grass by Blaxter (1973) but Demarquilly & Jarrige (1970) found digestibilities of twenty-one samples of dried grass were similar to those of the original herbage. The effects of silage fermentation on digestibility are generally considered to be small (Demarquilly & Jarrige, 1970), although some workers (Castle & Watson, 1970; Wilson & Wilkins, 1973) have obtained higher digestibilities of formic acid-treated silages than untreated butyrate silages. Brown & Valentine (1972) obtained significant reductions in DM digestibilities when formalin was used at >20g/kg. In our own studies with sheep over a period of years, the DM digestibilities of thirty-six different silages and the grasses from which they were made were 0·768 and

0·767, respectively. Methane losses on fresh grass diets have been reported by Ekern, Blaxter & Sawers (1965) to be equivalent to 7·5% of the GE while Blaxter & Graham (1956) and Armstrong (1964) reported methane losses on dried grass diets ranging from 7·3 to 9·2%. Ekern & Sundsøl (1974) found methane losses at maintenance of 8·1 and 8·3% of GE for two hays and 7·5 and 7·5% for two silages made from the same parent material. Smith, Wainman & Dewey (1975) quote methane losses of 8·6 and 7·7% for a silage fed at maintenance and twice maintenance, while Wainman (1976) found mean values of 7·2 and 7·5% for sixteen silages fed with oats and barley, respectively. It would appear that high-temperature drying, haymaking or ensiling has no significant effect on the magnitude of energy losses as methane.

Urinary energy losses on diets of grass products range from 3·5 to 8·3% of the GE (Blaxter & Graham, 1956; Armstrong, 1964; Ekern et al. 1965; Ekern & Sundstøl, 1974). Values for fresh grass are of the order of 4–5% (Ekern et al. 1965) and those for dried grass a little higher (Blaxter & Graham, 1956; Armstrong, 1964). Where direct comparisons are available, both drying (Ekern et al. 1965) and ensiling (Jentsch, Schiemann, Hoffman & Wittenburg, 1972; Ekern & Sundstøl, 1974) increase urinary loss. Our studies at the Edinburgh School of Agriculture confirm this and indicate that restriction of fermentation results in lower urinary losses. In one experiment with sheep, urinary energy losses (% GE) for grass, lactate, wilted and chemically restricted silages were 4·55, 6·32, 5·94, 5·51, in another values for lactate and chemically restricted silages were 8·86 and 5·97.

Owing to the reduction in digestibility during haymaking, the ME values of hays will be lower than those of grasses from which they are made. Dried grasses will have ME values similar to the original herbage. Because of the increases in GE during fermentation, lactate silages will have ME values higher than the original material. Where fermentation has been restricted, ME values may be slightly higher than in fresh herbage, except with wilted silages in which digestibility has been significantly reduced. In our experiment quoted above, mean ME values of ryegrass, lactate, wilted and formic acid treated wilted silages were 11·6, 13·6, 11·4 and 11·9 MJ/kg DM. Corresponding Q_m (ME/GE×100) values were 62·7, 65·6, 60·7 and 62·3.

Net energy. The efficiency of utilization of the ME of fresh grass ranges from 0·60 to 0·75 for maintenance (k_m) and from 0·28 to 0·52 for growth (k_g) (Ekern et al. 1965; Graham, 1965; Corbett, Langlands, McDonald & Pullar, 1966). Corresponding values for dried grass have been reported to range from 0·66 to 0·78 and 0·33 to 0·61, depending upon stage of maturity, season and processing (Blaxter & Graham, 1956; Armstrong, 1964; Blaxter, 1964; Wainman, Blaxter, Smith & Dewey, 1970). Ekern et al. (1965) and Jentsch et al. (1972) quoted lower k_g values for fresh than for dried grass while Graham (1965) found no difference in k_m and k_g between fresh grass and hay. Ekern & Sundstøl (1974) found similar k_m and k_g values for hays and silages made from the same source material. Smith et al. (1965) obtained k_g values for silages similar to those of Ekern & Sundstøl (1974). El Serafy, Goodrich & Meiske (1974) concluded that the extent of

fermentation had no effect on ME utilization in conserved forages. Van Es (1969), from a study of 280 balance trials, concluded that k_m and k_1 (efficiency of utilization of ME for lactation) did not differ for hays and silages of similar ME value. Only in drying does there appear to be conclusive evidence for an effect on energy utilization resulting from conservation. This may result from a change in site of digestion from rumen to small intestine (Beever, Thompson, Pfeffer & Armstrong, 1969).

Acetate is less efficiently used as a source of energy for tissue growth than propionate or butyrate (Armstrong & Blaxter, 1957), and mixtures of VFA are used more or less efficiently for gain depending upon the proportion of acetate present (Armstrong, Blaxter, Graham & Wainman, 1958; Blaxter & Wainman, 1964). Ørskov & Allen (1966), Poole & Allen (1970) and Tyrrell, Reynolds & Moe (1975) have shown that efficiency of utilization of acetate depends upon the supply of glucose precursors, and Ørskov (1975) has suggested a relationship between the non-glucogenic ratio (NGR) and productive efficiency. NGR is calculated as follows: NGR=(A+2B+V)/(P+V), where A, P, B and V are molar proportions of
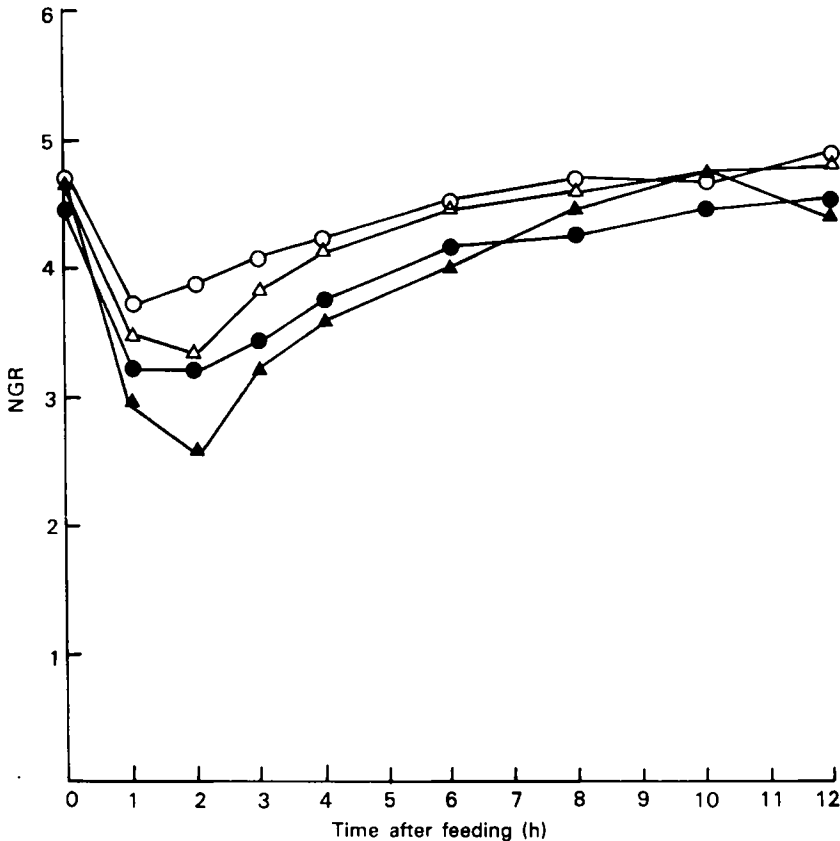

Fig. 1. Non-glucogenic ratios (NGR) (see above) for sheep given, daily at 09.00 hours, fresh grass (frozen) (O), directly made silage (●), wilted silage (△) or acid-treated, wilted silage (▲) made from the same grass crop.

rumen acetate, propionate, butyrate and valerate respectively. It is suggested that maximum k_g is obtained with NGR values between 2·25 and 3·00. Ørskov, Flatt, Moe, Munson, Henkess & Katz (1969) and Flatt, Moe, Munson & Cooper (1969) showed equal k_l values for acetate and propionate, but Elliot & Loosli (1959) and Coppock, Flatt & Moore (1964) found k_l to depend upon the relative proportions of rumen acetate and propionate. The association of low-fat milk production with high rumen propionate is well documented. Ørskov (1975) has suggested that k_l is reduced when the NGR exceeds 4·00 and that there is a danger of producing low-fat milk when the NGR is below 3·00.

Data accumulated at the Edinburgh School of Agriculture have been used to calculate NGR values after intake of silages at 09.00 hours daily. Fig. 1 shows these values for three silages and the fresh grass (frozen) from which they were made. Mean NGR values over the period were 4·29 for the grass, 4·15 for wilted silage, 3·85 for a directly made lactate silage and 3·76 for a formic acid-treated, wilted silage. None of these lies within the range given by Ørskov (1975) for maximal k_g but all could be regarded as satisfactory for acceptable k_l values. It might be more relevant to consider the NGR values at peak VFA production, c. 11.00 hours. The high NGR for the wilted silage resulted from an increase in butyrate instead of propionate, in response to lower acetate. Fig. 2 shows NGR values for a spring and
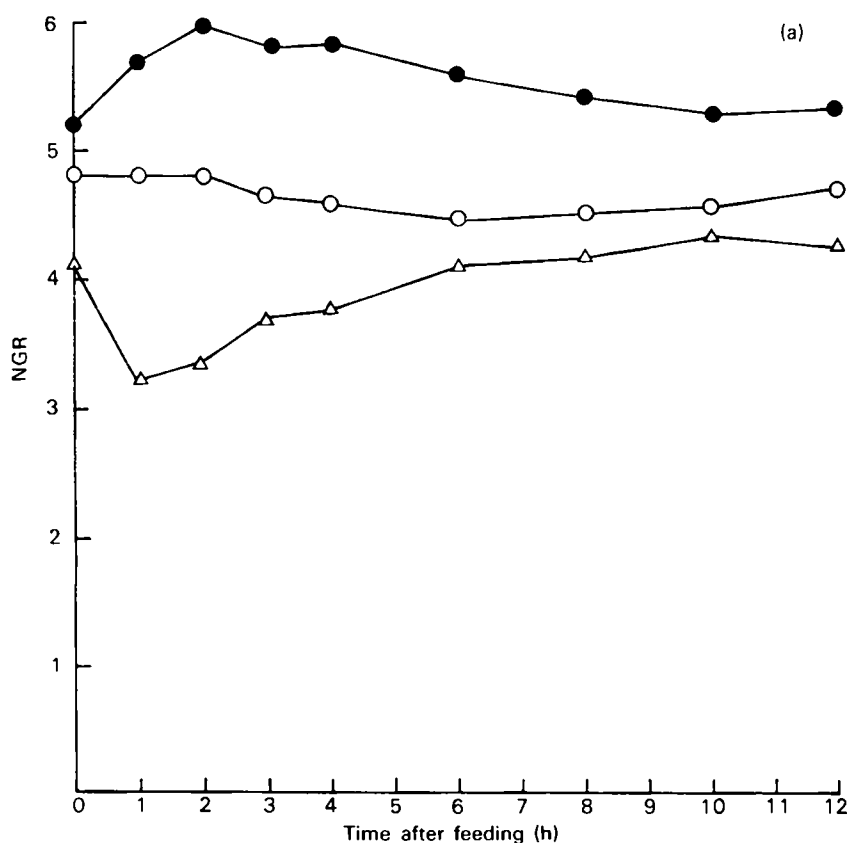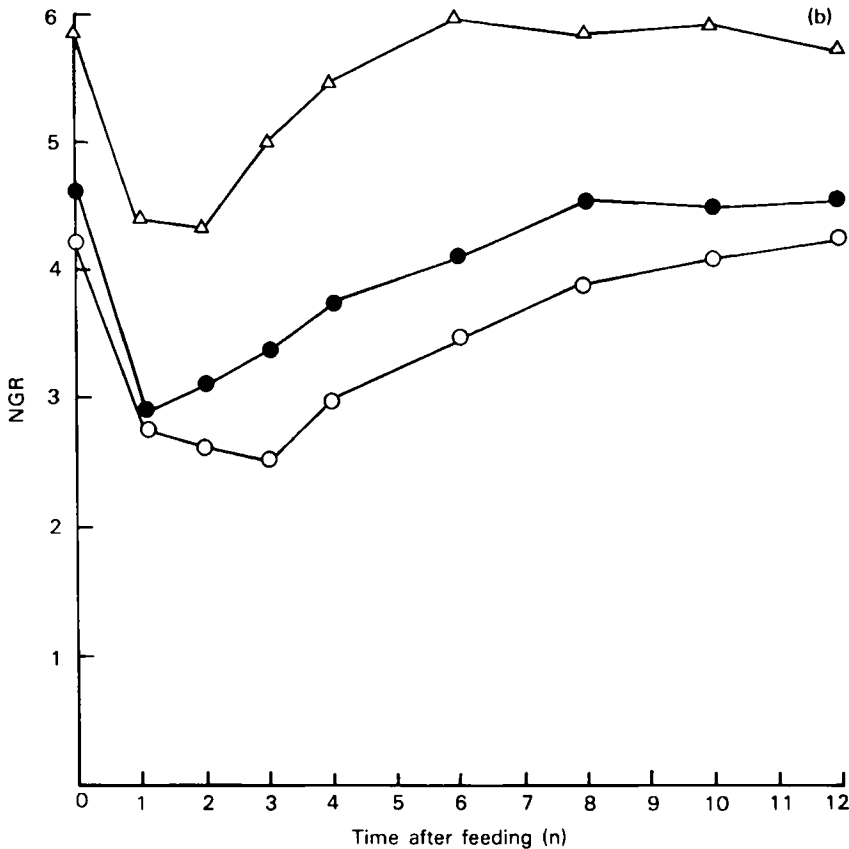

Fig. 2.

Fig. 2.   Non-glucogenic ratios (NGR) (see p. 205) for sheep given, daily at 09.00 hours, silages and dried grass made from (a) autumn and (b) spring cuts of the same pasture: (○), dried grass, (●), treated silage (sealing delayed) (△), control silage (sealed directly).

an autumn cut of dried grass from the same pasture, together with two silages made from each. In each instance one of the silages was directly made (control) and the other with delayed sealing of the silo (treated). The mean NGR values were 3·40, 3·92 and 5·39 respectively for the grass, treated and control silages from the spring materials and 4·67, 5·54 and 3·86 for the autumn cuts. Thus for the spring-cut materials the dried grass would be regarded as most suitable for promoting tissue gain, and both the grass and treated silage for milk production. For the autumn cuts the only acceptable material is the control silage, and that for milk production. The NGR values for the dried grasses are in keeping with the generally accepted productive capacity of spring-cut compared with autumn-cut materials.

N utilization. Forbes & Irwin (1968), Waldo, Miller, Okamoto & Moore (1965), Waldo, Smith, Miller & Moore (1969) and Durand, Zelter & Tisserand (1968) have shown low retentions of N in ruminants on diets of silage compared with fresh herbage or hay. Conrad, Hibbs & Pratt (1961) found that N utilizations were similar for hay, silage and fresh herbage when N content was below 24 g/kg DM.

Above this level, retention for silage but not for hay was lower than for fresh herbage. Fatianoff, Durand, Tisserand & Zelter (1966) concluded that ensilage reduced N retention in sheep only when the silage was badly preserved.

Armstrong (1974) quoted unpublished results of D. E. Beever & D. J. Thomson, who showed that N uptakes from the small intestine (g N/g N intake) were 0·53, 0·77, 0·61 and 0·76 for fresh, oven-dried, high-temperature dried and low-temperature-dried grasses respectively. Using unpublished results of C. J. Proud he calculated that the supply of amino acid-N (AAN) to the host on a silage diet was 0·76 of that when fresh grass was given and 0·58 of that for dried grass. It was suggested that differences in absorbed AAN were due to differences in rate of NH_3 production or microbial protein synthesis, or both, in the rumen.

Chalmers (1963) noted a correlation between protein utilization and rumen NH_3 production, and the importance of the degradability of dietary protein and the content of WSC. In our work, peak rumen NH_3 concentrations for six lactate found mean rumen NH_3 concentrations of 268, 275 and 182 mg NH_3-N/l for grass, lucerne (*Medicago sativa* L.) and clover (Trifolium sp.) and showed that concentrations were increased by ensiling, and decreased, to an extent depending upon the conditions, by drying. Concentrations of rumen NH_3 for hay diets were about 0·66 of those for diets based on silages. A number of workers, notably El Shazly (1952), Chalmers (1963) and Durand *et al.* (1968) have shown higher rumen NH_3 concentrations as a result of ensilage of diets, with peak NH_3 concentrations ranging from 300 to 500 mg NH_3-N/l. These high concentrations are in keeping with the highly degraded nature of the N of ensiled materials and their negligible content of WSC. In our work, peak rumen NH_3 concentrations for six lactate silages varied from 195 to 450 mg NH_3-N/l and there was a highly significant correlation between peak concentration and the non-protein-N and NH_3-N contents of the silages. The effect of WSC was non-significant but levels were low in all the silages, Ciszuk & Eriksson (1973) stated that net losses of N occurred when the rumen NH_3 concentration exceeded 150 mg NH_3-N/l, which supplies an explanation for the poor utilization of silage N.

In the present economic climate there is pressure for conserved forages to satisfy an increasing proportion of the needs of the animal, which requires that their N be utilized with greater efficiency. This involves protection of the original protein against breakdown, both during ensilage and in the rumen, and conservation of the WSC of the herbage. Wilting conserves WSC and limits amino acid breakdown by clostridia but does not completely inhibit proteolysis. Fatianoff *et al.* (1966) showed better N retention for wilted than for directly made silages. Durand *et al.* (1968) confirmed improved N retention owing to wilting and showed an accompanying reduction in rumen NH_3 concentrations. In a comparison of two wilted and lactate silages made from the same parent materials, E. Donaldson & R. A. Edwards (unpublished results) showed little or no effect of wilting on peak rumen NH_3 concentrations when stated as mg NH_3-N/l per g N intake. Waldo *et al.* (1969) and Saue (1970) have shown that use of formic acid in ensilage will reduce rumen NH_3 concentrations and improve N retention, probably by inhibition of

proteolysis in the cut crop and clostridial degradation of amino acids in the silo. The extent of the improvement will depend upon how soon the acid is added after cutting the crop. In three comparisons we have found peak rumen NH_3 concentrations of 16 mg NH_3-N/l per g N intake for wilted compared with 18 mg/l for materials where formic acid was added to the wilted material before ensiling.

Formaldehyde treatment of casein has been shown to prevent its degradation in the rumen while allowing digestion in the small intestine (Ferguson, Hemsley & Reis, 1967). Hemsley, Hogan & Weston (1970) showed lower N digestibility but greater absorption of N from the small intestine as a result of treatment of a dried-grass–clover mixture with formaldehyde. Mean peak rumen NH_3 levels for three formaldehyde-treated silages investigated by R. A. Edwards & E. Donaldson (unpublished results) were 10·7 mg NH_3-N/l per g N intake, compared with 20 for untreated lactate silages, which confirms the control of degradability of protein but probably also reflects the greater residual WSC in the treated materials. Use of formaldehyde in ensilage has resulted in a greater entry of amino acids into the small intestine (Beever, Thompson & Harrison, 1974) and improved animal performance (Brown & Valentine, 1972; Barry, Fennessy & Duncan, 1973; Valentine & Radcliffe, 1975; Waldo, 1975).

Voluntary food intake. Using sheep, Demarquilly & Jarrige (1970) measured the voluntary food DM intake (DMI) of 108 forages conserved as hay and twenty-one as dried grass. Reductions in DMI of 21·3 and 13·8% were obtained for swath-dried and barn-dried hays. The intake of forages dehydrated in an efficient drier is similar to that of the fresh forage (Jarrige *et al.* 1974).

The DMI of high-moisture lactate silages is lower than that of fresh or dried herbage (Moore, Thomas & Sykes, 1960; Campling, 1964; Jarrige *et al.* 1974; Wilkins, 1974). McLeod, Wilkins & Raymond (1970) showed that the adjustment of silage pH from 4 to 5·4 with sodium bicarbonate resulted in increases in DMI, while the addition of lactic acid to reduce the pH of a silage from 5·4 to 3·8 resulted in a decrease in DMI of 22%. There was a negative correlation between DMI and titratable acidity, total organic matter content, lactic acid content and pH of the silages. Demarquilly (1973) found DMI was significantly reduced as the lactic, acetic, propionic and total VFA of the silage increased.

The negative correlation of acetic acid content with DMI for a range of silages found by Wilkins, Hutchinson, Wilson & Harris (1971) and Brown & Radcliffe (1972), and the intraruminal infusion experiments of Rook, Balch, Campling & Fisher (1963) and Ulyatt (1965), would support the view that DMI of high-acetate silages would be low.

Reductions in DMI are associated with butyrate silages. Ulyatt (1965) found that intraruminal infusion of butyric acid depressed intake of fresh forages, while Wilkins *et al.* (1971) found close negative correlations between DMI and the concentrations of NH_3-N in such silages. The components of butyrate silages which may be responsible for reduced intake have not been identified.

The beneficial effects on DMI of increasing the DM content of forages before ensiling are well known (Thomas, Moore, Okamoto & Sykes, 1961; Jackson &

Forbes, 1970). In recent studies by Hinks, Edwards & Henderson (1976), DMI were 64·2 and 117·5 g/kg body-weight$^{0.75}$ for a lactate and a wilted silage made from the same crop of ryegrass, when fed to fattening Friesian steers.

Some workers have demonstrated increased intakes of formic acid-treated silages when compared with untreated butyrate-type silages (Castle & Watson, 1970; Wilson & Wilkins, 1973). When formalin is used in high concentrations, >13 g/kg fresh grass, severe restrictions in DMI have occurred (Wilkins et al. 1974). With more moderate levels of application, 8 g/kg (equivalent to 0·43 g formaldehyde/g N), to a cocksfoot–clover mixture, Barry et al. (1973) obtained increases in both DMI and live-weight gain in sheep, compared with untreated silages. At lower levels of usage, 2·5 g/kg (equivalent to 0·2 g/g N), Wilkins et al. (1974) recorded stimulation of clostridial activity.

REFERENCES

Alderman, G., Collins, F. C. & Dougall, H. W. (1971). *J. Br. Grassld Soc.* **26**, 109.
Armstrong, D. G. (1964). *J. agric. Sci., Camb.* **62**, 399.
Armstrong, D. G. (1974). *Proc. 5th gen. Mtg Eur. Grassld Fedn, Uppsala,* p. 114.
Armstrong, D. G. & Blaxter, K. L. (1957). *Br. J. Nutr.* **11**, 413.
Armstrong, D. G., Blaxter, K. L., Graham, N. M. & Wainman, F. W. (1958). *Br. J. Nutr.* **12**, 177.
Barry, T. N., Fennessy, P. F. & Duncan, S. J. (1973). *N.Z. Jl agric. Res.* **16**, 64.
Beever, D. E., Thompson, D. J. & Harrison, D. G. (1974). *Proc. 12th int. Grassld Congr., Moscow.*
Beever, D. E., Thompson, D. J., Pfeffer, E. & Armstrong, D. G. (1969). *Proc. Nutr. Soc.* **28**, 26A.
Blaxter, K. L. (1964). *Proc. Nutr. Soc.* **23**, 62.
Blaxter, K. L. (1973). *Proc. 1st int. Congr. Green Crop Drying, Oxford,* p. 64.
Blaxter, K. L. & Graham, N. McC. (1956). *J. agric. Sci., Camb.* **47**, 207.
Blaxter, K. L. & Wainman, F. W. (1964). *J. agric. Sci., Camb.* **63**, 113.
Brown, D. C. & Radcliffe, J. C. (1972). *Aust. J. agric. Res.* **23**, 25.
Brown, D. C. & Valentine, S. C. (1972). *Aust. J. agric. Res.* **23**, 1093.
Campling, R. C. (1964). *Proc. Nutr. Soc.* **23**, 80.
Castle, M. E. & Watson, J. N. (1970). *J. Br. Grassld Soc.* **25**, 65.
Catchpoole, V. R. (1972). *Aust. J. exp. Agric. Anim. Husb.* **12**, 269.
Chalmers, M. I. (1963). In *Progress in Nutrition and Allied Sciences,* p. 91 [D. P. Cuthbertson, editor]. Edinburgh: Oliver & Boyd.
Ciszuk, P. & Eriksson, S. (1973). *Swed. J. agric. Res.* **3**, 13.
Conrad, H. R., Hibbs, J. W. & Pratt, A. D. (1961). *J. Dairy Sci.* **44**, 85.
Coppock, C. E., Flatt, W. P. & Moore, L. A. (1964). *J. Dairy Sci.* **47**, 1359.
Corbett, J. L., Langlands, J. P., McDonald, I. & Pullar, J. D. (1966). *Anim. Prod.* **8**, 13.
Demarquilly, C. (1973). *Annls Zootech.* **22**, 1.
Demarquilly, C. & Jarrige, R. (1970). *Proc. 10th int. Grassld Congr.,* p. 733.
Durand, M., Zelter, S. Z. & Tisserand, J. L. (1968). *Annls Biol. anim. Biochim. Biophys.* **8**, 45.
Ekern, A., Blaxter, K. L. & Sawers, D. (1965). *Br. J. Nutr.* **19**, 417.
Ekern, A. & Sundstøl, F. (1974). *Publs Eur. Ass. Anim. Prod. no.* 11, p. 231.
Elliot, J. M. & Loosli, J. K. (1959). *J. Dairy Sci.* **42**, 843.
El Serafy, A. M., Goodrich, R. D. & Meiske, J. C. (1974). *J. Anim. Sci.* **39**, 3.
El Shazly, K. (1952). *Biochem. J.* **51**, 640.
Fatianoff, N., Durand, M., Tisserand, J. L. & Zelter, S. Z. (1966). *Proc. 10th int. Grassld Congr.,* p. 551.
Ferguson, K. A., Hemsley, J. A. & Reis, P. J. (1967). *Aust. J. Sci.* **30**, 215.
Flatt, W. P., Moe, P. W., Munson, A. W. & Cooper, T. (1969). *Publs Eur. Ass. Anim. Prod no.* 12, p. 235.
Forbes, T. J. & Irwin, J. H. D. (1968). *J. Br. Grassld Soc.* **23**, 299.
Graham, N. McC (1965). *Publs Eur. Ass. Anim. Prod. no.* 11, p. 231.
Hemsley, J. A., Hogan, J. P. & Weston, R. H. (1970). *Proc. 11th int. Grassld Congr.,* p. 703.

Henderson, A. R. & McDonald, P. (1971). *J. Sci. Fd Agric.* **22**, 157.
Henderson, A. R. & McDonald, P. (1975). *J. Sci. Fd Agric.* **26**, 653.
Hinks, C. E., Edwards, I. E. & Henderson, A. R. (1976). *Anim. Prod.* **21**, 217.
Jackson, N. & Forbes, T. J. (1970). *Anim. Prod.* **12**, 591.
Jarrige, R., Demarquilly, C. & Dulphy, J. P. (1974). *Proc. 5th gen. Mtg Eur. Grassld Fedn,* p. 98.
Jentsch, W., Schiemann, R., Hoffman, L. & Wittenburg, H. (1972). *Arch. Tierernähr.* **22**, 17.
Kemble, A. R. & Macpherson, H. T. (1954). *Biochem. J.* **58**, 46.
McDonald, P., Henderson, A. R. & Ralton, I. (1973). *J. Sci. Fd Agric.* **24**, 827.
McDonald, P. & Whittenbury, R. (1973). In *Chemistry and Biochemistry of Herbage,* vol. 3, p. 33 [G. W. Butler and R. W. Bailey, editors]. New York: Academic Press.
McLeod, D. S., Wilkins, R. J. & Raymond, W. F. (1970). *J. agric. Sci., Camb.* **75**, 311.
Moore, L. A., Thomas, J. W. & Sykes, J. F. (1960). *Proc. 8th int. Grassld Congr.,* p. 701.
Ørskov, E. R. (1975). *Wld Rev. Nutr. Diet.* **22**, 152.
Ørskov, E. R. & Allen, D. M. (1966). *Br. J. Nutr.* **20**, 519.
Ørskov, E. R., Flatt, W. P., Moe, P. E., Munson, A. W., Henkess, R. W. & Katz, I. (1969). *Br. J. Nutr.* **23**, 443.
Poole, D. A. & Allen, D. M. (1970). *Br. J. Nutr.* **24**, 695.
Rook, J. A. F., Balch, C. C., Campling, R. C. & Fisher, L. J. (1963). *Br. J. Nutr.* **17**, 399.
Saue, O. (1970). *Nord. JordbrForsk.* **52**, 447.
Shepperson, G. (1960). *Proc. 8th int. Grassld Congr.,* p. 704.
Smith, J. S., Wainman, F. W. & Davey, P. J. S. (1975). *Proc. Nutr. Soc.* **34**, 102A.
Sullivan, J. T. (1973). In *Chemistry and Biochemistry of Herbage,* vol. 3, p. 1 [G. W. Butler and R. W. Bailey, editors]. New York: Academic Press.
Thomas, J. W., Moore, L. A., Okamoto, M. & Sykes, J. F. (1961). *Proc. 8th int. Grassld Congr.,* p. 701.
Tyrrell, H. F., Reynolds, P. J. & Moe, P. (1975). *J. Anim. Sci.* **41**, 423.
Ulyatt, J. M. (1965). *N.Z. Jl agric. Res.* **8**, 397.
Valentine, S. C. & Radcliffe, J. C. (1975). *Aust. J. agric. Res.* **26**, 769.
Van Es, A. J. H. (1969). *Proc. 3rd gen. Mtg. Eur. Grassld Fedn, Braunschweig,* p. 275.
Wainman, F. W. (1976). *1st a. Rep. Fd Eval. Unit Rowett Res. Inst.*
Wainman, F. W., Blaxter, K. L., Smith, J. S. & Dewey, P. J. S. (1970). *Publs Eur. Ass. Anim. Prod.* no. 13, p. 17.
Waldo, D. R. (1975). *J. Anim. Sci.* **41**, 424.
Waldo, D. R., Miller, R. W., Okamoto, M. & Moore, L. A. (1965). *J. Dairy Sci.* **48**, 910.
Waldo, D. R., Smith, L. W., Miller, R. W. & Moore, L. A. (1969). *J. Dairy Sci.* **52**, 1609.
Wilkins, R. J. (1974). In *University of Nottingham Nutrition Conference for Feed Manufacturers:* 8, p. 167 [H. Swan and D. Lewis, editors]. London: Butterworth.
Wilkins, R. J., Hutchinson, K. J., Wilson, R. F. & Harris, C. E. (1971). *J. agric. Sci., Camb.* **77**, 531.
Wilkins, R. J., Wilson, R. F. & Woolford, M. K. (1974). *Proc. 5th gen. Mtg Eur. Grassld Fedn, Uppsala,* p. 197.
Wilson, R. F. & Wilkins, R. J. (1973). *J. agric. Sci., Camb.* **80**, 225.
Wilson, R. F. & Wilkins, R. J. (1975). *Rep. Grassld Res. Inst.* 1974, p. 57.
Woolford, M. K. (1975). *J. Sci. Fd Agric.* **26**, 219.

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