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Vacuum packing: a model system for laboratory-scale silage fermentations

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ABSTRACT

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Aims: To determine the utility of vacuum-packed polythene bags as a convenient, flexible and cost-effective alternative to fixed volume glass vessels for lab-scale silage studies.

Methods and Results: Using perennial ryegrass or red clover forage, similar fermentations (as assessed by pH measurement) occurred in glass tube and vacuum-packed silos over a 35-day period. As vacuum-packing devices allow modification of initial packing density, the effect of four different settings (initial packing densities of 0·397, 0·435, 0·492 and 0·534 g cm^{-3}) on the silage fermentation over 16 days was examined. Significant differences in pH decline and lactate accumulation were observed at different vacuum settings. Gas accumulation was apparent within all bags and changes in bag volume with time was observed to vary according to initial packing density.

Conclusions: Vacuum-packed silos do provide a realistic model system for lab-scale silage fermentations.

Significance and Impact of the Study: Use of vacuum-packed silos holds potential for lab-scale evaluations of silage fermentations, allowing higher throughput of samples, more consistent packing as well as the possibility of investigating the effects of different initial packing densities and use of different wrapping materials.

Keywords: inoculants, model system, modified atmosphere, packing density.

INTRODUCTION

Preservation of forages by fermentation is a core component of the ruminant feed supply chain as it provides conserved feed over periods when fresh forage is limited or unavailable. It has been estimated that, in western Europe, silage production accounts for almost 70% of the total forage preserved as ruminant feed (Jones 1998). As poor quality silage has lower nutritional value, and is often rejected by animals, much research has focused on studying the ensiling process and the factors affecting it (Woolford 1984; McDonald *et al.* 1991).

Herbage additives, such as acids, salts and more recently bacterial inoculants [in particular lactic acid bacteria (LAB); Done 1986], have been developed to improve the fermentation process and maintain forage nutritional value (Henderson 1993; Weinberg *et al.* 1993; Weinberg and Muck 1996). Other additives have been targeted at improving the aerobic stability of grass and whole-crop silage, which can be poor even when fermentation quality is high (Merry *et al.* 1997), and recently the ban on the use of animal protein in ruminant diets has renewed interest in ensilage of high protein alternative forages (Anil *et al.* 2000; Wilkins and Jones 2000; Fraser *et al.* 2001; Merry *et al.* 2001).

The efficacy of new additives and additive combinations is initially assessed using laboratory-scale silos. The high throughput of these smaller scale systems provides useful information to guide the design of more costly and labour-intensive farm-scale studies. A variety of laboratory systems have been developed, typically using fixed-volume vessels, such as glass boiling tubes [capacity *ca* 100 g fresh matter (FM); Henderson and McDonald 1984; Seale *et al.* 1986), plastic tubes (Lindgren *et al.* 1988] or preserving jars

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containing 1 kg or more of FM (Weinberg *et al.* 1993; Cussen *et al.* 1995).

Although these methods have all been shown to be suitable for modelling larger scale ensiling systems (Merry *et al.* 1995), several drawbacks have been identified. The packing and unpacking of fixed-volume silos is both labour intensive and time-consuming because, in order to achieve anaerobic conditions and a density approaching that of farm-scale silos, a considerable quantity of herbage must be pressed into a relatively small volume. Certain forages, such as red clover or kale, are more stemmy and heterogeneous, even after chopping, and thus difficult to pack evenly. These factors contribute to difficulties in controlling initial packing density among vessels and are also subject to variability between the packing density of individual silos. In addition, changes in the forage characteristics over time, both short-term (e.g. whilst setting up experiments) and long-term (e.g. because of seasonal variations), contribute to poor repeatability. In some circumstances, where the number of jars packed in an experiment is limited by time and labour constraints, it may be necessary to repeat work over time and thus seasonal influences can have a significant impact on the results.

It is known that the initial packing density affects silage fermentation (Ruppel *et al.* 1995; Muck and Holmes 2000) but there is little published information about its effect on silage quality. Furthermore, packing density is likely to change during the course of fermentation, because of physicochemical changes in the herbage, gas production and the efflux of liquids. Cai *et al.* (1997, 1998) have reported the use of plastic bags sealed with a household vacuum sealer but experimental details were not provided and no comparison made with glass tube silos.

The objective of this study was to investigate further the use of vacuum packing in polyethylene bags as a simple, convenient, flexible and cost-effective system for studying the ensiling process at laboratory scale. Specifically, comparison was made of vacuum-packed silos vs glass tube silos, both with and without added LAB inoculant, and the effect of different initial vacuum settings on silage quality and the accumulation of gases within vacuum-packed bags was investigated.

MATERIALS AND METHODS

Herbages

Pure swards of perennial ryegrass (*Lolium perenne*) and red clover (*Trifolium pratense*) were harvested on 12 July 2000 (grass; second cut), 30 September 2000 (clover; third cut) or 29 July 2001 (grass; second cut). All were ensiled as pure herbages from individual swards and were wilted overnight (14–16 h) over a cool-airbed drier [Institute of Grassland and Environmental Research (IGER), Aberystwyth, Wales, UK] prior to ensiling. The final dry matter (DM) content was *ca* 280 g kg^{-1} for grass and 250 g kg^{-1} for red clover. The wilted herbage was then chopped, once in the case of grass and twice in the case of the red clover, using a static precision-chop forage harvester to give a chop length of 2–4 cm (IGER design). The chopped herbages (either perennial ryegrass or red clover) were ensiled in glass boiling tubes or in polyethylene bags, either untreated, or treated with a silage LAB inoculant Powerstart®; Genus plc, Cheshire, UK), applied at the manufacturer's recommended rate.

Glass tube silos

Herbage (100 g FM) was packed into glass boiling tubes (200 mm × 33 mm diameter; volume 170 ml), compressed by hand with the aid of a Perspex rod, and the tubes were then sealed with a rubber bung equipped with a fermentation lock (Winters *et al.* 1998, 2000). The bung was inserted until just above the surface of the plant material to minimize the amount of air in the system. The packing density within a tube was *ca* 0·625 g cm^{-3}. The fermentation locks were filled with glycerol to allow release of fermentation gases whilst preventing air ingress. Tube silos were incubated in a constant temperature room at 18°C and destructively sampled at the specified opening times.

Vacuum-packed silos

Two commercial chamber vacuum-packing machines were used. A model MVS30 (Minipack Torre S.p.A.; 24044 Dalmine, Bergamo, Italy) device was used to compare glass tube and vacuum-packed silos with and without addition of LAB inoculant. The vacuum pump draws 10 m^3 h^{-1} air and the amount of air removed from the bag, which determines the packing density, is altered by changing the time (0–45 s, in 5-s increments) for which the air is pumped out of the bag. The second vacuum packer, model MVS35 [Minipack Torre (UK) Ltd, Corby, UK; Fig. 1], was used to study the effect of initial packing density on ensilage. In this model, the percentage vacuum to be drawn is user-defined over a range of 0–99·9% vacuum and this determines the proportion of air removed from the packing chamber, and hence from the bag. Therefore, a setting of 80% removes 80% of the air from the bag prior to sealing. Both machines are equipped with an automatic heat-sealing mechanism that seals the bag after air extraction.

In all experiments, the 100-g FM chopped herbage was packed into polythene bags (dimensions 300 × 150 mm; Kalle Nalo, Witham, UK). These bags are designed for vacuum-packing food produce and have the specification: gas permeability at 23°C is 40, 155 and 10 cm^3 m^{-2} day^{-1} bar^{-1}

Fig. 1 The MVS35 chamber vacuum-packing machine is shown here with a bag of forage in place ready for vacuum packing

for oxygen, carbon dioxide and nitrogen, respectively [Technical Data Sheet, Hi-vac Laminate (PA/PE), Kalle Nalo, UK]. All bags were heat sealed to provide a reliable seal without melting the plastic bag. Care was taken to seal bags at exactly 150 mm from the base and, following sealing, the vacuum packer automatically cut the remaining plastic 5 mm above the seal (Fig. 2). The vacuum bag silos were incubated at 18°C in an incubator and destructively sampled at the specified opening times. The volume of the vacuum bag silos was measured by water displacement, using a method similar to that described by Cai *et al.* (1997) in a 5-l graduated cylinder. Bags were submerged in the cylinder so that the top of the bag was 1–2 cm below the surface of the water a depth at which is a very small (*ca* 1%), but consistent, increase in pressure. Care was taken to equilibrate both the bags and the water to 18°C. Atmospheric pressure on the days when volumes were measured ranged from 1010 to 1020 mbar (<1% variation; Prof G. Vaughan, UW Aberystwyth, Department of Physics).

Analytical methods

Samples were taken from the fresh chopped herbage just prior to packing and from each silo after destructive sampling. Each sample was well mixed, subsampled and frozen at −20°C for subsequent analysis of DM content by freeze drying (20°C, 48 h), L- and D-lactic acid concentrations. The pH of each sample was measured prior to packing and at each opening time.

Lactic acid concentrations and pH were measured as described by Merry *et al.* (1995). DM content was determined by freeze drying the sample to a constant weight.

Fig. 2 Vacuum-packed herbage immediately after packing

Herbage inoculation

The commercial silage LAB inoculant 'Powerstart' (Genus Plc., Crewe, UK) based on *Lactobacillus plantarum* and *Lactococcus lactis* was used. The inoculant was prepared according to the manufacturers instructions and 11 ml was sprayed onto 5 kg of chopped herbage prior to mixing and packing (final inoculation rate of 10^6 CFU g^{-1} FM). The same volume of distilled water was applied to 5 kg of the control herbage.

Data analysis

Data analysis and graph plotting was performed using Microsoft Excel 2000. One- and two-way ANOVA was conducted using Minitab v12·23 (Minitab Inc., State College, PA, USA).

RESULTS

Optimization of vacuum-packing settings for ensiling perennial ryegrass

The aim of this experiment was to identify the vacuum setting that produced a comparable fermentation to that observed in the glass tube silos. Vacuum bag silos were

packed using four vacuum settings, at which the vacuum was drawn for 5, 10, 15 and 20 s respectively (model MVS30). Fermentation in these vacuum bag silos was compared with that obtained in the glass tube silos. All silos were destructively sampled after 1, 2 and 5 days. A decrease in pH and an increase in total lactate concentration occurred in all silos, irrespective of packing method or vacuum setting. The rate of these changes was positively correlated with increasing vacuum setting, showing that more air removed from the bag prior to packing resulted in a faster rate of fermentation, measured by pH decrease. The pH after 5 days incubation was 4·39 in the glass tube silos compared with 4·57 (5 s), 4·23 (10 s), 4·14 (15 s) and 4·09 (20 s) in the vacuum bag silos. Thus a bag evacuation time of 10 s was selected for further experiments.

A comparison between ensiling red clover in glass tube and vacuum bag silos, with and without the addition an LAB inoculant

The rate of change of pH over time was very similar in glass tube or vacuum bag silos containing red clover forage without added inoculant (Fig. 3). Addition of an LAB inoculant increased the rate of pH decline ($P < 0{\cdot}001$), relative to herbage in bags and tubes without inoculant addition (Fig. 3). However, the pattern of pH decline was very similar irrespective of packing method. After 35 days, the mean pH values in the glass tube and vacuum bag silages without inoculant treatment was 4·21 compared with 3·94 ($P < 0{\cdot}001$) in the inoculant-treated silos.

Effect of initial packing density on perennial ryegrass silage ensiled without LAB inoculant

Table 1 shows the initial relative density (ρ) of the herbage at each vacuum setting. The values for initial packing density fall within the range of 96–192 g DM l^{-1} (converted from t ft^{-3}; corresponding to 0·320–0·640 g cm^{-3}) for silages at different depths from within a silage clamp (Flynn 1974). In grass bale silos, values of 125–150 g DM l^{-1} (corresponding to relative densities of 0·357–0·428 g cm^{-3}) have been calculated (P. O'Keily, Teagasc, Grange Research Centre, Ireland, pers. comm.).

Table 1 Initial relative density, and initial/final volume for 100 g bag silos packed at four different vacuum settings

	% Vacuum					
	60	70	80	90	Significance	LSD
Initial relative density (g cm^{-3})	$0{\cdot}397^{a}$	$0{\cdot}435^{ab}$	$0{\cdot}492^{bc}$	$0{\cdot}534^{c}$	$P < 0{\cdot}01$	0·0849
Initial bag volume (ml)	253^{a}	230^{ab}	201^{bc}	189^{c}	$P < 0{\cdot}001$	33·9
Bag volume at 16 days (ml)	424^{a}	414^{ab}	370^{bc}	334^{c}	$P < 0{\cdot}05$	44·9

A decrease in pH was recorded in all vacuum-packed silos, irrespective of packing density. However, the rate of pH decline was significantly affected ($P < 0{\cdot}001$) by packing density, with the greatest divergence in the samples analysed at 4 and 7 days (Fig. 4). The rate of pH decline in bags packed at 158·7 g DM l^{-1} (90% vacuum) was markedly faster over days 2–4, than those packed using lower vacuum settings. However, by 16 days there was no significant pH difference (range 4·15–4·32) between treatments.

The faster pH decline in bags packed at 90% vacuum correlated with a more rapid increase in total lactate concentration (Fig. 5) from 4 days after packing ($P < 0{\cdot}05$) compared with bags packed at lower vacuum settings. There was no significant difference between the rate of lactate accumulation in bags packed at 60, 70 and 80% vacuum initially, although by 16 days bags packed at

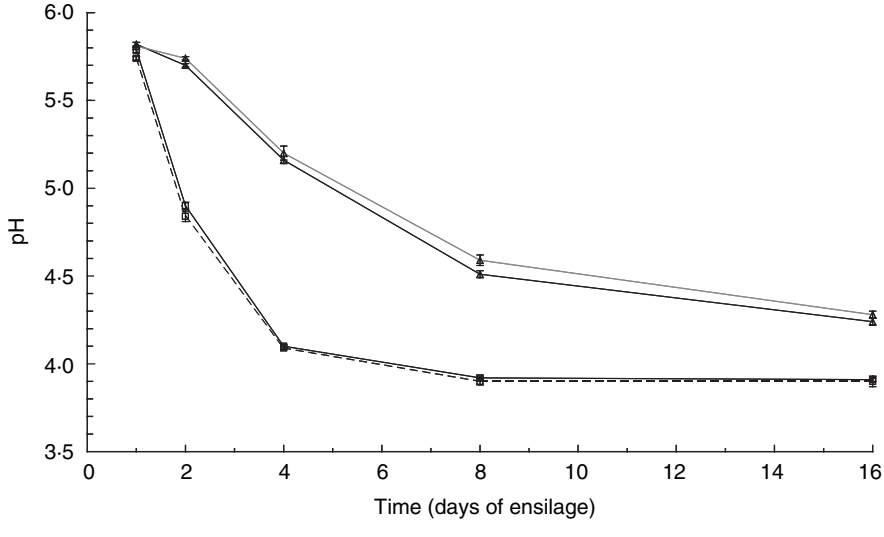

Fig. 3 A comparison between the pH profiles for red clover ensiled in both glass tube silos (full line) and vacuum bag (10 s evacuation) silos (dotted lines), either with (□) and without (△) the addition of LAB inoculant. Error bars show standard deviation ($n = 3$)

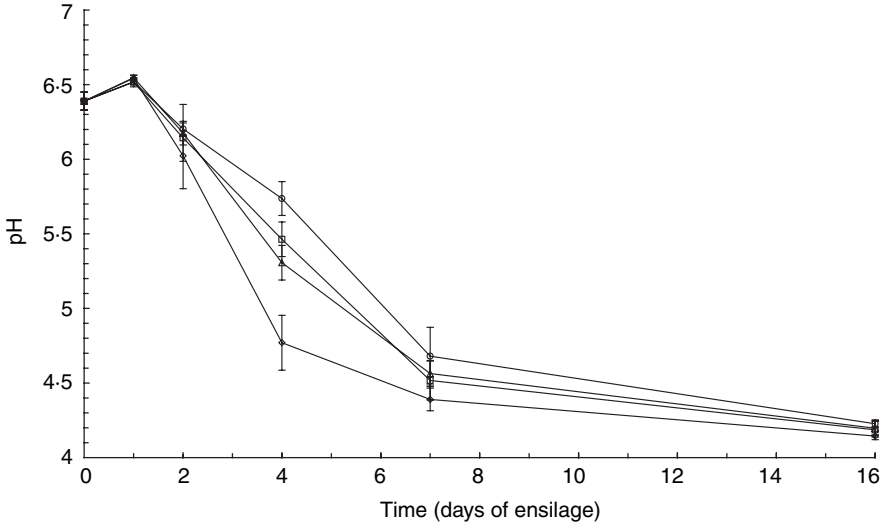

Fig. 4 The pH profile for perennial rye grass ensiled in vacuum bag silos at different packing densities. Vacuum settings: 60% (○), 70% (△), 80% (□) and 90% (◇). Error bars show standard deviation ($n = 3$)

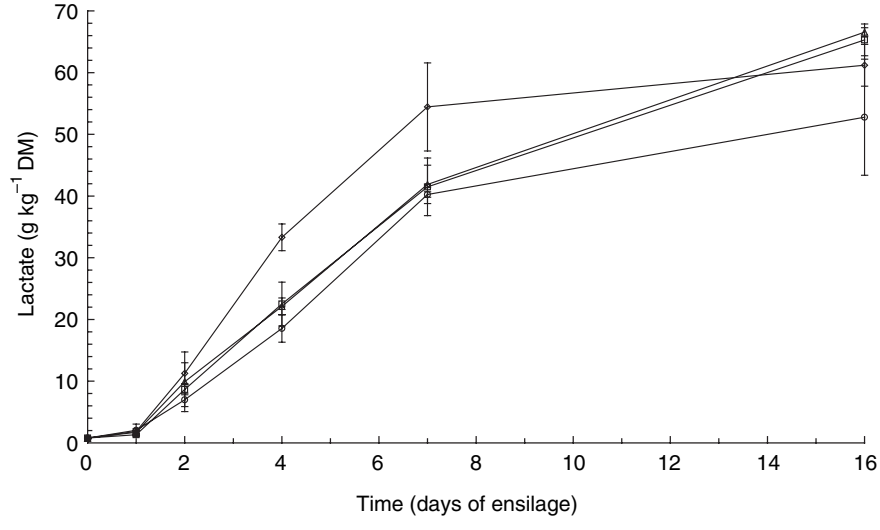

Fig. 5 The change in total lactate concentration during the ensilage of perennial rye grass in vacuum bag silos in relation to packing density. Vacuum settings: 60% (○), 70% (△), 80% (□) and 90% (◇). Error bars show standard deviation ($n = 3$)

60% contained lower lactate concentrations ($P < 0{\cdot}05$) than other treatments. Measurement of the abundance of the L- and D-lactate isomers (L : D ratio decreasing from *ca* 3·0 at 2 days to *ca* 1·2 at 16 days) did not reveal any significant differences between treatments.

The difference between the initial volumes of bags packed at different vacuum settings (189–253 ml) is indicative of the volume of gas remaining in the bags. It is important to note that the pressure inside the bags was initially below atmospheric in all cases (i.e. all bags were shrunken, as shown in Fig. 2). However, direct volume comparison is not possible as a result of these pressure variations and the fact that herbage was noticeably more crushed at higher vacuum settings.

In bags packed at all vacuum settings there was noticeable gas accumulation (Fig. 6), resulting in loosening of the fermenting herbage. However, only bags packed at 60% vacuum became turgid. The maximal volume of the bags was *ca* 430 ml, so higher volumes (e.g. after 7 days in bags packed at 60% vacuum) were the result of stretching of the bags caused by gas pressure greater than atmospheric within the bags. Increased partial pressure of the gases inside turgid bags led to increased outward gaseous diffusion and the observed reduction in volume between 7 and 16 days. It is frequently observed that plastic bale-wrap in big-bale silage exhibits transient distension during the early stages of ensilage when gas production by silage micro-organisms is greatest.

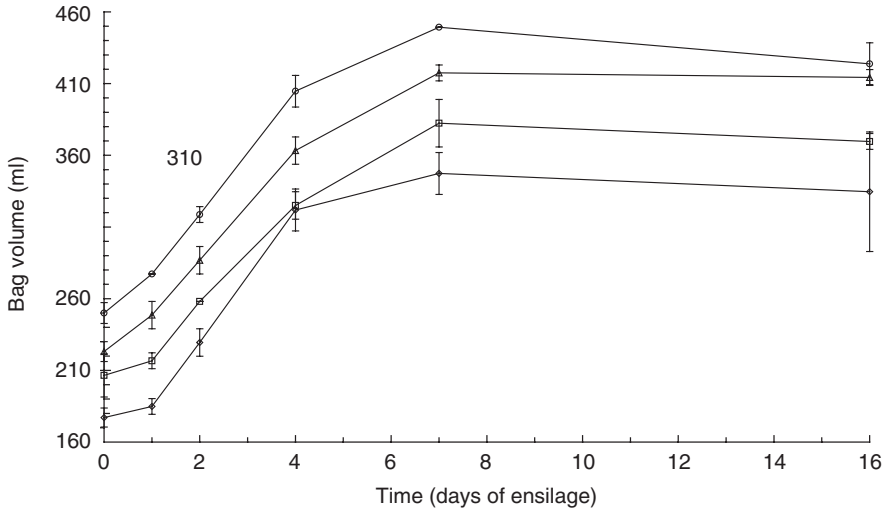

Fig. 6 The effect of initial packing density, determined by the percentage vacuum drawn at the time of packing, on the accumulation of gases within the vacuum bags during the fermentation of perennial rye grass. Vacuum settings: 60% (○), 70% (△), 80% (□) and 90% (◇). Error bars show standard deviation ($n = 3$)

DISCUSSION

During ensilage for up to 35 days we found that the pH change in glass tube silos and vacuum-packed plastic bags was similar. There are, however, some fundamental differences between these two systems, notably the fact that in glass tubes packing density does not change with time. Initial packing densities were similar (625 and 534 g FM l^{-1} for glass tubes and bags packed at 90% vacuum respectively) but it is important to note that the amount of residual oxygen in vacuum-packed bags (as all bags were initially at below atmospheric pressure) is much less because pressure does not deviate significantly from atmospheric pressure in glass tubes.

Nevertheless, vacuum-packed bags offer several advantages over glass tubes despite the initial outlay required (*ca* €1500). Not only does vacuum packing permit higher throughput, during silo packing (and in particular for unpacking) but consistency of initial packing density is more uniform and much less susceptible to operator differences, particularly when more heterogeneous substrates (e.g. clover or kale) are ensiled. Furthermore, herbage volumes of 10 g up to 2 kg could be studied (Helen Johnson, unpublished data) and as we have shown here initial packing densities can also be modified.

In the experiments reported here with vacuum-packed bags, no attempt was made to constrain the herbage during fermentation (as it would be in rigid-walled vessels such as glass), so the packing density decreased over time as gases accumulated. It was apparent that more efficient removal of air at initial packing improved the rate of pH decline and lactate accumulation. However, it was also apparent that increased vacuum settings caused more crushing of herbage and thus greater release of sap. We recognize that this could affect effluent production; however in these experiments the silages were mixed well after unpacking so that any effluent would have been evenly distributed throughout the sample (as is the case for most published lab-scale silage experiments).

The fate of gases within plastic-wrapped silos is complex and is affected by a combination of pressure differences and differential diffusion of gases through the bale-wrap (McGechan and Williams 1994). Precise measurement of gas flux requires the use of complex equipment and is time-consuming (Paillat and Gaillard 2001). Factors such as wrap colour which in the field affects the surface temperature of the bale and consequently the permeability of the wrap can have a significant effect (Snell *et al.* 2002), as can the thickness and chemical composition of the wrapping plastic (Keller *et al.* 1998). Of key importance in this respect, particularly with small-scale silages with a large surface area to volume ratio, is the differential permeability of plastics to different gases. The polythene bags used here are much more permeable to CO_2 than O_2 (155 cm^3 m^2 day^{-1} bar^{-1} vs 40 cm^3 m^2 day^{-1} bar^{-1} respectively) and in combination with the greater difference in CO_2 partial pressure between ambient air (0·03% CO_2) and inside a vacuum-packed silo (*ca* 100%), efflux of CO_2 will occur much more rapidly than ingress of O_2.

Using the system described here it is possible to model the dynamics of gas production during silage fermentation. Despite the fact that many factors can affect bag volume, volume measurement is simple and non-destructive. Our data show that changes in bag volume correlated well with accepted measures of silage quality such as pH and lactate concentration. Cai *et al.* (1997) also found an inverse correlation between the volume of gas accumulated and silage quality in vacuum-packed silos. A technique for venting gases during fermentation, such as a pressure sensitive valve similar to that used in rumen automated

pressure evaluation system (Davies *et al.* 2000) or as used with round bales (P. Lingvall, pers. comm.) could be a valuable addition. This would not only allow the release of gas as occurs in a large scale silo but also its collection for analysis and further characterization of the silage fermentation.

The MVS35 device also offers the possibility of modifying the atmosphere of the vacuum-packed bags (as is widely used for storage of food products). The effects of different initial gas concentrations (notably O_2 and CO_2) at initial packing could also be investigated by this method.

Vacuum-packed bags also offer the possibility of investigating the effects of different types of bale wrapping materials (varying in thickness, composition, etc.). Bags varying in thickness and gas permeability are commercially available (e.g. http://www.davisfreshtech.com/articles_whatmap.html) but sheet plastic used for silage wrapping can also be formed into bags by heat sealing. Furthermore, the localized effects of silage wrap puncture (by birds, etc.) could also be investigated at lab scale using this method.

In summary, the use of the vacuum-packed silos provides a highly flexible method for the study of the ensilage process and enables the study of packing density, herbage type and pretreatment on silage quality and stability.

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