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Comparison of the content of clostridial spores in wilted grass silage ensiled in either laboratory, pilot-scale or farm silos

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Abstract

Fifty-two laboratory silages (1.5 l), 108 samples of pilot-scale silos (2-4 tonnes), and 167 samples of farm silages were analysed for their contents of clostridial spores, proximate composition and concentrations of fermentation products. Numbers of clostridial spores in the silages ranged from 100 to more than 10 million per gram. Relationships between spore counts as dependant variable and dry matter, butyric acid and ammonia-N contents as independant variables were established for the three types of silages by regression analyses. Similarity existed in the relationships found for pilot-scale and farm silages, showing a gradual drop of spore counts with higher dry matter levels. Laboratory silages differed in showing either a pronounced clostridial fermentation with high levels of butyric acid, ammonium-N and spores or showing no clostridial fermentation at all. In contrast to the larger-scale silages, no clostridial fermentation was found at dry matter contents above 250 g kg^{-1}. In laboratory and in pilot-scale silages about 60 % of the variation in spore count could be explained from either the butyric acid or ammonia content. In farm silages this figure dropped to 20-30 % and it was concluded that the chemical parameters can not be used to predict spore counts in farm silages. The differences between the larger scale silages and laboratory silages are attributed to the heterogeneity of the pilot-scale and farm silages.

Keywords: wilted grass silage, clostridia, spores, butyric acid, ammonium-N

Introduction

The primary goal of ensiling is to preserve the feeding value of the crop by a lactic acid fermentation. When the crop is deficient in fermentable carbohydrates or when an inefficient fermentation takes place, clostridia develop, leading to high levels of ammonium-nitrogen, butyric and branched-chain volatile fatty acids and elevated pH values. Silages that undergo clostridial fermentation lose a considerable part of their nutritive value and contain high levels of clostridial spores. From the preservation point of view the metabolic activity of the vegetative cells is of primary interest. However, the dairy industry faces the negative effects of the spores on the quality of dairy products. The spores are taken in by the animal together with the silage and

pass the alimentary tract unaffected and enter the milk with manure contamination (Contrepois et al., 1971). Notably *Clostridium tyrobutyricum* can cause late blowing of hard cheeses at contamination levels below 1 spore per 100 ml. In farming practice such low levels are extremely difficult to obtain when feeding silage. In order to prevent the occurrence of problems due to contamination with clostridial spores, several solutions have been found. In Switzerland and Bavaria, silage is excluded from the ration of dairy cows. In Finland, a well-controlled system of silage making with the use of formic acid has developed (Ali-Yrrko & Antila, 1975). In the cheese factory, spore counts of the milk can be reduced by centrifugation (van den Berg et al., 1980; Lembke et al., 1984) or growth of clostridia in the cheese can be inhibited by adding small amounts of nitrate to the cheese milk (Galesloot, 1964).

In the Netherlands, grass for silage is wilted to dry matter (DM) contents over 350 kg^{-1} and often gathered with a self-loading forage wagon and ensiled in simple clamp or trench silos with a concrete floor (Spoelstra, 1985a). Increased DM contents reduces the butyric acid content of silage (Wieringa, 1958). Despite the generally high DM contents of Dutch silages the dairy industry is still confronted with elevated numbers of clostridial spores in the milk. In order to be able to make hard cheese, as Gouda, with for all markets acceptable low levels of nitrate, expensive removal of spores by centrifugation in combination with silages of a high microbiological quality is necessary. Against this background it is important for the dairy industry and the farmer to have information about the contamination level of silages with clostridial spores. Hereto it was investigated whether clostridial contamination of grass silages could be predicted from the chemical parameters which are routinely measured to assess silage quality. Because this research was carried out with laboratory, pilot-scale and farm silages a comparison of the relationships between silage quality parameters and the numbers of clostridial spores was made for the different silo types.

Materials and methods

Laboratory experiments

Plots of 12 × 6 m were marked out in one of the experimental permanent pasture fields of the institute. The pastures were used alternatively for grazing and mowing for silage. The fields received about 200 kg ha^{-1} of inorganic nitrogen fertilizer in the spring followed by 80 kg N ha^{-1} after grazing or cutting for silage. These fields with predominantly perennial ryegrass underwent routine farm practice for silage making (i.e. mowing with a disc mower and tedding once per day) during the wilting period. The plots were sampled daily during the wilting period by combining 10-15 grab samples (about 15 kg), which were subsampled by coring (diameter of the coring tube 40 mm). The first sample of a plot was taken immediately after mowing. After mixing the subsamples (2.5 kg) in a 150-l concrete mixer, samples were taken for chemical and microbiological analyses and for ensiling in 1.5-l preserving jars (in duplicate). The jars were stored for 3 months at 25 °C before opening and analysis.

In total, 13 plots with wilting periods varying from 2 to 7 days were followed. The grass ensiled was analysed for proximate composition and in addition ammonium-N and sugar contents, in vitro organic matter digestibility and numbers of lactic acid bacteria and enterobacteria. The silages were analysed for pH, ammonia, lactic acid, volatile fatty acids and clostridial spores. Chemical methods have been outlined before (Spoelstra, 1983). The trials were carried out in the years 1982-1985 throughout the season.

Pilot-scale experiments

To test the influence of different loading machines on silage quality, pilot-scale silages were made. Fields of permanent grassland with perennial ryegrass dominating were selected at four locations in the Netherlands. After cutting, the grass was tedded and wilted to dry matter contents not exceeding 450 kg^{-1}. After raking, the grass was loaded with either a self-loading forage wagon, a self-loading forage wagon equipped with about 30 stationary knives and a self-propelled metered chopper. Per treatment, one silage of 2-4 tonnes of grass was made on a concrete floor. Silages of one experiment were made in one clamp separated by a plastic sheet. The clamps were covered by two sheets of polyethylene (thickness 0.15 mm) and 10 cm of sand. Detailed results have been published elsewhere (Hengeveld, 1983). In this paper only the results of the 108 silages made with a self-loading forage wagon will be quoted for comparison between laboratory and farm silages.

Farm silages

Samples were taken from 167 farms in the northern part of the Netherlands in the period June to September 1982. The silages had been made of prewilted grass without the use of an additive and loaded with a self-loading forage wagon. The volumes of the clamp or trench silos varied from 30-150 tonnes. The clamps were typically covered with two sheets of polyethylene weighted with tyres or with a layer of 5-10 cm of sand.

Sampling

Laboratory silages were opened and the content of one jar was treated as a sample.

Pilot-scale and farm silages were not sampled before at least 2 months after making of the silage. They were sampled by drawing vertical cores. Three to five cores were taken at different spots and mixed to give a composite sample. The coring device consisted of a stainless steel tube with an inner diameter of 22 mm and a length up to 2 m. A conical coring head with an inner diameter of 18 mm was fitted by screw thread on the coring tube.

Microbiological procedures

Extracts of grass were prepared by treating 30 g of grass, to which 270 g of deionized

water had been added, for 5 min in a Stomacher (Seward Laboratory, London). From the extract a decimal dilution series was prepared in sterile quarter-strength Ringer solution. Per sample, two dilution series were prepared and Petri dishes inoculated in duplicate.

Spore counts

Decimal dilution series were prepared from the above described silage extract in quarter-strength Ringer solution. From each dilution, 1 ml was transferred to 3 culture tubes (150 × 16 mm) with 9 ml molten hand-warm lactate-acetate agar. The inoculated tubes were pasteurized (12 min, 78 °C) and an anaerobic atmosphere established by adding 0.5 ml of each 25 % pyrogallol solution and saturated soda to the cotton wool plug. The tubes were then sealed with a rubber stopper. Tubes showing gas formation after 7 days incubation at 37 °C were considered positive. The most probable number was read from a table.

The spore count with the described pyrogallol-soda method was used for the samples of pilot-scale silages only. In order to be able to work faster a miniaturized method was developed and used for counting the spores in the laboratory and farm silages. This method differed from the pyrogallol-soda method in the following aspects. From a pasteurized dilution series, 0.10 ml was transferred by a sterile disposable 1-ml syringe mounted on a dispensor in small culture tubes (Durham tube, 50 × 7 mm). Subsequently, 0.65 ml molten lactate-acetate agar was pipetted in the tubes using a continuous pipetting syringe (Becton and Dickinson). The racks with the Durham tubes were incubated anaerobically using the Gaspak system (Becton and Dickinson).

Forty paired comparisons of the two methods were made. Large culture tubes and Durham tubes were inoculated from the same dilution series. No statistically significant differences were found between results of the two methods (paired t test).

The standard error of the spore count as derived from the replicate spore counts in samples of laboratory silages amounted to 0.6 logarithmic units g^{-1}.

As a routine, 4 subsequent dilutions were tested in the miniaturized method. When no tubes or all tubes showed gas production the results were noted with smaller than (often <2.56 log units g^{-1}) of greater than (often >6.4) signs, respectively. In the statistical evaluation the signs were ignored.

Media

Lactate-acetate agar (g l^{-1}): peptone 5, yeast extract 10, 60 % solution of sodium lactate 25, sodium acetate·$3H_2O$ 8; pH value after autoclaving 6.5.

Violet-red-bile-glucose agar (Oxoid) and Rogosa agar (Difco) were purchased as ready-for-use media.

Chemical analyses

The dry matter losses in laboratory silages were calculated from the weight losses of the silos.

Proximate analyses were performed as outlined by van Es & van der Meer (1981).

Other chemical analyses were carried out as described before (Spoelstra, 1983). Chemical methods used for the pilot-scale silages were given by Hengeveld (1983).

Organic matter digestibility of grass and silages was estimated by the in vitro technique of Tilley & Terry (1963) as adapted by van der Meer (1986). The results were corrected to in vivo values by using standard grass or silage samples of known in vivo digestibility as estimated in digestibility trials with wethers fed at maintenance.

Net energy for lactation, expressed as VEM kg^{-1} DM (van Es, 1977) was calculated from the crude fibre content of the silages by using the regression equation in use for farming practice (Anonymous, 1977).

Results

Laboratory silages

In total, 52 duplicate laboratory silages were made and analysed. The grass used in these trials originated from 13 different field experiments with wilting performed in 1982-1985 (Table 1). The results of the analyses of the silages were treated as independent samples. A correlation matrix was calculated of the parameters indicatory for the fermentation quality of the silages. A mutual correlation of about 0.8 (range 0.78 to 0.86) was found between the ammonium-N contents (g kg^{-1} total-N), weight losses, butyric acid contents and spore counts. Correlation of these parameters with propionic acid was about 0.6 (range 0.56-0.69), whereas low correlations were found with acetic acid concentrations (0.09-0.30).

Linear regression showed a rather poor relationship of spore counts with the dry matter content. Better relationships were found with butyric acid and ammonium-N (Table 5).

Twenty-one silages (40 %) could be characterized as butyric acid silages (Table 2). These silages typically contained high levels of butyric acid, ammonium-N and low levels of lactic acid and elevated counts of clostridial spores and DM contents below 250 g kg^{-1}. DM contents of laboratory silages without a butyric acid fermentation ranged from 180 to 591 g kg^{-1}. Butyric acid silages did not contain any residual nitrate, this in contrast to the non-butyric acid silages which showed nitrate concentrations of 0-4 g kg^{-1}. The butyric acid silages had 2.5 times higher dry matter losses due to fermentation and an average reduction of the in vitro digestibility of the dry matter of 9.1 % compared to 1.6% in non-butyric acid silages.

Pilot-scale silages

Table 3 gives characteristics of the 108 pilot-scale silages analysed. Correlations of spore counts and the contents of butyric acid on a fresh weight basis and the ammonium-N/total-N ratio was both 0.67. The correlation between the butyric acid and ammonia amounted to 0.78. Linear regression was best suited to describe the relationship between spore counts and the dry matter content (45.6 % of the variation accounted for). For butyric acid and ammonium-N an exponential function suited best (Table 5).

Table 1. Characteristics of the grass ensiled in laboratory silos.

Parameter	Mean	Range
Dry matter (DM; g kg^{-1}	236	120 -591
Ash (g kg^{-1} DM)	103	73 -198
N-total (g kg^{-1} DM)	31.5	24.8 - 40.0
Crude fibre (g kg^{-1} DM)	265	201 -327
Sugar (g kg^{-1} DM)	93	19 -147
Nitrate (g kg^{-1} DM)	7.8	0.0 - 23.1
Wilting period (days)	2.4	0 - 8
Digestibility of the organic matter (%)	77.46	71.0 - 82.8
Lactic acid bacteria (log units g^{-1} DM)[1]	5.04	3.69- 6.43
Enterobacteria (log units g^{-1} DM)[2]	7.17	6.17- 8.21

[1] Counts on MRS-agar.
[2] Counts on violet-red-bile-glucose agar.

Table 2. Mean and standard deviation of silage quality parameters measured in laboratory silages with (n = 21) and without (n = 31) butyric acid fermentation.

Parameter	Without butyric acid fermentation		With butyric acid fermentation	
	mean	SD	mean	SD
Dry matter (DM; g kg^{-1})	268	-[1]	196	-[1]
Ash (g kg^{-1} DM)	97	12	122	34
DM loss (g kg^{-1} DM)	42	18	102	37
NH_3-N (g kg^{-1} total-N)	132	48	326	152
Clostridial spores (log units g^{-1})	2.75	0.40	4.89	1.52
Butyric acid (g kg^{-1} DM)	1.7	-[1]	34.0	13.4
Lactic acid (g kg^{-1} DM)	39.9	23.9	15.1	25.4
Fermentation products (g kg^{-1} DM)[2]	83	39	93	45
Decrease of digestibility of the organic matter (%)[3]	1.6	2.4	9.2	5.0
Nitrate (g kg^{-1})	1.1	1.4	0.0	0.0

[1] No standard deviation could be calculated because of skew distribution of data.
[2] Ethanol + lactic acid + volatile fatty acids.
[3] In vitro.

Farm silages

In total 167 silages were sampled and analysed. The majority (67 %) of the silages contained grass of different fields harvested at different days. The results (Table 4) show an average DM content of 503 g kg^{-1}. Only 6 samples had less than 350 g DM kg^{-1}. The average crude fibre content amounted to 256 g kg^{-1} DM. The mutual correlations between the spore count, butyric acid content and the ammonia-N fraction ranged from 0.45 to 0.72, being well below the values found for laboratory silages. By linear regression 31 % of the variation in the spore content was ac-

Table 3. Characteristics of samples taken of 108 pilot-scale silages (Hengeveld, 1983).

Parameter	Mean	Range
Dry matter (DM; g kg^{-1})	397	151 -693
Wilting period (days)	3.2	1 - 9
Ammonia-N (g kg^{-1} total-N)	131	20 -430
Butyric acid (g kg^{-1} DM)	17.4	0 -154
Clostridial spores (log units g^{-1})	5.12	2.12- 7.50

Table 4. Characteristics of samples taken of 167 farm silages.

Parameter	Mean	SD	Range
Dry matter (DM; g kg^{-1})	503	82	266 -689
Ash (g kg^{-1} DM)	130	32.7	84 -255
Crude fibre (g kg^{-1} DM)	256	26.4	160 -334
Crude protein (g kg^{-1} DM)	176	23.4	104 -246
Wilting period (days)	3.4	0.76	1 - 6
NEL (VEM kg^{-1} DM)[1]	793	67.1	497 -914
pH	5.4	0.34	4.8 - 6.6
Ammonium-N (g kg^{-1} total-N)	113	55.5	30 -430
Lactic acid (g kg^{-1} DM)	6.7	-[2]	0 - 27.8
Acetic acid (g kg^{-1} DM)	3.2	-[2]	0 - 24.7
Propionic acid (g kg^{-1} DM)	0.5	-[2]	0 - 8.0
Isobutyric acid (g kg^{-1} DM)	0.2	-[2]	0 - 3.4
Butyric acid (g kg^{-1} DM)	4.9	-[2]	0 - 41.8
Clostridial spores (log units g^{-1})	4.92	1.01	1.88- 7.04

[1] Net energy for lactation (van Es, 1978).
[2] No standard deviation could be calculated because of skew distribution of data.

counted for by the variation in the dry matter contents.

By exponential regression 20 % of the variation in the spore content of the silages could be explained by the contents of butyric acid and 30 % by the ammonia-N fraction (Table 5). Five percent of the variation in the spore counts could be explained by the ash content. No relationship could be established between the spore count and the concentration of residual nitrate. Multiple regression did not improve the relationships significantly.

Comparing laboratory, pilot scale and farm silages

In all three silage types the spore count declined with increased DM content (Table 5). For laboratory silages only 19 % of the variation in spore counts was explained by variation in the DM content in the linear regression equation, which is lower than for pilot-scale (46 %) and farm silages (31 %). The relationships for pilot-scale and farm silages were similar, whereas in the laboratory silages much lower spore counts were found at the same dry matter content.

Table 5. Comparison of linear (form $Y = aX + b$) or exponential (form $Y = c + d \times r^X$; equivalent to $Y = a + b \times e^{-kX}$ with $k = -\ln r$) relationships obtained by regression analyses between the spore counts (logarithmic units g^{-1} and DM (g kg^{-1}), butyric acid (g kg^{-1}) and ammonia-N (% of total-N) contents for laboratory, pilot-scale and farm silages.

Type of silages	Relationship	n[1]	V[2]	Estimate/standard error of estimate				
				a	b	c	d	r
X = dry matter (g kg^{-1}), Y = spore counts (log units g^{-1})								
Laboratory	$Y = 5.03 - 0.00613X$	51	23.1	12	−4.0	-	-	-
Pilot	$Y = 8.37 - 0.00819X$	108	45.8	23	−9.3	-	-	-
Farm	$Y = 8.45 - 0.00701X$	167	31.4	21	−8.8	-	-	-
X = butyric acid (g kg^{-1}), Y = spore counts (log units g^{-1})								
Laboratory	$Y = 2.64 + 0.3567X$	52	66.7	17	10	-	-	-
Pilot	$Y = 6.42 - 2.816 \times 0.657^X$	108	64.8	-	-	39	−14	11
Farm	$Y = 5.61 - 1.503 \times 0.529^X$	167	20.2	-	-	27	− 6.5	45
X = ammonium-N (% of total-N), Y = spore counts (log units g^{-1})								
Laboratory	$Y = 1.78 + 0.0898X$	51	70.0	8.5	11	-	-	-
Pilot	$Y = 7.16 - 5.387 \times 0.916^X$	108	55.3	-	-	15	−11	43
Pilot	$Y = 7.20 - 5.769 \times 0.910^X$	105	64.1	-	-	19	−13	50
Farm	$Y = 7.41 - 4.320 \times 0.949^X$	167	30.3	-	-	6.5	− 5.0	36
Farm	$Y = 6.91 - 4.344 \times 0.928^X$	163	39.0	-	-	11	− 9.9	37
X = butyric acid (g kg^{-1}), Y = ammonium-N (% of total-N)								
Laboratory	$Y = 11.56 + 3.269X$	52	64.1	7.4	9.5	-	-	-
Pilot	$Y = 7.97 + 0.995X$	108	60.7	13	13	-	-	-
Farm	$Y = 8.87 + 1.129X$	167	27.9	19	8.1	-	-	-

[1] Number of measurements used in the regression analyses.
[2] Percentage of variance explained by the regression equation.

In the laboratory and pilot-scale silages the contents of butyric acid and ammonium-N fraction explained 65 and 55 %, respectively, of the variation of the numbers of clostridial spores, whereas in farm silages only 29 % and 20 %, respectively, was explained by these parameters. Here again the exponential relationships for the larger scale silages were similar and differed considerably from the linear relationships for the laboratory silages. In the latter, lower spore counts were found at a given ammonium-N fraction, and lower counts at low butyric acid concentrations but higher counts at concentrations exceeding 10 g kg^{-1}.

The levels of spores at low butyric acid, low ammonia-N and high dry matter values were about 1 logarithmic unit per gram lower in the laboratory than in the larger scale silages.

Ammonium-N levels found in the laboratory silos were much higher than those in pilot-scale or farm silages.

Discussion

General

For the cheese-making industry as well as for the farmer it is important to have an estimate of the content of clostridial spores in silages. Routine analyses of spore contents of farm silages is time-consuming and expensive. It would be of advantage if an easily measurable chemical parameter would correlate well with spore counts in silages. Butyric acid and ammonium contents, being products of clostridial activity, seem to be the products of choice.

In this study, three sets of data of wilted silages were analysed and compared. The silages had in common that they were all made from grass typical for the silage making system in the Netherlands. The silages were characterized by high crude protein levels reflecting the general high gifts of fertilizer nitrogen, and also by crude fibre contents of on average 250 g kg^{-1} DM, which agrees well with the values reported for Dutch grass silages. The DM contents of the laboratory silages (mean 236 g kg^{-1}) and to a lesser extent of the pilot-scale silages (mean 373 g kg^{-1}) were lower than those of the farm silages (mean 503 g kg^{-1}). The latter values are in accordance with national annual averages for silages made without an additive (Spoelstra, 1985a).

The levels of clostridial spores in farm grass silage reported here are comparable to those published by Waes (1987), but generally higher than those reported for Finnish formic acid silages reported by Ali-Yrrko & Antila (1975). Differences can be explained on one side by the method of silage making and methods of microbial analyses on the other.

Basic contamination level

From the results of chemical analyses (notably the contents of butyric acid and ammonium-N contents) the laboratory silages could be divided in silages which underwent a clostridial fermentation and silages which were free from clostridial activity. The level of clostridial spores in the first group was below or around the detection threshold of 360 spores g^{-1} (2.56 log units g^{-1}; Table 2). It is expected that this level of spores reflects the original contamination level of the ensiled grass. From the obtained regression equations the spore counts can be predicted for well-fermented silages without butyric acid, with a low ammonium-N fraction or with a very high dry matter content. These calculations indicate basic spore contents approaching 10 000 spores g^{-1} in well-fermented pilot-scale and farm silages. This is more than ten times higher than found here in the laboratory silages and nearly ten times higher than reported by Hengeveld (1983) in prewilted grass just before ensiling. This basic contamination level of the grass with clostridial spores is thought to be due to soil contamination with possibly landspreading of manure being a main factor determining the spore content of the soil. But these data also indicate that even in a well fermented prewilted grass silage some increase in spore contents by clostridial growth and sporulation takes place.

Butyric acid and ammonium-N

Spore count increased with the butyric acid as well with the ammonium-N content. The levelling off of the spore count in farm silages at higher concentrations of butyric acid and ammonium-N was probably caused by the limits of the most probable number tests for clostridial spores. The 'greater than' signs were ignored in the statistical evaluation. However, the spore counts in the pilot-scale silages were assessed over the full range without this limitation. Nevertheless, the results for pilot-scale silages were very similar to those of farm silages, though levelling off occurred at a somewhat lower spore count in the pilot-scale silages. In laboratory silages this levelling off of the spore count at increased concentrations of butyric acid and ammonium-N was not found.

The relationships found by linear regression showed that the butyric acid content and the ammonia-N fraction could give an indication of the content of clostridial spores in laboratory and pilot-scale silages, but not in farm silages which show too much variation (Table 5).

Dry matter

In the laboratory silages no clostridial fermentation took place at DM contents over 250 g kg^{-1}, which agrees with earlier laboratory studies (Wieringa, 1958). However, it must be kept in mind that the inhibition of clostridia is caused rather by water activity than by DM content. But because DM and water activity are strongly related within a particular type of crop it is possible to use the DM content as a variable. When clostridial fermentation took place in the laboratory silos it was very pronounced with high levels of ammonium-N and butyric acid. This explains also the poor relationship found between spore counts and dry matter content and could be the cause of the rather good relationships found between spores and ammonium-N and butyric acid. In pilot-scale and even more pronounced in farm silages, clostridial spores occurred at much higher dry matter contents, and a somewhat better relationship was found by regression analyses (46 % and 31 % of the variation in spore count could be explained by the variation in dry matter of the pilot-scale and farm silages, respectively, versus 23 % for the laboratory silages). The general trend of lower levels of clostridial spores at higher dry matter contents was apparent in all three silage types.

Heterogeneity of the silages

The paradox between laboratory silages and larger scale silages was also observed for other parameters than influence of the dry matter content on spore counts. In laboratory silages no clostridial fermentation had taken place when the silage contained residual nitrate, A relationship that is explained by the inhibitory effect of nitrate on clostridia (Spoelstra, 1983; 1985b). Also Weissbach & Haacker (1988) concluded from ensiling experiments with immature whole crop cereals that the absence of nitrate in the crop was the cause of the butyric acid fermentation at relative-

ly high DM contents. In pilot-scale silages, clostridial fermentation did not occur when more than approximately 400 mg kg^{-1} residual nitrate was present in the silages (Spoelstra, unpublished results). In farm silages no such level of residual nitrate was found above which no clostridial fermentation had taken place.

The main difference between the silages considered here seems to be the heterogeneity. There is a clear increase in variation in the dry matter content from the laboratory silos to farm silos. Laboratory silages are made from a few kilograms of well-mixed chopped material and ensiled in a gas-tight container. The pilot-scale silages were made from 2-4 tonnes of grass from one field and ensiled in a clamp. The farm silages were often made from grass of different fields, often even ensiled at different days. So it is not surprising that extreme differences in dry matter content can occur within one silo. For example, Spoelstra (1985a), taking samples of about 1 g, found that the dry matter content within one farm silage ranged from 200 to 700 g kg^{-1}. Factors contributing to the heterogeneity at the farm level are uneven drying during wilting, differences in botanical and chemical composition of the crop ensiled and in-silo heterogeneity due to surface layers and differences in pressure, temperature and air influences. Also migration of water vapour and condensation against the walls contributes to the lower dry matter content of outer layers. In case no adequate drainage of rain water is present, water can be sucked up by capillary forces in the high-DM silage. Farm silages and, to a lesser extent, pilot-scale silages may be considered as to be composed of many small spots (or niches) with different fermentation patterns. Part of the spots consists of low dry matter malfermented silage with high levels of spores, butyric acid and ammonia and residual nitrate, other spots consist of well-fermented (high dry matter) often nitrate-containing silage. Fermentation patterns in these small spots could possibly be similar to those found in laboratory silages.

The general tendency of lower spore counts at higher DM contents and low levels of ammonium-N and butyric acid was clearly established but these chemical parameters can merely give a rough indication of spore counts in silages. The relationships presented in Table 5 are to inaccurate to allow reliable prediction of the spore counts in farm silages.

The results presented in this paper also demonstrate the difficulty in translating results from laboratory silos to full-scale farm silos. Apart from the differences in degree of heterogeneity also the effects of other essential differences should be considered. Farm silos are generally not air-tight, resulting in lower levels of CO_2, and they have not a constant temperature during the storage period. Also densities are generally higher in farm silos. Notably the different composition of the gas phase and the changes in temperature during storage could have an effect on the fermentation. In this study the lower ratio of spore counts to butyric acid content or to ammonium-N found in laboratory silages as compared to larger scale silages remains unexplained. Factors influencing sporulation of the vegetative cell might also be considered.

It must also be stressed that a close relationship between the metabolic products of clostridia in silage and the number of spores not necessarily exists. The method of counting spores does not differentiate between the gas producing saccharolytic

(*Clostridium tyrobutyricum* and *C. butyricum*) and the gas producing proteolytic (notably *C. sporogenes*) species in silage (Ali-Yrrko et al., 1975). The first group produces butyric acid from sugars and lactic acid and sporulation in pure culture is often difficult, whereas the latter produce ammonium and branched-chain butyric acids from protein in addition to butyric acid and easily form spores. In typical butyric acid silages both groups are present.

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