ID-LELYSTAD

Application of Effective Micro-organisms (EM) as Silage Improver

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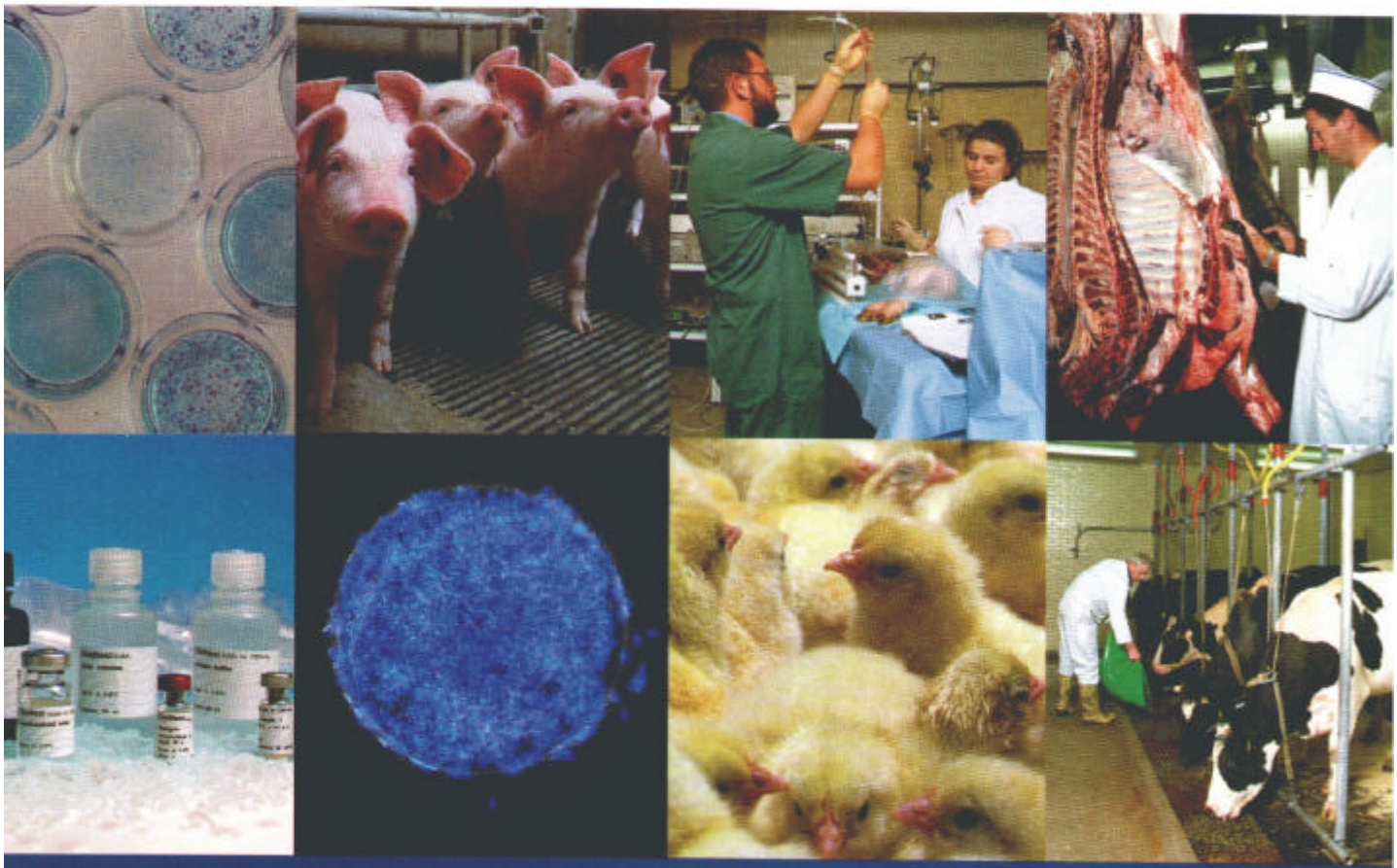

Summary

This study exists of two parts.

Part 1: Determining the minimal required incubation time and incubation temperature of EM-A as an inoculant for grass silage. The number of lactic acid bacteria in EM-A has been observed during 10 days incubation at three temperatures (15, 20 and 25^{o}C).

Part 2: To determine the positive effect of EM-A and EM-Silage during the ensilage process in order to conserve and preserve Dutch grass silage. Laboratory silages have been made and the effect of EM-A and EM-silage have been observed with respect to the initial fermentation (pH decrease after 6 days incubation) and the final product (micro flora composition, fermentation profile and aerobic stability of the silage after two months incubation).

At 1: The usual dosage for lactic acid bacteria as a silage inoculant is minimal 1.10.^{5} cfu (colony forming units) lactic acid bacteria per gram silage. The recommanded dosage for EM-A will show a minimal required number lactic acid bacteria of 1.10.^{8} cfu g^{-1} inoculant. According to Agriton the prescribed incubation time of 7 days appeared to be very sufficient to cultivate the required number of lactic acid bacteria. After 7 days all EM-A inoculants contained more than 5.10^{8} cfu lactic acid bacteria g^{-1}. At higher incubation temperatures of 20 and 25^{o} C an incubation time of 2 or 3 days was sufficient to cultivate about 1.10^{8} cfu lactic acid bacteria per gram inoculant. An inocubation time of more than 10 days was not meaningful, because the number of lactic acid bacteria in EM-A already decreased. (mortality phase).

At 2: EM-A as well as EM-Silage had a positive effect on the fermentation and the aerobic stability of grass silages. After two months the pH in the treated silages in comparison with the control silages was about 0.7 units lower (pH 4.4 towards pH 5.1). Only EM-A had also a positive effect on the initial acidification. After 6 days the pH of the EM-A treated silages in comparison with EM-Silage treated silages and the control silages was already about 1 pH-unit lower (pH 5.5 towards pH 6.5 / 6.6). As the effect of both additives regarding the velocity in acidification was greater for EM-A than for EM-Silage is possibly explainable due to the greater number of lactic acid bacteria, which has been added to the silages through the EM-A treatment in comparison with the EM-Silage treatment. Through EM-A 2.10^{5} cfu lactic acid bacteria g $^{-1}$ grass and through EM-Silage 3.10^{4} lactic acid bacteria g^{-1} grass.
Lactic acid, acetic acid and ethanol were the dominant fermentation products in all silages after 2 months incubation. The grade of conserving lactic acid and acetic acid was in the EM-A and EM-Silage treated silages much higher than in the control silage. Besides this high grades 1,2 propanediol and 1-propanol have been measured in EM-A as well as in EM-Silage treated silages, which could point to the activity of *Lactobacillus buchneri* and / or related lactic acid bacteria. These bacteria could play an important role in improving the aerobic stability of silages. EM-A and EM-Silage increased the aerobic stability of grass silages after two months inocubation. The control silages got already heated after 60 hours, whereas the treated silages kept 3 weeks stable. As a conclusion it can be stated that in this study EM-A as well as EM-Silage improved the fermentation quality of the grass silages and the aerobic stability.

Introduction

This study has been executed in cooperation with Agriton. Agriton is a company involved in creating sustainable agriculture. Effective Microorganisms (EM) play an important role. Effective Microorganisms is a product developed by Prof. Dr T. Higa from the Ryukyus University in Japan. EM contains a mix of microorganisms consisting of five groups: lactic acid bacteria, photosynthetic bacteria, yeasts, actinomycetes and moulds.
Lactic acid bacteria play a major role in the acidification of grass silage. The number of which in EM-1 are about 1.10^6 cfu (colony forming units/milliliter. EM-1 is the name of the starting material Effective Microorganisms, which Agriton delivers in plastic bottles of 1 liter and in jerrycans of 10 liters. In order to applicate these Effective Microorganisms as a silage inoculant one has to activate EM-1. Activating EM-1 take place by adding molasses (or sugar) to EM-1 and this solution has to be incubated in an air-tight container during 7 days at 20-35^o C. This activated EM is called EM-Active (EM-A) and will last for 14 days. Besides EM-1 Agriton has made a ready made product (no activation is necessary) namely EM-Silage. EM-Silage is a product to improve the silage process for grass and maïze silages. EM-Silage consists of a mix of Microorganisims among which are bacteria and yeasts, which not only cause an acceleration of a pH decrease (lactic acid bacteria) but also producing a number of bio-active substances.
These bio-active substances have according to Agriton an increase in taste and an inhibition of heat in silages.

In the first part of this study the optimal incubation time and incubation temperature of EM-A as an inoculant for grass silage has been researched. Starting from the principal that lactic acid bacteria play the most important role during the ensiling process.

In the second part of this study the positive effects of EM-A and EM-Silage in the ensiling process regarding the preparation and conservation of Dutch grass silage have been researched.

Materials and methods

3.1 Part 1, Optimalisation of the pre-culture

EM-1 has been activated mixing 0.6 liter EM-1 (as delivered by Agriton) and 0.6 liter sugar cane molasses (also delivered by Agriton) and 18.8 liters of water. This solution has been devided in 6 equal parts. These parts have been incubated twice (15, 20 and 25^{o} C.) in 5 liters glass bottles. On moment t = 0 and after 1, 2, 3, 5, 7 and 10 days incubation a sample of each bottle has been taken in order to determine the number of lactic acid bacteria.

3.2 part 2 Effect of EM-A and EM-Silage on grass silage

3.2.1 Ensiling

The ensiling experiment has been executed with grass, mainly consisting of English ray-grass, from a permanent meadow at the experimental farm of ID-Lelystad (plot number 122). The grass has been mowed with a cyclo-mower on May 7th and has been pre-dried during about 40 hours until a dry matter of plusminus 50% has been achieved. The pre-dried grass has been cut with a grass cutter and devided into three parts of about 15 kilograms. Each part got another treatment. The inoculant has been mixed with the grass thoroughly. The treatments were as follows:

Control (25 milliliter per kilogram grass)
Recommanded dosage EM-A (0,80 ml EM-A filled up to 25 ml water per kilogram grass) [(a)]
Recommanded dosage EM-Silage (0,08 ml EM-Silage filled up to 25 ml water per kilogram grass) [(b)]

[(a)]EM-1 has been activated by mixing 0,6 liter EM-1 (as delivered by Agriton) with 0,6 liter sugar cane molasses and 18.8 liters water and incubated during 15 days in an air-tight container at 20^{o} C (according to the application manual 7 days incubation is sufficient for a strong multiplication of microorganisms. The obtained EM-A will last for 14 days).
EM-A has been added into the silage according to the recommanded dosage. This is for an EM-A silage 33.3 liters per 100 m^{3}, 1 m^{3} silage has the equivalent of about 200 kilograms dry matter (IKC, 1993).
The pre-dried grass had a dry matter of 50%. So 33.3 liters of EM-A per 40 tons silage was necessary (= 0.8 liters EM-A per ton silage).

[(b)] EM-Silage has been added to the silage according to the recommanded dosage (= 0,08 liter EM-Silage per ton silage).

From each treatment one 1 liter preserving-bottle and one 2 kilograms plastic bag (2 layers) have been filled in quadruplicate. The silos have been stored at 20 plusminus 1^{o} C in the dark. After 1 week incubation 2 preserving-bottles have been emptied in order to determine the pH. After 2 months incubation 2 preserving bottles per treatment have been emptied to determine the pH, dry matter contents, contents of volatile fatty acids, lactic acids and ammonia and the number of yeasts and moulds present in the silage. At the same time 2 bags have been emptied in order to determine the aerobic stability.

3.2.2 Analyses

3.2.2.1 Chemical analyses

Dry matter has been determined according producedure NEN 3332 of the Dutch Normalisation Institute. Lactic acid, volatile fatty acids and alcohols have been determined with HPLC (Oude Elferink et al., 2001) Ammonia has been determined via the modified method of Berthold (Robinson et al., 1986). pH has been measured in a watery extract which have also been used fore the microbial analyses.

3.2.2.2 Microbial analyses

Microbial analyses have been executed with a watery extract of 30 grams silage and 270 grams of demineralised water which has been treated in a stomacher (Seward, London). Decimal dilution sequences have been made in PFZ (pepton 1.0 gram per liter^{-1}, sodium chloride 8.5 grams per liter $^{-1}$). Lactic acid bacteria have been counted on double casted plates with MRS agar (oxoid). Yeasts and moulds have been counted on double casted plates with Mald Extract Agar, acidified with lactic acid up to a pH 3.5. The plates have been incubated during 3 days at 30^{o}C.

3.2.3 Aerobic stability

Silage samples (100 grams) have been incubated in polyethylene foam trays at 20 plusminus 1^{o} C. The lid and the bottom of the trays have been perforated so that oxygen could enter and CO_2 could escape. The temperature has been measured continuously with a thermo-couple. The aerobic stability is defined as the time which is required to raise the temperature till 1^{o} C above the reference silage. The reference silage has been made by treating with a mixture of formic acid and propionic acid (6.6 and 10.7 grams kilograms silage, respectively).

Results and discussions

4.1 Part 1

The effect of the incubation temperature with respect to the numbers of lactic acid bacteria during the incubation piriod has been stated in figure 1. During the entire measuring period (10 days) an increase in the number of lactic acid bacteria (from plusminus 3.10^6 cfu ml^{-1} at day 0 till 1.10^9 cfu ml^{-1} at day 10) has been observed in all three incubation temperatures (15, 20 and 25 ^{o} C). At the highest temperature (25^o) the increase was the fastest.
But from day 2 the difference in increase has disappeared between 20^o C and 25^o C. The increase in the number of lactic acid bacteria at 15^oC was at the back until day 7. After that the increase was similar at 20 and 25^o C. After 7 days incubation there was not any longer a difference in lactic acid bacteria (plusminus 5.10^8 cfu ml^{-1}) between the 3 incubation temperatures. After 10 days the numbers of lactic acid bacteria has been increased till plusminus 1.10^9 cfu ml^{-1}. Probably this was the maximum because an extra count on day 15 at 20^o C showed a lower number (number of lactic acid bacteria decreased until plusminus 2.10^8 cfu ml^{-1}). According to the application manual EM-A after 7 days incubation would still be useful up to 14 days. It is clear that the numbers of lactic acid bacteria will be lower than after 7 days(at day 15 about a factor 2 lower than at day 7). It is definitely not optimal to use EM-A 21 days after preparation during ensiling, the lactic acid bacteria are then already in the mortality phase.
Realising that a minimal inoculation level of 1.10^5 cfu $gram^{-1}$ grass is required for a fast acidification, means that EM-A should contain minimal 1.10^8 cfu ml^{-1} (at an inoculation level of 0.8 ml EM-A per kilogram grass). At 20 and 25^o C this has already been achieved after 2 days and at 15^o C after 5 days.
The required incubation time could be shorter regarding the number of lactic acid bacteria (2-5 days instead of 7 days).

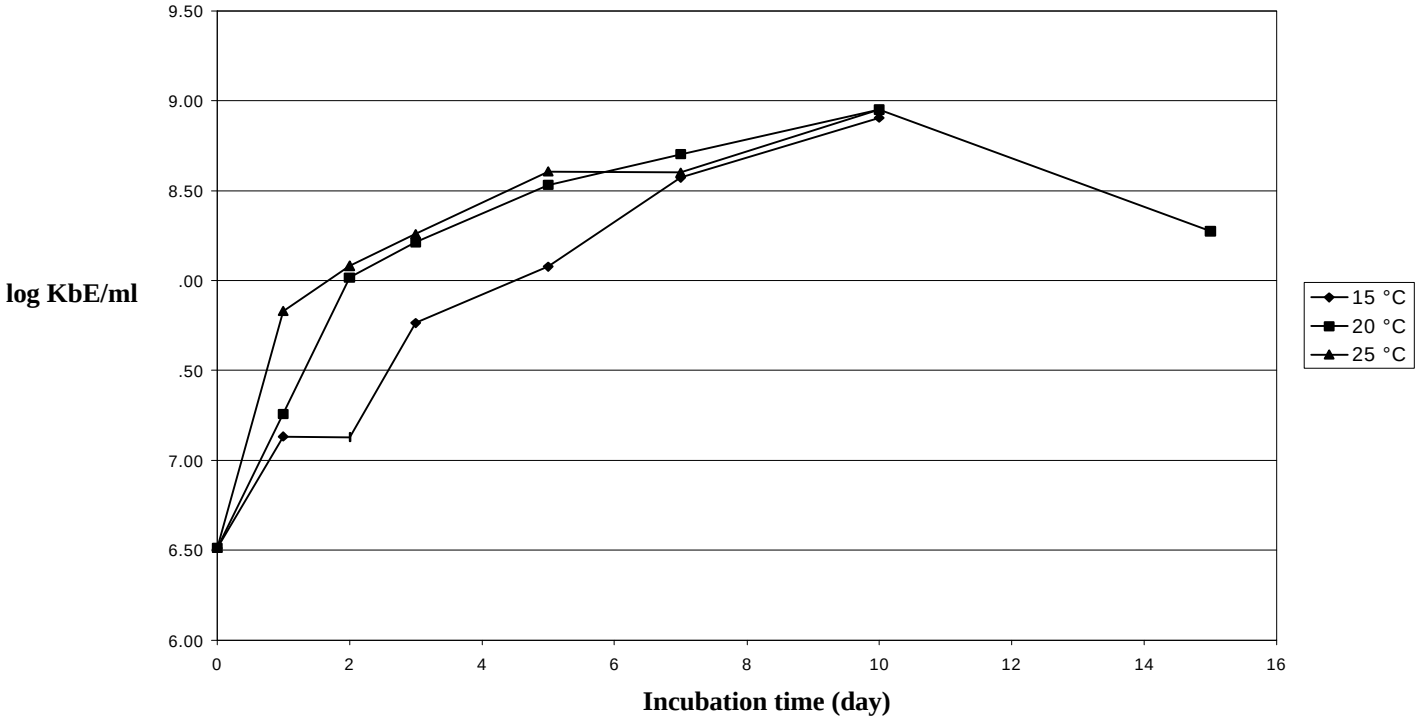

Figure 1. Growth of lactic acid bacteria in EM-A at different incubation temperatures.

4.2 Part 2

4.2.1 Dosage of Lactic Acid Bacteria

The number of added lactic acid bacteria on grass treated with EM-A was $2x10^5$ cfu lactic acid bacteria per gram grass and on grass treated with EM-Silage $3x10^4$ cfu lactic acid bacteria per gram grass.

4.2.2 Dry Matter

Dry matter content of the grass before ensiling was 480gram $kilogram^{-1}$.

4.2.3 pH

The effect of EM-A and EM-Silage on the pH of grass-silages after 2 month of incubation was equal. The pH in the treated silages in comparison with the control silages plusminus 0.7 units lower (pH 4.4 with regard to pH 5.1). This was concerned the silages in the preserving-bottles (table 2). Regarding the silages in bags (table 1) the difference in pH between the treated and control silages was still greater, viz. plusminus 1.4 units (pH 4.4 with regared to pH 5.8).
The effect of EM-A and EM-Silage on the pH of grass silages after 6 days incubation was not equal. With regard to the control silages only EM-A showed a decrease of the pH (pH 5.5 with regard to pH 6.5). Probably this has been caused by the initial number of lactic acid bacteria in the silage. The number of lactic acid bacteria added by EM-A (plusminus 2.10^5cfu $gram^{-1}$ grass) was a factor 7 higher than the number added by EM-Silage (3.10^4 cfu $gram^{-1}$ grass). The difference in inoculation level between EM-A and EM-Silage did not have any effect on the final pH after 2 months incubation, but still on the initial pH decrease velocity. A fast pH decrease in silage reduces the chance of unwished silage fermentations.

Table 1: effect of additives EM-A (EMA) and EM-Silage (EMS) with respect to weight losses, pH and aerobic stability of grass silages in 1 kilogram bags after 2 month incubation. The figures are averages of duplicate incubations.

	After 2 months incubation		
	Controle	EMS	EMA
Loss of weight (g kg^{-1})	39.0^a	25.8^b	23.9^b
PH	5.88^a	4.36^b	4.29^b
Aerobic stability (hours)	60^a	$>525^b$	$>525^b$

Averages in a row with different supscript letter code are significant different ($p<0.05$)

4.2.5 Aerobic stability and fermentation products

EM-A and EM-Silage increased the aerobic stability of grass silages after 2 months incubation. The control silages already started to get heated after 60 hours, as the treated

silages kept stable for more than 3 weeks (table 1). As a standard the aerobic stability test will be finished after 3 weeks because the test is not any longer representative for the silage situation (the material dry too much). Explaining the differencies in aerobic stability the number of putrefaction organisims (yeasts and moulds) and the amounts of organic acids (lactic acid, acetic acid) play an important role.
The number of putrefaction organisims were for all silages very low and were below the detection border ($<10^2$ cfu $gram^{-1}$) or just somewhat above (table 2).
Lactic acid, acetic acid and ethanol were in all silages the dominant fermentation products after 2 months incubation (table 2). The content of lactic acid and acetic acid was in the EM-A and EM-Silage treated silages much higher than in the control silage. Besides this in the with EM-A as well as with the EM-Silage treated silages high contents of 1,2-propandiol and 1-propanol have been found. This could mean that a stimulation of the activity of *Lactobacillus buchnerie* and/or related lactic acid bacteria .These bacteria play an important role in improving the aerobic stability of silages (Oude Elferink et al., 2001). The higher contents of ammonia in the with EM treated silages could be build up by lactic acid bacteria, but also by other bacteria in the EM-inoculant.
The positive effect of EM-A and EM-Silage on the aerobic stability of the treated silages with regard to the control silage harmonises well with the much higher concentrations conserving acids in the with EM treated silage.

Table 2 Effect of additives EM-A (EMA) and EM-Silage (EMS) on silage characteristics of grass silages in 1 liter preserving-bottles after 6 days and 2 months incubation.
The figures are the averages of incubations in duplicate.

	After 6 days incubation			After 2 month incubation		
	Control	EMS	EMA	Control	EMS	EMA
Dry matter (g kg^{-1})	nd[1]	nb	nb	451	440	436
Weight loss (g kg^{-1})	2.73[a]	3.00[a]	6.70[b]	11.5[a]	24.0[b]	21.2[b]
pH	6.55[a]	6.50[a]	5.49[b]	5.11[a]	4.42[b]	4.36[b]
Yeasts (log cfu g^{-1})	nd	nd	nd	2.15	<2	<2
Moulds (log cfu g^{-1})	nd	nd	nd	<2	<2	<2
Lactic acid (g kg^{-1} dry matter)	nd	nd	nd	41.9	79.3	85.2
Acetic acid (g kg^{-1} dry matter)	nd	nd	nd	7.6	36.2	39.2
Ethanol (g kg^{-1} dry matter)	nd	nd	nd	11.2	17.7	11.7
1,2- Propanediol (g kg^{-1} dry matter)	nd	nd	nd	0	10.0	9.0
2,3- Buthanediol (g kg^{-1} dry matter)	nd	nd	nd	0,3	0,3	0,3
Propionic acid (g kg^{-1} dry matter)	nd	nd	nd	2.2	2.4	2.7
1-Propanol (g kg^{-1} dry matter)	nd	nd	nd	0	2,3	2,9
Ammonia (g kg^{-1} dry matter)	nd	nd	nd	2.5	3.5	3.6

Averages in a row with different superscript letter code and the same incubation time are significant different ($p<0.05$)

nd[1]= not determined

5 Conclusions

Treatment of the grass with EM-A as well as EM-Silage during ensiling had a very clear positive effect on the final pH and the aerobic stability of grass silage in this experiment incomparison with non inoculated silage. EM-A had besides this also a clear positive effect on the initial acidification velocity.
Activation of EM-1 according to the recommanded method by Agriton was very satisfying to activate the inoculant, but this method can be improved (the recommanded activation time can be possibly decreased at higher incubation temperatures).

6 References

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