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BARD

FINAL REPORT
PROJECT NO. IS-3297-02

**The Effect of Lactic Acid Bacteria Silage Inoculants on
the Ruminal Ecosystem, Fiber Digestibility and Animal
Performance**

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Project Title: The effect of lactic acid bacteria silage inoculants on the ruminal ecosystem, fiber digestibility and animal performance.

Investigators

Principal Investigator (PI):

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USDA, Agricultural Research Service

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Keywords *not* appearing in the title and in order of importance. Avoid abbreviations.

Rumen microbiology

Abbreviations commonly used in the report, in alphabetical order:

CFU, colony forming units; LAB, lactic acid bacteria; RF, rumen fluid; VFA, volatile fatty acids.

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US: \$50,000

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Signature
Principal Investigator

Signature
Authorizing Official, Principal Institution

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Publication Summary (numbers)

	Joint IS/US authorship	US Authors only	Israeli Authors only	Total
Refereed (published, in press, accepted)	1			
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Invited review papers				
Book chapters				
Books				
Master theses				
Ph.D. theses				
Abstracts				
Not refereed (proceedings, reports, etc.)				

Postdoctoral Training: List the names and social security/identity numbers of all postdocs who received more than 50% of their funding by the grant.

Cooperation Summary (numbers)

	From US to Israel	From Israel to US	Together, elsewhere	Total
Short Visits & Meetings				
Longer Visits (Sabbaticals)				

Description of Cooperation:

The project started during a sabbatical leave of Zwi Weinberg at the U.S. Dairy Forage Research Center, with Richard Muck and Paul Weimer. During that time preliminary experiments on the survival of LAB silage inoculants in RF have been started. The same inoculants were used in both countries. In the U.S. the RF was obtained from lactating cows whereas in Israel it was obtained from dry cows. In the U.S. one level of inoculation was used and in Israel – two rates were applied. In Israel bacteriocin production by the inoculants was tested. In the U.S. the definite determination of the inoculated LAB in the RF samples will be accomplished by Real Time PCR techniques.

The results enable us to select the most promising LAB inoculants for the next steps of the research and to propose additional experiments in which the interaction of the ration of the cows and the LAB effect on the ruminal ecosystem will be studied.

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Patent Summary (numbers)

	Israeli inventor (s) only	US inventor (s) only	Joint IS/US inventors	Total
Submitted				
Issued (allowed)				
Licensed				

Abstract

The overall objective of the whole research was to elucidate the mechanisms by which LAB silage inoculants enhance ruminant performance. The results generated will permit the development of better silage inoculants that maximize both silage preservation and animal performance. For this one-year BARD feasibility study, the objectives were to: 1. determine whether lactic acid bacteria (LAB) used in inoculants for silage can survive in rumen fluid (RF) 2. select the inoculants that survived best, and 3. test whether LAB silage inoculants produce bacteriocins-like substances. The most promising strains will be used in the next steps of the research.

Silage inoculants containing LAB are used in order to improve forage preservation efficiency. In addition, silage inoculants enhance animal performance in many cases. This includes improvements in feed intake, liveweight gain and milk production in 25-40% of studies reviewed. The cause for the improvement in animal performance is not clear but appears to be other than direct effect of LAB inoculants on silage fermentation. Results from various studies suggest a possible probiotic effect. Our hypothesis is that specific LAB strains interact with rumen microorganisms which results in enhanced rumen functionality and animal performance. The first step of the research is to determine whether LAB of silage inoculants survive in RF.

Silage inoculants (12 in the U.S. and 10 in Israel) were added to clarified and strained RF. Inoculation rate was 10^6 (clarified RF), 10^7 (strained RF) (in the U.S.) and 10^7 , 10^8 CFU ml⁻¹ in Israel (strained RF). The inoculated RF was incubated for 72 and 96 h at 39°C, with and without 5 g l⁻¹ glucose. Changes in pH, LAB numbers and fermentation products were monitored throughout the incubation period.

The results indicated that LAB silage inoculants can survive in RF. The inoculants with the highest counts after 72 h incubation in rumen fluid were *Lactobacillus plantarum* MTD1 and a *L. plantarum*/*P. cerevisiae* mixture (USA) and *Enterococcus faecium* strains and *Lactobacillus buchneri* (Israel). Incubation of rumen fluid with silage LAB inoculants resulted in higher pH values in most cases as compared with that of un-inoculated controls. The magnitude of the effect varied among inoculants and typically was enhanced with the inoculants that survived best. This might suggest the mode of action of LAB silage inoculants in the rumen as higher pH enhances fibrolytic microorganisms in the rumen. Volatile fatty acid (VFA) concentrations in the inoculated RF tended to be lower than in the control RF after incubation. However, *L. plantarum* MTD1 resulted in the highest concentrations of VFA in the RF relative to other inoculants. The implication of this result is not as yet clear. In previous research by others, feeding silages which were inoculated with this strain consistently enhanced animal performance. These findings were recently published in Weinberg *et al.* (2003), J. of Applied Microbiology 94:1066-1071 and in Weinberg *et al.* (2003), Applied Biochemistry and Biotechnology (accepted).

In addition, some strains in our studies have shown bacteriocins like activity. These included *Pediococcus pentosaceus*, *Enterococcus faecium* and *Lactobacillus plantarum* MTD1.

These results will enable us to continue the research with the LAB strains that survived best in the rumen fluid and have the highest potential to affect the rumen environment.

Achievements

The specific objectives of the research were to: 1. determine whether LAB used in inoculants for silage survive in rumen fluid 2. select the inoculants that survived best, and 3. test whether LAB silage inoculants produce bacteriocins-like substances.

The results reveal that LAB can survive in rumen fluid, and in most experiments LAB counts were above the lowest detectable level (10^4 CFU ml⁻¹) after 72 or 96 h of incubation at 39°C. (LAB counts below that level were considered as non-surviving levels). In some cases LAB counts after incubation were comparable to their initial numbers or even slightly higher. In some cases there was a 2-3 log decrease in their numbers (in some experiments in Israel). Addition of glucose at 5 g l⁻¹ enhanced LAB counts. The most promising LAB silage inoculant strains were *Lactobacillus plantarum* MTD1, and a *L. plantarum*/*P. cerevisiae* mixture (USA) and *Enterococcus faecium* strains and *Lactobacillus buchneri* (Israel). These strains will serve in the next steps of the research.

A surprising observation was that the inoculants consistently resulted in higher pH values than the respective controls throughout the incubation period. This result is surprising because in the silage such inoculants usually cause a rapid decrease in pH as a result of their formation of organic acids, mainly lactic acid. Higher rumen pH might certainly enhance the activity of fibrolytic rumen microorganisms and hence rumen functionality, especially when the pH decreases following consumption of a high-energy diet. This result might, therefore, be a clue to the mode of action of LAB silage inoculants in the rumen. How the pH increases in the RF in the presence of LAB is not as yet clear and this topic should be further investigated. The volatile fatty acid profiles could not explain the reason for this phenomena.

Some inoculants had bacteriocins-like activity against a target microorganism *Micrococcus luteus*. These included *Pediococcus pentosaceus*, *Enterococcus faecium* and *Lactobacillus plantarum* MTD1.

The next immediate experiments which are designed include the incubation of inoculated and un-inoculated silage samples in RF, to test whether LAB from silage are washed into the RF. So far, alfalfa (U.S.) wheat and corn (Israel) silages have been prepared with and without the LAB inoculants in sealed glass jars. In addition, these silage samples will be checked for bacteriocin production.

Cooperation

The idea for this research was initiated during a previous sabbatic leave of Zwi Weinberg with Richard Muck in Madison (1995/1996). At that time both researchers co-authored a review article on inoculants for silage that was published in FEMS Microbiology Reviews (1996), 19: 53-68. In that review the authors suggested that the effect of LAB silage inoculant on ruminant performance should be further investigated.

The message that BARD has approved our proposal as a feasibility study for one year was received during a second sabbatical of Zwi Weinberg in Madison. This was an opportunity to initiate preliminary experiments on the survival of LAB in RF and to set up the methodology required. Later experiments continued in both the U.S. and Israel.

In both countries the same LAB silage inoculants were tested. The differences were that in the U.S. the RF was obtained from lactating cows fed a 22-23 kg DM ration comprised of 60% silage, 10% soybean meal and 30% ground shell corn, whereas in Israel the RF was obtained from dry cows that received daily 10 kg DM comprised of 40% hay and TMR with silage, soybean and sunflower meal and by-products (Weinberg *et al.*, submitted). This difference promoted us to suggest in the current BARD proposal an experiment in which the interaction of the cows' ration (high energy vs. high roughage) with the inoculant effect on the rumen environment.

Another difference between the experiments in the U.S. and Israel was that in Israel two rates of inoculation were used with strained RF (10^7 and 10^8 CFU ml⁻¹) whereas in the U.S. only 10^7 CFU ml⁻¹ was used. Higher inoculation rates tended to result in higher LAB counts. However, higher inoculation rates did not change the ranking of the inoculants. Definite determination of the LAB strains in the RF will be determined in the U.S. by Real Time PCR techniques.

Bacteriocin production by the LAB silage inoculants was tested in Israel. The presence of bacteriocin-like substances will be determined in control and inoculated silages (Israel).

Publications

1. Weinberg, Z.G., Muck, R.E. and Weimer, P.J. (2003).

The survival of silage inoculants lactic acid bacteria in rumen fluid.

Journal of Applied Microbiology 94, 1066-1071.

2. Weinberg, Z.G., Muck, R.E., Weimer, P.J., Chen, Y. and Gamburg, M.

Lactic acid bacteria used in inoculants for silage as probiotics for ruminants.

Applied Biochemistry and Biotechnology (accepted).

Appendix

Table of Contents:

1. Weinberg, Z.G., Muck, R.E. and Weimer, P.J. (2003).

The survival of silage inoculants lactic acid bacteria in rumen fluid.

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