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BARD

FINAL REPORT

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**Biosensors for On-Line Measurement of
Reproductive Hormones and Milk Proteins
to Improve Herd Management**

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BARD Final Scientific Report

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Project Title:

Biosensors for On-Line Measurement of Reproductive Hormones and Milk Proteins to Improve Herd Management

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Keywords:

progesterone, estradiol, urea, casein, lactose, MUN, estrus

Abbreviations:

MUN – milk urea nitrogen

TS – (milk) total solids

Budget:	IS	US	Total
	\$174,960	\$175,040	\$350,000

Signature
Principal Investigator

Signature
Authorizing Official

Publication Summary:

	Joint Authorship	US Authors	Israeli Authors	Total
Refereed		2	1	3
Submitted & in preparation		4	3	7
Invited review papers				
Book Chapters				
Books				
M.S. Theses				
Ph.D. Theses		2		2
Abstracts				
Conference - not refereed		7		7

Postdoctoral Training:

none

Cooperation Summary:

	From US to Israel	From Israel to US	Together, elsewhere	Total
Short Visits & Meetings	2	3	1 (in Oslo)	6
Longer Visits (Sabbaticals)				

Description of Cooperation:

Over the course of this research project the US and Israeli teams have exchanged ideas and information through numerous visits and email exchanges, which promoted work on both sides by setting the direction of effort and evaluation of progress.

Patent Summary:

	Israeli inventors only	US inventors only	Joint IS/US inventors	Total
Submitted	1	1		2
Issued (allowed)				
Licensed				

Abstract

The original objectives of this research project were to: (1) develop immunoassays, photometric sensors, and electrochemical sensors for real-time measurement of progesterone and estradiol in milk, (2) develop biosensors for measurement of caseins in milk, and (3) integrate and adapt these sensor technologies to create an automated electronic sensing system for operation in dairy parlors during milking. The overall direction of research was not changed, although the work was expanded to include other milk components such as urea and lactose.

A second generation biosensor for on-line measurement of bovine progesterone was designed and tested. Anti-progesterone antibody was coated on small disks of nitrocellulose membrane, which were inserted in the reaction chamber prior to testing, and a real-time assay was developed. The biosensor was designed using micropumps and valves under computer control, and assayed fluid volumes on the order of 1 ml. An automated sampler was designed to draw a test volume of milk from the long milk tube using a 4-way pinch valve. The system could execute a measurement cycle in about 10 min. Progesterone could be measured at concentrations low enough to distinguish luteal-phase from follicular-phase cows. The potential of the sensor to detect actual ovulatory events was compared with standard methods of estrus detection, including human observation and an activity monitor. The biosensor correctly identified all ovulatory events during its test period, but the variability at low progesterone concentrations triggered some false positives. Direct on-line measurement and intelligent interpretation of reproductive hormone profiles offers the potential for substantial improvement in reproductive management.

A simple potentiometric method for measurement of milk protein was developed and tested. The method was based on the fact that proteins bind iodine. When proteins are added to a solution of the redox couple iodine/iodide (I/I_2), the concentration of free iodine is changed and, as a consequence, the potential between two electrodes immersed in the solution is changed. The method worked well with analytical casein solutions and accurately measured concentrations of analytical caseins added to fresh milk. When tested with actual milk samples, the correlation between the sensor readings and the reference lab results (of both total proteins and casein content) was inferior to that of analytical casein.

A number of different technologies were explored for the analysis of milk urea, and a manometric technique was selected for the final design. In the new sensor, urea in the sample was hydrolyzed to ammonium and carbonate by the enzyme urease, and subsequent shaking of the sample with citric acid in a sealed cell allowed urea to be estimated as a change in partial pressure of carbon dioxide. The pressure change in the cell was measured with a miniature piezoresistive pressure sensor, and effects of background dissolved gases and vapor pressures were corrected for by repeating the measurement of pressure developed in the sample without the addition of urease. Results were accurate in the physiological range of milk, the assay was faster than the typical milking period, and no toxic reagents were required. A sampling device was designed and built to passively draw milk from the long milk tube in the parlor.

An electrochemical sensor for lactose was developed starting with a three-cascaded-enzyme sensor, evolving into two enzymes and $Co_2[Fe(CN)_6]$ as a mediator, and then into a microflow injection system using poly-osmium modified screen-printed electrodes. The sensor was designed to serve multiple milking positions, using a manifold valve, a sampling valve, and two pumps. Disposable screen-printed electrodes with enzymatic membranes were used. The sensor was optimized for electrode coating components, flow rate, pH, and sample size, and the results correlated well ($r^2 = 0.967$) with known lactose concentrations.

Achievements

Significance and Impact

Management of reproductive health is one of the most challenging activities facing operators of large commercial dairy herds. Significant time and effort is devoted to the detection of estrus, the time period just prior to ovulation, thus allowing successful artificial insemination. While a variety of methods based primarily on behavior are used for estrus detection, the most effective approach is to track the estrous cycle by periodic measurement of reproductive hormones in the blood plasma or milk. A biosensor for on-line measurement of progesterone was designed and calibrated. The goals were to develop an improved immunoassay sensitive enough to measure progesterone in milk at follicular phase concentrations in real-time, and to design a biosensor for implementation of the detection chemistry. Anti-progesterone antibody was coated on small disks of nitrocellulose membrane, which were inserted in the reaction chamber prior to testing, and the sensor was designed using micropumps and valves under computer control. Progesterone could be measured at concentrations low enough to distinguish luteal-phase cows (2-10 ng/ml) from follicular-phase cows (<1 ng/ml). Tests were conducted to evaluate the potential for detection of ovulatory events as compared with standard estrus detection methods. Daily measurements were made on 11 nonpregnant cows, 15-57 day post-partum, over a period of 2 months. True ovulatory events were determined by radioimmunoassay and compared with biosensor classification, herd manager observation of estrus behavior, and activity monitor classification. The biosensor system correctly identified all 14 ovulatory events during its period of operation, but the variability at low progesterone concentrations caused 5 false positives. The herd manager identified only 7 of 17 ovulatory events, probably due to a lack of overt estrus behavior, and had 4 false positives. The activity monitor detected slightly more events than the herd manager (9 out of 17), but was more prone to false positives (10). Based on the analysis of progesterone profiles, a decision strategy to assist with reproductive management over an entire lactation is presented. Direct on-line measurement and intelligent interpretation of reproductive hormone profiles offers the potential for substantial improvement in reproductive management.

The dairy industry faces an increasing demand for milk proteins, out of which caseins (80% of the total proteins) are the most significant component. The quality of milk protein is an important factor affecting fermentation of cheeses, yogurts, etc. The demand for high quality milk

is reflected in the increasing relative value of protein and in research efforts looking for ways to affect milk proteins through nutritional manipulation. On-line information regarding casein content in milk, on an individual cow basis, could help address this demand. A simple and fast method for measuring protein concentration in milk samples was developed. The method was based on the binding of iodine by proteins and the potential variations between electrodes immersed in a redox solution of iodine and iodide, when proteins were added. This method could be used as a tool for separation of milk, based on quality, into separate containers for various production purposes.

Milk urea is commonly analyzed in order to determine the efficiency of utilization of protein and nitrogen in the diet of dairy cows. This information allows the dairy producer to adjust the ration for savings in feed costs, reduction in nitrogenous waste to the environment, and possible improvement in animal health. The goal of this research was to develop an automated sensor to analyze milk urea on-site as cows are milked. A number of different technologies were explored and a manometric technique was selected for the final design based on the robustness and low cost of the sensing element. In the new sensor, urea in the sample was hydrolyzed to ammonium and carbonate by the enzyme urease, and subsequent shaking of the sample with citric acid in a sealed cell allowed urea to be estimated as a change in partial pressure of carbon dioxide. The pressure change in the cell was measured with a miniature piezoresistive pressure sensor, and effects of background dissolved gases and vapor pressures were corrected for by repeating the measurement of pressure developed in the sample without the addition of urease. Results were accurate (S.E. $\cong$ 1 mg/dl urea nitrogen) in the physiological range of milk (6-24 mg/dl urea nitrogen). The sensing element was robust, the reagent cost per sample was less than \$0.03, the assay was faster than the typical milking period (10 min), and no toxic reagents were required. A sampling device was designed and built to passively draw milk from the long milk tube so that the sensor could operate autonomously in the parlor. Based on the observations from the literature and from experiments conducted for this research, sampling 10 or more individuals from each production string every 1 or 2 days would allow more unambiguous interpretation of milk urea values than sampling weekly or monthly. This would allow a more proactive management of herd diet.

The normal concentration of lactose in cow's milk is approximately 5%, and other carbohydrates are also present, but in smaller amounts. Lactose is one of the major milk solids, and its frequent on-line measurement for individual cows, could contribute in two aspects: diagnostic and management. There are three components in milk total solids (TS): fat, protein, and lactose. For management purposes, it is desirable to know all of them. On-line measurement of any two of the milk solids yields the possibility to evaluate the last one by difference, provided TS is known. A microflow injection system using poly-osmium modified screen-printed electrodes was developed. The sensor was designed to serve multiple milking positions in an attempt to reduce cost per station. Disposable screen-printed electrodes were introduced. Screen printed electrodes can be mass-produced and, therefore, are relatively inexpensive and uniform in performance. The sensor performed well in analytical lactose and in measuring lactose added to actual milk samples.

The four different sensing methods developed by the US and Israeli teams present new options for the modern milking parlor. Use of on-line sensors for measurement of reproductive hormones, proteins, urea, and lactose would provide critical information for improving production and processing efficiency, thus increasing profit for the farmer. While these sensors have been demonstrated to work in the laboratory on calibrated standards and in limited field trials under controlled conditions, additional research is needed to reduce the complexity of the detection chemistry and increase the robustness of the sensing device, thus leading the way to commercial development.

Details of Cooperation

Over the course of this research project the US and Israeli teams have exchanged ideas and information through numerous visits (see table on cover page) and email exchanges, which promoted work on both sides by setting the direction of effort and evaluating results.

Publications

- Eshkenazi, I., E. Maltz, B. Zion, and J. Rishpon. 2000. Three-cascaded-enzymes biosensor for determination of lactose in raw milk. *J. Dairy Sci.*, 83:1939-1945.
- Jenkins, D. M., M. J. Delwiche, E. J. DePeters, and R. H. BonDurant. 1999. Chemical assay of urea for automated sensing in milk. *J. Dairy Sci.*, 82(9):1999-2004.
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