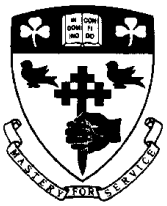


**McGill University**
**Macdonald Campus**

# *Department of Animal Science Research Reports*

**Faculty of Agricultural and Environmental Sciences**

**2006**

# Department of Animal Science

# Research Reports

Faculty of Agricultural and Enviromental Sciences

Macdonald Campus of McGill University

2006

# Table of Contents

## Introduction

It is with great pleasure that we at Department of Animal Science, McGill University bring you this annual report of research activities for 2005. This report was prepared thanks to the efforts of Professor Arif Mustafa as editor and Deborah Martin. This publication partially reflects the research activities of the department and I thank both staff and students for their contribution. Generous and continuous funding support from government funding agencies, industry sponsors and other organizations to our extensive research programs is also gratefully acknowledged.

Since our last annual research report in 1999, much has happened in the department. Several young, talent professors have joined the department. Dr. Mustafa was hired as an Assistant Professor in Dairy Nutrition and Dr. Bordignon joined the department as a molecular biologist/reproductive physiologist. Dr. Ruiz-Feria was appointed as a poultry specialist. A year ago, the department welcomed Dr. Sarah Kimmins on board to fill a position in Nutrition Genomics. During the same period, several professors left the Department. Dr. Laguë retired in 2002. Three other professors, Drs Buckland, Chavez and Downey, retired in 2005.

The significant improvement to our research facilities has been made possible through several grants from the Canadian Foundation of Innovation. The Donald McQueen Shaver Poultry Complex was officially opened in 2005, as a part of the Montreal Research Centre for the Development of Microbe-Free/Disease Resistant Poultry. The grant for the Development of a Dairy Information System Laboratory for Canada, led by Professor Wade, has finally solved the space problem for our Information Systems Group and provided the much-needed infrastructure for expanding our research in this area. Professor Bordignon received his CFI grant in 2004 to support the department's new exciting research directions in reproductive biotechnology. Last year, Professor Ruiz-Feria received his CFI grant to equip the poultry research laboratory. In addition, we were participants of other CFI grants.

After winning the 2003 Canadian Society of Animal Science's young scientist award, Professor Mustafa received 2004 Faculty teaching award. Professors Zhao and Mustafa were named William Dawson Scholars in 2002 and 2005, respectively. Professor Buckland was bestowed an Emeritus Professorship by McGill University in 2005. Professor Zhao is a recipient of the 2006 Canadian Society of Animal Science's award for Technical Innovation in Enhancing Production of Safe Affordable Food. Furthermore, our graduate and undergraduate students are continuing to receive fellowships and prizes.

As you read through the research reports, you will get a glimpse of a variety of research in the department. Please feel free to contact the authors of any report if you need further information. Copies of this report are available from the Department of Animal Science and may be obtained upon request. We would be pleased to have the press and others make use of the material contained herein, although acknowledgements of the source would be appreciated.

Xin Zhao, Chair and Professor
Department of Animal Science
McGill University, Macdonald Campus
Ste. Anne de Bellevue, QC, Canada H9X 3V9

# Department of Animal Science Staff and Graduate Students

## Academic

### Emeritus Professors

J.E. Moxley
R.B. Buckland

### Professors

J.F. Hayes
U. Kühnlein
K.F. Ng-Kwai-Hang
X. Zhao

### Associate Professors

R.I. Cue (Director, Swine Facilities)
H. Monardes
A. Mustafa
L.E. Phillip
K.M. Wade
D. Zadworny

### Assistant Professors

V. Bordignon
S. Kimmins
C. Ruiz-Feria
R-C Chian (part-time)

### Adjunct Professors

H. Baldassare
P. Lacasse
D. Lefebvre
B. Murphy

### Postdoctoral Fellows/ Research Associates

Y. Bing
J. Burchard
E. Ibeagha-Awemu
J. Moro
D. Pietersma

## Administrative

### Administrative Assistant

B. Stewart

### Secretaries

C. Horvath
D. Martin

## Technical

### Technicians/ Research Assistants

L. Che
D. Laurin
D. Praslickova

## Graduate Students

Abdukalykova, Saule
Alabri, Mohammed
Albornoz, Marcelo
Amini, Keyvan
Baurhoo,Bushansingh
Borucki, Sylvia
Chappaz, Eugenie
El-Beyrouthi, Nayla
Ferland, Marie-Claude
Hassanat, Fadi
Joeboy, Vinayaraj
Kawthekar, Sunil
Kgwatalala, Patrick
Lalonde, Annie
Leclerc, Benoit
Leduc, Mathieu (Leave of absence)
Levital, Tamir
Lorenzoni, Alberto Gino
Olaniyan, Benjamen
Perez Vargas, Einar
Praslickova, Dana
Ramirez, Jessica
Said, Kamal
Sindhwani, Madhu
Simon, Deeni
Squires, Sarah
Wan, Yonghong
Wang, Xiaoliang (Joanna)
Wysote, Marsha
Yeboah, Charles

# Associations of mastitis resistance traits with genetic markers in Canadian Holsteins

J. Moro-Méndez and J.F. Hayes


## ABSTRACT

Several genetic markers were found to be in association with incidence of clinical mastitis (ICM), occurrence of clinical mastitis, culling due to mastitis, and somatic cell scores in the Canadian Holstein population. Bulls which had daughters with lactation records were genotyped for polymorphisms of genes related to immune response (GH, GHR, ODC, IGF-1, ACTH, CRH and PRL). Estimated transmitting abilities for incidence of clinical mastitis, occurrence of clinical mastitis, culling due to mastitis, and somatic cell score were estimated for each bull, and were included as response variables in across-population analysis and within-family analysis to detect associations between the markers and several phenotypic indicators of mastitis resistance, and SCS, obtained from lactation records of daughters. Permutation tests were performed to reduce type I error. Significant associations were found within families for markers of IGF-1 (BTA5), ODC (BTA11), GH (BTA 19), GHR (BTA 20), and PRL (BTA 23) for ICM, OCM, CDM, and SCS in different lactations. These significant marker effects suggest the presence of QTL with effects on the traits under study.


**Keywords**: QTL mapping, mastitis resistance, dairy cattle, candidate genes

## INTRODUCTION

In Canada, the total annual consumption per capita of milk has decreased from 102.84 litres in 1980 to 85.31 in 2003 (Dairy Market Review 2003); however, yogurt consumption shows an increase during the same period from 1.61 to 6.23 litres per capita. Any effort in diminishing incidence of clinical mastitis by means other than administration of antibiotics (i.e. genetic selection) may help in avoiding residual antibiotics in milk that, in addition to public health concerns, is considered a major problem in yogurt production (Ruegg 2005). Research on the genetic control of mastitis may provide means to ensure the quality of highly demanded dairy products.

There is evidence of genetic variability in mastitis resistance (Rupp and Boichard 2003). Several studies have partially unveiled genetic factors underlying mastitis resistance. Most attempts in gene detection have been performed by analyzing associations between genes of the major histocompatibility complex or anonymous markers with mastitis traits. As a result, several quantitative trait loci with effect on somatic cell score and clinical mastitis have been mapped on the 29 bovine autosomes (Khatkar et al. 2004). However no study has reported the use of candidate genes. Complex traits are likely under control of many genes, and thus the role of candidate genes such as genes related to immune response must be explored. In this study we tested the hypothesis that genetic markers of genes related to immune function are associated with mastitis resistance in Canadian Holsteins.

## MATERIALS AND METHODS

### Genetic Markers

The data used in the present study contained information for Canadian Holstein bulls on DNA markers of genes related to immune response (Postel-Vinay et al. 1997; Clark et al. 1997; Carr et al. 1990): growth hormone (nine markers), growth hormone receptor (three), prolactin (two), ornithyne decarboxylase (two), adrenocorticotropic hormone (two), insuline-like growth factor-1 (one), and corticotropine releasing hormone (one). 721 bulls which had daughters with lactation records were genotyped

for these polymorphisms in a granddaughter design.

Details regarding the development of the DNA markers used in this study may be found in Aggrey et al. (1999), Yao et al. (1996), and Yao et al. (1998).

**Phenotypic Data**

Lactation records of cows enrolled in the PATLQ from 1980 to 1994 were used to generate estimated transmitting ability of mastitis resistance traits (incidence of clinical mastitis, occurrence of clinical mastitis and culling due to mastitis) and somatic cell scores for first, second, and third lactations. Incidence of clinical mastitis was defined as a binary trait (cow with clinical mastitis along the lactation=1, otherwise=0); occurrence of clinical mastitis was defined as the number of clinical mastitis cases reported along the lactation; culling due to mastitis was defined as a binary trait (cows culled before the end of lactation=1, otherwise=0), somatic cell counts available for each lactation were transformed to somatic cell score as proposed by Shook and Schultz (1994). From the phenotypic indicators of mastitis resistance, estimated transmitting ability were generated by restricted maximum likelihood using a model that included fixed effects of herd-year-season of calving, age at calving, genetic group, and random effect of sire.

**Statistical Analysis**

Two types of association analyses were performed between the genetic markers and the estimated transmitting ability: an across-population analysis with fixed effect of marker and random effect of bull. This model accounted for the additive relationships among bulls. The other analysis was a within-family analysis with a model with fixed effects of grandsire, marker nested within grandsire, and random effect of son nested within marker and grandsire. Permutation tests were performed according to procedures proposed by Churchill and Doerge (1994) to reduce type I error.

## RESULTS

No significant associations were found across population; however, significant associations were found within families. Table 1 shows the markers in association with the quantitative traits within families. The first two columns contain the identities for the marker and the bovine autosome where the marker is located.

Two quantitative trait loci were detected linked to ornithine decarboxylase 1 and growth hormone 61 in first lactation. Three quantitative trait loci were detected linked to insulin growth factor 390, growth hormone 61, and prolactin 152, respectively, in second lactation cows. At least three quantitative trait loci linked to insulin growth factor 390, growth hormone 61, growth hormone 1300 and growth hor-mone receptor AC were detected in third lactations. The effect of markers growth hormone 61 and growth hormone 1300 in third lactations may be produced by the same quantitative trait loci as the distance between markers is about 1583 bases.

## DISCUSSION AND CONCLUSIONS

A significant association between the marker and the trait in across population analysis would indicate the presence of linkage disequilibrium between the marker and a quantitative trait loci affecting the trait. A significant effect for the marker in the within-family analysis indicates that a quantitative trait loci is physically linked to the marker in some families.

Several markers were in association with the quantitative trait within families but not across population. Such an association suggests that there are quantitative trait loci physically linked to the markers in some families, but they appear to be in linkage equilibrium across the population. Marker allele differences (not shown) suggest that the marker-quantitative trait loci linkage phase is different in different families.

Some of these putative quantitative trait loci are located in regions where other quantitative trait loci for somatic cell score, incidence of clinical mastitis, or conformation traits have been reported (Khatkar et al. 2004). Mapping studies may be useful to combine with information collected from more complex methods of gene detection, such as gene expression profiles (de Koning et al. 2005). Further studies are needed in order to fine map the detected quantitative trait loci in the Canadian Holstein population.

Putative quantitative trait loci were detected affecting several mastitis resistance traits and somatic cell score in different lactations. These results may contribute to efforts in dissecting the genetic basis of mastitis resistance in dairy cattle.

**Table 1.** Significant markers for various mastitis resistance traits in Canadian Holsteins

| Marker | Chromosome | Parity | Trait | p-value |
|---|---|---|---|---|
| ODC1 | 11 | 1 | CDM | 0.0172 |
| GH61 | 19 | 1 | SCS | 0.0407 |
| | | | SCS | 0.0308 |
| IGF390 | 5 | 2 | CDM | 0.0322 |
| GH61 | 19 | 2 | ICM | 0.0002 |
| | | | OCM | 0.0001 |
| | | | CDM | 0.0039 |
| PRL152 | 23 | 2 | ICM | 0.0161 |
| IGF390 | 5 | 3 | ICM | 0.0029 |
| | | | OCM | 0.0038 |
| | | | CDM | 0.0055 |
| GH61 | 19 | 3 | ICM | 0.0114 |
| | | | OCM | 0.0058 |
| | | | CDM | 0.006 |
| | | | SCS | 0.008 |
| GH1300 | 19 | 3 | SCS | 0.0409 |
| GHRAC | 20 | 3 | ICM | 0.0145 |
| | | | OCM | 0.0028 |
| | | | CDM | 0.0092 |

## REFERENCES


Aggrey S. E., J. Yao, M. P. Sabour, C. Y. Lin, D. Zadworny, J. F. Hayes, and U. Kuhnlein. 1999. Markers within the regulatory region of the growth hormone receptor gene and their association with milk-related traits in Holsteins. J. Hered 90: 148-151.

Carr, D. J. J., Acosta, B. R., Jacobson, A. E. and Blalock, J. E. 1990. Corticotropin-releasing hormone augments natural killer cell activity through a naloxone-sensitive pathway. J. Neuroimmunology 28: 53-61.

Churchill, G. A. and Doerge, R. W. 1994. Empirical thresholds values for quantitative trait mapping. Genetics 138: 963-971.

Clark, R. 1997. The somatogenic hormones and insuline-like growth factor-1: stimulators of lymphopoiesis and immune function. Endocrine Reviews 18: 157-179.

De Koning, D. J., Carlborg, Ö. and Haley, C. S. 2005. The genetic dissection of immune response using gene-expression studies and genome mapping. Veterinary Immunology and Immunopathology 105: 343-352.

Harmon, R. J. 1996. Controlling contagious mastitis. Proceedings of the 1996 National Mastitis Council Meeting. National Mastitis Council, p.10.

Khatkar, M.S. Thomson, P. C., Tammen, I. and Raadsma, H. W. 2004. Quantitative trait loci mapping in dairy cattle: review and meta-analysis. Genet. Sel. Evol. 36: 163-190.

Postel-Vinay, M. C., De Mello Coello, V., Gagnerault, M.C. and Dardenne, M. 1997. Growth hormone stimulates the proliferation of activated mouse T lymphocytes. Endocrinol. 138: 1816-1820.

Ruegg, P. 2005. Relationship between bulk tank milk somatic cell count and antibiotic residues. Proceeding of the 2005 National Mastitis Council Meeting. National Mastitis Council, p. 28.

Rupp, R. and Boichard, D. 2003. Genetics of resistance to mastitis in dairy cattle. Vet. Res. 34:671-688.

Shook, G. E. and Schutz, M. M. 1994. Selection on somatic cell score to improve resistance to mastitis in the United States. J. Dairy Sci. 77: 648-658.

Yao, J, S.E. Aggrey, D. Zadworny, F.J. Hayes, and U. Kuhnlein. 1996. Sequence variations in the bovine Growth hormone Gene characterized by Single-Strand conformation polymorphism (SSCP) analysis and their association with milk production traits in Holsteins. Genetics 144: 1809-1816.

Yao J., S. E. Aggrey, D. Zadworny, U. Kuhlein, and J.F. Hayes. 1998. Restriction length polymorphisms at the ornithine decarboxylase locus associated with milk protein yield in Holsteins. DNA Sequence – J. sequencing and mapping, 8: 203.

# Exposure chamber for determining the biological effects of electric and magnetic fields on dairy cows

D.H.Nguyen,[1] L. Richard[1] and J.F. Burchard

[1]Hydro-Quebec Research Institute (IREQ),Varennes, Quebec, Canada

**ABSTRACT**

An exposure chamber was designed to study the effects of electric and magnetic fields (EMF) on dairy cattle. The chamber was 15 $_x$ 10 3m; and the control system was fully computerized so that the field intensities can be varied and monitored continuously, on site or remotely. The chamber characteristics allow use of a wide range of exposure such as electric fields (0– 30kV/m) and magnetic fields (0– 100 mT) at frequencies ranging from 45 to 3000 Hz.

**Keywords**: exposure system; ELF; dairy cattle; electric field; magnetic field

## INTRODUCTION

One of the concerns raised as part of Hydro Quebec's Action Plan on Biological Effects of EMF was the possible influence of EMF exposure dairy cattle. Since many dairy farms are located in close proximity of 735 kV transmission lines, Hydro Quebec was required to study the influence of EMF exposure produced by these lines dairy cows, with particular emphasis on milk production. Most of the early investigations on dairy cows were survey type studies in which the health of cattle was evaluated as a function of proximity to power transmission lines. (Williams and Beiler 1979). Hydro Quebec considered two approaches (1) selecting two groups of dairy farms, close to 735 kV lines and far away from the lines, measuring EMF exposure as well as animal health variables over a period of several months; (2) designing and building an EMF exposure chamber and studying the health of cattle under controlled exposure conditions. An expert Industry – Government Committee selected the second alternative, The first EMF exposure facility designed and built for studies on dairy cows is described in this report.

## MATERIALS AND METHODS

In order to design the exposure chamber for studies on dairy cattle, it was necessary to characterize the EMF environment near Hydro Quebec's 735 kV transmission lines. The lateral profiles of the EMF magnitudes at ground level are shown in Figure 1a. Typical statistical distribution (left vertical axis) as well as cumulative probability (right vertical axis) of load current (bottom horizontal axis) and the corresponding resulting magnetic field (top horizontal axis) over a period of time are shown in Figure 1b. It is seen that the EMF attain maximum values of 10 kV/m and 30 mT, respectively Exposure Chamber The exposure chamber was built in one of the metabolism rooms at the Cattle Complex (Macdonald Campus, McGill University)., The chamber dimensions were 15 x 10 3 m high (Fig. 2). Vertical electric fields and horizontal magnetic fields were generated, simulating the conditions under a transmission line. It contained eight wooden box stalls, each capable of holding one adult dairy cattle,

### Field Generation in the Exposure Chamber

The magnetic field was generated by 14 rectangular coils, each 10 m wide and 4 m high, distributed uniformly along the exposure chamber. The number of turns in each coil permit obtain a reasonably uniform magnetic field distribution along the chamber To

ensure vertical field uniformity in the direction of the space. the lower part of each coil was embedded in the concrete floor, A uniform electric field was generated using a parallel plate arrangement, with the insulated high voltage electrode that comprised two plates, each 9 m long and 6.5 m wide, suspended 0.4 m below the ceiling and a ground mat embedded in the concrete floor serving as the ground electrode

**Power Supply, Control, and Safety Systems**

A stable Elgar 112 V, 3 kVA, 45–45000 Hz power source was used to supply both, the parallel plate arrangement to generate the electric field and the system of coils to generate the magnetic field. A Messwandler-Bau step-up transformer was used to apply the high voltage to the plates, while a step-down transformer provided the current supply to the coils. The control system was fully computerized so that the field intensities could be varied and monitored continuously. A telephone link also allowed remote monitoring. The high voltage equipment was controlled via a fiber optic link. The system was equipped with integrated safety devices. Magnetic relays were installed in the three access doors to allow the power supply to be switched off, in case any of them was opened inadvertently during tests. The results of calculation of the uniformity of magnetic field distribution were verified by making systematic measurements using a Holaday model 3602 m. The measurements have shown a maximum deviation of 4% from a uniform magnetic field distribution. Placing animals, which may be considered essentially as conductive objects, in the exposure chamber may lead to a slight distortion of the magnetic field distribution. However, a similar distortion also takes place when the animals are exposed to magnetic fields under transmission lines. Placing animals in an electric field, either under a transmission line or in an exposure chamber leads to a significant distortion of the field, particularly in the immediate vicinity of the animal. However, since the dimensions of the exposure chamber are generally not much larger than those of the exposed animals, the field distortion may be much larger [Kaune, 1981].

Because of the limited height of the exposure chamber in our study compared to the height of the animals, the electric field enhancement, which attains a maximum on the back of the animal, would be higher than when it is located under a transmission line. Calculations were made using the Charge Simulation Method (Singer et al. 1974), with the body of the animal represented by a cylinder 0.6 m in diameter and terminated at both ends with a hemisphere, for a total length of 2.3 m. The animal height was assumed to be.
The EMF was monitored continuously at three locations in the chamber using probes calibrated prior to the exposure periods. The exposure chamber was heated during winter months. Temperature but not humidity controls were provided. The maximum ambient fields generated by the farm appliances were 2.5 V $m^{-1}$ for electric fields and 0.15 mT for magnetic fields. Every day when the animals were remotely milked for 15 min, the magnetic field close to the milking unit reaches 2.4 mT and the animal was locally exposed to a maximum field of 0.8 mT. The blood samples were taken with a catheter no more than 5 m long.

In order to prevent additional electric currents from entering the animal due to the induction effects of the electric field, the catheter was attached to the bottom of the animal's neck and was consequently shielded by its head. The catheter followed the wall of the chamber to the adjacent room where the blood sampler was located. With regard to the magnetic induction, we ensured that the catheter did not make a closed electrical loop. The maximum field during centrifugation of blood samples was detected at the bottom of the centrifuge where the intensity was 1.73 mT. The high voltage electrodes were designed to be completely corona-free.

Before introducing the animals, corona-free operation of the exposure chamber was checked first by visual inspection in the dark and then by monitoring long term ozone levels. To avoid a high circuit current from becoming grounded through the animal's mouth when drinking water, the bowl was made of non - conducting material and the water was supplied through nylon tubing (9.5 mm) in diameter and at least 5 m long. With a water conductivity of 100 mS $cm^{-1}$ (at 8 8C), the water line would limit any resulting short circuit current. The stray voltage measurements were performed under two

conditions: (1) between two locations in the rubber mat on which the animals stood or laid down; (2) between an aluminum plate (1 2 m), simulating the animal contact with the mat, and the water bowl. The stray voltage amplitudes were less than 1 mV peak. These measurements demonstrate that the metallic grid inserted in the concrete floor of the chamber was efficient. The stray voltage measurements were performed using the techniques suggested by Canadian Electricity Asssociation (1996).

## CONCLUSIONS

An indoor laboratory EMF exposure chamber was designed and built for biological studies on dairy cows. Electric fields were generated by a parallel plate arrangement, while magnetic fields were generated using a multi coil arrangement in which the number of turns in each coil was chosen to produce a uniform magnetic field distribution in the space occupied by the study animals. Uniformity of the electric field distribution and the field enhancement due to the presence of animals as well as the uniformity of magnetic field distribution were established through calculations and checked by measurements. Local and remote operation and control of the exposure chamber were achieved by using a computer controlled system. Calibration and verification tests were carried out before commissioning the exposure chamber to ensure its proper functioning.

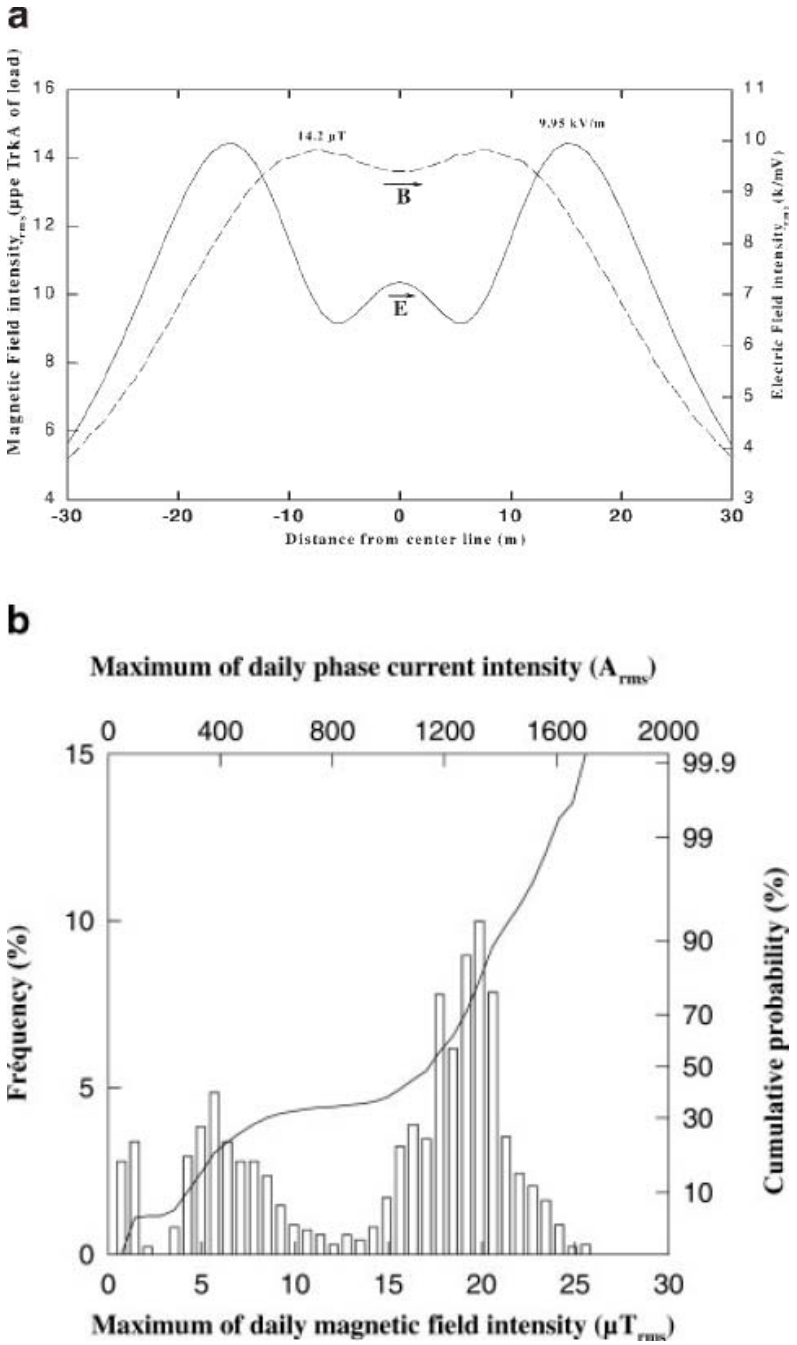


**Fig. 1. (a):** Lateral profiles of electric and magnetic fields below 735 kV line; (b) statistical distribution of line current and magnetic field of typical 735 kV line.

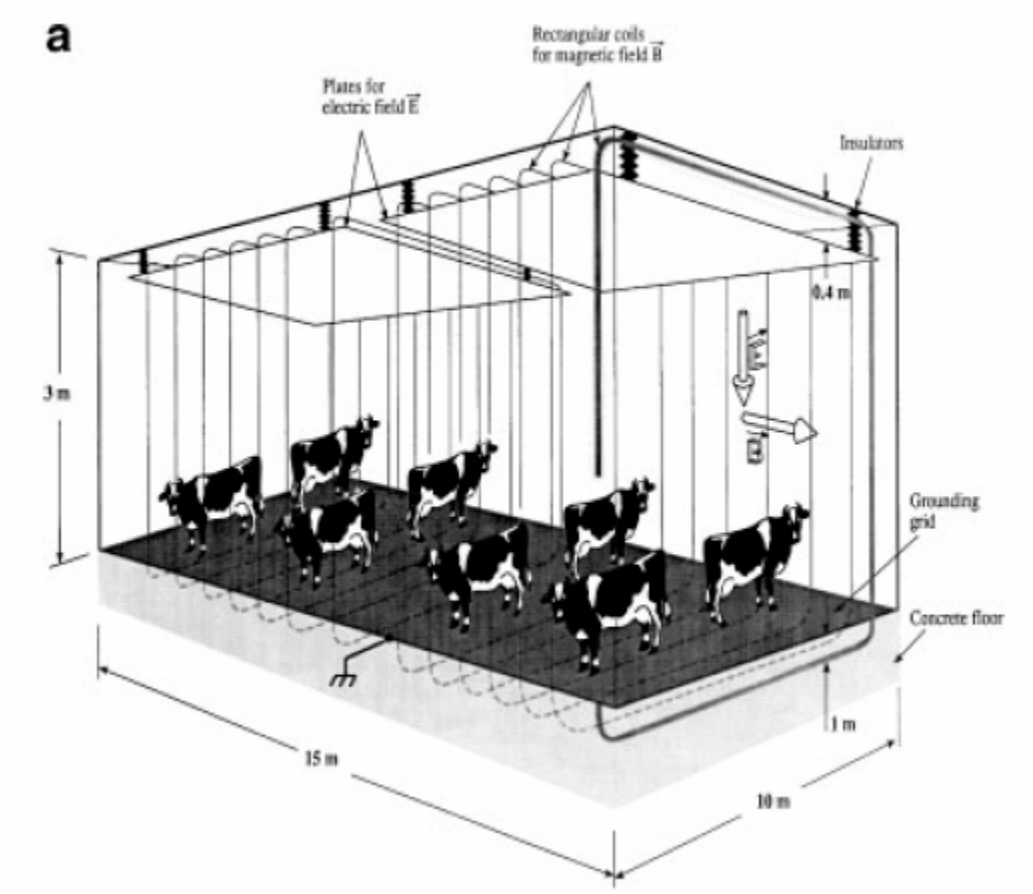


**Fig. 2:** A schematic of the livestock exposure chamber, showing electric field plates and magnetic field coils.

## REFERENCES

Canadian Electricity Asssociation. 1996. W.A. Chisholm, ‘‘Technique for Transient Stray Voltage Measurements in Livestock Barns’’, 9143 U 885/178 D 885.

Kaune WT. 1981. Interactive effects in 60 Hz electric field exposure systems. Bioelectromagnetics 2: 33- 50.

Singer H, Steinbigler H, Weiss P. 1974. Acharge simulation method for the calculation of high voltage fields IEEE Trans PAS 93: 1660 – 1668.

Williams JH, Beiler EJ. 1979. An investigation of dairy farm operations in association with 765 kV transmission in Ohio. Cleveland, Ohio, USA: Ohio Power Sitting Commission and Cleveland Electric illuminating Company.

# Blood melatonin and prolactin concentrations in dairy cows exposed to 60 Hz electric and magnetic fields during eight-hour photoperiods

M. Rodriguez, D. Petitclerc[1] J.F. Burchard, D.H. Nguyen[2] and E. Block[3]
[1]Agriculture and Agri-Food Canada, Dairy and Swine Research and Development Centre, Lennoxville, QC, Canada J1M 1Z3
[2] Institut de Recherche d'Hydro-Québec (IREQ), Varennes, QC, Canada J3X 1S1
[3]Church and Dwight Co. Inc. 469 Harrison St. Princeton, NJ 08543-5297, USA

## ABSTRACT

Two experiments were conducted to test the hypothesis that EMF exposure may result in endocrine responses similar to those observed in animals exposed to long days. In the first experiment, sixteen lactating, pregnant Holstein cows were assigned to two replicates according to a crossover design with treatment switch back. All animals were confined to wooden metabolic cages and maintained under short day photoperiods (8 h light/16 h dark). Treated animals were exposed to a vertical electric field of 10 kV/m and a horizontal magnetic field of 30 μT EMF for 16 h/d for 4 wk. In a second similar experiment, sixteen non-lactating, non-pregnant Holstein cows subjected to short days were exposed to EMF using a similar protocol, for periods corresponding to the duration of one estrous cycle. In the first experiment, circulating MLT concentrations during the light period showed a small numerical decrease during EMF exposure ($P<0.05$). Least square means for the 8 h light period were 9.9 vs. 12.4 pg/ml, SE=1.3. Melatonin concentrations during the dark period were not affected by the treatment. A similar trend was observed in the second experiment, where MLT concentrations during the light period tended to be lower (8.8 pg/ml vs. 16.3 pg/ml, $P<0.06$) in the EMF exposed group, and no effects were observed during the dark period. Plasma PRL was increased in the EMF exposed group (16.6 vs. 12.7 ng/ml, $P<0.02$) in the first experiment. In the second experiment, the overall PRL concentrations found were lower, and the mean plasma PRL concentration was not affected by treatment. These experiments provide evidence that EMF exposure may modify the response of dairy cows to photoperiod.

**Key words:** Electric field; magnetic field; cows, melatonin, prolactin.

## INTRODUCTION

In previous studies dairy cows exposed to 30μT, 10kV $m^{-1}$ EMF showed some biological responses (Burchard et al. 1996, Rodriguez et al. 2003). Light inhibits the activity of pineal N-acetyl transferase, a rate limiting enzyme in the synthesis of MLT from the neurotransmitter serotonin, decreasing MLT levels during the light period. Exposure to EMF suppresses MLT secretion or circulating levels (Reiter 1993). It has been suggested that EMF are interpreted by the SNC as light. Lactating, pregnant dairy cows exposed to a 30μT, 10kV $m^{-1}$ EMF continuously, did not show any change in nocturnal MLT. Diurnal concentrations were not measured. (Burchard et al. 1998). Long days have enhanced PRL secretion in cattle. (Bourne and Tucker 1975). These experiments tested the effects of EMF on PRL and MLT in dairy cows maintained under short day photoperiods (8 h of light).

## MATERIALS AND METHODS

### Exposure Chamber

Refer to other research report paper (Nguyen et al.) for Animals and exposure protocol

### Experiment 1

Sixteen, lactating, pregnant Holstein cows were divided into two replicates of eight animals each, and housed in the EMF chamber. Each replicate was exposed to EMF according to one of two sequences of three periods of 28 days each. Sequence 1 OFF-ON-OFF and sequence 2 ON-OFF-ON. During the last day of each treatment period, blood samples were collected every 4 h for 24 h for RIA analysis.

### Experiment 2

Sixteen non-lactating, non-pregnant, Holstein cows were housed in the EMF exposure chamber. The animals were distributed in replicates analogous to experiment 1. Each treatment period was one estrous cycle and the treatment sequence was: sequence 1 OFF-ON-ON and sequence 2 ON-OFF-OFF. All animals had ovarian activity confirmed and were synchronized for estrus. Blood samples were collected every other day for RIA analysis.

### Statistical Analysis

The experimental designs were crossover designs with treatment switchback. The results were analysed using the mixed model procedure of SAS (1996).

## RESULTS

The mean EMF exposure time was 14.01 ± 0.3 and 12.23 ± 0.4 h $d^{-1}$ for experiment 1 and 2, respectively.

### Melatonin

Serum MLT concentration was much higher in the dark period than in the light period, as expected. In Experiment 1, MLT concentrations showed a small decrease in the exposed animals during the light period (P = 0.04), but no significant differences were observed in the dark period. In Experiment 2 a similar trend was observed; overall, EMF treatment did not affect the mean serum MLT concentration (P = 0.18), but the difference in MLT concentrations between treatments during the light period tended to be significant (P = 0.06). No differences were detected in the dark phase. Mean MLT concentrations during the light period and during the dark period for the two experiments are shown in Table 1 and 2. The variability of nocturnal MLT levels was higher than that of diurnal values.

### Prolactin

In Experiment 1, exposure to EMF resulted in significantly increased PRL levels at the end of the exposure period (P = 0.014). Treatment least square means were 12.7 ng $mL^{-1}$ and 16.6 ng $mL^{-1}$ for control and exposed groups, respectively (SE = 0.8 ng/ml. No time trend in prolactin concentrations over the 24-h period was observed. Mean PRL concentrations over 24 h for each treatment are illustrated in Fig. 2.

## DISCUSSION

### Melatonin

Previous studies have not observed an effect of EMF exposure on MLT. (Lee et al. 1993; Lee et al., 1995; Burchard et al., 1998). The differences detected in the present trial were small and only surfaced when the heterogeneity of the variance between diurnal and nocturnal measurements was taken into account. In the control group, MLT begins to rise as soon as the lights turn off and peaks 6 h later, then declines. A similar situation is observed in the exposed group, although the diurnal baseline levels are higher (Fig. 1). However, when EMF is turned off, a second rise in MLT concentration appears to occur, conducive to a second maximum 4 h later.

The hypothesis that some of the observed effects of EMF may be explained by alteration of the response to photoperiod may contribute to explain some of this inconsistency. If EMF exposure could modify photoperiod response mimicking light, we hypothesize that this would be likely to occur under the above conditions.

### Prolactin

In Experiment 1, exposure to EMF appeared to be associated with a positive change of PRL over time, whereas during non-exposed periods there was no obvious time trend. The results in Experiment 2 also show a trend in PRL concentration to increase in exposed treatment (P = 0.10) illustrated in Fig. 4.

In general, PRL responses in the present experiments provide support to the hypothesis that EMF exposure results in effects analogous to those of long days. In cattle long days stimulate PRL secretion (Bourne and Tucker, 1975).

## CONCLUSIONS

Exposure to EMF may modify the response of dairy cows to photoperiod, but our model only begins to address the problem, and the results are far from definitive. The suppressive effect of EMF on MLT levels observed during the light period, when the concentrations are already low is interesting and should further.

**Table 1.** Mean serum melatonin concentrations during the light period and the dark period in lactating, pregnant Holstein cows exposed to EMF under short day conditions (Experiment 1).

| Treatment | Serum Melatonin (pg $mL^{-1}$) | | | |
|---|---|---|---|---|
| | Light period | | Dark period | |
| | LS mean | SE | LS mean | SE |
| Control | 12.4a | 1.3 | 55.6c | 5.2 |
| EMF exposed | 9.9b | 1.3 | 46.2c | 5.0 |

a-c Means with different letters differ ($P < 0.05$)

**Table 2.** Mean serum melatonin concentrations during the light period and the dark period in Holstein non-lactating, non-pregnant cows exposed to EMF under short day conditions (Experiment 2).

| Treatment | Serum Melatonin (pg $mL^{-1}$) | | | |
|---|---|---|---|---|
| | Light period | | Dark period | |
| | LS mean | SE | LS mean | SE |
| Control | 16.3a* | 2.3 | 51.2b | 6.0 |
| EMF exposed | 8.8a* | 2.3 | 46.1b | 6.0 |

a,b Means with different superscripts differ ($P < 0.01$)

* Means differ at P = <0.1

**Table 3.** Mean plasma prolactin concentrations of lactating, pregnant cows exposed to EMF for each period and sequence (Experiment 1)

| Sequence | Period | Plasma PRL ($n\ mL^{-1}$) | SE ($ng\ mL^{-1}$) |
|---|---|---|---|
| 1 | 1(OFF) | 6.7 | 1.6 |
| 1 | 2(ON) | 13.0 | 1.6 |
| 1 | 3(OFF) | 15.1 | 1.6 |
| 2 | 1(ON) | 22.7 | 1.6 |
| 2 | 2(OFF) | 13.3 | 1.6 |
| 2 | 3(ON) | 15.5 | 1.6 |

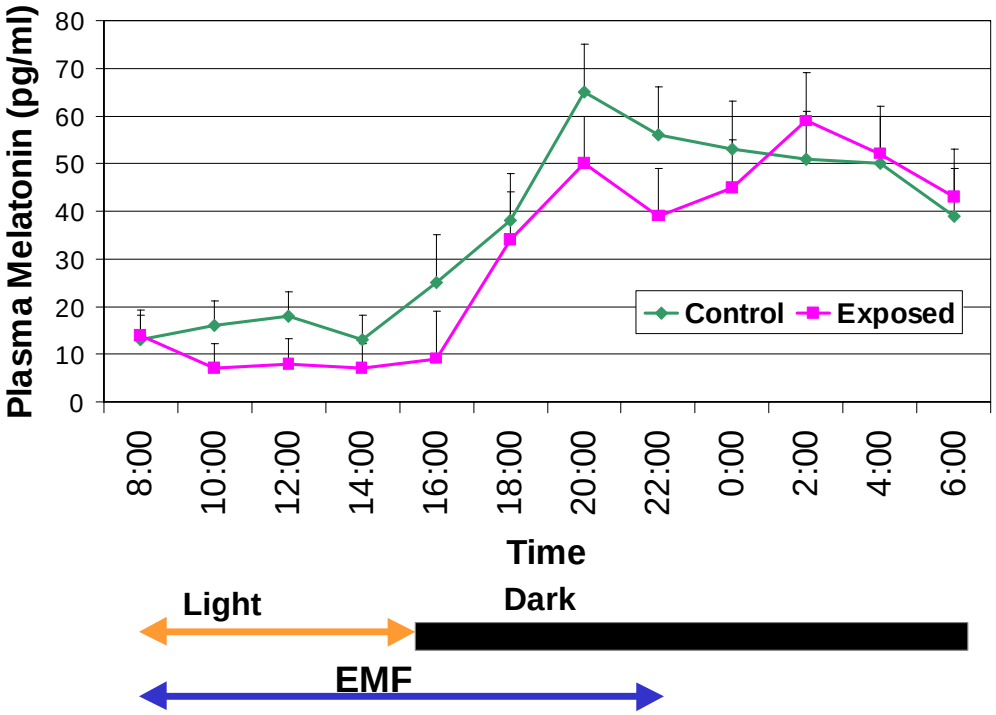


**Fig. 1.** Mean (±SEM) serum melatonin concentrations over 24 hours in lactating, pregnant dairy cows exposed and non-exposed to EMF under short day conditions (Experiment 1)

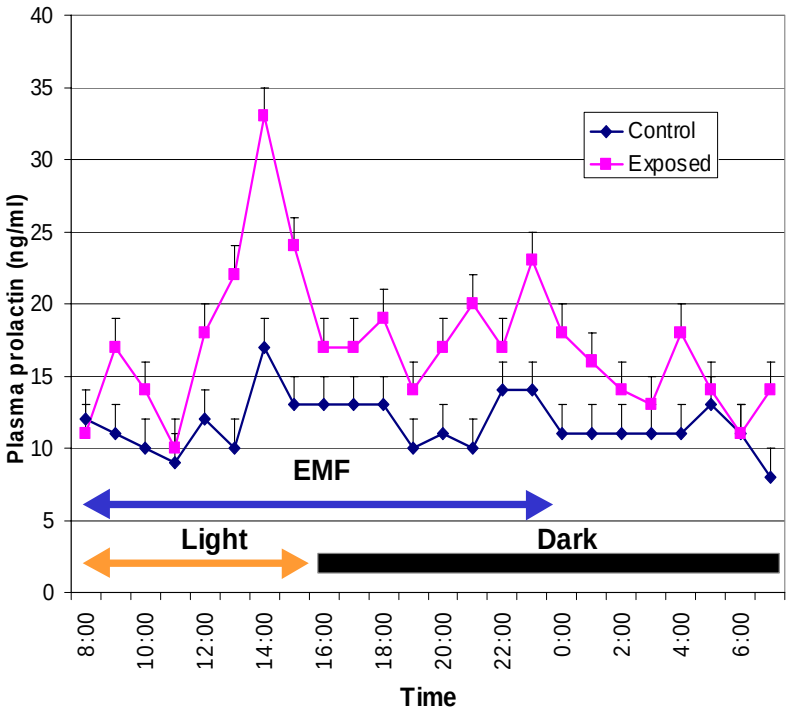


**Fig. 2.** Mean (±SEM) plasma prolactin concentrations over 24 hours in lactating, pregnant dairy cows exposed and non-exposed to EMF under short day conditions (Experiment 1)

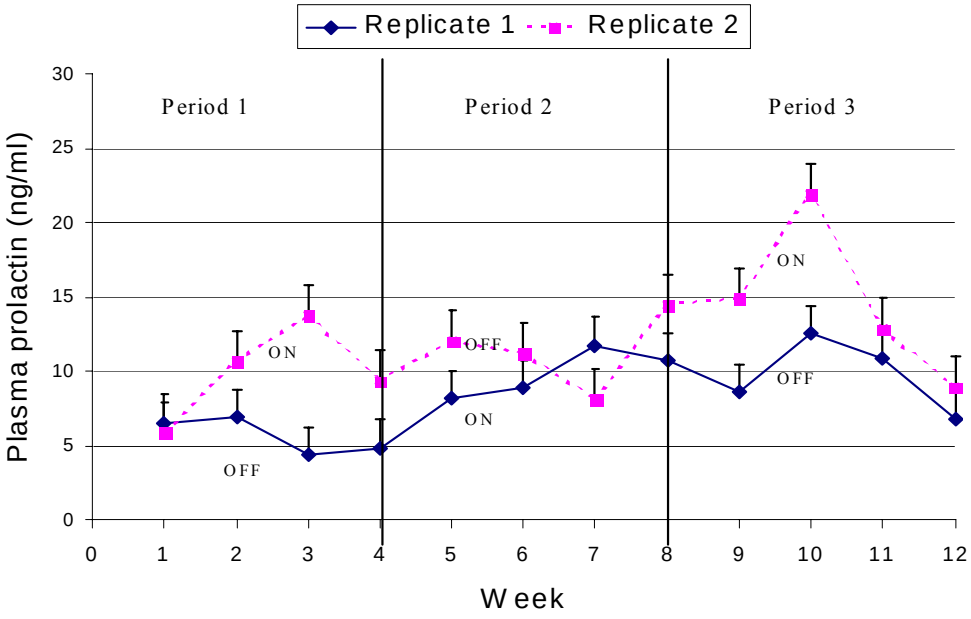


**Fig. 3.** Mean (±SEM) Weekly changes of plasma prolactin concentration in lactating, pregnant cows in the two sequences: Rep 1: OFF-ON-OFF; Rep 2: ON-OFF-ON.

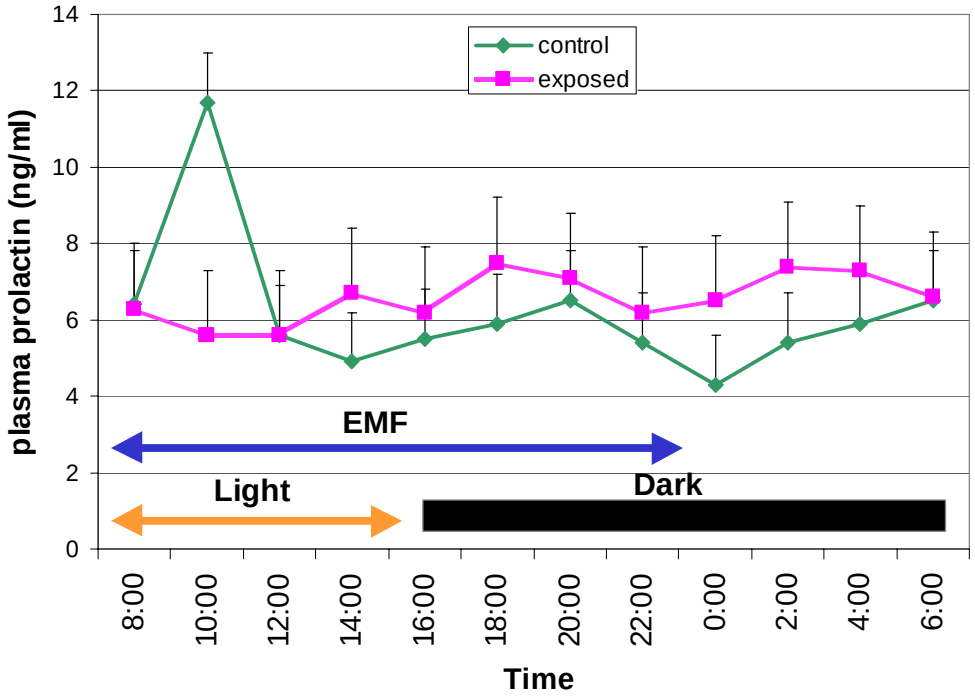


**Fig. 4.** Mean (+SEM) plasma prolactin concentrations over 24 hours in non-lactating, non-pregnant dairy cows exposed and non-exposed to EMF under short day conditions (Experiment 2)

## REFERENCES


Bourne, R. A. Tucker, H. A. 1975. Serum prolactin and LH responses to photoperiod in bull calves. Endocrinol 97: 473-475.

Burchard, J. F., Nguyen, D. H. and Block E. 1998. Effects of electric and magnetic fields on nocturnal melatonin concentrations in dairy cows. J. Dairy Sci. 81: 722-727.

Burchard, J. F., Nguyen, D. H, Richard, L. and Block, E. 1996. Biological effects of 60 Hz of electric and magnetic fields on productivity of dairy cattle. J. Dairy Sci. 79: 1549-1554.

Lee, Jr J. M., Stormshak, F., Thompson, J. M., Thinesen, P., Painter, L. J., Olencheck, E. G., Hess, D. L., Forbes, R. and Foster, D. L. 1993. Melatonin secretion and puberty in female lambs exposed to environmental EMF. Biol. Reprod. 49: 857-864.

Lee, Jr J. M., Stormshak, F., Thompson, J. M., Hess, D. L. and Foster, D. L. 1995. Melatonin and puberty in female lambs exposed to EMF: a replicate study. Bioelectromagnetics 16: 119-123.

Reiter, R. J. 1993. Static and extremely low frequency electromagnetic field exposure reported effects on the circadian production of melatonin. [Review] J. Cell. Biochem. 51: 94-403.

Rodriguez, M., Petitclerc, D., Burchard, J. F., Nguyen, D. H., Block, E. and Downey, B. R. 2003. Responses of the estrous cycle in dairy cows exposed to electric and magnetic fields (60Hz) during 8-h photoperiods. Anim. Reprod. Sci. 77: 11-20

SAS/STAT Software 1996. SAS Institute, Cary, NC

# Plasma concentrations of thyroxine in dairy cows exposed to electric and magnetic fields (60 Hz).

J.F. Burchard , D.H. Nguyen[1] and M. Rodriguez

[1] Institut de Recherche d'Hydro-Québec (IREQ), Varennes, QC, Canada J3X 1S1

## ABSTRACT

Two experiments were carried out to assess the effects of electric and magnetic fields (EMF) on blood thyroxine (T4) in dairy cattle. In experiment 1 and 2, 16 Holstein cows were divided into two groups of eight animals each. Each group was exposed to 10 kV/m, 30 μTesla (μT) EMF according to one of two sequences of three treatment periods. The light cycle emulated a short photoperiod (8 h light/16 h dark). During the ON periods, the animals were exposed to EMF for 16 h, 8 h of the light period plus the first 8 h of during the dark period (Figure 1). In experiment 1, 16 lactating, pregnant Holstein cows were used and each treatment period was 28 d. Sequence 1 was EMF OFF-ON-OFF and sequence 2 was EMF ON-OFF-ON. During the last day of each treatment period, blood samples were collected every 4 h for 24 h to estimate T4 plasma concentrations. In experiment 2, 16 non-lactating, non-pregnant, Holstein cows were used and each treatment period corresponded to one estrous cycle. Sequence 1 was EMF OFF-ON-ON and sequence 2 was EMF ON-OFF-OFF. Blood samples were collected every other d for T4 analysis. In experiment 1, exposed animals did not have any change in T4 plasma concentrations due to treatment but, the time of sample collection revealed a significant - difference. In experiment 2, the effect of days ($p = 0.0314$), period ($p=0.0002$) and the treatment by days interaction ($p=0.0018$) were statistically significant. Worst case scenario exposure of dairy cattle to 10 kV/m, 30 μT EMF influences, in a moderate fashion, the blood levels of thyroxine.

**Keywords**: thyroxine, electric field, magnetic field, dairy cattle.

## INTRODUCTION

High tension lines in the Province of Québec, Canada, generate extremely low frequency (ELF) electric and magnetic fields (EMF). Dairy products represent Quebec's main agricultural income. Continuous EMF (10 kV $m^{-1}$, 30 μT), caused a variety of biological effects in dairy cattle. (Burchard et al. 1998; Rodriguez et al. 2003) Thyroid hormones play a fundamental role in the regulation of vital functions animals (Capen and Martin 2003). Melatonin (MLT) interacts with thyroid hormones during the seasonal reproductive cycle in sheep (Champney 2001), decreases plasma triiodothyronine (T3) , T4 and TSH (Baltaci et al. 2004) and is with PRL. Considering that previous exposure to EMF has changed a variety of hormones that have association to T4 (see other related research reports), two experiment were designed to evaluate the effect of EMF exposure on T4 plasma concentrations in dairy cattle.

## MATERIALS AND METHODS

### Exposure Chamber

The EMF exposure chamber was built by Hydro-Québec in the Dairy Cattle Complex of Macdonald Campus, McGill University. The chamber allows simultaneous exposure of 8 cows to 30 μT, 10 kV $m^{-1}$ EMF. The EMF was monitored with three probes installed in the chamber. The intensity of the EMF in these experiments resembles the worst case scenario encountered by dairy cattle on pasture if standing continuously under an 735 kV

AC power line with a load of current of 2000 A (Nguyen et al. 2005).

**Animals and Exposure Protocols**

The EMF exposure treatment consisted, of a 10 kV/m electric field and a 30 μT magnetic field. During ON periods, EMF were activated at 08:00 h and deactivated at midnight and during the OFF period were deactivated 24 h/d. A short-day light regime (8 h light/16 h dark) was provided... Lights were turned on at 08:00 h and off at 16:00 h. During the ON periods, the animals were exposed to EMF for 16 h continuously, 8 h of the light period plus the first 8 h of the dark period (Fig 1). Procedures followed the recommendations of the Canadian Council on Animal Care (CCAC, 1984).

**Experiment 1**

Sixteen, lactating, pregnant Holstein cows were divided into two replicates of eight animals each, and housed in the EMF chamber. Each replicate was exposed to EMF according to one of two sequences of three periods of 28 days each. Sequence 1 OFF-ON-OFF and sequence 2 ON-OFF-ON. During the last day of each treatment period, blood samples were collected every 4 hours for 24 h for T4 RIA analysis.

**Experiment 2**

Sixteen non-lactating, non-pregnant, Holstein cows were housed in the EMF exposure chamber. The animals were distributed in replicates analogous to experiment 1. Each treatment period was one estrous cycle and the treatment sequence was: sequence 1 OFF-ON-ON and sequence 2: ON-OFF-OFF. All animals had ovarian activity confirmed and were synchronized for estrus. Blood samples were collected every other day for RIA T4 analysis.

**Statistical Analysis**

The experimental designs were crossover designs with treatment switchback. The results were analysed using the Mixed Model procedure of SAS (SAS Institute, Inc., Cary, NC).

## RESULTS

Magnetic and electric fields during exposure were 30 ± 1.5 μT and 10 ± 0.5 kV $m^{-1}$, respectively. Light intensity during the light and dark period was 321± 14 and 0 lux, respectively. Mean EMF exposure time was 14.01 ± 0.3 and 12.23 ± h/d for experiment 1 and 2, respectively. Mean temperatures in the chamber for the control and for the exposed treatments were 18.52 ± 6.41 $^{o}$C and 22.03 ± 9.92 $^{o}$C in experiment 1 and 22.91 ± 3.38 $^{o}$C and 17.14 ± 2.02 $^{o}$C in experiment 2, respectively. It was detected a slight increase in T4 plasma concentrations that could be attributable to EMF exposure (Table 1). In experiment 1, exposed animals did not have any change in T4 plasma concentrations due to the treatment ($p = 0.0968$) but the time of sample collection revealed a significant difference ($p = 0.012$). In experiment 2, the effect of days ($p = 0.0314$), period ($p = 0.0002$) and the treatment by days interaction ($p = 0.0018$) were statistically significant.

## DISCUSSION

Thyroxine plasma concentrations are presented in Table 1 and figures 2 and 3. In experiment 1 EMF exposure did not result in any T4 variation. The effect of sampling hour was significant. Both control and treated groups had T4 higher concentrations at 04:00 am which declined when the light where turn on at 08:00 am. Then the exposed group maintained same levels throughout the sampling period whereas the control animals showed a slightly different pattern than the exposed ones (Figure 2). In experiment 2 the significant interaction between treatment and days indicates that exposure to EMF produces a 3.8 % increase in T4 plasma concentrations and this variation is dependant on days within periods. Rats exposed to EMF showed decreased T4 in blood and changes in the thyroid gland (Matavulj et al. 1996; Rajkovic et al. 2003). Exposure of men to magnetic fields (MF) did not affect T4 (Selamaoui et al. 1997). Melatonin changed T4 blood concentration (Champney 2001) regulating TSH secretion (Sakamoto et al., 2000). Exposure to EMF (Burchard et al. 1996; Rodriguez et al. 2002), injections of PRL and T4 increased feed consumption (Ryg and Jacobsen 1982) in ruminants. The T4 increase in observed this experiment, the positive correlation between IGF-I and T4 (Ciccioli et al. 2003), the stimulatory effect of thyroid hormones on IGF (Rodriguez-Arnao et al., 1993) the increases in PRL (Rodriguez et al., 2004) and IGF-I (Rodriguez etal., 2002) in EMF exposed animals might contributet o explain the increases in feed intake, and P4 secretion observed previously in EMF exposed dairy cattle.

## CONCLUSIONS

Exposure of dairy cattle to EMF influences the blood levels of thyroxine. In light of the worst case scenario conditions, it is reasonable to speculate that, under the normal commercial conditions in the Province of Québec, this variation in T4 does not represent a health hazard for dairy cows.

**Table 1.** Thyroxine plasma concentrations (LS means ± SE) in pregnant, lactating (experiment 1) and non pregnant non lactating (experiment 2) dairy cattle exposed to EMF during short day emulation.

| Experiment | Control ($ng\ mL^{-1}$) | Exposed ($ng\ mL^{-1}$) | $p > t$ |
|---|---|---|---|
| 1 | 48.96± 2.76 | 46.66± 2.78 | 0.0968 |
| 2 | 48.06 ±2.42 | 49.87± 2.49 | 0.3602 |

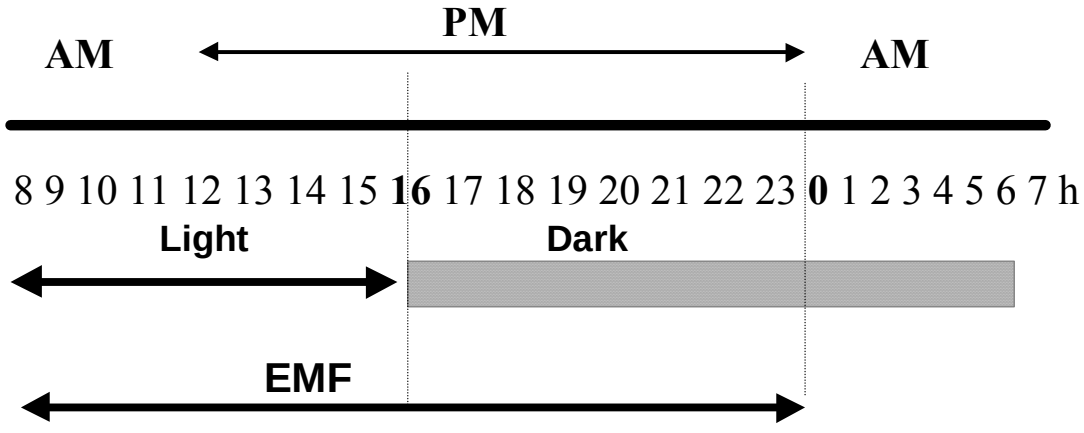


**Figure 1.** Daily light and EMF exposure protocol

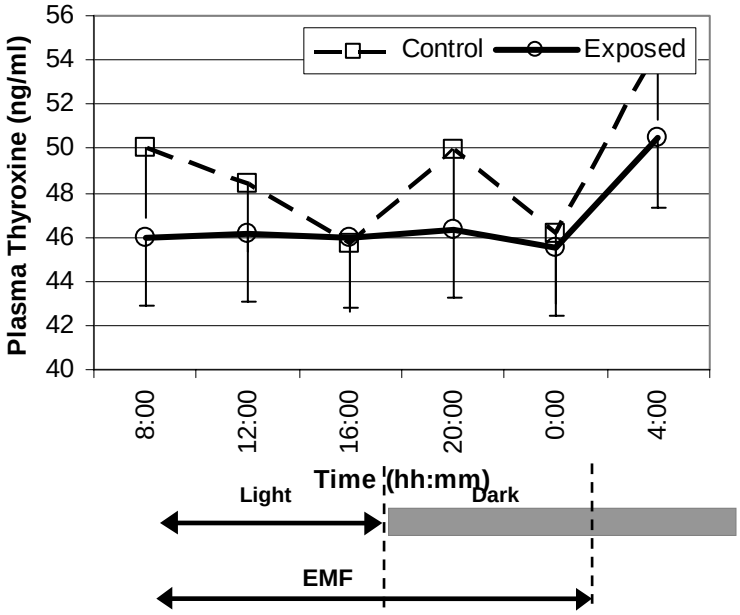


**Figure 2.** Thyroxine plasma concentration (LS means ± SE) in pregnant, lactating (experiment 1) dairy cattle not exposed ( control) and exposed (○) to EMF during short day emulation. Blood samples were collected every 4 h for 24 h at the end of each treatment period.

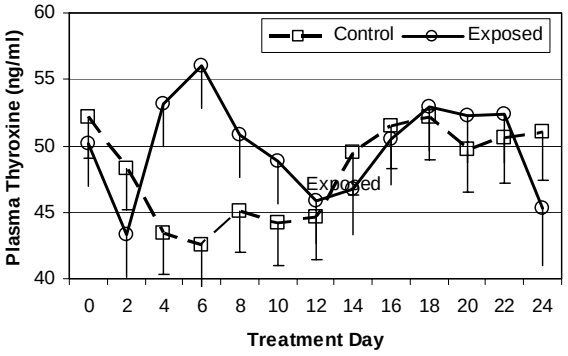


**Figure 3.** Thyroxine plasma concentration (LS means ± SE) in non pregnant, non lactating (experiment 2) dairy cattle not exposed ( control) and exposed (○) to EMF during short day emulation. Blood samples were collected every 2 days during each treatment period.

## REFERENCES


Baltaci, A.K., Mogulkoc, R., Kul, A., Bediz, C.S., Ugur A. 2004. Opposite effects of zinc and melatonin on thyroid hormones in rat. Toxicology 195: 69-75.

Burchard, J.F., Nguyen, D.H., Richard, L., Block, E. 1996. Biological effects of 60 Hz of electric and magnetic fields on productivity of dairy cattle. J. Dairy Sci. 79: 1549-1554.

Burchard, J.F., Nguyen, D.H., Block, E. 1998a. Progesterone concentrations during estrous cycle of dairy cows exposed to electric and magnetic fields. Bioelectromagnetics 19: 438-443.

Capen, C.C., Martin, S.L. 2003. The thyroid gland. In:McDonald's veterinary endocrinology and reproduction . Ed. Pineda M.H and Dooley M.P. 5th ed pp 35-70.

CCAC. 1984. Guide to the use and care of experimental animals Vol. 1 Ottawa: Canadian Council of Animal Care.

Champney, T.H. 2001. Reductions in hamster serum thyroxine levels by melatonin are not altered by changes in serum testosterone. Gen. Comp. Endocrinol. 123: 121-126.

Ciccioli, N.H., Wettemann ,R.P., Spicer, L.J., Lents ,C.A., White, F.J., Keisler, D.H. 2003. Influence of body condition at calving and postpartum nutrition and endocrine function and reproductive performance of primiparous beef cows. J. Anim. Sci. 81: 3107-3120.

Matavulj, M., Rajkovic, V., Uscebrka, G., Gudovic, R., Stevanovic, D., Blazetic, B. 1996. Stuctural and stereological analysis of rat thyroid gland after exposure to an electromagnetic field. Acta Veterinaria (Belgrade) 46: 285-292.

Nguyen D.H., Richard L., Burchard J. 2005. Exposure Chamber for Determining the Biological Effects of Electric and Magnetic Fields on Dairy Cows. Bioelectromagnetics 26: 138-144.

Raijkovic, V., Matavulj, M., Gledic, D., Lazetic B. 2003. Evolution of the rat thyroid gland morpho-physiological state after three months exposure to 50 Hz electromagnetic field. Tissue Cell 35: 223-231.

Rodriguez-Arnao, J., Miell, J.P., Ross, R.J.M. 1993. Influence of Thyroid Hormones on the GH-IGF-I Axis. TEM 4: 169-173.

Rodriguez, M., Petitclerc, D., Burchard, J.F., Nguyen, D.H., Block, E. 2004. Blood. Melatonin and Prolactin Concentrations in Dairy Cows Exposed to 60 Hz Electric and Magnetic Fields During Eight-Hour Photoperiods. Bioelectromagnetics 25: 508-505.

Rodriguez, M., Petitclerc, D., Burchard, J.F., Nguyen, D.H., Block, E., Downey, B.R. 2003. Responses of the estrous cycle in dairy cows exposed to electric and magnetic fields (60 Hz) during 8-h photoperiods, Anim, Repr, Sci, 77: 11-20.

Rodriguez, M., Petitclerc, D., , Nguyen, D.H., Block, E., Burchard, J.F. 2002. Effect of Electric and Magnetic Fields (60 Hz) on Production, and Levels of Growth Hormone and Insulin-Like Growth Factor 1 in Lactating, Pregnant Cows Subjected to Short Days. J. Dairy Sci. 85: 2843-2849.

Ryg, M., Jacobsen, E.1982. Effects of thyroid hormones and prolactin in food intake and weight changes in young male rein deer rangifer-tarandus-tarandus. Can. J. Zool. 60: 1562-1567.

Selmaoui, B., Lambrozo, J., Touitou, Y. 1997. Endocrine functions in young men exposed for one night to a 50-Hz magnetic field, a circadian study of pituitary, thyroid and adrenocortical hormones. Life. Sci. 61: 473-486.

# Lack of effect of 10 $kVm^{-1}$ 60 Hz electric field exposure on pregnant dairy heifer hormones.

J.F. Burchard, D.H. Nguyen [1], H. Monardes and D. Petitclerc [2].
[1]Institut de Recherche d'Hydro-Québec (IREQ), Varennes, QC, Canada.
[2]Agriculture and Agri-Food Canada, Lennoxville, QC, Canada

## ABSTRACT

Sixteen pregnant Holstein heifers weighing 521 ± 46 kg, at 3.3 ± 0.7 mo of gestation were confined to wooden metabolism cages and were exposed to a vertical electric field (EF) of 10 0 ± 0.4 kV $m^{-1}$ and an artificial light cycle of 12 h light and12 h dark. The heifers were divided into two replicates of eight each. Each replicate was divided into two groups of four animals each, one group becoming the non-exposed and the second the EF exposed group. The exposed group were housed in metabolism cages that were in an area where EF were generated, and the non-exposed group, in metabolism cages located in the adjacent area where the EF was less than 2% of that of the exposed area. The test animals were subjected to the different treatments for four weeks continuously. After four weeks, the animals switched treatment, the exposed group becoming the non- exposed group and vice versa. Then the treatment continued for four more weeks. Catheters were inserted into the jugular vein of the animals, and blood samples were collected twice weekly to estimate the serum concentration of progesterone (P4), melatonin (MLT), prolactin (PRL) and IGF-1. Feed consumption was measured daily and feed samples were collected twice a week. The results indicated that exposure of dairy cattle to EF similar to those encountered directly underneath a 735 kV high tension electrical power line carrying a maximum load of current, cannot be associated with any variation in the experimental variables mentioned above. An exception to this, the variation is MLT which was associated with the EF exposure. Due to the inconsistency of the MLT response in the different replicates, caution should be exercised in the interpretation of this phenomenon.

**Keywords**: Dairy heifers, electric field, hormones

## INTRODUCTION

Electric and magnetic fields (EMF) induce electric currents and fields in exposed subjects (Kaune and Gillis 1981). Dairy cows exposed to worst case scenario EMF conditions, directly under 735 kV AC high tension power lines carrying around 2000 A, show biological responses (Burchard et al. 1996). Previous research has established an association between the EMF exposure and changes in a variety of production and physiological variables in dairy cattle. The experiment reported here is an attempt to evaluate whether the established EMF effects are associated exclusively with the electric field exposure similar to that encountered, under worse case scenario exposure conditions, underneath 735 kV AC high tension power lines.

## MATERIALS AND METHODS

Sixteen pregnant Holstein heifers were confined to wooden metabolism cages in an EMF exposure chamber for the duration of the experiment. This chamber was designed and constructed to resemble a commercial tie stall barn. Further description of the chamber can be found elsewhere (Nguyen et al. 2005). The animals were exposed to artificial light (12 h darkness and 12 h of light)

This study was carried out in two replicates (eight animals each). Exposure to the EF was conducted using a crossover design. Sixteen heifers were

divided into two replicates of eight animals each. Each replicate was divided into two groups of four animals each, one group becoming the non-exposed and the second group, the EF exposed group. The exposed group was housed in the area where the EF was generated, and the non-exposed group was housed in the metabolism cages located in an area where the EF was less than 2% of that in the exposed area. The animals were subjected continuously to the different treatments for four weeks. After four weeks, the animals switched treatment. The non- exposed group became the exposed group and vice versa, continuing the treatment for four additional weeks. Blood samples were collected on Tuesdays at 9:00 h and on Thursdays at 9:00, 10:00, 11:00 and 12:00 h for the duration of experiment and stored at -20 $^{o}$C pending analysis. Feed samples were collected twice a week. Serum concentrations of P4, MLT PRL and IGF were estimated by RIA.

Comparisons between treatments were carried out using contrast between the differences of the means using a t- test. Significant differences between means were declared at $P < 0.05$.

## RESULTS

The control and treated animals were exposed to a vertical EF of $0.2 \pm 0.1$ or $10 \pm 0.4$ kV m$^{-1}$ for an average of $22.5 \pm 1.7$ h d$^{-1}$. Temperature and humidity during the experiment were $23 \pm 3$ $^{o}$C and $55 \pm 9$%, respectively. Exposure to EF did not change either feed consumption, body weight or body weight gain (Table 1). Results for PRL, P4, MLT and IGF-1 are presented in Table 2. All hormonal concentrations did not differ between heifers exposed or not exposed to EF. It is worthwhile to note that; overall, there was not an association of EF exposure and MLT. Nevertheless, when analyzed by replicate, the exposed animals in replicate 1 had a 10.4% decrease in MLT concentrations. Conversely, in replicate 2, an increase of 8.7% in MLT concentrations was detected (Fig. 1).

## DISCUSSION

The results obtained DMI, protein and energy intake, are in agreement with the expected intake according to the nutritional requirements for dairy cattle of the National Research Council (NRC 2000). The variations in dry matter consumption, P4, MLT, PRL and IGF-1 associated to EMF exposure observed in previous experiments (Burchard et al. 1996, 1998ab, 2003; Rodriguez et al. 1998) were not detected in this experiment. In this experiment, where dairy cattle were exposed only to EF similar to that encountered under high tension line, a MLT response associated with the EF exposure could not be detected when both replicates were analyzed together. However, there was a treatment by replicate interaction ($P < 0.002$). The statistical analysis by replicate detected a significant decrease in the concentration of MLT when the animals were exposed to EF during the first replicate. However, the effect of the electric field was reversed in the second replicate and an increase in MLT concentrations was observed during exposure. A similar situation has been observed in previous experiments where the nocturnal concentrations of MLT were measured in cows exposed to EMF (Burchard et al. 1998a).

The electric field used in this experiment did not show any association with variation in the concentration of IGF-1 and P4 in serum collected from dairy heifers.

## CONCLUSIONS

From this experiment it can be inferred that exposure of dairy cattle to electric fields of 10 kV/m, which are similar to those encountered, under worst case scenario conditions, directly underneath a 735 kV high tension electrical power line , does not cause any variation in the experimental variables mentioned above. An exception to this is MLT, which showed a variation associated to the EF exposure. However, due to the inconsistency of the MLT response in both replicates, caution should be exercised in the interpretation of this phenomenon. Another word of caution for these results is the less than optimal power of the test for PRL and those variables relate to body weight. Hence, the question remains as to whether the EMF effects observed in previous research are the result of the magnetic field exposure alone, or it is an effect that can only be achieved in the presence of both, electric and magnetic fields.

**Table 1.** Least-squares means for intakes and body weight change.

| Variable | EF Off | SE | EF On | SE | *p* |
|---|---|---|---|---|---|
| DM intake ( kg $d^{-1}$) | 11.68 | 0.19 | 11.63 | 0.19 | 0.853 |
| Protein intake (kg $d^{-1}$) | 1.86 | 0.03 | 1.84 | 0.03 | 0.707 |
| $Ne_L$ intake ( Mcal $d^{-1}$) | 14.42 | 0.24 | 14.49 | 0.24 | 0.856 |
| Boy weight (kg) | 566.54 | 3.09 | 563.13 | 3.09 | 0.397 |
| Body weight change ( kg) | 8.94 | 0.75 | 7.08 | 0.75 | 0.093 |

**Table 2.** Least-Square Means and standard error (SE) for melatonin (MLT), progesterone (P4), insulin growth factor 1 (IGF-1) and prolactin (PRL) in dairy heifers.

| Variable | EF Off | SE | EF On | SE | *p* |
|---|---|---|---|---|---|
| Melatonin (pg $mL^{-1}$) | 23.7 | 0.6 | 23.2 | 0.6 | 0.434 |
| Progesterone (ng $mL^{-1}$) | 4.0 | 0.2 | 3.7 | 0.2 | 0.100 |
| IGF-1 (ng $mL^{-1}$) | 254.0 | 4.4 | 248.9 | 4.4 | 0.244 |
| Prolactin (ng $mL^{-1}$) | 7.9 | 1.0 | 7.4 | 1.0 | 0.703 |

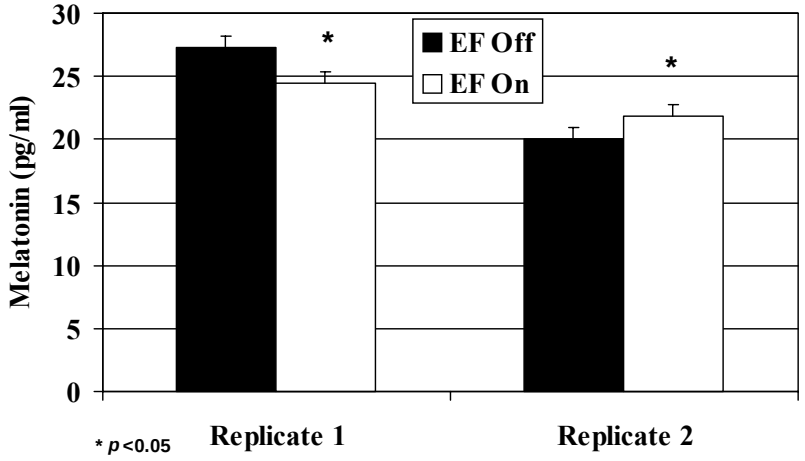


**Fig. 1.** Least-square means by replicate of serum concentration of melatonin in pregnant dairy heifers not exposed (off) or exposed (on) to 60Hz AC electric fields (EF).

## REFERENCES


Burchard, J.F., D.H. Nguyen, L. Richard and E. Block. 1996. Biological effects of 60 Hz of electric and magnetic fields on productivity of dairy cattle. J. Dairy Sci. 79: 1549-1554

Burchard, J.F., D.H. Nguyen and E. Block. 1998a. Effects of electric and magnetic fields on nocturnal melatonin concentrations in dairy cows. J. Dairy Sci. 81: 722-727.

Burchard, J.F., D.H. Nguyen and E. Block. 1998b. Progesterone concentrations during estrous cycle of dairy cows exposed to electric and magnetic field. Bioelectromagnetics 19: 438-443.

Burchard, J.F. D.H. Nguyen and E. Block. 1999. Macro- and trace element concentrations in blood plasma and cerebrospinal fluid of dairy cows exposed to electric and magnetic fields. Bioelectromagnetics. 20: 358-364.

Burchard, J.F H. Monardes and D.H. Nguyen. 2003. Effect of 10 kV, 30 μT, 60 Hz electric and magnetic fields on milk production and feed intake in nonpregnant dairy cattle. Bioelectromagnetics. 24: 557-563.

Canadian Council of Animal Care. 1984. Guide to the use and care of experimental animals Vol 1.Ottawa, ON, Canada.

Kaune W. T. and M. F. Gillis. 1981. General properties of the interaction between animals and ELF electric fields. Bioelectromagnetics. 2: 1-11.

Nguyen D. H., L. Richard, J. F. Burchard, and E. Block. 2005. Exposure Chamber for Determining the Biological Effects of Electric and Magnetic Fields on Dairy Cows. Bioelectromagnetics. 26: 138-144 .

Rodriguez M., D. Petitclerc, J. Burchard and E. Block. 1998. Effects of exposure to electric and magnetic fields on melatonin and prolactin levels in dairy cows subjected to short photoperiods. 3rd International Conference on Farm Animal Endocrinology, Brussels, Belgium

# Effects of a 10 –kV, 30 μt, 60-HZ electromagnetic field on milk production and feed intake in non-pregnant dairy cattle

J.F. Burchard, H. Monardes and D.H. Nguyen[1].
[1]Institut de Recherche d'Hydro Québec, Varennes, QC J3X 1S1 Canada.

## ABSTRACT

Milk production is the main agricultural income in the province of Quebec, and the electrical distribution network traverses rural land. This study evaluates the hypothesis that electromagnetic fields may affect dairy production. Sixteen multiparous non-pregnant lactating Holstein cows (weighing 662 ± 65 kg and with 150.4 ± 40 days in milk) were confined to wooden metabolic crates during the experiment with 12:12 hours light: dark cycle. The cows were divided into two replicates of eight cows each and exposed to a vertical electric field of 10 kV/m and a uniform horizontal magnetic field of 30 micro Tesla (μt) at 60 Hz. Replicate one was exposed for three periods. Each period was represented by an estrous cycle ranging from 24 to 27 days. During the first period, the electric and magnetic fields (EMF) were off; during the second period they were on; and during the final period, they were off. The second replicate was exposed for three periods also, but exposure protocol was reversed (first period, on; second period, off; last period, on). Exposure to EMF (ON) resulted in a decrease of 4.97%, 13.78%, and 16.39% in milk yield, fat corrected milk yield and milk fat, respectively; and an increase of 4.75% in dry matter intake.

**Key words**: feed intake; electric field; magnetic field; cows; extremely low frequency; alternating current.

## INTRODUCTION

Milk production represents the most important economic activity in agriculture in Québec. The electrical network of Hydro-Québec traverses through rural areas. Because of this exposure Hydro-Québec was motivated to study the effect of extremely low frequency alternating current (AC) electric and magnetic fields (EMF) on dairy cows. A chamber was constructed to generate EMF similar to those prevailing directly under alternating current 735- kV power lines and to resemble normal dairy farming conditions in a confined housing system.

Previous related studies were principally field studies based on surveys of farmers (Amstutz and Miller 1980; Busby et al. 1974; Williams and Beiler 1979), retrospective studies based existing data banks (Algers et al. 1981; Algers and Hennicks 1985; Martin et al. 1986; Williams and Beiler 1979) or in a semi-controlled environments (Algers and Hultgren 1987) and in the US (Raleigh 1988). Relatively few studies have been conducted in a controlled environment (Burchard et al. 1996; Burchard et al. 1998: Burchard et al. 1999, Rodriguez et al. 1998; Rodriguez et al. 2002). These studies suggest that dairy cows respond to EMF. This study evaluates the effect of continuous exposure to 60-Hz AC EMF similar to those generated by 735-kV power lines, on milk yield and composition, and feed intake in non pregnant dairy cows with estrous cycle activity

## MATERIALS AND METHODS

Sixteen multiparous non-pregnant lactating Holstein cows were confined to wooden metabolic crates in a EMF chamber during the experiment with a 12:12 h light: dark cycle. The cows were allocated into two replicates of eight cows each based in their parity and days in lactation. Each replicate was treated for three consecutive periods (three estrous cycles). During the first period of the treatment corresponding to replicate one, the electric and magnetic fields were off; during the second period they were on;

and during the final period, they were off. The second replicate was treated for three periods also, but the activity of the fields was reversed (first period, ON; second period, OFF; third period, ON). The number of days of each exposure period varied according to the estrous cycle duration. Since each treatment period is equivalent to an estrous cycle, and estrous cycle differ in duration, only the first three weeks of exposure within each treatment period were considered for data analysis.

**Exposure Chamber**
See Nguyen et al. (2005)

Estrous cycles were synchronized in order to obtain a homogeneous sample of animals regarding oestrous cycle status within each period of treatment.

The animals were fed twice daily a total mixed ration (TMR) formulated to meet NRC requirements (NRC, 1989) supplied within individual plastic containers. Total mixed rations were sampled and were chemically analyzed. Feed intake and milk production were measured daily. Milk Samples were submitted for component analysis.

**Statistical Analysis**
The data collected during the first three weeks of treatment within each treatment period were compiled and analysed using the Mixed Model Procedure of SAS.

## RESULTS

Room temperature and humidity were 18.2 ± 4.6$^{o}$ C and 57.1 ± 29.8%, respectively. Results for the production variables are presented in Table 1. Exposure to EMF (ON) resulted in a decrease of 4.97 %, in milk yield, 13.78% in fat corrected milk yield, and 16.39%, in milk fat content. An increase of 4.75% in dry matter intake was detected. An interaction between treatment and days of treatment within period was detected for the variables milk fat percentage ($P = 0.011$), and dry matter intake ($P < 0.001$) (Fig. 1).

## DISCUSSION

In this experiment milk production decreased and feed consumption increased during EMF exposure (Table 1). This partially agrees with previous results where EMF were associated with a 5.5% increase in feed consumption and a 9.0% increase in fat-corrected milk yield in pregnant dairy cows exposed to EMF (Burchard et al. 1996). The percentage of fat in milk was lower during EMF exposure. The interaction between treatment and days of exposure was significant for milk fat, (Fig. 1 D) and dry matter intake (Fig. 1 C). This suggests that the magnitude of EMF effect varied over the time of treatment. Long photoperiod stimulation of milk yield in dairy cows becomes statistically detectable after 28 days of treatment (Dahl et al. 1997). The increase in dry matter intake observed in the study reported herein is coincidental with an increase in milk fat content. Further statistical analysis of previous experiments (Burchard et al. 1996) revealed a significant week-by-treatment interaction for fat-corrected milk, dry matter intake and milk fat content. An analogous situation was observed when lactating pregnant dairy cows were exposed to EMF (Rodriguez et al. 2002). This agrees with the suggestion that the EMF exposure effect varies across time due to an adaptation response of the animals. The increase in dry matter intake associated to EMF has been observed previously concomitant with increases in milk yield (Burchard et. al. 1996) and no changes in milk yield but increased body weight (Rodriguez et al. 2002). It is possible that the increase in dry matter intake observed in this experiment, at this stage of lactation, resulted in weight gain rather than milk production

## CONCLUSIONS

From this study it can be concluded that EMF exposure (10 kV m$^{-1}$, 30 μt, 60 Hz) similar to that encountered under worst case scenario underneath a 735-kV transmission line, resulted in a moderate decrease in milk yield and milk fat percentage; and an increase in dry matter intake in non lactating non pregnant dairy cows.

**Table 1. Performance of lactating non-pregnant dairy cows during EMF non exposure (OFF) and exposure (ON) periods.**

| Variable | OFF | ON | SE | P |
|---|---|---|---|---|
| Milk yield (kg $d^{-1}$) | 23.76 | 22.58 | 0.30 | 0.0002 |
| 4% Fat-corrected milk yield (kg $d^{-1}$) | 24.66 | 21.26 | 0.71 | <0.0001 |
| Dry matter intake (kg $d^{-1}$) | 20.00 | 20.95 | 0.24 | 0.0002 |
| Milk fat content (%) | 4.27 | 3.57 | 0.23 | 0.0033 |

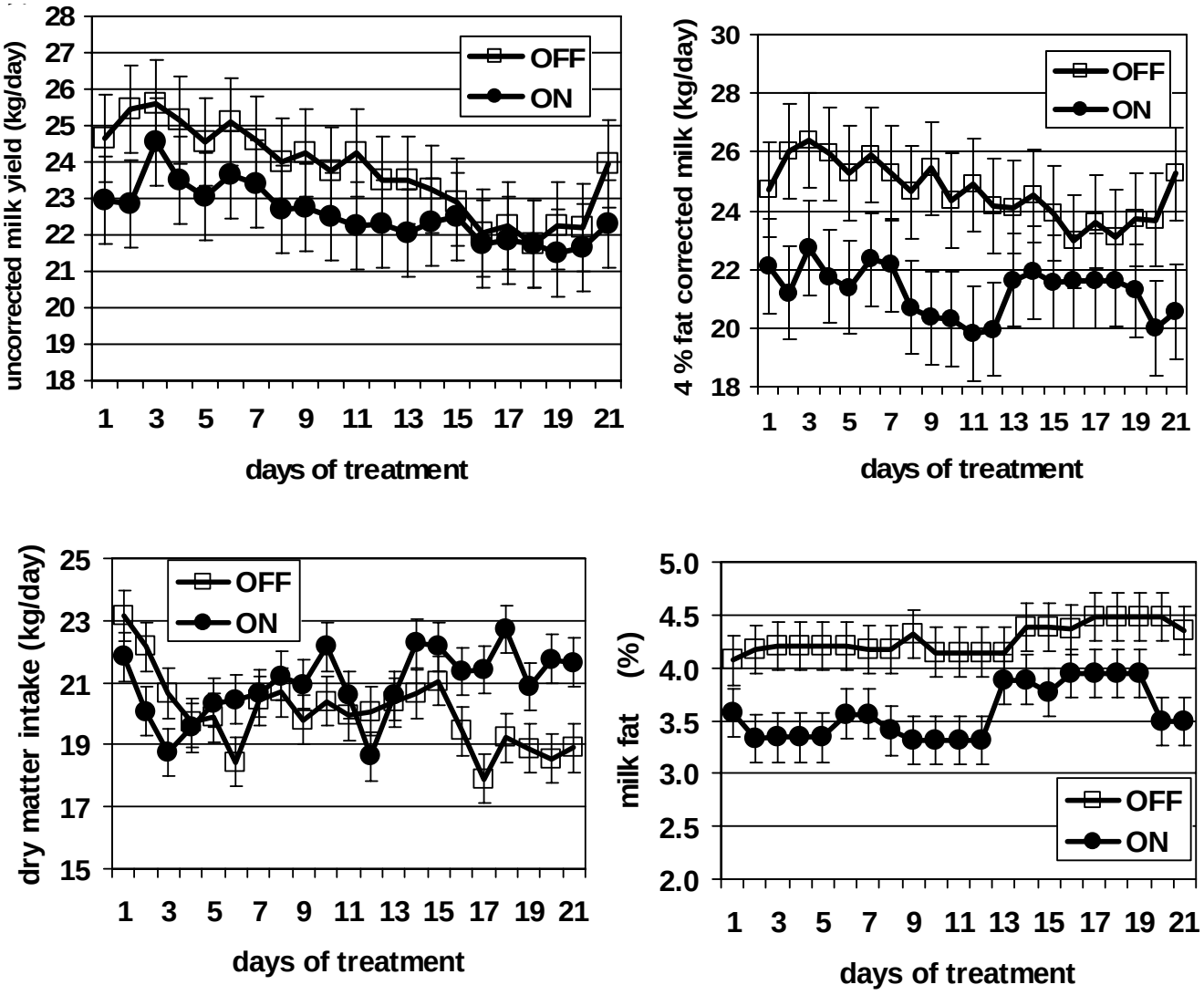


**Fig. 1.** Milk yield (A), fat corrected milk yield (B) , dry matter consumption (C) and milk fat percentage (D) by day of treatment within each period (estrous cycles) obtained in 15 lactating non-pregnant dairy cows during the 21 days in three consecutive periods during EMF non exposure (OFF) and exposure (ON) periods. Results are least-squares means and their standard errors.

## REFERENCES


Algers, B., Ekesbo, I. and Hennichs, K. 1981. The effects of ultra high-voltage transmission lines on the fertility of dairy cows. A preliminary study. Rep. No. 5 Swed. Univ. Agric. Sci. Dep. Anim. Hygiene with Farrier school, Skara, Sweden.

Algers, B. and Hennichs, K. 1985. The effects of exposure to 400 kV transmission lines on the fertility of cows. A retrospective cohort study. Prev. Vet. Med. 3: 352-361.

Algers, B. and Hultgren, J. 1987. Effects of long-term exposure to 400-kV, 50 Hz transmission line on estrous and fertility in cows. Prev. Vet. Med. 5: 21-26.

Amstutz, H. E., Miller, D. B. 1980. A study of farm animals near 765 kV transmission lines. Bovine Pract. 15: 51-62

Burchard, J. F., Nguyen, D. H., Richard, L., Block, E. 1996. Biological effects of 60 Hz of electric and magnetic fields on productivity of dairy cattle. J. Dairy Sci. 79: 1549-1554.

Burchard, J. F., Nguyen, D. H., Block, E. 1998. Progesterone concentrations during estrous cycle of dairy cows exposed to electric and magnetic field. Bioelectromagnetics 19: 438-443.

Burchard, J. F., Nguyen, D. H., Block, E. 1999. Macro- and trace element concentrations in blood plasma and cerebrospinal fluid of dairy cows exposed to electric and magnetic fields. Bioelectromagnetics 20: 358-364.

Busby K, Driscoll D, Washbon WE. 1974. A field survey of farmer experience with 765 kV transmission lines. Agric. Res. Commiss., State of New York, Albany, New York, NY.

Dahl, G.E., Elsasser, T. H., Capuco, A.V., Erdman, R. A., Peters, R. R. 1997. Effects of a long daily photoperiod on milk yield and circulating concentrations of insulin-like growth factor 1. J. Dairy Sci. 80: 2784-2789.

Martin, F. B., Bender, A., Steuernagel, G., Robinson, R. A., Revsbech, R., Sorensen, D. K., Williamson, N., Williams, A. 1986. Epidemiological study of Holstein dairy cow performance and reproduction near a high-voltage direct-current powerline. J. Toxicol. Environ. Health. 19: 303-324.

NRC. 1989. Nutrient Requirements of Dairy Cattle. 6th rev. ed. Nat. Acad. Sci., Washington DC.

Raleigh RJ. 1988. Rep. DOE/BP-21216-3. Joint HVDC agricultural study. Oregon State University, Boneville Power Administration Portland, Oregon. DOE. Portland, OR

Rodriguez, M., Petitclerc, D., Burchard, J., Block, E. 1998. Effects of exposure to electric and magnetic fields on melatonin and prolactin levels in dairy cows subjected to short photoperiods. 3rd International Conference on Farm Animal Endocrinology, Brussels, Belgium

Rodriguez, M., Petitclerc, D., Nguyen, D. H, Block, E. Burchard J. F. 2002. Effect of electric and magnetic fields (60 Hz) on production, and levels of growth hormone and insulin-like growth factor 1, in lactating, pregnant cows subjected to short days. J. Dary Sci. 85: 2843-2849.

Williams JH, Beiler JE. 1979. An investigation of dairy farm operations in association with 765-kV transmission in Ohio. Rep.Ohio Power Sitting Commission and Cleveland Electric Illuminating Co. Cleveland, OH.

# Evaluation of different methods of treating soybean meal to improve ruminal outflow and bioavalibilty of amino acids for lactating dairy cattle

S.I. Borucki Castro , L.E. Phillip , H. Lapierre [2], P.W. Jardon [1] and R. Berthiaume [2]
[1]Agriculture and Agri-Food Canada, Dairy and Swine R&D Centre, Lennoxville, QC and
[2]West Central Soy, Ralston, IA

## ABSTRACT

Four multiparous Holstein cows at 158 DIM equipped with ruminal and duodenal cannulae where used to determine, under standardized experimental conditions, the impact of multiple methods of treating soybean meal (SBM) on *in situ* ruminal degradability and *in situ* intestinal digestibility of CP and amino acids (AA). The 4 SBM studied were: solvent extracted SBM (SE), expeller SBM (EP), lignosulfonate treated SBM (LS) and heat treated SBM with soy hulls (HS). After 16 h of ruminal incubation, the contents of the nylon bags for each feed were transferred to 3.5 X 5.5 cm bags, soaked in HCl-pepsin solution to mimic abomasal digestion and then introduced through the duodenal cannula, and recovered in feces. The treatment of SBM (EP, LS, HS) protected the CP and AA from ruminal degradation increasing the value of RUP from 30% (SE) to a maximum of 78% (HS). Rumen undegradable CP and AA at 16 h of incubation were higher for HS compared to the rest of the treated SBM. In all feeds, degradation was higher for Lys and Glx and lower for Ile, Val, Leu and Tyr compared to other AA at 16 h. Considering very small and no statistical differences within treated SBM for intestinal digestion, differences on bioavailable AA were given by the differences on ruminal degradation. Non- enzymatic browning caused by the addition of soyhulls and mild heat was the treatment of SBM that rendered the highest value of bioavailable AA.

**Keywords**: Amino acids, heat treatment, soybean, ruminal and intestinal degradability

## INTRODUCTION

The objectives in diet formulation for high producing dairy cows are to provide rumen degradable protein (RDP) to ensure maximal synthesis of microbial protein, with adequate amounts of rumen undegradable protein (RUP) to optimize the amount of absorbable amino acids (AA). Given the North American and European ban on the use of animal protein supplements for ruminant animals there is growing emphasis on the use of plant protein concentrates in diets for lactating dairy cattle. Soybean meal (SBM) is the most commonly used protein supplement in dairy cattle diets in North America, and within oilseed meals, it has the highest content of essential AA (NRC 2001).

Although the quantity of RUP delivered to duodenum could be increased from 40 to 70% (Waltz and Stern 1989), there is still doubt as to the impact of the various methods of SBM treatment on the final AA profile of the protected protein and on the intestinal digestibility of the AA that have escaped ruminal degradation. The objective of this study was to determine, under standardized experimental conditions, the impact of multiple methods of treating SBM on ruminal degradability and intestinal digestibility of CP and AA in SBM.

## MATERIALS AND METHODS

### Animals and Treatments

Four multiparous Holstein cows averaging 649 (± 46.3 kg) BW and 158 (± 28.7) DIM at the start of the experiment were fed a total mixed ration (TMR) ad libitum twice daily (Table 1). The 4 SBM studied in this project were: solvent extracted SBM (SE), expeller SBM SoyPlus® West Central Soy (EP), lignosulfonate

treated SBM Surepro® Land O'Lakes Farmland Feed LLC (LS) and heat treated SBM with soy hulls Aminoplus® Ag Processing Inc. (HS). In EP, soybeans are fed into expeller presses, with a central revolving shift that creates pressure causing the mechanical extraction of oil from the cracked SBM. The expeller processing involves heating to a maximum of 163°C. In the LS treatment the SBM is treated with 8-25% $wt^{-1}$ sulfite liquor from hardwood or soft-wood processing; then is heated at 95°C for 1 h producing a partial Maillard reaction with xylose. The process of HS comprises the step of mixing SBM with soy hulls (10:1 wt ratio), adding water to 30-50% and further cooking at 95°C to final moisture content of 12-16% wt.

Cows were equipped with ruminal and duodenal cannulae. Each SBM (2 mm) was incubated in the rumen for 16 h in N free polyester bags (9 X 18 cm) with a pore size 50 ± 15 µm at 12.3 mg $cm^{-2}$. The contents were then transferred into 3.5 X 5.5 cm N free polyester bags. The bags were soaked into 0.1 N HCl solution containing 1 g $L^{-1}$ of pepsin for 1 h at 39°C to mimic abomasal digestion (Calsamiglia and Stern 1995). Three randomly chosen bags were frozen to determine acid-pepsin losses. The remaining bags were used to estimate intestinal digestibility using the mobile nylon bag technique (Hvelplund and Weisbjerg 2000). They were introduced through the duodenal cannula at a rate of 1 bag every 30 min. Upon recovery from feces, the bags were washed in an automatic washing machine and then frozen.

**Chemical Analyses**

Analytical DM and ash were determined with a Thermo-gravimetric analyzer TGA-601 (LECO Corporation, St. Joseph, MI). Fat was determined by gravimetric analysis using ISCO SFX™ 3560 supercritical fluid extraction (ISCO Inc., Lincoln, NE). Analyses of NDF and ADF were performed using the Ankom System (ANKOM 200, Fiber Analyzer, Fairport, NY) with heat stable alpha-amylase and without sodium sulfite. Nitrogen was determined by thermal conductivity (LECO model TruSpec v1.10 Nitrogen Determinator, LECO, St. Joseph, MI). Cornell Net Carbohydrate and Protein System (CNCPS) N fractions were determined on SBM according to Licitra et al. (1996).

For AA determination samples of feed were processed according to AOAC, 2000. The AA concentration of the hydrolysate was determined by the isotope dilution method (Calder et al. 1999) and quantified by Gas Chromatography Mass Spectrometry (HP 6890 GC System 5973 and Mass Selective Detector, Hewlett Packard, Palo Alto, CA).

**Statistical Analyses**

Data was analyzed using a randomized complete block design with the MIXED procedure of SAS. Feed (fixed) and cows (random) were used as main effects in the following model:

$$Y_{ij} = \mu + Feed_i + Cow_j + \varepsilon_{ij}$$

The preplanned orthogonal contrasts tested were:
(1) SE vs EP-LS-HS
(2) EP vs LS-HS
(3) LS vs HS

**RESULTS AND DISCUSSION**

The chemical composition of the original SBM studied is presented in Table 2.

At 16 h of incubation (Table 3), untreated SBM presented higher CP and AA degradation when compared with treated SBM *(*$P < 0.001$*)*. Within treated sources, EP presented higher CP and total AA rumen degradation *(*$P < 0.01$*)* compared to LS, HS (but for His); these differences were mainly explained by the lower *(*$P < 0.001$*)* rumen degradation values of HS compared to LS. When treated, SBM protein undergoes denaturation, racemization and cross-linking reactions, which are catalyzed by the presence of heat (EP), pH and reducing sugars (LS, HS) (Friedman et al. 1984). The presence of protein-protein or protein-sugar crosslink, a racemized AA or a misfolded protein difficult microbial attack and therefore reduce ruminal degradation (Stern 1984). The addition of soyhulls combined with heat in HS increased the protection from microbial degradation.

The differential extent of degradation between AA was never higher than 5.5% for essential or 12% for non essential AA after 16 h of rumen incubation. Lys and Glx showed higher degradation, while Ile, Val, Leu and Tyr showed lower degradation rates compared to the rest of AA in most feeds studied. This pattern was similar to that found by Prestl∅kken and Rise (2003) and LjØkjel et al. (2000) for SE and LS. Lys and His are very reactive

AA which makes them more susceptible to degradation (Gerrard 2000). On the other hand, the steric hindrance of the side chain of the aliphatic AA, and their hydrophobicity, reduces microbial accessibility and produces a slower release of Ile, Val and Leu (Liu 1999).

The pattern of 16h -AA degradation for SE was similar to that reported by Maiga et al. (1996) after 12 h of incubation. These authors also found that EP decreases His and increases Gly degradation when compared to untreated SE. The structure of the N-terminus of protein and peptides has a major influence on the rate at which it is hydrolyzed in rumen fluid; acidic amino acids and Gly at or near the N- terminus slow polypeptide hydrolysis and the liberation of AA (Wallace 1996). The treatment of EP, and protein denaturation, could have had a positive influence rendering Gly available for degradation. Contrary to the present study Maiga et al. (1996) found a slower release of Lys for EP.

The role of Pepsin-HCl digestion is to mimic abomasal digestion. In this study, this step was responsible for 36 to 50% of CP and AA post-ruminal digestion and from 15% (SE) to 28% (HS) for total tract SBM digestion (data not presented). CP and AA *in situ* intestinal digestion was similar (> 97.3%) in all SBM products (Table 4). In general, SE showed a lower digestion compared to treated SBM, but the difference was small (<2%). Although significant in most of the cases, differences in AA intestinal digestion (determined *in situ*) between treated sources were never higher than 1%. Compared to this study, O'Mara et al. (1997) found lower intestinal digestibility for SBM. These authors did not perform the pepsin-HCl step. Prestl∅kken and Rise (2003), found similar values of intestinal digestibility for untreated SE, LS and heat treated SBM using nylon bags with a smaller pore size (15μm vs. 45μm in this study). Both studies suggest that there are no biologically significant differences (>3%) in intestinal AA digestion between treated and untreated nor within treated SBM when digestion is estimated *in situ.*

## CONCLUSIONS

The treatment of SBM protected CP and AA from 30% to 78%. Rumen undegradable CP and AA after 30% to 78%. Rumen undegradable CP and AA after 16 h of ruminal incubation were higher for HS compared to the rest of the treated SBM. In all feeds, 16 h degradation was higher for Lys and Glx and lower for Ile, Val, Leu and Tyr compared to other AA. The treatment of SBM with the expeller press (EP) and non-enzymatic browning (LS, HS) reduced the degradation of Phe and His and increased the degradation of Gly, compared to untreated SBM. CP and AA intestinal digestion determined *in situ* was high for all feeds exceeding 97.5% with no relevant differences between treatments. Considering lack of difference within treated SBM for intestinal digestion, differences in the bioavailability of AA were result of differences in ruminal degradation. Non- enzymatic browning caused by the addition of soyhulls and mild heat was the treatment of SBM that yielded the highest value of AA bioavailability.

**Table 1**. Ingredients and chemical composition (% of DM) of the basal diet fed during the *in situ* experiment

| | |
|---|---|
| Ingredients | |
| Red Clover – timothy silage | 33.0 |
| Corn silage | 32.0 |
| High moisture shelled corn | 21.0 |
| Solvent extracted soybean meal | 11.0 |
| Vitamin – mineral supplement[1] | 3.0 |
| | |
| Chemical Composition | |
| DM | 40.0 |
| CP | 16.5 |
| Fat | 3.1 |
| NDF | 38.6 |
| ADF | 25.7 |
| Ash | 8.2 |

[1] 10.8% Ca; 5.5% P; 3.5% Mg; 13.2% Na; 1.4% K; 2.1% S; 370,000 IU/kg of Vitamin A; 65,000 IU/kg of Vitamin D ; 1,800 IU/kg of Vitamin E.

**Table 2.** Chemical composition and amino acid content of soybean meal obtained by different methods of treatment

| | Treatment method[1] | | | |
|---|---|---|---|---|
| | SE | EP | LS | HS |
| DM, % | 93.60 | 92.20 | 92.40 | 93.10 |
| CP, % of DM | 51.82 | 49.24 | 47.84 | 51.13 |
| Fat, % of DM | 0.85 | 4.23 | 0.97 | 0.75 |
| NDF, % of DM | 11.54 | 29.50 | 33.01 | 31.04 |
| ADF, % of DM | 6.30 | 13.77 | 12.34 | 10.10 |
| Ash, % of DM | 7.26 | 7.05 | 7.79 | 7.20 |
| NPN, %CP | 20.2 | 16.5 | 17.1 | 17.8 |
| Soluble CP, % CP | 29.0 | 18.6 | 13.9 | 15.6 |
| Neutral detergent insoluble CP, %CP | 4.0 | 29.5 | 38.6 | 29.3 |
| Acid detergent insoluble CP, %CP | 1.8 | 8.2 | 7.6 | 3.6 |
| | | | | |
| Essential AA, % of DM | | | | |
| His | 1.26 | 1.13 | 1.23 | 1.29 |
| Ile | 2.08 | 2.10 | 2.15 | 2.23 |
| Leu | 3.81 | 3.67 | 3.69 | 3.87 |
| Lys | 2.99 | 2.58 | 2.67 | 2.89 |
| Phe | 2.44 | 2.36 | 2.40 | 2.49 |
| Thr | 1.98 | 1.90 | 1.93 | 2.00 |
| Val | 2.12 | 2.16 | 2.20 | 2.28 |
| Non- essential AA, % of DM | | | | |
| Ala | 2.15 | 2.08 | 2.07 | 2.17 |
| Glu+Gln | 7.74 | 7.25 | 7.18 | 7.94 |
| Gly | 2.05 | 2.08 | 2.08 | 2.14 |
| Ser | 2.57 | 2.45 | 2.45 | 2.56 |
| Tyr | 1.81 | 1.78 | 1.79 | 1.80 |

[1] solvent extracted (SE), expeller (EP), lignosulfonate (LS), heat and soy hulls (HS)

**Table 3. E**ffects of different treatments of soybean meal on CP and AA degradation after 16 h rumen incubation[2]

| | Treatments[1] | | | | SEM | Contrasts | | |
|---|---|---|---|---|---|---|---|---|
| | SE | EP | LS | HS | n=16 | SE vs others | EP vs LS, HS | LS vs HS_ |
| CP, % | 70.2 | 37.8 | 34.9 | 22.0 | 1.57 | <0.001 | <0.001 | <0.001 |
| Essential AA,% | | | | | | | | |
| His | 68.4 | 33.2 | 36.9 | 22.6 | 1.52 | <0.001 | 0.065 | <0.001 |
| Ile | 63.7 | 32.1 | 33.1 | 18.6 | 1.53 | <0.001 | 0.008 | <0.001 |
| Leu | 65.0 | 33.9 | 33.6 | 17.8 | 1.36 | <0.001 | <0.001 | <0.001 |
| Lys | 68.2 | 37.0 | 35.3 | 21.8 | 1.39 | <0.001 | <0.001 | <0.001 |
| Phe | 66.0 | 33.5 | 34.0 | 18.4 | 1.36 | <0.001 | 0.002 | <0.001 |
| Thr | 65.7 | 36.4 | 35.6 | 19.6 | 1.57 | <0.001 | <0.001 | <0.001 |
| Val | 63.0 | 32.7 | 33.3 | 18.9 | 1.58 | <0.001 | 0.007 | <0.001 |
| Non- essential AA,% | | | | | | | | |
| Ala | 65.5 | 35.7 | 35.1 | 20.1 | 1.38 | <0.001 | <0.001 | <0.001 |
| Glu+Gln | 71.8 | 38.3 | 36.8 | 27.0 | 1.31 | <0.001 | 0.002 | <0.001 |
| Gly | 66.6 | 38.0 | 37.0 | 22.4 | 1.33 | <0.001 | <0.001 | <0.001 |
| Ser | 66.8 | 36.6 | 34.6 | 18.6 | 1.58 | <0.001 | <0.001 | <0.001 |
| Tyr | 64.2 | 33.5 | 33.4 | 15.0 | 1.47 | <0.001 | <0.001 | <0.001 |

[1] Treatments: solvent extracted (SE), expeller (EP), lignosulfonate (LS), heat and soy hulls (HS)

[2] % Rumen degradation of the treatments at 16 h incubation (*in situ* technique)

## REFERENCES


AOAC. Association of Official Analytical Chemists. 2000. Official Methods of Analysis 17th Ed. Arlington, VA.

Calder, A. G.; Garden, K. E.; Anderson, S. E. and Lobley, G. E. 1999. Quantification of blood and plasma amino acids using isotope dilution electron impact gas chromatography/mass spectrometry with U-13C amino acids as internal standard. Rapid Commun. Mass Spectrom. 13: 2080-2083.

Calsamiglia, S. and Stern, M. D. 1995. A three-step in vitro procedure for estimating intestinal digestion of protein in ruminants. J. Anim. Sci. 73: 1459–1465.

Ceresnaková, A.; Sommer, A., Chrenková, M. and Dolesová, P. 2002. Amino acid profile of escaped feed protein after rumen incubation and their intestinal digestibility. Arch. Anim. Nutr. 56: 409 – 418.

Friedman, M; Levin, C.E. and Noma, A.T. 1984. Factors governing lysinoalanine formation in soy proteins. J. Food Sci. 49: 1282-1288.

Gerrard, J.A. 2002. Protein-protein crosslinking in food: methods, consequences, applications. Trends Food Sci. & Technol. 13: 391–399.

Hvelplund, T. and Weisbjerg, M.R. 2000. In situ techniques for the estimation of protein degradability and post rumen availability. In: Forage Evauation in Ruminant Nutrition. Ed. By D.I. Givens, E. Owen, RFE Axford and HM Omed. CABI Publishing.

Licitra, G.; Hernandez, T.M. and Van Soest, P.J. 1996. Standardization of procedures for nitrogen fractionation of ruminant feeds. Anim. Feed Sci. Technol. 57: 347–358.

Liu, K. 1999. Chemistry and nutritional value of soybean components. In: Soybeans: Chemistry, technology and utilization. Ed. by Monsanto. Aspen Publishers, Inc. Gaithersburg, MD. USA. Chapter 2.

Maiga, H. A.; Schingoethe, D.J. and Henson, J. E. 1996. Ruminal degradation, amino acid composition and intestinal digestibility of the residual components of five protein supplements. J. Dairy Sci. 79: 1647-1653.

NRC. 2001. Nutrient requirements of dairy cattle. 7th Ed. by the National Research Council.

PrestlØkken, E. and Rise, O. 2003. Protein and amino acid digestibility in dairy cows measured with mobile nylon bags recovered in ileum or in faeces. Acta Agric. Scand. Sect. A, Animal Sci. 53: 11-20.

Stern. M.D. 1984. Effect of lignosulfonate on rumen microbial degradation of soybean meal protein in continuous culture. Can. J. Anim. Sci. 64 suppl: 27 – 28.

Wallace, R.J. 1996. Ruminal microbial metabolism of peptides and amino acids. J. Nutr. 126: 1326S -1334S.

Waltz, D.M. and Stern, M.D. 1989. Evaluation of various methods for protecting soya-bean protein from degradation by rumen bacteria. Anim. Feed Sci. Technol. 25: 111 – 122.

# Chemical composition and ensiling characteristics of normal and brown-midrib pearl millet forage harvested at two stages of development in southwestern Québec

F. Hassanat, A.F. Mustafa, and P. Seguin[1]
[1]Department of Plant Science, Macdonald Campus of McGill University

## ABSTRACT

This study was conducted to determine the effects of cultivar and stage of development on ensiling characteristics and chemical composition of forage millet. Regular and brown midrib millet were harvested at vegetative or heading stage then ensiled for 0, 2, 4, 8, 16, and 45d. Both millet types were well ensiled and had a pH below 4.2 after 45d of ensiling. Most proteolysis occurred between 0d and 8d post-ensiling, where 40 to 50% of TP was lost to NPN for the two millet types at any stage of development. Silages made from BM contained 2%, 6%, 27% less ($P<0.05$) NDF, ADF and ADL than silages made from RM, respectively. Heading stage harvest contained more ($P<0.05$) ADF and ADL (5% and 80% respectively) and 30% less ($P<0.05$) CP than silages harvested at VS. In vitro dry matter and NDF digestibilities were greater ($P<0.05$) for BM than RM silages. In conclusion, cultivar and stage of development at harvest can significantly forage yield and quality of pearl millet.

**Keywords:** forage quality, pearl millet, brown midrib, maturity, silage.

## INTRODUCTION

Pearl millet [*Pennisetum glaucum* (L.) R. Br.] is an annual forage that can be utilized for forage or grain production. It is highly drought tolerant, contains no prussic acid and resistant to many diseases affecting corn. Forage cultivars adapted to eastern Canadian conditions have recently been developed (Agricultural Environmental Renewal Canada 2004). Brown midrib cultivars of sorghum, corn, or millet produce less lignified fiber, and often more digestible (Oba and Allen 1999, Cherney et al. 1991) and generally improve animal performance when compared to regular cultivars. The objective of this study was to determine the effects of cultivar and stage of development at harvest on ensiling characteristics, chemical composition and in vitro digestibility of pearl millet grown under southwestern Québec conditions.

## MATERIALS AND METHODS

Two forage millet cultivars were evaluated: regular (RM, CFPM101) and brown midrib (BM, CFPBMR). Seeding was done on 3 June 2003 in Sainte-Anne-de-Bellevue, QC, Canada. Each millet cultivar was harvested at vegetative stage (VS) or at heading stage (HS). Harvested forage chopped, sampled, packed using a manual pestle PVC laboratory silos (7.6 cm diameter x 25 cm height), sealed with a plastic cap fitted with a small valve, stored at room temperature and allowed to ensile for 2, 4, 8, 16, and 45d. Silos were opened at the designated fermentation period and the ensiled forage was mixed thoroughly. Ensiled and thawed fresh forages were homogenized with distilled water. The extract was used to determine pH, volatile fatty acids, water soluble carbohydrates (WSC) and lactic acid. Sub-samples (500 g) of fresh and ensiled forages were dried in a forced air oven at 55 °C for 48 h and then ground through a 1-mm screen. Ground samples were analyzed for crude protein using a Leco Nitrogen Analyzer. Neutral (NDF) and acid (ADF) detergent fiber were determined according to Van Soest et al.

(1991) and AOAC (1990) respectively. Soluble protein (SCP), non-protein nitrogen (NPN), neutral (NDICP) and acid (ADICP) insoluble crude protein were determined according to Licitra et al. (1996). Dried and ground samples of fresh forage and d 45 silages were analyzed for dry matter, ash, ether extract, acid detergent lignin (AOAC 1990) and starch. In vitro DM disappearance (IVDMD) and IVNDFD was determined as in Holden (1999). Chemical composition data were analyzed as a 2 x 2 factorial design (two cultivars and two stage of development at harvest for each fermentation period) with three replications using the proc mixed procedure of the SAS Institute, Inc. (1989). When significant effects were detected ($P < 0.05$), least significant difference was used to determine differences among means.

## RESULTS AND DISCUSSION

There was a rapid decline in pH during the first 2 d of ensiling for all forage treatments (Fig.1). The pH continued to decline up to d 8 post-ensiling for both cultivars of forage millet harvested at the VS, and up to d 4 for those harvested at HS. Brown midrib and RM harvested at the VS had higher ($P < 0.05$) pH than the ones harvested at the HS at any fermentation period. All the 45 d silages had pH less than 4.2, which agrees with the range reported by Messman et al. (1992). Lactic and acetic acids were the main fermentation acids in all treatments, regardless of fermentation period. The concentration of lactic acid increased ($P < 0.05$) rapidly between 0d and 4d of ensiling, then increased slightly up to 45d post-ensiling for VS but stabilized between 4d and 8d for HS. Acetic acid concentration was very low until 2d and then increased rapidly in all samples until 16d for millet harvested at HS but continued to increase for VS. Fraser et al. (2001) reported that acetic acid concentration of silages declines as forage maturity progresses. Pre-ensiled BM contained more ($P < 0.05$) WSC than RM (Table 1). Both forage millet cultivars harvested at HS also had higher ($P < 0.05$) WSC concentrations than those harvested at VS. Water soluble carbohydrate concentrations for pre-ensiled forage treatments were over the minimum level recommended for optimum ensiling. Concentration of WSC declined ($P < 0.05$) rapidly between 0d and 4d post-ensiling and stabilized between 16d and 45d post-ensiling (Fig.1).

Soluble crude protein and NPN increased ($P < 0.05$) sharply during the first 2d of ensiling, continued to increase slowly up to 8d post-ensiling, and then stabilized (Fig. 2). Concentration of NDICP declined ($P < 0.05$) as ensiling progressed (Fig.2). Most of the decline in NDICP occurred between d 0 and d 8 post-ensiling with no further decline between d 16 and d 45 post-ensiling. At any given ensiling period, NDICP was higher ($P < 0.05$) for ensiled forages harvested at HS compared with those harvested at VS. Changes in ADICP during ensiling were small with RM harvested at HS containing higher ($P < 0.05$) ADICP than the other forage millet treatments at all fermentation periods. True protein concentration decreased ($P< 0.05$) rapidly between 0d and 4d for all forage treatments with no significant changes after d 8 post-ensiling. Regular millet harvested at VS had the highest ($P < 0.05$) level of TP at any given fermentation period. On average, 50 % of the TP was degraded during ensiling. During ensiling, plant proteins are rapidly converted to peptides, amino acids, and other N compounds (Papadopoulos and Mckersie 1983). The end of proteolysis after 8 d of ensiling indicated adverse conditions for action of proteases after that time. Changes in protein fractions during ensiling are similar to those observed for other cereal and legumes silages (Mustafa and Seguin 2003).

Chemical composition of pre-ensiled forages is shown in Table 1. Composition of pre-ensiled forages was similar to corresponding silages (Table 2) for most parameters. The DM concentration of silages was low for all treatments and was higher ($P < 0.05$) for silages harvested at HS than for those harvested at VS. Increasing stage of development at harvest increases DM concentration of silages (Meeske et al. 1993; Dawson et al. 2002; Johnson et al. 2003). Concentrations of NDF, ADF and ADL were greater ($P < 0.05$) for RM than BM 45-d silages (Table 2). With increasing maturity, ADL increased ($P < 0.05$) in both millet silage types, with no effect on NDF and ADF concentrations (Table 2). Brown midrib millet, corn, and sorghum have lower concentrations of fiber fractions than their normal counterparts (Cherney et al. 1988; Oba and Allen 1999). In our study, BM silage contained 57 and 24% less

ADL than RM silage at VS and HS, respectively which agrees with findings of Cherney et al (1991). A significant cultivar × stage of development interaction ($P < 0.05$) was observed for CP concentration in silages after 45 d of fermentation (Table 2), and was due to CP concentration of BM silage being higher at VS than that of RM, but not at HS. The difference in CP concentration between the two stages of development was greater for BM than RM silage. In agreement with our study, Cherney et al. (1988) and Mustafa et al. (2004) reported higher CP concentration for brown midrib than for normal forage millet. Differences in the CP concentration between the two stages of development in our study agree with others who reported a decline in CP concentration of forages and silages as maturity progress (Mustafa and Seguin 2003a; Gupta and Pradhan 1975). In vitro DM and NDF digestibilities were higher ($P < 0.05$) for BM than RM silages, and were higher ($P < 0.05$) for silages harvested at VS than for those harvested at HS. There was a significant ($P < 0.05$) cultivar × stage of development interaction for IVNDFD, which reflected a greater decline between VS and HS for BM than RM. Cherney et al. (1988) reported higher IVDMD of brown midrib than for regular forage millet. Similar results have also been reported for corn silage (Goto et al. 1994). The improved IVDMD and IVNDFD of BM silages relative to RM silages can at least in part be attributed to their lower ADL concentrations. The brown midrib trait reduces the amount of lignin and the extent of lignin cross linkage with cell wall carbohydrates through *p*-coumaric acid (Cherney et al. 1991).

## CONCLUSIONS

Despite low dry matter concentration, forage millet can be utilized to produce good quality silage. Ensiling characteristics and microbial changes in general were not greatly affected by millet cultivar or stage of development. Brown midrib millet contained 15% more CP concentrations, but 2%, 6% and 27% less NDF, ADF and ADL respectively, compared to RM. In vitro dry matter digestibility and IVNDFD were higher in BM compared to RM, by 4% and 10% respectively. Reduction in in vitro DM and NDF digestibilities for silages harvested at the heading stage was likely due to increased lignin concentration. Millet silages made from forages harvested at the vegetative stage may be less stable than those made from forages harvested at the heading stage.

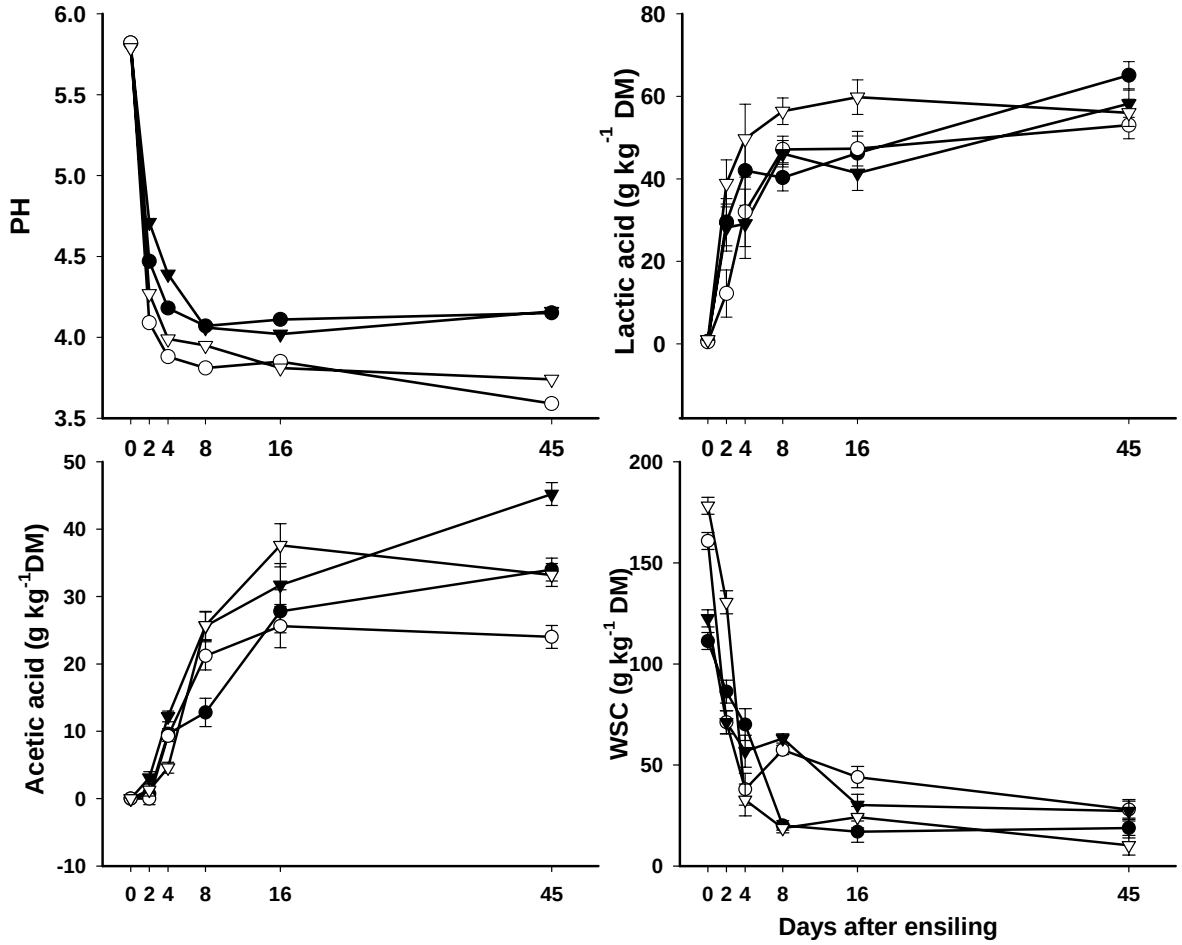


**Fig. 1.** Changes in pH, lactic acid, acetic acid, and water soluble carbohydrates (WSC) during ensiling of regular (RM), or brown midrib pearl millet (BM) harvested at the vegetative (VS) or heading stage (HS) (● RMVS, ○RMHS, ▼BMVS, ∇ BMHS). Vertical bars represent ± SEM.

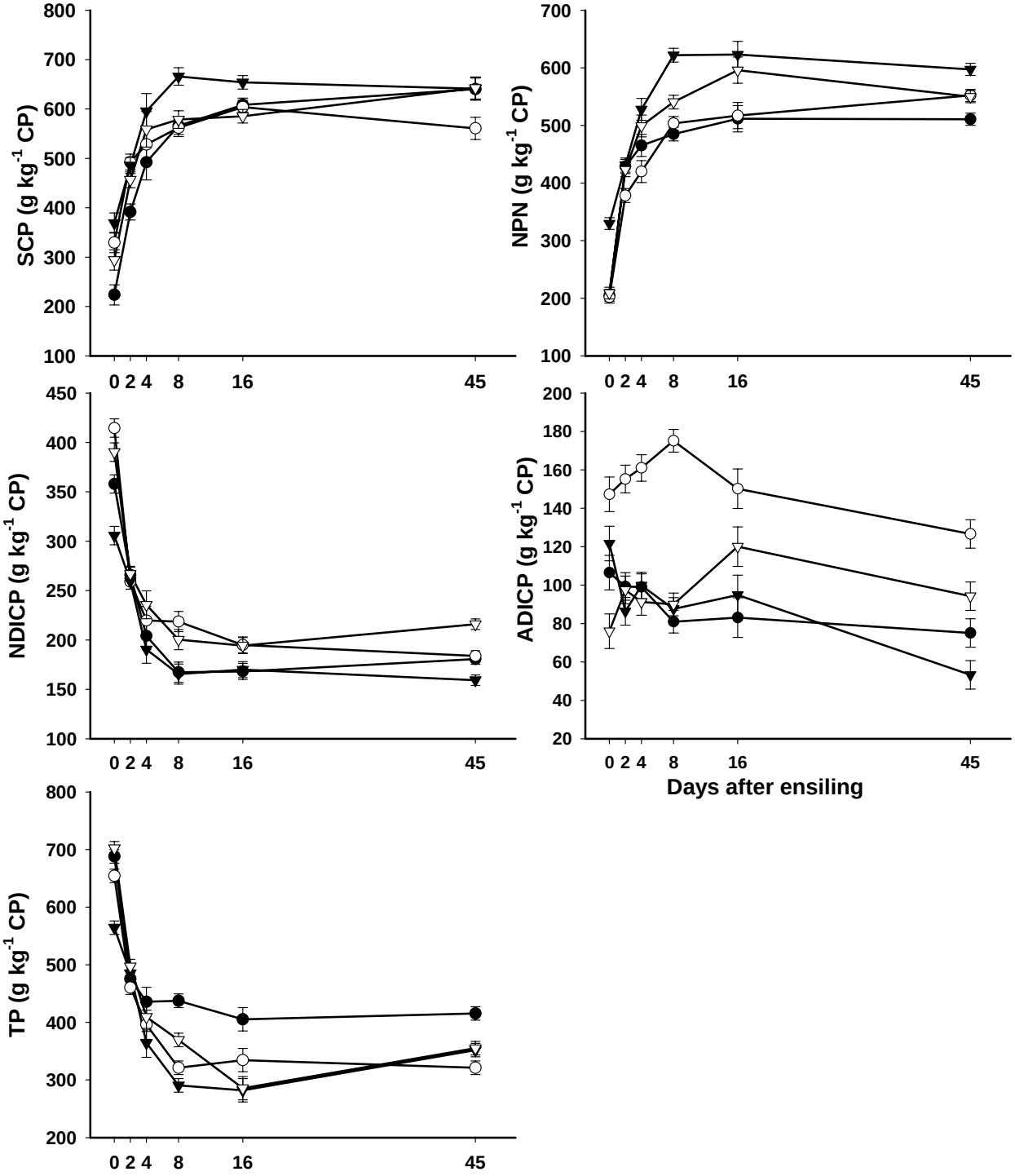


**Fig. 2.** Changes in soluble crude protein (SCP), non protein nitrogen (NPN), neutral detergent insoluble crude protein (NDICP), acid detergent insoluble crude protein (ADICP), and true protein during ensiling of regular (RM), or brown midrib pearl millet (BM) harvested at the vegetative (VS) or heading stage (HS) (● RMVS, ○RMHS, ▼BMVS, ∇ BMHS). Vertical bars represent ± SEM.

**Table 1. Effect of cultivar and stage of development at harvest on chemical composition of fresh pearl millet forage.**

| | Regular millet | | Brown midrib millet | | | Treatment effect (P value) | | |
|---|---|---|---|---|---|---|---|---|
| | Vegetative | Heading | Vegetative | Heading | SEM | Millet type | Stage of development | Millet type x stage of development |
| DM (g $kg^{-1}$) | 240 | 279 | 209 | 296 | 4.2 | 0.07 | <0.01 | <0.01 |
| Yield (ton $ha^{-1}$) | 3.65 | 9.03 | 2.50 | 5.67 | 0.23 | < 0.01 | < 0.01 | < 0.01 |
| Ash (g $kg^{-1}$ DM) | 75 | 61 | 77 | 66 | 1.6 | 0.06 | <0.01 | 0.20 |
| NDF (g $kg^{-1}$ DM) | 654 | 635 | 628 | 640 | 8.3 | 0.21 | 0.68 | 0.11 |
| ADF (g $kg^{-1}$ DM) | 333 | 339 | 302 | 327 | 4.3 | <0.01 | <0.01 | 0.07 |
| ADL (g $kg^{-1}$ DM) | 13 | 21 | 8 | 17 | 0.7 | <0.01 | <0.01 | 0.23 |
| Starch (g $kg^{-1}$ DM) | 14 | 19 | 19 | 9 | 0.6 | <0.01 | <0.01 | <0.01 |
| WSC (g $kg^{-1}$ DM) | 111 | 160 | 123 | 178 | 4.2 | <0.01 | <0.01 | 0.87 |
| Crude protein (g $kg^{-1}$ DM) | 98 | 65 | 107 | 83 | 3.1 | <0.01 | <0.01 | 0.18 |
| SCP (g $kg^{-1}$ CP) | 224 | 330 | 369 | 294 | 20.4 | 0.03 | 0.47 | <0.01 |
| NPN (g $kg^{-1}$ CP) | 205 | 202 | 330 | 209 | 10.2 | <0.01 | <0.01 | <0.01 |
| NDICP (g $kg^{-1}$ CP) | 358 | 415 | 306 | 390 | 10.3 | <0.01 | <0.01 | 0.21 |
| ADICP (g $kg^{-1}$ CP) | 107 | 142 | 76 | 122 | 9.0 | 0.02 | <0.01 | 0.59 |
| IVDMD (g $kg^{-1}$ DM) | 727 | 703 | 772 | 723 | 1.2 | <0.01 | <0.01 | 0.76 |
| IVNDFD (g $kg^{-1}$ DM) | 614 | 566 | 682 | 619 | 1.2 | <0.01 | <0.01 | 0.46 |

**Table 2. Effect of cultivar and stage of development at harvest on chemical composition of pearl millet silage**

| | Regular millet | | Brown midrib millet | | | Treatment effect (P value) | | |
|---|---|---|---|---|---|---|---|---|
| | Vegetative | Heading | Vegetative | Heading | SEM | Millet type | Stage of development | Millet type x stage of development |
| Dry matter (g $kg^{-1}$ fresh matter) | 201 | 248 | 190 | 241 | 4.0 | 0.05 | <0.01 | 0.60 |
| Yield (ton $ha^{-1}$) | 3.65 | 9.03 | 2.50 | 5.67 | 0.23 | < 0.01 | < 0.01 | < 0.01 |
| Ash (g $kg^{-1}$ DM ) | 85 | 66 | 88 | 70 | 2.0 | 0.18 | <0.01 | 0.87 |
| NDF (g $kg^{-1}$ DM) | 674 | 689 | 631 | 660 | 11.4 | 0.01 | 0.09 | 0.57 |
| ADF (g $kg^{-1}$ DM) | 365 | 358 | 336 | 346 | 94.4 | 0.05 | 0.90 | 0.37 |
| ADL (g $kg^{-1}$ DM) | 14 | 25 | 06 | 19 | 0.3 | <0.01 | <0.01 | 0.03 |
| Starch (g $kg^{-1}$ DM) | 16 | 12 | 22 | 11 | 0.8 | 0.84 | <0.01 | <0.01 |
| Crude protein (g $kg^{-1}$ DM) | 96 | 71 | 118 | 83 | 1.8 | <0.01 | <0.01 | 0.02 |
| Soluble protein (g $kg^{-1}$ CP) | 640 | 561 | 641 | 631 | 22.5 | 0.13 | 0.14 | 0.13 |
| NPN (g $kg^{-1}$ CP) | 510 | 552 | 597 | 548 | 10.5 | <0.01 | 0.80 | <0.01 |
| NDICP (g $kg^{-1}$ CP) | 181 | 184 | 159 | 216 | 5.2 | 0.33 | <0.01 | <0.01 |
| ADICP (g $kg^{-1}$ CP) | 75 | 127 | 64 | 96 | 7.4 | 0.22 | <0.01 | 0.23 |
| True protein (g $kg^{-1}$ CP) | 414 | 321 | 352 | 355 | 11.7 | <0.01 | 0.26 | <0.01 |
| IVDMD (g $kg^{-1}$ DM) | 737 | 670 | 772 | 696 | 1.2 | <0.01 | <0.01 | 0.57 |
| IVNDFD (g $kg^{-1}$ DM) | 597 | 548 | 661 | 561 | 1.2 | <0.01 | <0.01 | <0.01 |
| Dry matter recovery (%) | 99.2 | 98.6 | 98.8 | 98.2 | 0.3 | 0.86 | 0.94 | 0.98 |

## REFERENCES


Agriculture Environmental Renewal Canada. 2004. Canadian forage pearl millet 101 CFPM-101. Online. http://www.aerc.ca/foragepearl.html [verified 10 May 2004]

Association of Official Analytical Chemists (AOAC). 1990. Official Methods for Analysis. 15th ed. AOAC, Arlington, VI.

Barker, S. B. and Summerson, W. H. 1941. The colorimetric determination of lactic acid in biological material. J. of Biol. Chem. 137: 537-554.

Cherney, J. H., Axtell, J. D., Hassen, M. M., and Anliker, K. S. 1988. Forage quality characterization of chemically induced brown-midrib mutation in pearl millet. Crop Sci. 28: 783-787.

Cherney, J. H., Cherney, D. J. R., Akin, D. E., and Axtell, J. D. 1991. Potential of brown-midrib low-lignin mutants for improving forage quality. Adv. Agronomy 46: 157-197.

Dawson, L. E. R., Kirkland, R. M., Ferris C. P., Steen, R. E. J., Kilpatrick D. J., and Gordon, F. J. 2002. The effect of stage of perennial ryegrass maturity at harvesting, fermentation characteristics and concentrate supplementation, on the quality and intake of grass silage by beef cattle. Grass Forage Sci. 57: 255-267.

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., and Smith, F. 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. 28: 350-356.

Fraser, M. D., Winters, A., Fychan R., Davis, D R. and Jones, R. 2001. The effects of harvest date and inoculation on the yield, fermentation characteristics, and feeding value of kale silage. Grass Forage Sci. 56: 151-161.

Goto, M., Matsuoka, J., Sato, T., Ehara, H. and Morita, O. 1994. Brown midrib mutant maize with reduced levels of phenolic acids ether-linked to the cell walls. Anim. Feed Sci. Technol. 48: 27-38.

Gupta, P. C., and Pradhan, K. 1975. Effect of stage of maturity on chemical composition and in vitro nutrient digestibility of non-legume forages. Indian J. Anim. Sci. 45:433-437.

Holden, L. A. 1999. Comparison of methods of in vitro dry matter analysis of ten feeds. J. Dairy Sci. 82: 1791-1794.

Johnson, L. M., Harrison, J. H., Davidson, D., Manhanna W. C. and Shinners, K. 2003. Corn silage management: effect of hybrid, maturity, inoculation and mechanical processing on fermentation characteristics. J. Dairy Sci. 86: 287-308.

Licitra, G., Hernandez, T., M., and Van Soest, P. J. 1996. Standardization procedures for nitrogen fractionation of ruminant feeds. Anim. Feed Sci. Technol. 57:347-358.

Meeske, R., Ashbell, G., Weinber, Z. G. and Kipins, T. 1993. Ensiling forage sorghum at two stages of maturity with addition of lactic acid bacterial inoculants. Anim. Feed Sci. Technol. 43: 165-175.

Messman, M. A., Weiss, W. P., Henderlong, P. R., and Shockey, W. L. 1992. Evaluation of pearl millet and field peas plus triticale silages for mid lactation cows. J. Dairy Sci. 75: 2769-2775.

Mustafa, A. F., Hassanat, F. and Seguin, P. 2004. Chemical composition and in situ ruminal nutrient degradability of normal and brown midrib forage pearl millet grown in southwestern Québec. Can. J. Anim. Sci. 84: 737–740.

Mustafa, A. F. and Seguin, P. 2003. Effects of stage of maturity on ensiling characteristics and ruminal nutrient degradability of oat silage. Arch. of Anim. Nutr. 57: 347-358.

Oba, M. and Allen, M. S. 1999. Effects of brown midrib 3 mutation in corn silage on dry matter intake and productivity of high yielding dairy cows. J. Dairy Sci. 82: 135-142.

Papadopoulos, Y. A. and McKersie, B. D. 1983. A comparison of protein degradation during wilting and ensiling of six forage species. Can. J. Plant Sci. 63: 903-912.

SAS Institute, Inc., 1989. SAS user's guide: Statistics. SAS Institute, Inc., Cary, NC.

# Effects of inoculation on ensiling characteristics, chemical composition and aerobic stability of regular and brown midrib millet

F. Hassanat, A.F. Mustafa, and P. Seguin[1]
[1]Department of Plant Science, Macdonald Campus of McGill University

## ABSTRACT

This study was conducted to determine the effect of inoculation on ensiling characteristics and chemical composition of two millet cultivars. Regular (RM) and brown midrib millet (BM) was obtained, treated with commercial inoculum, or left untreated, and ensiled in laboratory silos for 2, 4, 8, 16, and 45 d. The pH of treated silage was below 4.0 after 2d of ensiling, while control silages reached that pH at 8d with pH at 45d for all treatments. Inoculation increased lactic acid and decreased acetic acid, causing the lactic: acetic acid ratio to double. Proteolysis during ensiling was not strongly affected by millet cultivar or treatment. Chemical composition and IVDMD of silages was affected by millet type, but not by inoculation. Lactic acid bacteria population was higher for inoculated silage compared to control at 0d and 2d post ensiling, then the count was similar across all treatments with populations of enterobacteria and yeast and molds declined sharply by 4d of ensiling. Inoculated silage of the two millet cultivars was less aerobically stable compared to control by an average of 44 hrs. Inoculation did not notably improve fermentation or chemical composition of silage and contributed to making it less aerobically stable.

**Keywords:** forage millet, inoculation, silage, aerobic stability

## INTRODUCTION

Silage making depends mainly on epiphytic lactic acid bacteria on forage (Pahlow et al. 2003). Adding lactic acid bacteria to forage is promising. It ensures presence of enough lactic acid bacteria to cause fast reduction in pH, reduce proteolysis, and eliminate undesirable fermentation in most studies (Kung et al 2003). Inoculation might reduce aerobic stability of silages during feed out stage (Weinberg et al. 1993, Kung and Ranjit 2001). These observations vary according to forage and inoculants type. Millet cultivars suitable for conditions in Canada have been developed by AERC (1998). Testing the suitability of these cultivars for ensiling with or without inoculation is important. Our objective is to observe effects of inoculation on ensiling characteristics, chemical composition, and aerobic stability of regular and brown midrib millet.

## MATERIALS AND METHODS

Two millet cultivars (regular (RM, CFPM101) and brown midrib (BM, CFPBMR) millet were grown on a sandy soil in L'Acadie,QC, Canada on the June, 4, 2003. Second cut were obtained 5 wk from the first cut and14 wk from seeding time. Harvested forages were chopped, welted for 3 hours, sampled, then treated with a commercial inoculum (Pioneer 1129 sorghum silage) at a rate of $2.5x10^6$ CFU $kg^{-1}$ fresh forage, or left untreated. Forage material was packed using a manual pestle PVC laboratory silos (7.6 cm diameter x 25 cm height), sealed with a plastic cap fitted with a small valve, stored at room temperature and allowed to ensile for 2, 4, 8, 16, and 45d as in Sebastian et al. (1996). Silos were opened at the designated fermentation period and silage was mixed thoroughly. Ensiled and thawed fresh forages were homogenized with distilled water (ratio 1:10) for 5 min. The extract was used to determine pH, volatile fatty acids water soluble carbohydrates (WSC) and lactic

acid. Sub-samples (500 g) of fresh and ensiled forages were dried in a forced air oven at 55 °C for 48 h and then ground through a 1-mm screen. Ground samples were analyzed for crude protein using a Leco Nitrogen Analyzer. Neutral (NDF) and acid (ADF) detergent fiber were determined according to Van Soest et al. (1991) and AOAC (1990) respectively. Soluble protein (SCP), non-protein nitrogen (NPN), neutral (NDICP) and acid (ADICP) insoluble crude protein were determined according to Licitra et al. (1996). Dried and ground samples of fresh forage and d 45 silages were analyzed for dry matter, ash, ether extract, acid detergent lignin (AOAC 1990) and starch (McCleary et al. 1997). In vitro DM disappearance (IVDMD) and IVNDFD was determined as in Holden (1999).

Forage and silage samples were pooled for each millet cultivar, treatment and fermentation period (0, 2, 4, 8, 16 and 45 d) across replicates and used for determining lactic acid bacteria, Enterobacteria and yeast and mold population Kung et al. (2000). Samples of 45d silage were used for aerobic stability determination (Kung et al. 2000). Silage material were thawed in plastic containers and were thoroughly agitated to ensure air exposure, packed loosely in 500-mL plastic containers with thermocouple probes inserted in the core of the container to detect temperature difference from the environment using a Hotmux data logger. Samples were incubated for 7 d at 25 ºC, and then analyzed for pH, lactic acid, yeast and mold count and DM loss. Chemical composition and aerobic stability data were analyzed statistically as a 2 x 2 factorial design (two cultivars and two treatments for each fermentation period) with three replications using the proc-mixed procedure of the SAS Institute, Inc. (1989). When significant effects were detected ($P<0.05$), least significant difference was used to determine differences among means

## RESULTS AND DISCUSSION

Silage pH dropped from 5.8 to 3.6 by the end of the ensiling period (Fig. 1). There was a rapid ($P < 0.05$) decline in silage during the first 4d in all treatments. Inoculated silages had lower pH ($P < 0.05$) than untreated silages up to 8d post ensiling with similar pH at 45d. Lactic and Acetic acid content increased steadily through out the ensiling period. No significant increase in lactic acid content after 8d in inoculated silage, while it continued to increase up to 16d in the untreated silages with higher concentration for the treated silages compared to control. Acetic acid was higher ($P < 0.05$) for untreated than inoculated silages at d 8, 16 and 45 post-ensiling (Fig.1). Our results for fermentation parameters agree with other studies that used homofermentative lactic acid bacteria as silage inoculants (Fraser et al. 2001, Rizk et al. 2005). Ratio of lactic to acetic acid increased from 1.5:1 in control silages to 3.4:1 in inoculated silages. Similar results have been reported for barley (Hristov and McAllister 2002) and wheat and sorghum (Weinberg et al. 1999). All pre-ensiled forages contained more than 2% WSC, which is adequate for producing good quality silage (Lunden Petterson and Lindgrin 1990). There was a sharp drop in WSC for all silages up to 4d (Figure 1), with minimal changes after 8d post ensiling.

Both soluble crude protein and NPN for all silage treatments increased significantly ($P < 0.05$) during ensiling (Fig. 2). The sharpest increase was during the first 2d of ensiling, followed by small increase up to 45d post ensiling. Almost 58% of the CP was in the form of SCP, 96% of which was NPN. Almost 50% of the NDICP and TP were degraded during ensiling which caused the increase in SCP and NPN. The two protein fractions declined significantly ($P < 0.05$) during the first 2 d of ensiling with small changes after 8d of ensiling Averaged across treatments, concentration of ADICP was 5.5% and 4.4% in fresh forage and silage respectively with minimal changes through out ensiling. One of the anticipated benefits of inoculation is reducing proteolysis during ensiling (Kung et al. 2003, Weinberg and Muck 1996). Inoculation failed to reduce proteolysis for barley (Kung and Ranjit 2001) and wheat (Froetschel et al. 1991). Reducing the action of plant and microbial proteases could be achieved by fast reduction in pH (Rooke and Hatfield 2003) which what happened in all silage treatments in our study.

Chemical composition of fresh forage and silage are shown in Table 1 and Table 2 respectively. Dry matter content varied little and averages 21% among all treatments. In general, inoculation had no effect on chemical composition of millet silage, nor in

IVDMD. Inoculation did not have any effect on fiber fractions of corn (Kung et al. 1993), or alfalfa silage (Rizk et al 2005). No effect of inoculation on IVDMD was observed in barley silage (McAllister et al. 1995), or wheat silage (Froetschel et al. 1991). Millet cultivar had stronger effect on chemical composition and IVDMD.

It was not possible to statistically analyze the microbiological data (Table 3) of silage because samples representing each day of ensiling were pooled for each treatment. There was an increase in lactic acid bacteria population up to 4 d of ensiling, and then it stabilized with higher count in 0d and 2d of ensiling in inoculated silage compared to control. This may explain the high concentration of lactic acid and low pH and WSC concentrations during the first days of ensiling in treated forages compared to control (Fig.1). Eneterobacteria and yeast and mould population count decreased in all treatments to a non detectable concentration and less than 2.0 by 8 d post ensiling respectively. Similar changes in microbial population were observed by Whiter and Kung (2001) and Kung et al (2000) on alfalfa and barley silages.

All silage types deteriorated after 7d of aerobic exposure (Table 4). Averaged across all treatments, pH increased from 3.6 to 7.5 after 7d of aerobic exposure. The increase in pH was mainly due to loss of 80% of lactic acid during aerobic exposure. Yeast and mold count increased from less than 2 log CFU $g^{-1}$ to an average of 7.4 after aerobic exposure in all silage treatments. Yeast and mould count and dry matter loss were higher ($P < 0.05$) for inoculated silages compared to control silages (Table 3). Control silages took longer time ($P < 0.05$) to heat above 2ºC than inoculated silages by an average of 40 hr. Similar findings were reported by Weinberg et al. (1999) for wheat and sorghum silage, and (Weinberg et al. 1993) in barley silage inoculated with homo-fermentative acid bacteria.

## CONCLUSIONS

Millet can be ensiled well under low dry matter conditions. Inoculation with homolactic acid bacteria cause faster reduction in pH and increase in lactic acid bacteria count, faster disappearance of the Eneterobacteria and yeast and mould populations in the first few days of ensiling, and double lactic: acetic acid ratio. Inoculation did not cause any differences between treatments in chemical composition. Aerobic stability was reduced by an average of 44 hours in inoculated silages compared to control. The two millet cultivars can produce good silage without the addition of inoculant.

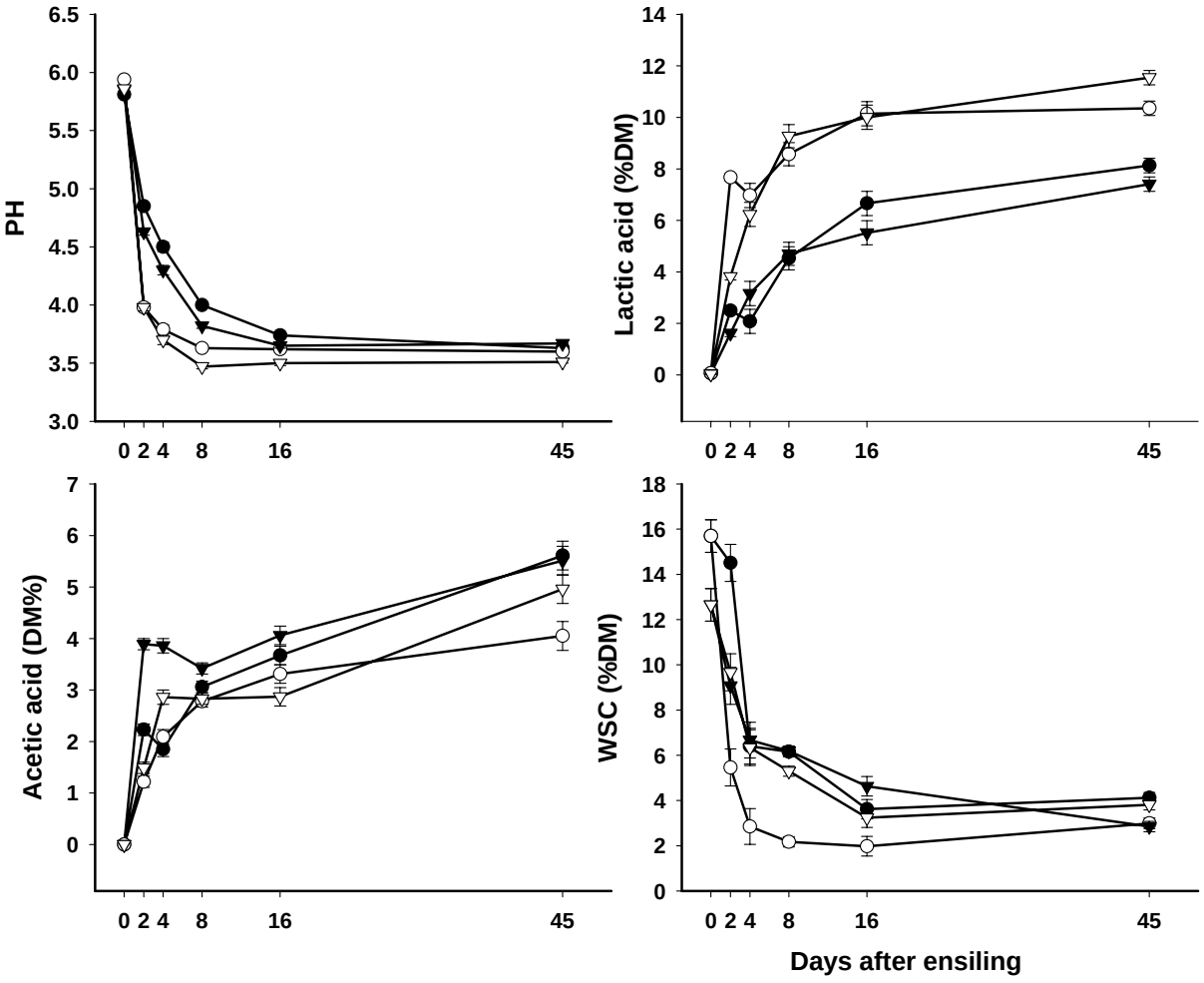


**Fig. 1**. Changes in pH, lactic acid, acetic acid and water soluble carbohydrates (WSC) during ensiling of regular (RM), or brown midrib millet (BM) treated with inoculum or untreated (control) (● RMCT, ○RMIN, ▼BMCT, ∇ BMIN). Vertical bar represent ± SEM.;l

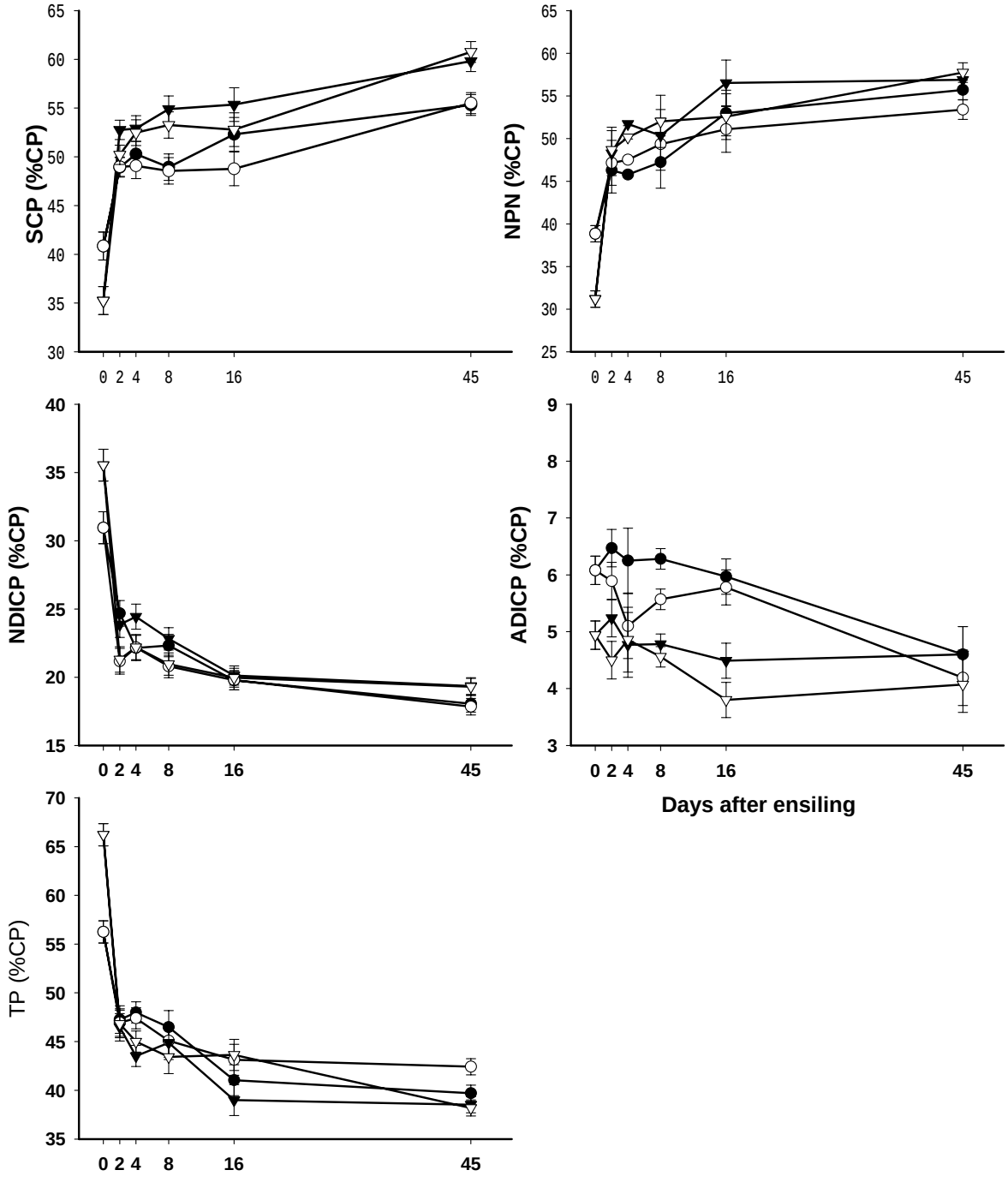


**Fig. 2**. Changes in soluble crude protein (SCP), non protein nitrogen (NPN), neutral detergent insoluble crude protein (NDICP), acid detergent crude protein (ADICP), and true protein (TP) during ensiling of regular (RM), or brown midrib millet (BM) treated with inoculum or untreated (control) (● RMCT, ○RMIN, ▼BMCT, ∇ BMIN). Vertical bar represent ± SEM.

**Table 1.** Chemical composition of regular and brown midrib millet forage

| | Regular Millet | Brown Midrib Millet | SEM |
|---|---|---|---|
| Dry matter (% DM) | 24.4 | 26.1 | 0.5 |
| Crude protein (CP) | 14.5 | 15.1 | 0.4 |
| Soluble protein ( % of CP) | 40.9 | 35.3 | 1.3 |
| Non-protein nitrogen (% of CP) | 38.8 | 31.2 | 1.4 |
| Neutral detergent insoluble protein (% of CP) | 30.9 | 34.2 | 1. 5 |
| Acid detergent insoluble protein (% of CP) | 6.1 | 4.9 | 0.2 |
| True protein (% of CP) | 55.8 | 63.9 | 1.7 |
| Neutral detergent fiber (% of DM) | 63.8 | 59.5 | 0.7 |
| Acid detergent fiber (% of DM) | 35.1 | 30.8 | 0.6 |
| Acid detergent lignin (% of DM) | 2.7 | 1.4 | 0.03 |
| Water Soluble Carbohydrates (% of DM) | 15.5 | 12.1 | 0.7 |
| Ash (% of DM) | 10.4 | 9.4 | 0.2 |
| In vitro dry matter digestibility (%) | 70.7 | 77.1 | 0.7 |

**Table 2.** Effect of inoculation on chemical composition of regular and brown midrib millet silage**.**

| | Regular millet | | Brown midrib millet | | | Effect | | |
|---|---|---|---|---|---|---|---|---|
| | Control | Inoculated | Control | Inoculated | SEM | Millet type | Inoculation | Interaction |
| DM (%) | 20.25 | 20.84 | 21.71 | 22.04 | 0.22 | <0.01 | 0.08 | 0.58 |
| Ash (% DM) | 11.46 | 11.11 | 9.92 | 9.56 | 0.19 | <0.01 | 0.10 | 0.97 |
| Neutral detergent fiber (% DM) | 63.24 | 60.66 | 59.82 | 58.83 | 0.96 | 0.03 | 0.09 | 0.47 |
| Acid detergent fiber (% DM) | 36.75 | 36.75 | 32.87 | 33.40 | 0.33 | <0.01 | 0.46 | 0.46 |
| Acid detergent lignin (% DM) | 3.14 | 3.27 | 1.54 | 1.19 | 0.14 | <0.01 | 0.42 | 0.12 |
| Water soluble carbohydrates (%) | 4.12 | 2.99 | 2.85 | 3.81 | 0.3 | 0.35 | 0.72 | <0.01 |
| CP (% DM) | 16.20 | 16.48 | 17.06 | 16.94 | 0.34 | 0.08 | 0.84 | 0.33 |
| Soluble protein, (% of CP) | 55.32 | 55.50 | 59.82 | 60.76 | 1.07 | <0.01 | 0.62 | 0.73 |
| Non-protein nitrogen (% of CP) | 55.70 | 53.39 | 56.88 | 57.74 | 0.79 | <0.01 | 0.38 | 0.08 |
| NDICP (% of CP) | 18.05 | 17.84 | 19.35 | 19.30 | 0.61 | 0.05 | 0.84 | 0.90 |
| ADICP (% of CP) | 4.60 | 4.19 | 4.60 | 4.07 | 0.41 | 0.88 | 0.29 | 0.89 |
| True protein (% of CP) | 39.70 | 42.42 | 38.52 | 38.19 | 0.83 | 0.01 | 0.19 | 0.10 |
| DM recovery (%) | 98.3 | 99.4 | 98.0 | 98.3 | 0.4 | 0.51 | 0.17 | 0.81 |
| IVDMD (%) | 73.6 | 75.5 | 79.1 | 77.4 | 0.7 | <0.01 | 0.90 | 0.20 |

**Table 3.** Changes in microbial population during ensiling of inoculated and uninoculated regular and brown midrib millet (log CFU $g^{-1}$ sample).

| | Days of ensiling | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 2 | 4 | 8 | 16 | 45 |
| Lactic acid bacteria | | | | | | |
| Regular millet control | 2.69 | 5.12 | 7.54 | 7.57 | 7.54 | 7.51 |
| Regular millet inoculated | 4.79 | 6.76 | 7.20 | 7.31 | 7.26 | 7.12 |
| Brown midrib millet control | 3.52 | 5.91 | 9.16 | 8.47 | 9.21 | 7.58 |
| Brown midrib millet inoculated | 5.10 | 7.93 | 9.17 | 8.67 | 7.56 | 7.52 |
| Enertobacteria | | | | | | |
| Regular millet control | 5.67 | 2.74 | ND | ND | ND | ND |
| Regular millet inoculated | 5.80 | <2.00 | ND | ND | ND | ND |
| Brown midrib millet control | 5.82 | 4.66 | 2.61 | ND | ND | ND |
| Brown midrib millet inoculated | 5.75 | <2.00 | ND | ND | ND | ND |
| Yeast and Molds | | | | | | |
| Regular millet control | 4.15 | 3.25 | <2.00 | <2.00 | <2.00 | <2.00 |
| Regular millet inoculated | 3.10 | 3.42 | <2.00 | <2.00 | <2.00 | <2.00 |
| Brown midrib millet control | 4.63 | 2.77 | <2.00 | <2.00 | <2.00 | <2.00 |
| Brown midrib millet inoculated | 4.79 | 2.20 | 2.02 | <2.00 | <2.00 | <2.00 |

*ND: not detected.

**Table 4.** Effect of aerobic exposure for 7 days on silages of regular and brown midrib millet silage harvested at stages of maturity.

| | Regular millet | | Brown midrib millet | | | Effect | | |
|---|---|---|---|---|---|---|---|---|
| | C* | I** | C | I | SEM | Millet type | C | I |
| pH | 7.4 | 7.5 | 6.9 | 8.1 | 0.4 | 0.82 | 0.12 | 0.18 |
| Lactic acid (% of DM) | 2.4 | 2.0 | 1.4 | 1.8 | 0.3 | 0.03 | 0.97 | 0.14 |
| Yeast and mold (log CFU $g^{-1}$) | 6.9 | 7.6 | 6.7 | 8.1 | 0.1 | 0.24 | <0.01 | <0.01 |
| Dry Matter loss (%) | 10.1 | 13.1 | 7.5 | 13.3 | 1.3 | 0.38 | <0.01 | 0.30 |
| Time to heat ≥ 2 ˚C (h) | 153 | 134 | 164 | 103 | 4.7 | 0.07 | <0.01 | <0.01 |

* Control; ** Inoculated

## REFERENCES


Agriculture Environmental Renewal Canada. 1998. Canadian forage pearl millet 101 CFPM-101. Online. http://www.aerc.ca/foragepearl.html [verified 10 May 2004]

Association of Official Analytical Chemists (AOAC). 1990. Official Methods for Analysis. 15th ed. AOAC, Arlington, VI.

Fraser, M. D., Fychan, R., and Jones, R. 2001. The Effects of Harvest Date and Inoculation on the Yeild, Ferementation Characteristics, and Feeding Value of Forage Pea and Feild Beans Silage. Grass Forage Sci. 56: 218.

Froetschel, M. A., Ely, L. O., and Amos, H. E. 1991. Effects of additives and growth environment on preservation and digestibility of wheat silage fed to Holstein heifers. J. Dairy Sci. 74: 546-556.

Hristov, A. N. and T. A. McAllister. 2002. Effect of inoculants on whole-crop barley silage fermentation and dry matter disappearance in situ. 80:510-516.

Kung, L. Jr., Martin, R. S., Lin. C. J. 2003. Silage additives. Pages 305-360 in D. R. Buxton, R. E. Muck , and J. H. Harrison, eds. Silage science and technology. American Society of Agronomy, Inc. Madison, Wisconsin.

Kung, L. Jr., Robinson, J. R., Ranjit, N. K., Chen, J. H., Golt, C. M., and Pesek, J. D. 2000. Microbial population, fermentation end products, and aerobic stability of corn silage treated with ammonia or propionic acid -based preservative. J. Dairy Sci. 83:1479-1486.

Licitra, G., Hernandez, T., M., and Van Soest, P. J. 1996. Standardization procedures for nitrogen fractionation of ruminant feeds. Anim. Feed Sci. Technol. 57:347-358.

McAllister, T. A., Selinger, L. B., McMahon, L. R., Bae, H. D., Lysyk, T. J., Oosting, S. J., and Cheng, K. J. 1995. Intake, digestibility and aerobic stability of barley silage inoculated with mixtures of lactobacillus plantarum and Enterococcus faecium. Can. J. Anim. Sci. 75:425-432.

Pahlow, G. R. Muc F. Driehuis S. Elferink and S. Spolestra. 2003. Pages 31-93. Microbiology of ensiling in D. R. Buxton, R. E. Muck , and J. H. Harrison, eds. Silage science and technology. American Society of Agronomy, Inc. Madison, Wisconsin.

Rizk, C., Mustafa, A. F., Phillip, L. E. 2005. Effects of Inoculation on high dry matter alfalfa silage on emsiling characteristics, ruminal nutrient degradability and dairy cow performance. J. Sci. Food Agric. 85:743-750

Rooke, J. A. and Hatfield R. D. 2003. Biochemistry of Ensiling. Pages 95-140. in D. R. Buxton, R. E. Muck , and J. H. Harrison, eds. Silage science and technology. American Society of Agronomy, Inc. Madison, Wisconsin.

SAS Institute, Inc., 1989. SAS user's guide: Statistics. SAS Institute, Inc., Cary, NC.

Van Soest, P. J., Robertson, P. J. and Lewis, B. A. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74: 3583–3597.

Weinberg, Z. G., Ashbell, G., Hen, Y., Azrieli A. 1993. The effect of applying lactic acid bacteria at ensiling on the aerobic stability of silages. J. Appl. Bacter. 75: 512-518.

Weinberg, Z. G., Ashbell, G., Hen, Y., Szalac, G. 1999. The Effect of Lactobacilluc buchneri and L. Plantarum, Applied AT Ensiling, on the Ensiling Fermentation and Aerobic Stability of Wheat and Sorghum Silages. J. Indus. Microbiol. and Biotech. 23: 218-222

Whiter, A. and Kung, L. Jr. 2001. The Effect of a Dry or Liquid Application of Lactobacillus plantarum MTD1 in the Fermentation of Alfalfa Silage. J. Dairy Sci. 84: 2195-2202.

# Performance of lactating ewes fed oilseeds: Effects on nutrient utilization

R.H. Zhang, A.F. Mustafa and X. Zhao

**ABSTRACT**

Sixteen lactating ewes were used to determine the effects of oilseed supplementation (i.e. canola seed, sunflower seed and flaxseed) on dry matter (DM) intake and total tract nutrient digestibilities. Ewes were fed diets containing no oilseed (C), canola seed (CS), sunflower seed (SF), or flaxseed (FS). Oilseed supplementation had no effect on DM intake or total tract fiber digestibility. Ewes fed FS had a higher ($P < 0.05$) DM digestibility than those fed CS or C. Oilseed supplementation improved ($P < 0.05$) total tract digestibility of $C_{18:1}$ while total fatty acid digestibility was improved ($P < 0.05$) by feeding FS and SF. It was concluded that oilseed supplementation had no effect on DM intake or nutrient utilization by lactating ewes.

**Keywords**: Ewes, nutrient digestibility, oilseeds

## INTRODUCTION

Sheep milk is commonly used for cheese and yogurt production due to its high fat and total solid contents. However, the high fat content in sheep milk might limit the demand for milk and milk products by health conscious consumers. Several studies have demonstrated the benefits of supplemental fat from oilseeds in increasing health-promoting fatty acids, such as conjugated linoleic acid and omega-3 fatty acid in milk of sheep (Zhang et al. 2006) and goats (Mir et al. 1999). A major concern with feeding high levels of polyunsaturated fatty acids (PUFA) to ruminants is the adverse effects on ruminal fermentation and total tract fiber digestion (Doreau and Chilliard 1997). However, feeding PUFA in the form of oilseeds might be less detrimental than feeding free oils. Gonthier et al. (2004b) showed that feeding flaxseed to dairy cows improved total tract nutrient utilization with no adverse effects on ruminal fermentation. Data on total tract nutrient utilization of ewes fed oilseeds are limited and therefore the objectives of this study were to determine the effects of feeding diets containing canola seed, sunflower seed and flaxseed on intake and total tract nutrient utilization by lactating ewes.

## MATERIALS AND METHODS

### Animals and Feeding

Four concentrates were formulated that contain no oilseed (CT), canola seed (CS), sunflower seed (SF), or flaxseed (FS). The concentrates were mixed with grass silage (50:50 dry matter, DM basis) and fed as total mixed diets (Table 1). Diets were formulated to be isonitrogenous and the oilseed diets were intended to contain 70 g $kg^{-1}$ fatty acids.

Sixteen lactating ewes were balanced for parity and days in milk and randomly allotted to one of the four dietary treatments (4 animals per treatment). Ewes were individually housed in pens with free access to water and were fed twice daily for ad libitum intake. Experimental periods consisted of 14 d of diet adaptation and 7 d of data collection.

Chromic oxide was used as an inert external marker to determine total tract nutrient digestibility. Six g of $Cr_2O_3$ were offered twice daily to each ewe starting on day 7 of the experimental period for 14 days. Grabbed fecal samples were collected three times daily during the last 3 d of the data collection period, composited by animal and kept frozen. Feed samples and orts were collected daily during the data collection period, composited by treatment and kept frozen.

**Chemical Analysis**

Feed, orts and fecal samples were dried at 55 $^{o}$C in a forced-air oven for 48 h and then ground in a Wiley mill. Dried feed, orts and fecal samples were analyzed for DM according to the procedures of the Association of Official Analytical Chemists (AOAC 1990). Crude protein was determined using a nitrogen determinator (Leco Corporation, MI). Neutral (NDF) and acid (ADF) detergent fiber were determined using the ANKOM System (Ankom Technology Corporation, Fairport, NY). Gross energy (GE) was determined using an oxygen bomb calorimeter. Chromic oxide concentration in feces was determined according to the procedure of Fenton and Fenton (1979).

Fatty acids (FA) of feed and fecal samples were extracted and methylated according to the one-step procedure (Sukhija and Palmquist 1988) using hexane instead of benzene. Methyl esters of FA were analyzed by gas chromatography as described by Gonthier et al. (2004a).

**Statistical Analysis**

Data were statistically analyzed using the General Linear Model of SAS Institute, Inc (1990) for a completely random design with the following model:

$Y = \mu + T_i + e_{ij}$

Where: $\mu$ is the overall mean; $T_i$ is the fixed effect of dietary treatment ($i = 1 \sim 4$); and $e_{ij}$ is the residual effect. Differences were considered significant if $P < 0.05$.

## RESULTS AND DISCUSSION

As expected, FA content was higher for the oilseed diets than for CT and the FA composition of the oilseed diets reflected the FA composition of the respective oilseed (Table 1). The CS was high in $C_{18:1}$ which accounted for 46% of the total FA, whereas $C_{18:2}$ and $C_{18:3}$ accounted for 57.6 and 35.2% of the total FA in the SF and FS, respectively.

Intakes of DM, NDF, ADF, CP, and GE were not influenced by oilseed supplementation (Table 2). Several researchers found no adverse effects of supplemental fat on DM intake of ewes (Bayourthe et al. 1994; Goulas et al. 2003). Feeding supplemental fat in the form of oilseeds is expected to have less detrimental effects on DM intake than if a similar amount was added as free oil (Kennelly 1996). As expected, ewes fed oilseed supplemented diets consumed more ($P < 0.05$) FA than ewes fed CT.

Dry matter digestibility was higher ($P<0.05$) for ewes fed FS than for those fed CT or CS (Table 2). However, no difference in DM digestibility was observed between ewes fed FS and those fed SF. Feeding CS or SF relative to CT had no effect on DM digestibility. Total tract digestibility of NDF, ADF and CP was not affected by oilseed supplementation and averaged 57.2, 54.7, and 62.1%, respectively (Table 2). Differences in total tract GE digestibility were similar those observed for DM digestibility (Table 2). Feeding sheep diets containing 60 g kg$^{-1}$ fat from Megalac, fish oil or whole flaxseed had no influence on total tract nutrient digestibilities (Wachira et al. 2000). Supplementing chopped ryegrass hay with up to 80 g kg$^{-1}$ calcium salt of FA had no effect on total tract digestibility of DM, CP, ADF and NDF by sheep (Bayourthe et al. 1994). In contrast to our results, Enjalbert et al. (1994) found that ruminal infusion of soy oil or calcium salts of soy oil fatty acids depressed total tract nutrient digestibilities by sheep. Discrepancies between various studies can be attributed to levels and physical forms of dietary fat and dietary fiber (Jenkins 1993).

Intakes of $C_{16:0}$, $C_{18:0}$, $C_{18:1}$ and $C_{18:2}$ were greater ($P < 0.05$) for ewes fed the oilseed supplemented diets than for ewes fed CT (Table 2). However, intake of $C_{18:3}$ was higher ($P < 0.05$) for ewes fed FS relative to ewes fed the other dietary treatments. Differences in FA intake between the oilseed treatments reflected the difference in FA composition between the three oilseeds. Oilseed supplementation increased total tract digestibility of $C_{18:1}$ (Table 2). Total tract FA digestibility was highest for ewes fed FS, intermediate for ewes fed SF and lowest for ewes fed CS or CT ($P < 0.05$). This is likely due to higher levels of FA intake for ewes fed the oilseed diets relative to those fed CT. The increased FA digestibility as a result of oilseed feeding is in agreement with other studies with sheep (Enjalbert et al. 1994) and dairy cows (Gonthier et al. 2004a). The lower FA digestibility for CS relative to SF and FS is likely due to the fact that significant level of fat in canola seed is attached to the seed hull (McKinnon et al. 1996).

Total tract digestibility of $C_{16:0}$ and $C_{18:2}$ was higher ($P < 0.05$) for ewes fed SF and FS than those fed CS or CT. In agreement with our findings, Gonthier et al. (2004a) found that feeding flaxseed to dairy cows increased total tract digestibility of $C_{16:0}$, $C_{18:2}$ and $C_{18:3}$. In other studies, feeding supplemental fat to ruminants increased (Romo et al. 2000), decreased (Hussein et al. 1996) or had no effect (Kalscheur et al. 1997) on total tract digestibility of individual FA. Differences in digestibility of FA can be attributed to several factors including level and source of fat fed, degree of unsaturation of FA, chemical and physical treatment of supplemental fat and level and source of forage (Jenkins 1993).

Total tract digestibility of $C_{18:0}$ was negative for all dietary treatments (Table 2). This is likely due to ruminal biohydrogenation of unsaturated $C_{18}$ FA which causes more total $C_{18:0}$ to be excreted in feces than was consumed. Furthermore, biohydrogenation of unsaturated $C_{18}$ FA may occur in the lower intestinal tract, which will further reduce total tract digestibility of $C_{18:0}$ and increased that of unsaturated FA (Plascencia et al. 2003). In general, total tract digestibility of $C_{18}$ FA tended to increased for more unsaturated $C_{18}$ FA.

## CONCLUSIONS

It can be concluded that oilseed supplementation had no adverse effects on DM intake or nutrient utilization. Total tract digestibility of most $C_{18}$ FA was improved as a result of oilseed supplementation, suggesting higher absorption of long chain FA which would be transferred to the mammary gland, thus improving the FA of ewe's milk.

**Table 1. Ingredient and chemical composition of the experimental diets (DM basis)**

| | Dietary treatments | | | |
|---|---|---|---|---|
| | Control | Canola seed | Sunflower seed | Flaxseed |
| Ingredients (g $kg^{-1}$) | | | | |
| Grass silage | 500 | 500 | 500 | 500 |
| Ground corn | 440 | 407 | 413 | 400 |
| Soybean meal | 40 | 0 | 0 | 0 |
| Oilseed | 0 | 73 | 66 | 80 |
| Premix[a] | 20 | 20 | 21 | 20 |
| Chemical Composition (g $kg^{-1}$) | | | | |
| Ash | 56 | 54 | 53 | 53 |
| CP | 156 | 147 | 143 | 153 |
| Total fatty acids | 40 | 68 | 72 | 75 |
| NDF | 362 | 360 | 364 | 360 |
| ADF | 261 | 259 | 272 | 256 |
| | | | | |
| Fatty acid (g $kg^{-1}$ of total fatty acids) | | | | |
| $C_{16:0}$ | 127 | 77 | 85 | 75 |
| $C_{16:1}$ | 88 | 45 | 43 | 43 |
| $C_{18:0}$ | 23 | 20 | 38 | 35 |
| $C_{18:1}$ | 228 | 460 | 215 | 213 |
| $C_{18:2}$ | 477 | 310 | 576 | 276 |
| $C_{18:3}$ | 28 | 70 | 19 | 352 |
| Others | 30 | 19 | 24 | 7 |

[a]Premix contained 120 g $kg^{-1}$ NaCl, 6 mg $kg^{-1}$ Co, 500 mg $kg^{-1}$ Cu, 1200 mg $kg^{-1}$ Fe, 22 mg $kg^{-1}$ I, 2200 mg $kg^{-1}$ Mn, 2200 mg $kg^{-1}$ Zn, 120000 IU $kg^{-1}$ vitamin A, 16000 IU $kg^{-1}$ vitamin D, 1300 IU $kg^{-1}$ vitamin E.

**Table 2. Dry matter intake and total tract nutrient utilization of ewes fed oilseed supplemented diets**

| | Dietary treatment | | | | SEM |
|---|---|---|---|---|---|
| | Control | Canola seed | Sunflower seed | Flaxseed | |
| ***Nutrient intake*** | | | | | |
| Dry matter (g $d^{-1}$) | 2690 | 2560 | 2540 | 2500 | 110.6 |
| Neutral detergent fiber (g $d^{-1}$) | 980 | 900 | 910 | 880 | 30.3 |
| Acid detergent fiber (g $d^{-1}$) | 703 | 664 | 690 | 641 | 40.9 |
| Fatty acids (g $d^{-1}$) | 108b | 173a | 185a | 192a | 5.2 |
| Crude protein (g $d^{-1}$) | 420 | 377 | 364 | 382 | 23.7 |
| Gross energy ( MJ $d^{-1}$) | 41.5 | 41.4 | 41.0 | 41.0 | 0.28 |
| ***Nutrient digestibility (%)*** | | | | | |
| Dry matter | 59.5b | 58.6b | 61.2ab | 63.3a | 0.86 |
| Neural detergent fiber | 56.3 | 55.6 | 57.8 | 58.9 | 1.20 |
| Acid detergent fiber | 55.4 | 50.6 | 56.9 | 55.7 | 1.98 |
| Fatty acids | 70.8c | 70.9c | 78.9b | 84.2a | 0.33 |
| Crude protein | 61.4 | 60.1 | 62.8 | 63.9 | 0.93 |
| Gross energy | 61.8b | 60.2b | 63.0ab | 63.8a | 0.64 |
| ***Fatty acid intake (g $d^{-1}$)*** | | | | | |
| $C_{16:0}$ | 16.1c | 18.8b | 20.6a | 18.9b | 0.28 |
| $C_{16:1}$ | 13.0 | 13.9 | 13.9 | 13.4 | 0.58 |
| $C_{18:0}$ | 2.7c | 4.0b | 6.5a | 6.3a | 0.15 |
| $C_{18:1}$ | 15.1c | 61.1a | 32.2b | 33.9b | 1.66 |
| $C_{18:2}$ | 33.8c | 48.1b | 86.5a | 48.2b | 2.04 |
| $C_{18:3}$ | 23.9b | 30.2b | 23.8b | 69.9a | 1.96 |
| Others | 18.3a | 18.4a | 19.1a | 14.8b | 0.71 |
| ***Fatty acid digestibility (%)*** | | | | | |
| $C_{16:0}$ | 77.5b | 77.9b | 83.6a | 87.8a | 1.46 |
| $C_{16:1}$ | 64.8 | 72.9b | 80.6a | 85.1 | 1.64 |
| $C_{18:0}$ | -231.5b | -461.8c | -243.2b | -161.3a | 14.01 |
| $C_{18:1}$ | 79.3b | 87.7a | 90.4a | 92.7a | 2.14 |
| $C_{18:2}$ | 94.7b | 94.7b | 98.0a | 98.3a | 0.65 |
| $C_{18;3}$ | 94.4b | 96.6ab | 97.3ab | 99.2a | 1.03 |
| Others | 41.2b | 33.2c | 52.84a | 53.98a | 2.20 |

a-c Means in the same row followed by different letters differ ($P < 0.05$)

## REFERENCES


AOAC 1990. Official Methods of Analysis, 15th ed. Association of Official Analytical Chemists. AOAC, Arlington, VA, USA

Bayourthe C., Vernay, M. and Moncoulon, R. 1994. Effect of calcium salts of fatty acids on rumen function and digestibility of rations by sheep. J. Sci. Food Agric. 64: 341-347.

Doreau, M. and Chilliard, Y. 1997. Effects of ruminal or postruminal fish oil supplementation on intake and digestion in dairy cows. Rep. Nutr. Dev. 37: 113-124.

Enjalbert, F., Moncoulon, R., Vernay, M. and Griess, D. 2004. Effects of different forms of polyunsaturated fatty acids on rumen fermentation and total nutrient digestibility of sheep and prairie hay based diets. Anim. Feed Sci. Technol. 14: 127-135.

Fenton, T. W. and Fenton, M. 1979. An improved procedure for the determination of chromic oxide in feed and feces. Can. J. Anim. Sci. 59: 631-634.

Gonthier, C., Mustafa, A. F. Berthiaume, R., Petit, H. V. and Ouellet, R. D., 2004a. Feeding micronized and extruded flaxseed to dairy cows: effects on digestion and ruminal biohydrogenation of long-chain fatty acids. Can. J. Anim. Sci. 84: 705-711.

Gonthier, C., Mustafa, A. F., Berthiaume, R., Petit, H. V., Marineau, R. and Ouellet, D.R. 2004b. Effects of feeding micronized and extruded flaxseed on ruminal fermentation and nutrient utilization by dairy cows. J. Dairy Sci. 87: 1854-1863

Hussein, H. S., Merchen, N. R. and Fahey Jr, G. C. 1996. Effects of chemical treatment of whole canola sed on digestion of long-chain fatty acids by steer fed high or low forage diets. J. Dairy Sci. 79: 87-97.

Jenkins, T. C.1993. Lipid metabolism in the rumen. J. Dairy Sci. 76: 3851-3863.

Kalscheur, K. F., Teter, B. B., Piperova, L. S. and Erdman, R. A. 1997. Effect of fat source on duodenal flow of trans-C18:1 fatty acids and milk fat production in dairy cows. J. Dairy Sci. 80: 2115-2126.

Kennely, J. J. 1996. The fatty acid composition of milk fat as influenced by feeding oilseeds. Anim. Feed Sci. Technol. 60: 137-152.

McKinnon, J. J., Mustafa, A. F. and Cohen, R. D. H. 1995. Nutritional evaluation and processing of canola hulls for ruminant. Can. J. Anim. 75: 231-237.

Mir Z., Goonewardene, L. A., Okine, E., Jaegar, S. and Scheer, H. D. 1999. Effect of feeding canola oil on constituents, conjugated linoleic acid (CLA) and long chain fatty acids in goats milk. Small Rum. Res. 33: 137-143.

Plascencia, A., Mendoza, G. D., Vasquez, C. and Zinn, R. A. 2003. Relationship between body weight and level of fat supplementation on fatty acid digestion in feedlot cattle. J. Anim. Sci. 81: 2653-2659

Romo, G. A., Erdman, R. A., Teter, B. B., Sampugna, J. and Casper, D. P. 2000. Milk composition and apparent digestibilities of dietary fatty acids in lactating dairy cows abomasally infused with cis or trans fatty acids. J. Dairy Sci. 83: 2609-2619.

SAS User's Guide: Statistics. 1989. Version 6. SAS Institute, Inc, Cary, NC, USA.

Sukhija, P. S. and Palmquist, D.L. 1988. Rapid method for determination of total fatty acid content and composition of feedstuffs and feces. J. Agric. Food Chem. 36: 1202-1206.

Wachira, A. M., Sinclair, L. A., Wilkinson, R. G., Hallett, K., Enser, M. and Wood, J. D. 2000. Rumen biohydrogenation of n-3 polyunsaturated fatty acids and their effects on microbial efficiency and nutrient digestibility in sheep. J. Agric. Sci. 135: 419-428.

Zhang, R. H., Mustafa, A. F. and Zhao, X. 2006. Effects of flaxseed supplementation to lactating ewes on milk composition, cheese yield and fatty acid composition of milk and cheese. Small Rumin. Res. 63: 333-341.

# Performance of lactating ewes fed oilseeds: Effects on milk and cheese yield and composition

R. H. Zhang, A. F. Mustafa and X. Zhao

**ABSTRACT**

Sixteen lactating ewes were used to determine the effects of oilseed supplementation on milk yield and composition, cheese yield and composition and fatty acid profile of milk and cheese. Four iso-nitrogenous diets were formulated: a control diet (CT) with no oilseed supplementation, a flaxseed supplemented diet (FS), a sunflower seed supplemented diet (SF), and a canola seed supplemented diet (CS). Feeding oilseeds had no effect on dry matter intake. Milk yield was similar for ewes fed CT, FS and SF and was higher ($P < 0.05$) than that of ewes fed CS. Ewes fed oilseeds supplemented diets produced milk with higher ($P < 0.05$) protein and total solid percentages than those fed CT. However, milk fat percentage was only higher ($P < 0.05$) in the milk of ewes fed FS and SF relative to those fed CT. Actual cheese yield was higher ($P < 0.05$) from milk of ewes fed oilseed-supplemented diets relative to those fed CT. However, cheese composition was not affected by dietary treatments.

**Key words:** Cheese, fatty acids, milk, oilseeds, ewes

## INTRODUCTION

Ewe's milk is mainly used for cheese production due to its high total solid content. However, the high fat content might limit the demand for dairy products made from ewe's milk. In a previous study, Zhang et al. (2006) showed that feeding flaxseed to lactating ewes increased milk fat and total solid percentage with no effect on milk protein and lactose percentages or on cheese yield and composition. Studies on the effects of oilseed supplementation on performance of dairy ewes are limited. It has been suggested that the response of milk yield and composition to fat supplementation vary considerably between lactating ruminants (Kitessa et al. 2003; Chilliard et al. 2003). Therefore the objectives of this study were to determine the effects of feeding sunflower seed, canola seed and flaxseed to lactating ewes on feed intake, milk composition, and cheese yield and composition.

## MATERIALS AND METHODS

### Animals and Feeding

Four concentrates were formulated that contain no oilseed (CT), canola seed (CS), sunflower seed (SF), or flaxseed (FS). The concentrates were mixed with grass silage (50:50 DM basis) and fed as total mixed diets (Table 1). Diets were formulated to be isonitrogenous and the oilseed diets were intended to contain 70 g $kg^{-1}$ fatty acids (DM basis).

Sixteen lactating pure Dorset ewes, averaging 39 ± 6.2 days in milk and 81 ± 10.1 kg body weight were used. Animals were balanced for parity and days in milk and were randomly assigned to one of the four dietary treatments (4 animals per treatment). Ewes were individually housed in pens with free access to water and were fed twice daily for ad libitum intake. Experimental period consisted of 14 d of diet adaptation and 7 d of data collection.

Milk yield was measured on d 20 and d 21 of the experimental period using the oxytocin method (Ortega-Jimenez et al. 2005). Pooled milk samples were used to make cheese using a laboratory cheese making procedure (Zhang et al 2006).

Diets were sampled daily during the collection period and were composited by treatment. The composited samples were oven dried at 55 °C for 48 h, ground

through a 1-mm screen using a Wiley mill (Arthur H. Thomas, Philadelphia, PA, USA), and stored at room temperature for later analysis. Orts were collected daily to determine daily intake of each ewe.

**Chemical Analysis**

Dried feed samples were analyzed for DM and ash according to the AOAC (1990), crude protein by a Nitrogen Determinator System (Leco Corporation, MI), neutral and acid detergent fibre using Ankom Fibre Analyzer. Milk samples were analyzed for fat, protein, and lactose by near-infrared analysis using a Milk-o-Scan (Foss Food Technology, Denmark) calibrated for sheep milk analysis (Programme d'Analyse des Troupeaux Laitiers du Québec, QC, Canada). Total solids, casein and non-protein nitrogen contents were analyzed according to the procedures of AOAC (1990), except that N was determined using a Nitrogen Determinator System.

**Statistical Analysis**

Statistical analyses were performed using SAS/STAT (1989) General Linear Model procedure for a completely random design with the following model:

$$Y = \mu + T_i + e_{ij}$$

Where: $\mu$ is the overall mean; $T_i$ is the fixed effect of dietary treatment ($i = 1 \sim 4$); and $e_{ij}$ is the residual effect. Differences were considered significant if $P < 0.05$.

## RESULTS AND DISCUSSION

**Feed intake, milk yield and composition**

Dry matter intake was not influenced by oilseed supplementation and averaged 2.6 kg $d^{-1}$ (Table 2). As expected, ewes fed the oilseed supplemented diets consumed more ($P < 0.05$) fatty acids than those fed CT. Several researchers found no adverse effects of supplemental fat on dry matter intake of ewes (Bayourthe et al. 1994; Goulas et al. 2003). Feeding supplemental fat in the form of oilseeds is expected to have less detrimental effect on DM intake than if a similar amount was fed as free oil (Kennelly 1996).

Milk yield was similar for ewes fed CT, SF and FS but higher ($P < 0.05$) than that of ewes fed CS (Table 2). Data on effects of fat supplementation on milk yield are inconsistent. Results from previous studies showed that fat supplementation increased (Casals et al. 1999) or had no effect (Kitessa et al. 2003) on milk yield of dairy ewes. The response of dairy ruminants to supplemental fat may be affected by factors such as source of fat, level of supplementation, and stage of lactation (NRC 2001).

Ewes fed SF produced milk with a higher ($P < 0.05$) fat percentage than those fed the other dietary treatments. Milk fat percentage was also higher ($P < 0.05$) for ewes fed FS relative to those fed CT. No difference in milk fat percentage was observed between ewes fed FS and CS or between ewes fed CS and CT. These results are consistent with our previous findings where feeding flaxseed to dairy ewes increased milk fat percentage (Zhang et al. 2006). Others also reported an increase in milk fat percentage of ewes (Casal et al. 1999) and goats (Bernard et al. 2005) as a result of fat supplementation. A review by Chilliard et al. (2003) indicated that fat supplementation increases milk fat percentage in dairy goats and ewes but not always in dairy cows in which it could often either decrease or not change.

Milk protein percentage was similar in milk of ewes fed oilseed supplemented diets and was higher ($P < 0.05$) than in milk of ewes fed CT. The positive effect of fat supplementation on milk protein percentage in our study is different from others where fat supplementation to ewes had negative (Rotunno et al. 1998; Casala et al. 1999) or no (Zhang et al. 2006) effect on milk protein percentage. However, our results agree with those of Bernard et al. (2005) who reported an increase in milk protein percentage of lactating goats as a result of flaxseed supplementation. Reasons for inconsistence response of milk protein percentage to fat supplementation are not fully understood. It is possible that the effects of fat supplementation on mammary metabolism of lipogenic, and aminogenic nutrients varies between species and therefore result in differences in milk fat and protein secretion Chilliard et al. (2003).

As a result of higher fat and protein percentages, milk from ewes fed SF and FS contained more ($P < 0.05$) total solids than that from ewes fed CT (Table 2). Feeding CS had no effect on milk

total solid percentage. Milk protein fractions were not affected by oilseed supplementation which is in agreement with our previous findings (Zhang et al. 2006).

**Cheese Yield and Composition**

Cheese yield and composition are presented in Table 3. Actual cheese yield was higher ($P < 0.05$) for milk from ewes fed oilseed supplemented diets than for milk from ewes fed CT. However, adjusted cheese yield (37% moisture) was higher ($P < 0.05$) for milk from ewes fed SF than for milk from ewes fed other diets. Differences in cheese yield between dietary treatments are likely due to differences in milk total solids. Cheese yield was significantly higher from milk of ewes fed 180 g $kg^{-1}$ flaxseed concentrate than from milk of ewes fed a control concentrate (Zhang et al. 2006). Fat supplementation has also been found to increase cheese yield form goat's milk as a result of a higher milk protein percentage (Chilliard et al. 2003). Cheese fat, protein and ash percentages were not affected by oilseed supplementation which is in agreement with our previous findings (Zhang et al. 2006). Our results are also consistent with Dhiman et al (1995) where no differences in cheese composition were found between oilseed diets and a control diet.

## CONCLUSIONS

Oilseeds can be included in lactating ewes' diets to increase milk yield without affecting feed intake. Oilseed supplementation also increased milk protein and total solid percentages and actual cheese yield.

**Table 1.** Ingredient and chemical composition of the experimental diets (DM basis)

| | Dietary treatments | | | |
|---|---|---|---|---|
| | Control | Canola Seed | Sunflower seed | Flaxseed |
| Ingredients (g $kg^{-1}$) | | | | |
| Grass silage | 500 | 500 | 500 | 500 |
| Ground corn | 440 | 407 | 413 | 400 |
| Soybean meal | 40 | 0 | 0 | 0 |
| Oilseed | 0 | 73 | 66 | 80 |
| Premix[a] | 20 | 20 | 21 | 20 |
| Chemical Composition (g $kg^{-1}$) | | | | |
| Ash | 56 | 54 | 53 | 53 |
| CP | 156 | 147 | 143 | 153 |
| Total fatty acids | 40 | 68 | 72 | 75 |
| NDF | 362 | 360 | 364 | 360 |
| ADF | 261 | 259 | 272 | 256 |
| | | | | |
| Fatty acid (g $kg^{-1}$ of total fatty acids) | | | | |
| $C_{16:0}$ | 127 | 77 | 85 | 75 |
| $C_{16:1}$ | 88 | 45 | 43 | 43 |
| $C_{18:0}$ | 23 | 20 | 38 | 35 |
| $C_{18:1}$ | 228 | 460 | 215 | 213 |
| $C_{18:2}$ | 477 | 310 | 576 | 276 |
| $C_{18:3}$ | 28 | 70 | 19 | 352 |
| Others | 30 | 19 | 24 | 7 |

[a]Premix contained 120 g $kg^{-1}$ NaCl, 6 mg $kg^{-1}$ Co, 500 mg $kg^{-1}$ Cu, 1200 mg $kg^{-1}$ Fe, 22 mg $kg^{-1}$ I, 2200 mg $kg^{-1}$ Mn, 2200 mg $kg^{-1}$ Zn, 120000 IU $kg^{-1}$ vitamin A, 16000 IU $kg^{-1}$ vitamin D, 1300 IU $kg^{-1}$ vitamin E.

**Table 2.** Effects of oilseed supplementation on intake, milk yield and composition.

| | Dietary treatment | | | | SEM |
|---|---|---|---|---|---|
| | Control | Canola seed | Sunflower seed | Flaxseed | |
| Intake (g $d^{-1}$) | | | | | |
| Dry matter | 2690 | 2560 | 2540 | 2500 | 110.6 |
| Fat | 108b | 174a | 183a | 188a | 5.2 |
| Milk composition (%) | | | | | |
| Fat | 7.6c | 7.8bc | 8.6a | 8.0b | 0.11 |
| Protein | 4.2b | 4.6a | 4.6a | 4.4a | 0.06 |
| Casein | 78.0 | 77.9 | 77.8 | 77.9 | 1.15 |
| True protein | 94.6 | 94.4 | 94.2 | 94.4 | 0.67 |
| NPN | 5.4 | 5.6 | 5.8 | 5.60 | 0.67 |
| Lactose | 5.1a | 4.8b | 5.0ab | 5.0ab | 0.07 |
| Total solids | 17.7b | 17.9b | 19.4a | 18.8a | 0.21 |
| Yield (g $d^{-1}$) | | | | | |
| Milk | 2600a | 2400b | 2700a | 2700a | 141.2 |
| Fat | 198.4b | 187.5b | 231.3a | 216.7a | 5.82 |
| Protein | 109.7b | 110.4b | 124.6a | 119.6a | 2.34 |
| Lactose | 132.4a | 114.1b | 134.3a | 134.6a | 3.49 |
| Total solids | 462.5b | 426.7b | 523.4a | 505.8a | 12.10 |

a-cMeans in the same row with different superscript differ ($P < 0.05$).
SEM = Pooled standard error of the mean.

**Table 3.** Effects of oilseed supplementation on cheese yield and cheese composition

| | Dietary treatment | | | | SEM |
|---|---|---|---|---|---|
| | Control | Canola Seed | Sunflower seed | Flaxseed | |
| Cheese yield (%) | | | | | |
| Actual | 15.8c | 18.3b | 19.4a | 17.6b | 0.37 |
| Adjusted yield | 16.0b | 16.5b | 18.9a | 16.9b | 0.42 |
| Cheese composition (%) | | | | | |
| Dry matter | 63.9a | 56.9b | 61.4ab | 60.9ab | 1.42 |
| Fat | 36.4 | 34.45 | 38.3 | 36.0 | 1.16 |
| Protein | 24.1 | 21.3 | 20.5 | 21.5 | 1.08 |
| Ash | 3.39 | 3.02 | 3.19 | 3.29 | 0.14 |
| Whey composition (%) | | | | | |
| Dry matter | 8.0a | 7.3ab | 6.8b | 7.7a | 0.21 |
| Fat | 1.2a | 0.5b | 0.4b | 1.2a | 0.10 |
| Protein | 1.1b | 1.4a | 1.2b | 1.1b | 0.05 |
| Lactose | 4.7 | 4.3 | 4.4 | 4.5 | 0.12 |

*a-c*Means in the same row with different superscript differ ($P < 0.05$).
SEM = Pooled standard error of the mean.

## REFERENCES


Association of Official Analytical Chemists 1999. Official Methods of Analysis. 16th ed. AOAC, Gaithersburg, MD, USA.

Bayourthe, C., Vernay, M. and Moncoulon, R. 1994. Effect of calcium salts of fatty acids on rumen function and digestibility of rations by sheep. J. Sci. Food Agric. 64: 341-347.

Bernard, L, Rouel, J. C., Leroux, A., Ferlay, Y., Faulconnier, Y., Legrand, P. and Chilliard, Y. 2005. Mammary lipid metabolism and milk fatty acid secretion in Alpine goats fed vegetable lipids. J. Dairy Sci. 88: 1478-1489.

Chilliard, Y., Ferlay, A., Rouel, J. and Lamberet, G. 2003. A review of nutritional and physiological factors affecting goat milk lipid synthesis and lipolysis. J. Dairy Sci. 86: 1751-1770.

Goulas, C., Zervas, G. and Papadopoulos, G. 2003. Effect of dietary animal fat and methionine on dairy ewes milk yield and milk composition. Anim. Feed Sci. Technol. 105: 43-54.

Kennelly J. J. 1996. The fatty acid composition of milk fat as influenced by feeding oilseeds. Anim. Feed Sci. Technol. 60: 137-152.

Kitessa, S. M., Peake, D., Bencini, R. and Williams, A. J. 2003. Fish oil metabolism in ruminants. III. Transfer of n-3 polyunsaturated fatty acids (PUFA) from tuna oil into sheep's milk. Anim. Feed Sci. Technol. 108: 1-14.

Mir, Z., Goonewardene, L. A., Okine, E., Jaegar, S. and Scheer, H. D. 1999. Effect of feeding canola oil on constituents, conjugated linoleic acid (CLA) and long chain fatty acids in goat milk. Small Rumin. Res. 33: 137-143.

NRC 2001. Nutrient Requirements of Dairy Cattle, 7th rev ed. National Academy of Sciences, Washington, DC, USA.

Ortega-Jimenez, E., Alexandre, G., Boval, M., Archimede, H., Mahieu, M. and Morand-Fehr, P. 2005. Intake, and milk production of suckling Creole goats reared at pasture in humid tropics according to the post-grazing residue management. Small Rumin. Res. 59: 217-227.

Rotunno, T., Sevi, A., Caterina, R. D. and Muscio, A. 1998. Effects of graded levels of dietary rumen-protected fat on milk characteristics of Comisana ewes. Small Rumin. Res. 30: 137-145.

SAS User's Guide: Statistics. 1989. Version 6. SAS Institute, Inc, Cary, NC, USA.

Zhang, R, Mustafa, A. F. and Zhao, X 2006. Effects of flaxseed supplementation to lactating ewes on milk composition, cheese yield, and fatty acid composition of milk and cheese. Small Rumin. Res. 63: 331-241.

# Performance of lactating ewes fed oilseeds: Effects on milk and cheese fatty acid composition

R.H. Zhang, A.F. Mustafa and X. Zhao

**ABSTRACT**

Sixteen lactating Dorset ewes were used in a completely randomized design to determine the effects of oilseed supplementation on fatty acid profile of milk and cheese. Four isonitrogenous diets were formulated: a control diet (CT) with no oilseed supplementation, a flaxseed supplemented diet (FS), a sunflower seed supplemented diet (SF), and a canola seed supplemented diet (CS). Oilseed supplementation increased ($P < 0.05$) concentrations of unsaturated fatty acids in milk fat. The order of increase was SF > FS > CS. Feeding SF, FS and CS increased ($P < 0.05$) conjugated linoleic acid concentration in milk by 83.5, 39.2 and 16.5%, respectively. Concentration of linolenic acid in milk was only increased ($P < 0.05$) by feeding SF. Changes in milk fatty acid profiles were reflected in the cheese.

**Keywords**: Cheese, fatty acids, milk, ewes

## INTRODUCTION

Oilseeds can also be used to manipulate milk fatty acid composition of dairy animals. In general, oilseed supplementation reduced concentrations of short (SCFA)- and medium (MCFA)-chain fatty acids and increased those of long chain polyunsaturated (PUFA) ones (Kennelly 1996). Concentrations of specific fatty acids with known health benefits to humans such as $C_{18:3}$ and conjugated linoleic acid (CLA) can also be increased by feeding specific oilseeds. Feeding vegetable oils rich in $C_{18:2}$ and $C_{18:3}$ significantly increased concentrations of $C_{18}$ fatty acids in goat's milk (Mir et al. 1999; Bernard et al. 2005). Studies on the effects of the effects of oilseed supplementation on performance of dairy ewes are limited.

Ewe's milk is mainly used for cheese production due to its high total solid content. However, the high fat content might limit the demand for dairy products made from ewe's milk. In a previous study, Zhang et al. (2006) showed that feeding flaxseed to lactating ewes increased concentrations of PUFA, CLA and $C_{18:3}$ in milk and cheese. Data on effects of feeding other oilseeds to lactating ewes on milk fatty acid composition are not available. Therefore the objectives of this study were to determine the effects of feeding sunflower seed, canola seed and flaxseed to lactating ewes on fatty acid composition of milk and cheese.

## MATERIALS AND METHODS

### Animals and Feeding

Four concentrates were formulated that contain no oilseed (CT), canola seed (CS), sunflower seed (SF), or flaxseed (FS). The concentrates were mixed with grass silage (50:50 DM basis) and fed as total mixed diets (see page 61 of this report). Diets were formulated to be isonitrogenous and the oilseed diets were intended to contain 7% fatty acids (DM basis). Sixteen lactating ewes were used in this study. Details regarding animals used and experimental procedures are described elsewhere (page 61).

### Chemical Analysis

Fatty acids in feed samples were extracted and methylated according to the one-step procedure (Sukhija and Palmquist 1988). Fat from milk samples was separated by centrifuging 10 mL of fresh milk at 2000 x g for 15 min, while fat from cheese was extracted with a mixture of methanol and methylene chloride as described

by Garcia-Lopez et al. (1994). Methylation of milk and cheese fats was conducted by a base-catalyzed procedure as described by Ward et al (2002). Methyl esters of fatty acids were separated and quantified by gas chromatography as described by Zhang et al. (2006).

**Statistical Analysis**

Statistical analyses were performed using SAS/STAT (1989) General Linear Model procedure for a completely random design with the following model:

$$Y = \mu + T_i + e_{ij}$$

Where: $\mu$ is the overall mean; $T_i$ is the fixed effect of dietary treatment ($i = 1 \sim 4$); and $e_{ij}$ is the residual effect. Differences were considered significant if $P < 0.05$.

## RESULTS AND DISCUSSION

**Fatty Acid Composition of Milk**

Supplementary oilseeds decreased ($P < 0.05$) milk concentrations of SCFA and MCFA and increased ($P < 0.05$) those of long-chain fatty acids (Table 1). Ewes fed oilseed supplemented diets produced milk with lower ($P < 0.05$) saturated and higher ($P < 0.05$) PUFA and mono-unsaturated fatty acids concentrations than ewes fed CT. Concentrations of SCFA and saturated fatty acids in milk were lower ($P < 0.05$) for ewes fed SF than for those fed CS or FS. However, the increase in concentration of unsaturated fatty acids in milk as a result of oilseed supplementation was highest for ewes fed SF, intermediated for those fed FS and lowest for those fed CS ($P < 0.05$). The higher concentrations of $C_{18:0}$ in milk of ewes fed the oilseed diets relative to those fed CT is likely due to the biohydrogenation of unsaturated $C_{18}$ fatty acids in the rumen while the higher concentrations of $C_{18:1}$ is likely a combination of ruminal biohydrogenation and desaturation of $C_{18:0}$ by $\Delta^9$-desaturase in the mammary gland.

Oilseed supplementation increased ($P < 0.05$) concentration CLA in milk (Table 1). The increase in milk CLA concentration was 38, 122 and 65% for ewes fed CS, SF and FS, respectively. The higher milk CLA concentration observed for ewes fed SF is likely due to the higher concentration of $C_{18:2}$ in sunflower seed relative to canola seed and flaxseed. Dietary $C_{18:2}$ is a major precursor for CLA synthesis in the rumen (Kelly et al. 1998). Trans-11 $C_{18:1}$ or vaccenic acid is formed during ruminal biohydrogenation of $C_{18:3}$ and is desaturated in the mammary epithelial cell by the $\Delta^9$-desaturase enzyme to produce CLA. Voigt and Hagemeister (2001) suggested that 33% of trans-11 $C_{18:1}$ taken up by the mammary epithelial cell is desaturated to cis-9, trans-11 $C_{18:2}$, the predominant isomer of CLA in milk. The $\Delta^9$-desaturase enzyme is not specific to vaccenic acid. Chilliard et al (2000) suggested that 40% of $C_{18:0}$ is converted to $C_{18:1}$ by $\Delta^9$-desaturase enzyme, contributing to 50% of total $C_{18:1}$ in milk.

Milk concentration of $C_{18:3}$ was only increased ($P < 0.05$) by feeding FS (Table 5). Feeding flaxseed and flaxseed oil has been shown increase milk $C_{18:3}$ concentration in ewes (Zhang et al. 2006) and goats (Bernard et al. 2005). It is well known that dairy ruminants fed oils rich in $C_{18:3}$ produce more $C_{18:3}$ in their milk than those fed oils rich in $C_{18:2}$ (Bernard et al. 2005).

Differences in daily yield of milk fatty acids (Table 2) between dietary treatments were similar to those reported for milk fatty acid profiles (Table 1). Compared with CT, diets supplemented with oilseeds decreased ($P < 0.05$) daily milk yields of SCFA and MCFA and increased ($P < 0.05$) those of LCFA (Table 2). Feeding FS resulted in the highest daily milk yield of $C_{18:3}$, followed by SF. There was no difference in daily milk yield between ewes fed CS and those fed CT. Daily milk yield of $C_{18:2}$ fatty acid and CLA was substantially increased ($P < 0.05$) by feeding SF, followed by feeding FS. However, feeding CS had no effect on daily milk yield of $C_{18:2}$ and CLA. Our results suggest that despite differences in milk composition responses, changes in milk fatty acid composition in lactating ewes as a result of oilseed supplementation are similar to those in cows.

Apparent transfer efficiency of unsaturated fatty acids was estimated by dividing the amount of individual unsaturated fatty acids in milk fat by the amount of individual unsaturated fatty acid consumed (Table 3). Oilseed supplementation reduced ($P < 0.05$) apparent transfer efficiency of $C_{18:1}$ with more reduction noted for ewes fed CS than for those fed SF or FS. Transfer efficiency of $C_{18:2}$ and $C_{18:3}$ was low with no consistent effect of oilseed supplementation. The low transfer efficiency of $C_{18:2}$ and $C_{18:3}$

suggest extensive ruminal biohydrogenation of these fatty acids.

**Fatty Acid Composition of Cheese**

Fatty acid profiles of cheeses were in general similar to those of the milks, suggesting that processing of milk into cheese did not alter fatty acid composition (Table 4). The higher concentrations of CLA and $C_{18:3}$ in chesses made from milk of the oilseed treatments suggest that cheese with high levels of CLA and $C_{18:3}$ can be produced from milk that contains high concentrations of these fatty acids. Similar findings have also been reported for cheese made from ewe's (Zhang et al. 2006) and cow's (Dhiman et al. 1999) milk.

## CONCLUSIONS

Dietary supplementation of oilseeds is an effective way to increase ovine milk concentrations of long chain and polyunsaturated fatty acids. Concentrations of health-promoting fatty acids such as $C_{18:3}$ and CLA in ewes' milk and cheese can be increased by selective feeding of oilseeds. The highest concentration of $C_{18:3}$ and CLA can be achieved by feeding flaxseed and sunflower seed, respectively. Results indicate that oilseed supplementation can be used as a nutritional strategy to increase concentrations of human health-promoting fatty acids in ewes' milk and cheese.

**Table 1.** Effect of oilseed supplementation on milk fatty acid composition of lactating ewes (g $kg^{-1}$ of total fatty acids)

| | Dietary Treatments | | | | SEM |
|---|---|---|---|---|---|
| | Control | Canola seed | Sunflower seed | Flaxseed | |
| $C_{6:0}$ | 33 | 32 | 33 | 33 | 2.1 |
| $C_{8:0}$ | 29a | 23b | 23b | 23b | 0.6 |
| $C_{10:0}$ | 87a | 72b | 66c | 67c | 1.4 |
| $C_{12:0}$ | 41a | 34b | 29c | 3.2b | 1.6 |
| $C_{12:1}$ | 4a | 2b | 2ab | 3ab | 0.3 |
| $C_{14:0}$ | 97a | 83b | 72c | 74c | 1.8 |
| $C_{14:1}$ | 8a | 4b | 3b | 4b | 0.4 |
| $C_{15:0}$ | 10a | 9ab | 7c | 8b | 0.5 |
| $C_{16:0}$ | 232a | 186b | 175c | 185b | 4.1 |
| $C_{16:1}$ | 22a | 19b | 19b | 19b | 0.6 |
| $C_{18:0}$ | 132b | 192a | 200a | 194a | 06.1 |
| $C_{18:1}$ | 227b | 269a | 263a | 265a | 07.2 |
| $C_{18:2}$ | 18b | 18b | 36a | 19b | 01.5 |
| $C_{18:3}$ | 7b | 8b | 8b | 11a | 0.6 |
| CLA[z] | 11d | 16c | 24a | 19b | 0.7 |
| SCFA[y] | 193a | 162b | 152c | 157bc | 01.7 |
| MCFA[x] | 368a | 300b | 276c | 290b | 06.1 |
| LCFA[w] | 394c | 494b | 530a | 509b | 09.5 |
| Saturated[g] | 670a | 639b | 613c | 626bc | 4.5 |
| Unsaturated[h] | 285d | 317c | 345a | 310b | 3.0 |

a-d Means in the same row with different superscript differ ($P < 0.05$)

[z]Sum of cis-9, trans-11 and trans-10, cis-12 conjugated linoleic acid.

[y]Short-chain fatty acid: $C_{4:0}$ to $C_{12:1}$.

[x]Medium-chain fatty acid: $C_{14:0}$ to $C_{16:1}$.

[w]Long-chain fatty acid: sum of $C_{18}$ fatty acids.

[g]Sum of saturated fatty acids.

[h]Sum of unsaturated fatty acids.

**Table 2.** Effects of oilseed supplementation on milk fatty acid yield of lactating ewes (g $d^{-1}$)

| Fatty acids | Dietary treatment | | | | SEM |
|---|---|---|---|---|---|
| | Control | Canola seed | Sunflower seed | Flaxseed | |
| $C_{6:0}$ | 6.2b | 5.7c | 7.2a | 6.8a | 0.09 |
| $C_{8:0}$ | 5.4a | 4.0c | 5.0b | 4.8b | 0.08 |
| $C_{10:0}$ | 16.3a | 12.8c | 14.3b | 13.8bc | 0.11 |
| $C_{12:0}$ | 7.7a | 6.0c | 6.2b | 6.6b | 0.05 |
| $C_{12:1}$ | 0.7a | 0.3c | 0.5b | 0.6ab | 0.02 |
| $C_{14:0}$ | 18.1a | 14.7c | 15.7b | 15.3b | 0.19 |
| $C_{14:1}$ | 1.4a | 0.6c | 0.7bc | 0.8b | 0.02 |
| $C_{15:0}$ | 1.9a | 1.6bc | 1.6c | 1.7b | 0.02 |
| $C_{16:0}$ | 43.4a | 33.0c | 38.1b | 38.3b | 0.51 |
| $C_{16:1}$ | 4.0a | 3.4c | 4.2a | 3.9b | 0.04 |
| $C_{18:0}$ | 24.6d | 34.0c | 43.6a | 40.0b | 0.35 |
| $C_{18:1}$ | 42.4d | 47.7c | 57.5a | 54.8b | 0.65 |
| $C_{18:2}$ | 3.3c | 3.3c | 7.8a | 3.9b | 0.03 |
| $C_{18:3}$ | 1.3c | 1.4c | 1.7b | 2.3a | 0.04 |
| CLA[c] | 2.1c | 2.9c | 5.7a | 4.0b | 0.03 |
| SCFA[d] | 36.2a | 28.7c | 33.2b | 32.6b | 0.21 |
| MCFA[e] | 68.9a | 53.3c | 60.3b | 59.9b | 0.79 |
| LCFA[f] | 73.9d | 87.8c | 115.6a | 105.1b | 0.52 |
| Saturated[g] | 125.5c | 113.5d | 133.8a | 129.3b | 0.47 |
| Unsaturated[h] | 53.5c | 56.3c | 75.2a | 68.3b | 0.49 |

a-d Means in the same row with different superscript differ ($P < 0.05$)

[z]Sum of cis-9, trans-11 and trans-10, cis-12 conjugated linoleic acid.

[y]Short-chain fatty acid: $C_{4:0}$ to $C_{12:1}$.

[x]Medium-chain fatty acid: $C_{14:0}$ to $C_{16:1}$.

[w]Long-chain fatty acid: sum of $C_{18}$ fatty acids.

[g]Sum of saturated fatty acids.

[h]Sum of unsaturated fatty acids

**Table 3.** Transfer efficiency of fatty acids from feed to milk fat in lactating ewes fed oilseeds

| Fatty acids | Dietary treatment | | | | SEM |
|---|---|---|---|---|---|
| | Control | Canola seed | Sunflower seed | Flaxseed | |
| $C_{18:1}$ | 280.9a | 78.1c | 178.5b | 161.7b | 0.73 |
| $C_{18:2}$ | 16.2a | 11.8c | 14.4b | 15.2a | 0.25 |
| $C_{18:3}$ | 5.6b | 4.5bc | 7.2a | 3.3c | 0.15 |

a-c Means in the same row with different superscript differ ($P < 0.05$).

**Table 4**. Effect of oilseed supplementation on fatty acid composition of cheese (g $kg^{-1}$ of total fatty acids)

| | Dietary Treatments | | | | SEM |
|---|---|---|---|---|---|
| | Control | Canola seed | Sunflower seed | Flaxseed | |
| $C_{6:0}$ | 29a | 26b | 25b | 27b | 0.8 |
| $C_{8:0}$ | 24a | 22b | 21b | 21b | 0.5 |
| $C_{10:0}$ | 86a | 71b | 70b | 70b | 1.2 |
| $C_{12:0}$ | 42a | 34b | 31b | 31b | 1.3 |
| $C_{12:1}$ | 3a | 2b | 2b | 2b | 0.3 |
| $C_{14:0}$ | 97a | 87ab | 76c | 77c | 2.2 |
| $C_{14:1}$ | 5a | 4b | 4b | 4b | 0.4 |
| $C_{15:0}$ | 12a | 9b | 9b | 10b | 0.4 |
| $C_{16:0}$ | 222a | 187b | 175c | 181b | 3.0 |
| $C_{16:1}$ | 22a | 22a | 18b | 19b | 0.6 |
| $C_{18:0}$ | 138c | 184b | 199a | 192a | 3.2 |
| $C_{18:1}$ | 236b | 274a | 270a | 278a | 5.1 |
| $C_{18:2}$ | 18b | 18b | 34a | 19b | 0.5 |
| $C_{18:3}$ (omega-3) | 7b | 7b | 7b | 10a | 0.3 |
| CLA[z] | 10d | 15c | 24a | 18b | 0.3 |
| SCFA[y] | 184a | 154b | 149c | 150bc | 2.3 |
| MCFA[x] | 357a | 308b | 282c | 290c | 2.9 |
| LCFA[w] | 408d | 495c | 530a | 515b | 5.7 |
| Saturated[g] | 650a | 618b | 605c | 608c | 3.2 |
| Unsaturated[h] | 300b | 339a | 356a | 348a | 3.4 |

a-d Means in the same row with different superscript differ ($P < 0.05$)

[z]Sum of cis-9, trans-11 and trans-10, cis-12 conjugated linoleic acid.

[y]Short-chain fatty acid: $C_{4:0}$ to $C_{12:1}$.

[x]Medium-chain fatty acid: $C_{14:0}$ to $C_{16:1}$.

[w]Long-chain fatty acid: sum of $C_{18}$ fatty acids.

[g]Sum of saturated fatty acids.

[h]Sum of unsaturated fatty acids

## REFERENCES


Association of Official Analytical Chemists 1999. Official Methods of Analysis. 16th ed. AOAC, Gaithersburg, MD, USA.

Bernard, L, Rouel, J. C., Leroux, A., Ferlay, Y., Faulconnier, Y., Legrand, P. and Chilliard, Y. 2005. Mammary lipid metabolism and milk fatty acid secretion in Alpine goats fed vegetable lipids. J. Dairy Sci. 88: 1478-1489.

Chilliard, Y, Ferlay, A., Mansbridge, R. M. and Doreau, M. 2000. Ruminant milk fat plasticity: nutritional control of saturated, polyunsaturated, trans and conjugated fatty acids. Ann. Zootech. 49: 181-205.

Dhiman, T. R., Helmink, E. D., Mcmahon, D. J., Fife, R. L. and Pariza, M. W. 1999. Conjugated linoleic acid content of milk and cheese from cows fed extruded oilseeds. J. Dairy Sci. 82: 412-419.

Garcia-Lopez, S, Echeverria, E.,Tsui, I. and Balch, B. 1994. Changes in the content of conjugated linoleic acid (CLA) in processed cheese during processing. Food Res. Int. 27: 61-64.

Kelly, M. L., Berry, J. R., Dwyer, D. A., Griinari, J. M., Chouinard, P. Y., Van Amburgh, M. E. and Bauman, D. E. 1998. Dietary fatty acid sources affect conjugated linoleic acid concentrations in milk from lactating dairy cows. J. Nutr. 128: 881-885.

Kennelly J. J. 1996. The fatty acid composition of milk fat as influenced by feeding oilseeds. Anim. Feed Sci. Technol. 60: 137-152.

Mir, Z., Goonewardene, L. A., Okine, E., Jaegar, S. and Scheer, H. D. 1999. Effect of feeding canola oil on constituents, conjugated linoleic acid (CLA) and long chain fatty acids in goat milk. Small Rumin. Res. 33: 137-143.

SAS Institute, Inc., 1989. SAS user's guide: Statistics. SAS Institute, Inc., Cary, NC.

Sukhija, P. S. and Palmquist, D. L. 1988. Rapid method for determination of total fatty acid content and composition of feedstuffs and feces. J. Agric. Food Chem. 36: 1202-1206.

Voigt, J. and Hagemeister, H. 2001. Dietary influence on a desirable fatty acid composition in milk from dairy cattle. J. Anim. Feed Sci. Technol. 10: 87-103.

Ward, A.T., Wittenberg, K. M. and Przybylski, R. 2002. Bobine milk fatty acid profiles produced by feeding diets containing solin, flax and canola. J. Dairy Sci. 85: 1191-1196.

Zhang, R, Mustafa, A. F. and Zhao, X 2006. Effects of flaxseed supplementation to lactating ewes on milk composition, cheese yield, and fatty acid composition of milk and cheese. Small Rumin. Res. 63:.233-241

# Expression of rumen microbial fibrolytic enzyme genes in intestinal *Lactobacillus reuteri*

J.R. Liu, B. Yu[1], F.H. Liu[2], K.J. Cheng[2], and X. Zhao
[1]Department of Animal Science, National Chung-Hsing University, Taiwan; [2]Institute of BioAgricultural Sciences, Academia Sinica, Taiwan

**ABSTRACT**

This study was aimed at evaluating the cloning and expression of three rumen microbial fibrolytic enzyme genes in a strain of *Lactobacillus reuteri*, and investigating the probiotic characteristics of these genetically modified lactobacilli. The *Neocallimastix patriciarum* xylanase gene *xynCDBFV*, the *Fibrobacter succinogenes* β-glucanase (1,3-1,4-β-D-glucan 4-glucanohydrolase [EC 3.2.1.73]) gene, and the *Piromyces rhizinflata* cellulase gene *eglA* were cloned in a strain of *L. reuteri* isolated from the gastrointestinal tract of broilers. The enzymes were expressed and secreted under the control of *Lactococcus lactis lac* A promoter and its secretion signal. The *L. reuteri* transformed strains not only acquired the capacity to break down soluble CMC, β-glucan, or xylan, but also showed high adhesion efficiency to mucin and mucus, and resistance to bile salt and acid.

**Keywords***:* Cellulase; β-Glucanase; Xylanase; *Lactobacillus reuteri*

## INTRODUCTION

Animal agriculture relies heavily on antibiotics, both for treatment of diseases and for growth promotion. With increasing public concerns associated with antibiotic resistance, the ban on subtherapeutic antibiotic usage in Europe and the potential for a ban in other regions of the world, there is increasing pressure to reduce the use of antibiotics in feed. Addition of probiotics to feed is one of the alternatives to be used as a replacement for antibiotics. There is sufficient evidence to show that probiotics are effective in enhancing the immune system, increasing body weight gain, reducing diarrhea, and improving feed conversion efficiency (Patterson and Burkholder 2003). The currently used or researched probiotics are mostly selected from native gut microflora and the selection of optimal strains is often been largely empirical. It has been speculated that more efficacious probiotics can be developed through genetic modification (Steidler 2003).

Cereals, such as barley, wheat, rye and oats, are major feed components for monogastric animals. The cell walls of cereals are primarily composed of carbohydrate complexes referred to as nonstarch polysaccharides (NSP), which include β-glucans in barley and wheat, and arabinoxylans in rye and oats (Englyst et al. 1989). It has been demonstrated that the anti-nutritive effect of NSP is related to their low digestibility and their propensity to form high molecular weight viscous aggregates in the gastrointestinal tract (Choct and Annison 1992). This reduces the rate of passage, decreases diffusion of digestive enzymes, promotes endogenous losses, and stimulates bacterial proliferation (Bedford and Schulze 1998). Therefore, it is not surprising that addition of specific enzymes such as xylanase or β-glucanase into wheat- or barley-based diets for non-ruminant animals decreases viscosity and consequently reduces the anti-

nutritional effect of NSP, leading to better production performance (Mathlouthi et al. 2003). Enzyme supplementation increases the cost of feed and is only used for a short-term solution in enhancing digestion of cereals. An alternative and less expensive strategy might be to develop probiotics with the capacity to digest plant cell wall structural carbohydrates by introduction of heterologous genes encoding fibrolytic enzymes (Cho et al. 2000).

In the present study, we describe the cloning and expression of the rumen microbial xylanase, β-glucanase, and cellulase genes in a strain of *Lactobacillus reuteri* isolated from the gastrointestinal tract of healthy broilers. We also examined the probiotic characteristics such as adherence capability, acid tolerance, and bile-salt tolerance of these genetically modified *L. reuteri* strains.

## MATERIALS AND METHODS

**Construction of xylanase, β-glucanase, and cellulase expression plasmids.**

The DNA sequences encoding *Neocallimastix patriciarum* xylanase gene *xynCDBFV* (GenBank accession number AF123252), *Fibrobacter succinogenes* β-glucanase gene (GenBank accession number M33676), and *Piromyces rhizinflata* cellulase gene *eglA* (GenBank accession number AF094757) were amplified by PCR, respectively. The PCR fragments encoding xylanase, β-glucanase, and cellulase were ligated into pNZ3004 (van Rooijen et al. 1992) to generate pNZJ021 (xynCDBFV), pNZJ023 (glu), and pNZJ068 (eglA), respectively.

**Transformation of plasmid DNA**

The *E. coli* transformants were selected on LB agar plates containing erythromycin (100 μg/mL). Plasmids expressing the desired enzyme activity in *E. coli* were electroporated into *L. reuteri* Pg4 as described by Serror et al. (2002). After electroporation, the *L. reuteri* Pg4 transformants were spread on MRS agar plates containing erythromycin (10 μg $mL^{-1}$) and incubated at 37°C until the appearance of transformants.

**Xylanase, β-glucanase and CMCase production by *Lactobacillus reuteri* Pg4 transformed strains.**

The xylanase, β-glucanase, and CMCase activity of each *L. reuteri* cell culture were determined by measuring the amounts of reducing sugar liberated by culture supernatant or whole-cell extract fractions incubated with 0.5% (w $v^{-1}$) xylan, β-glucan, and CMC in 50 mM sodium citrate buffer (pH 5.0), respectively. The reducing sugar produced was estimated by the dinitrosalicyclic acid reagent (Konig et al. 2002). One unit of the fibrolytic enzyme activity was defined as that releasing 1 μmoL of reducing sugar equivalents per minute from the respective substrate under the assay conditions.

**In vitro adhesion assay.**

Both small intestinal mucus and gastric mucin were conjugated with horseradish peroxidase (HRP) by the method of Rojas and Conway (2001). Ninety-six-well microtiter plates (Nunc, Roskilde, Denmark) were coated with 200 μl of formaldehyde-killed *L. reuteri* cells. After blocked with 250 μl of blocking buffer (PBS containing 1% BSA), the plates were incubated at 37°C for 1 h with 200 μL of HRP-labeled mucin and mucus solution (1 μg $μL^{-1}$ of blocking buffer). Following three washes with PBST, 100 μL of TMB (Sigma) solution was added to each well. Finally, the absorbance of each well at a wavelength of 450 nm was measured.

**Tolerance to acidic pH and bile-salt.**

Acid and bile-salt tolerance of *L. reuteri* Pg4 strains was determined according to the method of Ehrmann et al. (2002).

## RESULTS AND DISCUSSION

**Cloning of rumen microbial xylanase, β-glucanase, and cellulase genes in *L. reuteri* Pg4.**

In the culture of *L. reuteri* pNZJ021 (xynCDBFV), more than 61 % of total xylanase activity was detected in the extracellular fraction (Table 1). In the culture of *L. reuteri* pNZJ023 (glu), approximately 62 % of β-glucanase activity was detected in the extracellular fraction (Table 1). Both β-glucanase, and CMCase activities were observed for *L. reuteri* pNZJ068 (eglA). More than 50 % of the total β-glucanase and CMCase activity of the culture of *L. reuteri* pNZJ068 (eglA) was present in the extracellular fraction (Table 1). No xylanase activity was detected from *L. reuteri* pNZJ068 (eglA) culture.

**Adhesion of *L. reuteri* Pg4 transformed strains to small intestinal mucus and gastric mucin.**

All *L. reuteri* Pg4 strains adhered efficiently to both pig gastric mucin and chicken small intestinal mucus. There was no significant difference between the adherence ability of *L. reuteri* Pg4 transformed strains and that of *L. reuteri* Pg4 wild type (Fig. 1). This result indicated that the introduction of a heterologous fibrolytic enzyme gene into *L. reuteri* Pg4 did not alter its adherence ability.

**Tolerance of *L. reuteri* Pg4 transformed strains to different pH values and growth with bile salt.**

The results on acid tolerance showed that approximately 35% or 50% of all the *L. reuteri* Pg4 strains tested survived after 180 min incubation at pH 2.0 and 3.0, respectively. There was no significant difference between the acid tolerance of *L. reuteri* Pg4 transformed strains and that of *L. reuteri* Pg4 wild type (Table 2).

As shown in Fig. 2, there was no significant difference between the viable counts of *L. reuteri* Pg4 and that of *L. reuteri* pNZ3004 after cultured in MRS broth containing 0.5% ox gall for 10 h. The bile-salt resistance of the other *L. reuteri* Pg4 transformed strains [*L. reutrei* pNZJ021 (xynCDBFV), *L. reutrei* pNZJ023 (glu), *L. reutrei* pNZJ068 (eglA)] was also similar to that of *L. reuteri* Pg4 wild type (data not shown). All of the *L. reuteri* Pg4 strains tested in this study survived at an incubation period of 10 h in MRS broth containing 0.5% ox gall.

To date, several previous studies have investigated the expression of bacterial β-glucanase genes in lactobacilli. Most of these studies described the genetic manipulation in *L. plantarum*, which is the primary bacterium used in silage fermentation. However, the level of the heterologous β-glucanase gene expressed in *L. plantarum* was usually low (Rossi et al. 2001; Scheirlinck et al. 1990; Scheirlinck et al. 1990). Only a few studies were focused on expression of bacterial β-glucanase genes in intestinal lactobacilli strains (Cho et al. 2000; Heng et al. 1997). To the best of our knowledge, however, expression of a xylanase gene in intestinal lactobacilli has not been reported previously. This is the first report of successful expression of xylanase in intestinal lactobacilli. In addition, both rumen fungal cellulase gene *eglA* and rumen bacterial β-glucanase gene were expressed in *L. reuteri*. Interestingly, the extracellular β-glucanase activity of *L. reuteri* pNZJ068 (eglA) was much higher than *L. reuteri* pNZJ023 (glu) or those in previous studies in which the bacterial β-glucanase gene was transformed into *Lactobacillus*.

## CONCLUSIONS

We not only cloned the rumen microbial xylanase, β-glucanase, and cellulase genes in *L. reuteri* Pg4 but also demonstrated that the introduction of these heterologous fibrolytic enzyme genes into cells did not affect their adherence to mucin and mucus, and tolerance to acid and bile salts. New studies for evaluating the ability of these *L. reuteri* Pg4 transformed strains to colonize the gastrointestinal epithelium and secrete the fibrolytic enzymes into gastrointestinal tract of chickens are now in progress.

**Table 1.** The activity of fibrolytic enzymes in transformants of *L. reuteri* Pg4.

| Strain | Enzyme activity (U $mL^{-1}$)[a] | | | | | |
|---|---|---|---|---|---|---|
| | Xylanase | | β-Glucanase | | CMCase | |
| | Intracellular | Extracellular | Intracellular | Extracellular | Intracellular | Extracellular |
| *L. reuteri* pNZ3004 | nd[b] | nd | nd | nd | nd | nd |
| *L. reuteri* pNZJ021 (xynCDBFV) | 1.48 ± 0.42 | 2.33 ± 0.24 | nd | nd | nd | nd |
| *L. reuteri* pNZJ023 (glu) | nd | nd | 1.03 ± 0.07 | 1.66 ± 0.23 | nd | nd |
| *L. reuteri* pNZJ068 (eglA) | nd | nd | 3.14 ± 0.75 | 3.15 ± 0.11 | 1.13 ± 0.16 | 1.36 ± 0.10 |

[a] Enzyme activity was defined as that releasing 1 μmoL of reducing sugar equivalents per minute from the respective substrate.
[b] Not detectable.

**Table 2.** Survival of *L. reuteri* Pg4 strains after incubation at various pH values

| Strain | Survival (%) after incubation at | | | | | |
|---|---|---|---|---|---|---|
| | pH 2.0 | | | pH 3.0 | | |
| | 30 min | 60 min | 180 min | 30 min | 60 min | 180 min |
| *L. reuteri* Pg4 | 83.2 ± 0.6 | 53.6 ± 6.2 | 34.1 ± 0.5 | 87.0 ±1.7 | 73.3 ± 1.3 | 53.5 ± 1.7 |
| *L. reuteri* pNZ3004 | 83.9 ± 1.4 | 57.0 ± 3.3 | 35.4 ± 1.6 | 86.8 ± 3.8 | 74.9 ± 3.5 | 55.8 ± 3.1 |
| *L. reuteri* pNZJ021 (xynCDBFV) | 81.8 ± 2.3 | 55.4 ± 3.4 | 34.6 ± 3.8 | 86.4 ± 2.9 | 73.3 ± 0.8 | 54.3 ± 3.2 |
| *L. reuteri* pNZJ023 (glu) | 82.6 ± 2.5 | 53.5 ± 0.5 | 35.8 ± 1.9 | 86.3 ± 1.6 | 72.9 ± 2.3 | 53.1 ± 1.9 |
| *L. reuteri* pNZJ068 (eglA) | 81.9 ± 2.9 | 55.5 ± 1.3 | 35.7 ± 2.4 | 86.6 ± 0.9 | 71.2 ± 1.8 | 52.1 ± 2.5 |

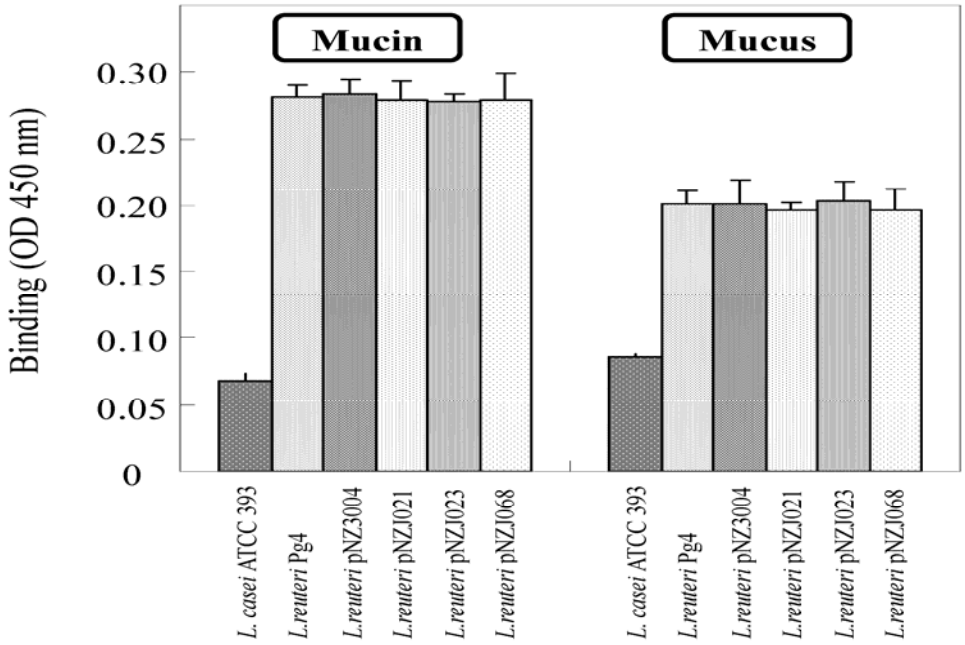


**Fig. 1.** Adhesion of *L. reuteri* Pg4 strains to immobilize porcine gastric mucin or chicken small intestinal mucus.

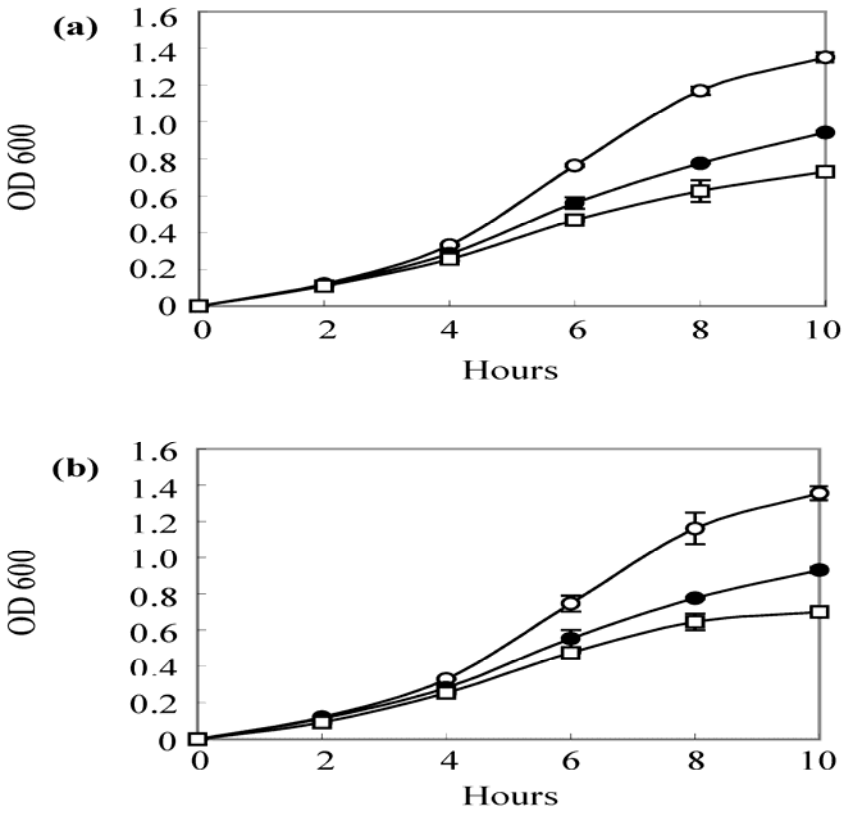


**Fig. 2.** Growth of lactobacilli cultured in MRS medium (○), MRS containing 0.3 % oxgall (●) and MRS containing 0.5 % oxgall (□). (a) *L. reuteri* Pg4; (b) *L. reuteri* pNZ3004.

## REFERENCES


Bedford, M. R. and Schulze, H. 1998. Exogenous enzymes for pigs and poultry. Nutr. Res. Revs. 11: 91-114.

Cho, J. S., Choi, Y. J. and Chung, D. K. 2000. Expression of *Clostridium thermocellum* endoglucanase gene in *Lactobacillus gasseri* and *Lactobacillus johnsonii* and characterization of the genetically modified probiotic *Lactobacilli*. Curr. Microbiol. 40: 257-263.

Ehrmann, M. A., Kurzak, P., Bauer, J. and Vogel, R. F. 2002. Characterization of lactobacilli towards their use as probiotic adjuncts in poultry. J. Appl. Micorbiol. 92: 966-975.

Englyst, H. N., Bingham, S. A., Runswick, S, A., Collinson, E. and Cummings, J. H. 1989. Dietary fibre (non-starch polysaccharides) in cereal products. J. Hum. Nutr. Diet. 2: 253-271.

Heng, N. C. K., Jenkinson, H. F. and Tannock, G. K. 1997. Cloning and expression of an endo-1,3-1,4-β-glucanase gene from *Bacillus macerans* in *Lactobacillus reuteri*. Appl. Environ. Microbiol. 63: 3336-3340.

Konig, J., Grasser, R., Pikor, H. and Vogel, K. 2002. Determination of xylanase, β-glucanase, and cellulase activity. Anal. Bioanal. Chem. 374: 80-87.

Mathlouthi, N., Mohamed, M. A. and Larbier, M. 2003. Effect of enzyme preparation containing xylanase and β-glucanase on performance of laying hens fed wheat/barley- or maize/soybean meal-based diets. Brit. Poultry Sci. 44: 60–66.

Patterson, J. A. and Burkholder, K. M. 2003. Application of prebiotics and probiotics in poultry production. Poult. Sci. 82: 627-631.

Rojas, M. and Conway, P. L. 2001. A dot blot assay for adhesive components relative to probiotics in microbial growth in biofilms. Methods Enzymol. 336: 389-402.

Rossi, F., Rudella, A., Marzotto, M., and Dellaglio, D. 2001. Vector-free cloning of a bacterial endo-1,4-β-glucanase in *Lactobacillus plantarum* and its effect on the acidifying activity in silage:use of recombinant cellulolytic *Lactobacillus plantarum* as silage inoculant. Antonie van Leeuwenhoek 80: 139-147.

Scheirlinck, T., Mahillion, J., Joos, H., Dhaese, P. and Michiels, F. 1990. Integration and expression of α-amylase and endoglucanase genes in the *Lactobacillus plantarum* chromosome. Appl. Environ. Microbiol. 55: 2130-2137.

Scheirlinck, T., de Meutter, J., Arnaut, G., Joos, H., Claeyssens, M. and Michiels, F. 1990. Cloning and expression of cellulase and xylanase genes in *Lactobacillus plantarum*. Appl. Microbiol. Biotechnol. 33: 534-541.

Serror, P., Sasaki, T., Ehrlich, S. D. and Maguin, E. 2002. Electrotransformation of *Lactobacillus delbrueckii* subsp. *Bulgaricus* and *L. delbrueckii* subsp. *Lactis* with various plasmids. Appl. Environ. Microbiol. 68: 46-52.

Steidler, L. 2003. Genetically engineered probiotics. Best Pract. Res. Cl. Ga. 17:861-876.

van Rooijen J. R., Gasson, M. J. and de Vos, W. M. 1992. Characterization of the *Lactococcus lactis* lactose operon promoter: contribution of flanking sequences and LacR repressor to promoter activity. J. Bacteriol. 174: 2273-2280.

# Factors affecting the activation of porcine oocytes matured in vitro during micromanipulation by the Piezo-electric device

Y. Bing[1], T. Nagai[1] and X. Zhao
[1]National Institute of Livestock and Grassland Science, Ibanaki, Japan

## ABSTRACT

Direct injection of a somatic cell nucleus into the cytoplasm of matured oocytes by the Piezo-electric device is a method of nuclear transfer. However, some factors resulting in the premature activation of oocytes during micromanipulation will affect the success rate. In this study, we evaluated the effects of $Ca^{2+}$ in the operation media and oocyte maturation periods on the development of nuclear transferred embryos. The extracellular $Ca^{2+}$ in the operation media augmented oocyte activation at manipulation. The nuclear remodelling and developmental competence of reconstructed oocytes matured for 38 h after nuclear transfer were significantly lower compared to those matured for 44 h. These results demonstrate that removal of $Ca^{2+}$ from the media and using complete matured oocytes as recipient cytoplasts would improve the in vitro development of nuclear transferred embryos.

**Keyword**: Cloning, oocytes, porcine

## INTRODUCTION

Somatic cell cloning has been successful in many animals and most of them were generated by the cell fusion method. Recently, direct injection of a somatic cell nucleus into the cytoplasm of matured oocytes by the Piezo-electric device as a method of nuclear transfer has been developed. The live cloned mice (Wakayama et al. 1998; Ogure et al. 2000) and piglet (Onishi et al. 2000) had been produced by this method. However, the overall efficiency of live offspring obtained in all reports regardless of either method is < 5%.

The protocol of nuclear transfer usually includes two micromanipulation processes: enucleation and injection. When donor nuclei are exposed to the cytoplasm of the non-activated oocyte for a few hours, nuclear envelope breakdown (NEBD) and premature chromosome condensation (PCC) occur under the high activity of maturation promoting factor (MPF). On the other hand, exposing donor nuclei to the cytoplasm of the activated oocytes, NEBD and PCC do not happen, but DNA replication might occur (Campbell et al 1996). Thus, methods which induce NEBD and PCC in reconstructed oocytes can increase developmental potential and may enhance reprogramming of gene expression of the donor cells in recipient oocytes (Cibelli et al. 1998). "Premature" activation of oocytes before or during nuclear transfer, however, might abolish this advantage. In addition, the elevated cytoplasmic $Ca^{2+}$ levels influence the activity of the MPF (Hashimoto and Kishimoto 1988) and result in oocyte activation (Machaty et al. 1996). During the procedure of micromanipulation, intracellular $Ca^{2+}$ might increase due to the entry of calcium ions from extracellular space caused by cell impalement, or injection of a small amount of calcium containing medium together with somatic nuclei. The objective of this study was to search the optimal oocyte maturation and microinjection conditions by the Piezo-electric device to reduce porcine oocyte activation before nuclear transfer.

## MATERIALS AND METHODS

In vitro maturation (IVM) of porcine oocytes and preparation of cumulus cells as donor cells were described in our previous study (Bing et al. 2000). After maturation, oocytes at metaphase II (M II) stage were used for micromanipulation. Selected oocytes were transferred to the microinjection solution (TL-Hepes) with or without $Ca^{2+}$ and 5

μg $mL^{-1}$ cytochalasin B (CB), and zona pellucida and cytoplasmic membrane were punctured by the injection pipette. After microinjection, oocytes were cultured in mNCSU23 with or without $Ca^{2+}$ for different periods according to different experimental designs, and then fixed and stained using 25% acetic alcohol and 1% acteoorcein, respectively. The chromosome changes and pronuclear formation were investigated.

**Experiment 1: Effects of extracellular $Ca^{2+}$ from both media (TL-Hepes and mNCSU23) on the activation of porcine oocytes during micromanipulations.**

Oocytes matured for 44 h were punctured by the injection pipette in TL-Hepes with or without $Ca^{2+}$. Then oocytes were incubated in mNCSU23 with or without $Ca^{2+}$ for additional 2 h before the zona pellucida and membrane in oocytes were punctured again with a small amount of TL-Hepes injected into cytoplasm. Two hours after the second microinjection, oocytes were assessed for activation.

**Experiment 2. Effects of maturation periods on the activation of oocytes during microinjection, and the remodelling of cumulus nuclei in reconstructed oocytes after nuclear transfer.**

The oocytes matured for 38 or 44 h were punctured by the pipette in TL-Hepes without $Ca^{2+}$. Then oocytes were incubated in mNCSU23 without $Ca^{2+}$ for 2 h. Afterwards, oocytes were punctured again and then cultured for an additional 2 h before assessing the activation of oocytes. In another experiment, the polar body and the M II plate with small amount of cytoplasm of oocytes in each group were enucleated and incubated for 2 h. Then, the cumulus cells were injected into the cytoplasm of oocytes and cultured for an additional 2 h. The remodelling of cumulus nuclei in transferred oocytes was assessed.

**Experiment 3. Effects of maturation periods on the development of reconstructed embryos after microinjection of cumulus cells into the cytoplasm of oocytes.**

After cumulus cells were injected into the cytoplasm of oocytes matured for 38 or 44 h, reconstructed oocytes were incubated in mNCSU23 with 4 mg/ml BSA and 0.1 mg/ml cysteine for 30 min, and then they were activated by exposure to 200 μM thimerosal for 10 min, followed by incubation in mNCSU23 containing 8 mM dithiothreitol (DTT) for 30 min (Machaty et al. 1997). The reconstructed oocytes were cultured in mNCSU23 for 6 days. Some oocytes matured for 38 or 44 h were simply activated parthenogenetically by thimerosal and DTT and cultured as controls. The developmental competence and reconstructed embryos and cell number of blastocysts were assessed.

## RESULTS

**Experiment 1**

As shown in Table 1, the rates of activated oocytes or activated oocytes with PN after the second penetration were significantly lower when oocytes were operated in both microinjection solution (TL-Hepes) and culture medium (mNCSU23) without $Ca^{2+}$ in comparison with other two groups. Thus, $Ca^{2+}$ in the operation media affected the percentage of oocyte activation during micromanipulation.

**Experiment 2**

Oocytes matured for 38 or 44 h were used to test the effects of maturation periods on the activation of oocytes after microinjection and remodelling of cumulus nuclei in oocytes after nuclear transfer. There was a significantly higher activation rate in oocytes matured for 44 h after microinjections in comparison with oocytes matured for 38 h (30.6% vs. 21.0%, $P < 0.05$) (Table 2). The nuclear remodelling at 2 h after directly injecting cumulus cells into enucleated oocytes was significantly different between oocytes matured for 38 or 44 h (41.9% vs. 63.3%, $P < 0.05$, respectively). The rates of non-changed cumulus at 2 h after injection were 58.1% vs. 36.7% ($P < 0.05$) for oocytes matured for 38 or 44 h, respectively. The cumulus cells did not further develop to pronuclear structure stage after injecting cumulus nuclei into 38 h matured oocytes (Table 3).

**Experiment 3**

As shown in Table 4, the rate of cleaved oocytes matured for 38 h in parthenogenetic activation (control) group was significantly lower than that of oocytes matured for 44 h (62.1% vs. 83.7%, $P < 0.05$, respectively). However, no differences in nuclear transfer (NT) group (36.4% vs. 28.5%). There was a tendency for increased blastocyst formation using oocytes matured for 44 h in both control and NT

groups in comparison with using oocytes matured for 38 h (10.9% vs. 1.3% and 5.5% vs. 0).

## DISCUSSION

The present study evaluated that effect of $Ca^{2+}$ in the media and oocyte maturation periods on the activation of oocytes during manipulation, nuclear remodelling and subsequent development of nuclear transferred embryos. The results indicated that extracellular $Ca^{2+}$ in the operation media augmented premature oocyte activation during micromanipulation. In addition, the nuclear remodelling and developmental competence of reconstructed oocytes matured for 38 h after nuclear transfer were significantly lower compared to those of 44 h matured oocytes, although its activation rate was also lower during microinjection.

In several studies on successful production of cloned animals, exposure to the cytoplasm of non-activated oocytes was crucial for the remodelling of the differentiated somatic nuclei (Cibelli et al. 1998; Tani et al. 2001; Dominko et al. 1999). In the protocols of nuclear transfer, nuclei of recipient oocytes were removed before fusion or injection of donor nuclei. The $Ca^{2+}$ from the external medium during microinjection causes oocyte activation, however, this activation is insufficient for porcine oocytes to develop to term (Machary et al. 1996). When transferring somatic nuclei into non-activated oocytes, NEBD and PCC occurred due to active MPF in the cytoplasm. When transferring into activated oocytes with decline MPF activity, the membranes and chromosomes of donor cells remain intact and decondensed, respectively. These results indicated that the cytoplasm of activated oocytes was inefficient for nuclear remodelling. Maintenance of recipient oocytes at non-activation might play an important role in the nuclear reprogramming. Our results in this study demonstrated that removal of $Ca^{2+}$ in the media decreased oocyte activation during micromanipulation.

Remodelling of an exogenous nucleus in M II oocyte involves the disassembly of the nucleus and the reconstitution of a new nucleus. The whole processes occur in the cytoplasm of oocytes. The cytoplasmic environment directs the extent of remodelling of transferred nuclei. Porcine oocytes reach the M II stage after IVM culture for 36 h (Yoshida et al. 1989). However, the developmental potential after IVF was significantly lower for oocytes matured for 36 h compared with oocytes cultured for 44 h (Grupen et al. 1997) due to the immaturity of the metaphase spindle and/or inadequate cytoplasmic maturation. Our previous study indicated that the developmental potential of oocytes cultured for 48 h was higher than that cultured for 36 h after the activation treatment (Bing et al. 2002). Taking together, these results suggest that the complete nuclear and cytoplasmic maturation of oocytes need additional culture after reaching the M II stage.

In conclusion, the present study demonstrated that removal of $Ca^{2+}$ in the media could decreases oocyte activation during micromanipulation. However, although the activation rate of the newly matured oocytes was lower during manipulation, the nuclear remodelling and development competence of reconstructed oocytes was also lower compared with those cultured for 44 h. It is important to use complete matured oocytes as recipient cytoplasts would improve the in vitro development of nuclear transferred embryos.

**Table 1.** Effect of $Ca^{2+}$ in the TL-Hepes and mNCSU23 on the activation of porcine oocytes after microinjection

| Injection Solution | Culture medium | No. of oocytes | No (%). of oocytes* | | | |
|---|---|---|---|---|---|---|
| | | | with M II | activated | with PCC | with PN |
| W Ca2+ | WCa2+ | 83 | 33 (39.8)[a] | 50 (60.2)[a] | 21 (25.3) | 29 (34.9)[a] |
| W/O Ca2+ | W Ca2+ | 85 | 42 (49.4)[a] | 43 (50.6)[a] | 16 (18.8) | 27 (31.8)[a] |
| W/O Ca2+ | W/O Ca2+ | 58 | 42 (72.4)[b] | 16 (27.6)[b] | 11 (19.0) | 5 (8.6)[b] |

*values with different superscripts differ ($P < 0.05$).

**Table 2.** Effect of maturation ages on the activation of porcine oocytes at microinjection

| Maturation periods (h) | No. of oocytes used | No (%). of oocytes activated* | No. of oocytes with PN |
|---|---|---|---|
| 38 | 95 | 20 (21.0)[a] | 1 |
| 44 | 85 | 26 (30.6)[b] | 4 |

*values with different superscripts differ ($P < 0.05$).

**Table 3.** Effect of culture periods on the remodelling of cumulus nuclei in nuclear transferred oocytes matured in vitro for 38 or 44 h

| Maturation | No. of oocytes | No. (%) of oocytes* with | | |
|---|---|---|---|---|
| periods (h) | examined | PCC | PN or like-PN | non-changed cumulus |
| 38 | 31 | 13 (41.9) | 0 | 18 (58.1)[a] |
| 44 | 30 | 7 (23.3) | 12 (40.0) | 11 (36.7)[b] |

* values with different superscripts differ ($P < 0.05$).

**Table 4.** Development of reconstructed embryos after microinjection of cumulus cells into the cytoplasm of oocytes matured in vitro for 38 or 44 h

| Maturation | No. of oocytes | No (%) of oocytes | No (%). of blastocysts | No. of nucleus |
|---|---|---|---|---|
| Periods (h) | used | cleaved* | formation** | per blastocys |
| 38 (control) | 74 | 46 (62.1)[b] | 1 (1.3) | 21.0 |
| 38 (NT) | 42 | 12 (28.5)[c] | 0 (0.0) | |
| 44 (control) | 92 | 77 (83.7)[a] | 10 (10.9) | 30.3 (26-33) |
| 44 (NT) | 55 | 20 (36.4)[c] | 3 (5.5) | 19.3 (8-28) |

*at 48 h after injection; **at 6 days after injection; *** values with different superscripts differ ($P < 0.05$).

## REFERENCES


Bing, Y. Z., Che, L. M., Hirao, Y., Takenouchi, N., and Nagai, T. 2000. Development of porcine embryos reconstructed by nuclear transfer of cultured cumulus cells into in vitro matured and enucleated oocytes. J. Reprod.. Dev. 46: 375-379.

Bing, Y. Z., Che, L. M., Hirao, Y., Takenouchi, N., Kuwayama, M. and Nagai, T. 2002. In vitro development of porcine oocytes activated by electric pulse: effect of maturation culture period. Cloning and Stem cell 3: 209.

Campbell, K. H. S., Loi, P., Otaegui, P. J. and Wilmut I. 1996. Cell cycle coordination in embryo cloning by nuclear transfer. Rev Reprod. 1: 40-45.

Cibelli, J. B., Stice, S. L., Golueke, P. J., Kane, J. J., Jerry, J., Blackwell, C. *et al.* 1998. Cloned transgenic calves produced from nonquiescent fetal fibroblasts. Science 280: 1256–1258.

Dominko, T., Mitalipova, M., Harley, B., Beyhan, Z., Memili, E., McKusick, B. and First, N. L. 1999. Bovine oocyte cytoplasm supports development of embryos produced by nuclear transfer of somatic cell nuclei from various mammalian species. Biol. Reprod 60: 1496-1502.

Grupen, C. G., Nagashima, H. and Nottle, M. B. 1997. Asynchronous meiotic progression in porcine oocytes matured in vitro: a cause of polyspermic fertilization? Reprod. Fertil. Dev. 9: 187-191.

Hashimoto, N. and Kishimoto, T. 1988. Regulation of meiotic metaphase by a cytoplasmic maturation promoting factor during mouse oocytes maturation. Dev Biol. 126: 242-252.

Machaty, Z., Funahashi, H., Mayes, M. A., Day, B. N. and Prather, R. S. 1996. Effects of injecting calcium chloride into in vitro matured porcine oocytes. Biol. Reprod 54: 316-322.

Machaty, Z., Wang, W. H., Day, B. N. and Prather, R. S. 1997. Complete activation of porcine oocytes induced by the sulfhydryl reagent, thimerosal. Biol. Reprod., 57: 1123 - 1127.

Ogura, A., Inoue, K., Ogonuki, N., et al., 2000. Production of male cloned mice from fresh, cultured, and cryopreserved immature sertoli cells. Biol. Reprod. 62: 1579-1584.

Onishi, A., Iwamoto, M., Akita, T., Mikawa, S., Takeda, K., Awata T., *et al.*, 2000. Pig cloning by micro-injection of fetal fibroblast nuclei. Science 289: 1188–1190.

Tani, T., Kato, Y. and Tsunoda, Y. 2001. Direct exposure of chromosomes to nonactivated ovum cytoplasm is effective for bovine somatic cell nucleus reprogramming. Biol. Reprod. 64: 324-330.

Wakayama, T., Perry, A. C. F., Zuccotti, M., Johnson, K. R. and Yanagimachi, R. 1998. Full-term development of mice from enucleated oocytes injected with cumulus cell nuclei. Nature 394: 369–374.

Yoshida, M., Bamba, K. and Kojima, Y. 1989. Effects of gonadotropins and estradiol-17 β on the timing of nuclear maturation and cumulus mass expansion in pig oocytes cultured in vitro. Jpn. J. Anim. Reprod. 35: 86-91.

# Effects of vitamin E and arginine on cardiopulmonary function in broilers.

G.A. Lorenzoni and C.A. Ruiz-Feria

**ABSTRACT**

Pulmonary hypertension (PH) is characterized by elevated production of free radicals and reductions in pulmonary artery relaxation. Supplemental Vitamin E (VE) reduces oxidative stress and Arginine (Arg) supplementation improves pulmonary capacitance, but its effects on reducing PH have not been consistent. The objective of this experiment was to evaluate the effect of Arg, VE, and its combination on cardiopulmonary response to an Epinephrine challenge (Epi). One d-old male broilers were fed a commercial corn-soybean meal based diet (1.2% Arg and 40 IU VE $kg^{-1}$). Birds were provided tap water (CTL), water with 0.3% Arg (HArg), water with 400 IU VE $L^{-1}$ (HVE), or a combination of both (AR-VE). At d 18, temperature was reduced to 16°C to amplify the incidence of PH. BW and hematocrit were recorded weekly throughout the experiment. From d 28 to d 42, pulmonary arterial pressure (PAP), mean arterial pressure (MAP), and heart rate (HR) were measured in clinically healthy anesthetised birds (n= 6 per treatment). After cannulation birds were allowed to recover for 10 min (basal), an IV injection of Epi was applied (1 mg $kg^{-1}$ BW), and a second dose was applied 20 min later. PAP, MAP, and HR were continually recorded and analyzed. Right/Total ventricular weight ratio (RV/TV) was recorded at the end of the experiment. No differences among treatments for BW, hematocrit or RV/TV were found. PAP peaked ($P < 0.01$) within 30 s after Epi in all treatments (23.9±1.1, 31.0±1.4 mmHg, basal and peak, respectively), and returned to basal levels within 60 s and 120 s after peak in the CTL, HArg and HVE groups, but it took more than 5 min for the AR-VE group to return to basal levels. In the second challenge, PAP peaked within 30 s for all treatments, HArg group returned to basal values within 180 s but it took 300 s for the other groups to return to basal levels. These results suggest that water soluble VE may not improve the effects of Arg on pulmonary arterial relaxing capacity.

**Keywords**: Arginine, pulmonary hypertension, vitamin E

## INTRODUCTION

Modern broilers achieve the market weight in 60% or less time than its ancestor, nevertheless its pulmonary and cardiac capacity remains very similar, which makes the cardiopulmonary system work close to its physiological limit. Thus, Pulmonary hypertension syndrome (PHS, ascites) is a common problem in poultry farms producing considerable losses due mortality. PHS starts with any increase in metabolic rate which implies a higher oxygen requirement. As a consequence a higher cardiac output is propelled through a non compliant pulmonary system, increasing PAP and right ventricle work. Increased PAP leads to right-sided congestive heart failure, central venous congestion and liver cirrhosis. By reducing pulmonary vascular resistance, it is possible to decrease the PAP needed to propel the cardiac output that matches with the metabolic demands (Wideman, 1995). Nitric oxide (NO) is a potent endogenous pulmonary vasodilator. NO is produced in the pulmonary endothelium using L-arginine as a substrate (McQueston et al, 1993). L-arginine is an essential amino acid in avian species, so it has to be added in the diet. There is evidence showing that L-arginine

levels supporting maximal growth rate are not enough for maximal NO production (Dietert et al., 1994). Supplemental L-arginine has helped in reducing cool temperature induced-ascites mortality (Wideman et al., 1995). Oxidative stress is also involved in the pathophysiological progression to ascites. Increased levels of $\cdot O_2^-$ . cause a loss of NO bioavailability leading to decrease endothelial vasodilation. Furthermore, the reaction of $\cdot O_2^-$ with NO leads to the production of peroxynitrite, a potent oxidant agent responsible for tissue injury (Beckman et al., 1990). Vitamin E is an antioxidant molecule that could help reducing oxidative stress in lung vessels decreasing in this way the endothelial damage. The combined effects of Arginine and vitamin E on PHS have not been studied. It is hypothesized that both components may have a synergistic effect on pulmonary vasodilation and endothelial integrity.

## MATERIALS AND METHODS

100 d-old Cobb male broilers were randomly distributed in 4 environmental-controlled chambers. Standard brooding temperatures were used during weeks 1, 2 and the beginning of the third week. At day 18$^{th}$ temperature was reduced from 27°C to 16°C to amplify the incidence of PHS. Birds were provided tap water (CTL), water with 0.3% Arg (HArg), water with 400 IU VE $L^{-1}$ (HVE), or water containing both compounds (AR-VE). Feed and water were provided ad-libitum. Birds were fed a commercial corn-soybean meal based diet (1.2% ARG and 40 IU VE $kg^{-1}$) meeting or exceeding the NRC (1994) requirements. BW and hematocrit were recorded weekly throughout the experiment. From day 28 to day 42, clinically healthy birds were selected for evaluation of cardiopulmonary performance (n= 8 per treatment). Birds were anesthetised by injecting Ketamine (70mg $kg^{-1}$ IM.) and Xylasine (4 mg $kg^{-1}$ IM.) and restrained in dorsal recumbency. Brachial artery was cannulated with a heparinized PE-50 polyethylene tubing. Brachial vein was cannulated using heparinized Silastic® tubing (0.012 I.D X 0.025 E.D), the proximal end was advanced though the vein until it reached the pulmonary artery. Distal ends of both tubing were attached to a blood pressure transducer interfaced with a Trasbridge preamplifier to a Biopac MP100 system for the continuous measurement of MAP, PAP and HR.

Once the birds were cannulated they were allowed to recover for 10 min. During this time three sampling points were taken at 1, 2 and 3 min (basal levels) previous to an IV. Epinephrine injection (Epi, 1 mg $kg^{-1}$ BW). The PAP, MAP, and HR response was determined at 0.5, 1, 2, 3, 5, 10 and 20 min after Epi. After the 20 min recovery period a second Epi challenge was applied and the same points described for the first challenge were recorded.

After surgery, birds were killed by aesthetic overdose. Right/total ventricular weight ratio was recorded at the end of the experiment as an index of pulmonary hypertension. eNOS activity in isolated lung arteries was determined by the conversion of L-[$^{14}$ C] ARG to L-[$^{14}$ C] citrulline. Sampling points for PAP, MAP and HR were analyzed by repeated measures ANOVA.

## RESULTS AND DISCUSSION

Epinephrine acts on adrenoreceptors of vascular smooth muscle and exerts a strong vasonstrictive effect (Smith et al. 2000), increasing MAP and PAP. Nitric oxide is released when PAP is higher than normal, as a mechanism to protect the gas exchange area. It has been shown that dietary arginine reduces PAP in birds in which one pulmonary artery was occluded, presumably through the action of NO (Wideman et al. 1996). However, arginine supplementation does not reduce ascites mortality, and increases the production of free radicals (Zalba et al. 2001). In this experiment we measured the response of PAP and MAP to a challenge of epinephrine in birds fed high levels of arginine, the precursor of NO, and vitamin E, an antioxidant and free radical scavenger. In all four treatments PAP peaked ($P < 0.01$) within 30 s after Epi (23.9±1.1, 31.0±1.4 mmHg, basal and peak, respectively, Fig. 1). In the first Epi challenge, the PAP in Ctl group returned to basal (pre-Epi) levels within 60 sec, while the PAP in the VE, Arg and VE-Arg groups returned to basal levels within 120, 180 and 1200 sec respectively. After 20 min recovery from the first Epi challenge, PAP in the Arg group was lower than basal levels. In the second Epi challenge it took 5 min for PAP to return to basal levels in all groups except the Arg group that returned to basal levels within 3 min.

These results agree with previous reports indicating that Arg supplementation improves pulmonary arterial relaxation, however, water soluble vitamin E does not improves the response of arginine to vasodilation under acute challenges. The fact that the Arg-VE group had the longest recovery period after Epi challenge suggests that VE at the levels used in this experiment may have detrimental effects on pulmonary relaxation. Heart rate decreased and MAP increased after the Epi challenge in all treatments as expected and returned to basal values within 10 min with no appreciable differences among treatments. Body weight, hematocrit, and RV/TV ratio was not affected by dietary treatment. The in vitro activity of eNOS in isolated pulmonary arteries was low (0.35µg arginine conversion per min ±0.17) and not different among treatments. These results suggest that extra arginine improves pulmonary vasodilation by increasing eNOS substrate, and that water soluble vitamin E does not improve vascular relaxation capacity. Further research is needed to determine the oxidative status of lung tissue under these conditions .

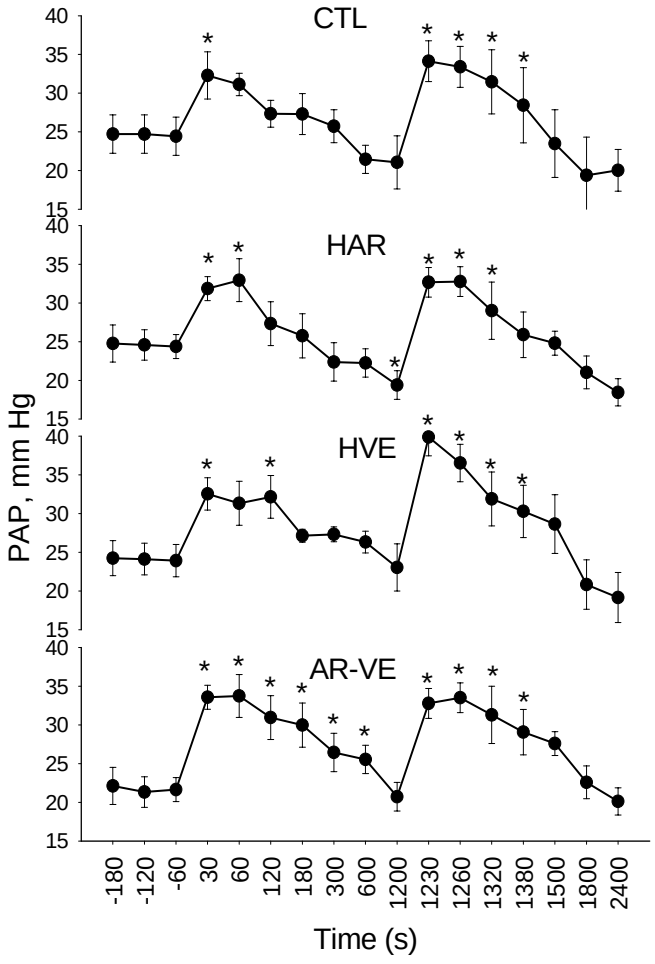


**Figure 1.** Pulmonary arterial pressure (PAP) after two epinephrine challenges (1 mg / kg BW) in male broilers.

## REFERENCES


Beckman J.S., Beckman T.W., Chen, J. Doan, R., Marshal P.A., Freeman B.A.,1990. Apparent hydroxyl radical production by peroxynitrite: implication for endothelial injury from nitric oxide and superoxide. Proc Natl Acad Sci 87: 1620-1624.

Dietert, R.R., R.E. Austic, 1994. Environment-immune interactions. Poultry Sci. 73:1062-1076.

McQueston, J.A., D.N. Cornfield, I. F. McMurtry and S.H. Abman, 1993. Effects of oxygen and exogenous L-arginine on EDRF activity in fetal pulmonary circulation. Am J.Physiol. 264: H865-H871.

Wideman,R. F., Kirby Y. K., Ismalil M., Bottje, W., Moore, R and Vardeman, R. 1995. Supplemental L-Arginine Attenuates Pulmonary Hypertension Syndrome (Ascites) in Broilers. Poultry Sci. 74: 323-330.

Wideman,R. F., Kirby, D. Tackett., N. E. Marson., C. J. Tressler and R. W. Mcnew. 1996. Independent and Simultaneous Unilateral Occlusion of the Pulmonary Artery and Extra-Pulmonary Primary Bronchus in Broilers. Poultry Sci. 75: 1417-1427.

Smith, F. M, N. H. West, and D. R. Johnes, 2000. The cardiovascular system. In: Sturkie's avian physiology. (Ed. G. C. Whittow). Academic press.

Ulrich Pohl and Cor de Wit.,1999. A Unique Role of NO in the control of Blood Flow. News Physiol. Sci. vol. 14, April. pp. 74-80.

Zalba,G., San Jose G., Moreno M. U.., Fortuno M.A., Fortuno A., Beaumont F. J. and Diez J. 2001. Oxidative stress in arterial hypertension: role of NAD(P)H oxidase. Hypertension 38: 1395-1399.

# Students who Graduated
# 2005-2006

| Name | Supervisor | Thesis Title |
|---|---|---|
| **M.Sc. Degree** | | |
| Gayatri Boda | K. Wade | Benchmarking dairy information using interactive visualization for dairy farm decision making |
| Gao Yongchun | K. Wade | The application of web ontology language for information sharing in the dairy industry |
| Ming-Kai Ho | D. Zadworny | Characterization of glycosylation of prolactin in galliformes |
| Karoline Lauzon | X. Zhao | Prevention of the neutrophil-induced mammary epithelial damage during bovine mastitis |
| Melanie Roy | X. Zhao | Identification and characterization of differentially expressed genes in response to *Escherichia coli* and *Staphylococcus aureus* in bovine mammary epithelial cells and mammary gland |
| **Ph.D. Degree** | | |
| Runhou Zhang | A.F. Mustafa | Manipulating fatty acids in sheep milk |

# Acknowledgements

The Department of Animal Science, Macdonald Campus, McGill University, wishes to thank the following for research grants and/or products used in conducting research in our Department.

Canadian Dairy Commission

Canadian Foundation for Innovation (CFI)

Canadian International Development Agency (CIDA)

Co-op Federée

Dairy Farmers of Canada

Faculty of Agricultural and Enviromental Sciences, McGill University

Faculty of Graduate Studies and Research, McGill University

Fédération des producteurs de bovines du Québec

Fonds Québécois de la recherche sur la nature et les technologies (FQRNT)

.

Natural Science and Engineering Research Council

Poultry Industry Council

Programme d'Analyse des Troupeaux Laitiers du Québec (PATLQ)

Saskatchewan Poultry Council Development Fund

Shaver Poultry Breeding Farms

William Dawson Fund, McGill University

# Publications

## Publications in Refereed Journals

**Burchard, J.F.**

Nguyen D.H., L. Richard.and **J. Burchard.** 2005. Exposure chamber for determining the biological effects of electric and magnetic fields on dairy cows. Bioelectromagnetics 26:1 38-144.

**Chavez, E.**

Shen, Y., Y. Yin, **E. R. Chavez** and M.Z. Fan. 2005. Methodological Aspects of Determination of Phytase Activity and Phytate Phosphorus Content with Selected Cereal Grains and Intestinal Samples of Pigs. J. Agr. Food Chem. 53 (4):853-859.

Shen, Y., D. Feng, M.Z Fan and **E.R Chavez**. 2005. Performance, carcass cut-up and fatty acids deposition of broilers fed different levels of pellet-processed flaxseed J. Sci. Food Agric. 85:2005-2014.

Shen, Y., D. Feng, T. F. Oresanya and **E.R. Chavez**. 2005. Fatty acid and nitrogen utilization of processed flaxseed by adult chickens. J. Sci. Food Agric. 85:1137-1142.

**Cue, R.I.**

Molento, C.F.M., E. Block, **R.I. Cue**, P. Lacasse and D. Petitclerc. 2005. Effects of insulin, recombinant bovine somatotrophin (rbST) and their interaction of DMI and milk fat production in dairy cows. Livestock Prod. Sci. 97. 173-182.

**Kimmins, S**.

Ruiz-Cortés, T., **S. Kimmins**, L. Monaco, K.L. Burns, P. Sassone-Corsi and B.D. Murphy. 2005. Estrogen receptor-alpha mediates estrogen-induced phosphorylation of histone H3 in ovarian follicle cells via the mitotic kinase, Aurora B. Molecular Endocrinology 19: 2991-3000.

Lapointe, J., **S. Kimmins**, L.A. MacLaren and J.F. Bilodeau. 2005. Estrogen selectively up-regulates the phospholipid hydroperoxide glutathione peroxidase (PHGPx) in the oviducts. Endocrinology 146: 2583-2592.

**Kimmins, S.** and P. Sassone-Corsi . 2005. Chromatin Remodeling and Epigenetic Features of Germ Cells. Nature. 434: 583-589.

**Kuhnlein, U.**

---------- See Zadworny, D.

**Monardes, H.G.**

Ribas, N.P., W. Hartmann, **H.G. Monardes** and U.V. Cotarelli de Andrade. 2004. Total milk solids in bulk tank samples of the States of Parana, Santa Catarina and Sao Paulo. R. Bras. Zootec. 33(6): 2343-2350. (*This paper was not included in the 2004-2005 report due to a delayed printing*).

**Mustafa, A.**

**Mustafa, A.F.**, P. Seguin and O. Marois-Mainguy. 2005. Ensiling characteristics, nutrient composition, and in situ ruminal and whole tract digestibilities of brown midrib and leafy corn silage. Arch. Anim. Nutr. 59:353-363.

Santschi, D.E., R. Berthiaume, J.J. Matte, **A.F. Mustafa** and C.L. Girard. 2005. Fate of supplementary B-vitamins in the gastrointestinal tract of dairy cows. J. Dairy Sci. 88:2043-2054.

Santschi, D.E., J. Chiquette, R. Berthiaume, J.J. Matte, **A.F. Mustafa** and C.L. Girard. 2005. Effects of methods of collection and sample preparation on the concentrations of B-vitamins in ruminal fluid of dairy cows. Can. J. Anim. Sci. 85:417-420.

Santschi, D.E., J. Chiquette, R. Berthiaume, R. Martineau, J.J. Matte, **A.F. Mustafa** and C.L. Girard. 2005. Effects of the forage to concentrate ratio on B-vitamin concentrations in different ruminal fractions of dairy cows. Can. J. Anim. Sci. 85:389-399.

Gonthier, C., **A.F. Mustafa**, R. Berthiaume, H. Petit and D. Ouellet. 2005. Effects of feeding extruded and micronized flaxseed on blood profile and milk fatty acid composition of dairy cows. J. Dairy Sci. 88:748-756.

Rizk, C., **A.F. Mustafa** and L. Phillip, L. 2005. Effect of inoculation of high dry matter alfalfa silage on ensiling characteristics, ruminal nutrient degradability and dairy cow performance. J. Sci. Food Agric. 85:743-750.

**Phillip, L.E.**

---------- See Mustafa, A.

**Wade, K.M.**

Pietersma, D., J.P.H. Holthuis, R. Lacroix and **K.M. Wade**. 2005. Interactive visualization of multi-dimensional data in dairy production. Applied Engineering in Agriculture 21 (6): 1081-1088.

**Zadworny, D.**

Kansaku, N., T. Ohkubo, H. Okabayashi, D. Guémené, U. Kuhnlein, **D. Zadworny** and K. Shimada. 2005. Duck prolactin: molecular and biochemical characterization. Gen. Comp. Endocrinol. 141: 39-47.

**Zhao, X.**

Lauzon, K., **X. Zhao**, A. Bouetard, L. Delbecchi, B. Paquette and P. Lacasse. 2005. Antioxidants to prevent bovine neutrophil-induced mammary epithelial cell damage. J. Dairy Sci. 88:4295-4303.

Liu, J-R., B. Yu, F-H. Liu, K-J. Cheng and **X. Zhao**. 2005. Expression of rumen microbial fibrolytic enzyme genes in probiotic lactobacillus reuteri. Appl. Environ. Microbiol. 71:6769-6775.

Gokavi, S., L. Zhang, M-KHuang, **X. Zhao** and M. Guo. 2005. Oats based symbiotic beverage containing L. plantarum, L paracasei and L. acidophilus. J. Food Science 70:M216-M223.

Lee, J.W., C.N. O'Brien, A.J. Guidry, M.J. Paape, K.A. Shafer-Weaver and **X. Zhao**. 2005. The effect of a trivalent vaccine against Staphylococcus aureus mastitis on lymphocyte subpopulations, antibody production and neutrophil phagocytosis. Can. J. Vet. Res. 69:11-18.

Tian, S.Z., C.J. Chang, M.C. Hsu, H.C. Peh, M.C. Huang; J-W. Lee and **X. Zhao**. 2005. Comparison of morphology, viability and functions between blood and milk neutrophils from peak lactating goats. Can. J. Vet. Res. 69:39-45.

Li, Y., Z. Yu, **X. Zhao** and S-H. Shen. 2005. Identification and Characterization of Hepsin/-TM, a non-transmembrane hepsin isoform. BBA-Gene Structure and Expression 1681:157-165.

Mehrzad, J., C. Desrosiers, K. Lauzon, K., G. Robitaille, **X. Zhao** and P. Lacasse. 2005. Proteases involved in mammary tissue damage during endotoxin-induced mastitis in dairy cows. J. Dairy Sci. 88:211-222.

**Publications Presented to Scholarly Conferences (Abstracts & Proceedings)**

**Bordignon, V.**

Che, L., A. Lalonde and **V. Bordignon**. 2005. The occurrence of phosphorylated histone H2A.X in porcine embryos produced by somatic cell nuclear transfer and pharthenogenetic activation. Biology of Reproduction (special issue): 671.

Lalonde, A., L. Che and **V. Bordignon**. 2005. Development of porcine embryos produced by nuclear transfer from somatic cells treated with protein synthesis and cyclin-dependent-kinase inhibitors. Biology of Reproduction (special issue): 704.

Bastos, G.M., P.B.D. Gonçalves and **V. Bordignon**. 2005. Development and DNA damage in parthenogenetically activated bovine embryos produced from oocytes derived from small and large follicles. Biology of Reproduction (special issue): 280.

**Hayes, J.F.**

Moro-Mendez, J. and **J.F. Hayes**. 2005. Detection of QTL affecting mastitis resistance traits and SCS in Canadian Holsteins. Presented at the 2005 ADSA/ASAS.CSAS Joint Annual Meeting, July 24-28, Cincinnati, OH. J. Dairy Sci. 88 (Suppl 1): 201.

**Kimmins, S.**

**Kimmins, S**., C. Crosio, N. Kotaja, J. Hirayama, C. Höög and P. Sassone-Corsi. 2005. Unique meiotic functions of the aurora-B kinase in mammalian spermatogenesis. Biology of Reproduction, Special Issue.

**Kuhnlein, U.**

**Kuhnlein, U.** 2005. Gene networks and the challenge of improving economic traits in poultry by DNA based selection. Poultry Breeder's Roundtable. St. Louis, May 5-6.

**Mustafa, A.F.**

Zhang, R., **A.F. Mustafa** and X. Zhao. 2005. Effects of feeding oilseeds on total tract nutrient utilization and milk composition of lactating ewes. J. Dairy Sci. 88:225 (suppl).

**Mustafa, A.** and P. Seguin,. 2005. Effects of variety on chemical composition and ruminal nutrient degradability of forage soybean. J. Dairy Sci. 88:385 (suppl).

**Ruiz-Feria, C.A.**

Lorenzoni, G.A. and **C.A. Ruiz-Feria**. 2005. Effects of Vitamin E and arginine on cardiopulmonary function in broilers. Poultry Sci. Suppl. 1: 87 (Abs. 223).

**Wade, K.M.**

Pietersma, D., R. Lacroix, R. Cue, D. Lefebvre and **K M. Wade**. 2005. Use of data mining to characterize dairy herds with poor heifer growth. Pages 1013-1018 in Proceedings of the Joint Conference of the 5th Conference of the European Federation for Information Technology in Agriculture, Food and Environment and the 3rd World Congress on Computers in Agriculture and Natural Resources. Vila Real, Portugal. CD-ROM communication n° (ISBN: 972-669-646-1 – Legal Deposit 228821/05) Publisher: Universidade de Trás-os-Montes e Alto Douro.

Fievez, V., J. Van Looveren, W. Wanzele, B. Vlaeminck, W. Van Straalen, **K.M. Wade** and R. Lacroix. 2005. Knowledge discovery in milk fatty acid databases applied to suboptimal milk production. Pages 1019-1023 in Proceedings of the Joint Conference of the 5th Conference of the European Federation for Information Technology in Agriculture, Food and Environment and the 3rd World Congress on Computers in Agriculture and Natural Resources. Vila Real, Portugal. CD-ROM communication n° (ISBN: 972-669-646-1 – Legal Deposit 228821/05) Publisher: Universidade de Trás-os-Montes e Alto Douro.

**Zhao, X.**

Tomita, G.M., B.G. Talbot, P. Lacasse, A.A Potter, **X. Zhao**, J. Lee and D. Scholl. 2005. Mastitis vaccine: past, present, and future. J. Dairy Sci. 88(Suppl.1):2.

**Zhao, X.** and P. Lacasse. 2005. Mammary tissue damage during mastitis: causes and controls. J. Dairy Sci. 88 (Suppl.1):2.

Scholl, D., G. Tomita, S. Messier, H. Barkema, P. Lacasse, **X. Zhao**, and É. Bouchard. 2005. A national research network to promote mastitis control in Canada. The 4th IDF International Mastitis Conference, 2005 Maastricht, the Netherlands.

Mehrzad, J., **X. Zhao** and C. Burvenich. 2005 Free radicals during E. coli mastitis: friends or foes? The 4th IDF International Mastitis Conference, 2005 Maastricht, the Netherlands.

Lee, J-W., M-K. Huang, M. Roy and **X. Zhao**. 2005. Identification of novel genes expressed in bovine mammary epithelial cells in response to Staphylococcus aureus cell wall components by differential display. The 4th IDF International Mastitis Conference, 2005 Maastricht, the Netherlands.

---------- See Mustafa, A.